A Field and Laboratory Study of the Epidemiology of Fascioliasis

(One Volume)

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ABSTRACT

The close association that exists between warm wet summers and large increases in the incidence of fascioliasis (a parasitic disease of sheep and cattle) has been recognised for several centuries.

Lymnaea truncatula, the intermediate host of the liver fluke, Fasciola hepatica, and all the free living stages of the parasite require wet soil surface conditions and adequate soil surface temperaures in order to survive, grow and develop. Several previous studies have demonstrated the general way in which the abundance of the parasite depends upon variations in spring and summer rainfall and the present annual "fluke forecast" is based upon the length of time that soil water content is maintained at field capacity during the warmer months. Variations in air temperature are largely ignored in the assessment of risk.

In the work reported here variations in the density of all the accessible stages of the parasite and its intermediate host were compared directly with variations in the microclimate at the soil surface of the habitat of L.truncatula. As expected, soil surface moisture was found to be of particular importance but the influence of variations in soil surface temperatures was also significant. It was found that the mean temperature experienced by organisms at the soil surface of the habitat was greater than the corresponding air temperatures

between April and October and, with the exception of the spring months when the desiccatory influence of soil surface temperatures was extreme, the habitats afford a relatively sheltered environment for both fluke and snail. There was no consistent relationship between fluctuations in general weather conditions and the microclimates of the habitats studied. This was attributed to the differences in topography, soil water and vegetation that exist between one habitat and the next.

The work reported here was intended to provide a comprehensive set of field data against which a coputer model of the epidemiology of fascioliasis could be tested. It was intended at least to describe the system being modelled and the thesis concludes with a speculative discussion concerning the stability of Fasciola populations in the field and the mechanisms which might regulate and control their density.

CHAPTER 1. FASCIOLIASIS - AN INTRODUCTION

1. FASCIOLIASIS

Fascioliasis is the name given to the disease caused by the Liver Fluke, Fasciola hepatica, a digenean endoparasite of herbivorous mammals. The intermediate host of the parasite throughout the Palaeartic region and the highland areas of N. Africa and the Middle East is the snail Lymnaea truncatula. (Wright, 1971).

Animals infected with adult flukes experience a range of symptoms depending upon the intensity of the infection. The flukes invade the liver capsule from the peritoneal cavity of the primary host, migrating through the parenchyma, sometimes in great numbers, until becoming established finally in the ducts of the biliary system. The symptoms of fascioliasis arise as a result of the parenchymal damage that occurs during the invasive phase and the subsequent disruption of normal liver function that accompanies the infestation of the bile ducts. The course of the disease in its two most common domesticated primary hosts, sheep and cattle, has been summarised by Sinclair (1962), Boray (1967), Sinclair (1971), Honer (1971) and Vink (1971).

The first symptoms in sheep are generally inappetance and lethargy. A reduction in live-weight gain has been reported though the evidence is equivocal. In cases where large numbers of flukes invade the liver capsule simultaneously there follows evidence of abdominal pain and ascites, the visible mucus membranes

become very pale and very little faeces is produced. The animals become emaciated and die between 6 and 10 weeks after the initial infection. In this form of the disease, known as acute fascioliasis, death is due to the profound anaemia and liver dysfunction that arises out of the extensive haemorrhage and parenchymal damage caused by the migration of the young flukes through the liver. More often though, the invasive phase involves small numbers of flukes over an extended period in which case the symptoms characteristic of the chronic form of fascioliasis become apparent. There is muscular weakness, oedema in the intermandibular space (the swelling known as "bottle jaw"), ascites, marked eosinophilia and severe anaemia. Scouring is sometimes seen and the wool lacks lustre and is easily pulled out. The untreated condition is progressive and death generally occurs after several months.

Fascioliasis in cattle is usually far less catastrophic. The resistance of these animals to infection with F. hepatica is well established (Ross 1966a,1967) and accounts for the vague symptomatology of bovine fascioliasis as compared with the dire manifestations of the disease seen in sheep, which have little if any resistance to invasion (Sinclair 1962). Severe infestations are rare in cattle: immunity may be only one reason. Vink (1971) points out that the immature flukes must travel a much greater distance in cattle than in sheep to reach the liver capsule.

Erratic flukes, e.g. in the lungs or uterus, occur more frequently in cattle than in sheep. In cattle it is the immature parasites that are important (Kendall and Parfitt, 1975). Major parenchymal damage occurs during the first 10 or 12 weeks of the invasive phase of a primary infection, many of the flukes are rejected at the adult stage and there is now an acquired resistance to subsequent challenge infections. The bile ducts become thickened and lined with calcium deposits leading to the typical "pipe-stem" liver and there is extensive fibrosis of the parenchyma. Acute fascioliasis is a disease of young cattle only; chronic disease often indicates a liver damaged during a primary infection rather than the continued presence of adult parasites.

A fatal concomitant of fascioliasis is black disease or infectious necrotic hepatitis. (Ollerenshaw, 1971). This is a bacterial infection caused by Clostridium oedematiens and may arise in the presence of even a few immature flukes. Losses from black disease generally occur slightly in advance of losses from acute fascioliasis.

2. THE ECONOMICS OF FASCIOLIASIS

There are three methods of controlling fascioliasis (Boray 1971).

(a) Eliminating the flukes from the primary host by antihelmintic treatment ("Dosing").

(b) Eliminating the intermediate hosts by draining the snail habitats or applying a molluscicide.

(c) Reducing the chances of infection by appropriate farm management. (Black disease can be prevented by vaccination).

Ollerenshaw and Rowcliffe (1961) found a general reluctance on the part of farmers to admit to losses from fascioliasis except in "bad fluke years" and noted that farmers were often unaware of the alternatives to closing stock even if fascioliasis was acknowledged to be a problem. Less than 10% of the farmers questioned by Ollerenshaw and Rowcliffe dosed their cattle and although in many cases sheep were dosed as a matter of routine the frequency of dose was usually inadequate to exert full control of the disease. A survey carried out by Reid (1973) over ten years later showed that attitudes to control measures had scarcely changed: less than 25% of the farmers questioned dosed their flocks adequately and cattle generally remained untreated.

Abbatoir surveys indicate that fascioliasis occurs over the whole of the United Kingdom and inadequate control measures have been estimated to lead to a total annual loss of £20 million. (Merck, Sharp and Dohme Research Laboratories - Technical Bulletin) Much of this financial loss occurs as a result of subclinical or chronic fascioliasis. The deterioration of wool quality ("wool break") in sheep has already been mentioned and though the evidence

for a reduction in mean live-weight gain is equivocal it may be that there is a reduction in food conversion efficiency as Hope-Cawdery and Conway (1971) have demonstrated in cattle. Affected store lambs or calves, for example, would take longer to reach their market weight so destroying their profit margin. Donker (1971) found that dairy cattle routinely treated with flukicide produced more milk than untreated animals. Ross (1970a) estimated that subclinical infections in dairy cattle resulted in an 8% reduction in milk yield, while Black and Froyd (1972) showed that infestations of F. hepatica reduce milk quality. On intensive dairy farms where a properly constituted diet is given the effects of fascioliasis may be largely camouflaged but the cost of foodstuffs is correspondingly higher (Vink 1971).

The insidious influence of the subclinical or chronic forms of the disease, particularly in cattle, is not easily recognised at the level of the individual farm but outbreaks of acute fascioliasis are devastating in their effect. The acute form of the disease is usually diagnosed at post mortem so rapid is its course and entire flocks have been known to succumb in a matter of weeks. Smith and Ollerenshaw (1969) have listed the years in which deaths from acute fascioliasis have exceeded the average: severe outbreaks are relatively infrequent. The most recent epidemic occurred during the winter of 1958/59 (Ollerenshaw, 1971a) when it is estimated that of the 1,500,000 sheep in N. Wales a total of 80,000 died of fascioliasis, amounting to a loss of around £560,000.

3. THE LIFE HISTORY OF F. HEPATICA

The life history and ecology of the liver fluke was summarised by Ollerenshaw (1959). He suggested that it might be of value to regard the life cycle as an alternation of developmental and transport phases. The first developmental phase occurs within the body of the primary host. Here, the immature flukes migrate through the tissue of the liver to the bile ducts where maturation is completed and egg laying begins. A transport phase follows: the eggs are released into the alimentary canal of the host and passed out with the faeces. Given suitable conditions, ciliated larvae, called miracidia, hatch from the eggs. These mobile larvae penetrate the tissue of the intermediate host, L. truncatula and a second developmental phase begins. The miracidium sheds its ciliated epithelium and assumes the sac-like form of the sporocyst. Propagative cells within the sporocyst give rise to further larvae, the rediae, which pass out of the sporocyst sac and migrate to the columella surface of the digestive gland where they feed upon the tissue of the snail. Propagative cells within each rediae give rise to further generations of daughter rediae or else to the final larval forms, the cercariae. When these cercariae are mature they are released from the rediae through a birth pore into the body of the snail and are eventually shed onto the mud or vegetation of the snail habitat. This marks the end of the second developmental phase. The transmission of fluke from snail to primary host is accomplished by the encysted cercariae.

These are called metacercariae and are ingested by grazing primary hosts. The flukes excyst in the alimentary canal of the primary host, pass through the gut wall and begin their migration to the liver.

The details of this life cycle were worked out independently by Thomas and Leuckart in 1882 following the "noted outbreak of 1879-80" (Walton, 1923), though the exact route by which the excysted metacercariae reached the liver was not described until ~~nearly~~ over fifty years later by Schumacher (1938). However, apart from papers by Walton (1918, 1923) which followed two severe outbreaks of the disease in Wales, the study of the epidemiology of fascioliasis languished in Britain until the Second World War, when Peters and Clapham (1942) conducted an abbatoir survey in order to establish the number of cattle that were infested with the parasite. After the two severe epidemics that occurred during the winters of 1946-7 and 1947-8 a concerted programme of research into the epidemiology of the disease was carried out by Taylor, Parfitt, Kendall and Ollerenshaw at the Central Veterinary Laboratory, Weybridge. This work continued for two decades and coincided with probably the most extensive outbreak of fascioliasis that has occurred in recent times, the epizootic of 1958-9. The British effort was paralleled by an investigation of "invasive pasture diseases" initiated in 1953 by the Parasitological Committee of the Polish Academy of Science. Fascioliasis was studied in particular

and most notably by Styczynska-Jurewicz, Bednarz, Chowaniec and Crozdz. More recent epidemiological studies include those by Boray and others in Australia, Ross in Northern Ireland, Over, Jansen, Honer and Vink in the Netherlands and Armour, Urquhart, Reid and others in Scotland.

4. EPIDEMIOLOGICAL STUDIES

Each of the main epidemiological studies will be considered in detail and particular attention will be given to the development of ideas concerning the relationship between variations in climate and variations in disease incidence.

(i) The Intermediate Host

In the years between 1913 and 1921 Walton (1918, 1923) surveyed several hundred farms in the Welsh counties of Dyfed, Gwynedd, and Clwyd. He began with the premise that the distribution of fascioliasis was entirely dependent upon the distribution of L. truncatula and the factors governing the latter were given particular attention in his first paper. He found, for example, that all of the snail habitats he studied were situated upon clay. By careful observation of some 73 sites over two or three years he was able to define the periods of maximum and minimum growth of the snails, the approximate size at which they became mature and the period during which oviposition commenced (March-May). He provided also a considerable amount of information on typical oviposition

sites and the preferred food of the snails (diatoms.)

A combination of laboratory experiment and field observation led Walton to the conclusion that though both the snails and their egg masses were to some extent resistant to dessication (and that the mortality of either depended upon the particular microclimate of each habitat) the timing of the life cycle and the ultimate abundance of the snails was heavily dependent upon the prevailing rainfall. Consolidating his argument he compared the annual rainfall in the Aberystwyth area between 1905 and 1915 with the timing of the outbreak of fascioliasis that just preceded his investigation. He found that the wettest years were 1910, 1912 and 1913,

> "that is, the period leading up to and including the attack of rot"

He remarks in passing that the relative distribution of rainfall and evaporation should also be borne in mind since a winter rainfall of

> "three inches distributed over 20 days would in all probability keep L. truncatula alive whilst in August falling in thunderstorm showers of brief duration it would probably amount to a physiological drought sufficient to kill a considerable percentage."

Walton suggested that in the areas in which he worked all winters were sufficiently wet to allow the snails to reach breeding size by spring and that it is wet summers which lead up to outbreaks of fascioliasis. The clear implication of his first paper (1918) that an increase in the range and density of the intermediate host

is a prerequisite of an outbreak found a more explicit formulation in his second (1923) where he wrote:

> "It is those years in which rainfall is general and persistent throughout the spring and summer that lead up to marked extensions of range of L. truncatula, and to its further increase by uninterrupted breeding on the ground gained. Given the infection of such snails by means of the normally present chronic or mild cases of Fluke infection usually present among the flocks, we have the conditions which precede and cause an epidemic of 'Rot'."

During the period described by Walton careful grazing management was the only effective control measure available to farmers but even after the introduction of the anthelmintic, tetrachloromethane, by Montgomerie in 1926 and its subsequent widespread use losses due to the acute form of the disease remained relatively undiminished and the depradations of chronic fascioliasis though reduced were not abolished. On the one hand, tetrachloromethane was not effective against the immature flukes that are responsible for acute disease and, on the other, an imprecise knowledge of the dynamics of the infestation of the primary host led to an arbitrary routine of dosing that rendered a treatment designed to control the chronic disease rather less than effectual.

Though Walton and others had been concerned with fascioliasis in sheep, a wartime abbatoir survey of some 73,000 cattle, all slaughtered within the same month, demonstrated that bovine fascioliasis was as serious a problem. Annual retail loss was estimated at £200,000. (Peters and Clapham, 1942).

During the post war years in Britain the ecology of L. truncatula and the intramolluscan stages of the parasite was the subject of much research (.e.g. Taylor and Mosely, 1948; Kendall, 1949a, 1949b; Taylor, 1949; Roberts, 1950 and Kendall and McCullough, 1951). A finding of particular significance was that the development of the parthenitae within the snail was negligible below 10°C and that redial growth though reduced continued even within an aestivating host provided the temperature exceeded this minimum. It was also discovered that the snails could resist dessication for periods far in excess of the three or four months suggested by Walton though the egg masses succumbed within a few hours. These results were largely confirmed by research that was carried out in Poland a few years later (Stycynska-Jurewicz, 1956; Chowaniec and Drodz, 1959; Bednarz 1960) and it is clear that during this time most authors still regarded variations in the abundance and distribution of the intermediate host as the prime determinant of variations in the incidence of the disease. Nevertheless, a series of papers which appeared in the late fifties and early sixties marked a considerable shift in emphasis (Ollerenshaw 1958, 1959 Ollerenshaw and Rowlands 1959 and Kendall and Ollerenshaw, 1963). The predominant role of the intermediate host was discounted and considerably more attention was given to the effect of climatic variation on the viability of the fluke eggs.

(ii) The Fluke Eggs

In a paper describing work carried out a few years earlier

Ollerenshaw and Rowcliffe (1961) reported that the eggs of F. hepatica were not at all resistant to dessication. The fragility of the fluke egg provided the centre piece of a new hypothesis that Ollerenshaw elaborated in a series of papers between 1958 and 1963. He suggested that the intensity of the infection reaching the primary host depended not upon the abundance of the intermediate snail hosts, as had been commonly assumed, but instead upon variations in the mortality of the fluke eggs. Ollerenshaw's argument may be summarised as follows:

(a) Under normal farming conditions in Britain there is considerable movement of stock from one area to another. Many of the animals are infected with F. hepatica and most are inadequately treated to remove the flukes. Therefore the supply of fluke eggs is rarely limiting.

(b) Herbage infestations (metacercariae) of a level dangerous to stock can in fact arise from snail populations as low as 5-50 per m^2 and infestations have been recorded in habitats where the density of snails following repeated treatment with molluscicide was so low that snails could not be found.

(c) In contrast to the fluke eggs the snails are extremely tolerant of prolonged drought but relatively slow to respond to improved conditions. It is quite possible

to have fluke eggs hatching and infecting snails before there has been any increase in the snail population.

(d) The mortality of the flukes at each stage of their life cycle will vary from time to time. These variations in mortality will be responsible not only for the absence or presence of the disease in an area but also for the varying incidence of the disease when it does occur. It is to be expected that the variations of greatest magnitude in time will exert the greatest effect on the parasite.

(e) Variations in the number of fluke eggs hatching are likely to be greater and more rapid than the variations in the number of snails.

(Ollerenshaw, 1958; 1959; Ollerenshaw and Rowlands, 1959; Kendall and Ollerenshaw, 1963). In essence, Ollerenshaw assumes that there are enough snails to produce a serious outbreak of fascioliasis at all times provided conditions are suitable for the hatching of the fluke eggs. While allowing that the chance of a miracidium successfully locating an individual snail depends upon the density of the snail population (Ollerenshaw, 1959) he maintains that the main feature of significance is the size of the infected snail, there being a direct relationship between the shell length and the number of rediae the intermediate

host can support (Kendall and Ollerenshaw, 1963). Thus he writes that an

> "increase in the number of snails is not considered important in the epidemiology of the disease unless favourable conditions have been maintained for about 5 months during the period May-October. After this period of time snails born following the onset of favourable conditions have had time to grow to a reasonable size and, if infected, are capable of producing a meaningful infestation on the herbage, but in summers where these conditions have occurred it has been found that dangerous infestations on the herbage were recorded before the infection in these snails reached maturity." (Ollerenshaw, 1959)

In other words, the infestation on the herbage is thought to be independent of the density of the snails but not necessarily of the size-class structure of the snail populations.

The value of Ollerenshaw's hypothesis lies in its dismissal of the simplistic notion of "more snails, more fluke." The epidemiological model that it generates, is concerned mainly with that phase of the fluke life cycle whose mortality follows climatic fluctuations with the very minimum of lag and has afforded a convenient basis from which to formulate a method of forecasting the incidence of fascioliasis. The comparative success of Ollerenshaw's fluke forecasts over the last decade and a half seems to have deterred a critical appraisal of the epidemiological ideas which provide its foundation but three objections at least ought to be mentioned.

First, the central tenet of the hypothesis, that the yearly mortality of fluke eggs controls[1] the abundance of F. hepatica as represented by the density of metacercariae on the pasture, is based upon the expectation that

> "the variations (in mortality) of greatest magnitude in time will exert the greatest effect on the parasite." (Ollerenshaw, 1959).

While the mortality of fluke eggs probably accounts for the bulk of the individual parasite deaths throughout the whole life cycle this is not sufficient to control the abundance of the fluke population if the proportion of fluke eggs failing to hatch is not consistently relatively greater than the proportions of parasites killed at any of the other stages of the life cycle, (e.g. if 0.1, say, of the fluke eggs survive to hatch but only 0.01 of the resulting miracidia succeed in penetrating a snail then, although the number of eggs failing to hatch is greater than the number of miracidia failing to find a snail, it is the mortality of the miracidia that has exerted the greater control of the number of parasites that became established in the snails). It has not yet been demonstrated that fluke eggs do consistently suffer a proportionately greater

[1]The word "control" is used here in the sense of determining population change. (See Varley et al 1973, p.93). The processes involved are independent of the density of the population. The word "regulate" will be reserved for the effect of density dependent processes the effect of which increases proportionately with the density of the population.

mortality than any other stage in the fluke life cycle. Such an undertaking requires a "key-factor" analysis of several consecutive years of fluke population data covering all major phases of the life cycle, (Varley and Gradwell, 1960) and is complicated by the reproductive stage that occurs in the snail.

Second, while the absence of any simple relationship between disease incidence and snail density is well established (Kendall and Ollerenshaw, 1963) it is a nonsense to imply, as Ollerenshaw does, that the fluke population is controlled by variations in fluke egg mortality rather than variations in snail density merely because variations in the first are greater (even proportionally greater) and more rapid than the second. Ollerenshaw (1971), points out that the bulk of the redial population is contained within a very few large snails (even in the epidemic year of 1958 0.5% of all snails found on habitats in Anglesey accounted for 85% of the redia - Ollerenshaw, loc cit). In consequence, the redial population could be decimated by the death of a relatively few hosts especially if infected snails are more susceptible to drought conditions than uninfected snails.[2] The proportion of rediae killed

[2]Kendall (1949a) could find no evidence for the enhanced susceptibility of infected snails in laboratory cultures though Stycynska-Jureviz (1965) found that snails collected in the field and then infected and subjected to dessication in the laboratory did succumb more rapidly than uninfected controls.

in this way might exceed even the proportion of fluke eggs which fail to hatch.

The third point concerns the relationship between snail density and the number of rediae an infected snail can support. Boray (1961) found that there was a marked inverse relationship between the density of L. tomentosa, the molluscan host of F. hepatica in Australia, and the number of cercariae released by each infected snail. He attributed this to the smaller size of the snails in the populations maintained at the higher densities. In Britain, Kendall (1949b) and Kendall and Ollerenshaw (1963) found that the maximum number of rediae that could be supported by the snail host depended upon the size of the snail and hence upon its nutritional state. The biomass of the infected snails represents the carrying capacity of the redial environment and competition for food within the host regulates the redial density within each snail. Should intraspecific competition for food in populations of L. truncatula operate so as to reduce the mean size of the snails ("scramble" competition, see Nicholson, 1954) then the carrying capacity of the redial environment will be determined by the density of the snails.

In nearly all respects Ollerenshaw's hypothesis remains untested and his repudiation of the "more snails, more fluke" argument has obscured the means by which the population processes of the intermediate host might alternatively control the density of metacercariae on the pasture.

(iii) The Fluke Cysts

More recent epidemiological work in Britain has established more precisely the chronology of primary host infection. The first such study was carried out by Ross (1967a, 1967b) in County Down, Northern Ireland. Tracer sheep were grazed almost continuously in a paddock (0.404ha) containing a single snail habitat. Observations were made for nearly two years between August 1964 and June 1966. The rate of metacercarial ingestion was ascertained by post mortem examination of the tracer sheep which were removed from the paddock at regular intervals throughout the study. These results were compared with spot estimates of cyst availability obtained using a guinea pig bioassay technique (Ross and O'Hagan, 1966). The relative density of snails on the habitat was recorded monthly during the spring, summer and autumn and the number of infected snails was found by dissection. An improved method of snail sampling using a "soil scrape" method was introduced late in the study (Ross and O'Hagan 1968). Ross reported that most of the infection was picked up by the sheep between August and February in 1964-65 and between August and December 1965-66. The size of the paddock was more than doubled (0.909ha) during the second season by the addition of a well-drained and therefore 'fluke-free' area of pasture. Though the number of sheep was correspondingly increased from 9 to 18 this resulted in a slightly reduced stocking rate and a consequent decrease in the likelihood of metacercarial uptake. Nevertheless, Ross found that the mean fluke burden of the tracer sheep was higher in

1965-66 than in 1964-65. This result corresponded with the generally higher incidence of fascioliasis forecast by the method of Ollerenshaw (see later) based upon data obtained from the nearest meteorological station and was consistent with the findings of a survey of cattle liver infection carried out at the Belfast abbatoir which indicated that the level of infection in October 1965 was approximately three times that of October 1964.

Close observation of the tracer sheep provided a comprehensive record of the physiological concommitants of naturally acquired fascioliasis so supplementing Sinclair's (1961) work on laboratory infections but Ross's overall design permitted only the most general conclusions about the epidemiology of the disease. His investigation of the snail habitat served merely to establish that snails were present and that a proportion of them were infected with F. hepatica. It would not have been reasonable to compare the snail population data with the infection rate of the tracer sheep nor was there any reported attempt to associate the estimated metacercarial densties of 1966 with the fluke egg output of the first season or the micro-climate of the paddock during the second.

The fact that Ross had used only small numbers of tracer sheep over an extended period has been criticised by Armour et al (1970) who comment that greater numbers of tracer sheep grazed on the pasture for monthly rather than two monthly intervals (as in Ross's study) not only takes account of variations in susceptibility to

infection among the sheep but provides a far greater resolution in terms of the periodic fluctuations in metacercarial availability. Using this improved design they monitored cyst uptake from a single field between April 1967 and March 1968. The mean number of flukes recovered from sheep grazed between mid August and mid September was at least three times greater than during any other period.

(iv) The Flukes in the Primary Hosts

Elsewhere in Europe Honer and Vink (1963a) determined the typical annual pattern of disease incidence in lambs. Deviations from this pattern were attributed to climatic changes particularly with respect to rainfall and temperature when these departed markedly from average seasonal values. In the same paper Honer and Vink proposed a mathematical model of fascioliasis in lambs but according to Jansen (1964) the assumptions underlying the model were based upon a misreading of a book by Hutyra and Marek (1959) and he dismisses their conclusions as "useless."

5. MATHEMATICAL MODELS OF FASCIOLIASIS

Mathematical models of fascioliasis have been developed in order to forecast the extent of the risk to grazing stock in any particular year. A forecasting system is necessary because there is no adequate cure for acute fascioliasis (Ollerenshaw, 1974) and if losses are to be kept to a minimum then appropriate prophylatic action must be taken to prevent stock becoming infected in the first place. An accurate forecast, if it is to be of most use, must be

available well in advance of the period of maximum risk so that changes in grazing management and molluscicide treatment can be implemented in plenty of time. Even in years of minimal risk the forecast is still useful since anthelmintics are expensive and occasionally prove fatal to a small proportion of stock. When the probability of infection is low it may well be less costly not to dose!

All models of fascioliasis depend upon the assumption that the provision of adequate soil moisture is essential if the development of the fluke from egg to cyst is to proceed to completion. It has been known for centuries that a "bad fluke year" was associated with a wet summer and even as imperfect an index of soil moisture as monthly rainfall expressed as a percentage of the long term average has proved to be a reasonable predicter of disease incidence. (Ollerenshaw and Smith, 1969).

The first two of the models described here model hydrological rather than biological processes. Both have proven predictive validity and both generate an index of soil moisture which forms the basis of a fluke forecast. Though each requires meteorological information neither pays more than cursory attention to the influence of temperature being applicable only during a previously defined sequence of months when the mean monthly temperature exceeds $10^{o}C$. The third model has not yet been adequately tested in the field. It sets out to calculate the pattern of cercarial shedding that

would occur if moisture conditions were optimal and temperature was the only rate limiting factor. The resulting pattern of incidence is then modified in terms of the moisture deficiencies that actually prevailed.

(i) The M_t Index

The M_t index, described by Ollerenshaw and Rowlands (1959) has been used to forecast the incidence of fascioliasis in North Wales since 1958. It has subsequently been applied to the whole of the British Isles and, with some modifications, to large areas of France and Holland (Ollerenshaw, 1966; 1971a; 1971b; 1971c; 1974). Its generality, despite considerable differences in terrain and farming management practices offers considerable advantages. The particular assumptions that form the basis of the model are these:

(a) Development of the extramammalian stages of the parasite is only possible when suitable moisture conditions prevail - that is when the snail habitats are covered with a little free water.

(b) Suitable conditions do not occur in any area until the soil attains field capacity. Field capacity is attained when rainfall exceeds evaporative losses.

(c) Metacercariae will be deposited on the herbage when rainfall exceeds evaporation for three months during that period when temperature does not limit development.

(d) The effect of temperature on the course of development is broadly similar from year to year and so provided monthly means are in excess of $10^{o}C$ further variation can be neglected.

(Ollerenshaw, 1958).

A monthly index of soil moisture (M) is provided by the difference between monthly rainfall (R in inches) and evapotranspiration (P).

i.e. $M = R - P$ (1)

In the driest months (R - P) approaches -5 inches and so a positive value of M is ensured by the addition of an arbitrary constant:

i.e. $M = R - P + 5$ (2)

Finally, the number of rain days (N) occurring each month is introduced to represent the distribution of rain fall.

i.e. $M_t = N ((R-P) + 5)$ (3)

Ollerenshaw (1959) suggested that when the monthly value of M_t exceeded 100 most habitats were sufficiently wet for the development of the parasite to proceed. Accordingly, the maximum value of M_t was fixed at 100. The index is calculated for every month in which the mean temperature is above $10^{o}C$ and then summed. Ollerenshaw (loc.cit.) guessed that the rate of parasitic development in May and September is about half that in the intervening months and so the M_t value for

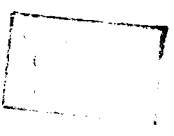
these two months is halved before summation.

The maximum value of M_t is 500. Retrospective analysis of meteorological data and disease incidence indicated that losses from acute fascioliasis begin when $M_t > 300$ and that an outbreak of serious proportions is likely when $M_t > 400$. Though this relationship which was worked out for N. Wales in particular, does not necessarily pertain over the whole of the United Kingdom. (Ollerenshaw, 1966).

The combined M_t values for September and October nearly always amount to 150 (Ollerenshaw, 1971a). The M_t index for August often approaches the maximum value of 100 and so Ollerenshaw argues that it is possible to provide an estimate of the likely value of M_t by the middle of this month at least. The fluke forecast is based upon this estimate of the likely value of M_t.

In fact the forecast depends upon the soil moisture conditions that prevail in May, June and July although some adjustment is made if mean temperatures deviate markedly from the long-term average. The risk from an overwintering infection can be estimated by summing the M_t values from August to October of one year with those of May and June of the next though in practice this is considered less important. The forecast is published each year in the first two weeks of August, which allows about a fortnight for appropriate prophylactic action. The risk is expressed in terms of the long term average, i.e. high, above average, average, below average and low.

Fascioliasis is not a notifiable disease in the United Kingdom and so the actual incidence of disease cannot be gauged with certainty. Ollerenshaw has had to rely upon the reports of fluke-related deaths (i.e. from acute or chronic fascioliasis or from black disease) provided by the M.A.F.F. Veterinary Investigation Centres. The extent to which these reports are related to actual incidence is of course unknown and the justification for their use is largely intuitive. Not surprisingly, therefore, the correlation between M_t values and these indirect indices of incidence provided by the V.I. Centres is barely significant (Craigon, pers. comm.) However, even when a particularly reliable index of incidence is available the correlation remains weak. For example, Ollerenshaw (1971c) compared lamb liver condemnation records from an abbatoir in the Netherlands with the corresponding M_t values for May, June and July. Visual inspection suggested a reasonably close relationship but the variations in M_t account for less than one third of the total variation in incidence (Craigon, pers. comm.). Even so, the forecast itself has been extremely successful. The M_t index is merely a guide and though central to the process of forecasting it is supplemented by a national programme of snail collection and faecal analysis. The results of this labour intensive activity all contribute to the final estimate of risk.



(ii) The Stormont "Wet Day" Forecasting System

Ross (1970) found that in N. Ireland abnormally dry conditions in June resulted in a very reduced incidence of fascioliasis irrespective of subsequent weather conditions and argued that a summed M_t index would not take account of this. Instead, Ross suggested an alternative index of soil moisture based upon the number of "Wet Days" that occur between June and September. A "Wet Day" is a day in which 1 mm. or more of rain falls. The months of May and October were ignored on the grounds that the mean monthly temperature is generally below $10^{o}C$. Overwintering infections are also ignored.

The number of Wet Days that occur within a single week forms the basic unit of the forecast. These weekly units are plotted on an accumulative graph thus indicating both the amount and distribution of rainfall during the summer months. The graph is then compared with similar plots for years in which the disease incidence has been rated as 'disastrous', above average, average or below average. As in the case of the M_t index the accumulative graph of Wet Days is merely a guide, other features are taken into account before the final forecast is published. The advantage of the method is that it requires only measurements of daily rainfall and, according to Ross, offers the possibility of a reasonably accurate forecast by the end of June or mid-July. So far at least, the forecasts have corresponded well with actual incidence but it may

be that the method is limited in its application to the region for which it was devised.

(iii) Temperature Models

Nice and Wilson (1974) described a model of redial development based upon the rate limiting effect of environmental temperatures. The relationship between temperature and redial growth was established by regression analysis and then using iterative methods it was possible to estimate the duration of an infection from penetration of the intermediate host by a miracidium to maturity. For the purposes of simulation standard daily temperature records were obtained from the Meteorological Office at Bracknell. The pattern of maturing infections that resulted was consistent with published descriptions of the field situation.

Gettingby et al (1974) proposed a similar model but considered the fluke eggs as well as the rediae within the snail. The model proceeded in three stages to arrive finally at a qualitative estimate of the pattern of cercarial shedding. The first step entailed the calculation of the average time period between deposition of the fluke eggs on the pasture and the subsequent shedding of cercariae from the intermediate host. Then the temperature dependent mortality of the fluke eggs was estimated as well as their distributional mortality (i,e. the proportion falling outside a snail habitat). Grass maximum and minimum temperatures were used at each stage and, like the Nice and Wilson model, moisture conditions

were assumed to be optimal throughout. The pattern of cercarial shedding predicted by the model was compared with estimates of metacercarial availability determined by the post-mortem examination of tracer sheep in a field study (Hope-Cawdery and Moran, 1971). The correspondence was only fair though the field study had not been designed to test the model and so could not provide a truly adequate yardstick.

Hope-Cawdery and Gettingby (1974) extended this model to include the effects of varying soil moisture conditions. The parasites were assumed to be ingested by sheep as soon as they were shed from the snails. An accumulative graph of metacercarial "pick-up" was constructed and compared with a similar graph of actual "pick-up" determined as before by a field study using tracer sheep. The difference between the predicted and observed "pick-up" was found to be related to a measure of soil moisture deficit based upon ground water levels. The combination of the temperature model and the modification due to variations in soil moisture provided an estimate of the rate at which the metacercariae were ingested by grazing stock. The predicted rates generated in this way corresponded reasonably well with the actual rates observed in the field early in the season but became progressively more unreliable towards the end. (Hope-Cawdery, pers.comm.) The poor predictive power of the model is due in part to the incorporation of assumptions regarding the miracidial phase in particular

(see for example, Gettingby 1974) which are actually untrue (Wilson, pers.comm.).

Hope-Cawdery and Gettingby (1974) suggest that as the model is refined it will provide a means of determining when metacercariae first appear on the pasture and, with the moisture modification, allow some approximate estimate of the severity of the risk to stock.

6. A CRITIQUE OF THE VARIOUS MODELS OF FASCIOLIASIS

Two kinds of model have been described. The fluke forecasts generated by the moisture models of Ollerenshaw, Rowlands and Ross depend upon the assumption that the risk of stock becoming infected is (linearly) related to the duration of optimal moisture conditions. Temperature is recognised as a rate limiting factor but regarded as a conservative influence from year to year and largely ignored. Soil moisture, a permissive factor, is held to be of paramount importance; the longer optimal conditions persist the greater the chance of cercariae being shed onto the pasture in densities that would be dangerous to stock. The summated indices of soil moisture were intended to indicate only the extent of the risk but when optimal conditions prevail for an extended period early in the season it seemed reasonable to conclude also that cercariae will be shed earlier than usual.

The simplicity of the method is deceptive, undue emphasis on the various indices of soil moisture conceals the extent to which

other factors such as snail infection rates contribute to the final estimate of risk (nearly 200 snail habitats are visited every month during spring and summer) and the mathematical basis of the models disguises the subjective nature of the forecasts.

When discussing the M_t index Ollerenshaw (1974) distinguished between two approaches to forecasting disease incidence:

> "an experimental approach starting with simple laboratory observations and developing these to explain events in the field, and the empirical approach which starts with incidence and attempts to relate this directly with climate."

The experimental approach generates a model whose predictive power, though subsequently ratified by comparison with the field situation, depends in the first instance upon the accurate simulation of natural processes. The empirical approach involves the identification of a readily accessible parameter which is highly correlated with the variable under consideration and requires no assumptions regarding the way in which the two are connected. Ollerenshaw (loc.cit.) cites the forecasting system based upon the M_t index as an example of the first approach and the demonstrated association between monthly rainfall and disease incidence as an example of the second (Ollerenshaw and Smith, 1969). In fact, the use of the summed M_t index involves elements of both approaches. The model describes soil moisture conditions rather than disease incidence and the extent to which one depends upon the other has been determined by graphical comparison. The precise way in which soil moisture operates to

control the parasite population is unknown. Ollerenshaw assumed that the moisture dependent mortality of fluke eggs in May, June and July is the key factor (Ollerenshaw's argument has been criticized in a previous section). If this were the case it would be convenient in two respects. First, all of the significant events would have occurred before mid-August, which is the last practical opportunity to publish a forecast, and second, one would expect there to be a high correlation between disease incidence and soil moisture since the latter exerts its effect, without complication, directly upon the parasite. Unfortunately, the correlation turns out to be weak.

The temperature models of Gettingby, Hope-Cawdery, Nice and Wilson simulate biological rather than hydrological processes. Their output describes the pattern of cercarial release given optimal moisture conditions. The accumulation of cysts on the herbage is represented in relative rather than absolute terms since the models are not intended to estimate actual densities. In the first place, the proponents of the temperature models argue that a more precise estimate of the period of maximum risk to stock would be a useful supplement to the present fluke forecast. However, the general method of simulating biological processes offers several further advantages. A comprehensive model of the parasite life cycle which incorporated both temperature and moisture components provides the means to a greater understanding of the epidemiology

of the disease. Such a model would embody a series of hypotheses concerning the way in which changes in temperature and soil moisture affect the density of the parasite. The validity of these hypotheses could be tested by comparing the output of the model with actual events in the field. Additional manipulation of the model's input would generate further hypotheses about the behaviour of the system in the field. For example, even the relatively simple model proposed by Nice and Wilson (1974) led to the suggestion that in S. Britain at least there are two overlapping periods of cercarial shedding resulting from short summer and long winter infections rather than two complete life cycles of the parasite per annum.

7. THE AIMS AND OBJECTIVES OF THE RESEARCH PRESENTED IN THIS THESIS

Fluke forecasts have been published in Britain since 1958 but a growing awareness of the problem of fascioliasis has resulted in only a marginal increase in the proportion of farmers that implement adequate control measures (Ollerenshaw and Rowcliffe, 1961; Reid, 1973). An accurate and discriminating fluke forecast is contingent upon a thorough understanding of the way in which the density of the parasite is regulated and controlled in the field. A comprehensive model of the sort described above which excludes neither temperature nor moisture is a convenient investigative tool for the identification of the most significant processes and would subsequently provide a sound quantitative basis for a reliable system of fore-

casting disease incidence. Control measures against fascioliasis will not be fully implemented unless farmers and veterinary practitioners have reasonable confidence in the forecasts of incidence. It is suggested here that any improvement in the reliability of the forecasting technique can only increase this confidence and thereby improve the chances that adequate control measures will be taken.

In 1972 the fascioliasis research group at the University of York undertook the construction of a mathematical model of fascioliasis which incorporated both temperature and soil moisture. The aim of the research reported here is to provide a detailed account of the variations in population density of the parasite, F. hepatica, and its intermediate host, L. truncatula, on a few selected habitats over a number of seasons together with appropriate meteorological data. At the very least this would provide a description of the system to be modelled and, more usefully, a comprehensive set of criteria against which a mathematical model of fascioliasis derived from laboratory data could be tested.

CHAPTER 2. HENDY AND THORNEYTHWAITE - A DESCRIPTION OF THE TWO FARMS AND A SURVEY OF THE MAIN L. TRUNCATULA HABITATS

1. INTRODUCTION - THE HABITATS OF L. TRUNCATULA

The mud snail, L. truncatula, and the free living stages of F. hepatica require more or less the same environmental conditions to survive and grow. (Styczynska-Jurewicz, 1965; Ollerenshaw, 1974). The distribution and population density of L. truncatula is determined largely by soil-surface moisture (Ollerenshaw 1971) and a potential snail and fluke habitat can be described in terms of a complex of characters that maintain or are concomitant with wet conditions. That particular features of topography, soil type, vegetation and p.H. are commonly associated with habitats is nicely illustrated by the French word "douve" which means 'liver fluke', 'ditch' and 'buttercup' (Over 1962).

(i) Typical Habitats

L. truncatula has been found in shallow pools, hoof prints, wheel nuts, neglected drainage ditches, small depressions, the environs of watering places, river shoals with a slow water current, sandy or muddy banks of shallow brooks, road-side ditches, swampy meadows, springs and, rarely, in mountain torrent beds (Walton, 1918; Walton and Rees-Wright 1926; Okland, 1938; Chowaniec and Drozdz, 1959; Bednarz, 1960; Styczynska-Jurewicz, 1965; Sosiptrov and Shumakovich, 1966; Over and Damon-Van Hapert, 1967; Ricom, 1971). Some authors distinguish between "permanent" and "temporary" habitats, the former providing suitably wet conditions for the snail

and fluke all year round while the latter dries out for at least a few weeks of every year (e.g. Bednarz, 1960; Hepplestton, 1972). However, in those habitats most likely to be of the permanent sort, i.e. ditches and streams, the snails are most often found on the ditch sides or stream banks. Indeed, they rarely occur in permanent water of a greater depth than 15cm. (Over and Damon-Van Hapert, 1967; Ollerenshaw 1971).

(ii) Soil Type and Topography

Habitats are more common on clay rather than sandy soils and over impervious rather than porous rocks. Any topographical feature that impedes natural drainage, or inadequate man made drainage, will create and maintain suitably wet conditions. (Walton, 1918; Okland, 1938; Styczynska-Jurewicz, 1965; Ollerenshaw, 1971).

(iii) Vegetation

Over (1962) attempted to characterise potential habitats in terms of vegetation cover, arguing that the plant species provide more permanent indicators of suitability than (say) soil or water p.H. He conducted a literature survey from which he compiled a list of those plant species which have been found on L. truncatula habitats. Over concluded that plant communities which are characteristic of the liver fluke - snail habitat are associations of the alliance Agropyro-Rumicon crispi (Nordhagen, 1940) and cited as

examples of this particular species combination first Glyceria fluitans, Alopecurus geniculatus and Ranunculus repens and second Glyceria declinata, Juncus inflexus and Ranunculus repens. Moens (1968) and Sosiptrov and Shumakovich (1966) list several other species not included by Over i.e. Ranunculus flammula, Veronica scutellata, Sagittaria sagittiflora and Conium maculatum.

(iv) Chemical Aspects

Atkins and Lebour (1924), Walton and Rees-Wright (1926) and Roberts (1950) measured the p.H. of the water in various snail habitats. The ranges obtained were 6.4-7.8, 6.0-8.6 and 6.9-8.2 respectively. Atkins and Lebour also noted that the p.H. of the underlying soil could be as low as 5.8 and Walton and Rees-Wright found that snails most commonly occurred in water of p.H. 7.2-7.6. Styczynska-Jurewicz (1965) reviewed the results of other workers and reported that snails have been found in situations where the p.H. was as low as 5.6 though it is not clear whether this is soil or water p.H.

It is characteristic of L. truncatula habitats that the water p.H. value fluctuates considerably as the quantity of water covering the habitat is itself so variable a feature. The typically shallow bodies of water heat up and cool down with great rapidity affecting the rate at which mineral salts become dissolved and their often small capacity quickly results in oxygen deficit in the presence of even slight amounts of rotting organic matter such as faeces.

2. THE CHOICE OF FARMS AND HABITATS

The field work described in the chapters that follow was carried out on two farms. Both farms are situated in areas of high rainfall and both farms, for different reasons of soil type, topography and management, include large areas of pasture which manifest those characteristics of a liver fluke- snail habitat given above.

There follows an account of the topography and management of each farm together with a description of the soil types and plant species found in selected habitats.

3. A DESCRIPTION OF THE FARMS

(i) Hendy (100 ft. above sea level)

A lowland farm, situated upon a small dome in the Caernarvon coastal strip (Gwynedd, SH 475613). The farmer operated a mixed farming system - 200 ewes (mostly Welsh Mountain crosses) and a variable number of dairy and beef cattle (mostly Welsh Blacks.) Management was typical of lowland sheep farms.

A small farm, "Muriau", (SH 482605) almost adjacent to Hendy and situated on the S.E. slopes of a similar dome had recently been acquired by the farmer. This was used as an adjunct to the main farm and so in the chapters that follow no distinction is drawn between Muriau and Hendy, the latter name being used to refer to both.

A "wet" farm, with a history of fascioliasis, it contained large areas of water logged pasture which despite recent attempts at drainage provided more than 30 sites potentially suitable for the mud snail, Lymnaea truncatula. The snail populations were first sampled in 1972 by Veterinary Assistants and have figured ever since in the "fluke forecasts" provided by the Central Veterinary Laboratories, Weybridge.

(ii) Thorneythwaite (400 ft. above sea level)

A hill farm, situated in the Borrowdale valley system (Cumbria, NY 246134). A flock of about 500 ewes (mainly Herdwicks with some Welsh Mountain crosses) ranged for much of the time on the fell sides which rise up to the S.E. of the main field system located on the valley floor. One or two of the fields were rented out for cattle grazing in the summer. Management typical of hill farms.

Thorneythwaite received more rainfall than most other places in England and its snail habitats have been visited in connection with the "fluke forecast" scheme for some time. Moderate levels of fascioliasis had been demonstrated in the resident flock in each of the five years leading up to this study.

4. A GENERAL SURVEY OF THE SNAIL HABITATS

A general survey of all the potential snail habitats on each farm was made in February 1973 with a view to selecting particular

sites for further study. Two categories of habitat were recognised; these were ditches or their adjoining flooded areas and field sites which consisted of isolated areas of wet pasture. Also noted was the size of the habitat, the extent of the herbage cover (particularly the Juncaceae) and the amount of poaching (trampling) that had occurred. A ten minute count for L. truncatula was made in each area.

(i) Hendy

The distribution of potential and actual habitats on Hendy is given in Figures 2.1 and 2.2. There were 18 ditch habitats and 16 field habitats. The largest measured 90 x 16m and the smallest 5 x 5m. Mud snails were found on 12 of the ditch habitats and 9 of the field habitats. 6 habitats (A -F) were selected for further study.

(ii) Thorneythwaite

The distribution of potential habitats on Thorneythwaite is given in Figure 2.3. 9 habitats of each type were found. Only two of them (B+D) contained any mud snails. This pair, and two other sites (A+F), were selected for further study.

5. A DETAILED DESCRIPTION OF THE SNAIL HABITATS

Several criteria were involved in the choice of habitat. Those likely to fall into the category of "temporary" were rejected and wherever the information was available it was the permanent

Fig. 2.1 Hendy Farm

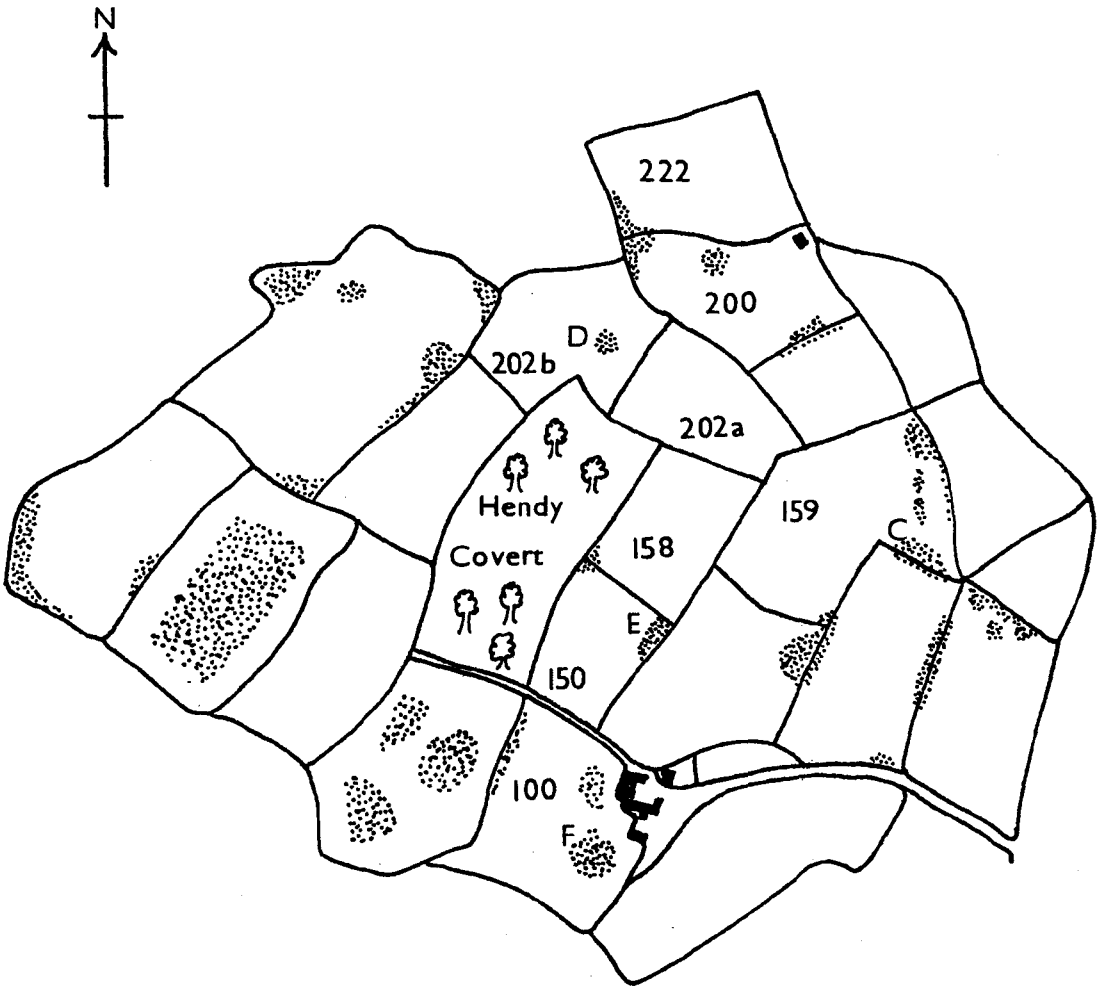


Fig. 2.2 Muriau Farm

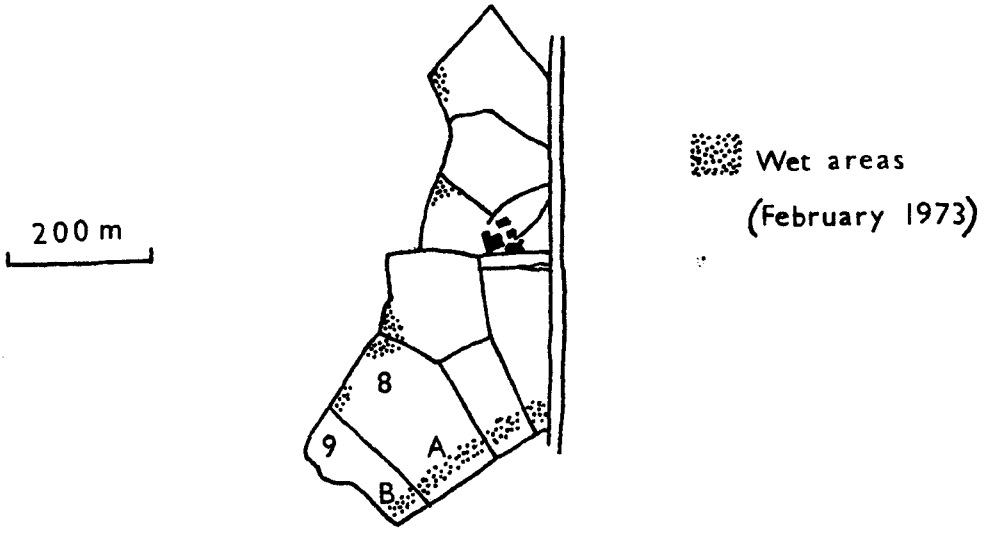
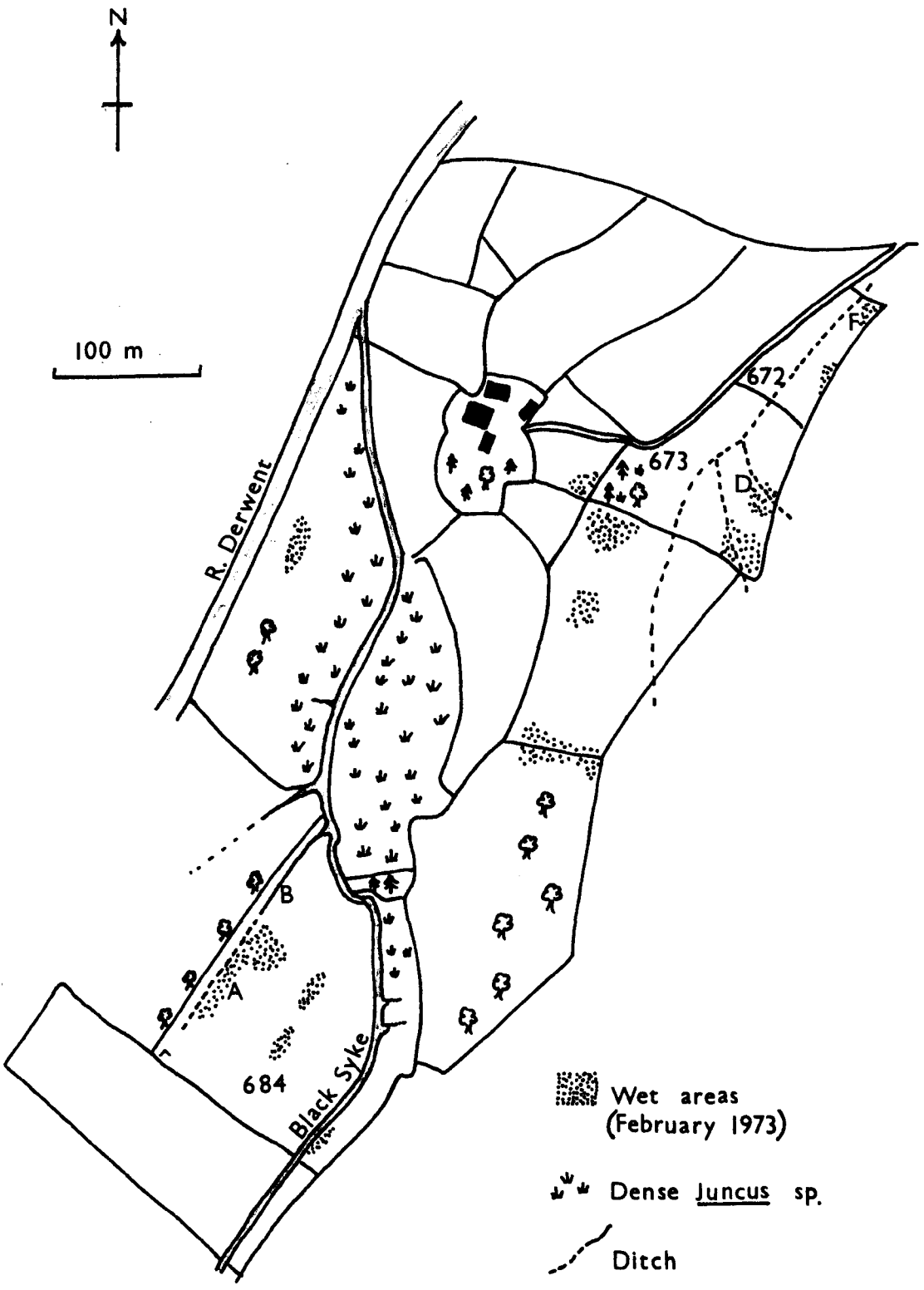


Fig. 2.3 Thorneythwaite Farm



sites which had supported populations of L. truncatula for a number of years previously that were chosen. Otherwise, the presence of a large number of snails at the time of the survey, a moderate size ($20m^2$) and the minimum of Juncus cover determined the selection of habitat.

(i) Hendy

Habitat A (Field No. 8)

Extensive area of very poached turf, 90 x 16m, running S.W. to N.E. at the base of a steep slope and bounded on the S. side by a wide flowing stream. (Sparse to moderate Juncus sp.) Habitat dips below stream water level in many places. Completely flooded to a depth of 10cm on several occasions.

Habitat B (Field No. 9)

Small area of flooded poached turf, 20 x 2m, running N.W. to S.E. along a dry stone wall. (Very sparse Juncus sp) The habitat is part of an extensive wet area which becomes continuous with Habitat A on the other side of the wall.

Habitat C (Field No. 159)

Very poached and muddy depression 40 x 5m widening to 30m, bounded on the S. side by a willow hedge (Salix caprea). Sheltered position. (No Juncus sp.) Typically flooded up to 10cm due to blocked drainage pipe. Became permanently flooded to a depth of over 1m in November 1974.

Plate I. Site D, Hendy

Habitat D (Field No. 202b)

Isolated area of poached turf, 15 x 13m, (very sparse Juncus sp.) situated on a gentle convex N. facing slope. In times of high rainfall similar wet areas appear all along the same contour line.

Habitat E (Field No. 150)

Large poached area in N.E. corner of field, 65 x 15 m (Juncus sp. sparse). Most snails found in two deep tractor ruts. Water spills over from a shallow neighbouring ditch.

Habitat F (Field No. 100)

An exposed area of poached turf, 30 x 20m, in a low lying and generally waterlogged field. (Very sparse Juncus sp.) Field drained in August 1974 and habitat dried out.

(ii) Thorneythwaite

Habitat A (Field No. 684)

An area 3 x 55m comprising a shallow, grass-bottomed ditch (1m wide) together with an accompanying strip of ditch spoil and heavily poached turf. (Moderate Juncus sp.) Habitat very exposed, running S.W. to N.E. Standing water in patches.

Habitat B (Field No. 684)

Extension of ditch described under Habitat A, 80m in length and approximately 1m wide. Grass-bottomed, alternating with stretches of gravel; water typically between 10cm and 25cm deep

Plate 2.

Site B, Thorneythwaite

Site D, Thorneythwaite

along most of length. Very gentle flow, draining at N. end into "Black Syke." Habitat flooded in July 1974 to a depth of 1m.

Habitat D (Field No. 673)

A triangular area of poached turf about 30 x 30 x 50m, on a gentle concave N.W. facing slope. Bounded on one side by a shallow stream and situated at the entrance to a fell gate. In places habitat dips belowwater level of stream and after moderate rainfall water issues from a point at the S.E. apex of the triangle and flows over the surface. (Sparse Juncus sp.)

Habitat F (Field No. 672)

A triangular habitat in the N.E. corner of the field, about 10 x 10 x 15m. (Considerable Juncus sp.) Water overflows onto the habitat from a shallow ditch on the fell sides.

6. THE PLANT SPECIES AND SOIL TYPES AT HENDY AND THORNEYTHWAITE

A list of plant species found on the fields and snail habitats of the two farms was compiled. Specimens were collected from Hendy on the 13th and 29th July 1975, and from Thorneythwaite on the 28th June and 30th July 1975. In order to make a detailed comparison of a snail habitat and the pasture which surrounded it 25 randomly placed quadrat samples (20 x 20cm) were taken from Habitat D, Hendy, and field 202b, Hendy. In each case the presence or absence of a species was noted and the results expressed as percentage frequency ocurrence.

Two pits (30–40cm deep) were dug at Hendy in August 1975. The pits were within 4m of one another, one being placed in the centre of site D and the other, outside the habitat, in field 202b. In October, 1975, a single pit(30–40cm deep) was dug in field 673, Thorneythwaite, adjacent to but not within site D. The soil profiles were described at each location and up to 20g of soil were removed from each identifiable horizon and returned to the laboratory. Here, the proportion of sand, silt, and clay in each sample was determined using a standard sedimentation technique (Pippette method - Tinsley, J. A Manual of Experiments for Students of Soil Science; para. 5.13; University Dept. of Soil Science, Aberdeen). The soil particles were classified according to the International Scale and the results were expressed as percentage by weight of the whole oven dried sample. Organic matter content was not measured. The p.H. of the soil samples from the Hendy profiles was determined with a p.H. meter (1 part of soil dispersed in 2.5 parts distilled water; Hall (1945), The Soil, pub. J. Murray).

The sites were visited again between the 7th and 10th April 1977. Fresh soil pits were dug in the approximate locations described above and the profiles were photographed. In addition, two new pits were dug, and the profiles examined, in situations not previously investigated in this way. The first pit was in site D, Thorneythwaite, and the second in field 684, Thorneythwaite, about 5m from the ditch habitat, site B.

(i) The Plant Species Found at Hendy

A list of plant species found on habitats A, D and E, and on field 202b is given in Tables 1 and 2.

GRAMINEAE - Alopecurus geniculatus, (Marsh Foxtail), was abundant in each of the habitats examined, only occasional representatives of this species were found in field 202b. Glyceria fluitans, (Flote grass) was found in the two wettest habitats, A and D; it was absent from habitat E. Two species were characteristic of the field, Lolium perenne, (Perennial Rye-grass), and Cynosurus crystatus, (Crested Dogs-tail).

JUNCACEAE- Juncus effusus, (Soft Rush), was found in each of the habitats. Juncus bufonius, (Toads Rush), was found in habitat D. No representatives of this family were found in field 202b.

DICOTYLEDONES - Ranunculus repens, (Creeping Buttercup), was abundant in all of the snail habitats examined being only occasionally represented in the surrounding pasture. Here, Trifolium repens (White Clover), Ceriastium fontanum, (Common Mouse Ear) and Plantago lanceolata (Ribwort) were the most common species.

The relative abundance of the plant species found in site D and on the surrounding pasture is given in Figure 2.4 ~~and Table 3. Only those species with a percentage frequency of at least 20% were represented in the figure.~~

Table 1

PLANT SPECIES FOUND ON HENDY

(a) GRAMINEAE

		HABITAT			FIELD
		A	D	E	202b
Alopecurus geniculatus	Marsh Foxtail	A	A	A	0
Glyceria fluitans	Flote grass	C	A		
Holcus lannatus	Yorkshire Fog	C	C	C	C
Poa trivialis	Rough Meadow Grass		C	C	C
Poa annua	Annual Meadow Grass		C		
Anthoxanthum odoratum	Sweet Vernal Grass				0
Phleum pratense	Timothy Grass			0	
Lolium perenne	Perennial Rye Grass		0	0	A
Cynosurus cristatus	Crested Dogs-tail	0		0	C
Agrostis tenuis	Common Bent				C

A = Abundant C = Common 0 = Occasional

1(b) JUNCACEAE AND CYPERACEAE

		HABITAT			FIELD
		A	D	E	202b
Juncus effusus	Soft Rush	x	x	x	
Juncus bufonius	Toad Rush		x		

x = Present

Table 2

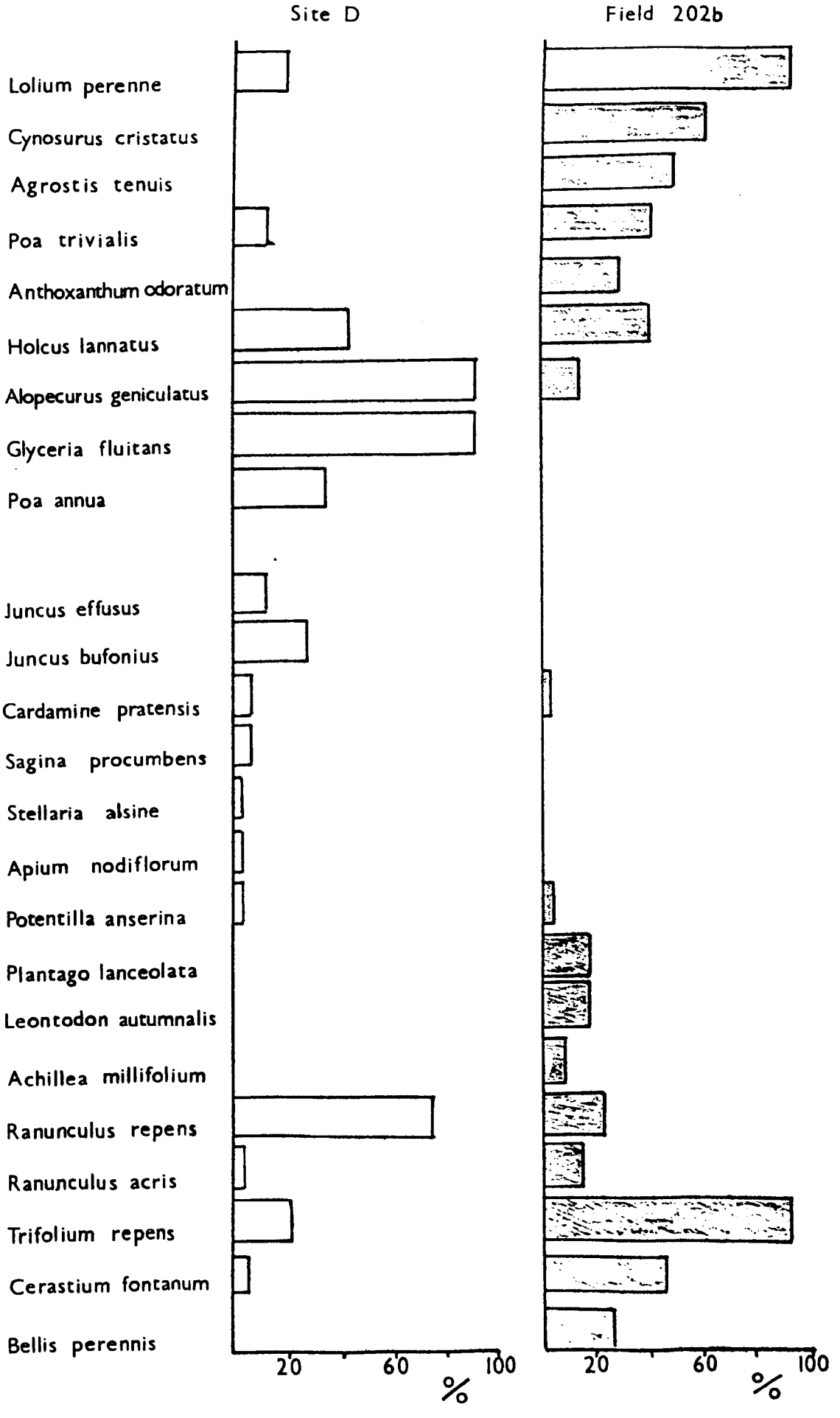
PLANT SPECIES FOUND ON HENDY

DICOTYLEDONES

		HABITAT			FIELD
		A	P	E	a b
Ranunculus repens	Creeping Buttercup	A	A	A	0
Trifolium repens	White Clover	0	0	0	A
Cerastium fontanum	Common Mouse Ear	0	0		C
Ranunculus acrie	Meadow Buttercup	0	0		0
Cardimine praetensis	Cuckoo Flower	0	0		0
Apium nodiflorum	Fools Watercress	0	0		
Ranunculus flammula	Lesser Spearwort	0			
Senecio aqualicus	Marsh Ragwort	0			
Polygonum persicaria	Persicaria	0			
Plantago lanceolata	Ribwort	0			C
Lotus corniculatus	Birds foot trefoil	C	C		
Myosotis scorpiodes	Water forget-me-not	0	0		
Rumex crispus	Curled dock			0	
Sagina procumbens	Procumbent pearlwort		0		
Stellaria alsine	Bog stickwort		0		
Potentilla anserina	Silverweed		0		0
Bellis perennis	Daisy				0
Leontodon autumnalis	Autumnal Hawkbit				0
Achillea millefolium	Yarrow				0
Prunella vulgaris	Self Keal				0
Veronica serpyllifolia	Thyme-leaved speedwell				0

A = Abundant C = Common 0 = Occasional

Fig. 2.4 Percentage Frequency Occurrence – Common Plant Species



The most frequently observed species in the pasture surrounding the snail habitat were Lolium perenne (91%), Cynosurus crystatus (60%) and Trifolium repens (91%). These species were presumably the main constituents of the seed mixture with which the ley was sown.

The habitat was characterised by two species of Juncaceae, neither of which were found on the field (Juncus effusus (12%) and Juncus bulbosus (28%)) and by a trio of species which belong to the Agropyro-Rumicon crispi alliance: Alopecurus geniculatus (91%) Glyceria fluitans (91%) and Ranunculus repens (76%). Over (1962) found that combinations of plant species belonging to this alliance are typical of the habitats of L. truncatula.

(ii) The Plant Species Found at Thorneythwaite

A list of the plant species found on habitats B and D and on field 673 is given in Tables 3 and 4.

GRAMINEAE - Alopecurus geniculatus and Glyceria fluitans were found exclusively in the trough of the ditch habitat (B). The four other grass species associated with this habitat, Poa trivialis (Rough Meadow grass), Agrostis tenuis (Common Bent), Molinia caerulia (Purple Moor grass), and Deschampsia cespitosa (Tufted Hair grass) occurred on the ditch sides. Habitat D had more in common with the pasture in which it was situated than with the ditch habitat B, the grass species found there (habitat D) being either common throughout the British Isles or characteristic of acid grassland.

Table 3

PLANT SPECIES FOUND AT THORNEYTHWAITE

(a) GRAMINEAE

		HABITAT		FIELD
		B	D	673
Alopecurus geniculatus	Marsh Foxtail	A		
Glyceria fluitans	Flote Grass	A		
Poa trivialis	Rough Meadow Grass	C		
Agrostis tenuis	Common Bent	C		
Molinia coerulia	Purple Moor Grass	0		
Deschampsia coespitosa	Tufted Hair Grass	0		0
Holcus lannatus	Yorkshire Fog	C	C	C
Cynosurus cristatus	Crested Dogs Tail		C	C
Nardus stricta	Mat Grass		0	0
Dactylis glomerata	Cocks foot			C
Festuca rubra	Red fescue			C
Anthoxanthum odoratum	Sweet Vernal Grass		0	0

A = Abundant C = Common 0 = Occasional

(b) JUNCACEAE AND CYPERACEAE

		HABITAT		FIELD
		B	D	673
Juncus effusus	Soft Rush	x	x	x
Juncus articulatus	Jointed rush	x	x	
Juncus filiformis	Slender rush		x	
Rynchospora fusca	Brown Bead Sedge		x	
Carex binerris	Ribbed Sedge		x	
Carex flacca	Carnation Grass		x	

x = Present

Table 4

PLANT SPECIES FOUND AT THORNEYTHWAITE

DICOTYLEDONES

		HABITAT		FIELD
		B	D	673
Filipendula ulmaria	Meadow Sweet	x		
Polygonum hydropiper	Water Pepper	x		
Ranunculus repens	Creeping Buttercup	x		
Epilobium palustre	Marsh Willow Herb	x		
Rumex acetosa	Sorrel	x	x	x
Ranunculus flammula	Lesser Spearwort	x	x	x
Galium palustre	Marsh Bedstraw	x	x	x
Potentilla erecta	Common Tormentil	x	x	x
Lotus ulinginosus	Large Birds foot trefoil	x	x	
Cardimine pratensis	Cuckoo Flower	x	x	
Leontodon autumnalis	Autumnal Hawkbit		x	x
Myosotis secunda	Water forget-me-not		x	x
Bellis perennis	Daisy		x	x
Cerastium fontanum	Common Mouse Ear		x	x
Trifolium repens	White Clover		x	x
Ranunculus acris	Meadow Buttercup		x	x
Lychnis flos-cuculi	Ragged Robin		x	
Taraxacum palustre	Marsh Dandelion		x	
Polygala (sp.)	Milkwort		x	
Hypochaeris radicata	Catsear			x
Pedicularis sylvatica	Lousewort			x
Achillea millifolium	Yarrow			x
Plantago lanceolata	Ribwort			x
Galium saxatile	Heath Bedstraw			x
Veronica (sp.)	Speedwell			x
Trifolium pratense	Red Clover			x
Euphraeia officionalis	Eye Wright			x
Stellaria (sp.)	Stickwort			x

x = present

JUNCACEAE AND CYPERACEAE - Juncus effusus occurred generally over the whole area although it was more common along the sides of ditches and in the wetter areas than elsewhere. Juncus articulosus occurred infrequently on habitats B and D but not field 673. DICOTYLEDONES - Four species were found on the sides of the ditch habitat (B) and nowhere else: Filipendula ulmaria (Meadow Sweet), Polygonum hydropiper (Wetter Pepper), Ranunculus repens and Epilobium palustre (Marsh Willow Herb). As before, a large proportion of the species found on habitat D were found on field 683. Only four of these were common to all three locations: Rumex acetosa (Sorrel), Ranunculus flammula (Lesser Spearwort), Galium palustre (Marsh Bedstraw), and Potentilla erecta (Common tormentil). Lotus uliginosus (Large birdsfoot trefoil) and Cardamine protensis (Cuckoo flower) were the only two species that were found in both habitats but not the field.

The ditch habitat at Thorneythwaite was characterised by the Alopecurus-Glyceria-Ranunculus repens combination of species. Site D on the other hand differed from the field which surrounded it by having fewer species of grasses and more species of Juncaceae and Cyperaceae.

(iii) The Soil Profiles at Hendy

The soil at Hendy is derived from drift and acid igneous rocks and belongs to the Deiniol soil series. The soil type is a gleyed brown earth.

A diagram of the soil profiles of the pasture and snail habitat is given in Figure 2.5. A detailed account of the soil horizons follows.

Field 202b

0–6cm

Ap(g) Firm, brown horizon; not stoney, red-orange mottles common and distinct (up to 3cm diameter); abundant, medium, fibrous roots; p.H. 5.3; narrow boundary.

6–20cm

B(g) Friable, lighter brown horizon; slightly stoney (up to 2cm diameter) becoming very stoney (up to 6cm diameter) as depth increases; red-orange mottles common and distinct (up to 1cm diameter); p.H. 5.8; merging boundary.

20+cm

Bg Friable, light brown horizon; not stoney; red, orange, and blue mottles abundant and prominent; p.H. 6.6.

Site D

As for field 202b except:

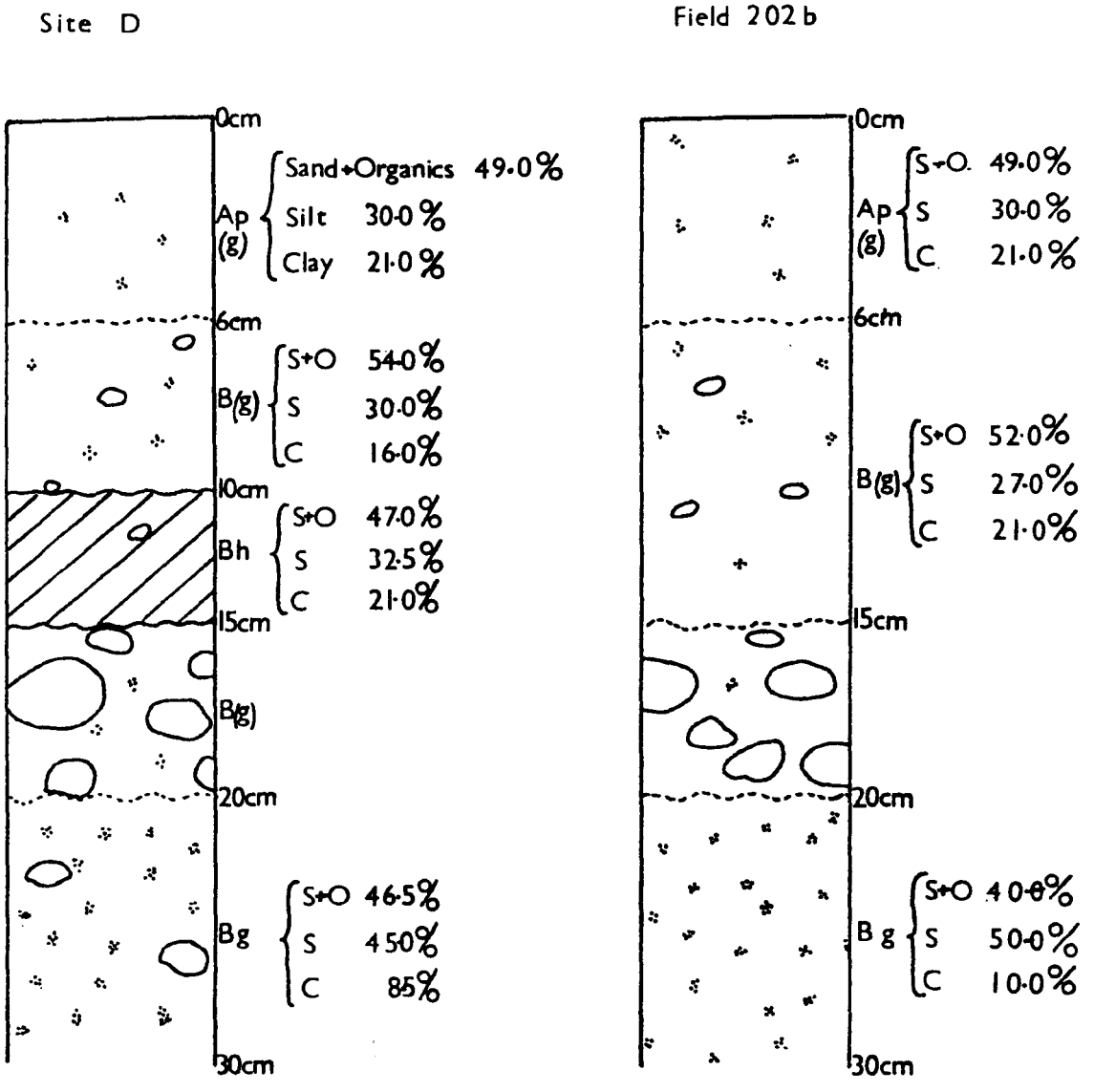
0–6cm

Ap p.H. 5.6

6–10cm

B(g) Generally dark brown; p.H. 5.6; sharp boundary

Fig. 2·5 Soil Profiles – Hendy



Gley Mottles

Plate 3

Soil Profile , Field 202 b
Hendy

10-15cm

B Distinct black acid horizon; p.H. 5.1; texture generally soapy; sharp boundary.

20+cm

Bg Slightly stoney (up to 4cm diameter); p.H. 6.4.

In both profiles gleying was apparent in all of the horizons, a characteristic of soils with impeded drainage. The most significant difference between the two profiles was in the apparent illuviation of organic material into the B horizons of the site profile. In addition, the B horizon of this profile contained a slightly greater proportion of clay and silt than either the horizon that overlaid it or the equivalent horizon in the field profile - again, suggesting illuviation from above.

(iv) The Soil Profiles at Thorneythwaite

Field 673

The soil type is a stagnopodzol (Curtis et al 1976).

A diagram of the soil profile is given in Figure 2.6. A detailed account of the soil horizons follows.

0-9cm

Ah Black horizon; soapy-gritty texture; not stoney; abundant, medium, fibrous roots; merging boundary.

9-23 cm

A /Ea Transitional horizon; generally black with a few faint grey-sandy coloured mottles (up to 1cm diameter) becoming common and distinct as depth increased; extremely stoney (up to 10cm diameter), sub angular, becoming stoney (up to 5cm diameter) as depth increased; frequent, fine fibrous roots to a depth of approximately 15 cm; sharp, irregular boundary.

23-31cm

Ea Grey-sandy coloured horizon; slightly stoney (up to 2cm diameter); narrow boundary.

31+cm

Bfe Bright orange horizon; very gritty texture; not stoney.

36+cm Level of water table (October 1975).

A podzolic soil profile is a characteristic of soils with good drainage in areas of high rainfall.

Site D

This site occurs in field 673. The soil pit very rapidly filled up with water and so only a very limited description of the horizons can be given. The main differences between the site and field profiles were these: first the soil in the site profile was uniformly black, there being no evidence of a bleached (Ea)

horizon, and second all the horizons, even the most superficial, were extremely stoney (sub angular, up to 10cm diameter).

Field 684

The ditch site B occurs in this field which differs from field 673 mainly in the fact that it is situated on the valley floor rather than on the lowest slopes of the fell side.

A diagram of the soil profile is given in Figure 2.7. A detailed account of the soil horizons follows.

0–15 cm

Ap Firm, dark brown horizon; not stoney; orange mottles occasional and distinct (up to 1cm diameter); abundant, fine–medium fibrous roots; narrow boundary.

15–25cm

Ag/Bg Transitional horizon; lighter brown; not stoney; orange mottles abundant and distinct (up to 1cm diameter); frequent, medium roots; orange colouration extends in streaks along lines of root penetration, irregular merging boundary.

25–45cm

Bg Dark brown horizon (as Ap); not stoney; orange mottles occasional and distinct (up to 1cm diameter); merging boundary.

Fig 2·6 Soil Profile – Field 673, Thorneythwaite

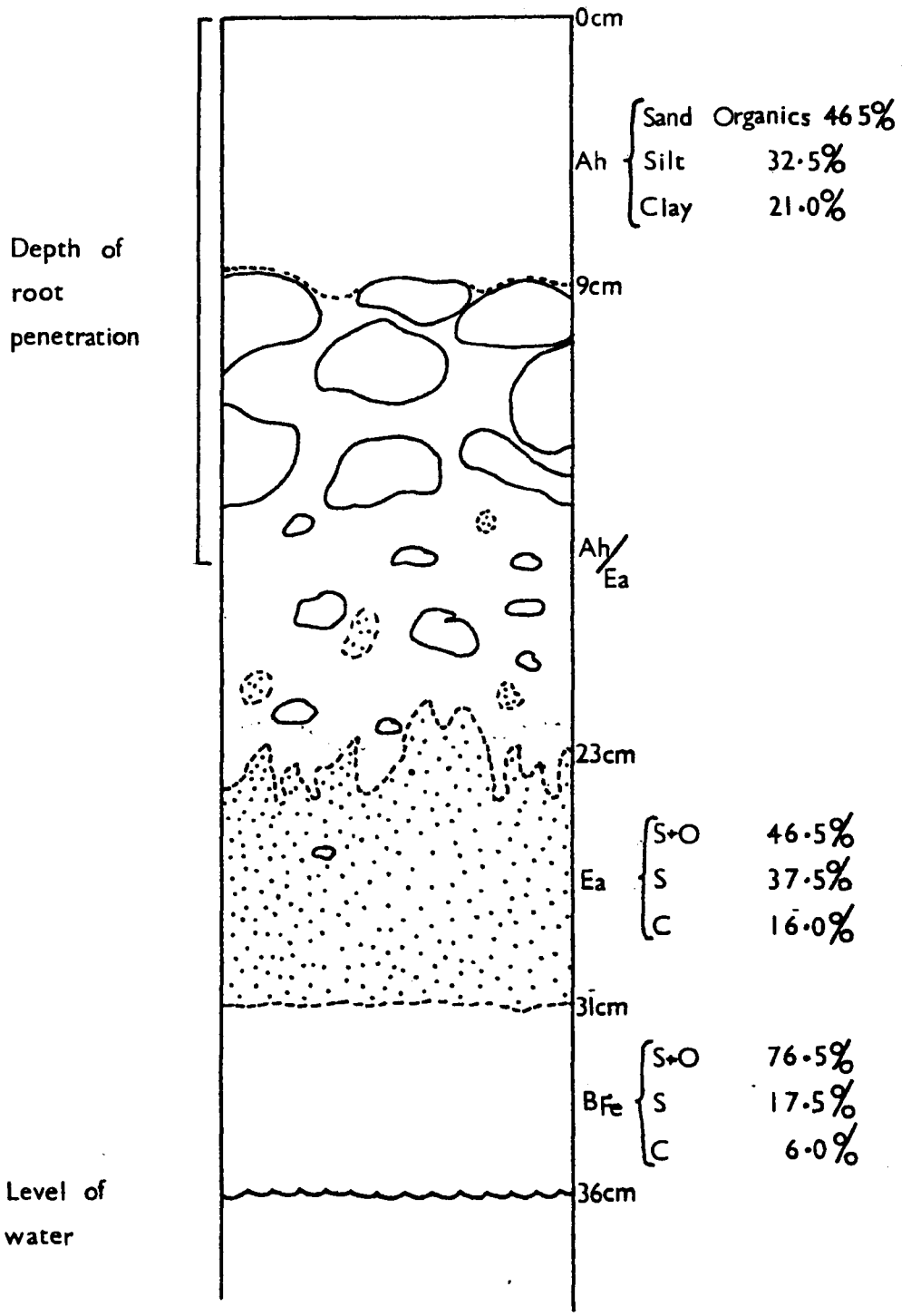


Fig 2·7 Soil Profile – Field 684 , Thorneythwaite

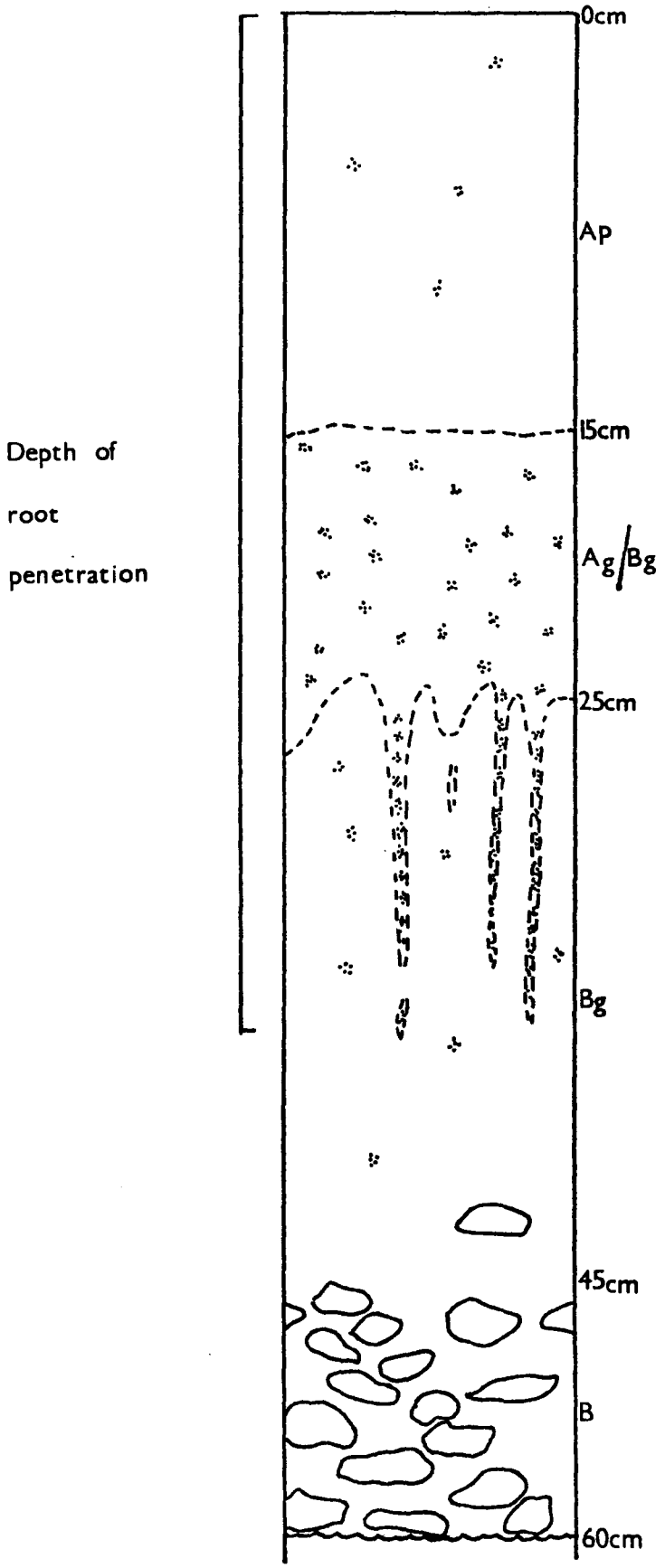


Plate 5. Soil Profile, Field 684, Thorneythwaite

Plate 4. Soil Profile, Field 673, Thorneythwaite

45-60cm

B Dark brown horizon; extremely stoney (up to 5cm diameter) angular to sub angular.

7 DISCUSSION

The snail habitats exhibited a considerable diversity of form, as much within the farms as between them. Nevertheless, plant species typical of the Agropyro-Rumicon crispi alliance were abundant on all but one of the five sites examined. (Site D, Thorneythwaite). These species are characteristic of an ecological assemblage that occupy contact zones (Over, 1962). That is to say, at the junction of wet and dry areas or in a situation that frequently alternates between one condition and the other. Even so, the division between the habitats and surrounding pasture was not clear cut. The mere presence of an 'indicator' species is not in itself sufficient to define a snail habitat since some occur in both habitat and field, e.g. Alopecurus geniculatus, Ranunculus repens, and Juncus effusus. However, all of these species are more abundant on the habitats than the pasture which sets them apart from others which are ubiquitous but more or less evenly distributed. A large degree of overlap is inevitable; fields which support snail habitats are likely to be rather wet over their whole area anyway, providing a suitable habitat for many helophytic species, and snail habitats will contain at least some representatives of the original ley

with which the pasture was sown.

The evidence of the soil profiles at Hendy indicates that the drainage characteristics of the soil here were poor. The soil type, a brown earth, is characteristic of areas with moderate rainfall, nonetheless, the extensive gleying apparent in all horizons suggests that the soil frequently became waterlogged. Any local increase in the normal water load caused flooding. Site C, for example, was situated above a blocked and fractured drain, and site D, site E and several other habitats not sampled were all located along the same contour line suggesting seepage off an impervious layer.

The mean annual rainfall at Thorneythwaite (3342mm) is about three times that at Hendy. The podzolic profile in field 673 indicates that drainage here was good; the habitats in this field (including site D), and presumably those others on the lower slopes of the fell, were maintained by a large amount of direct rainfall and more importantly by the continuous run-off and overspill from the fell sides. The character of the soil profile in field 684 indicated that the ditch habitat (site B) in this field was rather different. The site was situated on the valley floor between the Derwent river to the west and the Black Syke to the east. All of the run-off from the fell sides drained into one or other of these water courses before reaching the ditch. The morphology of the soil profile in this field placed it in a position intermediate between the "imperfectly" and "poorly" drained classes described

by Curtis et al. (1976), and so it was reasonable to assume that it was the poor drainage characteristics of the soil together with very high direct rainfall and gradual seepage from the surrounding field that maintained the ditch in its flooded condition rather than the run-off which sustained the other habitats.

It is interesting to note that all of the sites which depended upon impeded drainage had representatives of the Agropyro-Rumicon crispi alliance, whereas site D, Thorneythwaite, which relied upon a continuous inflow of water onto a quickly draining area, did not. Here the most abundant species were representatives of the Juncaceae and Cyperaceae.

CHAPTER 3. THE GENERAL WEATHER CONDITIONS AT HENDY AND THORNEYTHWAITE AND THE MICROCLIMATE OF THE HABITATS OF L. TRUNCATULA

1. INTRODUCTION

> "The conditions of climate to which young growing plants (and animals) are exposed cannot be deduced directly from the figures for climate published for the network of official stations." (Geiger, 1973)

The general relationship between standard weather features such as rainfall and temperature and the prevalence of fascioliasis is well known but these climatic variables exert their influence at second hand and are significant only in the extent to which they alter the microclimate of the habitats of L. truncatula. So far as I am aware the work reported in this chapter represents the first sustained attempt to investigate the relationship between macroclimatic variables and the microclimate of the snail habitats. The aim was fourfold:

(a) to devise a convenient measure of the soil moisture conditions on snail habitats which could be interpreted directly in terms of its effects on the snails and extramammalian stages of F. hepatica,

(b) to find the macroclimatic parameter or group of parameters that was most closely associated with this measure of habitat wetness,

(c) to investigate the relationship between air temperature at 1m and the temperature at the soil surface of a habitat,

(d) to provide a continuous record of the microclimate at two snail habitats over three consecutive seasons.

2. THE MEASUREMENT OF GENERAL WEATHER CONDITIONS AND HABITAT MICROCLIMATE

(i) The Weather Stations

Weather conditions were measured at one habitat on each farm. Site D, Hendy, and Site B, Thorneythwaite were chosen because the preliminary survey indicated that these habitats were likely to support a moderate to high population of L. truncatula throughout the whole period of observation. In addition, neither of the habitats was in a field which was continuously grazed by cattle. Trial weather stations had suffered considerable damage from farm stock within hours of construction and so the permanent stations were established within fenced enclosures. The fences consisted of 10 cm square wire mesh which afforded as open an aspect on all sides of the instruments as possible. There were two enclosures at each site. One housed an automatic tipping bucket rain gauge and the other a padlocked metal case which contained a Grant paper chart temperature recorder.

(ii) The Measurement of Temperature

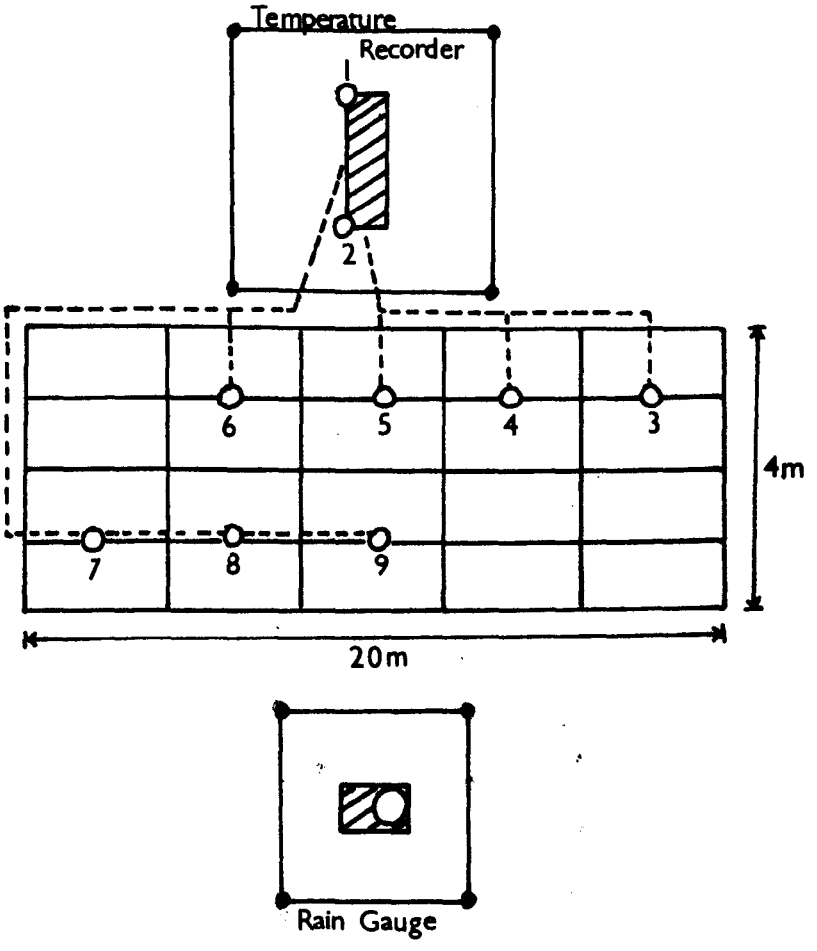
The temperature at the soil surface of the habitats was measured with thermistors. Each thermistor was enclosed within a stainless steel case (4 x 50 mm) and was held in place at the soil surface by a metal staple. The thermistors were connected

to a Grant paper chart recorder (Type D, 9 channels, Grant Instruments, Cambridge) and readings were taken on the hour at hourly intervals. Air temperature at a height of 1 m above the uncut pasture was measured by a pair of thermistors fixed to the scaffolding pipes which supported the metal case. The leads running from the thermistors to the recorders were each 10m long and buried for most of their length to a depth of 20cm. The paper chart recorders were powered by a rechargeable Nickel-Cadmium battery which required replacement once a month.

Each of the habitats was divided into blocks to facilitate the estimation of snail population density. On the Cumbrian site 7 thermistors were allocated to particular blocks using random number tables. On the Welsh site the 7 thermistors were distributed systematically (Figures 3.1 and 3.2). Recordings of the temperature at the soil surface were begun in June 1973. The placement of the thermistors was checked 8 months after installation in January 1974 and then once more in April 1975. The process caused considerable disturbance to the site but was necessary to relocate those sensors which had been trampled underground by the stock. In practice this applied to not much more than one or two thermistors on each occasion.

The thermistors at the soil surface became rapidly covered by a fine layer of silt and were soon overtaken by the growth of herbage. They were left unshielded by convention. However, shields

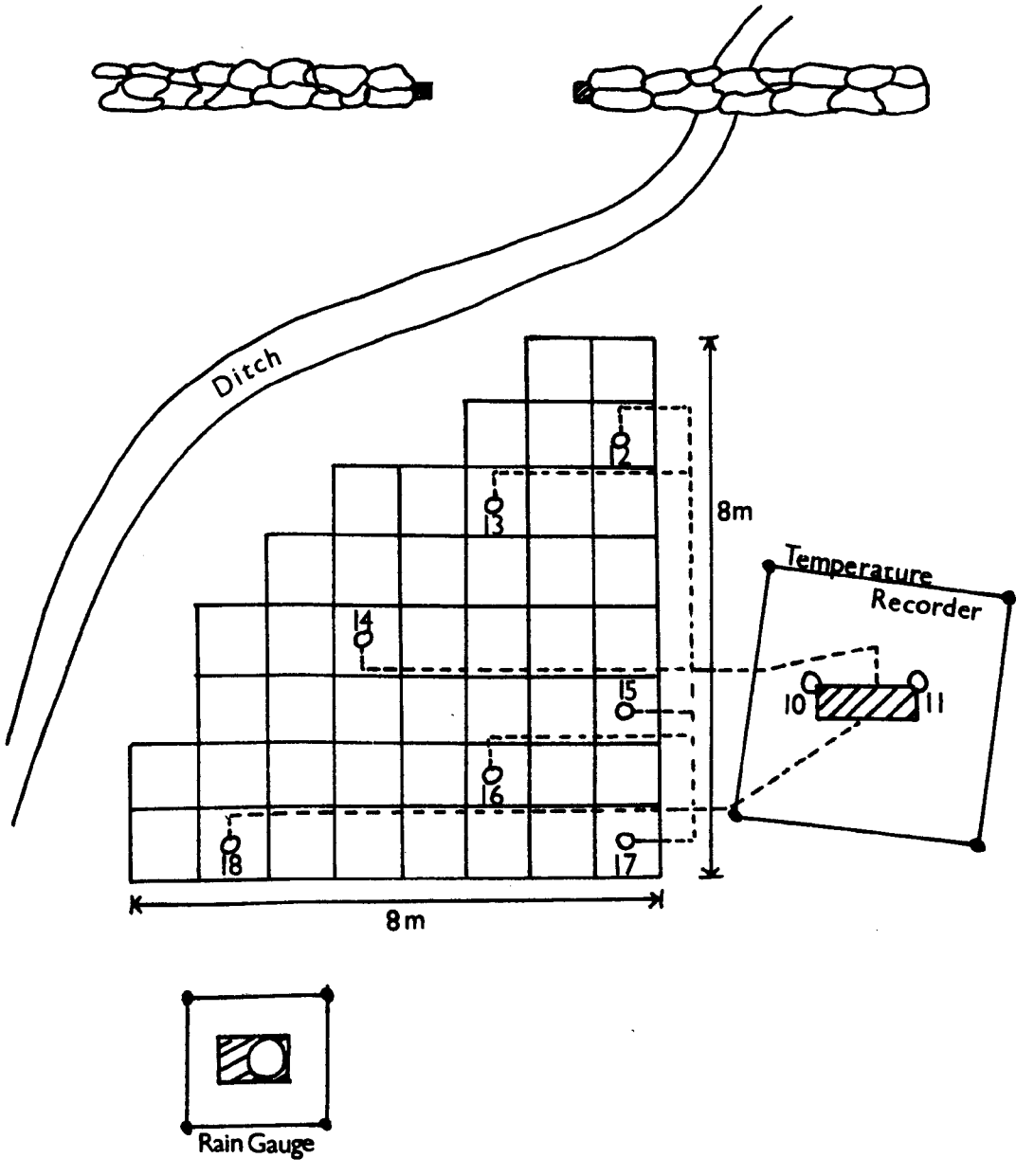
Fig. 3·1 Thermistor Placement – Site D, Hendy



o Thermistor

---- Cable

Fig. 3.2 Thermistor Placement – Site D, Thornethwaite



were used for the pairs of thermistors at each site that measured the air temperature. The shields comprised an arch of wire gauze held rigidly by a U-shaped wire frame that fixed into a perspex base plate. The thermistor was clamped in place beneath the gauze and the whole unit was attached to the scaffolding mast by a large terry-clip. The thermistors and their shields were positioned so that they pointed due south. The white paint of the shields was renewed as necessary. The relative efficiency of the shields is discussed in Appendix II.

Supplementary records of daily temperatures for the period between January 1973 and December 1975 were obtained from Glynllifon Park (Glynllifon Agricultural Institute, SH(23)455555) and Newton Rigg Farm (Cumbrian Farm School, NY(35)493310). Glynllifon Park is 6.5km from Hendy and situated in the same coastal strip. Newton Rigg Farm is 33km from Thorneythwaite. The records comprised daily maxima and minima measured by a dry bulb mercury thermometer in a Stevenson screen.

(iii) The Measurement of Rainfall

A tipping bucket raingauge was installed at each weather station. The raingauges were designed and constructed in the electrical workshops of the Biology Department at York. The bucket was calibrated to record each 0.01 inch of rainfall; its tipping activated a reed switch which initiated a short pulse recorded on

magnetic tape. A similar switch, operated by a battery driven clock, superimposed longer pulses on the record at noon and midnight of each day. The magnetic tapes were replaced at monthly intervals. The switch system was powered by two Nickel-Cadmium cells in parallel. These were removed monthly as were the batteries that powered the tape recorder. The clock battery was replaced every three months.

The rim of the 8 inch collecting funnel was set at a height of 12 inches above the soil surface. The Hendy raingauge was in a very exposed position, the nearest dry stone wall being over 100m away. The raingauge at Thorneythwaite occupied a more sheltered position within 30m of a dry stone wall some 1.5 metres high. The grass immediately surrounding the raingauges was continually cut to reduce splash and the upper surface of the instrument case was covered with wire gauze for the same reason.

Supplementary records of daily rainfall were obtained from three permanent rainfall stations. Two of them, Glynllifon Agricultural Institute (station number 57-530007) and Borrowdale C. of E. School (station number 44-592488), provided records of daily rainfall from January 1973 until December 1975 and September 1975 respectively. The raingauges were the standard non-recording Meteorological Office type. The third station, Seathwaite Farm (station number 44-592446), provided records of rainfall from February 1973 until September 1975. The raingauge was an automatic

tipping bucket gauge (Plessy M.M.46 gauge and Epsilon EDL10 data logger) which recorded rainfall in 0.2mm increments to the nearest minute G.M.T. Both Cumbrian rainfall stations were within 2km of Thorneythwaite Farm and in the same valley system.

(iv) Estimation of the Moisture at the Soil Surface

An attempt was made to measure the amount of moisture occurring on the soil surface of the snail habitats by an electrical resistance method (see for example Bouyoucos, 1949) It proved impossible to calibrate the sensors when they were placed at the soil surface and the method was subsequently abandoned. Instead a visual estimation method was devised.[1] A four point scale was used.

State	Description
1. Standing water	Soil surface covered by a layer of water that is more than a mere surface film.
2. Wet	Soil dark in colour. An obvious surface film of water present.
3. Damp	Soil dark in colour. No obvious surface film of water.
4. Dry	Soil pale in colour. Dry to the touch.

[1]The reliability of visual estimates of soil water has been demonstrated by Verigo and Razumova (1967).

Several habitats on each farm had been divided into blocks to facilitate the sampling of the snail populations. The state of the soil surface in each block was estimated as the sampling proceeded. A uniform condition of wetness over the whole soil surface of a block was indicated by the initial letters of the appropriate state (i.e. S, W, D or Dr.) A non-uniform condition of wetness was expressed in terms of the two predominant states e.g. S-W would indicate: "mostly standing water but some large wet areas." In general the least extensive of the two states needed to occupy about one third of the area of the block before it was recorded. In this way it was possible to estimate approximately the total area of a habitat over which a particular condition of wetness prevailed.

(v) The Measurement of Soil Water

Core samples of soil were taken from fields at Hendy and Thorneythwaite during the last 9 months of the field work. The cores were taken in batches of 5 allocated at random (core size 4.5mm diameter, 50-70mm deep). Samples were removed from Site D, Hendy, and the surrounding field area every month between January and September 1975. At Thorneythwaite the sampling began one month earlier in December 1974 and continued until August 1975, a batch of cores being taken from field 673 every month. In the laboratory the cores were weighed and oven dried at 105^{o}C to constant weight. The percentage soil water in each core was

calculated as:

$$\frac{\text{weight of water}}{\text{weight of dry soil}} \times 100 = \% \text{ soil water}$$

The results were expressed as the mean value for each batch of samples.

(vi) The Relationship between % Soil Water and Suction Pressure (pF)

The relationship between % soil water and suction pressure was determined for the soil type of each farm using tensiometers. The tensiometers comprised a P.V.C. tube with a ceramic porous cup at one end and a rubber bung at the other. A nylon manometer tube passed through the rubber bung and was led to a mercury cup. Water filled the P.V.C. tube and the manometer tube. Tensiometers work from saturation (suction pressure nil) to about 0.7 atmospheres.

Blocks of surface soil (50 x 30 x 10cm approx.) were removed intact from field 202 and Site D, Hendy, and field 673 Thorneythwaite. A pair of tensiometers was fixed in the superficial layers of each block and the blocks were immersed in water for up to an hour. Each block was then allowed to drain freely at room temperature for 24 hours and all herbage cover was cropped to the soil surface. Daily tensiometer readings were commenced immediately afterwards. The corresponding % soil water was determined for each reading by a gravimetric method. Measurement continued until the mercury columns collapsed. In order to obtain measurements for dry soil at suction pressures in excess of 1atm. samples were sent to the Department

of Forestry and National Resources, University of Edinburgh where Dr. David Whitehead continued the experiment using a pressure membrane apparatus.

All measurements of suction pressure were converted to the logarithmic pF scale before comparison with % soil water ($pF = \log_{10} cmH_2O$).

(vii) Other Weather Records

Complete copies of the monthly returns of daily observations from two manual stations were obtained. The records, which covered the years 1973, 1974 and 1975, were supplied by the Gwynedd River Authority who maintain a station at Allaw Reservoir, Anglesey, and by the Cumbrian Farm School at Newton Rigg.

3. RESULTS

England and Wales has been divided into 52 "agroclimatic" areas (M.A.F.F. Technical Bulletin, No. 35, 1976). The areas were initially defined in terms of farming practice on the assumption that farmers had correctly assessed which type of farming is suited to the prevailing conditions of climate and soil. The climate of each area has been described in terms of the monthly averages of a number of parameters including rainfall and temperature. These areal averages were calculated from data that cover the period between 1941 and 1970 and provided a convenient baseline from which to describe the weather conditions

Fig. 3·3 Agroclimatic Area 47

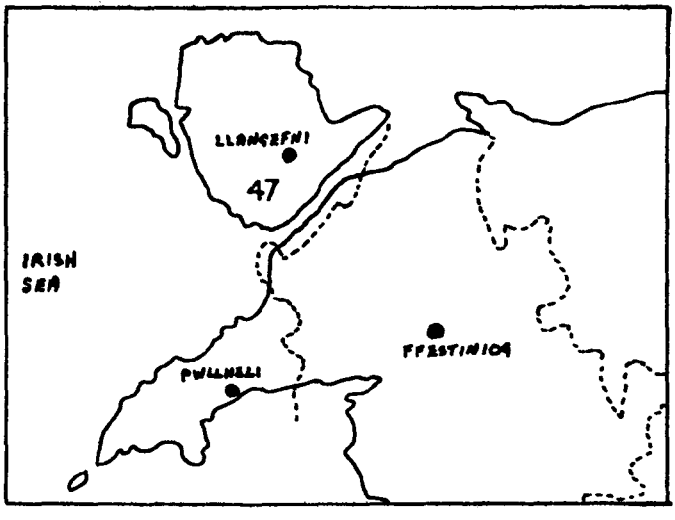
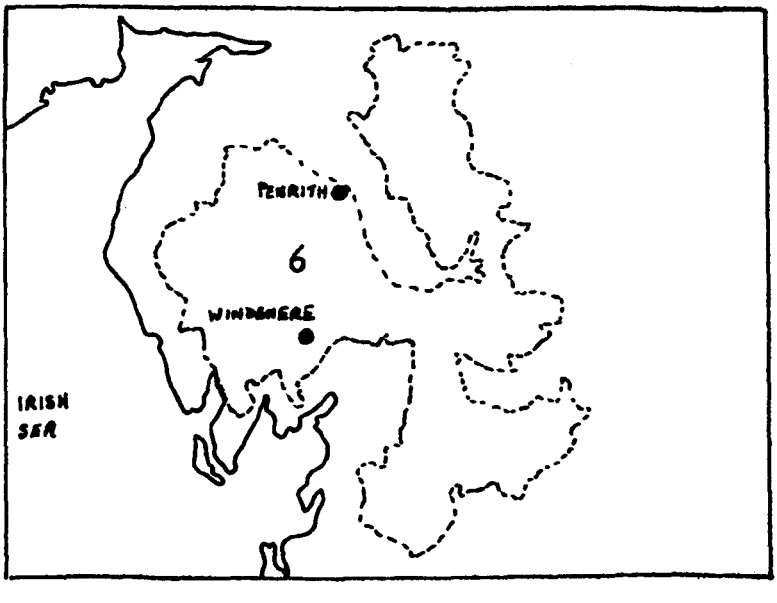


Fig. 3·4 Agroclimatic Area 6



that prevailed in the vicinity of Hendy and Thorneythwaite during the 30 months of study. The Welsh farm is in area number 47 and the Cumbrian farm is in area number 6. (Figures 3.3 and 3.4).

(i) Rainfall

A series of technical difficulties, some of which were never resolved, rendered the information obtained by the on-site rain gauges fragmentary at best. Nevertheless, sufficient information was obtained to establish that the monthly rainfall recorded by the Glynllifon station was significantly correlated with that recorded by the gauge at Hendy ($r=0.66$, $p<0.05$). The data provided by the gauge at Thorneythwaite were insufficient to permit a similar comparison but there was a highly significant correlation between the weekly rainfall recorded by the raingauges at Seathwaite and Borrowdale ($r=0.92$, $p<0.01$) and it was assumed that local variations in rainfall within the Borrowdale valley could be ignored. Henceforth, all estimates of rainfall at Hendy and Thorneythwaite were taken from the data provided by the Glynllifon and Seathwaite raingauges respectively.

The rainfall at each farm is presented as the deviation from the monthly areal average in Figures 3.5 and 3.6. In both cases the rainfall that occurred in the early half of the winters of 1973-74 and 1974-75 was well above average. At Hendy the rainfall in spring and early summer showed a progressive decline from year

Fig 3.5 Monthly Mean Rainfall—Hendy

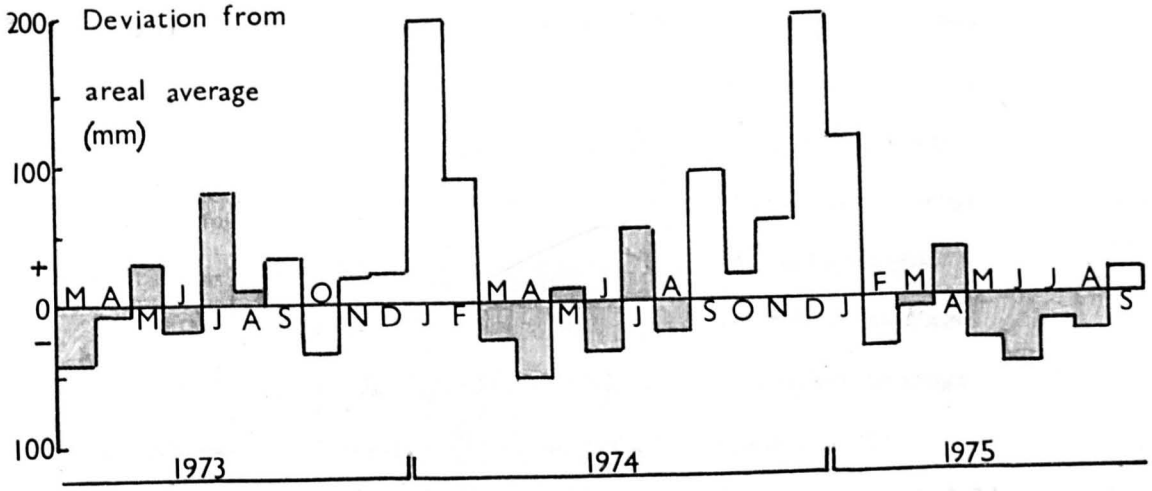
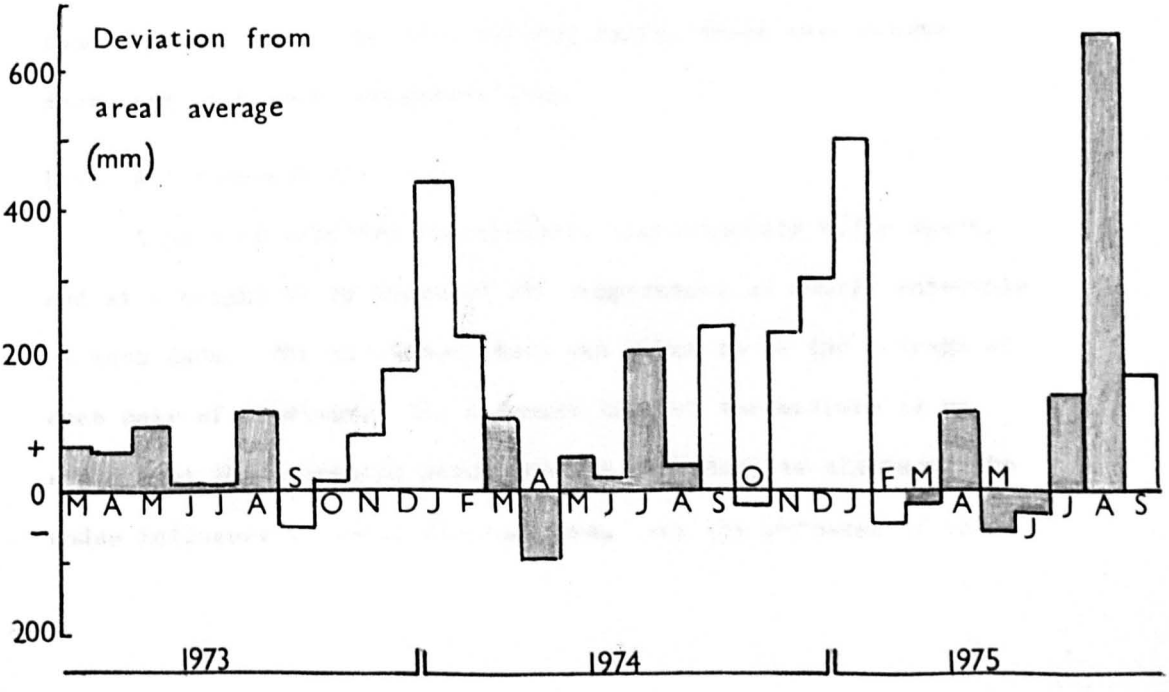


Fig. 3.6 Monthly Mean Rainfall—Thorneythwaite



to year, the summed monthly deviations for this period being 17.7mm in 1973, -55.5mm in 1974 and -67.2mm in 1975. The rain that falls in April is particularly significant since it is this that largely determines the date at which the amount of water in the soil falls below field capacity (M.A.F.F. Tech.Bull.No.35). At Hendy the rainfall in April 1973 was barely below the average figure whereas that in April 1974 was well below indicating an early end to field capacity. Though rainfall was above average in April 1975 this month had been preceded by two below average months and was followed by four below average months. The situation at Thorneythwaite was very similar; the spring rainfall declined from year to year and the rainfall in April 1974 was considerably below average. However, above average rainfall in July and August of 1973, 1974 and 1975 at Thorneythwaite indicated that at this farm the return to field capacity would be more rapid than at Hendy where the rain falling during these same months decreased with each successive year.

(ii) Air Temperature

A pair of shielded thermistors, approximately 0.75m apart, and at a height of 1m measured air temperature at hourly intervals at each farm. The air temperature was taken to be the average of each pair of readings. The response time of thermistors is so rapid that the averaging procedure was necessary to eliminate the undue influence of local fluctuations. For the purposes of these

records it was assumed that a day was the period between 1 a.m. and 12 midnight unlike the standard meteorological day which begins at 9 a.m. Similarly, a week was taken to be the period between 1 a.m. Sunday and 12 midnight on the following Saturday.

The highest of the 24 averaged temperature readings that occurred between 1 a.m. and 12 midnight was assumed to be the daily maximum temperature and the lowest of the 24 readings was assumed to be the daily minimum temperature. Since these measurements were made at discrete intervals rather than continuously they do not necessarily correspond to the daily maxima and minima that might have been recorded by a standard mercury maximum and minimum thermometer at the same location.

Weekly mean maximum temperatures and weekly mean minimum temperatures for each climate station are presented in Figures 3.7 and 3.8. In both cases the highest air temperatures were recorded during the last week of August 1973 and the first week of July 1975. At Hendy, the lowest air temperature was recorded during the first week of December 1973; there is no information for the corresponding week at Thorneythwaite.

The mean monthly air temperatures at each farm were calculated as the average of all the hourly measurements taken during the month and are expressed as deviations from the areal averages in Figures 3.9 and 3.10. In both cases the temperatures experienced in the winters of 1973-4 and 1974-5 were well above normal. In

Fig. 3·7 Mean Weekly Temperature Maxima and Minima – Hendy

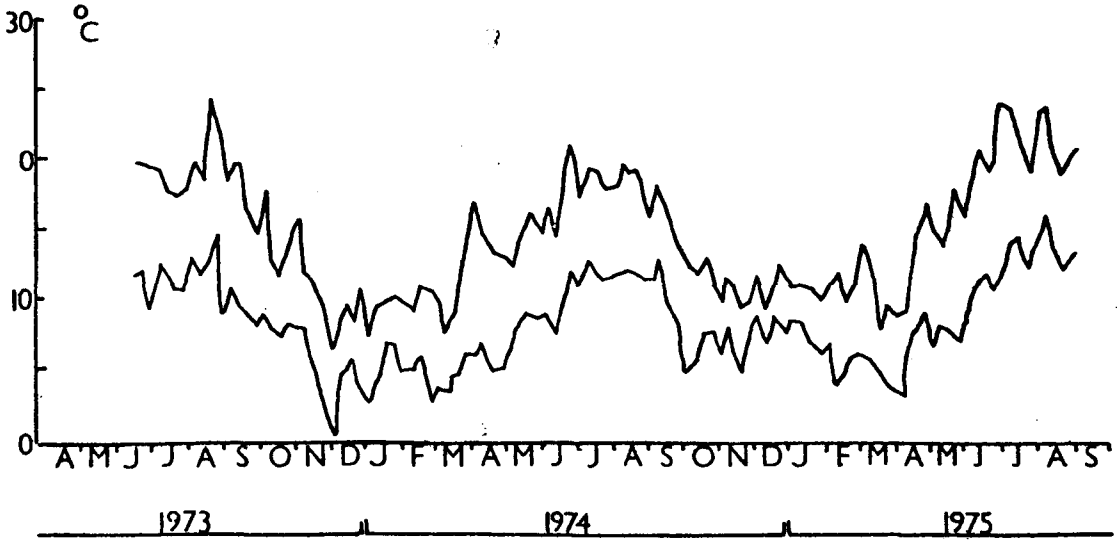


Fig. 3·8 Mean Weekly Temperature Maxima and Minima – Thorneythwaite

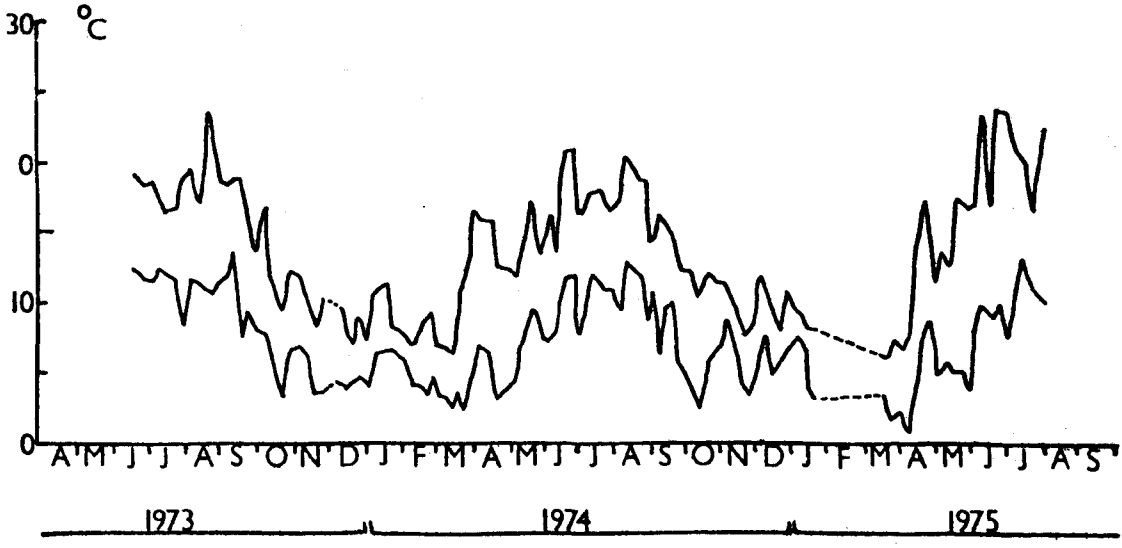


Fig.3.9 Monthly Mean Temperatures – Hendy

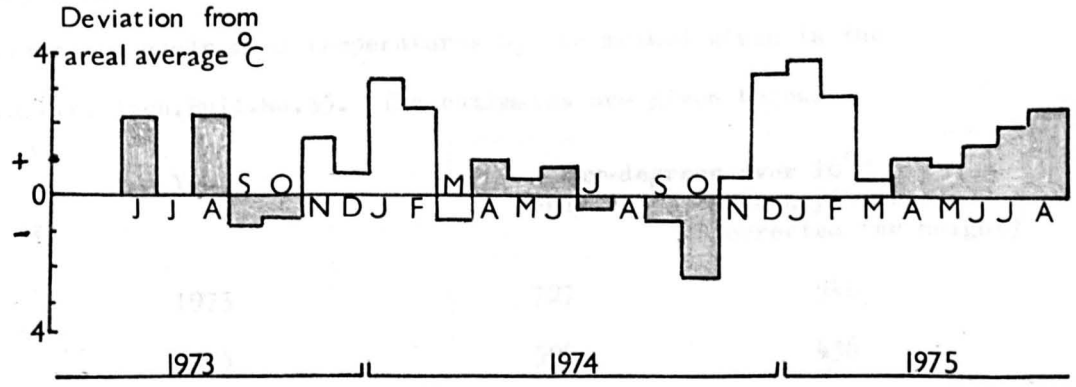
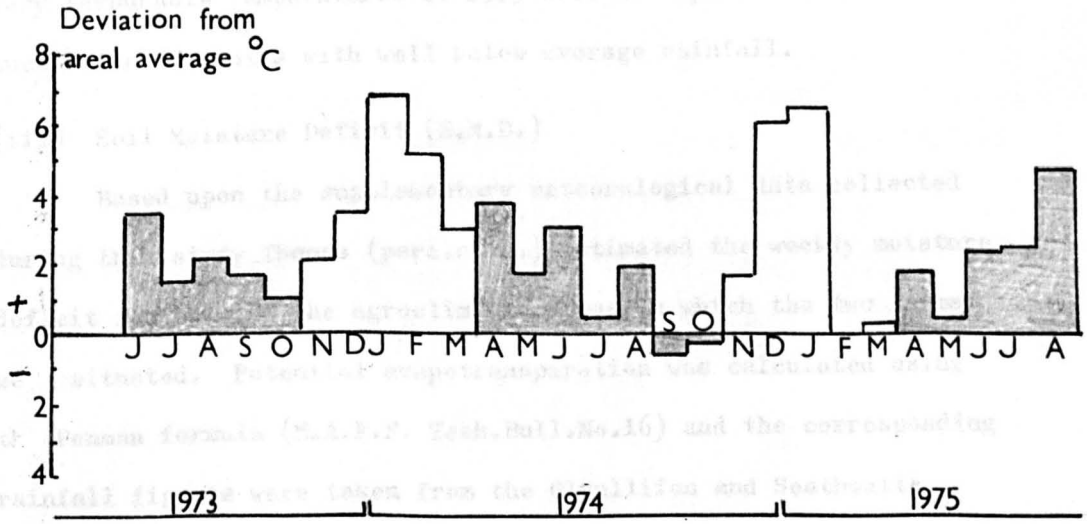


Fig.3.10 Monthly Mean Temperature – Thorneythwaite



order to assess the potential for the development of F. hepatica during the spring and summer of each year the accumulated temperatures (day-degrees) over 10°C for the May to October season were estimated from the monthly mean temperatures by the method given in the M.A.F.F. Tech.Bull.No.35. The estimates are given below.

Year	Day-degrees over 10°C	
	Hendy	Thorneythwaite (corrected for height)
1973	727	548
1974	590	456
1975	800	570
Published areal average	635	425

As expected Thorneythwaite provided a cooler environment than Hendy. The 1974 season on both farms was not only colder than the previous one but drier as well, particularly in September and October and the very favourable temperatures of 1975 were accompanied by a whole succession of months with well below average rainfall.

(iii) Soil Moisture Deficit (S.M.D.)

Based upon the supplementary meteorological data collected during this study Thomas (pers.comm.) estimated the weekly moisture deficit for each of the agroclimatic areas in which the two farms were situated. Potential evapotranspiration was calculated using the Penman formula (M.A.F.F. Tech.Bull.No.16) and the corresponding rainfall figures were taken from the Glynllifon and Seathwaite records respectively.

The variations in soil moisture deficit for each area are given in Figures 3.11 and 3.12. For the purposes of comparison the "end of capacity date" was defined as that period in which occurred the first sustained increase in soil moisture deficit over 5 mm. The "return to capacity date" was similarly defined as that period in which occurred the first sustained fall in soil moisture deficit below 5 mm. The dates so obtained are matched with the areal medians below.

End of Capacity Date

	Thorneythwaite		Hendy	
Year	Observed	Areal Median	Observed	Areal Median
1973	-	4 June (quartile range 12 May -	8–14 April	2 May (quartile range 15 April - 2 June)
1974	17–23 March		24–30 March	
1975	4–10 May		4–10 April	

Return to Capacity Date

	Thorneythwaite		Hendy	
Year	Observed	Areal Median	Observed	Areal Median
1973	23–29 Sept.	10 July (quartile range - 5 Aug.	23–29 Sept.	9 Oct. (quartile range 5 Sept.- 30 October
1974	25–31 Aug.		8–14 Sept.	
1975	7–13 Sept.		Later than 27 Sept.	

Fig.3·11 Weekly Mean S.M.D. – Hendy

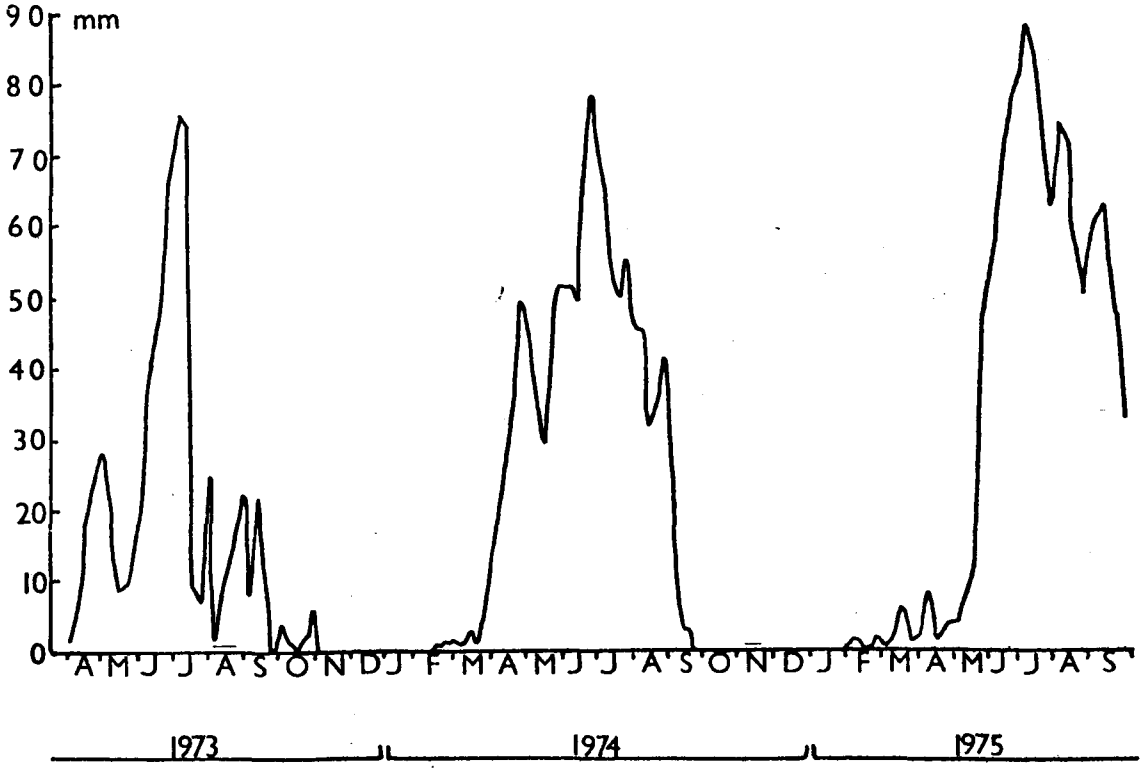
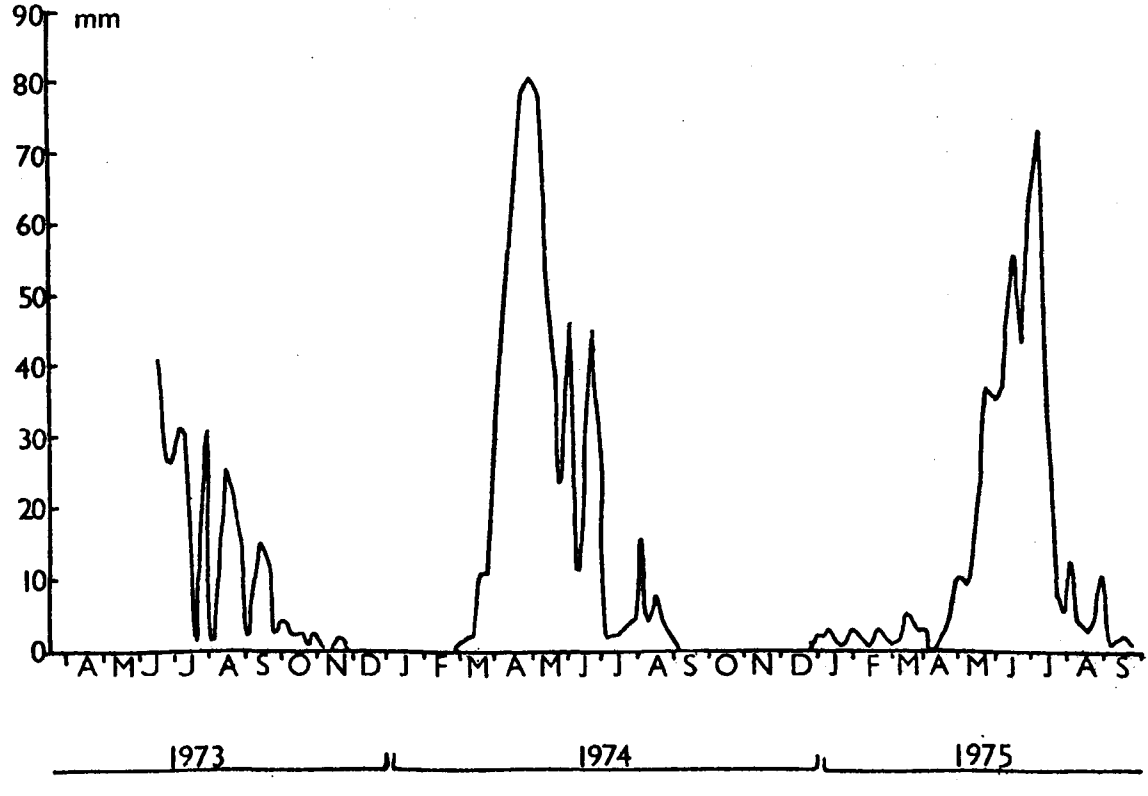


Fig.3·12 Weekly Mean S.M.D. – Thorneythwaite



The end of capacity dates in any particular year were very similar though much earlier than the areal medians and in fact just outside the quartile ranges in each case. The end of capacity in 1974 was particularly early and the rise in soil moisture deficit abrupt and sustained. In contrast the end of capacity dates in 1975 were less clear cut; transient soil moisture deficits were estimated to have occurredvery early in the year on both farms.

The return to capacity dates revealed the principal difference between the farms. In 1974 and 1975 Thorneythwaite returned to full capacity at least two weeks earlier than Hendy though the difference may in fact have been even larger since the soil moisture deficit at Thorneythwaite generally approached zero from mid-July onwards. In consequence the fields at Hendy spent about twice as long at an estimated soil moisture deficit in excess of 10mm than did Thorneythwaite. 1973 was an exceptional year at Hendy for the return to capacity followed the same course as that at Thorneythwaite, near zero values occurring at the beginning of August.

(iv) Soil-Surface Moisture

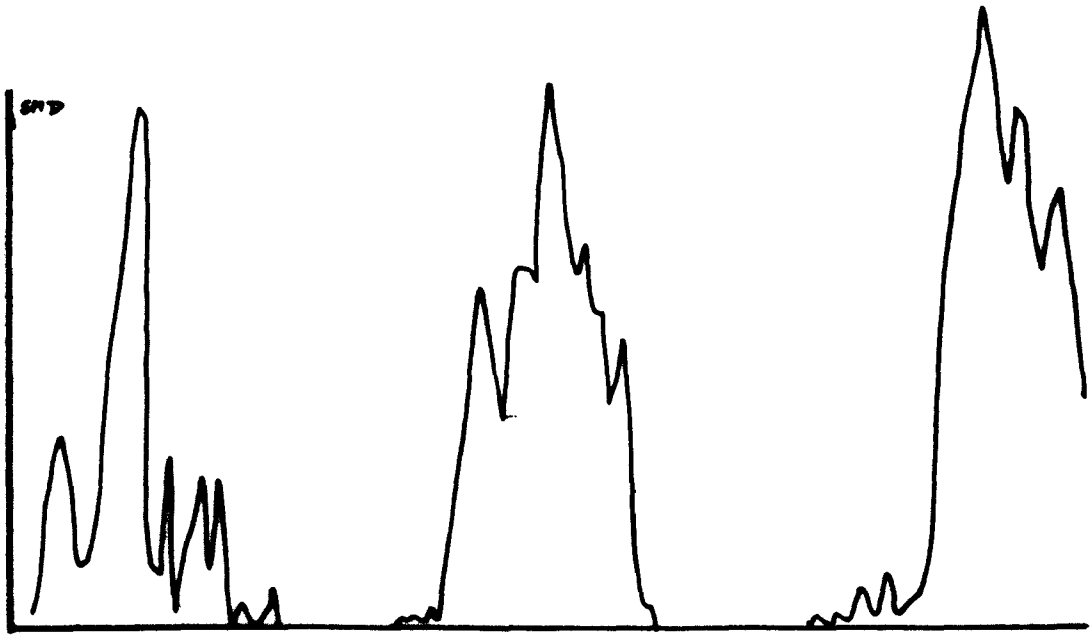
The visual estimates of soil moisture were used to calculate the proportion of each site that was covered with "standing water" and the proportion that was "damp" or "dry". On both farms the habitats dried out much earlier in 1974 than in either 1973 or 1975. The dry spring period became progressively longer with each passing year and in 1975 there was no sign of standing water on

any of the habitats even in late July and early August. These similarities apart, there were some important differences. Two sites, one on each farm, can be distinguished from the others. These were Site D, Hendy, and Site B, Thorneythwaite. In both cases the annual dry period was consistently either less intense or shorter than at any of the other sites at which soil moisture was estimated. However, standing water conditions were always re-established sooner at Site B, Thorneythwaite, than at Site D, Hendy. In 1974, for example, more than half of Site B was under standing water by July whereas Site D did not recover appreciably until September and did not approach maximal coverage until October. This instance reflects the more general tendency of the Cumbrian habitats to become wet again sooner than at Hendy.

The visual estimates of soil-surface water on all sites have been plotted together with a transparent overlay of the corresponding estimates of S.M.D. in Figures 3.13 and 3.14. The qualitative relationship is obvious but with so few sites and with such variation between sites a quantitative relationship could not be established.

(v) % Soil Water

The relationship between S.M.D. and the variations in % soil water and soil-surface water that occurred in and around Site B, Thorneythwaite, and Site D, Hendy, during 1975 is represented in Figures 3.15 and 3.16. % soil moisture varied


SND

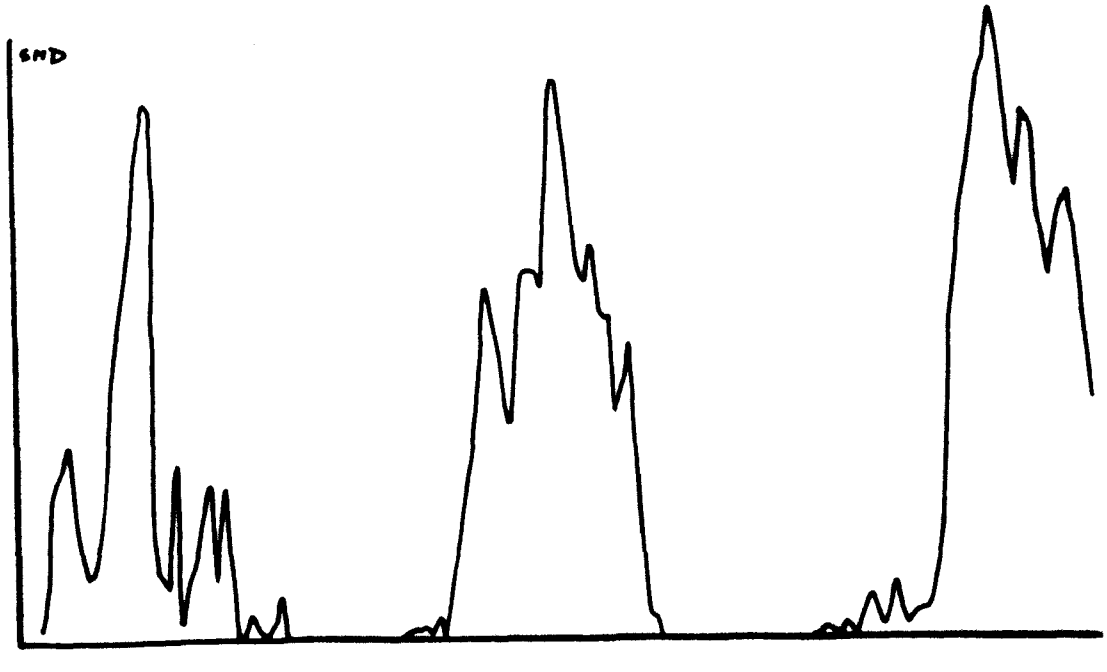

SND

Fig. 3·13a Proportion of Habitat; "Standing Water" — Hendy

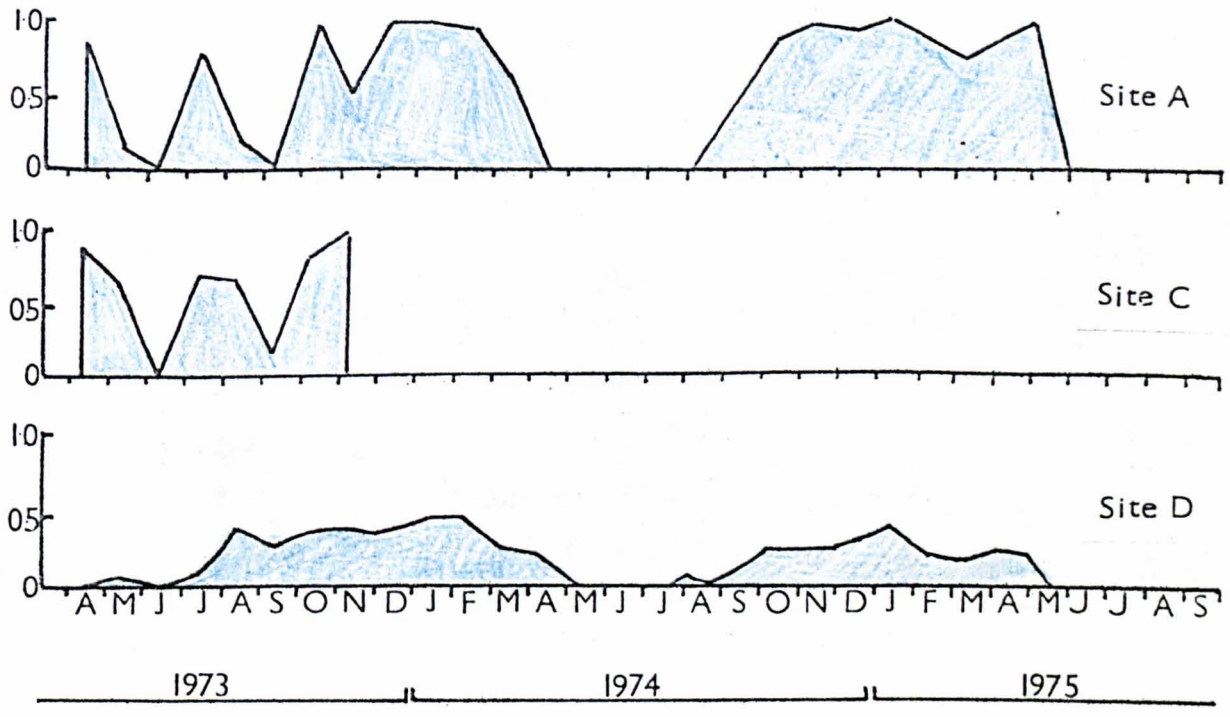


Fig. 3·13b Proportion of Habitat; "Damp or Dry" — Hendy

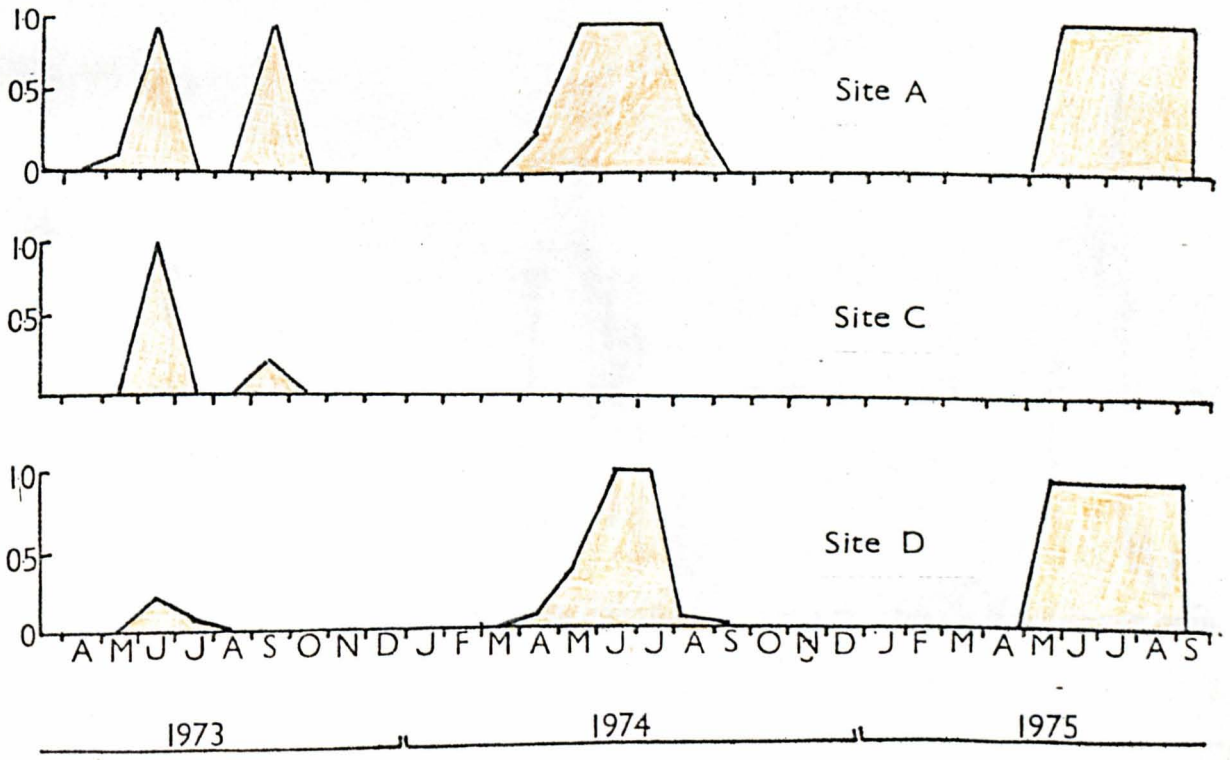


Fig 3·13a Proportion of Habitat; "Standing Water" — Hendy

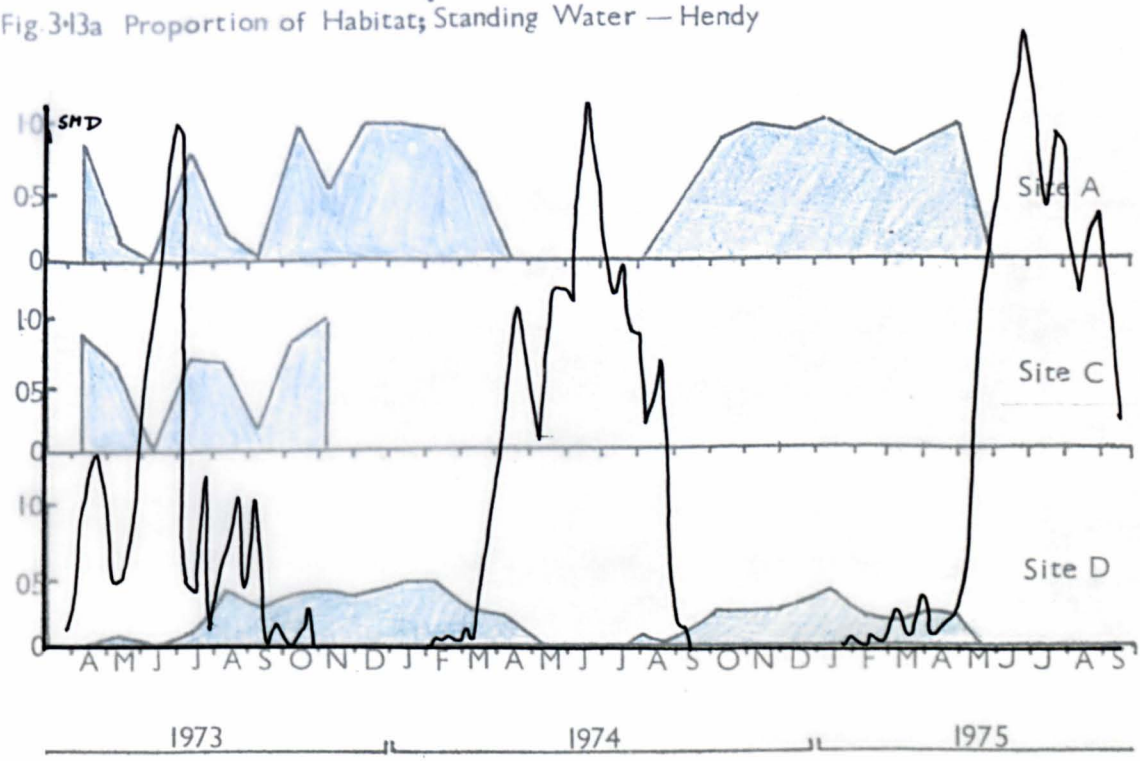
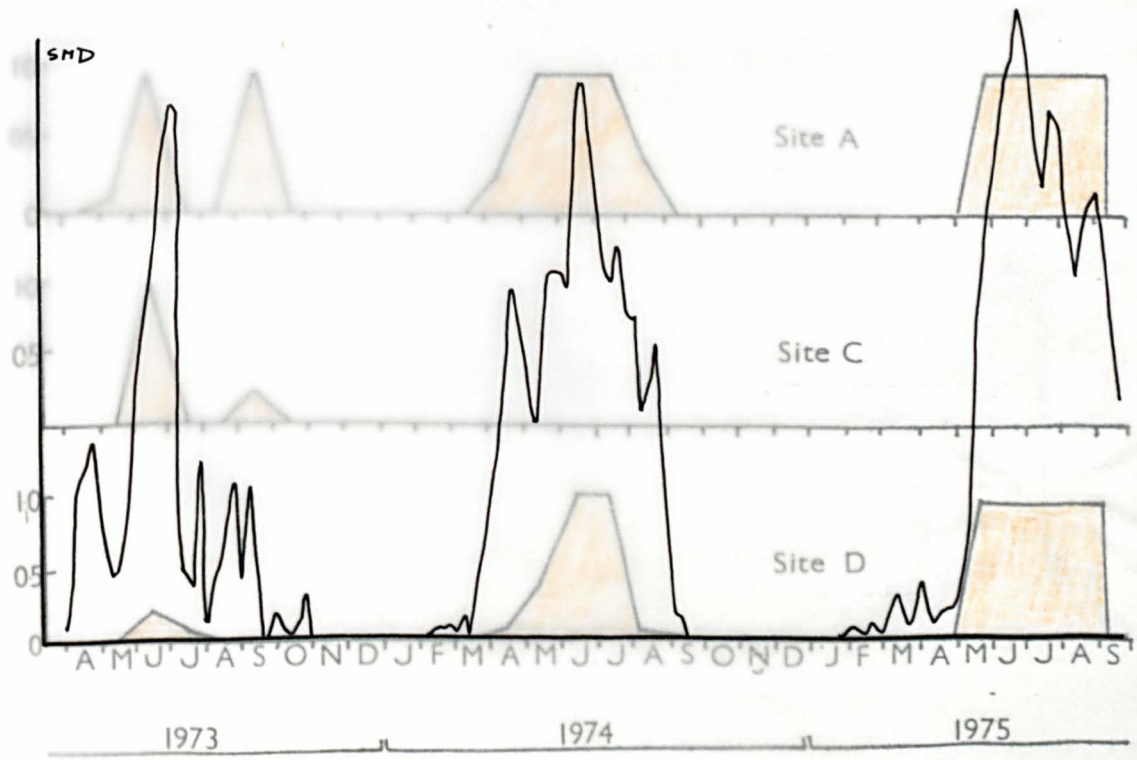
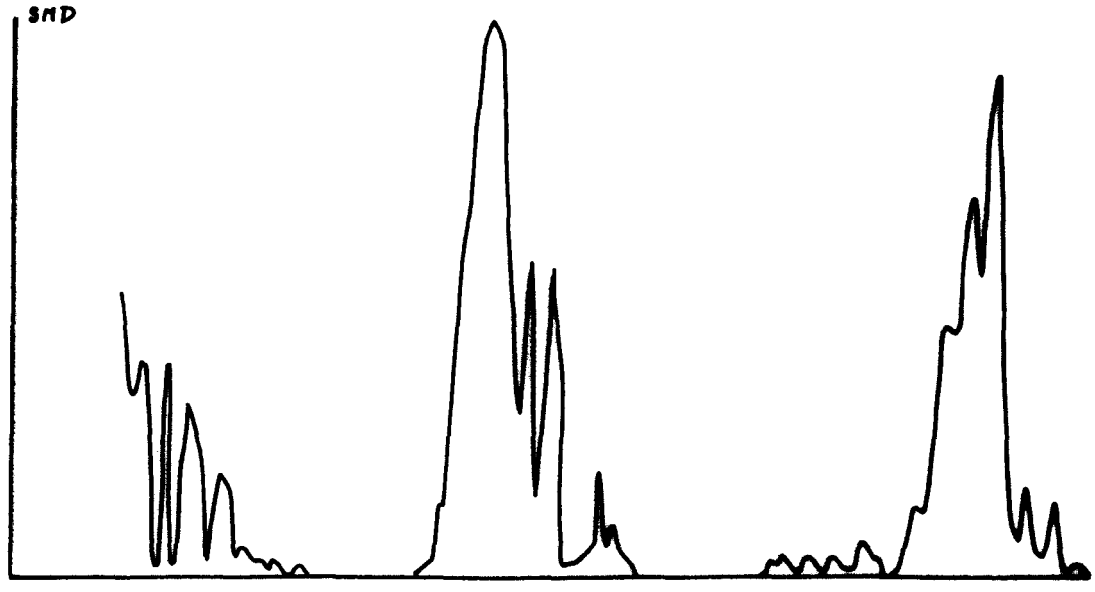


Fig 3·13b Proportion of Habitat; "Damp or Dry" — Hendy

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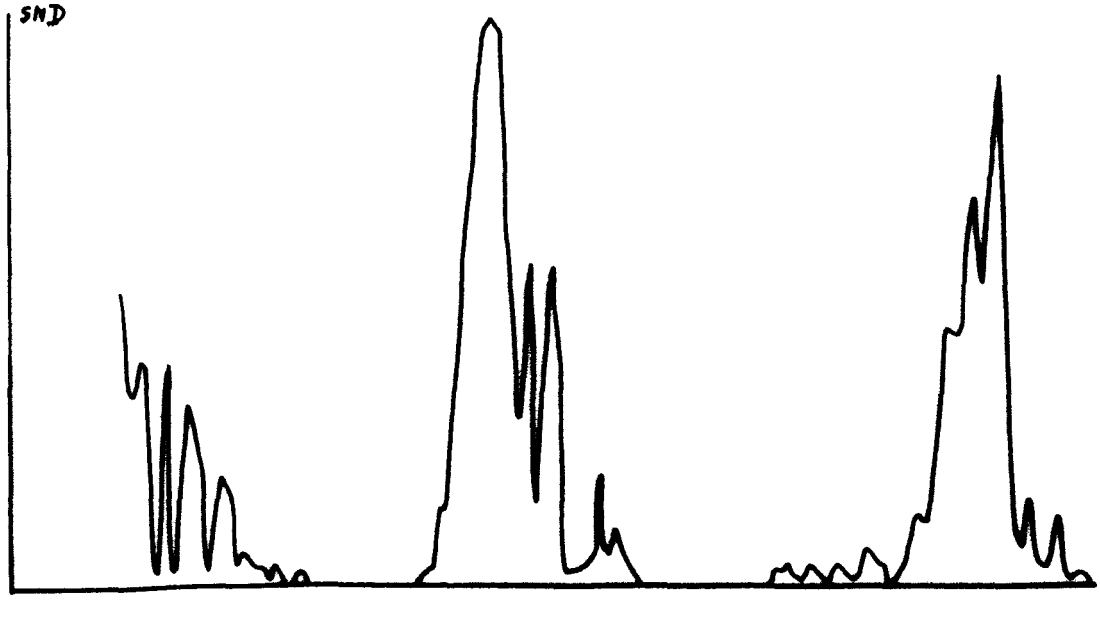

SND

Fig. 3·14a Proportion of Habitat · Standing Water —Thorneythwaite

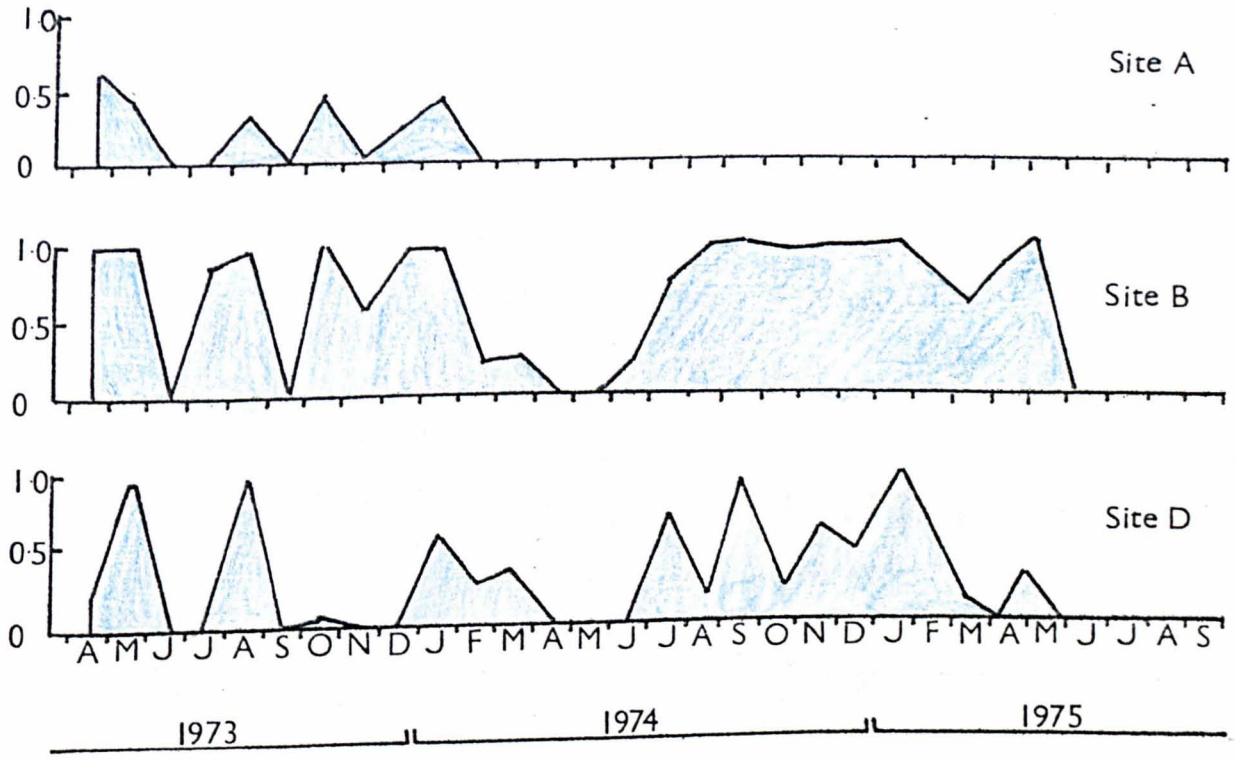


Fig · 3·14b Proportion of Habitat · Damp or Dry —Thorneythwaite

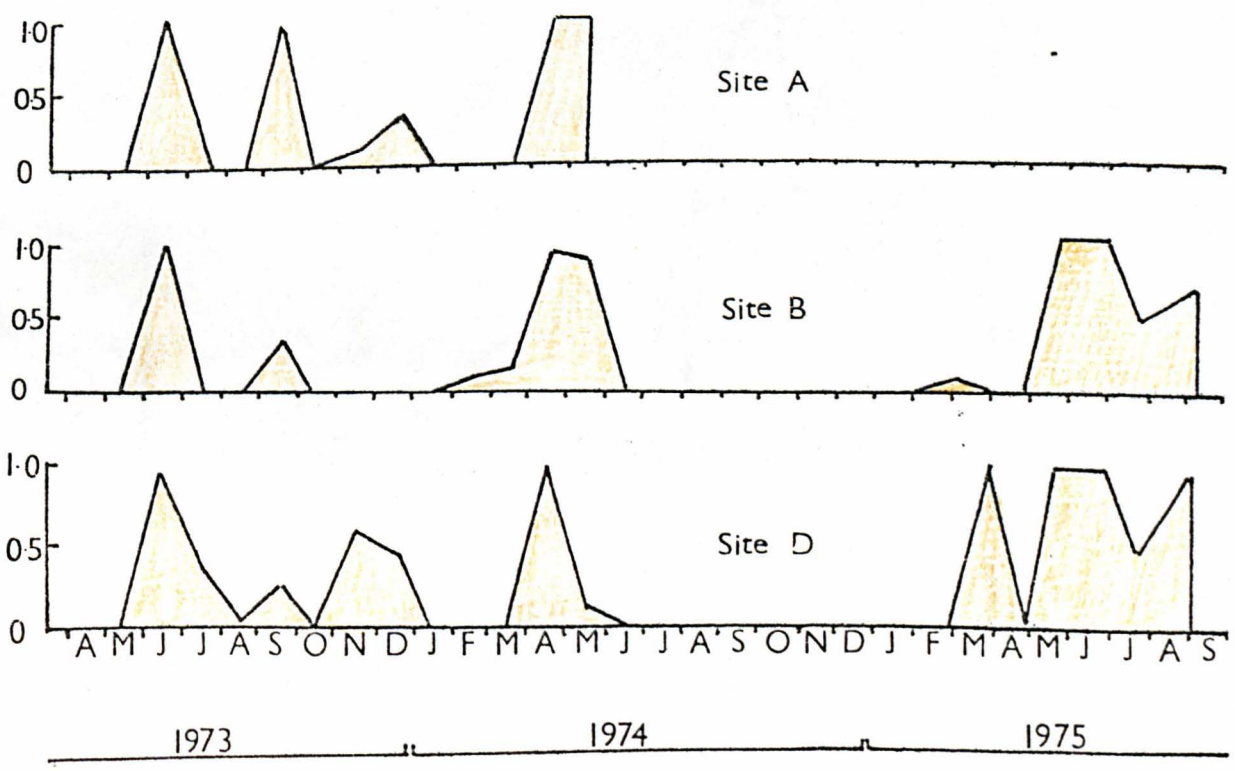


Fig. 3·14a Proportion of Habitat · Standing Water —Thorneythwaite

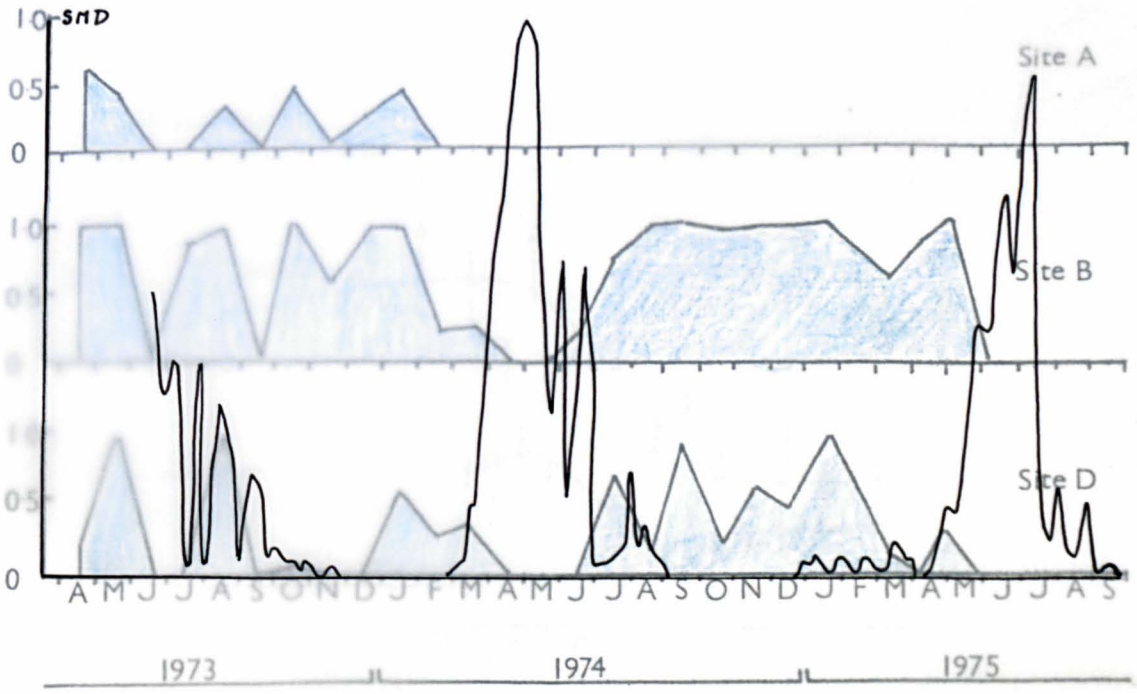
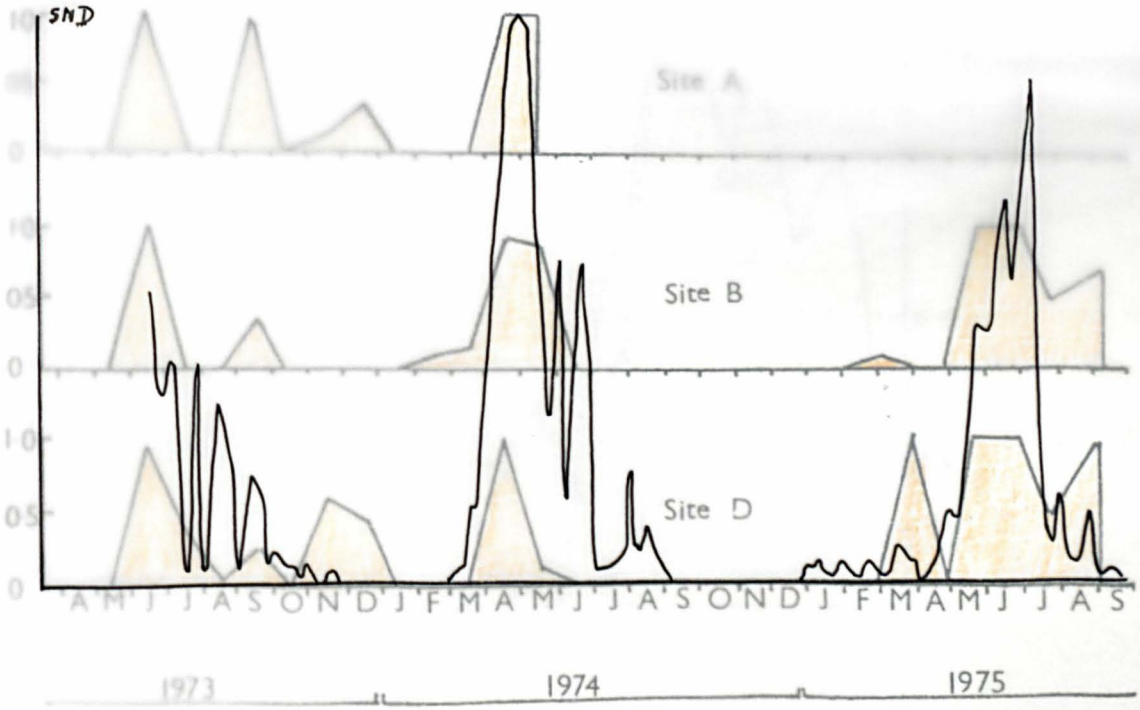
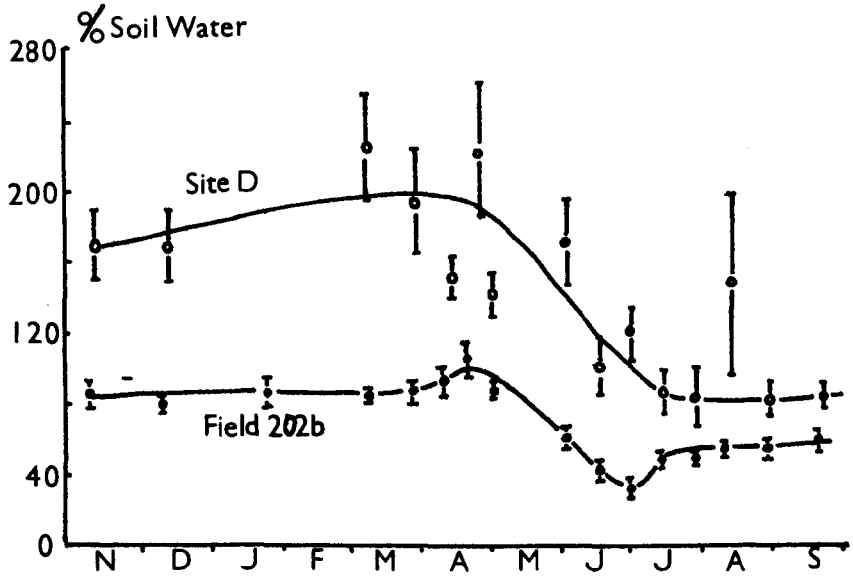


Fig. 3·14b Proportion of Habitat · Damp or Dry —Thorneythwaite




% Soil Water
280
200
120
40
0
Site D
Field 202b
N
D
J
F
M
A
M
J
J
A
S

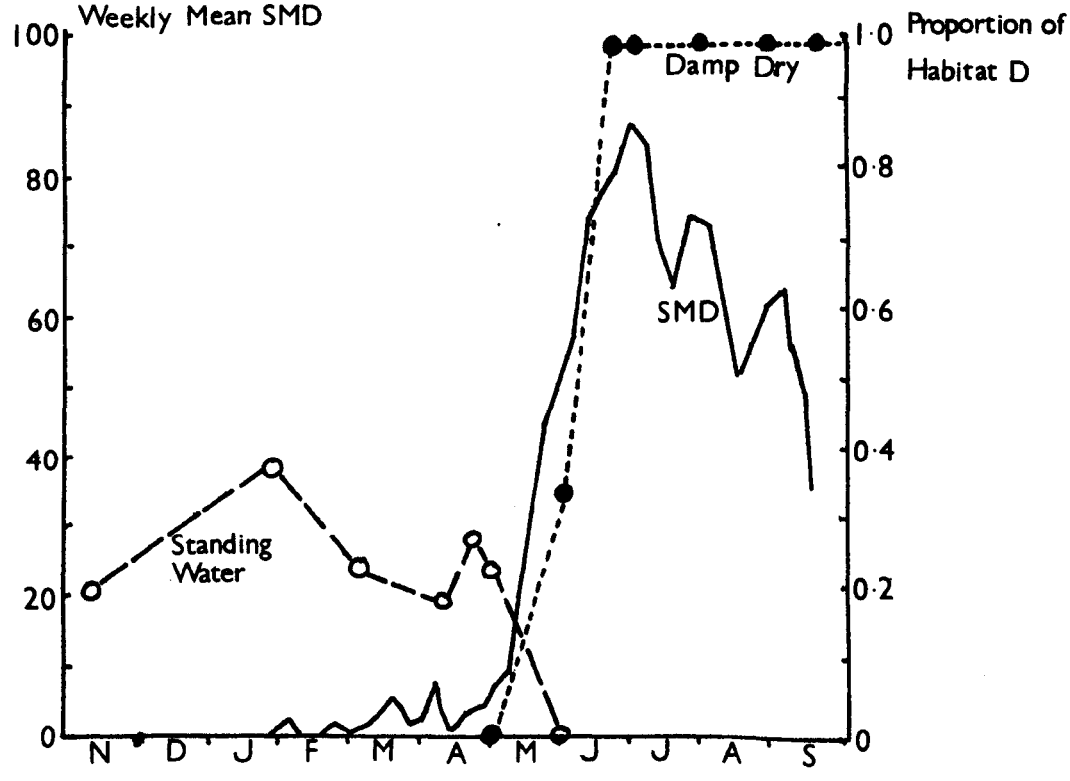
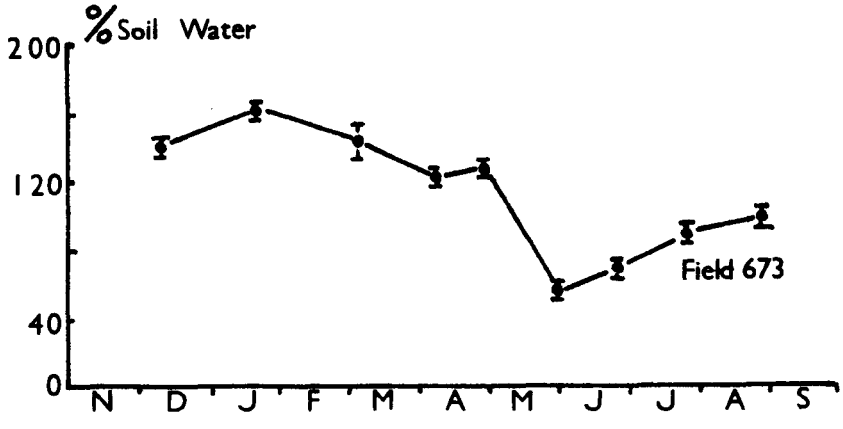
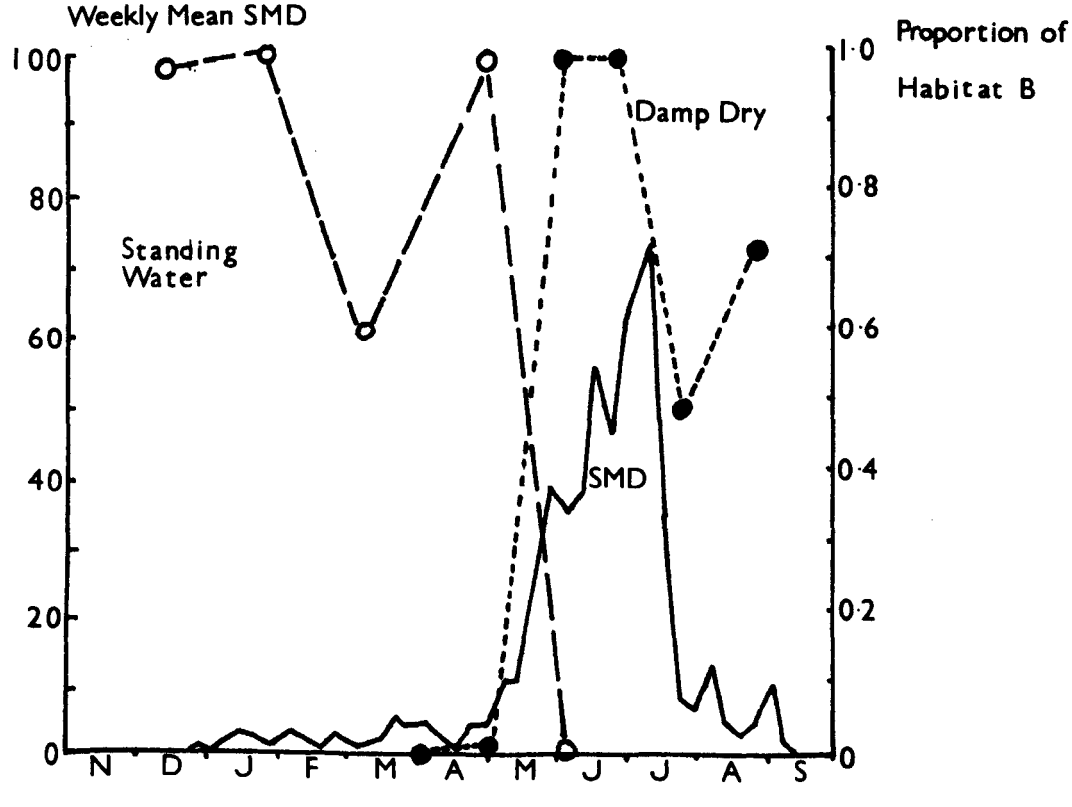

Weekly Mean SMD
100
80
60
40
20
0
Standing Water
SMD
Damp Dry
1·0
0·8
0·6
0·4
0·2
0
Proportion of Habitat D
N
D
J
F
M
A
M
J
J
A
S

Fig. 3·16 Site B, Thorneythwaite (Nov. 1974 – Sept 1975)





in the expected way with S.M.D. (Figures 3.17 and 3.18). The changes in soil-surface moisture which occurred during the spring drought were concurrent with the changes in % soil water and S.M.D. beginning and ending at the same time. However, once the habitats had dried out, unless the subsequent changes in S.M.D. and % soil water were relatively large (e.g. Site B, Thorneythwaite), then the state of the soil surface remained unchanged (e.g. site D, Hendy). Experience obtained while subjecting soil blocks to successive wetting and drying cycles in the laboratory suggests that the difference between a "wet" and a "damp" soil surface in terms of the % soil water in the first 10cm is very small indeed, whereas the difference between a "wet" and "dry" soil surface may be considerable depending, of course, on the extent of the drying that has occurred. In consequence, a small decrease in % soil water can render the soil surface sub-optimal for L. truncatula but once the soil has dried out a relatively large increase in % soil moisture is necessary to restore optimal conditions at the surface. In this respect, it is interesting to note that even when Site D was rated as "damp" or "dry" it contained at least as much water as did the surrounding field area at full field capacity. In addition, the large standard errors associated with the mean values of % soil water at this site reveal the considerable differences in moisture content that there were in soil from different parts of the habitat. Such heterogeneity appeared to be typical of most of the habitats examined.

Fig. 3·17 Relationship between % Soil Water and S.M.D —Hendy

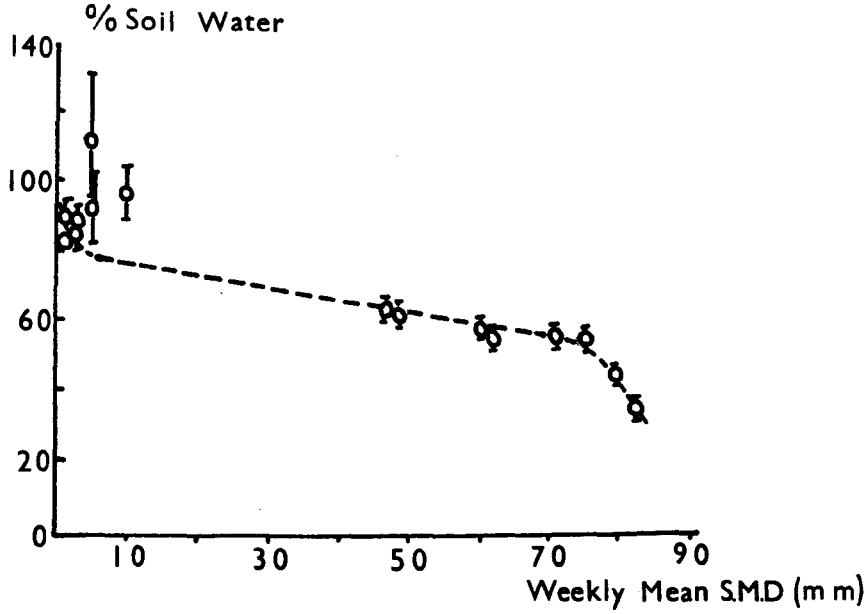


Fig. 3·18 Relationship between % Soil Water and S.M.D —Thorneythwaite

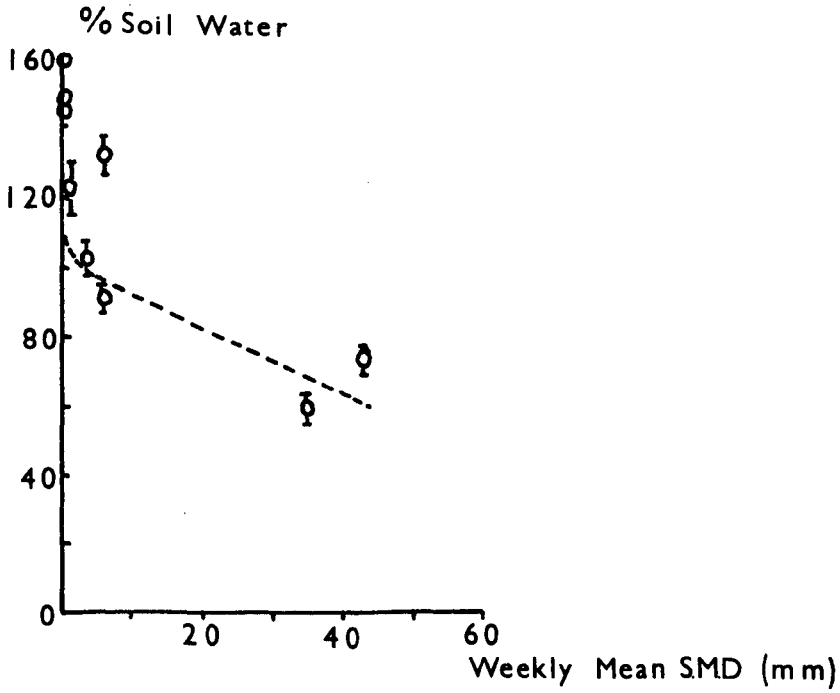


Plate 6. Site D , Hendy ,

Heterogeneous soil-surface moisture conditions

(vi) % Soil Water, Suction Pressure and Soil Characteristics

The soil moisture-characteristic curves for each farm are plotted in Figures 3.19 and 3.20. A sigmoid curve is typical of the relationship between percentage soil water and suction pressure (pF). Since suction pressure is inversely proportional to the effective radius of the soil pores containing the air-water menisci the shape and position of the soil-moisture characteristic curve is determined in part by the size distribution of the pores in the soil. The point of inflection on the drying curve corresponds to the emptying of the coarser pores between the crumbs (Russel, 1974) The points of inflection occur at about pF 2.25 at Thorneythwaite and pF 2.6 at Hendy. These correspond to suction pressures of 177 cm H_2O and 398 cm H_2O respectively, and suggests that the coarser pores at Thorneythwaite are about twice the diameter of the coarser pores at Hendy. The conclusion that the Thorneythwaite soil upon which the measurements were made should drain more rapidly is consistent with the interpretation of the respective soil profiles given in Chapter 2.

On the soil moisture-characteristic curve for Hendy both the snail habitat and the surrounding field are represented. The scatter of points is such that it is not possible to discern any difference between the two areas which is a surprising result since the snail habitat was heavily poached. This generally leads to compaction with a concomitant reduction in the number

Fig. 3.19 Soil Moisture-characteristic Curve ; Hendy

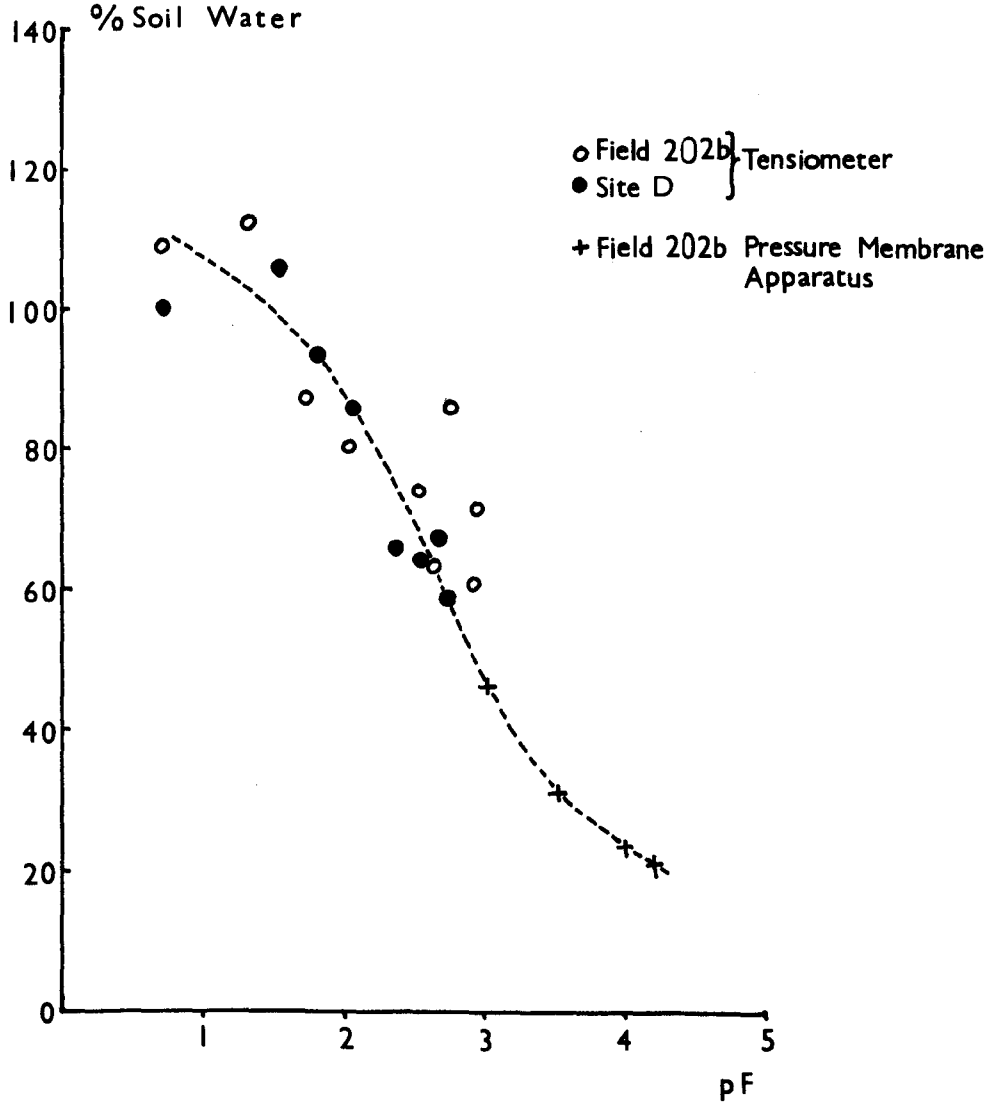
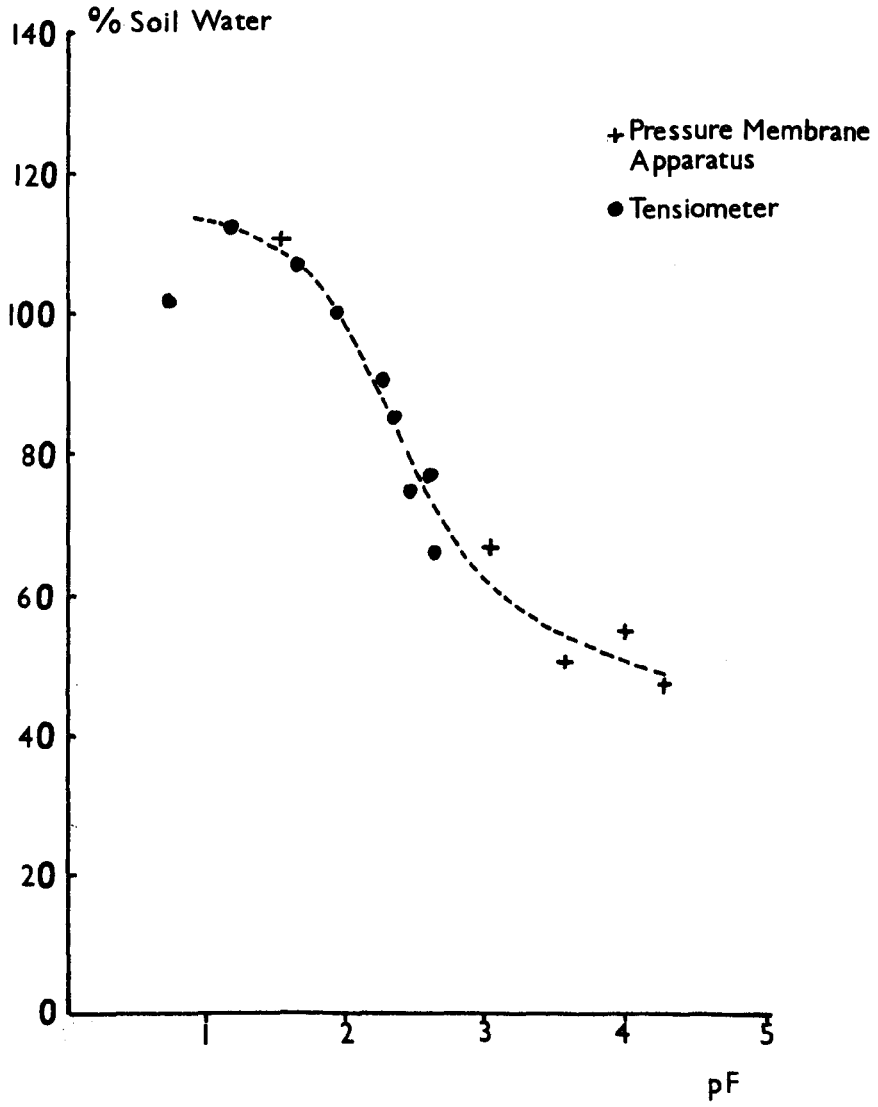


Fig. 3·20
Soil Moisture–characteristic Curve ; Field 673, Thorneythwaite



of coarse pores and an increase in the amount of water held at moderate suctions.

The moisture content of the soil block from Thorneythwaite ranged between 100-112% during the first week of draining and drying in the laboratory suggesting an approximate value for the field capacity of this soil (105%, pF 1.25). It is interesting to notice that the moisture content of the actual field soil at Thorneythwaite did not drop below 120% throughout the winter and early spring. The rainfall over this period must have been sufficient to maintain the field in a permanently waterlogged condition. In contrast the normal moisture content of the soil at Hendy was around 82% during the winter months. Values between 80-90% were obtained in the draining soil block from Hendy by the end of the first week and, taking into account the poor drainage of this soil, the moisture content measured during the winter months in the field at Hendy seems a fair estimate of the field capacity of this soil (82%, pF 2.2).

(vii) Soil-Surface Temperature (0-1cm depth)

The monthly mean temperatures at the soil surface were consistently higher than the corresponding mean air temperatures between April and October and consistently lower than the corresponding monthly mean air temperatures between November and March. (Figures 3.21 and 3.22). The difference between the weekly mean temperature maxima and minima (weekly mean temperature range) was

Fig.3·21 Monthly Mean Temperatures: Site D , Hendy

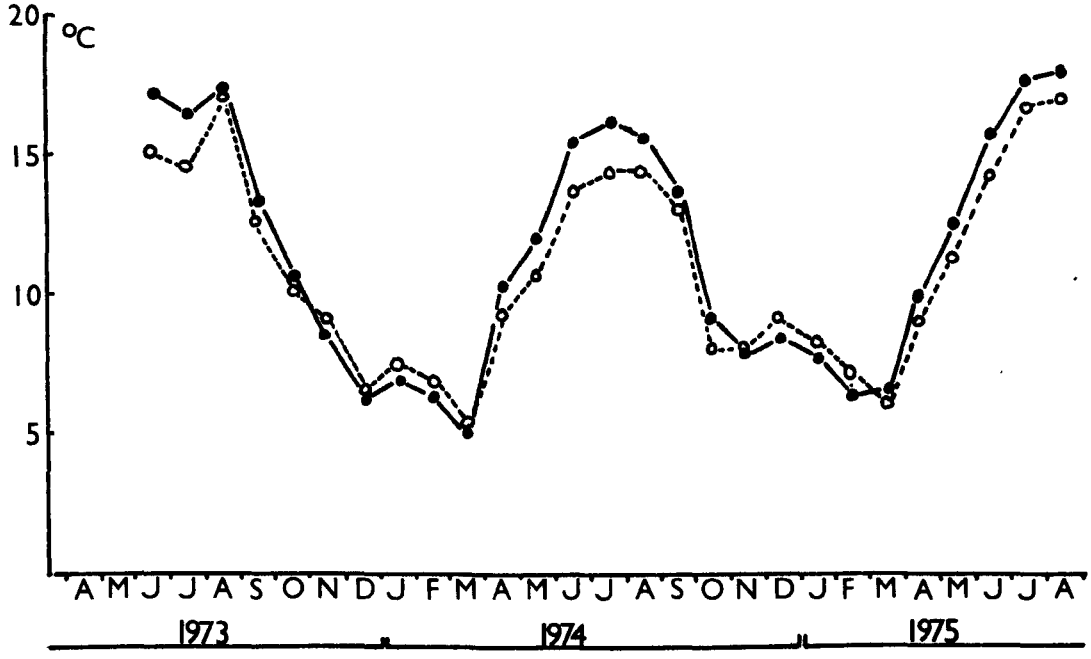
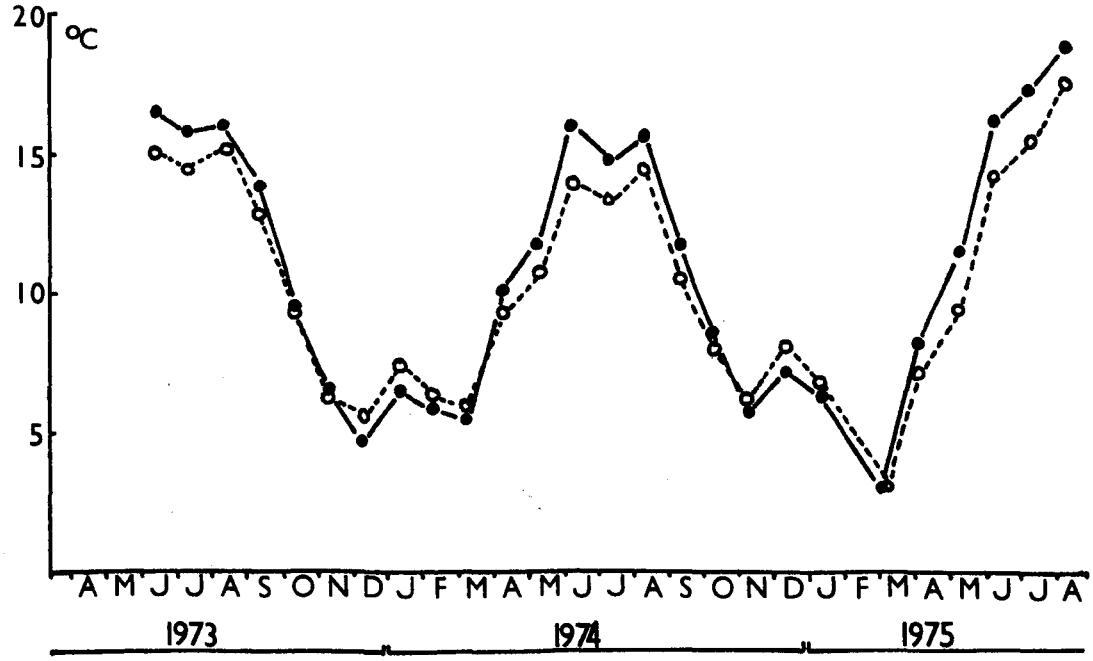


Fig.3·22 Monthly Mean Temperatures: Site D, Thorneythwaite



• Soil Surface Temperature
o Air Temperature

less for the soil temperatures than the air temperatures. In particular, on both farms, from about mid August of each year until the following spring the weekly mean soil temperature range was considerably less than the corresponding range for air temperatures (Figures 3.23 and 3.24). During an investigation of fallow soil plots Perman (1943) found that he could estimate the maximum and minimum temperatures of the soil surface from air temperature records if he divided the year into two parts. The two parts corresponded to a wet cold period during which the evaporation from the soil was nearly equal to the evaporation from an open water surface and a drier warmer period when the evaporation was less than that from an open water surface similarly exposed. On both Hendy and Thorneythwaite the increase in soil temperature range that occurred in the spring appeared to coincide with the end of capacity date. The midsummer decrease in range was not reliably associated with the return to field capacity but did occur at about the same time that the vegetation on the habitats approached maximum density. (The pasture around both climate stations was heavily grazed during the early spring months). Accordingly, the year was divided about these two points: the end of capacity date and mid-August. The period between the end of capacity date and mid-August was referred to as period I and the period between mid-August and the subsequent end of capacity date in the following year as period II. A linear relationship between soil and air temperatures was assumed for each period and the

Fig.3·23 Weekly Mean Temperature Maxima and Minima Hendy

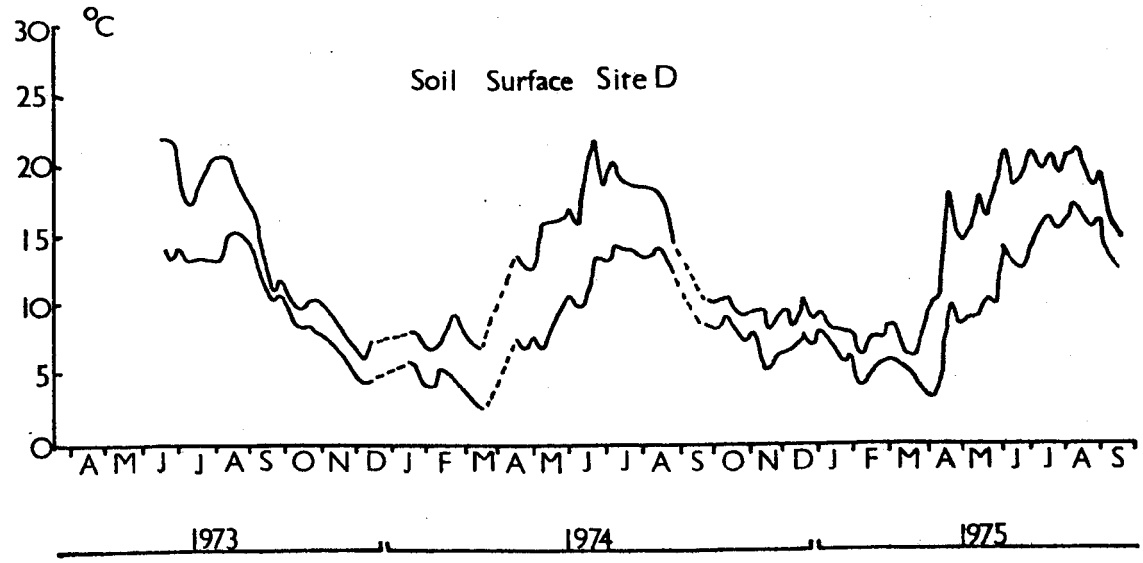
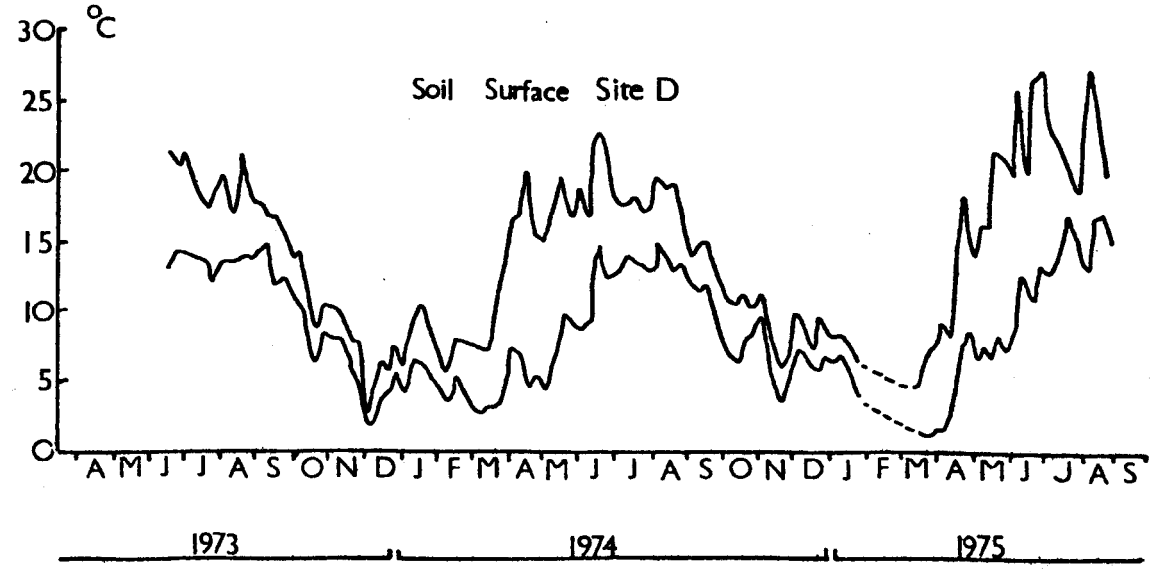


Fig 3·24 Weekly Mean Temperature Maxima and Minima Thorneythwaite



regression equations estimated for maximum and minimum temperatures in turn. The predicted values of soil surface temperature calculated from the equivalent values of air temperature for each case are plotted in Figures 3.25 and 3.26. The equations for each regression line are presented in Table 5. The question arose whether corresponding regression lines were the same and if not in what respects did they differ? Taking temperature minima and maxima in turn two comparisons were possible, between farms or between periods I and II. In each case the residual variances (mean square deviations from the regressions), slopes and elevations of the regression lines were compared by an analysis of covariance (Snedecar and Cochran, 1967, section 14.6, p.433).

Temperature minima were considered first. There was no significant difference between the slopes of the lines either between farms or between periods on either farm. There was a significant difference in the elevation of the lines between periods I and II at Hendy ($F=21.96$; $d.f.=1.92$; $p<0.01$) but not at Thorneythwaite. In other words the relationship between soil and air minima at Thorneythwaite was the same throughout the year in contrast to Hendy where soil minima were proportionately greater during period II. In addition it appeared that the regression line for minimum temperatures at Thorneythwaite during periods I and II was just as good a description of the situation at Hendy during period I.

Taking temperature maxima next, it was found that the residual variances between farms were not homogeneous for either period so

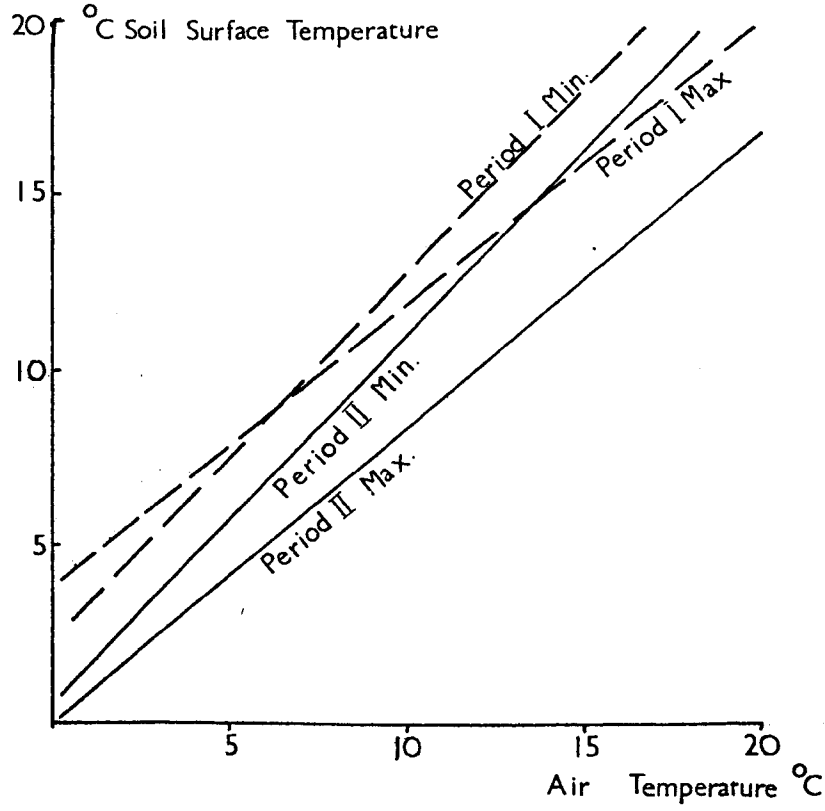


Fig. 3.26 Predicted Soil Surface Temperatures Thorneythwaite

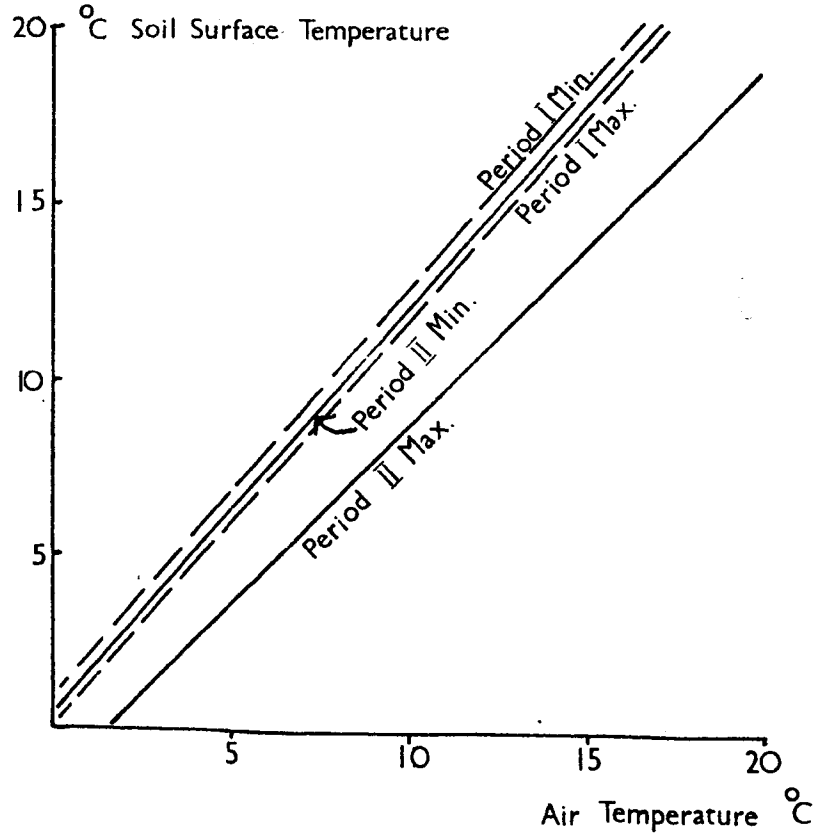


Table 5

THE RELATIONSHIP BETWEEN MEAN WEEKLY MAXIMUM AND MINIMUM TEMPERATURES AT THE SOIL SURFACE (y) AND THE CORRESPONDING TEMPERATURES AT A HEIGHT OF 1 m.

		Hendy	Thorneythwaite
Minima	Period I	$y = 1.05x + 2.25$	$y = 1.13x + 0.96$
	Period II	$y = 1.05x + 0.78$	$y = 1.13x + 0.86$
Maxima	Period I	$y = 0.80x + 4.16$	$y = 1.06x + 0.65$
	Period II	$y = 0.85x - 0.007$	$y = 1.03x - 1.64$

further comparison along these lines was abandoned, though it was noted that the actual values for slope and elevation of the lines differed considerably from one farm to the other. The residual variances between periods were homogeneous, however, and it was found in this case that the elevations but not the slopes of the lines differed considerably. (Hendy: $F=58.04$; d.f.= 1,92; $p < 0.01$. Thorneythwaite: $F = 116.29$; d.f. 1,92; $p < 0.01$).

As a result of this analysis it was concluded that a general quantitative relationship between air temperatures and the corresponding soil temperatures of the habitats of L. truncatula could not be established. Nevertheless, there was a consistent qualitative relationship. Weekly mean soil maxima during period I (from mid-August to the end of capacity date) were less than the corresponding air maxima. In all other cases weekly mean soil maxima and minima were higher than the corresponding air temperatures over the range considered.

4. DISCUSSION

(i) General Weather Conditions, 1973-1975

Ollerenshaw has summarised on a national scale those particular features of the prevailing weather between 1973 and 1975 which were assumed to be of special relevance to the survival and development of F. hepatica and L. truncatula. (Monthly snail reports and fluke forecast, 1973-75; Central Veterinary Laboratories, Weybridge.) He

noted that above average rainfall in May and June of 1973 followed by alternating wet and dry periods throughout July and August meant that most habitats remained wet until July at least. There followed a rapid recovery from the dry state during August and the habitats were maintained in a wet condition by the above average rainfall of September and October. Ollerenshaw suggested that the snail populations were little affected by the brief period of drought that occurred in July and the above average temperatures and wet conditions that prevailed in early August were sufficient to promote rapid development of both snails and fluke eggs. The winter of 1973-74 was mild and very wet but rainfall was well below average in March 1974 and subsequent months. Most habitats were dry by April. So severe was this spring drought that few habitats became wet enough to support active snails until the end of August. The late summer and autumn of that year was generally cold and the rate of development of snail and parasite was rather less than over the same period of 1973. However, the winter was again rather mild and wet allowing the snail populations to recover somewhat. Ollerenshaw noted that the average size of snail was greater than normal for this time of year and a few very large individuals had been found. In May 1975 rainfall was well below average and most habitats had completely dried out by June. They remained dry well into September.

Clearly then, according to Ollerenshaw's analysis, 1973 was generally favourable to the growth and development of both the snail populations and the fluke eggs on the pasture. In 1974 there was a prolonged period of drought early in the year with insufficient opportunity later on to recover entirely from its effects. The drought of 1975 was extensive and decimated the snail and fluke alike.

The local weather conditions at Hendy and Thorneythwaite were so similar to the national conditions described by Ollerenshaw that it was reasonable in the first instance to assume that the fluke and snail populations on each farm would also follow the national trends. What was of most interest then was the way in which these populations deviated from the national trends. It was expected that such deviations could be explained largely in terms of local differences in habitat microclimate.

(ii) Habitat Microclimate - Soil Surface Moisture

Thorneythwaite was generally wetter and colder than Hendy. The monthly rainfall at the Cumbrian farm was up to three times that at Hendy and the return to field capacity during the summer months was always earlier. In consequence the habitats at Thorneythwaite became wetter again sooner than at Hendy, though not uniformly so. Site B, Thorneythwaite, was situated in a low lying waterlogged area and, being a ditch, its bottom was below the usual level of the water table. This site always became

wetter sooner and dried out later than any of the other habitats on this farm most of which were situated on soil with good drainage characteristics and so depended upon a continual influx of large amounts of water from direct precipitation or run-off to maintain their wet condition. At Hendy also, though the timing of the annual cycle of drying and rewetting was broadly similar for every habitat, some habitats consistently dried out later and became wetter sooner, site D in particular. There were differences too in the extent of each habitat that became covered with standing water during the autumn or the proportion of it that dried out during the spring. No doubt such differences are explicable in terms of local variations in topography, drainage and plant cover but they impose a severe restraint of predictions of habitat state based upon some single areal average like soil moisture deficit. Even though the visual estimates had been made at intervals as along as four weeks this was not enough to smooth out the differences between some of the habitats.

Of the three methods used to describe soil water (% soil water, S.M.D. and visual estimates of soil surface moisture) only the visual estimates can be interpreted directly in terms of the effect on fluke and snail. Observations in the laboratory and field suggested that the categories "standing water" and "wet" were optimal and approaching optimal respectively (Appendix III). Given suitable temperatures most snails would have emerged from

their shells and be active under these conditions of surface moisture. Egg masses would be plentiful. Faecal dissipation would be rapid and fluke eggs would be in no danger of dessication. The categories "damp" and "dry" represented sub-optimal and probably lethal conditions respectively. Under such circumstances the snails would be withdrawn into their shells and the apertures would be pressed closely to the ground.

The visual estimates represented an attempt to describe exactly the surface moisture conditions to which the snails were actually exposed. The method leads to an estimate of the area of each habitat that is optimal or sub-optimal for the snail or fluke and when applied to the sites on Hendy and Thorneythwaite revealed how great was the heterogeneity of the habitats in this respect. This fact alone precluded a simple relationship between the moisture at the soil surface of the habitats and the two other estimates of soil water (% soil water and S.M.D.) During a drying phase all three measures change concurrently but as the ground becomes wet again the changes in soil surface moisture lag behind the changes in the other two.

Nevertheless, if a large enough number of habitats is considered it is reasonable to assume that it would be realistic to speak of an "average condition of soil-surface moisture." In such a case it would be useful to know, even approximately, when most of the habitats begin to dry out, when most become wet again, and, if

possible, the extent of the change that occurs. Measurements of % soil water in the pasture surrounding the habitats do at least indicate the timing of the wetting and drying cycle but could not be used in any absolute sense to compare one area with another since % soil water could be converted to equivalent suction pressures (pF) by reference to the soil moisture-characteristic curves specially constructed for each area and thus expressed in terms of a parameter which is independent of soil type and which is also related to the ease or difficulty with which a soil yields up its water for evaporation at the surface. Unfortunately most soils do not possess a unique curve (Russel, 1974). The moisture content at a particular suction depends upon the past history of wetting and drying and there is a marked hysteresis effect (pronounced in sandy soils, less so in clay soils.) Though it is possible to define the extremes of the hysteresis loop by laboratory measurement, a soil can be in equilibrium with moisture at a particular suction pressure for any moisture content between the extremes. In addition, the relationship between pF and % soil water varies with temperature and the concentration of dissolved salt.

On the other hand, measurements of % soil water can be made over the whole range of soil moisture experienced in the field, whereas S.M.D. figures refer only to the range below field capacity (S.M.D. being the amount of water required to bring the soil back to capacity.) Even so the end of capacity and return to capacity dates do at least define the duration of the period when the soil

surface moisture conditions are likely to be sub-optimal. Bruce et al (1973) have shown that the density of L. truncatula on various habitats in Scotland was related to the actual value of the monthly S.M.D. and the incidence of fascioliasis on Anglesey between 1951 and 1960 has been shown to be related to the maximum S.M.D. in each year (Wilson, pers.comm). It seems then that successive estimates of S.M.D. do provide an index of both the duration and intensity of the sub-optimal period for fluke and snail but as an index it has yet to be properly calibrated. Such a calibration was beyond the scope of this study but what has been demonstrated is the enormity of the task. Two aspects must be taken into account: the considerable differences in soil surface moisture that exist between habitats even within quite small areas and the lag that occurs during the wetting phase.

Calculation of S.M.D. (Rainfall-Evapotranspiration), of course, forms the basis of the M_t index described by Ollerenshaw and Rowlands (1959). The maximum monthly M_t index was set at 100 on the rule of thumb basis that in Anglesey "most habitats were wet enough for development to procede" when the index exceeded this value. The suitability of habitats was then assumed to vary in a linear fashion with values less than 100. Until the relationship between S.M.D. and the state of the soil surface on a representative sample of habitats has been worked out and this calibration tested over all the areas where fascioliasis is likely to be a problem

the assumptions essential to the use of the M_t index will remain unproven.

(iii) Habitat Microclimate - Soil-Surface Temperature

Ollerenshaw (1958) wrote that "the effect of temperature will be broadly similar in different years," by which he meant that temperature was a conservative parameter that could be ignored. Both the statement and conclusion are false (as Ollerenshaw himself later implied - see Ollerenshaw, 1971c, p.52). In the present case for example it was found that the number of day-degrees above 10°C could change by as much as 200 from one year to the next (i.e. about 25% of the maximum value recorded). In addition Ollerenshaw has necessarily considered only air temperatures which as this study has shown are up to 4°C cooler in the summer months than the soil-surface of the snail habitats. Since a mean increase in temperature of just 2.5°C (from 15°C to 17.5°C) reduces the total development time of the fluke from egg to cyst by six weeks (Ollerenshaw, 1971a) predictions based upon air temperature data may well considerably underestimate the rate of development of the parasite in the field.

The relationship between the temperature at the soil surface of a habitat and the equivalent temperature at a height of 1m is an interesting one. It is worth considering in some detail why it was that a quantitative relationship between these two parameters could not be established even though there was a simple and consistent qualitative relationship between them on both the habitats

where the situation was examined.

The range of temperatures experienced at the surface of a uniform area of bare earth often greatly exceeds that encountered in the air a few metres higher (Blanc, 1958). Geiger (1973) provided several examples of studies in which temperature maxima and minima at the ground surface were consistently more extreme than those measured, for example, at a height of 1 metre. The temperatures experienced at the soil surface of the snail habitats did not conform to this pattern because the habitats were not merely bare earth; for most of the year and over most of their area they were covered by a more or less dense layer of vegetation.

The density and height of the vegetation covering the ground exerts a profound influence upon the radiation received and emitted by the soil surface. During the daylight hours a substantial amount of the incident radiation is absorbed by the upper layers of vegetation. In a meadow consisting of Dactylis glomerata, Angstrom (1925) found that only one fifth of the incoming radiation actually reached the ground surface. Conversely at night, since the ground is shielded from the open sky by the vegetation that covers it, the heat lost by radiation from the surface is very much less than that lost from bare earth. (Geiger 1973). In consequence, the soil surface is relatively cooler during the day and relatively warmer during the night. If the soil is wet as is usually the case in a snail habitat then the effect is even more pronounced. The thermal conductivity of soils generally increases

with increasing water content. During the day the heat received by the ground surface is more rapidly carried downward to sub surface layers in a wet soil than a dry one and so the ground surface remains cooler. At night, a wet soil conducts heat more rapidly back to the surface and so the surface stays relatively warmer. For these reasons, the soil surface of a wet snail habitat that is covered with a dense growth of herbage would be expected to exhibit a much smaller diurnal range of temperature than the surface of an equivalent area of bare earth. As the vegetation cover is reduced and the soil dries out a greater range of diurnal temperature variation would be expected. Muller-Stoll and Freitag (1957) demonstrated just such a transition in a comparative study of six different types of meadow community. Peacock (1975) found that the temperature range at the soil surface under a stand of Lolium perenne depended upon the height of the crop. The taller the crop the smaller the range and in this particular study the range was always smaller than that experienced at a height of 1m.

Heat losses due to evapotranspiration contribute significantly to the maintenance of lower day time temperatures in wet soils though the actual evaporation from the ground at least is moderate since the temperature of the evaporating surface is kept relatively low under the shelter of the vegetation. However, the elevated temperatures at the surface of a drier soil with reduced vegetation cover do not necessarily bring about an increase in heat loss by

evaporation since the formation of a shallow dried out surface layer presents a considerable barrier to the upward movement of water (Perman 1943).

In short the habitat afforded the organisms within it a sheltered environment for all of the year except the spring months when the soil was driest and the vegetation cover reduced. During this period the weekly mean maximum temperatures at the ground surface were greater than the equivalent air temperatures and subjected the snails and fluke eggs to a desiccatory influence more formidable than would have been anticipated merely from a consideration of the air temperatures alone. At all other times of the year the habitat shelters its occupants from excessive cold and the drying influence of excessive heat. The considerable differences that exist between one habitat and the next in terms of the density and height of the vegetation covering them result in a unique quantative relationship between soil and air temperature for each site for each particular month.

In all subsequent chapters mean weekly soil surface temperatures will be used rather than mean monthly measurements since the latter "smooth" the data too much for more than very general comparisons.

CHAPTER 4. L. TRUNCATULA AND THE INTRAMOLLUSCAN STAGES OF F. HEPATICA - VARIATIONS IN THE DENSITY AND SIZE CLASS STRUCTURE OF THE POPULATIONS

1. INTRODUCTION

L. truncatula is ubiquitous in all the counties of England and Wales (Kerney, 1976) and though it requires a wet situation in order to grow it is not wholly aquatic. Typical habitats have been described in a previous chapter. Natural enemies include ducks and geese, though it appears that the snails are consumed incidentally as the birds search for other food (Bednarz, 1958). Other proven predators include the larvae of Sciomyzid flies (Knutson and Berg, 1964; Berg, 1964) and caddis flies of the genus Limnophilus (Bednarz, 1958). The extent to which predation limits natural populations of the snail is entirely unknown and after the field work of Walton (1918) most research has concentrated upon the influence of abiotic factors in the environment such as temperature and soil moisture content (Walton, 1923; Walton and Norman-Jones, 1926; Walton and Rees-Wright, 1926; Kendall 1949a; Roberts, 1950; Kendall, 1953; Styczynska, 1956; Chowaniec and Drozdz, 1959; Bednarz, 1960; Ollerenshaw 1971a, 1971b). In recent years this work has been extended to include the influence of the same environmental factors on the growth and development of the parasite larvae within the snail (e.g. Taylor, 1949; Kendall, 1949a, b, c; Kendall and McCullough, 1951; Styczynska, 1956; Chowaniec and Drozdz, 1959; Kendall and Ollerenshaw, 1963; Styczynska-Jurewicz, 1965; Ollerenshaw, 1971a; Over, 1971; Nice and Wilson, 1974; Wilson and Draskau, 1975.)

Variations in the abundance of L. truncatula were previously held to determine the incidence of fascioliasis from year to year (see Chapter 1.). This idea is no longer current (e.g. Over and Damou Van Hapert, 1967) but it is thought reasonable to assume that there is a minimum snail population density below which the probability of the parasite being transmitted from one generation of primary hosts to the other will approach zero. Accordingly the population dynamics of the snail populations has been studied specifically with the application of molluscicides in mind (Ollerenshaw, 1971a; Heppleston, 1972) or else as part of actual molluscicide trials (Crossland, 1970; Ross, Taylor and Morphy, 1970; Bruce et al., 1973). Alternatively the variations in density of L. truncatula have been monitored for some larger epidemiological study in order to follow the course of the infection from year to year (Ollerenshaw, 1971b) or to establish the character of the interaction between the intermediate host and the fluke in a particular geographical region (Sosiptrov and Shumakovich, 1966).

With the exception of the surveys conducted by Ollerenshaw (1971a, 1971b) none of these studies lasted more than 18 months and in most of them the data from several habitats were combined before presentation. Only Sosiptrov and Shumakovich (1966) provided continuous records for a single habitat but these workers made no attempt to explain the variations they observed. Heppleston (1972) included information on the prevailing weekly mean air temperatures and rainfall, Ollerenshaw (1971b) computed the M_t index for the

areas concerned and Bruce et al. (1973) reported the monthly soil moisture deficit. A general relationship between the observed variations in snail density and the prevailing climate and soil moisture conditions was demonstrated in each case but hitherto there has been no attempt to interpret the population dynamics of individual populations of L. truncatula directly in terms of the microclimates of the habitats.

A minimum requirement of the present study was a comprehensive account of the way in which natural populations of L. truncatula and the larval flukes within them respond to fluctuations in the microclimates of the snail habitats. In order to encompass the largest range of possible responses habitats were investigated for as long a period as was feasible (3 seasons). Variations in the density and size-class structure of individual snail populations are reported in this chapter together with a parallel account of the rediae and cercariae within the snails and a description of the variations in habitat microclimate.

2. MATERIALS AND METHODS

The results of a preliminary survey of the available snail habitats were presented in Chapter 2. During this survey the extent and nature of each habitat was noted and the presence of L. truncatula confirmed by visual search. Ten habitats were selected for further study. Six of these were in Gwynedd (Hendy) and four of them were in Cumbria (Thorneythwaite). Each of the habitats was at least $20m^2$

in area, easily accessible, and known to have sustained large snail populations in previous years.

(i) The Sampling Procedure

Each habitat was sampled monthly from April 1973 until September 1975. One habitat, Hendy Site D, was sampled every fortnight during the summer months of 1974 and 1975.

The sampling scheme is summarised in Table 6. Three methods were tried: quadrats, ten minute counts and soil cores. Sampling by quadrats and ten minute counts was continued throughout the period of study, sampling by soil cores was abandoned after the first few trials.

(ii) Quadrats

Quadrat samples were taken from 7 of the habitats. Only a portion of each habitat was sampled. This portion was termed a site and its corners were permanently marked by wooden stakes. The sites encompassed the bulk of the habitats but excluded the transition zones to drier permanent pasture.

In order to minimise the variance of the sample means the method of stratified random sampling was adopted (Southwood, 1966). The sites were subdivided equally into a number of blocks. One sample was taken from every block on each of the visits to the sites. The quadrat was placed within the block according to coordinates obtained from random number tables. Care was taken to avoid trampling unsampled blocks.

Table 6

THE SAMPLING SCHEME

FARM	HABITAT	TEN MIN. COUNTS	NUMBER OF QUADRATS	BLOCK SIZE (m)
HENDY	A	✓	20	16x16
	B	✓	30	1x1
	C	✓	20	1x1
	D	✓	20 then 40	1x2 then 1x1
	E	✓	-	
	F	✓	-	
THORNEYTHWAITE	A	✓	24	1x4
	B	✓	10 then 20	1x8 then 1x4
	D	✓	23	1x2
	F	✓	-	

A square wire quadrat (20 x 20cm) was used on all but one of the sites. The exception was a ditch habitat, (Thorneythwaite, site B) and here the sample unit was a "metre length of ditch."

Each quadrat was searched until it became apparent that further search would prove unrewarding. An average search time for the small quadrat was between 3 and 6 minutes. On the other hand, the metre length of ditch was usually examined for at least 10 minutes. The snails were removed from the site with curved forceps and preserved in 2.5-5% formalin. (Stronger solutions severely weakened the snails' shells).

(iii) 10 Minute Counts

Each habitat was searched for 10 minutes on every sampling occasion. In the case of those habitats from which quadrat samples were taken only the area within the site was searched. The snails were collected and preserved as before.

(iv) Soil cores

Soil cores were taken from 4 sites (Thorneythwaite, sites A, B and D; Hendy, site C) in May, June and July of 1973. The circular core was 10cm in diameter and 5cm deep. A core was taken at random from each block and sealed in a plastic bag. The cores were broken up by hand in the laboratory and sieved in a gentle flow of water (sieve mesh number 80). The material retained by sieve was searched for snails.

(v) The Laboratory Treatment of the Snail Collections

Each snail was examined under a low power stereo microscope

(8.75 magnification). Occasionally Potamopyrgus jenkinsi or Physa acuta were inadvertently collected and individuals belonging to either of these species were discarded at this stage. Specimens identified as L. truncatula were placed on a damp wad of tissue paper in order to set the longitudinal axis of the shell at right angles to the optical axis of the microscope. Viewing the snails through the eyepiece of the microscope, the shells were manipulated with mounted needles until the tip of the spire and the base of the aperture were in the same focal plane. The total length of the shell was then measured using a calibrated graticule eyepiece. In the case of the 10 minute count collections the body whorl and aperture length together with the breadth and aperture breadth were also measured (Figure 4.1).

Each snail was then dissected in a drop of water. The digenean larvae from infected snails were carefully examined under a low power stereo microscope (x25 magnification). The rediae of F. hepatica were prised free from the tissue of the snail and classified according to length and contents (Table 7). The larvae of other digenean parasites were identified if possible and discarded. The sporocysts of Cercaria cambrensis I were frequently encountered but never together with the rediae of F. hepatica. (For a description of C. cambrensis see Rees, 1932). Initially, the redial contents were viewed through the walls of the rediae without dissection. However, towards the end of the study the rediae were dissected and

Fig 4.1

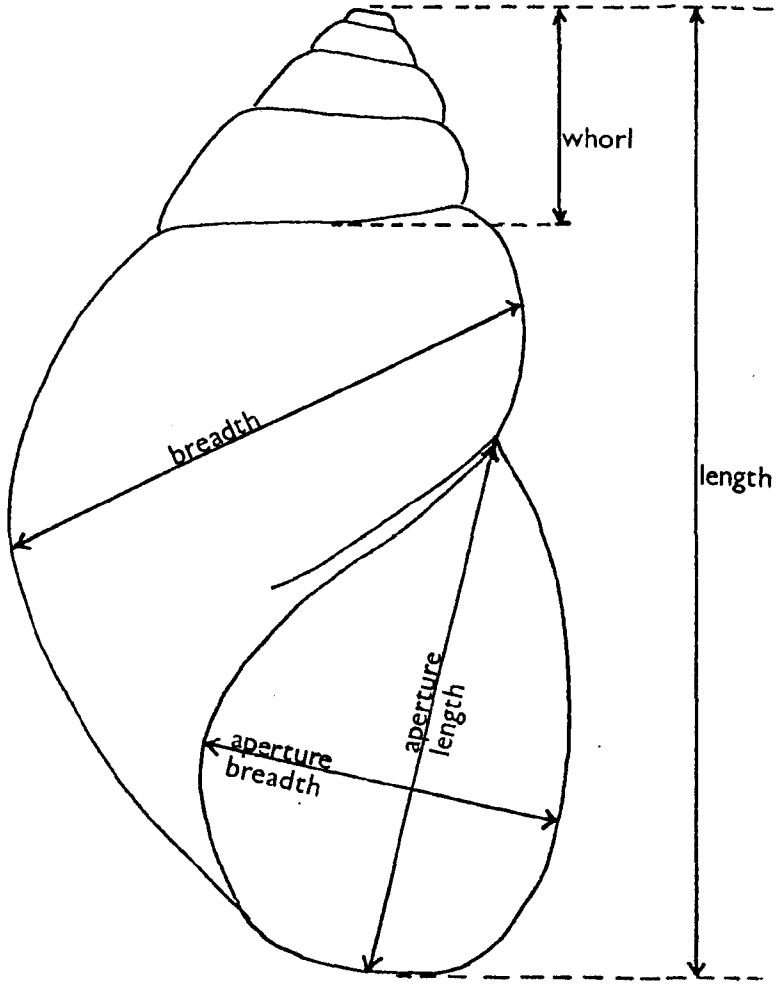


Table 7

CATEGORIES USED TO DESCRIBE INFECTION IN SNAIL

CODE	DESCRIPTION
1	Free cercariae within the body of the snail
2	Rediae 2.6 mm length
3	Rediae 1.8 - 2.6 mm length
4	Rediae < 1.8 mm length
A	Rediae containing mainly germ balls
B	Rediae containing mainly daughter rediae
C	Rediae containing mainly immature cercariae
D	Rediae containing mainly mature cercariae

an exact tally was made of the larvae developing within them. Dissection was easiest when the snails had been preserved in very dilute formalin (2.5%).

(vi) Transformation of the Snail Population Data

The numbers of L. truncatula that are found by visual search techniques are determined to some unknown extent by factors which are unrelated to the true population density of the snails and whose influence varies from collection to collection. Prevailing weather conditions and variations in the amount of plant cover are particularly important (Ollerenshaw 1971b; Hairston et al., 1958). In order to distinguish between real and apparent variations in the populations successive estimates of population density were transformed into three month running means before presentation.

The results from both the 10 minute counts and quadrat samples were treated in this way but in the case of the quadrat collections a prior transformation was necessary to render the variance independent of the mean. It was found that the logarithm of the variance was linearly related to the logarithm of the mean. A regression line was fitted to a set of data from each farm (Figures 42 and 43) and following the argument of Southwood (1966) the value of the parameter "p" was computed ($p = 1 - \frac{1}{2} b$, where b = the regression coefficient in each case). On both farms the value of "p" was between 0 and 0.5 (Hendy, P = 0.33; Thorneythwaite, p = 0.16). A logarithmic transformation was therefore appropriate. Since the data contained a number of zero counts the transformation was of the form $\log_e(x+1)$.

Fig 4·2 Quadrat Samples
(Hendy)

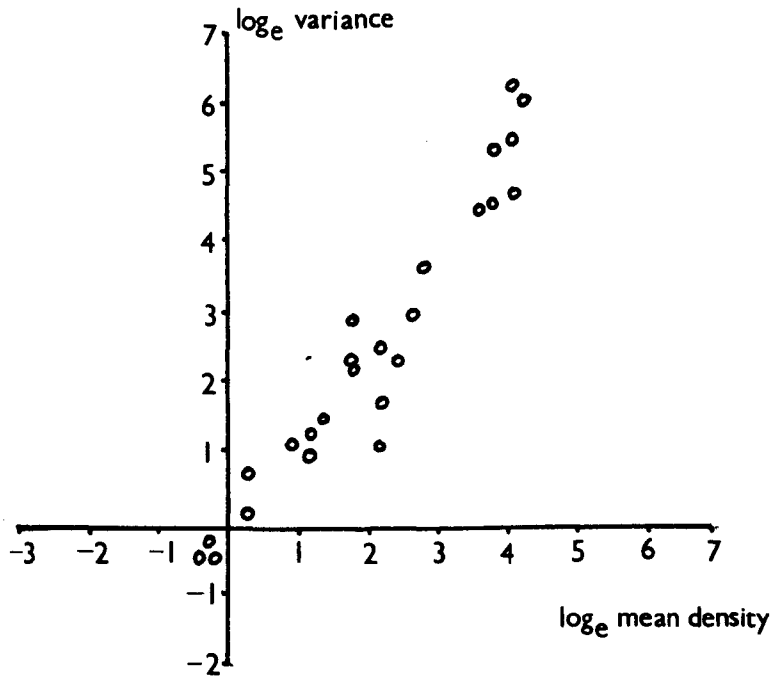
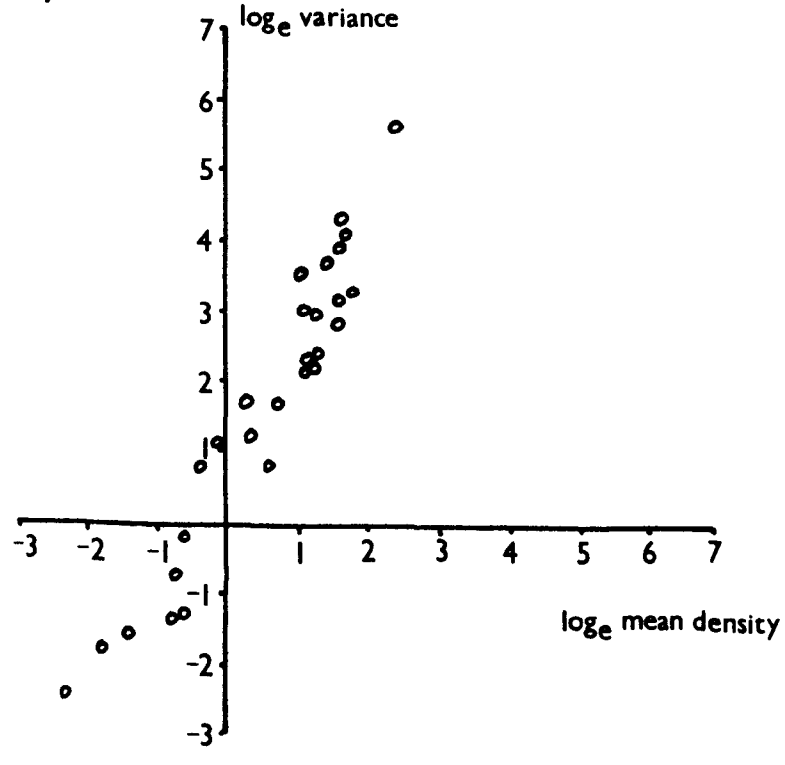


Fig 4·3 Quadrat Samples
(Thorneythwaite)



The percentage error of the derived means was estimated by analysis of the variance (Snedecor and Cochran, 1967, p.58). At site D, Hendy, for example, the error was reduced from less than 20% (95% probability level) to less than 10% when the number of samples taken was doubled in June, 1974. Similarly, at site B, Thorneythwaite, the error was reduced from less than 50% (95% probability level) to less than 20% when the number of samples was doubled in April 1974.

(vii) "Constant Effort Search"

In order to reveal the general way in which each group of populations varied over the 30 months of study the data from all of the habitats on each farm were pooled and adjusted so as to represent the results of a constant effort search. Data from both 10 minute counts and quadrat samples were included. Account was taken of any changes in the number of quadrats used and of any deviation from the normal sampling schedule of one visit a month to each farm. The results were grouped into five 6-monthly periods (April-September; October-March) and for every period the snails were each assigned to one of eleven size classes.

3. RESULTS - LYMNAEA TRUNCATULA

The field collections of L. truncatula are summarised in Table 8. A total of 2,652 snails was found, 123 of these were infected with F. hepatica and a further 26 were infected with other

Table 8

SUMMARY OF FIELD COLLECTIONS
(APRIL 1973 - SEPTEMBER 1975)

HENDY

METHOD	HABITAT	NO. OF SNAILS FOUND	NO. OF SNAILS INFECTED. F.hepatica	Others	COMMENT
QUADRATS	A	39	2	0	Abandoned Dec 73
	B	31	0	0	Flooded Nov 73
	C	72	7	3	Quadrats doubled June 74.
	D	382	11	12	
10 MINUTE COUNTS	A	65	7	0	
	B	26	1	0	
	C	82	1	0	
	D	405	19	8	
	E	208	20	2	Field drained Aug 74
	F	62	1	1	

THORNEYTHWAITE

METHOD	HABITAT	NO. OF SNAILS FOUND	NO. OF SNAILS INFECTED. F.hepatica	Others	COMMENT
QUADRATS	A	9	1	0	Abandoned June 74.
	B	740	37	1	Quadrats doubled April 74.
	D	46	1	0	
10 MINUTE COUNTS	A	37	3	0	
	B	287	9	1	
	D	57	2	1	
	F	104	1	0	

digenean parasites. Site D, Hendy, and site B, Thorneythwaite, accounted for more than half the number of snails found. Of the 1,814 snails removed from these habitats 76 were infected with F. hepatica.

(i) Core Samples

No snails at all were found by the method of core sampling. The technique entailed considerable disturbance to the habitat and required a prohibitive amount of laboratory time to sieve and sort the samples.

(ii) 10 Minute Counts Compared with Equivalent Quadrat Estimates

The method of 10 minute counts revealed large numbers of snails for a relatively small search effort (Table 9). However, the technique tended to underestimate the numbers of immature snails and overestimate the number of mature snails when compared with equivalent estimates derived from quadrat samples (Figure 4.4). The trend was consistent in all but one of the 7 habitats considered. (Habitat B, Thorneythwaite).

Successive density estimates obtained from quadrat samples were compared with their equivalent 10 minute counts in each of 6 sites. In only two cases was there a significant correlation: site D, Hendy and site B, Thorneythwaite (Table 10).

(iii) 10 Minute Counts by Other Collectors on Hendy and Thorneythwaite

Both Hendy and Thorneythwaite figure in the monthly snail records provided by the Central Veterinary Laboratories, Weybridge,

Table 9

RELATIVE SEARCH EFFORTS - QUADRATS AND 10 MINUTE COUNTS

FARM	METHOD	SEARCH EFFORT	NO. OF SNAILS FOUND	NO. OF SNAILS INFECTED	PERCENTAGE OF SNAILS INFECTED
HENDY	Quadrat	Approx 176 hrs. (1,940 Quads.)	524	20	3.8
	10 Min. counts	21 hrs. 20 mins.	848	49	5.8
THORNEYTHWAITE	Quadrat	Approx. 148 hrs. (1,407 Quads.)	795	39	4.9
	10 Min. counts	19 hrs. 40 mins.	485	15	3.1

Fig 4.4 Size Class Structure of the Total Population of Snails Collected Between 1973 and 1975

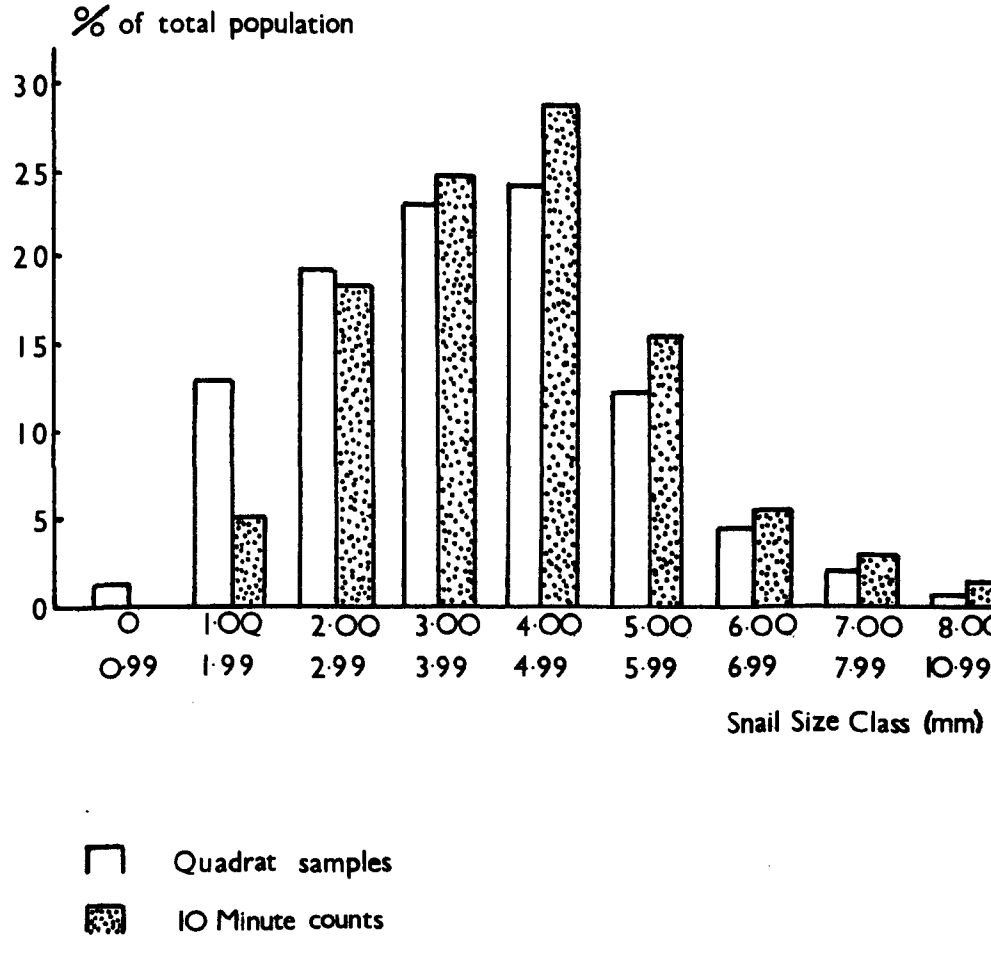


Table 10

THE CORRELATION BETWEEN 10 MINUTE COUNTS AND EQUIVALENT ESTIMATES OF DENSITY OBTAINED FROM QUADRAT SAMPLES

FARM	HABITAT	CORRELATION COEFFICIENT	DEGREES OF FREEDOM	p.VALUE
HENDY	A	-0.50	15	Not sig.
	C	0.69	5	Not sig.
	D	0.70	22	<0.001
THORNEYTHWAITE	A	-0.44	9	Not sig.
	B	0.85	25	<0.001
	D	0.24	13	Not sig.

as part of the "Fluke Forecast." Collections were made during the summer months from a number of habitats on each farm. Some of the habitats were the same as those sampled in this study. The monthly mean 10 minute counts for Hendy and Thorneythwaite were compared with means calculated from the monthly snail records. The comparison provided an approximate measure of two things: first, the inter-collector reliability of the 10 minute count method and second, the extent to which the particular habitats sampled in this study were representative of other habitats on the farms. In fact, there was a significant correlation only in the case of the Hendy collections. ($r = 0.69$, d.f. = 18, $p < 0,01$; Figures 4.5 and 4.6).

(iv) 10 Minute Counts from Individual Habitats

The complete record of 10 minute counts is presented in Figures 4.7 and 4.9. The percentages of immature snails (< 2.99mm) found on each farm are given in Figures 4.8 and 4.10. The counts were generally low (less than 10) but reference to the "Monthly Snail Reports" issued by the M.A.F.F. confirms that this is usual. It is noteable that there was little relationship between the variations in snail density and the proportion of immature snails found but an increase in either, where it did occur, was mostly during the autumn or winter. At Hendy, the proportion of immature snails was greatest during the autumn and winter of 1973-4. There appeared to be very little breeding activity during the latter months of 1974 despite a general increase in the number of snails

Fig 4.5

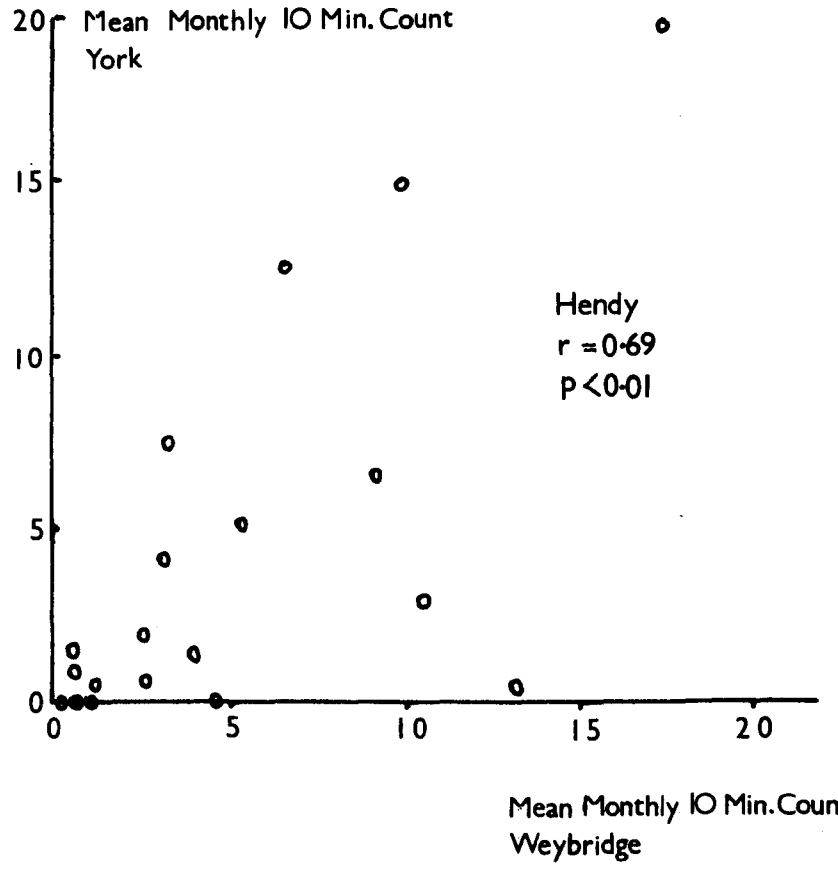

Mean Monthly 10 Min. Count
York
20
15
10
5
0
0
5
10
15
20
Hendy
r = 0·69
p < 0·01
Mean Monthly 10 Min. Count
Weybridge

Fig 4.6

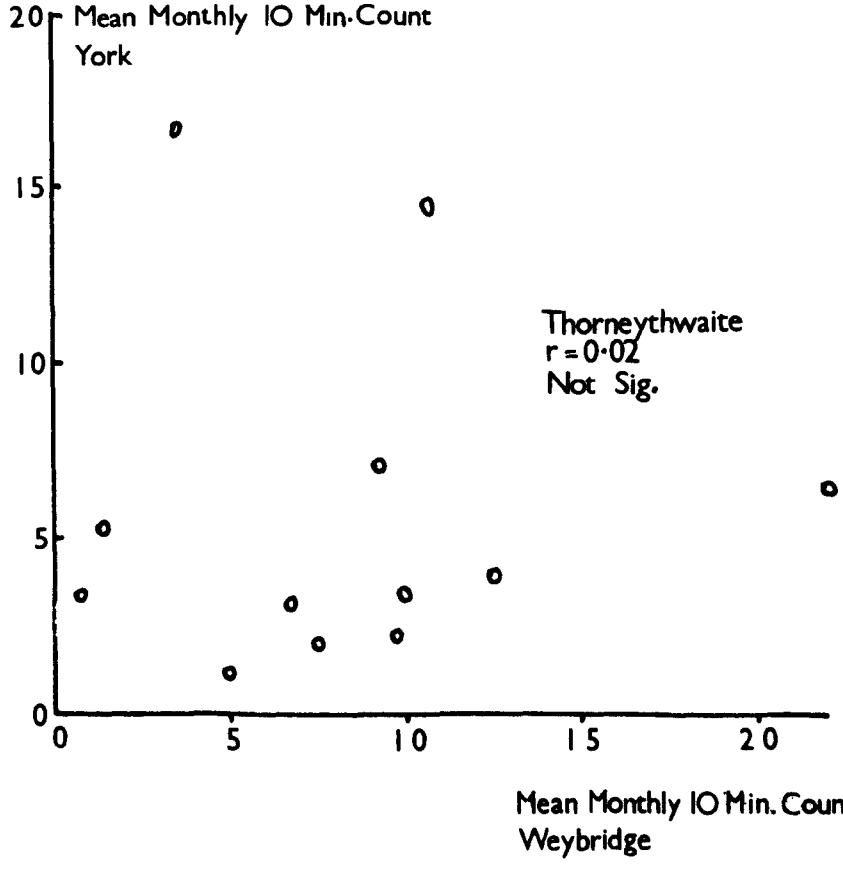

Mean Monthly 10 Min. Count
York
20
15
10
5
0
0
5
10
15
20
Thorneythwaite
r = 0·02
Not Sig.
Mean Monthly 10 Min. Count
Weybridge

Fig. 4·7

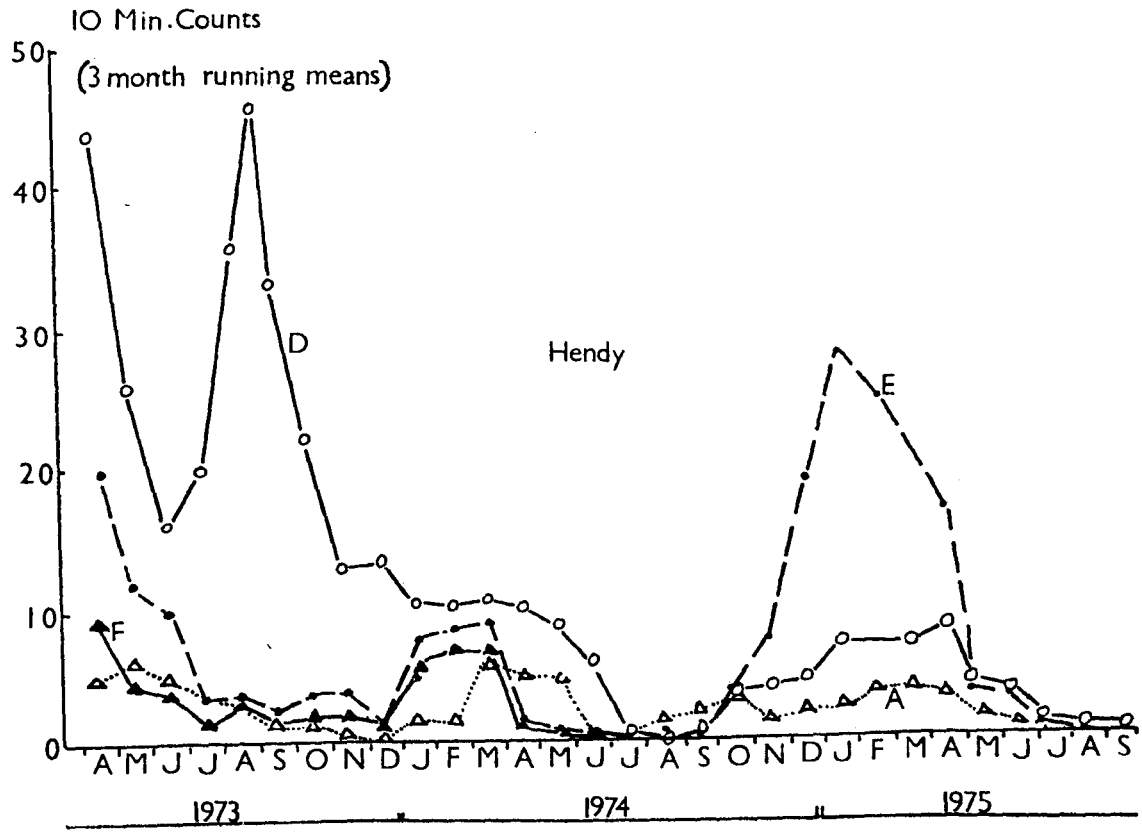

10 Min. Counts
(3 month running means)
50
40
30
20
10
0
D
Hendy
E
F
A
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1973
1974
1975

Fig. 4·8

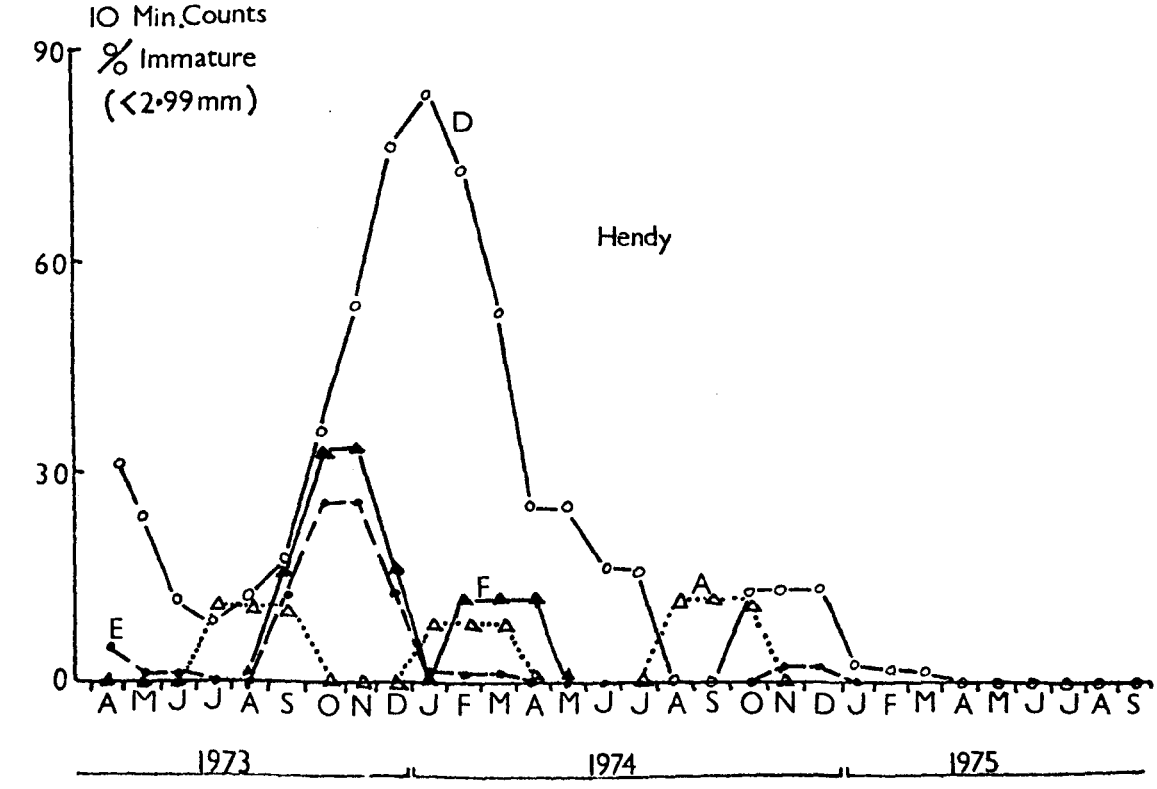

10 Min. Counts
% Immature
(<2·99 mm)
90
60
30
0
D
Hendy
E
F
A
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1973
1974
1975

Fig. 4·9

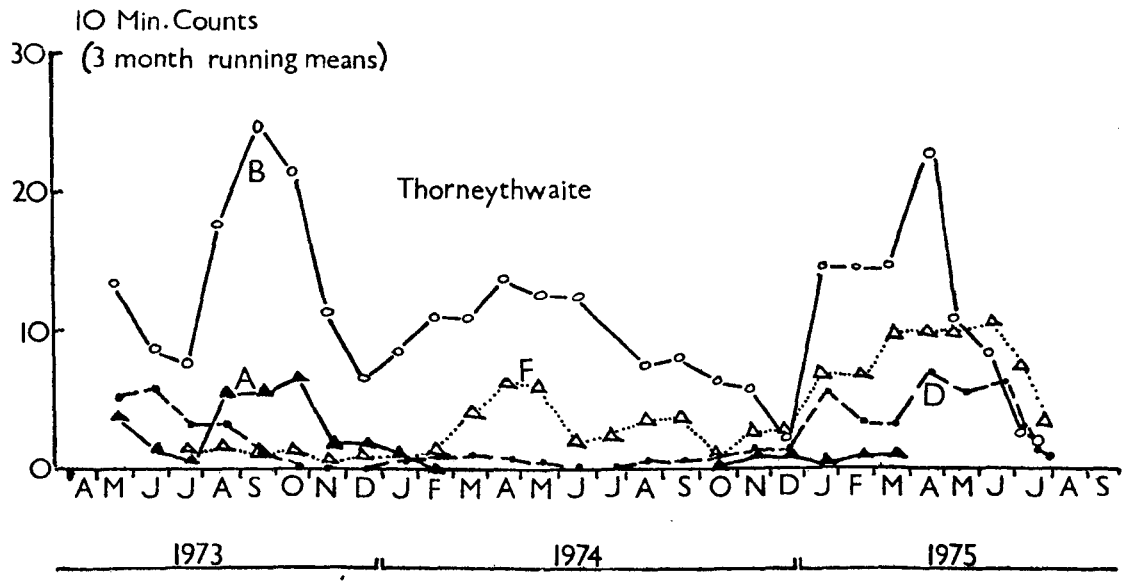
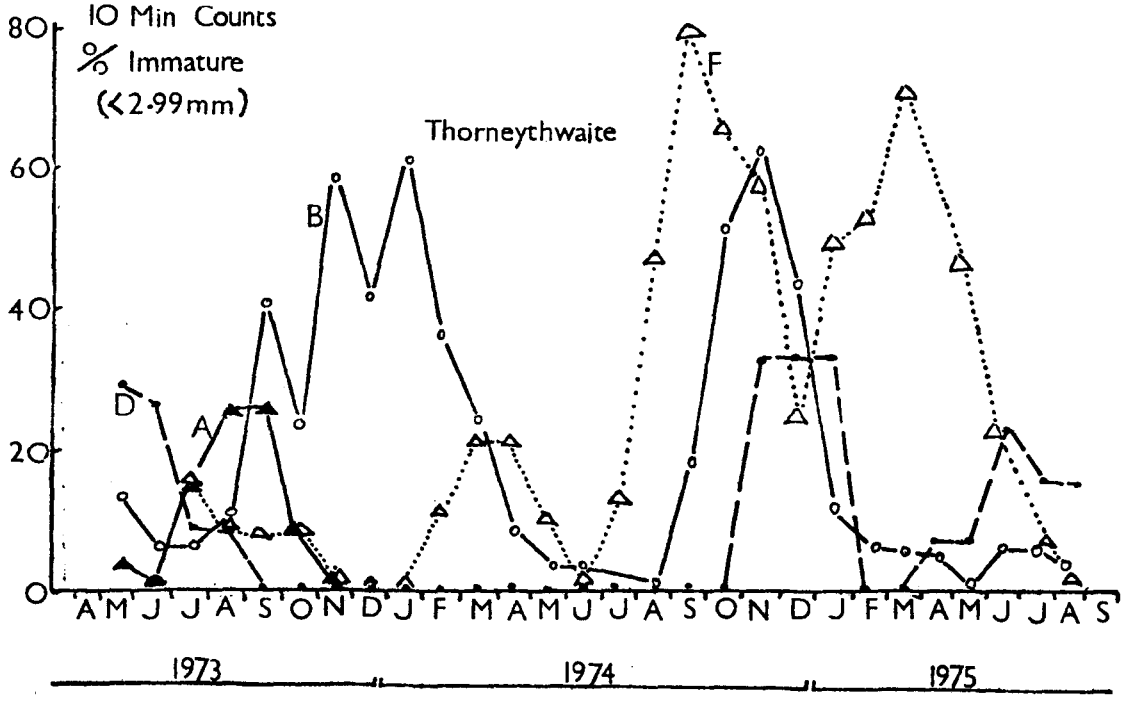


Fig. 4·10



counted. At Thorneythwaite, in contrast, large proportions of the populations consisted of immature snails during the autumn and winter months of 1974-5 as well as 1973-4.

(v) Quadrat Samples

Only two sites will be considered in detail. These are site D, Hendy, (a field habitat) and site B, Thorneythwaite, (a ditch habitat). As previously reported these two sites provided more than half of the snails found during this study and in only these two cases was there a significant correlation between the 10 minute counts for each site and the equivalent density estimates obtained from quadrat samples. The consistency with which the respective estimates of population density agreed was taken as a measure of their reliability.

(vi) Variations in the Mean Population Density of L. truncatula

The derived mean densities of L. truncatula on each site are presented in Figures 4.11 and 4.12. The 3 month running means are given in Figures 4.13 and 4.14.

The initial densities of snails on the Hendy site were between 6 to 10 times those on the Thorneythwaite habitat. Both populations were at high levels in the spring of 1973. In each case there was a decrease in population density which was halted in the early summer of 1973 and followed by a period of rapid population increase during the months of August and September. The increase continued on the Hendy site until November after which the number of snails

Fig. 4•13 3 Month Running Mean Snail Density

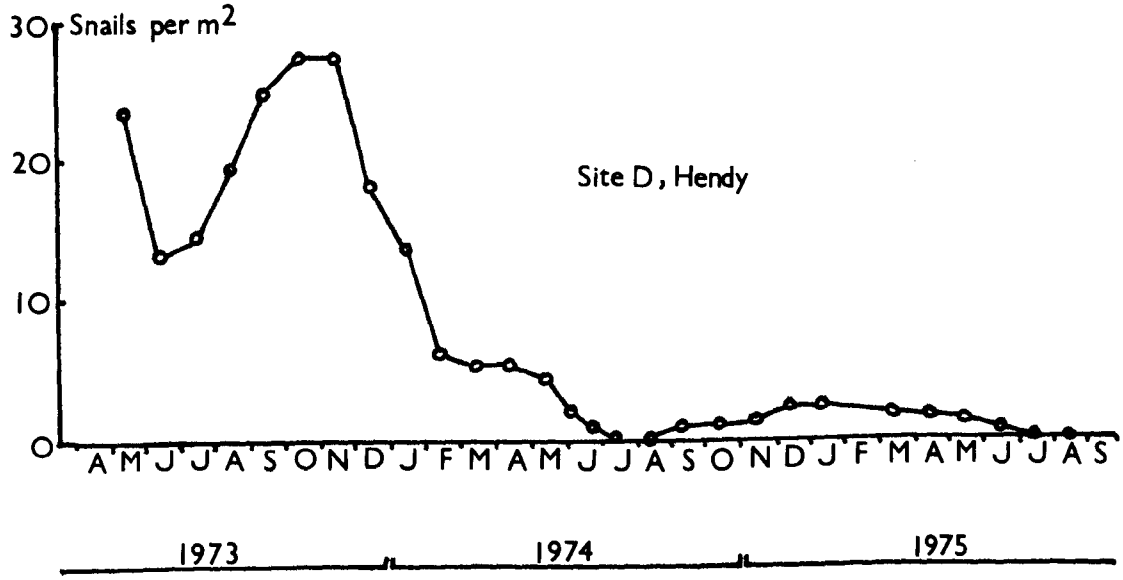
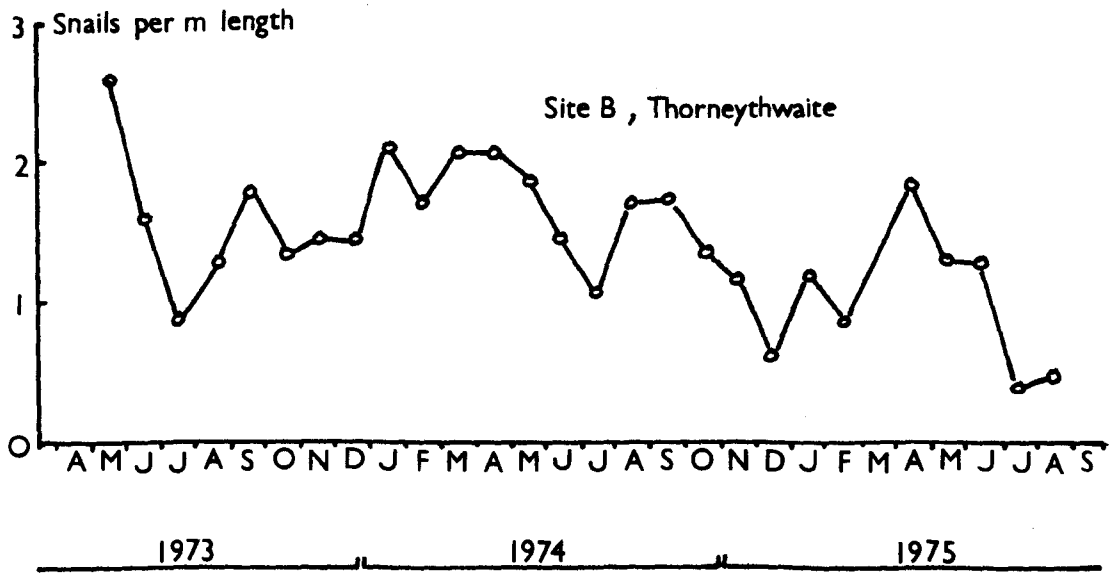


Fig. 4•14 3 Month Running Mean Snail Density



found rapidly declined again, this time to zero after some slight recovery in the autumn of 1974. By contrast, the population of snails in the ditch habitat at Thorneythwaite continued to fluctuate about a mean density of 1.43 snails per m throughout the whole period of observation. There was a slight suggestion of an overall downward trend but this proved to be statistically insignificant.

It is very unlikely that the variations in population density reported here could be accounted for by the regular removal of snails that was a necessary feature of the sampling procedure. The variations of greatest magnitude occurred in site D, Hendy. The total area of habitat searched was small (2%) and only about 4% of the total population was removed during each 10 minute count which is negligible when compared with the average monthly mortality (64%) that has been estimated for other populations of L. truncatula (Heppleston, 1972). The population on this site was probably little influenced by the sampling procedure. In any case, new poach holes were created every time the habitat was sampled so increasing the number of refuges within the site in which the snails were protected from dessication. Around 12% of the total length of the ditch site (site B, Thorneythwaite) was sampled but it is unlikely that more than a small proportion of the snails available in each quadrat was ever recovered since the ditch water often exceeded 10cm depth and the ditch bottom was covered with a dense growth of Marsh Fox-tail (Alopecurus geniculatus) and Flote Grass (Glyceria sp.) It follows that the estimates of population density obtained for this

site were relative in character whereas on site D, Hendy, the estimates approached absolute values since it is likely that most of the snails present within the small quadrats were actually found.

(vii) The Size-Class Structure of the Snail Populations

The number of immature snails (< 2.99mm) found on each sampling occasion was expressed as a percentage of the total number of snails found. (Figures 4.15 and 4.16). The size-class structure of the Thorneythwaite site population changed in an apparently seasonal way. The smallest proportion of immature snails was found each year in June or July. The proportion increased in August and September reaching a maximum in midwinter and declining thereafter to the low levels of late spring and early summer. On this site immature snails never constituted more than half the total snail population though this may have been due to the particular search difficulties of the ditch habitat. By comparison the percentage of immature snails on the habitat at Hendy exceeded 70% on four successive occasions during the winter of 1973-4. However, such high winter levels were never again repeated. After the winter decline no immature snails were found until the autumn of 1974 and after this the levels only once rose above 10% declining finally to zero in May 1975.

A more detailed analysis of the size class structure of the population of snails on site D and site B is given in Figures 4.17, 4.18, 4.19, and 4.20. The biomass of snails in each size-class

Fig.4·15 % of Snails that were Immature

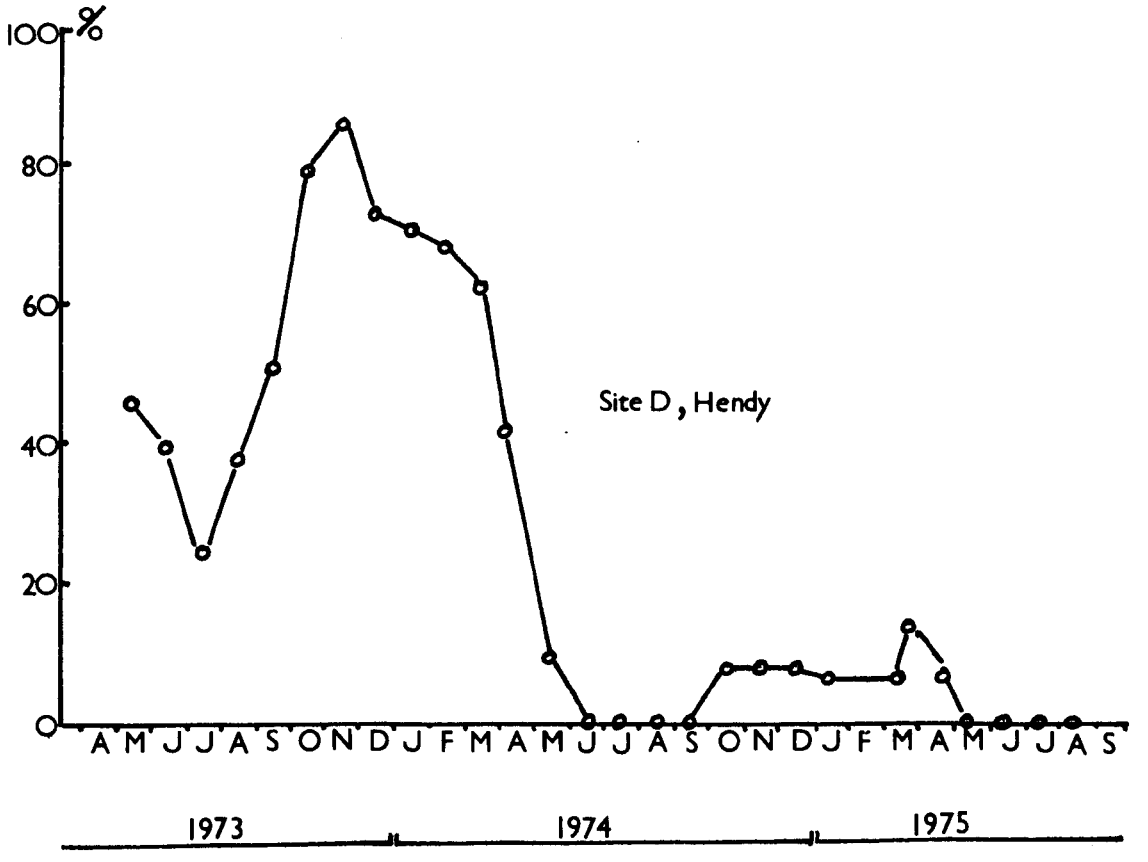


Fig.4·16 % of Snails that were Immature

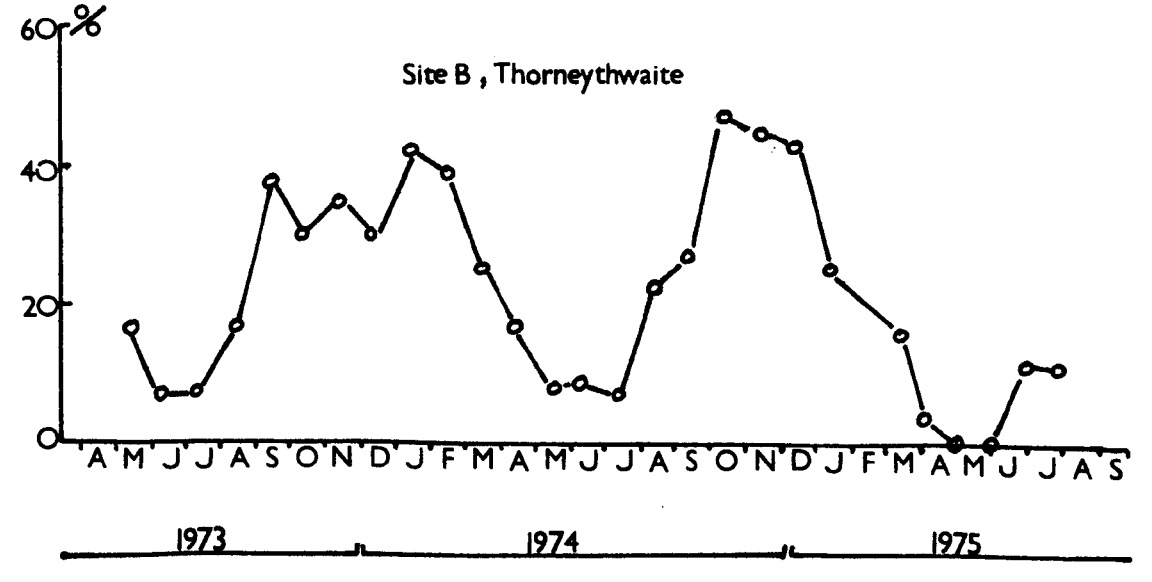
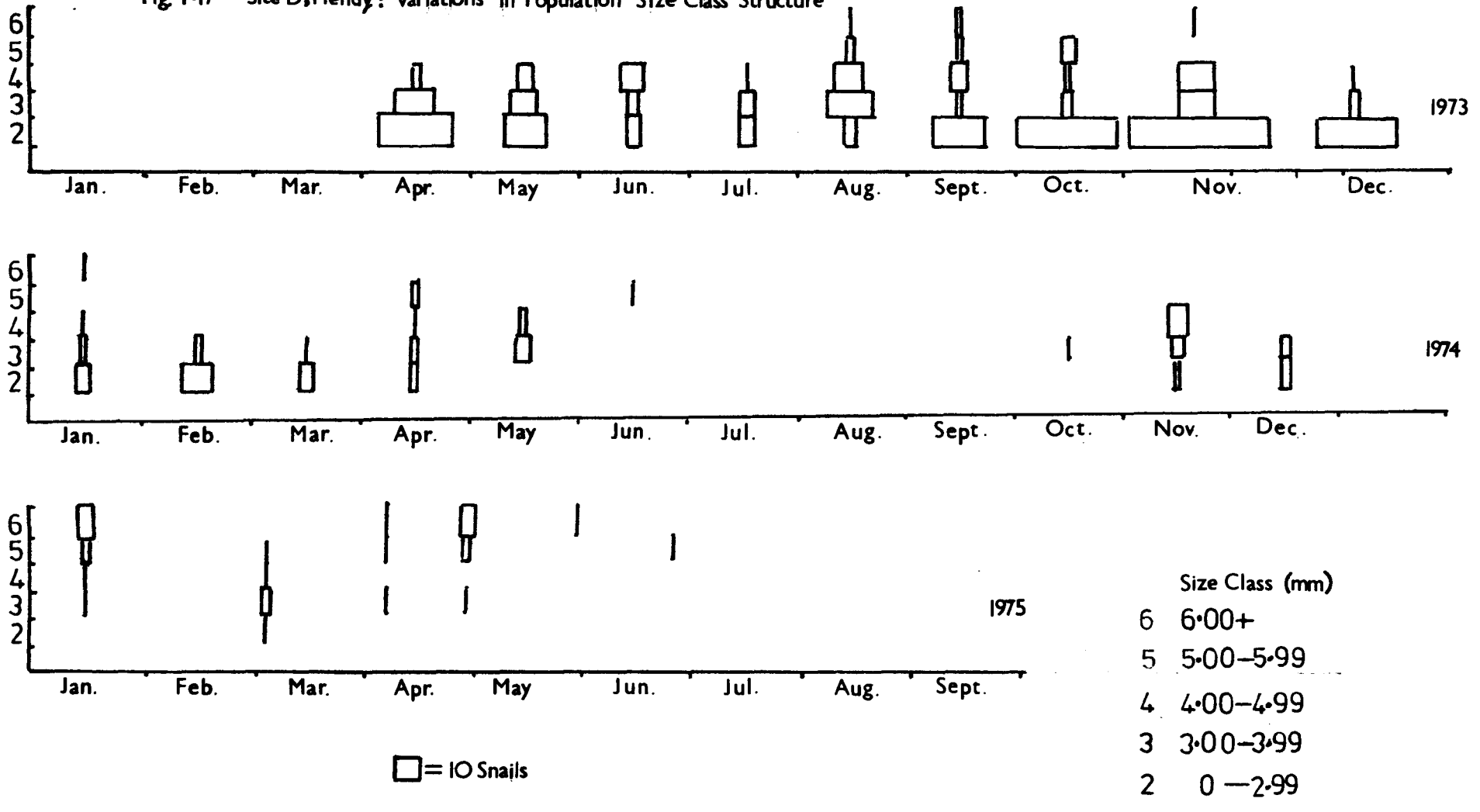


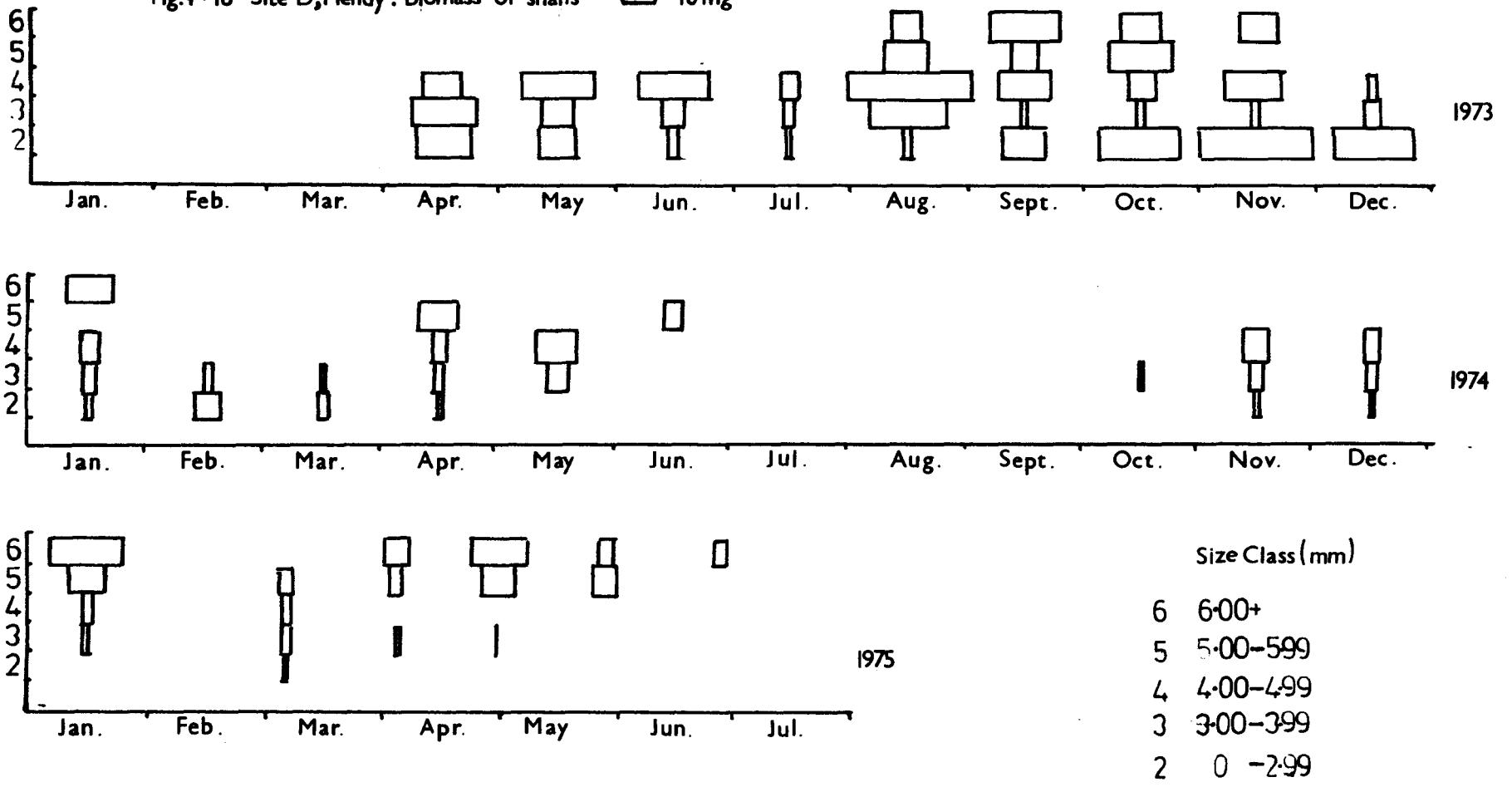
Fig. 4·17 Site D, Hendy: Variations in Population Size Class Structure



Size Class (mm)

6	6·00+
5	5·00–5·99
4	4·00–4·99
3	3·00–3·99
2	0 —2·99

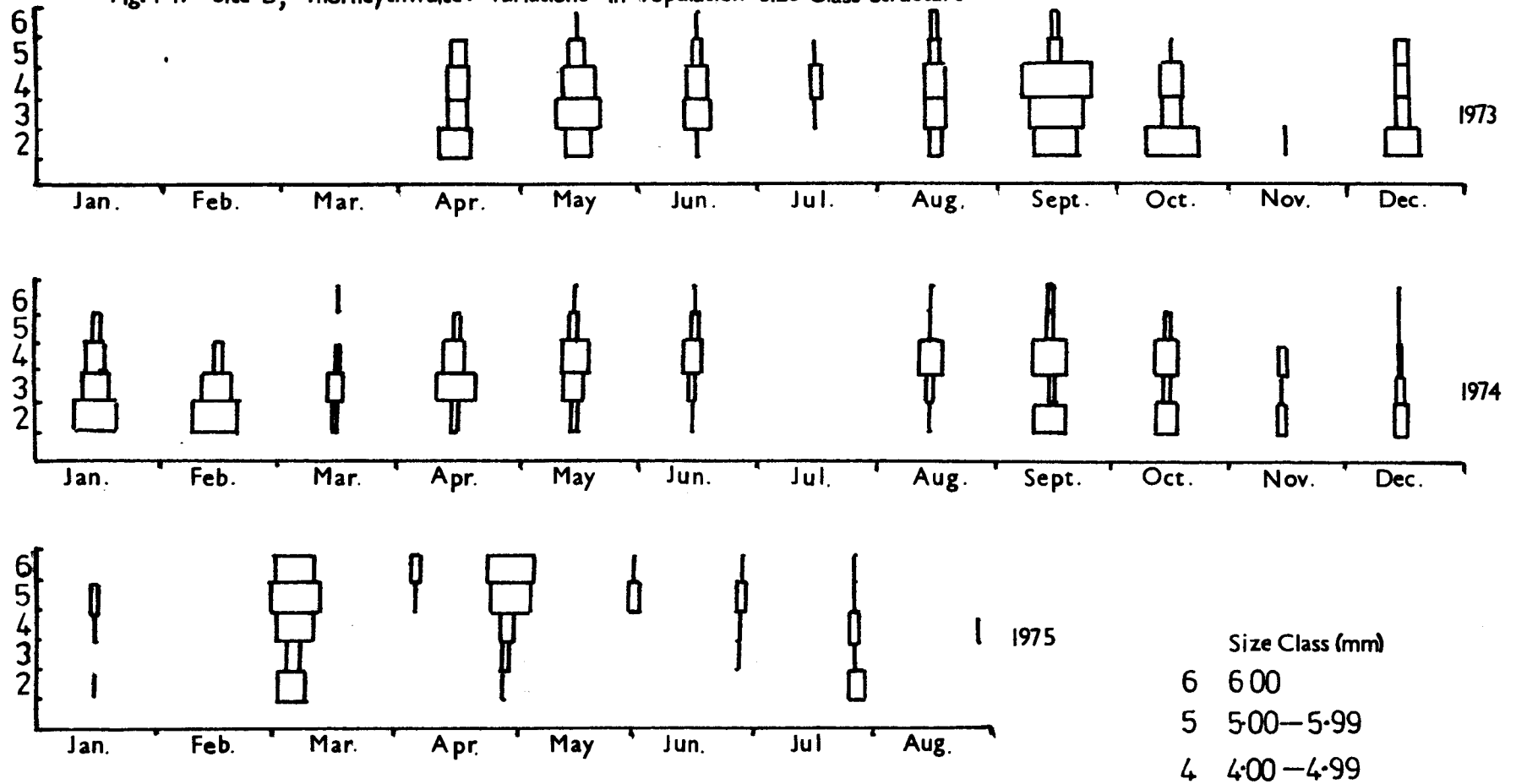
Fig.4 • 18 Site D, Hendy : Biomass of snails □=10mg



Size Class (mm)

6	6·00+
5	5·00–5·99
4	4·00–4·99
3	3·00–3·99
2	0 –2·99

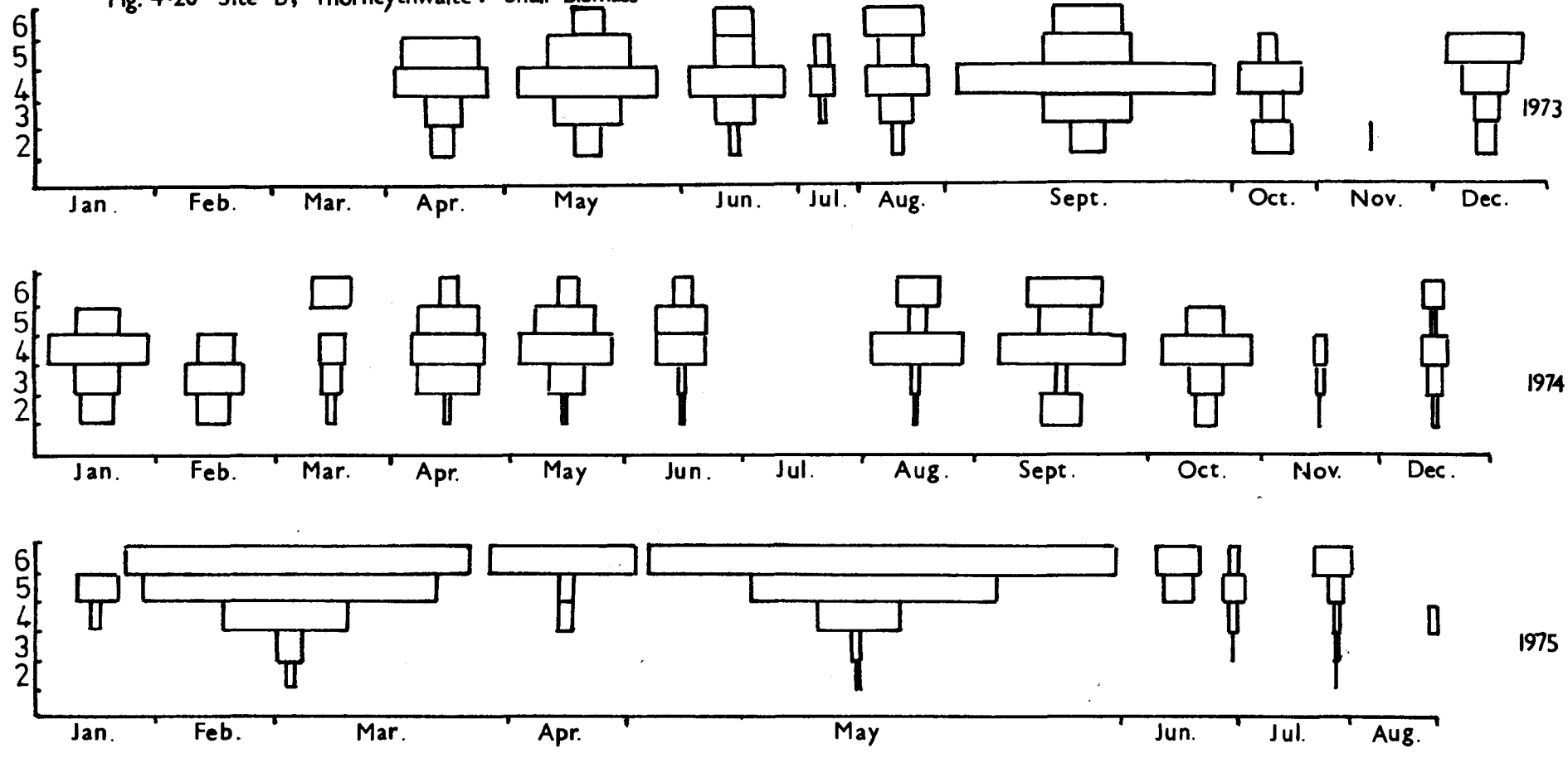
Fig.4·19 Site B, Thorneythwaite: Variations in Population Size Class Structure



Size Class (mm)

6	6·00
5	5·00—5·99
4	4·00—4·99
3	3·00—3·99
2	0 —2·99

Fig. 4·20 Site B, Thorneythwaite: Snail Biomass



□=10 mg

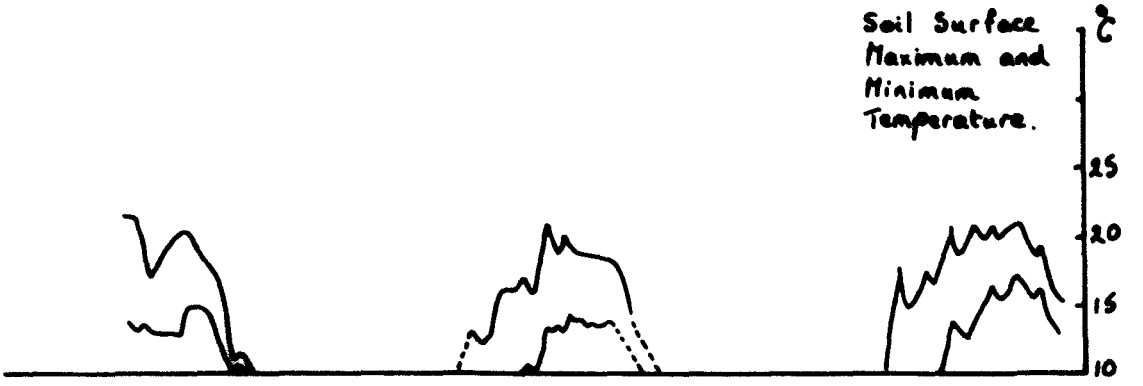
	Size Class (mm)		Size Class (mm)
6	6·00 +	3	3·00–3·99
5	5·00–5·99	2	0 –2·99
4	4·00–4·99		

(which represents the relative carrying capacity of the redial environment) was estimated from data supplied by Denison and Nice (pers.comm.) who measured the dry weight biomass of snails of various shell lengths ($y = 0.29x^2 - 1.84$; where y = dry wt. biomass, mg (shell + soft parts) and x = shell length, mm).

There was a marked decline in the density of immature snails at the Welsh site during the late winter of 1973/4 and recruitment to the smaller size classes in the autumn of 1974 was feeble by comparison with the previous year. Nevertheless, a preponderance of large snails in the early months of 1975 meant that the actual biomass of snails present was at least as great as in the equivalent months of 1974, though very much down on 1973 levels. For the same reason there was a striking increase in the biomass of snails present on site B during March and April of 1975. There appeared to be a smaller rate of recruitment to the immature size classes in 1974 than was observed in the previous year but the difference was slight and may not be significant.

(viii) Variations in Snail Population Density and the Microclimate of the Habitats

The association between soil-surface temperature, soil-surface moisture and the population dynamics of L. truncatula is clearly demonstrated when the microclimates of site D and site B are compared (Figures 4.21 and 4.22). In both cases the annual spring drought


Soil Surface
Maximum and
Minimum
Temperature.
°C
25
20
15
10

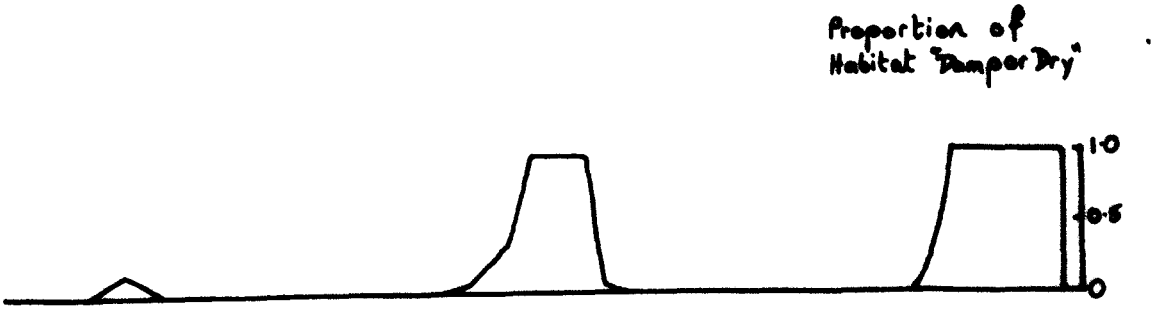

Proportion of
Habitat "Damper Dry"
1·0
0·5
0

Fig. 4·21 Site D, Hendy

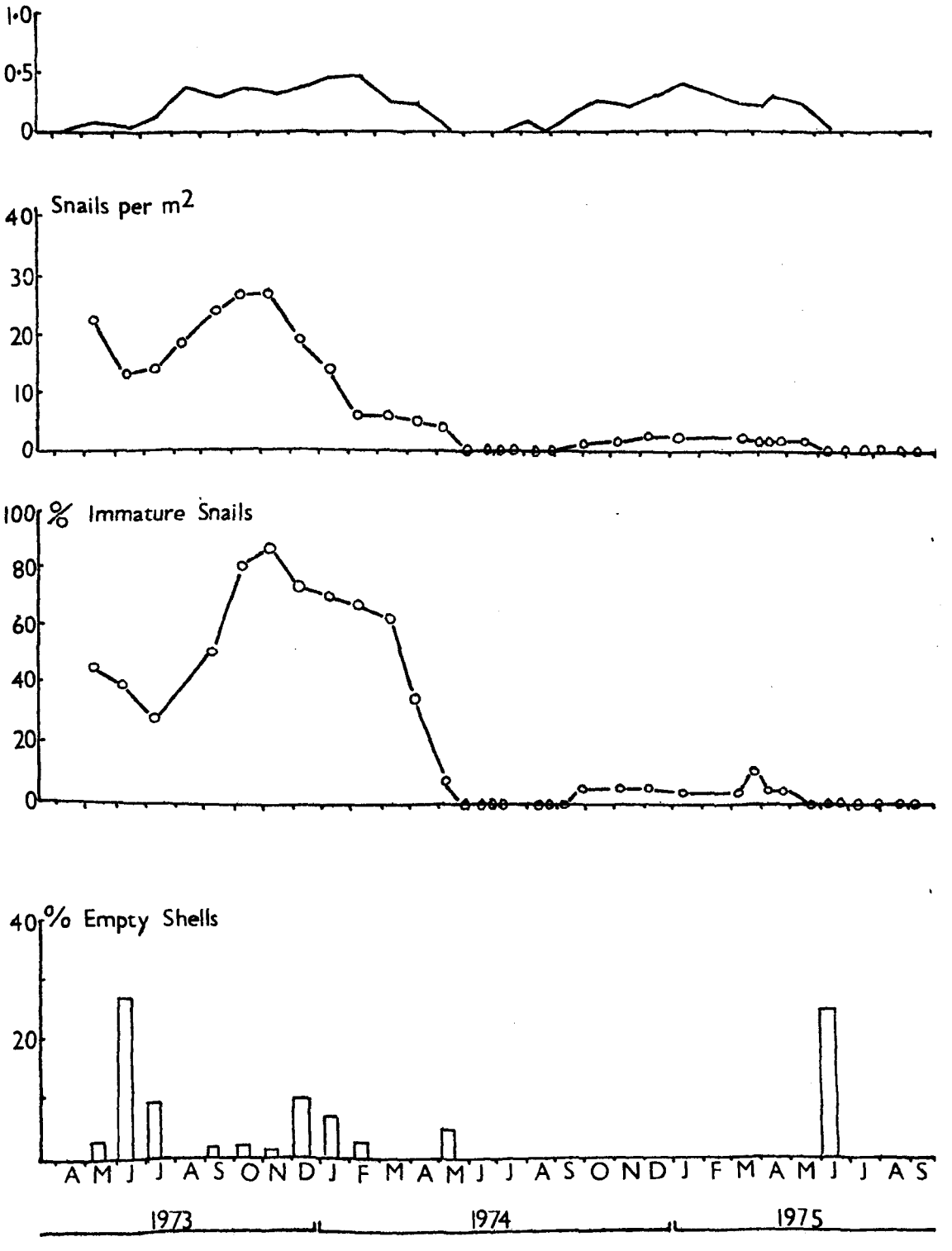
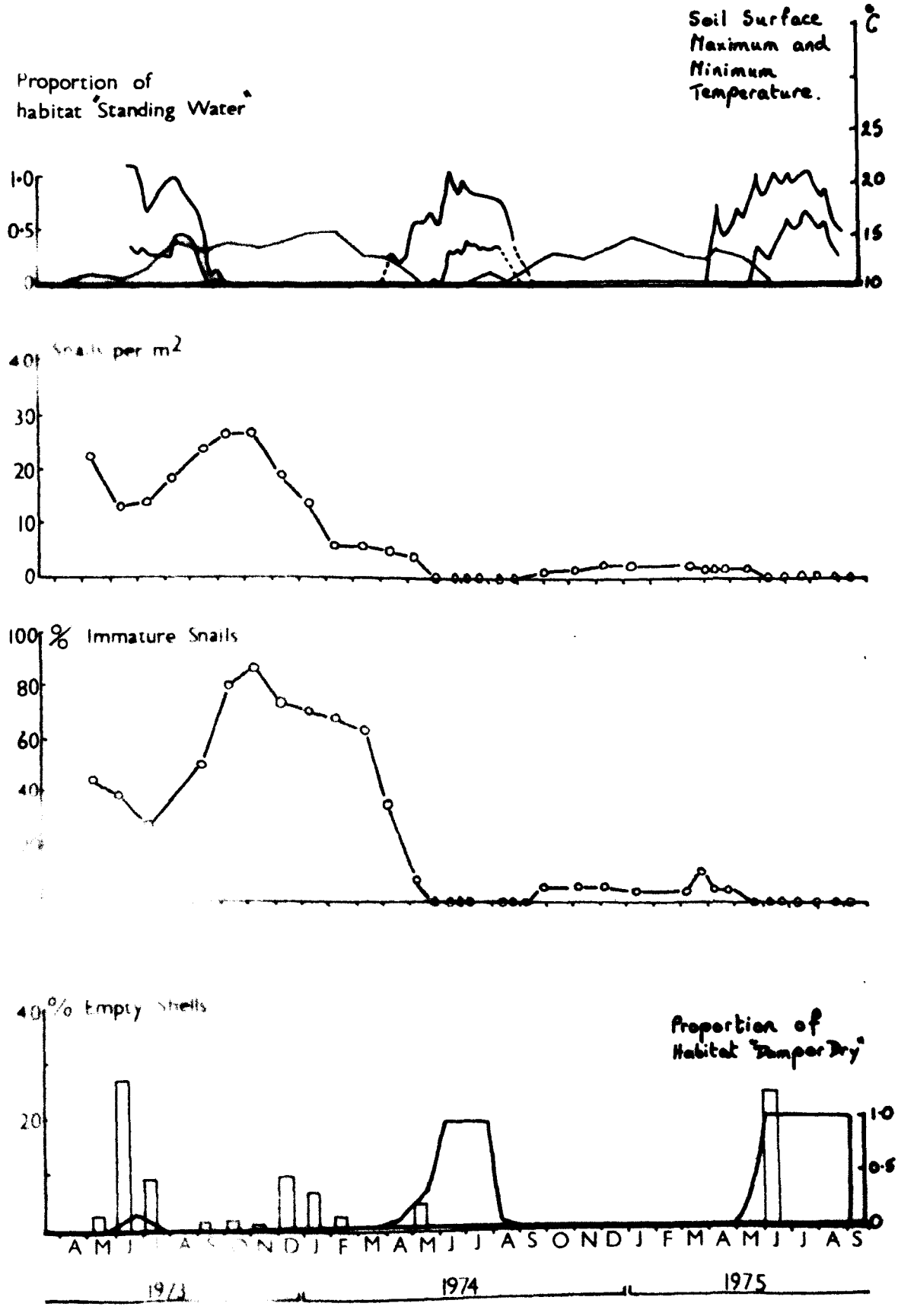
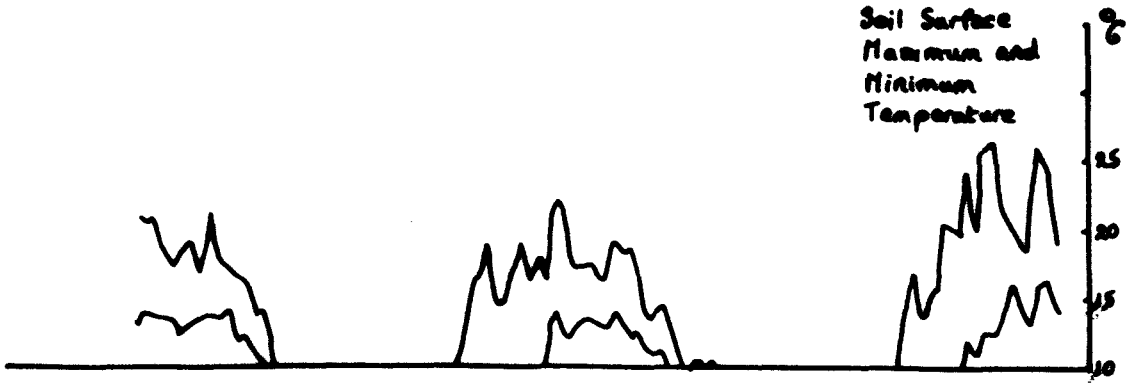


Fig. 4.21 Site D, Hendy




Soil Surface
Maximum and
Minimum
Temperature
°C
25
20
15
10

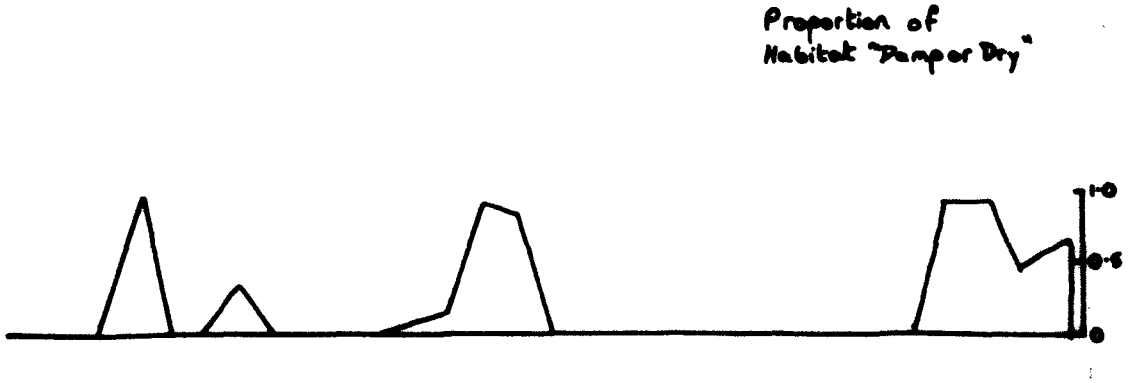

Proportion of
Habitat "Damp or Dry"
1·0
0·5
0

Fig. 4·22 Site B, Thorneythwaite

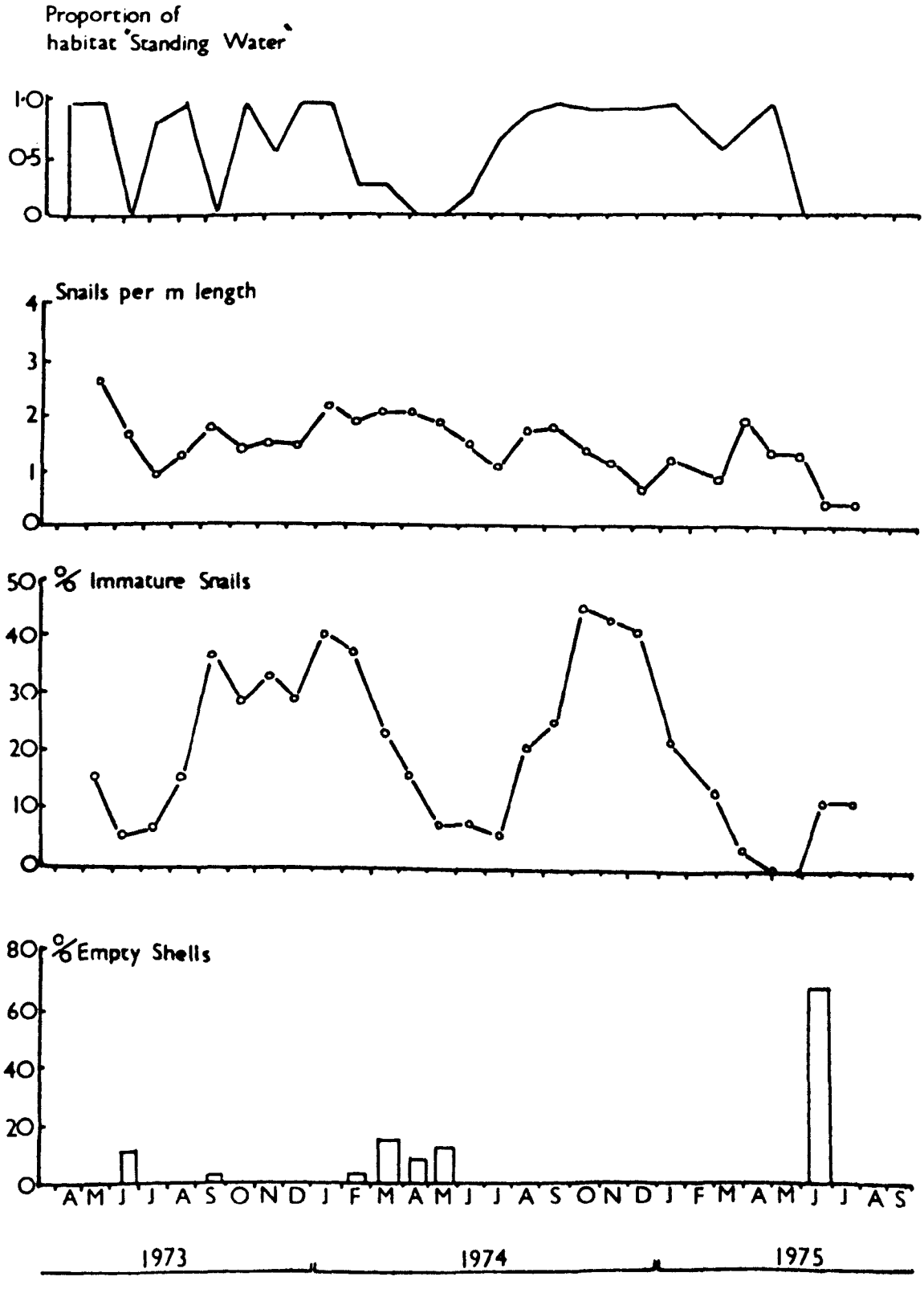
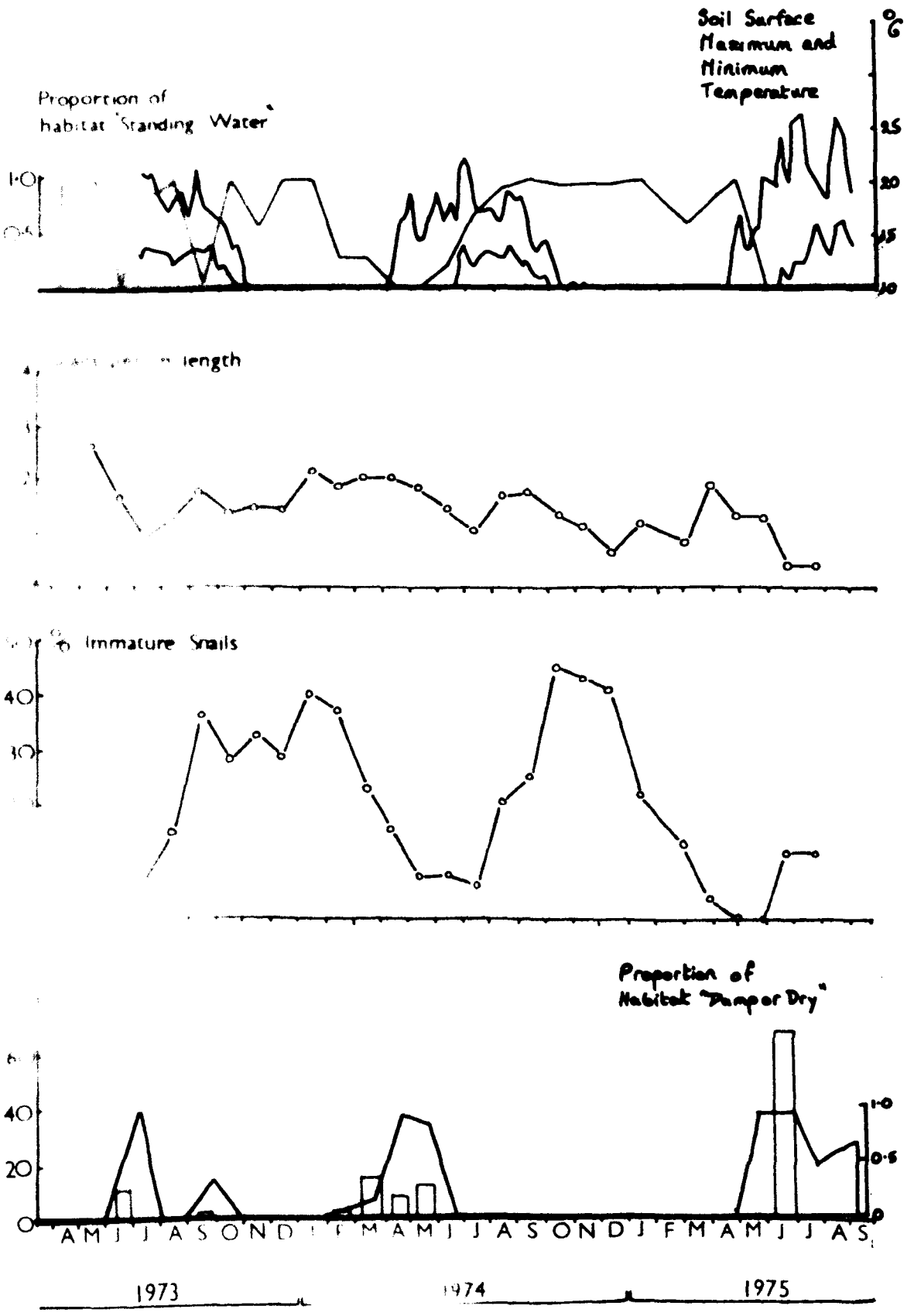


Fig 4-22 Site B, Thorneythwaite



increased intensity and duration with each successive year. In 1974, for example, the whole of site D was rated as "damp" or "dry" throughout June and July leaving only a few weeks in April and September when temperatures in excess of 10°C were coincident with "standing water" conditions. This was in marked contrast to 1973 when most of the habitat was rated as "wet" during the drier spring period and "standing water" conditions prevailed throughout July, August and September. The mortality of the snails (measured as the percentage of shells that were empty) was greatest on this site during the spring droughts and in 1974 there was too little opportunity in the late summer months for the population to recover. Not only was there a very small area of habitat which was suitably wet but the weekly mean temperatures were generally less than in 1973 (Table 11). No snails at all were found after the onset of the very severe spring drought of 1975.

All of the empty shells found between April and November 1973 were over 3.00 mm in length (mean 4.78mm, range 3.00-6.59) whereas all of the empty shells found in December of that year belonged to immatures (mean 2.16mm, range 1.85-2.68mm). The cause of the mortality of these immature snails was not at all clear. Minimum air temperatures during the first few days of December fell below 0°C but the few soil surface temperatures that were recorded during this period (the temperature recorder malfunctioned) were not below 4°C. The small number of empty shells found during the subsequent months

Table 11

MEAN WEEKLY SOIL SURFACE TEMPERATURES °C

HENDY

	JULY				AUGUST				
1973	16.8	15.8	16.7	—	17.25	18,45	17.5	16.65	-
1974	17.5	16.7	16.5	16.2	16.25	16.3	15.75	14.5	13.7
	SEPTEMBER				OCTOBER				
1973	16.0	13.85	11.9	12.55	11.0	9.35	9.8	9.4	
1974	-	-	-	9.47	9.4	10.15	8.8	8.45	

THORNEYTHWAITE

	JULY				AUGUST				
1973	17.45	16.4	15.35	15.55	16.6	15.25	17.65	15.9½	15.95
1974	15.2	15.6	15.4	14.7	14.8	17.05	16.2	15.45	14.95
	SEPTEMBER				OCTOBER				
1973	15.65	14.3	18.85	12.0	12.25	10.5	7.59	9.38	
1974	12.7	12.95	13.2	10.7	8.75	8.2	9.3	9.25	

ranged in size from 1.34mm to 7.21mm in length.

The population of snails on site B was obviously little affected by the spring drought of 1974. Though the habitat began to dry out much earlier than in 1973 an early return to field capacity restored at least a quarter of its area to "standing water" conditions by June and most of the habitat was under water by August. In other words, although surface temperatures[1] were generally lower in 1974, optimal moisture conditions and suitable temperatures prevailed for about 4 months in both 1973 and 1974. The mean density of the population varied little over these two years and the oviposition and hatching rates may be assumed to have remained more or less constant. Here also the mortality of the snails was associated with the dry periods, the greatest mortality being recorded at the height of the most severe drought in 1975. Empty shells of less than 3.00mm were hardly ever found on this site and so the mean length of the empty shells that were found was generally in excess of the mean length of shells in the population as a whole. A sample of the data is given below:

Month	Feb.	March	April	May	(1974)
Empty shell length (mm)	2.9	3.72	5.13	4.54	
Population shell length (mm)	3.07	3.70	3.92	4.22	

[1]The soil surface temperatures reported were measured at site D, Thorneythwaite, not site B, but it can be assumed that the relative pattern of temperatures on adjacent sites did not differ though the absolute values probably did.

The paucity of empty shells from the smaller size classes was taken to be an artefact of the search method.

There is some evidence to suggest that the density of snails on site B would not have been maintained at the previous levels during the autumn of 1975. Although the surrounding pasture approached field capacity during September of that year there were still no parts of the habitat that were covered by "standing water". The small increase in the percentage of immature snails that was seen at the end of June probably reflected a brief period of oviposition during April and May when at least the maximum temperatures exceeded $10^{o}C$. Indeed, the transformed mean densities of snails found on this site between August and September 1975 were significantly less that the mean densities observed in August and September of both 1973 and 1974 ($p < 0.01$ in all cases, d.f. 16) and the usual late summer increase in snail density was entirely absent.

(ix) Constant Effort Search - Pooled Data

The data were pooled and the snails assigned to size classes as described in 'Materials and Method.' It had been intended to compare the size class distribution of snails in successive 6 month periods however, application of the heterogeneity Chi-squared test to each set of pooled data demonstrated a marked heterogeneity in each case and further analysis was abandoned. Nevertheless, the total number of snails found during each 6 month period is presented below.

Hendy Farm

	April-Sept.	Oct.-March	
1973	475	298	1973/4
1974	67	190	1974/5
1975	35	-	

Thorneythwaite Farm

	April-Sept.	Oct.-March	
1973	323	196	1973/4
1974	184	217	1974/5
1975	147	-	

There was a fall in the total number of snails found in each successive April-September period. The transition from the October-March period to the April-September period was also marked by a fall in snail numbers, this being most pronounced at Hendy.

The percentage of empty shells was estimated for the pooled data and the results are presented in Figures 4.24 and 4.25 together with the weekly mean estimates of S.M.D. for each farm. The mortality rate was greatest when S.M.D. approached maximum values. Inspection of the raw data indicates that the empty shells found during each spring drought belonged to snails which were either mature or approaching maturity (i.e. shell length in excess of 3.00mm). These snails hatched out in the previous year and would have constituted the parental generation of the year in which they perished. The highest percentage of empty shells was found during Spring, 1975

Fig. 4·24 Percentage empty shells. (Hendy–all habitats)

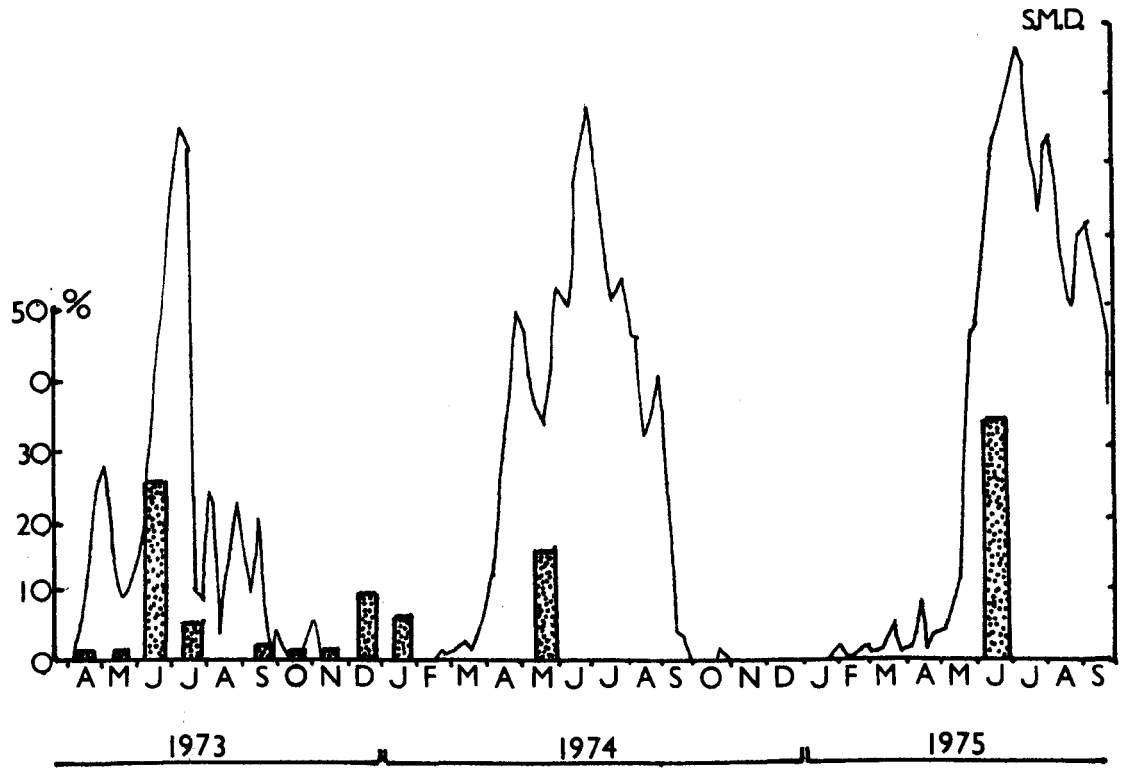
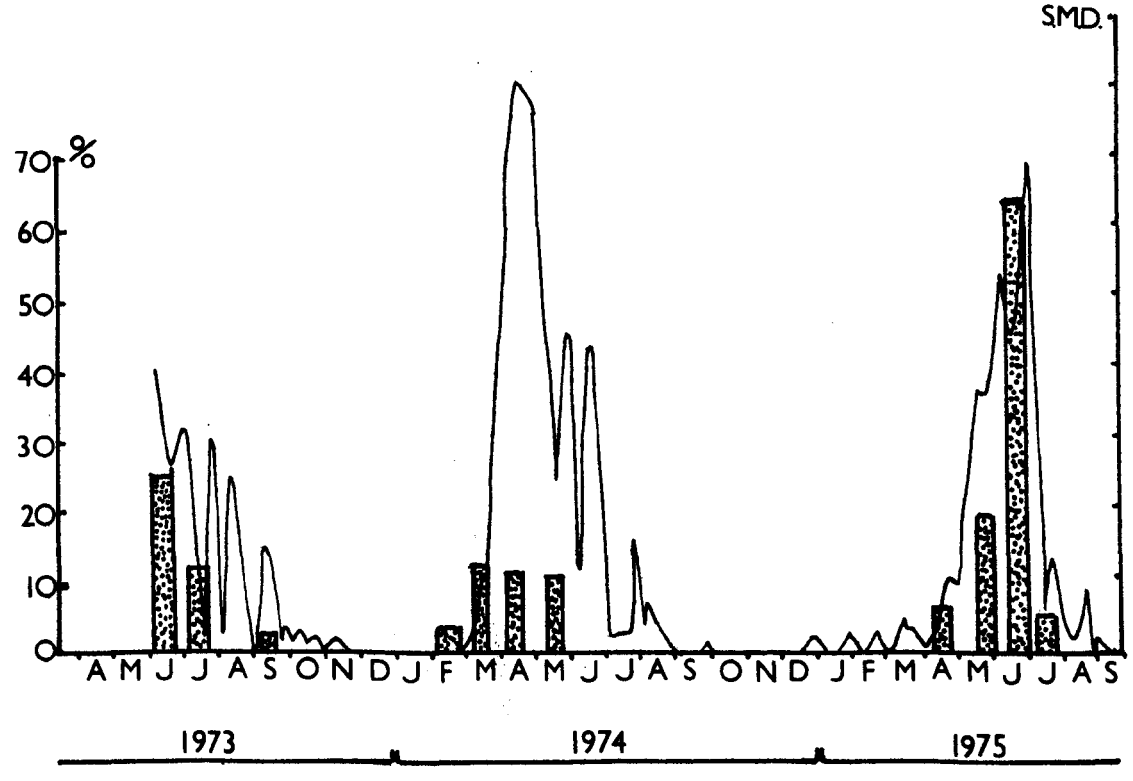


Fig. 4·25 Percentage empty shells (Thorneythwaite–all habitats)



which coincided with a marked decrease in total snail number, especially at Hendy.

4. RESULTS - THE INTRAMOLLUSCAN STAGES OF F. HEPATICA

(i) The Course of the Infection, April 1973 - September 1975

Tables 12 and 13 show the number of infected snails found during the course of 10 minute counts. Most of these snails were found during the late summer and autumn of 1973 and the winter of 1974. On both farms, in each successive year, there was a decrease in the number of infected snails collected. If site D, Hendy and site B, Thorneythwaite are once more considered separately from the rest, and the results of quadrat collections are also taken into account, then only site D conforms to the general pattern described above. Here all but two of the infected snails were found during the period from August 1973 to March 1974. Site B on the other hand was a consistent source of infected snails throughout the whole period of observation. The highest densities of infected snails were found in August 1973 on site D, Hendy and in September 1973 on site B, Thorneythwaite. (Tables 14 and 15).

In Figures 4.26 and 4.27 the mean length of the infected snails is compared with the mean length of the entire population of snails. The mean length of the snails on both site D Hendy, and Site B, Thorneythwaite varies in a seasonal manner, but there is also an overall increase in mean length between 1973 and 1975. The mean

Table 12

10 MINUTE COUNTS (HENDY) - NUMBER OF SNAILS INFECTED WITH F. HEPATICA

	1973 HABITAT			1974 HABITAT			1975 HABITAT		
	A	D	E	A	D	E	A	D	E
Jan									
Feb					1	5			
Mar							1		
Apr			3				1		
May		1	8			1			
Jun								1	
Jul									
Aug		12							
Sept		2		1					
Oct	2	1	2	1					
Nov		1	1	1					
Dec									

Table 13

10 MINUTE COUNTS (THORNEYTHWAITE) - NUMBER OF SNAILS INFECTED WITH F.HEPATICA

	1973 HABITAT				1974 HABITAT				1975 HABITAT			
	A	B	D	F	A	B	D	F	A	B	D	F
Jan						1						
Feb						1	1					
Mar						1						
Apr		1						1				
May												
Jun		1									1	
Jul												
Aug	1					1						
Sep	2	2										
Oct		1										
Dec												

Table 14

SITE D (HENDY) - DENSITY OF INFECTED SNAILS AND DENSITY OF REDIAE

	1973 Mean Density per m^2		1973 10 Min count		1974 Mean Density per m^2		1974 10 Min count		1975 Mean Density per m^2		1975 10 Min count	
	S	R	S	R	S	R	S	R	S	R	S	R
Jan												
Feb							1	30				
Mar												
Apr												
May			1	7								
Jun											1	42
Jul												
Aug	11.25	65.0	12	62								
Sep	1.25	27.2	2	42								
Oct			1	2								
Nov	1.25	30.0	1	17								
Dec												

Table 15

SITE B (THORNEYTHWAITE) - DENSITY OF INFECTED SNAILS AND DENSITY OF REDIAE

	1973 Mean Density per m		1973 10 Min count		1974 Mean Density per m		1974 10 Min count		1975 Mean Density per m		1975 10 Min count	
	S	R	S	R	S	R	S	R	S	R	S	R
Jan					0.16	1.16	1	15				
Feb					0.33	0.66	1	12				
Mar					0.16	3.5	1	12	0.25	15.58		
Apr	0.16	1.66	1	24	0.25	1.66						
May	0.16	2.00										
Jun			1	19	0.16	8.5			0.08	0.83		
Jul									0.16	5.33		
Aug					0.08	4.42	1	95				
Sep	2.0	23.33	2	26	0.08	8.83						
Oct				2	0.16	2.75						
Nov												
Dec	0.16	0.66										

S = Snails R = Rediae

Fig.4·26 Mean Shell Length : Site D , Hendy

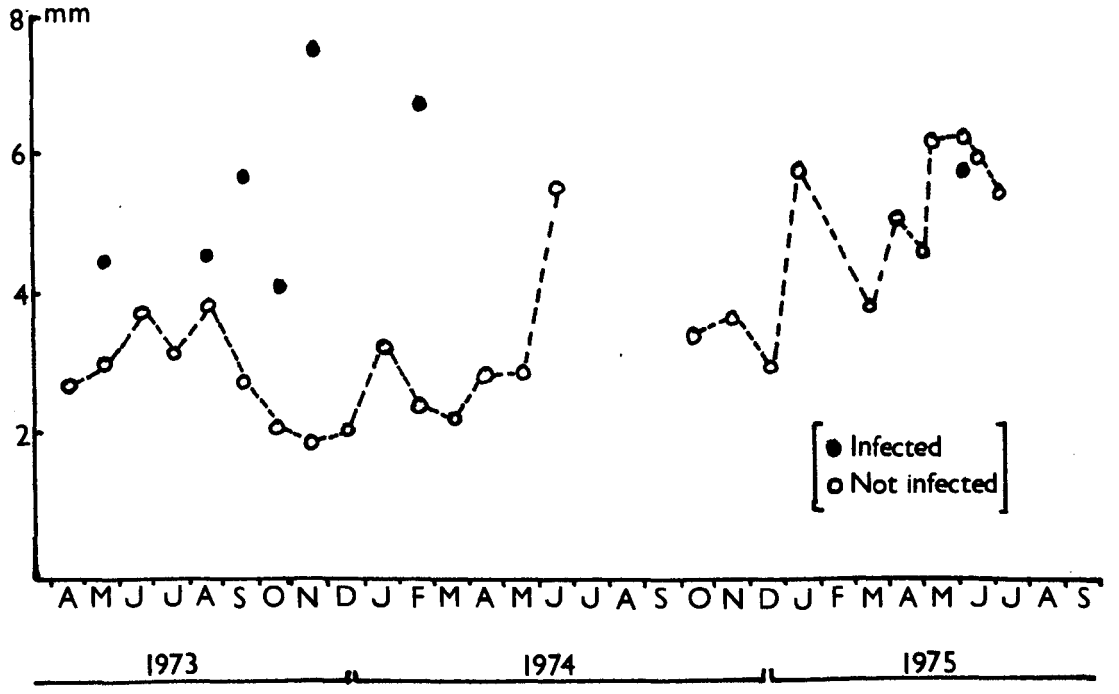
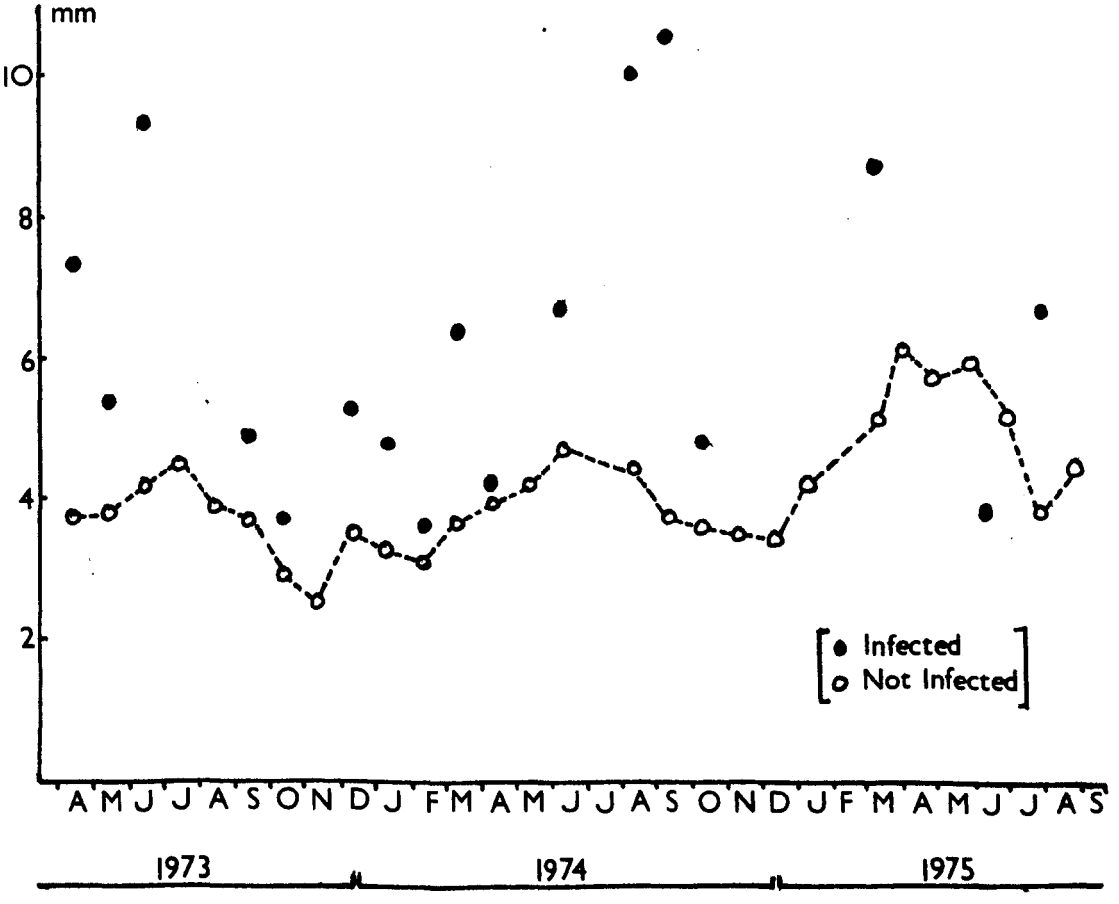


Fig.4·27 Mean Shell Length : Site B , Thorneythwaite



length of infected snails, while not following this trend, exceeded the mean length of the population as a whole in all but two cases.

In Tables 16 and 17 the rediae found on each sampling occasion have been classified according to their contents, i.e. those containing mainly germ balls, those containing mainly immature cercariae and those containing mainly mature cercariae. At 20°C rediae emerge from the sprocysts from 11 days after infection onwards (Wilson and Draskau, 1976) and more than 50% of the parthenitae within the rediae are recognisible as cercariae from about 35 days post infection. Using this information and the redial development times provided by Ollerenshaw (1971a) and Nice and Wilson (1974) it was possible to describe the approximate course of infection on each of the two sites, site D and site B.

Site D, Hendy, will be considered first. In May 1973 the infection was represented by a single snail. Although the number of rediae within it was small (7) all of them contained mainly mature cercariae suggesting that the infection had been acquired sometime in the previous year. No further cases of infection were found until August 1973 when the mean density of infected snails was estimated at 11.25 snails per m^2, the highest recorded on this site. In general the number of rediae per snail was low (mean 6) and the redial contents were predominantly immature cercaria. The monthly mean soil temperatures in June, July, and August were 17.4°C, 16.5°C and 17.4°C respectively. These infections must have been at least 4 weeks old

Table 16

SITE D (HENDY) - % OF REDIAE CONTAINING MAINLY GERM BALLS, IMMATURE CERCARIAE OR MATURE CERCARIAE

	1973			1974			1975		
	% of rediae			% of rediae			% of rediae		
	Germ Balls	Imm Cerc	Mat Cerc	Germ Balls	Imm Cerc	Mat Cerc	Germ Balls	Imm Cerc	Mat Cerc
Jan									
Feb				0	53	47			
Mar									
Apr									
May	0	0	100						
Jun							21	0	79*
Jul									
Aug	0	100	0*						
Sep	0	51	49*						
Oct	0	100	0						
Nov	0	41	59*						
Dec									

Table 17

SITE B (THORNEYTHWAITE) - % OF REDIAE CONTAINING MAINLY GERM BALLS, IMMATURE CERCARIAE OR MATURE CERCARIAE

	1973			1974			1975		
	% of rediae			% of rediae			% of rediae		
	Germ Balls	Imm Cerc	Mat Cerc	Germ Balls	Imm Cerc	Mat Cerc	Germ Balls	Imm Cerc	Mat Cerc
Jan				6	56	39			
Feb				0	100	0*			
Mar				0	6	94	41	22	37
Apr	63	29	8	85	0	15*			
May	92	8	0						
Jun	0	100	0	26	54	20	100	0	0
Jul							27	43	30
Aug				0	1	99			
Sep	8	92	0*	11	0	89*			
Oct	0	100	0	48	42	10			
Nov									
Dec	0	100	0*						

* Cercariae found outside the rediae

indicating that the snails were infected in the first or second week of July at the latest when the predominant soil-surface moisture condition was "wet" rather than "standing water." Nevertheless, the fact that cercariae were found free within the bodies of two of the snails suggests that amongst the infected snail population there were at least some L. truncatula that had acquired their infection long before this. The infections found in September and November probably represent the maturation of the infection acquired early in the summer: the mean size of the infected snails was high at a time when that of the population as a whole was low, the mean number of redia per snail was also high (25.3 and 20.5 respectively) and about half of the rediae contained mainly mature cercariae (free cercariae were noted on both occasions). Only a single infected snail was found in October of that year on this site and its small shell length and low number of rediae (2) suggest that it was infected during the late summer. No other infected snails were found until June 1975, when about 200 free cercariae and 42 rediae, most of which contained mature cercariae, were found in a single snail. It is likely that so mature an infection was acquired during the previous year and if so is the only example of an infection occurring in one of the generation of snails that hatched in 1974.

The situation at site B, Thorneythwaite was considerably more complex and is best interpreted as the result of several overlapping

waves of miracidial invasion. The infected snails found in April and June of 1973 were very large (in excess of 9mm length), the mean number of rediae per snail was moderately high (17 and 19 respectively) and in one case some of the rediae contained mature cercariae. These infections had overwintered from the summer and autumn of 1972. The only infected snail found in May 1973 was host to 12 rediae, 11 of which contained mainly germ balls; it is not certain whether this infection had overwintered or not. The highest density of infected snails was recorded in September 1973. The number of rediae per snail ranged from 1 to 33 (mean 11), all of the rediae contained mainly immature cercariae but free cercariae were found in one case. The data suggest that these snails were infected over a fairly broad interval of time earlier the same year. The maturation of this infection is probably represented by the free cercariae noted in October and December of 1973, and in February 1974, and by the rediae containing mainly mature cercariae that were found in January and March of 1974. Thereafter and throughout 1974 it is not possible to distinguish newly acquired infections from overwintering ones. In 1975 infections were found in March, June, and July. Probably only the June infection, represented by one snail containing 6 very small rediae, originated in that year. All of the rediae contained germ balls suggesting that the snail was infected in May when the whole habitat was rated as "wet." The other infections had almost

certainly overwintered from 1974: the mean number of rediae was high (62.3 and 83 respectively) and about 0.3 of the total number of redia contained mainly mature cercariae.

The predominance of "wet" and "standing water" conditions combined with the relatively high soil surface temperatures and high snail densities which prevailed during the spring and summer months of 1973 was particularly favourable to the parasite on both habitats resulting in a large increase in the proportion of infected snails in August and September. Though rather colder in 1974 there was sufficient surface moisture throughout the spring and a high enough density of snails to maintain the proportion of new infections at site B whereas, in the same year, at site D the spring drought of June and early July, together with a much reduced density of L. truncatula provided conditions unfavourable enough for the fluke eggs and miracidia to result in an almost complete absence of new infections. The prolonged "dry" period and the high soil surface temperatures on both habitats in 1975 in addition to the reduced snail densities were associated with a total absence of infections during August and September of that year.

It should be noted that in each year most of the new infections arose before the daughter generation of snails hatched out. The miracidia entered snails that were already of mature or nearly mature size and so established themselves in an environment with an already large carrying capacity.

(ii) The Relationship between the Size Class of an Infected Snail and the Number and Maturity of the Fasciola Larvae within it

The infection data for all the habitats on both farms were pooled. The mean number of rediae per infected snail was calculated for each of 8 size classes of snail. The results are presented in Figure 4.28 and demonstrate that the larger the size class of snail the more rediae it is likely to contain. Similarly, the mean number of rediae containing mainly mature cercariae and the mean number containing mainly immature cercariae and germ balls was calculated for each snail size class (Table 18, Figure 4.29). The proportion of rediae containing mainly mature cercariae increased with the size class of snail. The actual mean number of rediae containing mature cercariae remained negligible until the length of the infected snail exceeded 6mm. In general, then, the larger the size class of an infected snail the more rediae it was likely to contain and the more mature the infection within it.

If, finally, the total numbers of snails, the total numbers of infected snails and the total numbers of rediae are classified according to snail size class and presented as a log plot (Figure 4.30) then it becomes plain that as the size class of snail increased so did the proportion of infected snails it contained.

These results have two consequences: first, that the total number of rediae making up the parasite population in any particular population of snails is not related merely to the total number of

Table 18

THE MEAN NUMBER OF REDIAE PER INFECTED SNAIL (± ONE STANDARD ERROR)

Snail Size Class (mm)	0-2.99	3.00-3.99	4.00-4.99	5.00-5.99
Rediae containing Immature Cercariae and Germ Balls	2.67±0.65	4.81±1.1	9.42±1.99	10.19±1.54
Rediae Containing Mature Cercariae	0.67±0.36	0	1.06±0.45	2.15±1.27
n.	3	21	33	27
Snail Size Class (mm)	6.00-6.99	7.00-7.99	8.00-8.99	10.00-10.99
Rediae containing Immature Cercariae and Germ Balls	11.68±2.25	23.86±7.73	27.00±12.41	20.13±7.29
Rediae containing Mature Cercariae	8.09±2.28	6.00±2.25	13.00±13.04	34.63±14.62
n.	22	7	2	8

Fig. 4·28

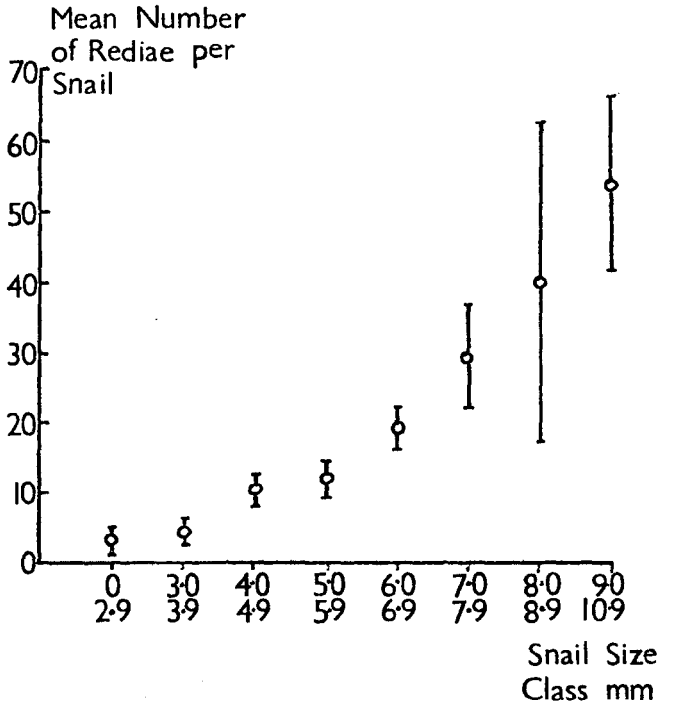


Fig. 4·29

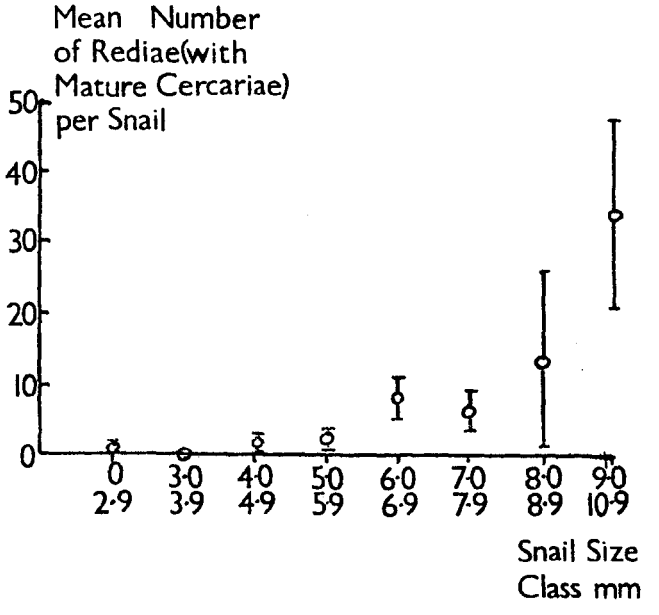
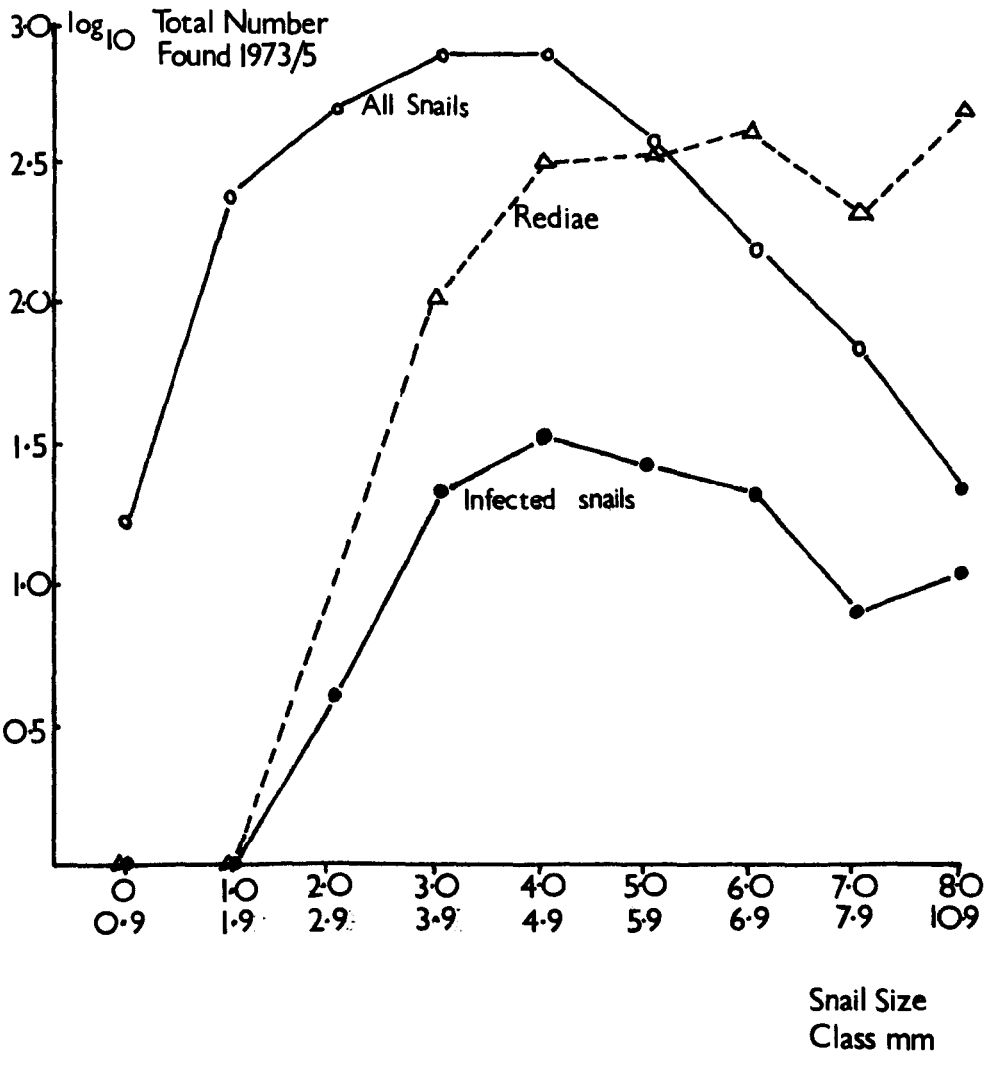


Fig. 4·30


log10 Total Number Found 1973/5
All Snails
Rediae
Infected snails
3·0
2·5
2·0
1·5
1·0
0·5
0 0·9
1·0 1·9
2·0 2·9
3·0 3·9
4·0 4·9
5·0 5·9
6·0 6·9
7·0 7·9
8·0 10·9
Snail Size Class mm

infected snails and second, that the bulk of the redial population was contained within a few large snails.

In order to examine the significance of these findings the pooled infection data were adjusted as described in 'Materials and Methods' so as to represent a constant effort search. Taking each farm in turn, the number of infected snails, the mean size of infected snails and the total number of rediae found are presented in Figures 4.31 and 4.32).

At Hendy, the highest numbers of rediae and infected snails were found in 1973. There followed a decline in the size of both these parameters during 1974. Though the number of infected snails remained at a low level for the rest of the time there was a small general increase in both the size of the infected snails and the number of rediae found in the first half of 1975. At Thorneythwaite, the number of infections remained at the same low level throughout the three years of observation apart from an exceptionally high number of infected snails (and rediae) in September 1973 and a four-month gap between November 1974 and March 1975 when no infected snails were found at all. There was a general increase in the mean size of the infected snails between 1974 and 1975. This was accompanied by a similar increase in the total number of rediae found.

Examination of Figures 4.31 and 4.32 indicated that the number of rediae was related to the total biomass of the infected snails. The dry weight biomass of an individual snail is proportional to the square of its shell length (Denison and Nice, pers.comm. as

Fig 4·31

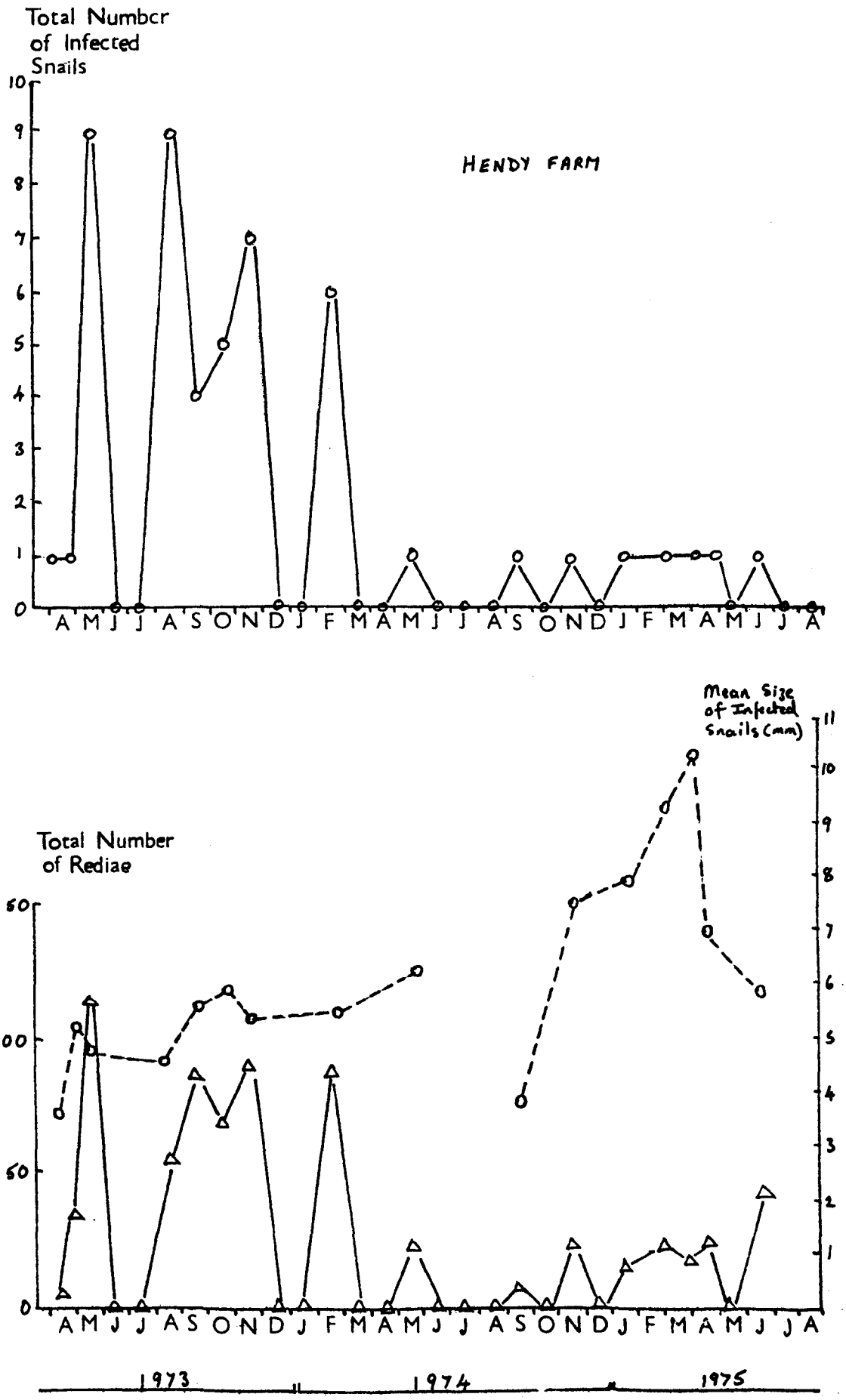
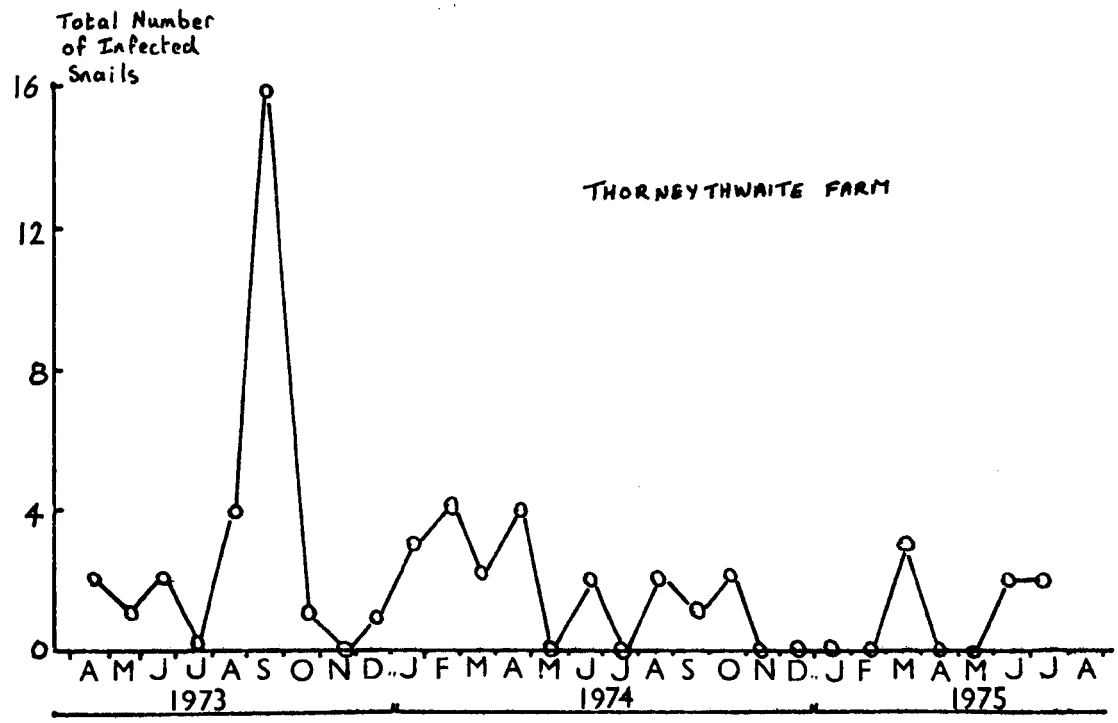
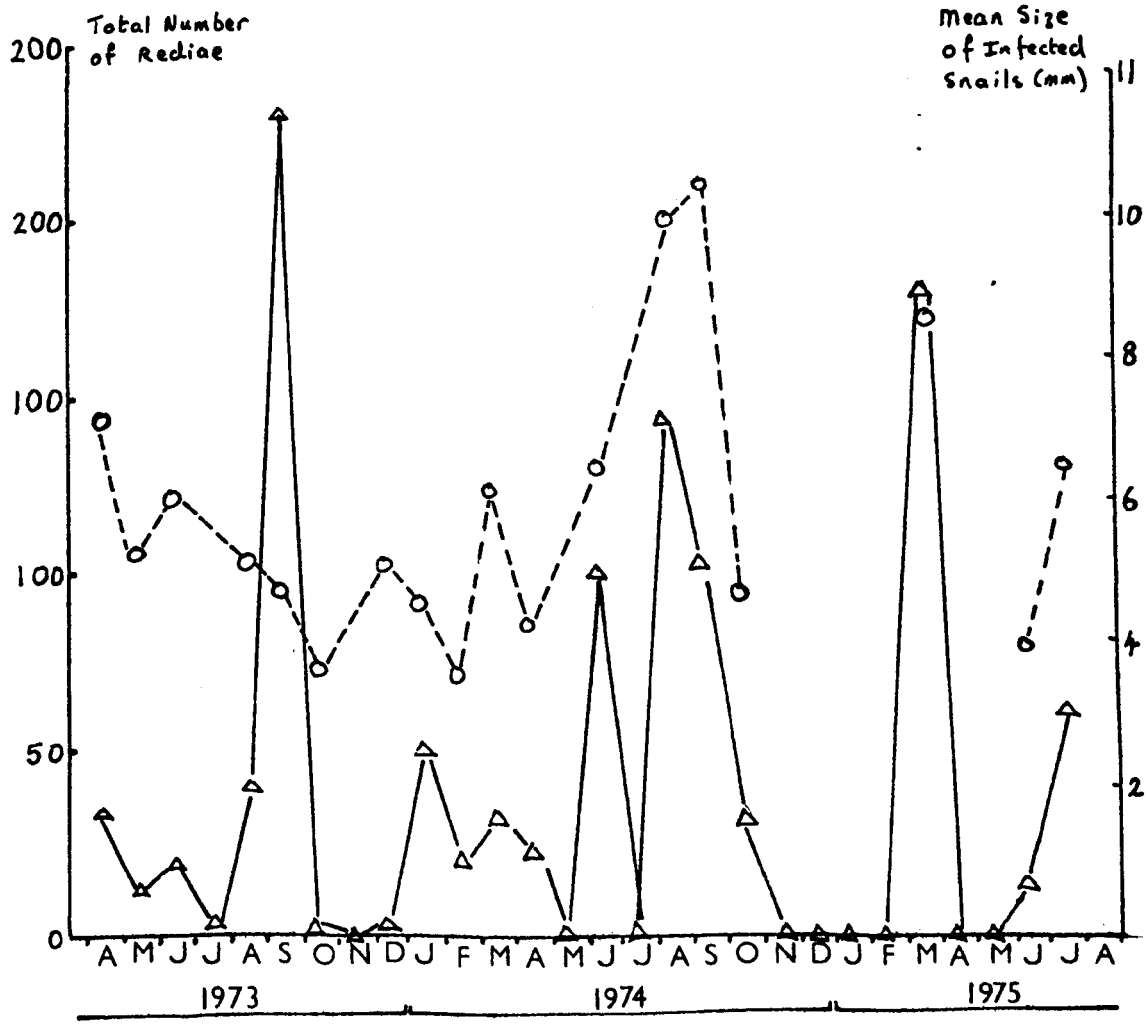

Total Number of Infected Snails
HENDY FARM
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A
Total Number of Rediae
Mean Size of Infected Snails (mm)
1973
1974
1975

Fig 4.32


Total Number of Infected Snails
16
12
8
4
0
THORNEYTHWAITE FARM
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A
1973
1974
1975


Total Number of Rediae
Mean Size of Infected Snails (mm)
200
200
100
100
50
0
11
10
8
6
4
2
A M J J A S O N D J F M A M J J A S O N D J F M A M J J A
1973
1974
1975

reported earlier). The simplest model was therefore:

$$r \propto n\, l^2 \qquad (1)$$

Where r = total number of rediae

n = total number of infected L. truncatula

l = mean shell length (mm)

For each farm the values of $n\, l^2$ were estimated from the pooled data and plotted against the corresponding values of r. Casual inspection suggested a linear relationship and the linear regression equation was derived for each set of results. The regression was highly significant on both farms (Hendy, $p < 0.005$, d.f. = 14; Thorneythwaite, $p < 0.005$ d.f. = 16). An analysis of covariance (Snedecar and Cochran, 1967, p.433) revealed that there was no significant difference between the two regressions. Accordingly the data were pooled and the slope and elevation recalculated (Figure 4.33). The regression was highly significant ($p < 0.005$, d.f. = 32). The equation for the regression line was of the form:

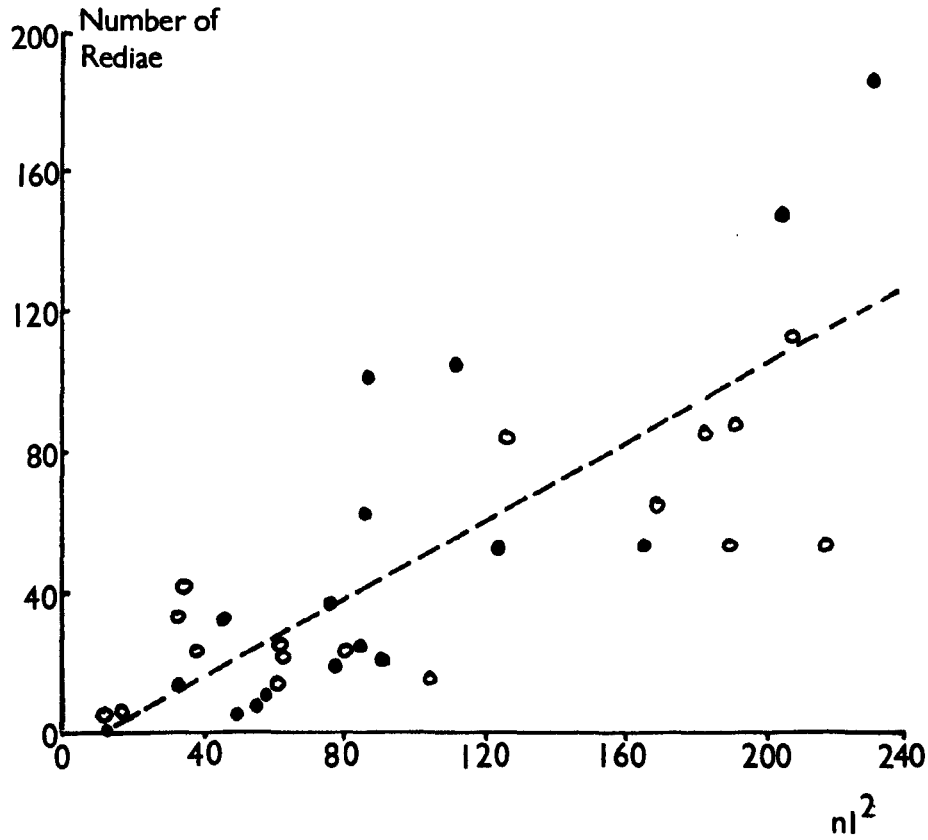
$$r = 0.56\, n\, l^2 - 5.09 \qquad (2)$$

slope = 0.56 ± 0.19 (95% confidence limits)

elevation= 5.09 ± 12.76 (95% confidence limits)

The regression line crosses the x axis at 9.09 which accords reasonably well with the expected intercept of 6.25 (the smallest snails in which redia were found were in the 2.00-2.99mm size-class; $2.5^2 = 6.25$).

Fig. 4·33



o Hendy

• Thorneythwaite

5. DISCUSSION

(i) Sampling Methods

The various methods of estimating the densities of mollusc populations have been reviewed and evaluated mainly with respect to the snail vectors of bilharziasis (Hairston et al., 1958; also W.H.O. technical report No. 120, p. 34 et seq.). Only Morphy and Ross (1970) appear to have compared and contrasted most of the major techniques available for the special case of L. truncatula. These include visual search - ranging freely over the habitat with or without a time limit (Roberts, 1950; Bednarz, 1960; Sossipatrov and Shumakovich, 1966; Crossland et al., 1967; Ollerenshaw, 1971; Bruce et al., 1973) or else within randomly placed quadrats (Heppleston, 1972) - nearest neighbour techniques (Keuls et al., 1963) and soil sampling (Ross and O'Hagan, 1968; Over and Damen Van Hapert, 1967).

Heppleston (1972) has shown that an increasingly significant proportion of the snail population is to be found under the mud surface as the mean air temperature declines. For this reason soil sampling is likely to be the most accurate of all the methods particularly if used in conjunction with an effective extraction technique (Morphy and Ross, loc.cit). However, the size of each sample is necessarily small and so the total number of samples required each time is correspondingly great. This results in the destruction of much of the habitat being sampled. In the present study, the time spent processing each sample in the laboratory proved to be prohibitive, an experience common to other workers in the field (Ollerenshaw,

pers. comm.), and so visual search methods were used instead. The subjectivity of these techniques is well known. As the sampling proceded at Hendy and Thorneythwaite it became plain that a successful search was contingent upon the activity of the snails (and thus upon the ambient temperature), the density of the herbage, whether or not it was raining and the experience and disposition of the searcher. More subtly, the "knowledge" that a collector believes he possesses concerning a habitat may in fact restrict his search. Too much time may be spent on those areas which had previously proved productive even when no snails are found and a systematic search would probably be more fruitful. This was not a problem, of course, when quadrats were searched. The minute inspection of small areas of habitat required by this method resulted in the collection of a greater proportion of immature snails than was revealed by the ten minute counts, which missed up to 10% of the smaller size classes of snail. Heppleston (1972) obtained exactly the same result in his study of L. truncatula in the Orkneys. Both the density of the snail population and its size-class structure were more accurately estimated from quadrat collections but the ten minute count method provides many more snails for considerably less effort and is particularly useful when so few snails are infected with the parasite of interest. However, there is one disadvantage of ten minute counts that has not yet been considered. It is obvious that there is a maximum number of snails that can be collected in ten minutes (probably not more than 100) and so variations in the relative

densities of snails above a certain limit cannot be distinguished by this method. More importantly as snail densities increase the amount of time spent handling each snail becomes significant (See Holling, 1965, for a discussion of handling time in predator-prey systems). In other words the relationship between the actual density of snails and the number collected is different depending upon whether there are many or a few specimens present.

(ii) Variations in the Populations of L. truncatula and F. hepatica at Hendy and Thorneythwaite Compared with the National Trends

During the 30 months which span the interval between April 1973 and September 1975 the populations of L. truncatula and F. hepatica at Hendy and Thorneythwaite underwent a progressive decline. It is illuminating to compare the events at these farms with the more general trends in Wales and Cumbria over the same period as described by Ollerenshaw (Monthly Snail Reports and Fluke Forecasts, 1973-75, M.A.F.F.) Figures 4.34 and 4.35 represent the variations in mean density of over 90 snail populations in Wales during the summer months of 1973, 1974 and 1975. Figure 4.36 shows the number of snails containing immature infections of F. hepatica over the same period. There is a decrease in numbers from one year to the next in all three figures, the decline experienced between 1974 and 1975 being the most severe. In 1974 the spring reduction in overall snail density occurred in June, a month earlier than in either 1973 or 1975. Ollerenshaw attributed this to below average rainfall in March, April and May 1973. The number of snails containing immature

Fig. 4·34

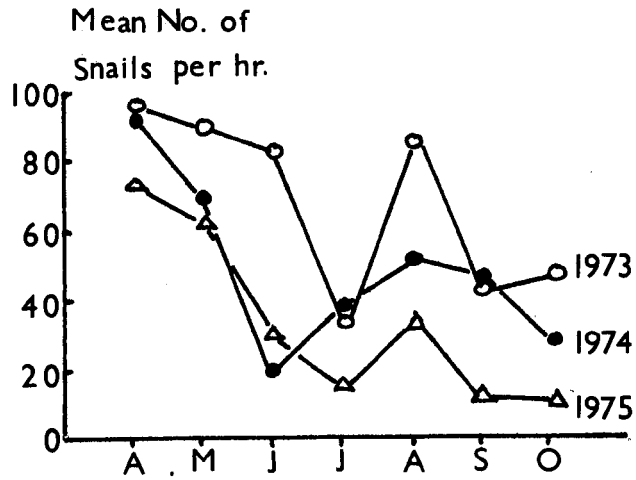


Fig. 4·35

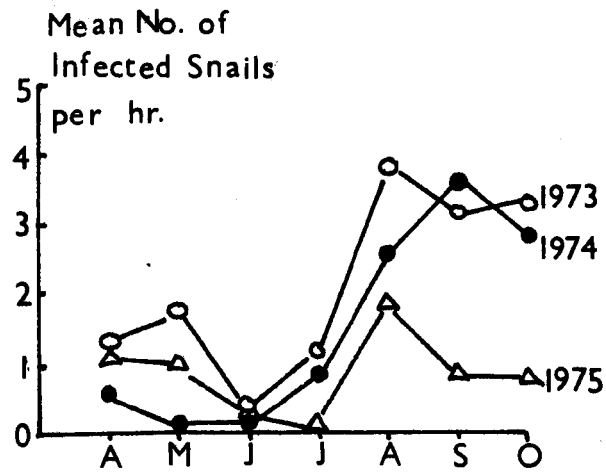
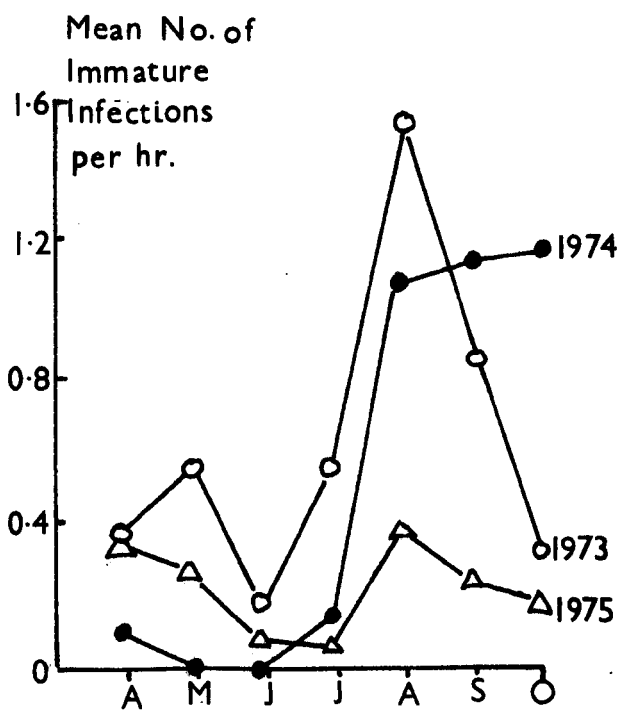


Fig. 4·36



Data from the 'Monthly Snail Counts' (Wales), Ollerenshaw 1973/5

infections increased between 3and 4 fold in August 1973 and August 1974. The infection rate in August 1975 barely rose above the levels of the previous winter. The snail collections from Cumbria were less regular and have not been figured here. Nevertheless, the results were broadly similar to those already described except that the major increase in infection rate occurred in September rather than August.

In nearly all essentials Ollerenshaw's account of the general population trends in Wales and Cumbria applies also to the populations of snails and flukes on Hendy and Thorneythwaite. The constant effort summary clearly showed that a general reduction in the density of L. truncatula occurred in each successive year. There was also a concomitant increase in the mean size of the snails. At Hendy, the number of infected snails underwent a considerable decline in the Spring of 1974, never to recover, whereas at Thorneythwaite the number of infected snails changed little over the three seasons. Nevertheless on both farms the greatest number of infected snails was found in August and September 1973 just as Ollerenshaw described for his Welsh and Cumbrian populations respectively. Ollerenshaw mentioned that in 1973 and 1975 most of his habitats had dried out by July whereas in 1974 an early spring drought accelerated the process and most habitats were dry by April. On both Hendy and Thorneythwaite empty shells were found mainly in June or July of each year except in 1974 when they were found in March, April or May. The mortality rate was greatest in spring 1975.

When particular habitats were considered separately it was found that site D, Hendy, largely conformed to the general pattern described above whereas site B, Thorneythwaite, did not. The difference was associated with differences in the microclimate of each habitat.

(iii) The Importance of Habitat Microclimate

From the outset in this thesis it has been assumed that soil-surface moisture and soil surface temperature were the most significant features of the microclimate of the snail habitats. The argument was developed in Chapters 1 and 3 and the relevant papers have already been listed in the introduction to the present chapter. Accordingly the main points will be merely summarised here.

Soil-surface moisture is a permissive factor. The length of time for which favourable moisture conditions persist determines the period that the snails remain active, feed, grow and reproduce. As the surface layers of the soil dry out the snails aestivate, growth and oviposition cease. A prolonged or severe drought causes the death of the snail eggs and is eventually responsible for the mortality of the snails themselves. When an infected snail aestivates the effect upon the parasites is the same as if the snail is starved. The rate of development of the rediae is less and the actual number of rediae and cercariae found in each snail is much reduced (Kendall, 1949a, 1949b; Chowaniec and Drozdz, 1959; Styczynska-Jurewicz, 1965).

When soil surface moisture is not limiting it is the temperature of the soil surface which controls the rate at which the parasites and host snails grow and develop. L. truncatula remains active at temperatures as low as 1°C (Kendall and McCullough, 1951). At Thorneythwaite, for example, L. truncatula was observed crawling along the undersurface of the ice sheets which covered the habitat. Snail growth and oviposition continues until temperatures fall below about 5°C (Roberts, 1950; Kendall, 1953; Nice and Thomas, pers. comm.) The actual rate of oviposition appears to be related to the ambient temperature (Appendix V). The development of snail eggs continues at least until temperatures fall to 10°C (Roberts 1950) but there have been no investigations below this temperature. The growth of the redia within the snails has been measured at various temperatures by Nice and Wilson (1974) and Ollerenshaw (1974). The development of the intramolluscan stages of F. hepatica is inhibited below 10°C but Wilson and Draskau (1976) have shown that short exposures to temperatures below this stimulate the production of daughter redia when growth is resumed.

During drought conditions the importance of soil-surface temperature lies in its relationship with evaporation rates. Kendall (1949a) was able to maintain L. truncatula under drought conditions for several months at temperatures of 12-17°C (provided the shells were not dislodged from the substratum and the apertures exposed to the air) whereas Roberts (1950) who repeated the experiment at higher temperatures (up to 25°C) found that survival was measured in days

rather than months. The drought-related mortality of the snails need not necessarily be a matter of simple dessication. It may be that if aestivation is sufficiently prolonged L. truncatula eventually dies of starvation. This happens in the case of Helicella virgata for example (Andrewartha, 1970). Since there is an inverse logarithmic relationship between metabolic rate and body size in poikilotherms (Prosser and Brown, 1961) it is reasonable to suppose that when drought conditions are prolonged the immature snails will incur a proportionately higher mortality than the mature snails. Indeed, in Kendall's (1949a) experiments the mature snails survived about five times longer than newly hatched specimens. It is certainly the case that the extra metabolic demand made upon an aestivating snail by its burden of parasites shortens the length of time that it can withstand drought conditions. Styczynska-Jurewicz (1965), for example, found that the mortality rate of a population of infected snails that had been aestivating for two months was twice that of an uninfected control group.

The drought induced mortality of a population of snails is likely to depend upon the proportion of the habitat that is dry and the intensity and duration of the dry spell. The relationship will not be a simple one because of the overdispersed distribution of the snails and the behavioural mechanisms the species has evolved to counter dessication. At any rate the mortality incurred will be independent of the density of snails or egg mass but not necessarily of the size-class structure of the population particularly when the

drought is prolonged. The drought induced mortality of infected snails is in addition likely to depend upon the intensity of the infection.

(iv) The Snail Populations on Site D, Hendy and Site B, Thorneythwaite

Favourable soil-surface moisture conditions persisted in at least some parts of site B until June 1975. The dessicatory effect of the early spring drought in 1974 was not nearly so damaging to the moisture conditions on this habitat as it was on site D where the whole habitat was rated as "damp" or "dry" for at least six weeks. It has been shown that the temperatures at the soil surface of the habitats during the drier periods were greater than the air temperatures measured at screen height and it was also argued that the drier the ground the warmer its surface was likely to be. In consequence the surface temperatures during the drier spring periods at Thorneythwaite on site B were not only cooler due to the difference in latitude and height above sea level than at site D but proportionately cooler due to the higher moisture content of the soil. As a result the snails were much less likely to suffer dessication at this site particularly since optimum moisture conditions were usually quickly restored over most of the area of the habitat after three or four weeks.

Even so the drier periods on both habitats were associated with an increase in the proportion of empty shells that were found. The spring decrease in population density is a characteristic of L. truncatula populations (see, for example Ollerenshaw, 1971) and, though an increase in search difficulty is partly responsible, the phenomenon also occurs when a soil-core sampling method is used as an adjunct

to normal visual search techniques (Sosiptrov and Shumakovitch, 1966). It is probable that there was a real decline in snail densities in the late winter and early spring of each year due firstly to the senescence of the few remaining snails of the parental generation of the previous year (Heppleston, (1972) estimated a maximum life span of 9-10 months for L. truncatula) and secondly to the dessication of the daughter snails that had overwintered. During this same period the daughter snails that survived grew slowly to maturity. Estimates of the shell length at which L. truncatula attains maturity vary between 3.0-4.5mm (Appendix V) but by June most of the snails were over 3.0mm in length.

Allowing at least three weeks between oviposition and hatching under field temperatures (Roberts, 1950) it was possible to estimate approximate oviposition dates from the date at which the percentage of immature snails began to increase. Egg laying on site D, Hendy, began during late July in 1973 (68% "wet", 11% "standing water") and late August in 1974 (100% "wet"). There was no increase at all in the percentage of immature snails during 1975. On site B, Thorneythwaite, egg laying began in late July, 1973 (8% "wet", 88% "standing water") early August, 1974 (4% "wet", 96% "standing water") and possibly early June, 1975 (100% "dry") Oviposition in 1974 was not delayed for so long as on the Welsh site and the subsequent increase in the proportion of immature snails was exactly comparable to that in 1973. Bruce et al. (1973) stated that in Ayrshire the pattern for L. truncatula populations was "clearly two generations per year"

and Heppleston (1972) reported that there were two generations on the habitats he examined but only in a year when the autumn was exceptionally warm. Inspection of the size class structure of the population of snails from site B, Thorneythwaite, in October 1973 revealed a preponderance of immature snails which might have hatched from egg masses laid by daughter snails in August of that year but otherwise there is no evidence at all that there was other than one generation of snails per year on either site.

The populations of L. truncatula on both sides were extremely aggregated requiring the application of a logarithmic transform to the sampling distribution before further analysis. Few habitats are uniformly suitable but the effect of habitat heterogeneity was enhanced by the behaviour of the snails. L. truncatula lays its eggs in a single mass consisting of up to 19 eggs (Walton and Jones, 1926) Laboratory evidence suggested that egg masses tend to be laid in situations where the light intensity is low (Appendix V) and it was a matter of field experience that the egg masses were usually found in small clumps. The rate of dispersal of newly hatched snails from these egg masses is probably rather small. Heppleston (1972) found that the snails exhibited marked site fidelity even when conditions were optimal over large areas. Ultimately the dispersal of the snails will be limited by the distribution of areas of suitable soil-surface moisture within each habitat. As the habitat dries out the number of favourable spots will decrease and it is reasonable to assume that the distribution of the snails will vary accordingly.

However, the situation is not entirely straightforward. The change in size of a habitat under varying weather conditions should not be thought of as an extension or retraction of its outer limits for unless the circumstances are exceptional, these are defined by features of drainage and topography which remain static, rather the habitat becomes more or less patchy. The visual estimates of soil surface moisture were made on an areal basis. Only that moisture condition which prevailed over most of the block was recorded. In consequence, very small areas which might have acted as refuges for the snails were largely ignored. There were several occasions on deeply poached habitats when an overall estimate of "dry" concealed the fact that at the bottom of a few of the deepest poach holes there were several centimetres of standing water. Even so it was not ~~usual~~ unusual to find inactive L. truncatula withdrawn into their shells around the rim of such refuges. It appeared that the snails did not follow a receding water line. (A similar observation was made by Walton, (1918)). Kendall (1949a) has shown that aestivating snails can survive for several months under very dry conditions provided that the shell apertures are kept closely pressed to the substratum. Exposure quickly resulted in dessication. A behavioural mechanism which required the snail to remain active and exposed until a suitable refuge was reached (like, for example, the orthokinetic response to a moisture gradient that is shown by Parcellio scaber - see Hinde, 1966) may have less survival value under extreme conditions than aestivation.

(v) The Mortality Rate of Infected Snails

Redial densities at both site D, Hendy, and site B, Thorneythwaite, were at a maximum in the summer months of 1973. The parasites infected 13% of the snail population at site D in August and 16% of the population at site B in September. In the months immediately following the proportion of the snail population that was infected fell to 5% at site D and 2% on site B. The summer maxima on both sides occurred before the majority of snail egg masses began to hatch and so all of the infected snails belonged to the mature parent generation that had overwintered from 1972. As the egg masses hatched there was extensive recruitment to the immature size classes of snail and a progressive dilution of the infected population. When the numbers of infected snails were expressed as proportions of the total numbers of snails in excess of 3.00mm in length the proportional decline from one month to the next was rather less. (16% to 12% site D; 21% to 7% site B). The residual difference was due to the actual mortality of the infected snails. In the interval between one sampling session and the next (i.e. Aug Sept., site D; Sept Oct., site B) the actual density of infected snails fell to about one tenth of its maximum value on each of the sites. Assuming that no new infections occured during the month that elapsed then approximately 90% of the infected snails perished together with their burden of rediae. For the sake of comparison, the approximate mortality rate of the uninfected snails over the same period was estimated by the method of Pesigan et al. (1958). It was assumed that all the immature snails

recorded on the first sampling date would have grown to maturity in the interval to the next sampling date. (A not unreasonable assumption since all the immatures were in the 2.00–2.99mm size class and the weekly mean soil temperatures were in excess of 16°C at Hendy and 14°C at Thorneythwaite). The number of mature snails actually found on the second sampling date was subtracted from the number of mature snails expected and the difference expressed as the percentage mortality. The mortality estimated for uninfected snails at site B was 100% and the mortality estimated for the uninfected snails at site D was 33%. (This compares with a mortality rate of 68% recalculated from data provided by Heppleston (1972) who used the same method for populations of L. truncatula in the Orkneys). Clearly then the mortality of infected and uninfected snails on site B was more or less the same but the mortality of infected snails on site D was much greater than the mortality of uninfected snails. The reason for the difference is unknown. Infected snails are particularly prone to die during prolonged aestivation (Styczynska-Jurewicz, 1965) but the soil moisture conditions precluded any possibility of desiccation. The density and total biomass of the snails on site D during August and September 1973 were at nearly the highest levels recorded. It may be that food had become limiting and the infected snails were at a competitive disadvantage.

The extensive parasitic invasion of the snail populations that was observed between July and September, 1973, was not repeated in the subsequent years of the study. The moisture conditions on the

habitats were quite exceptional during this particular summer. At site D, in particular, even at the height of the spring dry period, more than a third of the habitat was rated as "wet" a circumstance that did not occur again in either 1974 or 1975. It is probable that the mortality of the fluke eggs was lower in 1973 than 1974 or 1975 and that proportionately fewer snails were aestivating so increasing the chances that individual hosts would become infected.

(vi) Snail Biomass and Redial Density

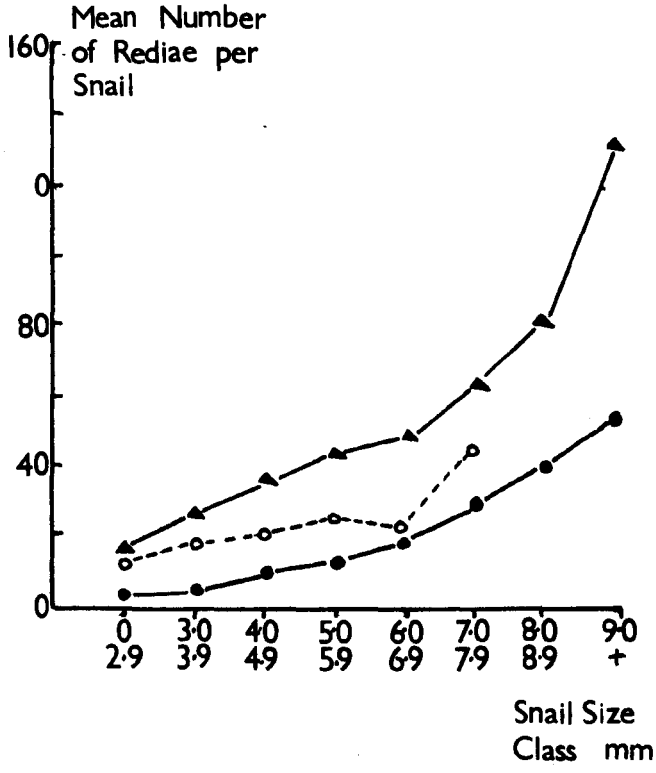
If parasites are to be randomly distributed among the hosts then every parasite must have the same probability of infecting a host and each host must have the same probability of being infected. Aggregated distributions are likely to occur whenever there is heterogeneity in the probability of occurrence of parasite and host. (Kennedy, 1975). The populations of L. truncatula studied at Hendy and Thorneythwaite all exhibited overdispersed distributions and since batches of fluke eggs entered the habitats contained in discrete faecal pats then the probability of occurrence of the miracidia was also certainly heterogeneous. The very variable soil surface moisture conditions that prevailed on the sites in addition to the tendency of the snails to be aggregated in poach holes further diversified the probability of individual snails becoming infected. It was to be expected then that even within snails of a particular age group the level of parasite infection would be

different in different individuals.

The older a host is the longer it has had to make contact with a parasite and the likelihood is that the older the host the longer the parasite has been developing within it. So it was not surprising to find that the distribution of rediae within the snails was complicated further by the relationship between the average number of rediae per snail and the size of the snail. The nature of the relationship was first suggested by Kendall (1949) and then convincingly demonstrated in field populations from Anglesey by Ollerenshaw (1959). The results reported here confirmed that the number and maturity of the rediae found in an infected snail in the field varies with the biomass of the snail but the actual figures differed considerably from those obtained by Ollerenshaw (1959). The two sets of results are compared in Figure 4.37. The average number of rediae per snail recorded by Ollerenshaw was generally more than twice that reported for Hendy and Thorneythwaite. Ollerenshaw collected his snails during the summer of 1958, when the incidence of fascioliasis was the highest this century. All the results obtained on redial abundance at Hendy and Thorneythwaite refer to a succession of years when the national incidence of fascioliasis was below average.

It is interesting to consider the mechanism by which the average redial density was increased in the epidemic year of 1958 and also the consequences in terms of cercarial output. Wilson and Draskau (1976) found that in snails maintained at 20°C the rediae emerge

Fig. 4·37



Anglesey 1958 Ollerenshaw's data
▲ Mature Infection
o Immature Infection

Thorneythwaite and Hendy 1973/5
● All Stages

from the sporocysts from 11 days post infection. Roberts (1950) reports finding rediae only 7 days p.i. at 25°C and 15 days p.i. at 16-19°C. The rate at which the rediae emerge from the sporocysts is thus related to temperature. The redial burden of infected snails would therefore increase more rapidly at elevated temperatures.

Ollerenshaw has provided no information concerning the temperatures at the habitats on Anglesey but the year was one of the wettest on record and the increase in soil moisture alone would tend to reduce temperatures at the soil surface (see Chapter 3) even if the cooling effect of direct precipitation is discounted. The infections found at Hendy and Thorneythwaite probably represent encounters with a single miracidium but it may be that the high levels of redial density noted by Ollerenshaw on Anglesey were the result of multiple infections.[1] However, multiple infections do not necessarily result in a proportional increase in cercarial output. Kendall (1949b) showed that multiple infections can increase the redial burden fourfold but also result in a reduction in the number of mature cercariae per redia. Nice (pers.comm.) has further demonstrated that in snails infected with more than two miracidia there is no concomitant increase in the cercarial output. In this case the density of cysts on the herbage would depend mainly upon

[1] There appears to be no resistance to multiple infection by F.hepatica in L. truncatula. (Kendall, 1965).

the density and size of the infected snails. The densities of the snail populations at Anglesey were certainly high and the very considerable herbage infestations that were inferred from the incidence data probably owe more to the high proportion of snails that were infected (16%)[2] than to the intensity of the intermediate host infection.

The question arose whether the distribution of rediae within the snail populations was markedly different in epidemic years. The overall distribution of rediae within snail populations is most conveniently shown by means of a cumulative percentage frequency plot of rediae and snails. The data for Hendy and Thorneythwaite were obtained by pooling all the infection records and the data for Anglesey were obtained from the frequency distribution diagrams presented by Ollerenshaw (1971a). The curves corresponding to the epidemic and below average years are compared in Figure 4.38. There were no major differences in the way that the rediae were distributed amongst the snails. In both cases about 25% of the whole population of snails (comprising size classes 5.00mm and above) accounted for 80% of the rediae.

There remained one further consideration. It has been pointed out earlier in this chapter that the mean size of infected snails was generally greater than the mean size of the snails in the population as a whole. This has been interpreted as a reflection

[2]This compares with the much smaller overall infection rate of 5% recorded at Hendy and Thorneythwaite between 1973 and 1975.

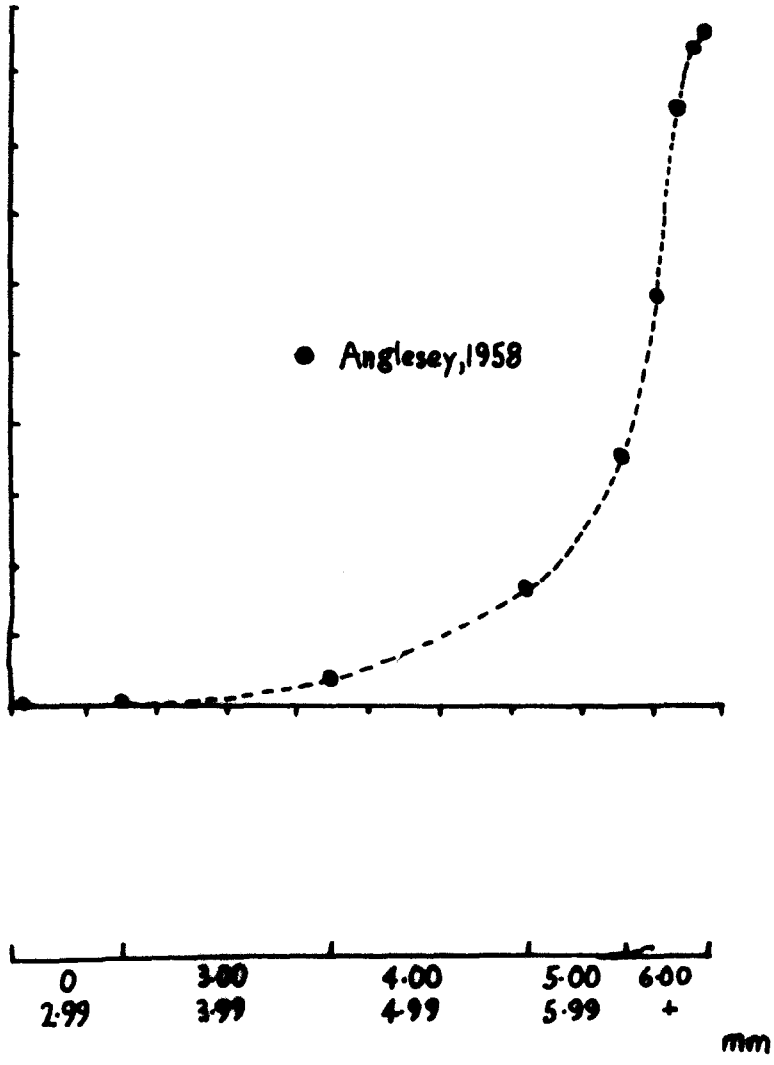
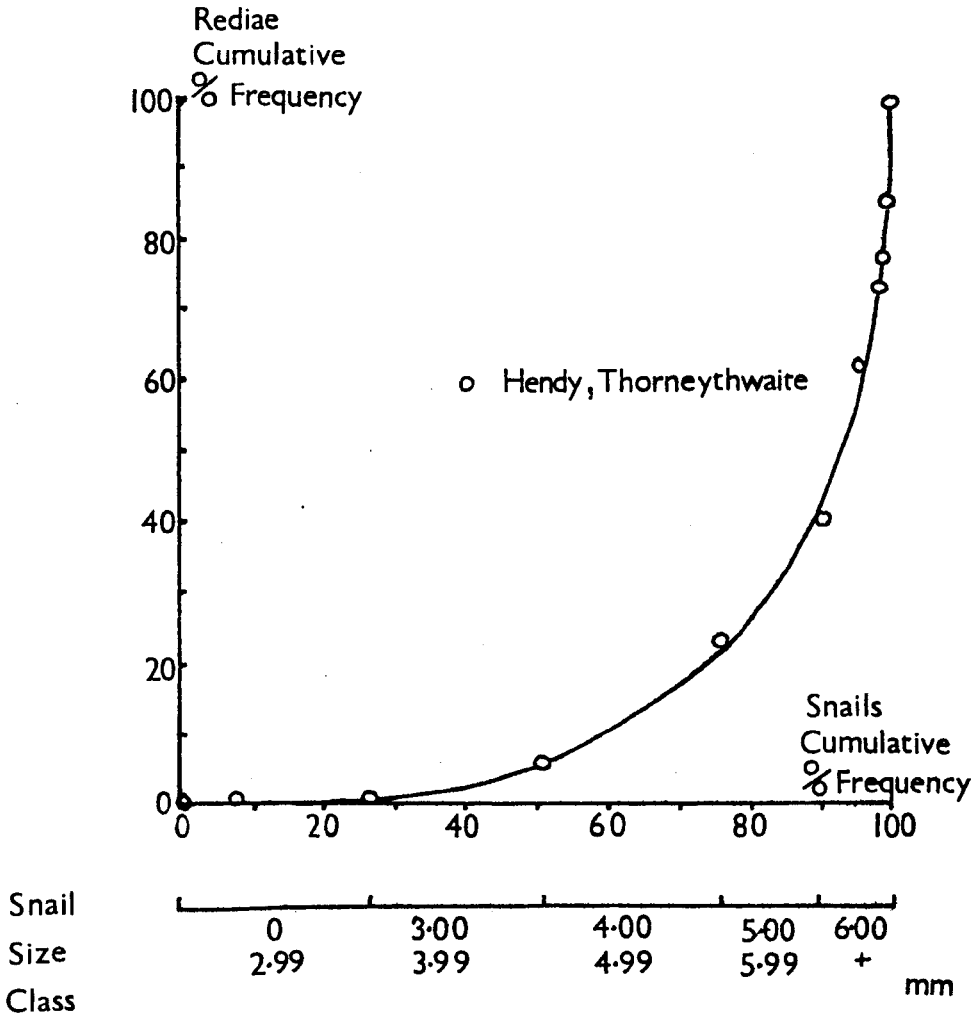

Anglesey, 1958
0
2·99
3·00
3·99
4·00
4·99
5·00
5·99
6·00
+
mm

Fig. 4·38


Rediae
Cumulative
% Frequency
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80
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0
o Hendy, Thorneythwaite
Snails
Cumulative
% Frequency
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40
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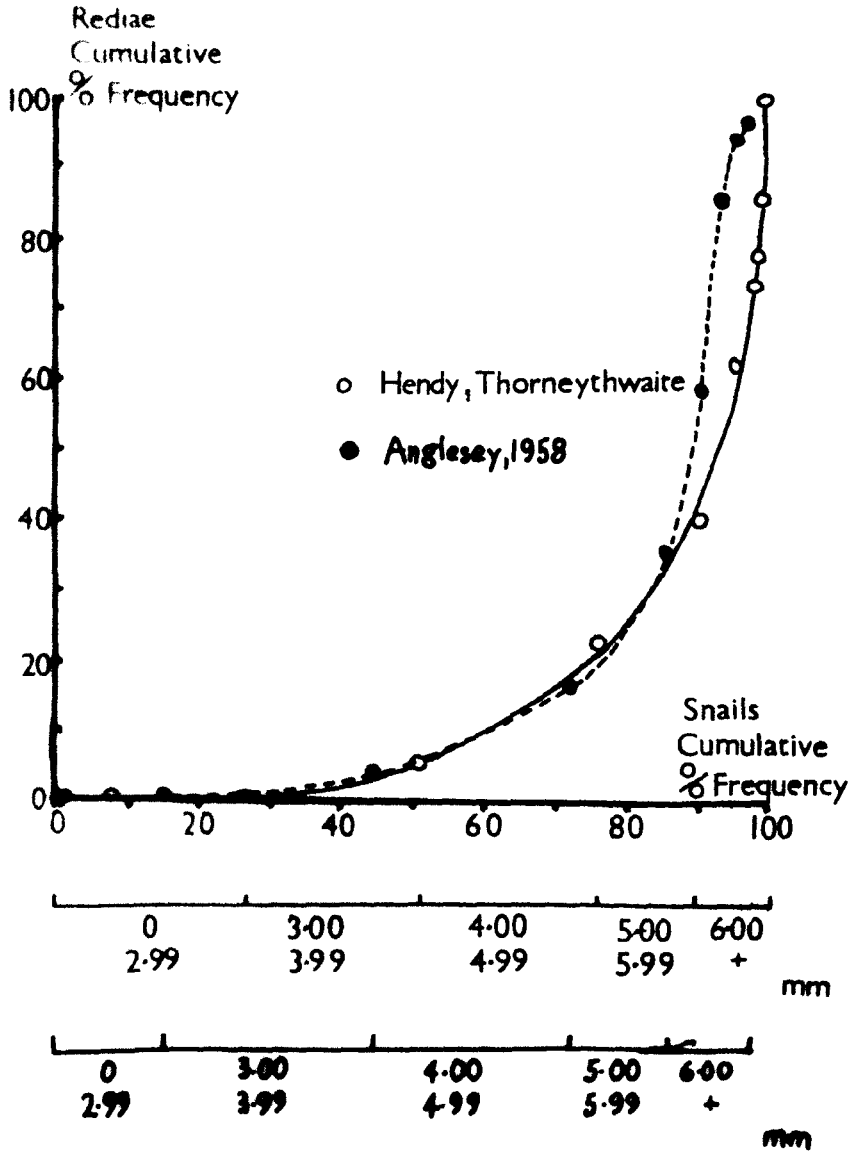
Fig. 4·38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o Hendy, Thorneythwaite
● Anglesey, 1958
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of the fact that the older a host is the longer it has had to make contact with a parasite. However, this interpretation is complicated by the effect of the parasite itself upon the growth of the snail. Cheng (1971) found that for various mollusc species one of the consequences of parasite infection was an enhanced growth rate. This certainly occurs in the case of L. truncatula. Infected specimens have been found to grow faster than those in an uninfected control group (Nice, pers. comm.). In consequence, the purely probabalistic ~~interpretion~~ interpretation of redial distribution within particular size classes of snail is probably overly simplistic.

CHAPTER 5. THE OCCURRENCE OF METACERCARIAE ON THE SNAIL HABITATS

1. INTRODUCTION

(i) The Fluke Cysts

As the *Fasciola* infection within the intermediate snail host matures it passes through two larval stages, the redia and the cercaria. The transfer of the parasite from the intermediate to the primary host is accomplished by the metacercariae. These are the encysted form of the cercariae which are ingested by the grazing livestock. Those parasites whose larval maturation, from the invasion of the tissue of the snail to release onto the pasture, is completed entirely during the summer months give rise to the "summer" infection of the primary host. Those parasites whose larvae overwintered in the snail, completing their development in the following spring, give rise to the "winter" infection of the primary hosts. (Ollerenshaw and Rowlands, 1959).

(ii) Indirect Measures of the Abundance of Fluke Cysts on the Pasture

The relative abundance of metacercariae on the pasture can be inferred from the variations in the number of sheep and cattle that are infected by *F. hepatica*

Losses from acute fascioliasis attributable to a summer infection generally start in October or November, rise to a maximum in December and January, and rapidly decline to a low level by the following June. The number of diagnosed cases of chronic fascioliasis begins to increase in October but reaches its peak in March or April before declining to minimal levels in June. (Ollerenshaw, 1971c). When a winter infection of significant proportions does occur its effect is to bring

forward to August the date at which losses first occur. Averaged estimates of disease incidence obtained from lamb liver condemnation records in the Netherlands (Honer and Vink, 1963a) and variations in the number of fluke eggs found in the faeces of cattle from Wales and the north of England (Ollerenshaw 1971d, Sloan, 1971) conform in general to the pattern already described for chronic fascioliasis. The maximum levels in all cases occurring in March or April.

In the laboratory sheep begin to die about 6 weeks after the administration of large numbers of metacercariae (Ollerenshaw, 1971c) and eggs are first detected in the faeces after about 9 weeks (Happich and Boray, 1969b). Since the number of cases of acute fascioliasis increases from about October or November it would seem that the fluke cysts first appear on the pastures in large numbers sometime in August of each year. After this there will be continual recruitment to the numbers of metacercariae until the mean temperature falls below $9^{o}C$, when the emergence of cercariae ceases (Kendall and McCollough 1951). This is normally in September or October.

(iii) Direct Measures of the Abundance of Fluke Cysts on the Pasture

The variations in metacercarial density that are implied by the pattern of disease incidence just described can be more directly measured. Three methods have been used. The first is a direct mechanical and chemical method of recovering and counting the metacercariae contained in samples of herbage taken from infected pastures. The second and third exploit the principle of bioassay.

(a) The Sieve Method (Tech. Bull. No. 18, p.33; Min. Agr. Fish and Food)

A herbage sample is finely shredded and sieved. Those remaining fragments of grass which still obscure the cysts are oxidised with concentrated sulphuric (VI) acid and the residue sieved once more. A recovery rate of about 90% is suggested but this varies with the skill of the operator and the method is time consuming and laborious. The samples that can be processed in this way are necessarily small and a large number is required since there is evidence to suggest that even in an area in which the infected snails are evenly dispersed the distribution of cysts is far from uniform. (Ollerenshaw, 1971c). Indeed, in previous studies most samples have contained no cysts at all. (Ollerenshaw, pers.comm.)

(b) Tracer Sheep

This well established method is used to assess the risk of infection to which a primary host is exposed,(i.e. Olsen, 1947; Ross, 1967a; Ross, 1967b; Crossland et al., 1969; Armour et al., 1970; Hope-Cawdery and Moran, 1971). It provides an estimate of the number of viable, infective metacercariae present on the pasture which are available and which were ingested at a specific stock grazing density over a given period of time. A number of fluke-free sheep are exposed to flukey pasture for a fixed period; they may be free ranging or tethered. The sheep are then removed from the pasture, maintained on fluke-free feed (usually indoors) for a minimum of four weeks and then slaughtered. The livers are removed and examined for flukes.

The method requires much less laboratory time than direct counting procedures, and the metacercariae can be sampled over a long period by an appropriate primary host in a way which conforms to the natural situation. However, large numbers of sheep are required to take account of individual variations in susceptibility to fluke, and grazing behaviour. For example, Hope-Cawdery and Moran (1971) found that there were differences in the number of flukes picked up by free ranging wethers and ewes on the same pasture and attributed this to behaviour differences in the sheep. In addition Armour et al. (1970) concluded that free running tracers do not necessarily mimic the grazing of the permanent flock. Initially, at least, they graze apart from the main flock and their feeding preferences are in part determined by the quality of pasture and the nature of the foodstuffs upon which they were maintained prior to exposure.

(c) Laboratory models

Ross and O'Hagan (1966) list several laboratory animals which in the past have been used as models of the primary host in the field. They include mice, rabbits and guinea pigs. In the same paper, the authors describe a technique whereby the density of viable, infective metacercariae can be estimated. The method involves feeding herbage samples to guinea pigs and assessing the extent of the infection six weeks later at post mortem. The grass samples are taken at random from within an identified snail habitat and bulked before feeding to the animals. Only those metacercariae which are both alive

and infective are detected by this method, the total number of cysts in each sample being estimated by the application of a calibration coefficient.

The procedure may be criticised on several grounds. The percentage of ingested cysts that become established as adult flukes in the liver is termed the 'percentage take'. This depends first upon the susceptibility of the laboratory model to infection - which may not be the same as that of the primary host in the field - and second upon the percentage of viable infective cysts that are present in the ingested sample. To estimate the actual number of cysts in the sample by means of a calibration coefficient, as did Ross and O'Hagan (1966) involves the assumption that not only is the proportion of infective cysts found in laboratory preparations the same as that found in the field but that the field value does not change. This last assumption, at any rate, has been undermined by Ollerenshaw's work on the decline in viability and infectivity of groups of metacercariae which were encysted in turf boxes (Ollerenshaw 1971c). Finally, all the laboratory models are smaller than the normal primary hosts and so the size of herbage sample that can be conveniently eaten by each animal is relatively small.

(iv) Variations in the Abundance of Fluke Cysts on the Pasture

Three separate studies in particular have measured the variations of metacercarial abundance on infected pastures. Each was carried out in the United Kingdom, and each used tracer sheep. The first, by Ross (1967a), though methodologically the least satisfactory (see

Chapter 1), identified the months of August and September as the period when sheep are most likely to ingest the cysts, noting also that the risk between February and June was negligible. A similar result was obtained by Crossland and Hope Cawdery (1969) who found that a batch of sheep exposed to an infected pasture between the end of July and the beginning of October picked up 268 flukes. This compares with an infection rate of 11 per batch in the three months previous and 85 per batch in the three months following. The period between late August and early September was firmly established as a time of maximum risk by Armour et al. (1970) who found that tracer sheep picked up more than three times as many flukes during these few weeks than at any other time.

The pattern of disease incidence and the results from investigations involving tracer sheep suggest that maximum densities of viable cysts are usually encountered during August and September. The number of viable metacercariae then declines to a moderate level which is maintained until January when a more severe decline begins, the months of lowest metacercarial densities being April, June and July.

(v) Aims

The culmination of the intermolluscan stage of the parasite is the release of cercariae onto the pasture. Though the intermediate stages of the infection within the intermediate host were accessible to dissection the end of this phase of development was signalled only by the appearance of metacercariae on the snail habitats. At first, it was hoped to measure the actual densities of cysts on the snail

sites (using the sieve method outlined above) comparing these figures in turn with the preceding densities of infected L. truncatula and the developmental history of the infection. However, after the autumn and winter seasons of 1973-74 and a series of technical difficulties this too optimistic an aim was replaced by the more modest intention to confirm at least that there were viable metacercariae on the habitats and so establish that the intermolluscan stages of the infection had been successfully completed. An indirect method was adopted, similar to that described by Ross and O'Hagan (1966). New Zealand white rabbits were used as the laboratory models of the primary host and a portion of the work reported here examines the suitability of these animals as host models.

2. MATERIALS AND METHOD

Herbage samples were taken monthly from site D, Hendy and site D, Thorneythwaite between September 1973 and April 1974. In order to isolate the metacercariae of F. hepatica the samples were treated by the method outlined in the Technical Bulletin No. 18 of the Ministry of Agriculture Fisheries and Food (p.33). No metacercariae were ever found, not even in samples deliverately "seeded" with fluke cysts in the laboratory, and the method was eventually abandoned.

In the following months the presence or absence of F. hepatica on the snail habitats was demonstrated by feeding herbage samples to New Zealand white rabbits (3-4 months old, and 3-4 kg weight at the time of the assay).

First the suitability of the New Zealand White as a primary host was tested.

(i) The New Zealand White Rabbit as Primary Host to F. hepatica

Batches of L. truncatula were exposed to the miracidia of F. hepatica and the infected snails were maintained until the infection was judged mature. The culture dishes were then allowed to dry out. A week later, fresh grass, collected from the grounds of the University was loosely packed into crystalising dishes. The dishes were filled with aerated tap water and to each dish were added between 10 and 20 of the infected L. truncatula. Cercariae were shed over night and the snails were removed from the dishes next morning. Individual blades of grass were examined under the microscope and the number of metacercariae on each blade was counted. The blades were cut into short lengths so that there were about 5 cysts to the length and mixed in with batches of grass free of fluke. Two samples containing a known number of metacercariae were made up. Each was weighed (wet weight) and fed to a rabbit. The grass was eaten over a weekend when it was normal to feed greenstuffs to the rabbits and the residue was collected on the Monday morning and weighed.

After a period of 8 weeks the rabbits were killed and the liver was removed and dissected. First, the major bile ducts were exposed and opened and then the whole liver was sliced across into small pieces. Any flukes or parts of flukes that were found at this stage were teased out and stored in 70% ethanol. More flukes were induced to leave the small bile ducts by immersing the liver pieces in distilled water for up to an hour. The total number of flukes in each liver was recorded. Parts of flukes were not counted unless the whole of the

anterior section was present.

The experiment was repeated with two more rabbits six weeks later.

(ii) The Collection and Treatment of Herbage Samples from the Snail Habitats

Herbage samples were collected from two snail habitats: site D, Hendy and Site B, Thorneythwaite. The samples were collected monthly between October 1974 and June 1975. Wherever possible, the samples were taken from those areas within the sites where L. truncatula was most numerous, otherwise they were taken from the wettest areas. The herbage was cut as close to the soil surface as possible and on each site sufficient was collected to fill two 9 x 11 inch polythene bags. The polythene bags were sealed with plastic tape and returned to the laboratory. Occasionally the rabbits were not immediately available and so the samples were stored at $4^{o}C$. They were never stored for more than 14 days.

The entire herbage sample from a site was fed to a single rabbit over one weekend. The rabbits were maintained and the flukes recovered from the livers as described above.

3. RESULTS

(i) The New Zealand White Rabbit as a Primary Host

Table 19 shows the number of F. hepatica recovered from each of the rabbits. In the first trial, the rabbits ate all of the grass offered and it can be assumed that all of the metacercariae were

Table 19

THE SUITABILITY OF NEW ZEALAND WHITE RABBITS AS PRIMARY HOSTS OF F. HEPATICA

	WT. OF GRASS OFFERED (g)	WT OF GRASS RESIDUE (g)	% OF GRASS EATEN	NO. OF *F. HEPATICA* FOUND IN LIVER	NO. OF METACERCARIAE IN GRASS SAMPLE
TRIAL I	61	Negligible	100%	4	41
	62	Negligible	100%	0	46
TRIAL 2	50	28	44%	9	40
	30	14	53%	0	12

Table 20

Site B (THORNEYTHWAITE)

DATE OF HERBAGE COLLECTION	WT. OF HERBAGE OFFERED (g)	WT. OF HERBAGE RESIDUE (g)	% OF HERBAGE EATEN	NUMBER OF F. HEPATICA FOUND IN THE LIVER
3 Oct 74	714	29	96	9
10 Nov 74	417	32	92	5
14 Dec 74	637	46	93	0
25 Jan 75	751	70	91	6
8 Mar 75	655	45	93	2
9 Apr 75	403	13	97	2
30 Apr 75	466	13	97	0
1 Jun 75	167	42	75	0

Table 21

Site D (Hendy)

DATE OF HERBAGE COLLECTION	WT. OF HERBAGE OFFERED (g)	WT. OF HERBAGE RESIDUE (g)	% OF HERBAGE EATEN	NUMBER OF F. HEPATICA FOUND IN THE LIVER
5 Oct 74	835	40	95	0
9 Nov 74	970	96	90	0
12 Dec 74	685	118	83	0
25 Jan 75	1100	358	67	0
4 Mar 75	765	67	91	0
8 Apr 75	381	43	89	0
29 Apr 75	302	17	94	0
31 May 75	204	42	79	0

swallowed. Only one rabbit of this pair was found to be infected, the percentage recovery in this case being 9.8%. In the second trial, each rabbit consumed only about a half of all the grass offered. Again, flukes were found in only one of the pair. Assuming that in this case only half the metacercariae were swallowed, then the percentage recovery was 45%. All of the flukes recovered were between 1 cm and 2 cm in length.

(ii) The Metacercariae on the Snail Habitats

Tables 20 and 21 show the number of flukes recovered from the test rabbits eight weeks after presentation of the herbage samples. Most of the herbage was eaten[1]. The presence of viable, infective metacercariae was confirmed in 5 of the 8 samples from the Thorney-thwaite site. None of the rabbits fed with herbage from the Hendy site were found to be infected. All of the flukes recovered were between 1 cm and 2 cm in length.

4. DISCUSSION

Boray (1971) listed 36 mammals and marsupials that are able to act as primary hosts to F. hepatica. Amongst them was the rabbit, Oryctolagus cuniculus, but Boray expressed doubts about its ability to maintain viable adult flukes for more than a limited period.

[1]The given weight of the herbage is the wet weight. As the weather became drier the measured weight of the samples became less although the approximate volume of herbage collected remained the same.

Nevertheless, Ollerenshaw (1959) cited the rabbit as an important host of F. hepatica in the British Isles and Ross and O'Hagan (1966) mention it as one of several laboratory animals that have been used to detect the presence of metacercariae. Indeed, rabbits have been frequently used in the experimental investigation of the immunology and physiology of fascioliasis. For example, Movesisjan and Cuperlovic (1973) obtained a 13% recovery of flukes after oral administration of 50 Fasciola cysts to chinchilla rabbits and Kendall et al. (1967), who used New Zealand Whites, obtained a 16.3% mean recovery after oral administration of 30 cysts and post mortem examination after 8 weeks and a 56% mean recovery if post mortem examination was delayed for 20 weeks. The rabbits' susceptibility to infection by F. hepatica appears to be similar to that of the sheep where recoveries of between 17% and 51% have been reported with the mean around 36% (Sinclair, 1962; Boray, 1967).

The recovery rates given in Table 19 range from a minimum value of 0% to a maximum value of 45%. It is probable that the rabbits varied in their susceptibility to infection and, since not all of the grass was eaten, quite likely that each rabbit consumed a different proportion of the available cysts. While the average percentage recovery of flukes was not dissimilar to the recovery rates reported by other workers there seemed to be no justification for using a calibration coefficient based upon a set of results that varied so much. Nonetheless, the rabbits could consume relatively large amounts of grass in a very short time and did provide a convenient means of establishing that cysts were present on a habitat if not a way of

estimating their actual numbers.

There were two opportunities to study the metacercarial populations during the period spent in the field. The first was in the winter of 1973-74 but as a result of the technical difficulties already described in "Materials and Methods" there is no record of the presence or absence of cysts for this period. The second was in the winter of 1974-75 when cysts were found on the herbage of the Thorneythwaite site (Site B) but not on the Hendy site (Site D). This implies that there were fewer cysts on the Welsh site though not that they were necessarily absent and is entirely consistent with the fact that there were no infected snails found here during the previous summer and autumn. At Thorneythwaite (Site B), on the other hand, infected snails containing relatively large numbers of rediae were found throughout most of this period. A more detailed consideration of the significance of these findings will be deferred until the next chapter.

CHAPTER 6. VARIATIONS IN THE PREVALENCE OF FASCIOLIASIS AT HENDY AND THORNEYTHWAITE AND ESTIMATES OF THE NUMBERS OF FLUKE EGGS DEPOSITED IN THE SNAIL HABITATS

1. INTRODUCTION

An important feature of the epidemiology of fascioliasis is the seasonal variation in the prevalence of the disease in cattle and sheep (Honer and Vink, 1963(a) and (b); Ross, 1966; Ollerenshaw 1971). In the British Isles, for example, outbreaks of fascioliasis are normally confined to a period which begins during the late summer and ends in the following spring. The level of disease is related to the density of Fasciola cysts on the herbage (Ollerenshaw, 1962; Ross, 1967; Armour et al., 1970) but given good management practices and weather conditions inimical to the viability of the metacercariae the chances of infection can be considerably reduced (e.g. Olsen, 1947). Prevalence levels, then, reflect the degree of success with which the parasite has transferred from intermediate to primary host.

Animals infected with mature F. hepatica contaminate pastures with fluke eggs. A single fluke may lay up to 50,000 eggs each day and in serious chronic cases of fascioliasis the primary host may have over 300 flukes in its liver (Happich and Boray, 1969). It is to be expected that the numbers of fluke eggs deposited on the pastures follow a pattern determined by the prevalence and severity of infection in the stock. However, it is not known whether this pattern of deposition has any significant influence on the subsequent course of infection in the intermediate host. Ollerenshaw (1959, 1971) suggests that the supply of fluke eggs is rarely limiting and

that the climatic variations which affect the hatching rate of the eggs are of more importance.

The work reported in this chapter was designed to establish the fate of the rediae and cercariae which had infected the snail populations during the summer and autumn months of each year and to investigate the extent to which the pattern of fluke egg deposition determined the course of reinfestation in these populations. It was necessary, therefore, to obtain an index of the prevalence of fascioliasis within the stock of the two farms, Hendy and Thorneythwaite, and to estimate the numbers of fluke eggs falling onto the habitats of the intermediate host, L. truncatula.

The methods by which the prevalence of fascioliasis is usually determined fall into three categories. First, there is the post mortem examination of animals brought into veterinary investigation centres (e.g. Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1971). This method typically underestimates incidence since only acute and serious chronic cases are brought to the attention of the veterinary centres. Second, there is the analysis of liver condemnation records from abbatoirs (e.g. Honer and Vink 1963(a) and (b)). Such records, while including animals with sub clinical infections, often reflect the cumulative effect of several years exposure to infection. Third, there is the examination of faecal samples for fluke eggs (e.g. Geeraerts, 1971; Sloan, 1971) This last method, particularly if the faeces have been obtained by direct rectal sampling, seemingly provides a convenient means of diagnosing the disease in the living

animal and of estimating egg output into the pasture. However, techniques of faecal examination that are sufficiently sensitive to detect even the lightest infections are both laborious and time consuming (e.g. Dorsman, 1958) and the continued collection of faeces by direct rectal sampling may lead in time to hypersensitivity of the rectal membranes and thus to abnormal defaecation activity (Honer, 1971). Nevertheless, of the three methods described, only faecal examination is suited to small scale epidemiological surveys and with some modifications, it was the method adopted here.

2. MATERIALS AND METHODS

(i) The Collection and Storage of Faecal Samples

Faecal samples were collected monthly and usually from the fields in which the snail habitats were located. Samples were taken from only the freshest pats and each pat was sampled once only. In wet conditions, when the appearance of the droppings gave no clue to their age, samples were taken from those pats which were infested with Scatophaga stercoraria (the Yellow Dung Fly), since observations had suggested that these flies are most likely to be found on the fresher pats. When stock were denied access to the study fields for more than just a few days it was necessary to make collections in other fields to ensure the freshness of the sample. For both sheep and cattle between 12 and 30 samples were collected on each occasion.

The samples were sealed in polythene bags and stored at $4^{o}C$. Whenever possible the collections were processed within three weeks of their return to the laboratory. Happich and Boray (1969(a)) suggest that samples may be stored in a refrigerator for periods up

to a month without harm.

(ii) Counting the Fluke Eggs

Honer (1971) and Boray (1969) have reviewed the various techniques of faecal examination. Generally, the quantitative methods involve, singly or in combination, sieving, flotation and sedimentation. The method used here was a modification of the sieving and sedimentation technique of Happich and Boray (1969(a)). The eggs of F. hepatica are from 130μm in length and between 70 and 90μm wide. They have a specific gravity of between 1.05 and 1.10. The faecal samples were broken up and passed through a sieve (mesh aperture 180μm). The filtrate was allowed to sediment. Since the eggs sink in water about 100mm per minute faster than most of the unwanted faecal debris a short sedimentation time of 4 minutes was chosen. The eggs, together with the remaining faecal material, were collected and preserved on filter paper.

(a) Homogenising the Sample

About 2g of each faecal sample were accurately weighed in a $30cm^3$ screw top flask. The flask was half filled with water and the sample was homogenised at moderate speed until the faecal pellets had entirely disintegrated. The mouth of the flask was sealed by a screw top fastening on the homogeniser so that there was no spillage. Any faecal material retained by the blades of the homogeniser was washed into the flask. The homogeniser blades were thoroughly cleaned between each sample.

(b) Sieving

The faecal suspension was filtered through a bronze sieve (mesh number 85) into a graduated 250cm^3 beaker. The washings from the screw top flask were also poured into the sieve and the faecal material was agitated by a fine jet of water from a wash bottle until 200cm^3 of filtrate had been collected.

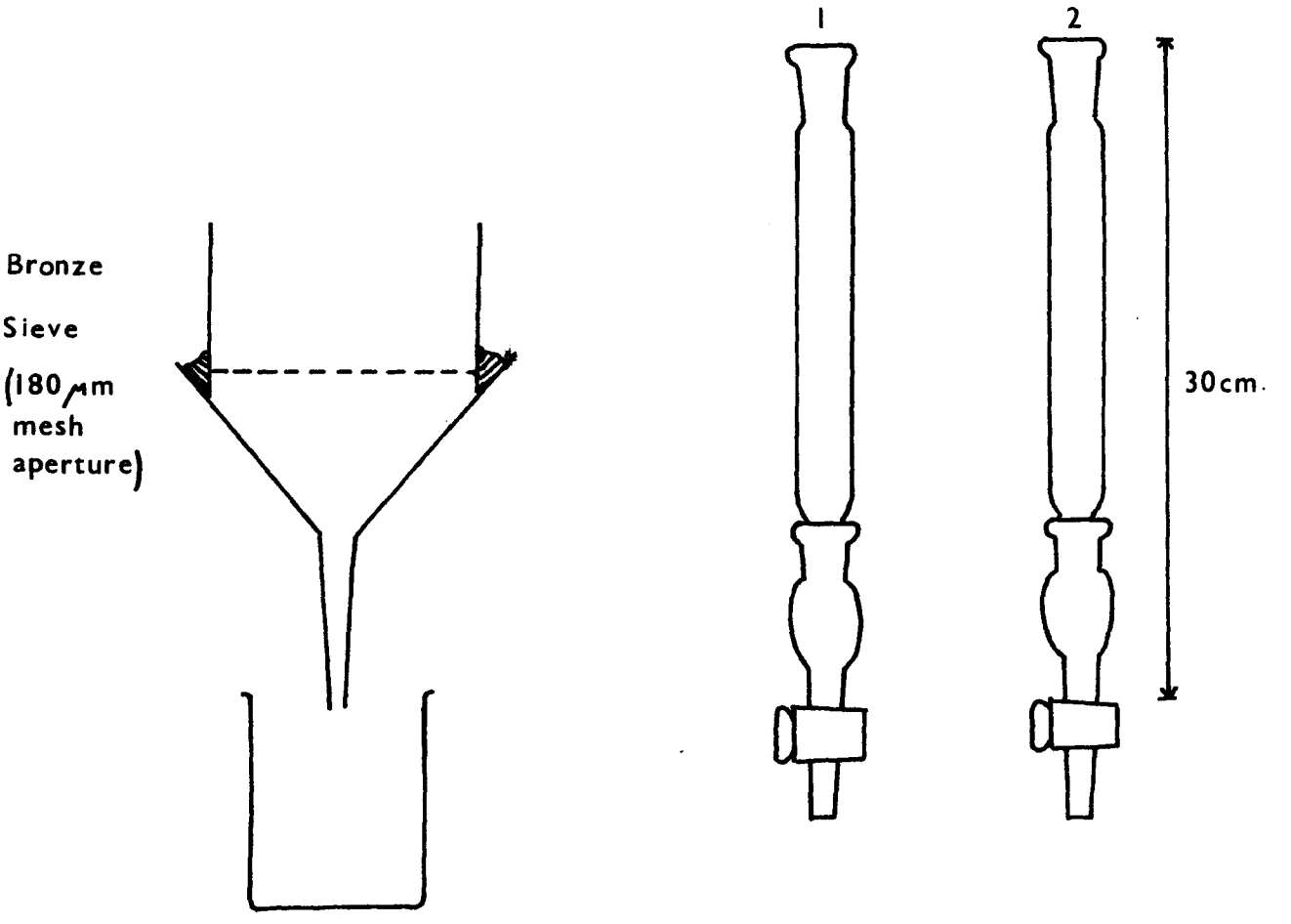
(c) Sedimentation

The contents of the beaker were allowed to sediment for 4 minutes. The supernatant was discarded by means of a water driven suction pump and the sediment was transferred to the first of two sedimentation columns (Figure 6.1). Each column consisted of a "quickfit" air condenser and a detachable ground glass stop-cock which when assembled formed a tube 30cm tall and 1.5cm wide. The first column was a quarter filled with water, and the sediment, together with the washings from the beaker, were poured directly into this until the level of liquid rose to the top of the column. The suspension was allowed to sediment for 4 minutes and the supernatant was discarded as before. The second column was three quarters filled with water. The sediment was transferred from the first column to the second by means of the detachable stop-cock and sedimentation proceeded as before.

(d) Treatment of the extract

The remaining sediment was stained with a few drops of methylene blue solution. The stained extracts were collected on Whatman No. 1 filter papers (9cm) by vacuum filtration using a Buchner funnel mounted

Fig. 6.1


Bronze
Sieve
(180 μm
mesh
aperture)
Sedimentation Columns.
1
2
30cm.

upon a suction flask. The filter paper was first wetted so that no air leaks occurred at the sides and was then flooded with water to a depth of 2 or 3cm. Filtration was started immediately to ensure that the filter paper did not subsequently become detached. The extract was quickly washed into the volume of water and dispersed. This procedure ensured an even spread of extract over the paper.

When filtration was complete, the filter paper was removed from the Buchner funnel and placed in the lid of a 9cm plastic petri dish. After gentle drying at room temperature for about half an hour the preparation was sprayed with Letraset varnish and allowed to dry once more. The extracts could be stored like this for periods up to 6 months without noticeable deterioration. The technique is a modification of that described by Rowan and Gram (1959) and later by Bell (1963) who were concerned with the eggs of Schistosoma mansoni.

(e) Counting the eggs

A perspex counting grid, marked off in 4 x 4mm squares was placed over each filter paper and the preparation was examined under a low power (24 x magnification) stereomicroscope. The straw coloured fluke eggs were easily distinguishable against the blue-stained faecal debris. The eggs had usually collapsed by this time but were readily differentiated from the eggs of other species by their size, colour, outline and texture. The results were expressed as the number of eggs per g. (e.p.g.) of faeces. By convention variations in the consistency of the original faecal samples were ignored (see Honer

1971; also Tech. Bull. No. 18, Min. of Agr. Fish and Food.)

The apparent e.p.g. was converted to an estimated actual figure by means of a calibration coefficient (see Appendix I).

(iii) The Location of the Stock and Management Practices

The presence or absence of stock in fields adjacent to or containing the major snail habitats was noted at each visit to the farms in 1973. Subsequently, the actual number of animals was recorded and this method was continued until the end of the study.

Complementary information regarding the management of the stock was obtained during discussions with the farmers. In particular, a record was kept of the dates upon which the cattle and sheep were dosed with flukicides.

3. RESULTS

(i) The Location and Management of Farm Stock at Hendy

The numbers of sheep and cattle found on field system 202b during the period of observation are presented in Tables 22 and 23. The sheep grazed these fields throughout the year, with the exception of four or five weeks during lambing (February and March) when the ewes were moved to fields nearer the farm buildings. The flock was largest just after lambing (258 sheep in 1974, 180 sheep in 1975) and was reduced during the summer and autumn to reach a mean over wintering size of 115 ewes. The field system was grazed by cattle at intervals throughout the year; mainly young stock and beef cattle,

Table 22

NUMBER OF SHEEP ON FIELD SYSTEM "202" (HENDY)

1973		1974					1975				
DATE	202 FIELD SYSTEM	DATE	FIELD 202a	202b	200	222	DATE	FIELD 202a	202b	200	222
		16 Jan		(112)			25 Jan	35	0	73	33
		21 Feb	0	0	0	0					
		11 Mar	0	0	0	0	4 Mar	0	0	0	0
4 Apr	Present						8 Apr	0	25	12	100
		15 Apr	8	70	18	0	20 Apr	55	25	50	50
							29 Apr	115	15	25	25
12 May	"	19 May	10	103	77	0	31 May	Shearing, no count			
16 Jun	"	16 Jun	93	29	30	0	15 Jun		(157)		
		30 Jun	59	34	48	0	27 Jun		(154)		
17 Jul	"	14 Jul	88	120	25	25	27 Jul		(136)		
11 Aug	"	11 Aug	0	0	0	0					
		24 Aug	0	83	0	0	28 Aug		(134)		
14 Sept	"	8 Sept	0	6	100	0	19 Sept		(100)		
12 Oct	"	5 Oct	50	48	0	0					
8 Nov	"	9 Nov	14	0	75	50					
6 Dec		12 Dec	0	56	28	28					

Figures in brackets represent total number of sheep in field system

Table 23

NUMBER OF CATTLE ON FIELD SYSTEM "202" (HENDY)

1973		1974					1975				
DATE	202 FIELD SYSTEM	DATE	FIELD 202a	202b	200	222	DATE	FIELD 202a	202b	200	222
		16 Jan		(0)			25 Jan	0	0	0	0
		21 Feb	0	12	0	0					
		11 Mar	0	20	0	0	4 Mar	0	0	0	0
4 Apr	(o)						8 Apr	0	0	0	0
		15 Apr	13	0	0	0	20 Apr	0	0	0	0
							29 Apr	0	0	0	0
12 May	(0)	19 May	0	0	0	0	31 May		(15)		
16 Jun	(0)	16 Jun	12	0	0	0	15 Jun		(4)		
		30 Jun	4	20	0	0	27 Jun		(5)		
17 Jul	PRESENT	14 Jul	0	0	17	18	27 Jul		(25)		
11 Aug	"	11 Aug	0	0	0	0					
		24 Aug	0	12	0	0	25 Aug		(30)		
14 Sept.	"	8 Sept	23	0	0	0	14 Sept		(21)		
12 Oct	"	5 Oct	0	20	0	0	11 Sept				
8 Nov	"	9 Nov	0	0	0	0					
6 Dec	(0)	12 Dec	0	0	0	0					

Figures in brackets represent total number of cattle in field system.

Table 24

FLUKICIDE ADMINISTRATION AT HENDY

SHEEP

YEAR	DATE DOSED WITH FLUKICIDE
1973	Not dosed between April-December incl.
1974	12th January, end of December
1975	Not dosed between January-September incl.

CATTLE

YEAR	DATE DOSED WITH FLUKICIDE
1973	Not dosed between April-December incl.
1974	12th January, 4th December.
1975	Late January.

their numbers varied between 4 and 35 animals.

The ewes were dosed with flukicide in January and December, 1974. The young stock and beef cattle were dosed in January and September, 1974 and then again in January 1975. (Table 24).

(ii) The Location and Management of Farm Stock at Thorneythwaite

The numbers of sheep and cattle found on field 684 during the period of observation are presented in Tables 25 and 26. The ewes spent January, February and March on the fell sides. Each year they were brought down at the end of the first week in April and enclosed in field 684. The first lambs were born in the last week of April and lambing continued throughout May. As lambing progressed the sheep were gradually transferred to neighbouring fields and by mid May field 684 was generally empty of stock. It was then grazed for irregular periods during June, July and August by between 3 and 15 cattle. An increase in the number of cow pats suggests that some cattle were also there in late September, 1974 although they were gone by the time the farm was visited again on the 3rd October. Sheep returned to field 684 at only two other times of the year: generally for a few weeks during November and December, when they were tupped (mated), and then for short periods in the last two weeks of July, when they were collected together for shearing.

The sheep were routinely dosed with flukicide before lambing (April), during shearing (July) and either before or after tupping

Table 25

NUMBER OF SHEEP ON FIELD 684 (THORNEYTHWAITE)

1973		1974		1975	
DATE	SHEEP	DATE	SHEEP	DATE	SHEEP
		14 Jan	0	26 Jan	0
		20 Feb	4		
		9 Mar	4	8 Mar	
1 Apr	0	12 Apr	190	9 Apr	2
				30 Apr	0
9 May	Present	16 May	0		
				1 Jun	50
14 Jun	0	17 Jun	0	28 Jun	0
14 Jul	0	13 Jul	0	28 Jul	0
8 Aug	0	12 Aug	0	29 Aug	0
12 Sept	0	5 Sept	0		
10 Oct	0	3 Oct	0		
6 Nov	0	10 Nov	0		
4 Dec	Present	14 Dec	63		

Table 26

NUMBER OF CATTLE ON FIELD 684 (THORNEYTHWAITE)

1973		1974		1975	
DATE	CATTLE	DATE	CATTLE	DATE	CATTLE
		14 Jan	0	26 Jan	0
		20 Feb	0		
		9 Mar	0	8 Mar	0
1 Apr	0	12 Apr	0	9 Apr	0
				30 Apr	0
9 May	0	16 May	0		
				1 Jun	0
14 Jun	Present	17 Jun	15	28 Jun	0
14 Jul	Present	13 Jul	0	28 Jul	9
8 Aug	0	12 Aug	8	29 Aug	10
12 Sept	3	5 Sept	0		
10 Oct	0	3 Oct	0		
6 Nov	0	10 Nov	0		
4 Dec	0	14 Dec	0		

Table 27

FLUKICIDE ADMINISTRATION AT THORNEYTHWAITE

SHEEP

YEAR	DATE DOSED WITH FLUKICIDE		
1973	6th March	-	Hoggets only
	7-14th April	-	Prior to lambing
	15-22nd July	-	During shearing
	Mid November	-	Prior to tupping
1974	Mid February	-	Tups and Hoggets
	8-14th April	-	Prior to lambing
	15-22nd July	-	During shearing
	Late December	-	After tupping
1975	Not dosed between January -August inc.		

No data available for cattle

(Mid November or late December). A few sheep, mainly tups (rams) and hoggets (store animals) were dosed in February or March (Table 27). The cattle were from a neighbouring farm and it is not known when or if they were dosed.

Thorneythwaite farm changed ownership in the spring of 1975 and none of the sheep were dosed in that year.

The location and management of the Hendy and Thorneythwaite flocks is summarised in Figure 6.2.

(iii) The Proportion of Faecal Pats Sampled

The numbers of faecal pats found on field 202b, Hendy, and field 684, Thorneythwaite, are given in Tables 28 and 29. Although the results are presented as number counted per 10 minutes they may be regarded as whole counts in all but a few cases. Between 12 and 30 pats were sampled on each occasion and since the number of samples taken was not related to the number of pats present the proportion of pats sampled varied as follows: on Thorneythwaite farm, 0.12$\pm$ 0.05 of the sheep droppings were sampled and 0.13$\pm$0.06 of the cow pats; on Hendy farm the figures were 0.10$\pm$0.02 for sheep and 0.17$\pm$0.13 for cattle.

(iv) The Percentage of Samples Containing Fluke Eggs

The numbers of faecal samples containing fluke eggs was expressed as a percentage of the total number of samples collected each month. The results in this form provide an approximate index of the prevalence of fascioliasis.

Fig. 6·2 Farm Management Practice

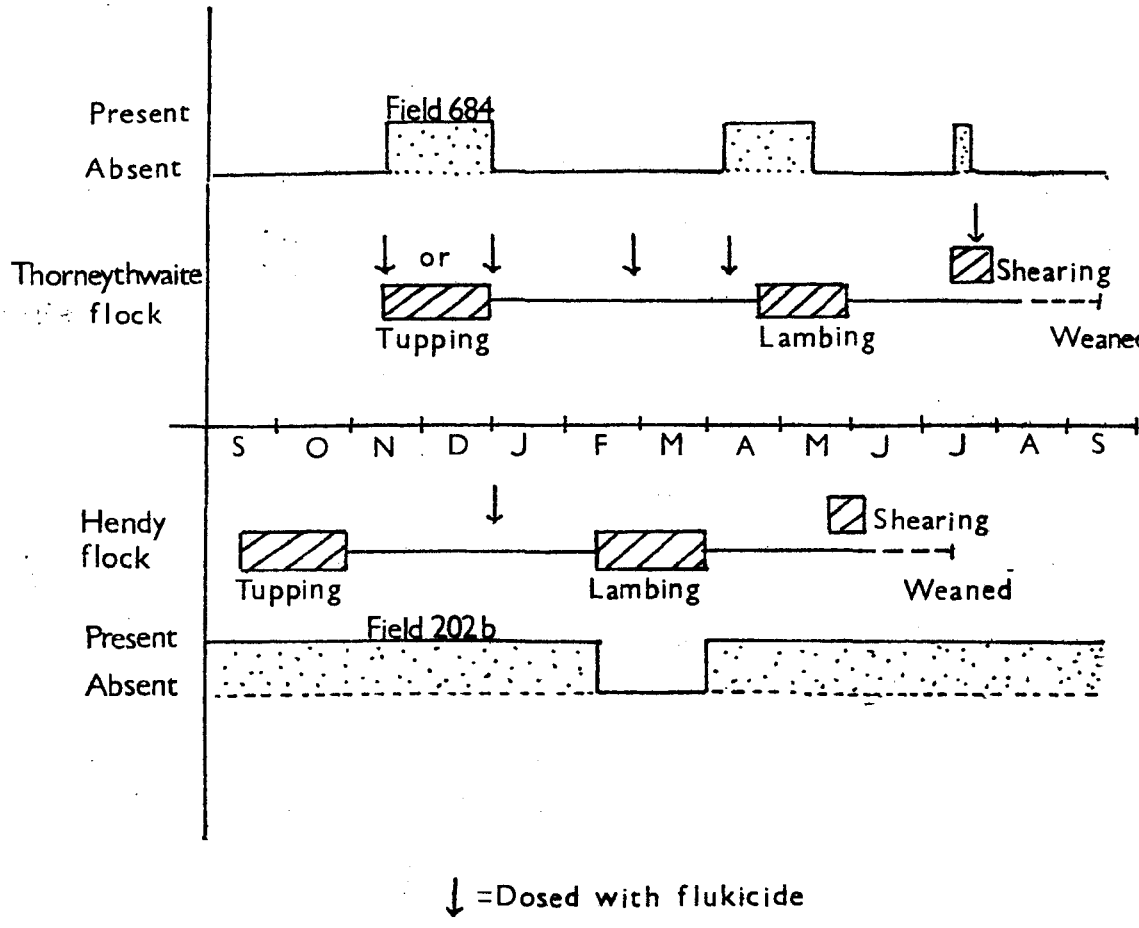


Table 28

NUMBER OF FAECAL PATS PER 10 MINUTES ON FIELD 202b (HENDY)

1974			1975		
DATE	FAECAL PATS PER 10 MIN.		DATE	FAECAL PATS PER 10 MIN.	
	SHEEP	CATTLE		SHEEP	CATTLE
16 Jan	8	0	25 Jan	269	0
21 Feb	5	65			
11 Mar	2	157	4 Mar	0	0
			8 Apr	161	0
15 Apr	93	2	20 Apr	508	0
			29 Apr	760	0
19 May	246		31 May	720	-
16 Jun	272	-	15 Jun	581	-
30 Jun	307	-	27 Jun	1059	-
14 Jul	225	137	27 Jul	451	140
24 Aug	145	330	28 Aug	91	235
8 Sep	211	89	14 Sept	-	-
5 Oct	192	46			
9 Nov	419	15			
12 Dec	419	0			

Table 29

NUMBER OF FAECAL PATS PER 10 MINUTES ON FIELD 684 (THORNEYTHWAITE)

1974			1975		
DATE	FAECAL PATS PER 10 MIN.		DATE	FAECAL PATS PER 10 MIN.	
	SHEEP	CATTLE		SHEEP	CATTLE
14 Jan	10	0	26 Jan	0	0
20 Feb	0	0			
9 Mar	0	0	8 Mar	-	-
12 Apr	500	0	9 Apr	472	0
			30 Apr	-	-
16 May	409	0			
			1 Jun	370	0
17 Jun	-	80	28 Jun	0	0
15 Jul	0	0	28 Jul	0	20
12 Aug	0	163	29 Aug	0	50
5 Sep	0	72			
3 Oct	0	271			
10 Nov	0	80			
14 Dec	63	0			

(a) Sheep

Generally, there was a greater chance of finding fluke eggs in the droppings of sheep from Hendy than in the droppings of the Cumbrian flock (Figures 6.3a and 6.3b). The proportion of samples containing fluke eggs exceeded 25% in 10 of the 22 collections from Hendy, the highest levels recorded being 66% in December, 1973, and 70% in May, 1974. This is in sharp contrast to the situation at Thorneythwaite where the proportion of positive samples exceeded 25% in only 2 of the 23 collections, the highest levels recorded here being 33% and 40% in May and July respectively of 1975.

The sheep at Hendy were dosed only twice during the period of study and on each occasion this was accompanied by a sharp decline in the percentage of samples containing fluke eggs. Another considerable fall in the percentage of positive samples was recorded between May and June in 1974. The sheep at Thorneythwaite were dosed 6 times between May 1973 and December 1974 and then not at all throughout the rest of the study period. The increase in the percentage of samples containing fluke eggs that occurred between January and August, 1975, coincided with the change in farm ownership and dosing practice.

(b) Cattle

The cattle faeces at Hendy were sampled on too few occasions to provide an adequate indication of the prevalence of fascioliasis throughout the year and so the results represented here (with permission) are those obtained by the Bangor V.I. Centre for the same period. The

Fig. 6.3a. Percentage of Faecal Samples Containing Fluke Eggs

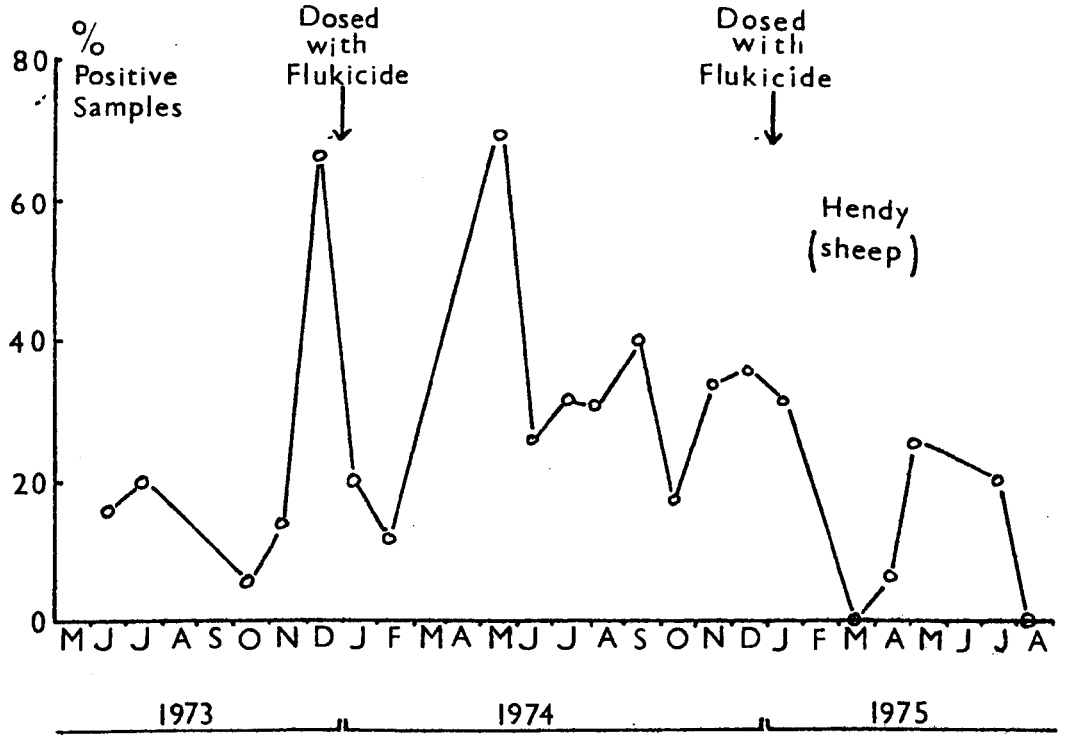


Fig. 6.3b.

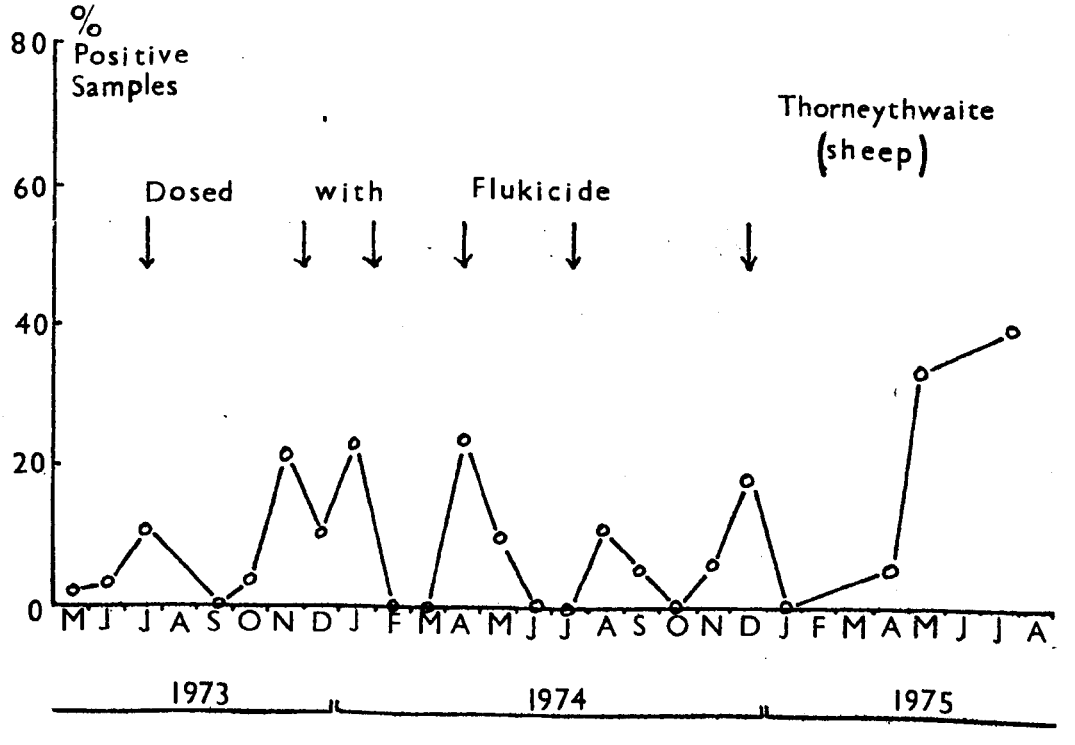
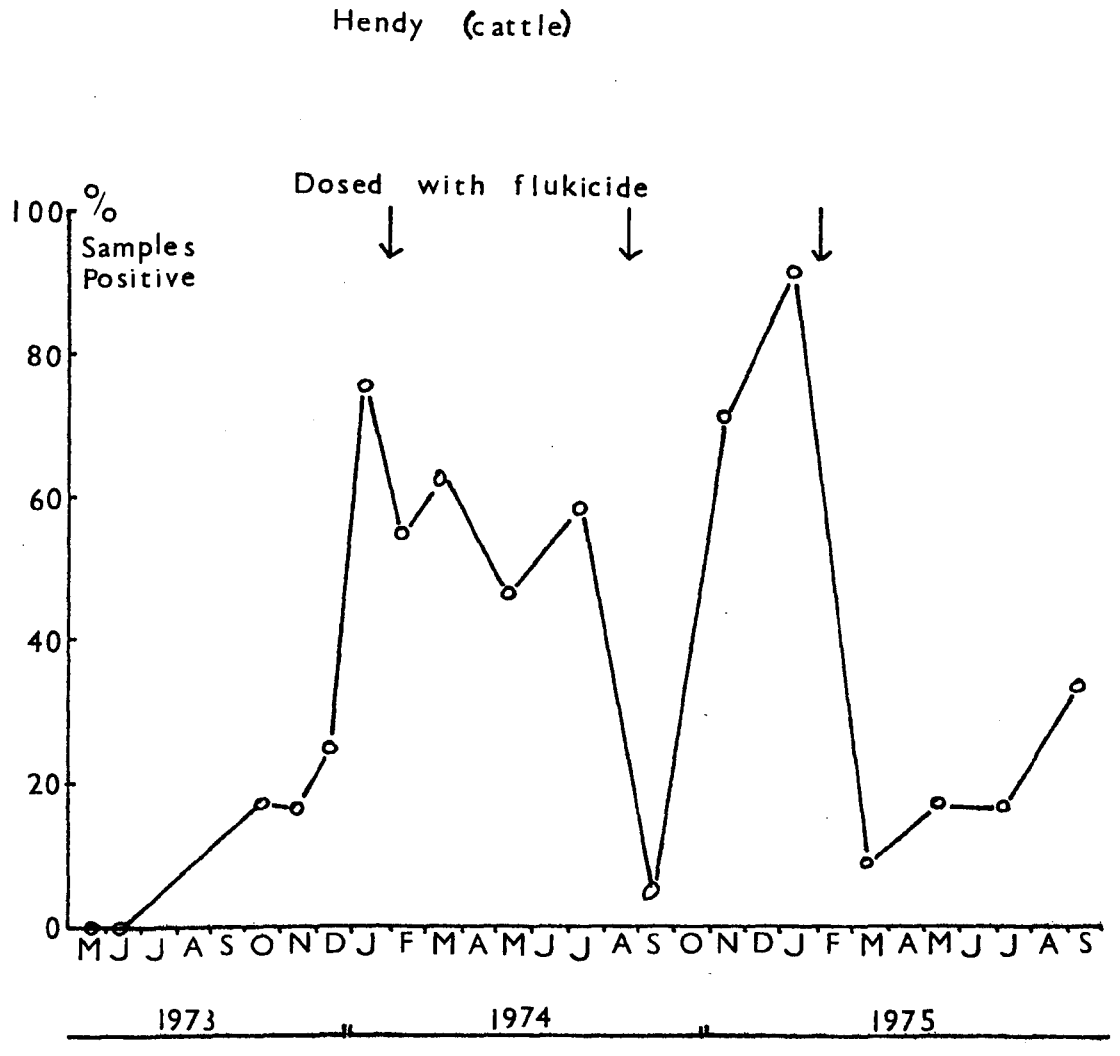


Fig. 6·4 Percentage of Faecal Samples Containing Fluke Eggs.



Calculated from data provided by the Bangor V.I. Centre.

figures given are for beef cattle and young stock and have been plotted in Figure 6.4. The proportion of samples containing fluke eggs exceeded 25% in 8 of the 18 collection from Hendy, the highest levels being 75% in January 1974 and 92% in January 1975. The cattle were dosed three times. There was a decline in the percentage of positive samples after each treatment.

There was no significant correlation between the cattle and sheep on Hendy with respect to the proportion of faecal samples containing fluke eggs.

Between 3 and 15 cattle were noted on field 684, Thorneythwaite, on 7 occasions; of the 4 faecal collections that were made only 1 (July 1973) contained any positive samples (2 out of 15). The results suggest a very low level of prevalence amongst a small number of animals.

(v) The Mean Number of Eggs per Gramme of Faeces

For all samples extracted by the sedimentation method, the actual number of eggs per g. of faeces was estimated by

$$y = 0.06x$$

where y = number of fluke eggs per g. found

x = number of fluke eggs per g. actually present.

(See Appendix I).

In the case of the egg counts provided by the Bangor V.I. Centre, where a flotation method of extraction was used, it was

Fig.6.5. Geometric Mean E.P.G. for sheep.

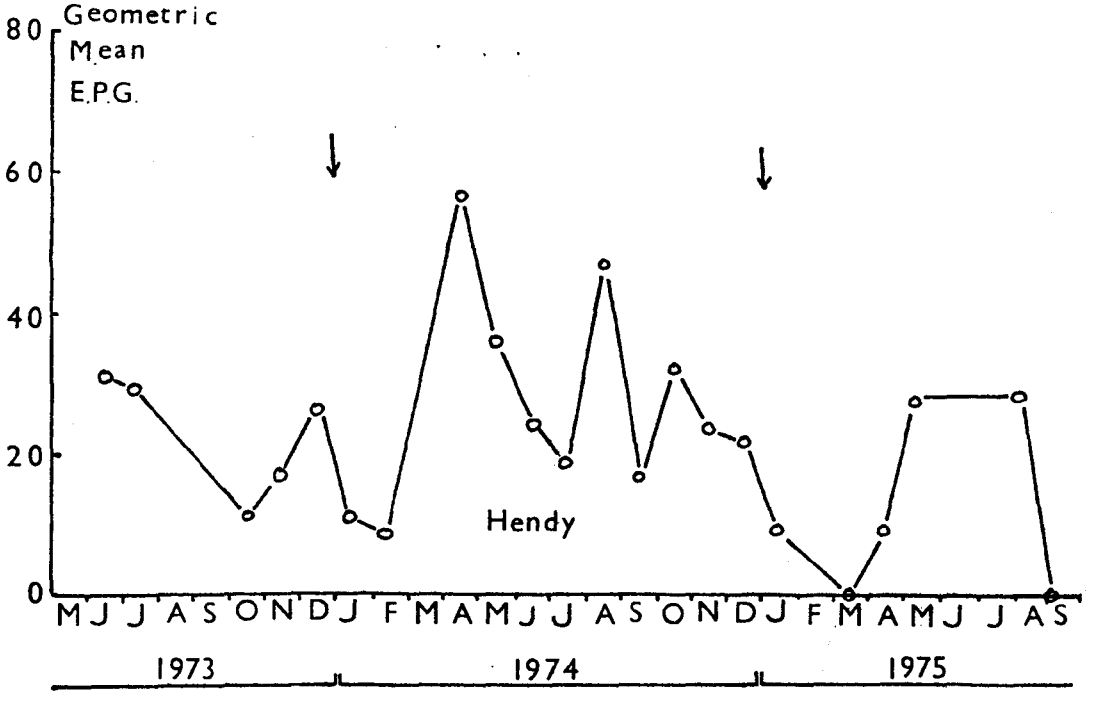


Fig.6.6.

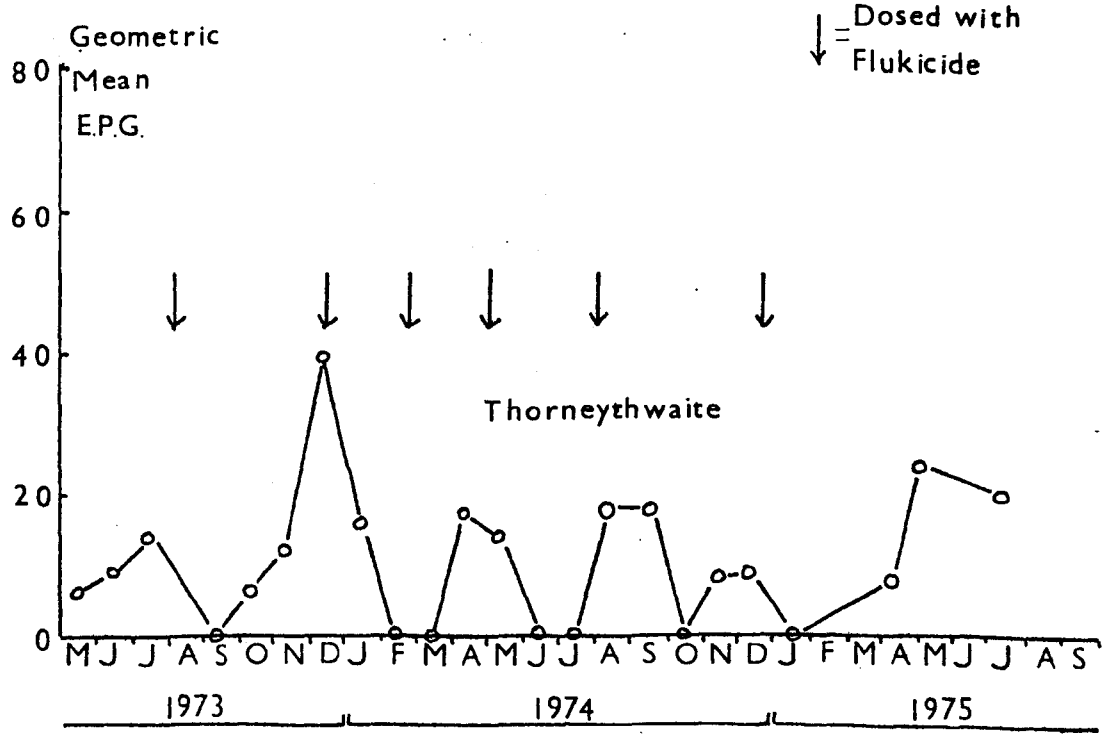
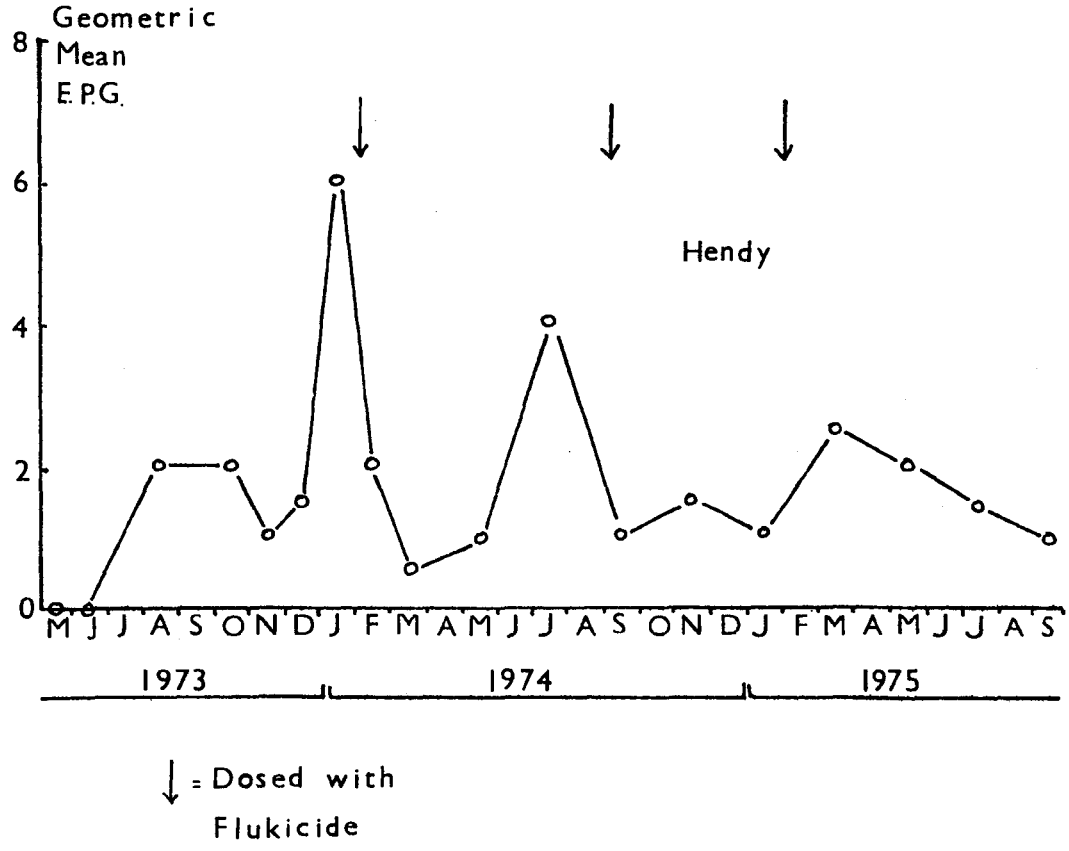


Fig. 6:7. Geometric Mean E.P.G. for cattle.



Calculated from data provided by the Bangor V.I. Centre.

assumed that 5/6 of the eggs were recovered (Parfitt, 1958; Tech. Bull. 18, Min. of Agr. Fish and Food) and the data treated accordingly.

Ignoring all zero counts, the geometric mean e.p.g. was calculated for each collection of samples (~~Table~~). The results are plotted in Figures 6.5, 6.6, and 6.7.) Generally, more fluke eggs were found in the faeces of the sheep from Hendy than in the faeces of the sheep from Thorneythwaite. The number of eggs estimated to be in the faeces of the cattle from Hendy was considerably lower than the equivalent estimates for sheep on either farm. The maximum values recorded for the Welsh flock were 57 e.p.g. and 47 e.p.g. in April 1974 and August 1974, respectively. These compare with 6 e.p.g. and 4 e.p.g. recorded in January 1974 and July 1974 for the cattle on Hendy and 37 e.p.g. recorded in December 1973 for the sheep on Thorneythwaite.

There was no consistent relationship between the changes in mean e.p.g. from month to month and the treatment of the stock with flukicide and only in the case of the Welsh flock was there a significant correlation between mean e.p.g. and the percentage of samples containing fluke eggs ($r = 0.46$, p 0.05, d.f. 18).

(vi) The Total Daily Fluke Egg Output.

The total number of fluke eggs that reached the pasture in the faeces of the stock (E) was estimated as the product of four parameters.

i.e. $E = n.f.p.e.$ (1)

where n = number of primary hosts

f = daily faecal output per animal (in grams)

p = proportion of faeces containing fluke eggs

e = geometric mean e.p.g.

The successive values of n, p, and e were given in the previous section. The figure for the daily output of faeces per animal (f) was estimated to be 1,000g for sheep and 15,000g for cattle. Happich and Boray (1967) measured the daily faecal output of 7 merino wethers over a period of 14 weeks. The mean value calculated from their data was 1347±93g. This compares with a mean value of 860g reported by Halnan et al. (1966) for a much smaller breed of sheep. The figure used in this study (1,000g per day) was chosen as a convenient compromise between these results. Gowan (1972) estimated that the mean daily faecal output of adult beef and dairy cattle was 33lbs and 66lbs respectively. The reported variation was considerable, the range being approximately equal to the value of the mean in each case. The cattle encountered on Hendy and Thorneythwaite were mostly young stock and beef cattle in varying proportions. The large variation reported by Gowan was considered to be sufficient to incorporate any differences that there might have been between the young and old stock and the value 15,000g (33.11lbs) was chosen for computational convenience.

Successive values of E are presented in Tables 30, 31 and 32 ~~and are plotted in Figures 6.5, 6.6, and 6.7.~~ The contribution of the

Table 30

POINT ESTIMATES OF THE TOTAL DAILY FLUKE EGG OUTPUT (E) FROM SHEEP ON FIELD SYSTEM "202" (HENDY)

1973		1974		1975	
DATE	$E(x10^5)$	DATE	$E(x10^5)$	DATE	$E(x10^5)$
		16 Jan	2.5	25 Jan	3.8
		15 Apr	27.4	8 Apr	1.0
		19 May	47.9	29 Apr	13.6
16 Jun	7.2	16 Jun	8.7		
17 Jul	11.4	14 Jul	15.7	27 Jul	10.4
		8 Sept	6.8	28 Aug	0
12 Oct	0.5	5 Oct	5.3		
8 Nov	2.4	9 Nov	11.0		
6 Dec	17.2	12 Dec	8.9		

Table 31

POINT ESTIMATES OF THE TOTAL DAILY FLUKE EGG OUTPUT (E)

FROM CATTLE ON FIELD SYSTEM "202" (HENDY)

1973		1974		1975	
DATE	$E(x10^5)$	DATE	$E(x10^5)$	DATE	$E(x10^5)$
		21 Feb	6.0		
		11 Mar	1.0	31 May	0.7
		14 Jul	12.2	27 Jul	0.9
12 Sept	0	8 Sept	1.6	19 Sept	1.0
10 Oct	0.2				
6 Nov	0.6				

Table 32

POINT ESTIMATES OF THE TOTAL DAILY FLUKE EGG OUTPUT (E) FROM SHEEP ON FIELD 684 (THORNEYTHWAITE)

1973		1974		1975	
DATE	$E(x10^5)$	DATE	$E(x10^5)$	DATE	$E(x10^5)$
		20 Feb	0		
		9 Mar	0		
		12 Apr	7.4	9 Apr	0.1
9 May	0.2				
6 Nov	1.6				
4 Dec	2.6	14 Dec	1.0		

Thorneythwaite cattle was assumed to be neglibible and so was not considered.

(vii) The Proportion of the Total Daily Fluke Egg Output that Fell into Site D, Hendy and Site B, Thorneythwaite

Site D, Hendy was considered first. Field system 202b consisted of four separate but linked fields, each of a different size. Site D was in field 202b. It was necessary to estimate how many of the eggs falling into the whole field system were deposited in the particular field containing the habitat. Initially, it was assumed that the sheepwere dispersed randomly and so in the long run were equally distributed throughout the field system. In order to test this assumption the data in Table 21 were pooled and the total numbers of sheep observed in each field over the whole period of study were compared. The comparison proved to be invalid, however, as the heterogeneity Chi-squared for the pooled data was estimated to be 1649.49 (39 d.f., $p < 0.001$). None of the individual Chi-squared values calculated for each of the separate observations confirmed the null hypothesis. The result is a consequence of the tendency of sheep to graze in social groups and so attention was shifted from the individual sheep to the flock. The location of the flock during each visit to the farm was defined as that field which contained the greatest number of sheep. The frequencies with which the flock was observed in particular fields are presented in Table 38 and are consistent with the assumption that there is an equal probability of the flock occurring in any of

Table 33

THE FREQUENCY WITH WHICH THE FLOCK AT HENDY WAS OBSERVED IN THE FIELDS OF FIELD SYSTEM "202"

FIELD	202a	202b	200	222
Frequency (Number of visits to farm)	5	5	3	1

Chi-squared = 3.14, d.f. = 3, $0.5 > p > 0.1$, Not Significant.

(Snedecor and Cochran (1967) suggest that the Chi-squared test is accurate enough if the smallest expectation is at least 1.)

the four fields. Therefore, the proportion of eggs falling in the field containing site D was taken to be a quarter of the total. There were insufficient observations to investigate the distribution of the cattle throughout the field system and so it was arbitrarily assumed that in this case too there was an equal probability of the herd occurring in any of the four fields.

In order to estimate the proportion of the total daily fluke egg output that fell into Site D (E_D) it was assumed that the stock ranged freely over the field and habitat alike.

i.e. $$E_D = 0.25\ E.\ a_1/a_2 \qquad (2)$$

where a_1 = area of site D (0.004 ha)

a_2 = area of field 202b (3.64 ha)

The calculations for site B, Thorneythwaite, were not complicated by flock movements within an interconnecting field system and so the number of flukes falling daily into the habitat (E_B) was estimated directly by

$$E_B = E.\ a_3/a_4 \qquad (3)$$

where a_3 = area of site B (0.0048 ha)

a_4 = area of field 684 (2.86 ha)

The daily rate of fluke egg input onto both sites is presented in Figures 6.8, 6.9 and 6.10. In order to obtain the estimates for 1973 the density of grazing stock was assumed to be the same as in 1974. Discussion with the farmers indicated that this was not an

Fig. 6·8 Estimated Daily Input of Fluke Eggs onto Site D, Hendy (sheep)

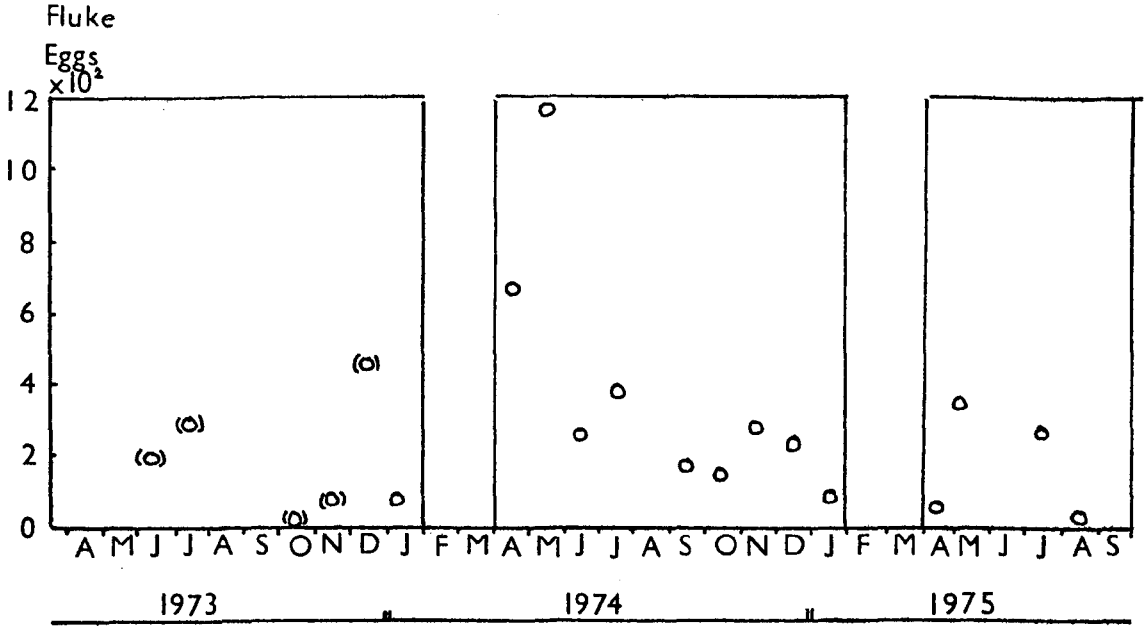
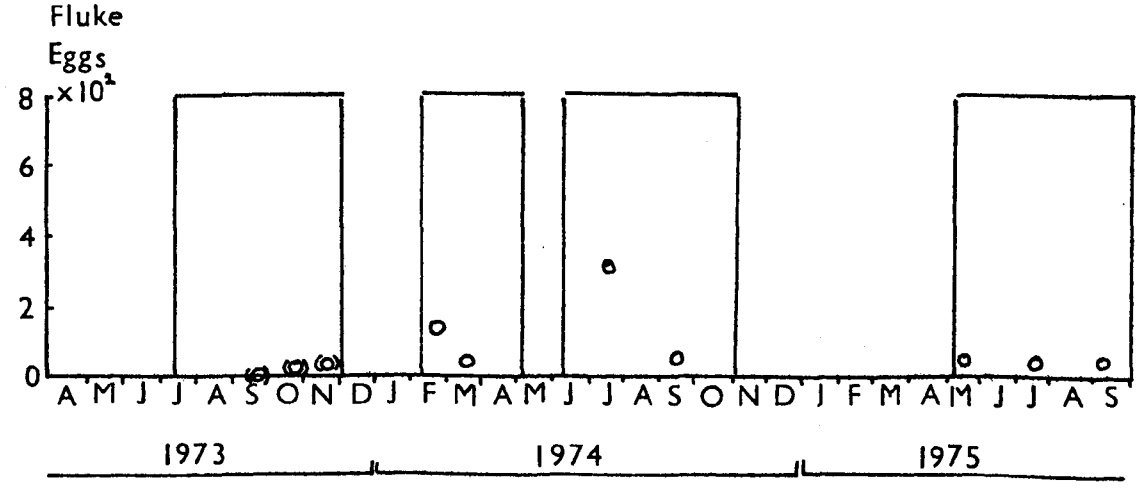
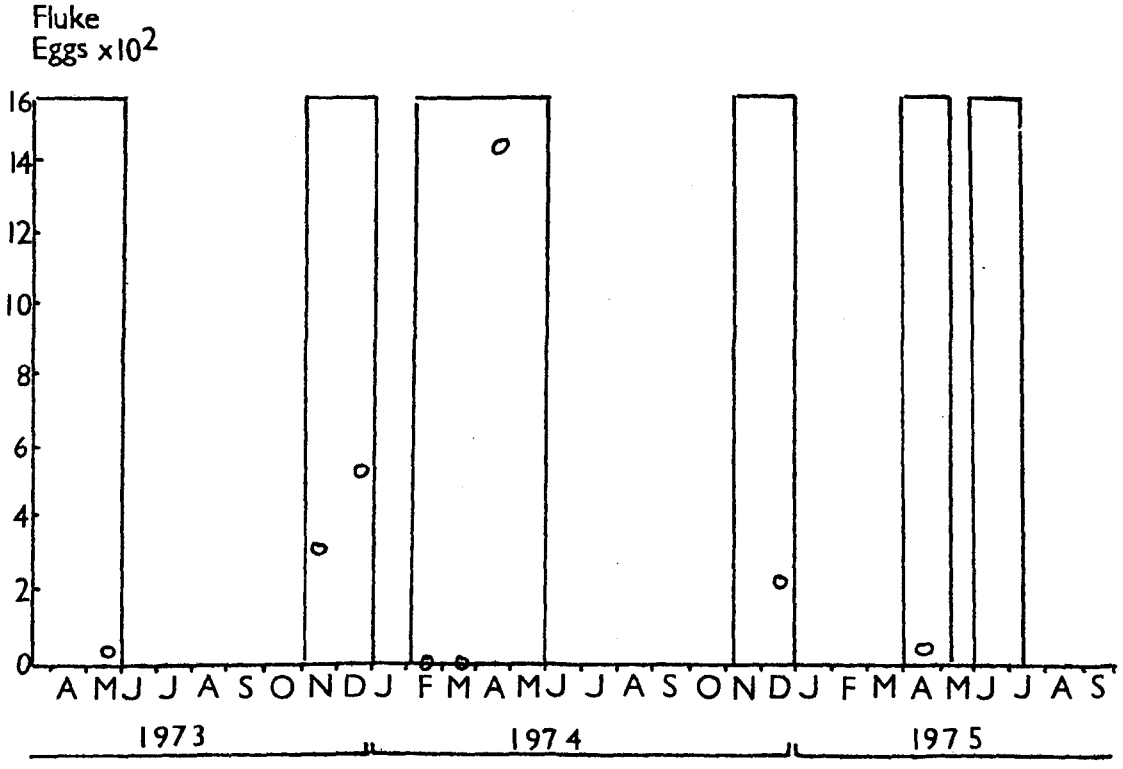


Fig. 6·9 Estimated Daily Input of Fluke Eggs onto Site D, Hendy (cattle)



Boxed areas indicate times when field system 202b was grazed.

Fig. 6·10 Estimated Daily Input of Fluke Eggs to Site B, Thorneythwaite (sheep)



Boxed areas indicate when field 684 was grazed

unreasonable supposition. An examination of the significance of these results will be postponed until later in this chapter when they will be compared with the corresponding redial densities and the microclimate of the two habitats.

(viii) The Distribution of Flukes in the Primary Host Population

A large proportion of the fluke eggs was contained in the faeces of only a small proportion of the farm stock (Figures 6.11 and 6.12). Assuming that the e.p.g. figures are roughly proportional to the number of mature flukes in the liver of the host then it may be concluded that distribution of the mature flukes in the primary host population was over dispersed. Such sample distributions are typical of parasite-host systems and are often adequately described by the negative-binomial distribution (Crofton, 1971a). The data were pooled and the values of the exponent 'k' of the negative-binomial distribution were computed for each farm. In both cases 'k' turned out to be fractional (Hendy, k = 0.06; Thorneythwaite, k = 0.03) indicating that a logarithmic distribution might fit the data better.

The non-random distribution of the mature parasites in the primary hosts mimics that of the larvae in the intermediate host (Chapter 4). Individual differences in diet, behaviour and susceptibility of the farm stock ensure that some individuals have a greater chance of becoming infected than others. Kennedy (1975) suggested that in some cases it is the single heavily infected host that is most significant in the maintenance and transfer of the

Fig. 6·11 Hendy Sheep

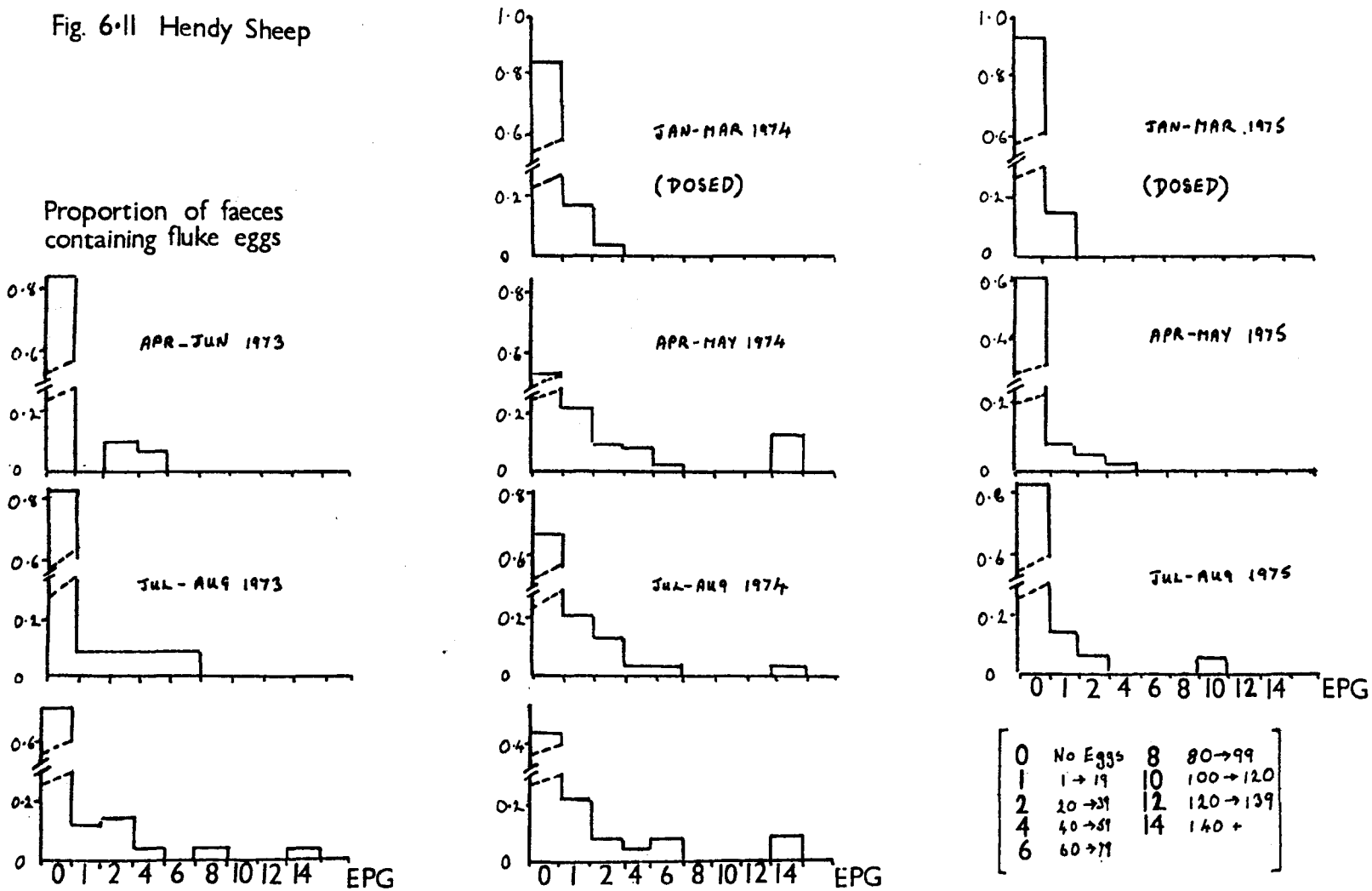
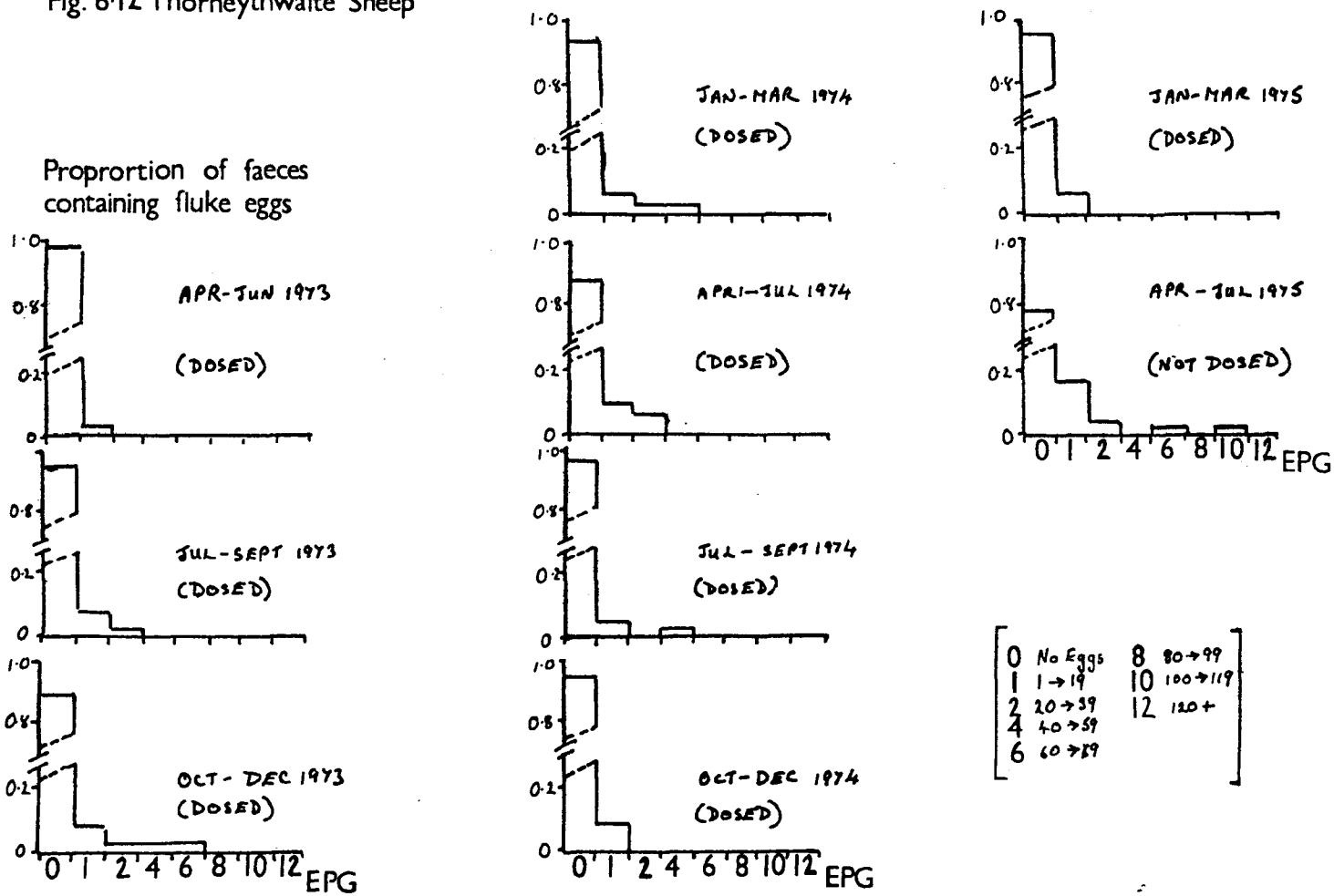


Fig. 6·12 Thorneythwaite Sheep



parasite and, certainly, an animal that tends to graze preferentially in flukey areas will not only ingest larger numbers of metacercariae than others but will also eventually depost larger numbers of eggs into a favourable environment.

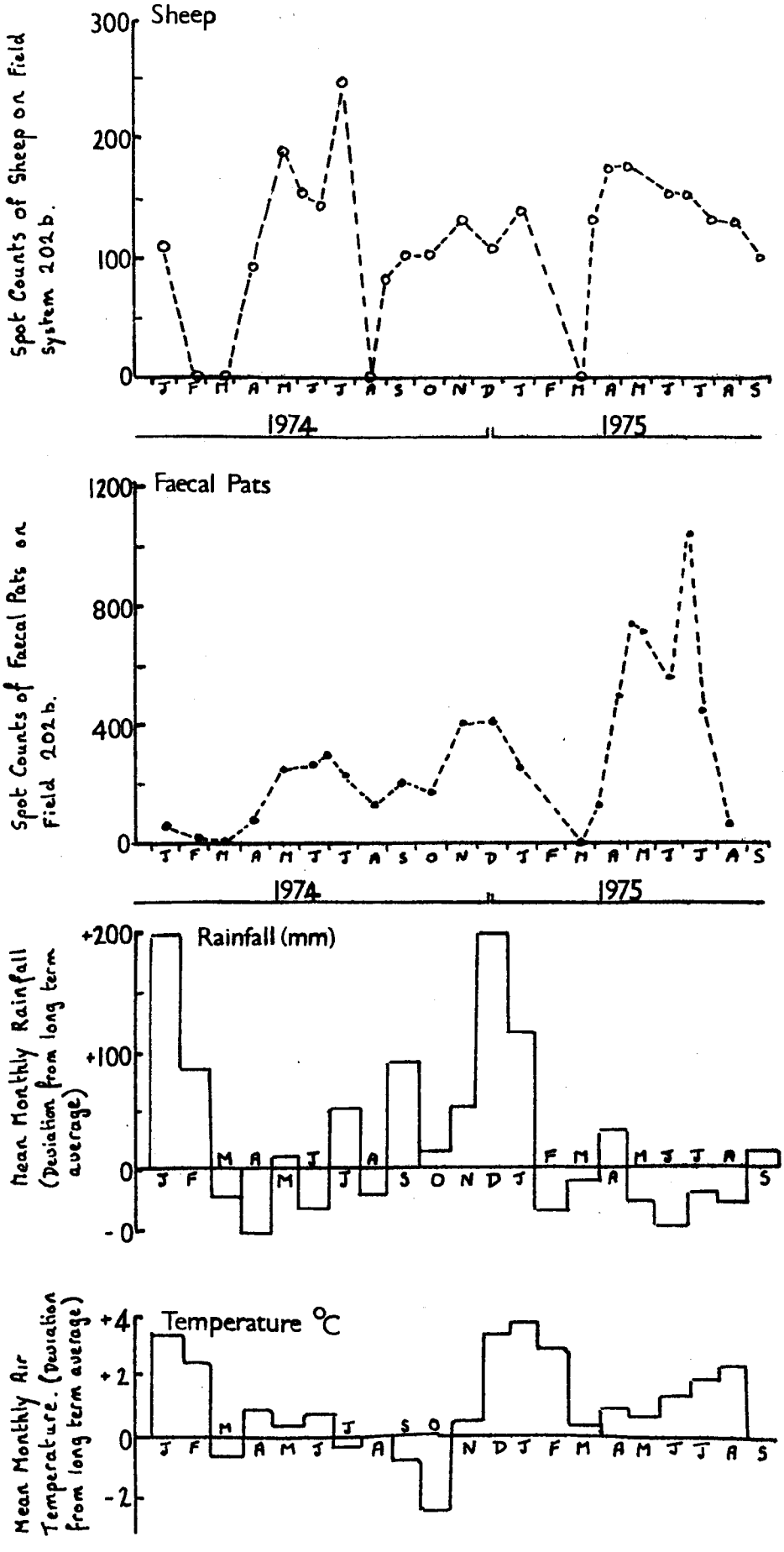
It is noted also that the application of a flukicide to an infected population tends to moderate the skew of the distribution.

(ix) The Dissipation of the Faeces

A fluke egg must be washed clean of faecal material before further development can procede (Rowcliffe and Ollerenshaw, 1960). The dissipation of the faeces containing the eggs is an essential prerequisite for the transmission of the parasite to the intermediate host. Helmsby (pers.comm.) has demonstrated under laboratory conditions using a rain simulator that the rate of dissipation of fresh sheep faecal pellets is related to the intensity and duration of the rain fall. In the field this simple and obvious relationship may be complicated by the desiccatory action of elevated temperatures and the formation of a surface crust. If the eggs are to remain viable then they must be washed clean of the faeces within a month. If the faeces are protected from rainfall altogether then under summer temperatures all of the eggs die within about three weeks (Rowcliffe and Ollerenshaw, 1960).

The density of faecal pats and the number of sheep housed on field system 202b at Hendy were recorded throughout 1974 and 1975. Examination of Figure 6.13 reveals an accumulation of faecal pats in

Fig. 6·13


Sheep
Spot Counts of Sheep on Field System 202b.
300
200
100
0
J F M A M J J A S O N D J F M A M J J A S
1974
1975
Faecal Pats
Spot Counts of Faecal Pats on Field 202b.
1200
800
400
0
J F M A M J J A S O N D J F M A M J J A S
1974
1975
Rainfall (mm)
Mean Monthly Rainfall (Deviation from long term average)
+200
+100
0
-0
J F M A M J J A S O N D J F M A M J J A S
Temperature °C
Mean Monthly Air Temperature. (Deviation from long term average)
+4
+2
0
-2
J F M A M J J A S O N D J F M A M J J A S

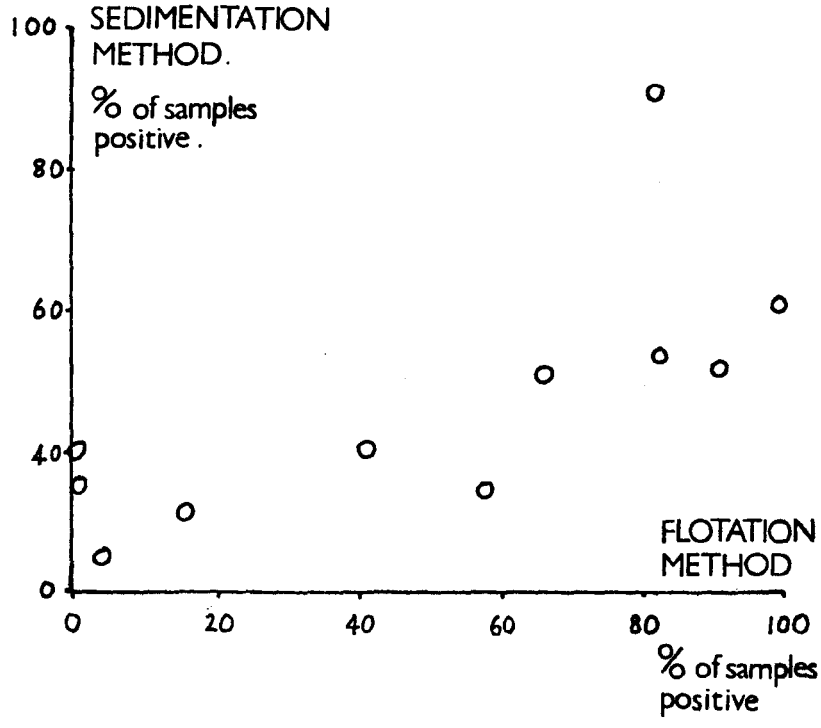
the spring and summer of 1975 that approached values two and three times greater than the densities recorded in the previous year. The densities of sheep were no higher but the mean monthly rainfall, particularly in May and July 1975, was well below that of the previous year and mean monthly air temperatures were correspondingly higher. It is reasonable to suppose that the proportion of fluke eggs washed free of faeces in 1975 was less than that in 1974.

4. DISCUSSION

(i) The Prevalence of Fascioliasis

An index of the proportion of stock acting as host to F. hepatica is provided by the percentage of faecal samples that contained fluke eggs. It was possible to ratify the results presented in section (iv) by comparison with values obtained independently, at least in the case of the Welsh farm. The Veterinary Investigation Centre, Bryn Adda, Bangor, provides a diagnostic service for farms in Gwynedd. The Centre uses a zinc flotation technique to extract fluke eggs from faeces (M.A.F.F. Tech. Bull. No. 18) and had examined samples from Hendy on various occasions throughout the period between April 1973 and September 1975. The samples were not necessarily from the fields containing the snail habitats under study and the discrepancy in the timing of corresponding collections was sometimes as much as three weeks, nevertheless, there was a significant correlation (corr.coef. = 0.71, $p < 0.05$, d.f.9) between the two series of estimates of the percentage of faecal samples containing fluke eggs (Figure 6.14). Samples collected in those months when the sheep were dosed were excluded from the comparison.

Fig. 6·14


100
SEDIMENTATION METHOD.
% of samples positive.
80
60
40
0
0
20
40
60
80
100
FLOTATION METHOD
% of samples positive

Frequent application of flukicides at Thorneythwaite kept the proportion of sheep infected with flukes down below 25% until the farm changed hands in 1975 when dosing was discontinued for a time. The marked increase in the proportion of infected animals noted in May and June of 1975 was probably due entirely to the influx of untreated ewes onto the fields containing the habitats. These sheep, brought down from the fell in time for lambing, would normally have been dosed within a week. This was not done and so the infection picked up between January and April from habitats on the fell was undiminished.

At Hendy, where the sheep were dosed with flukicide only once a year, the proportion of infected animals was generally higher. A large seasonal increase in prevalence during the winter of 1973-4 was not observed the following year indicating that the uptake of viable metacercariae in the autumn of 1973 was considerably more than in the autumn of 1974. In the winter of 1973-4 also, viable cysts persisted on the pastures in sufficient numbers after the application of a flukicide in January to permit the proportion of infected animals to rise once more in excess of 50%. The summer decline that followed and which occurred also in 1973 and 1975 was probably due to the progressive dilution of the ewes' faeces with those of the lambs. A large seasonal increase in the proportion of infected cattle at Hendy occurred not only in the winter of 1973-4, as was the case for sheep, but also in the following winter of 1974-5. However, in 1973-4 the infection persisted in a large proportion of the animals even after the January dosing, this did not occur in 1974-75 suggesting, again, that in the autumn and winter of 1973-74 viable metacercariae were ingested in

higher numbers and over a much longer period than in the corresponding months of 1974/5.

At Hendy then, the variations in the proportion of stock that were infected with flukes conformed to the seasonal pattern expected of successive "summer infections" (Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1971). At Thorneythwaite the expected variations were eradicated by the frequent application of flukicide. In neither farm was there any evidence that a "winter infection" contributed significantly to the variations in disease incidence. (The spring increase seen at Thorneythwaite in 1975 occurred much too early to be accounted for in this way (Ollerenshaw 1971) and is best explained by the influx of untreated stock as above).

(ii) The Intensity of Infection

An estimate of the relative intensity of infection with F. hepatica was provided by the geometric mean e.p.g. recorded for each month in Figs. 6.5, 6.6, and 6.7. The frequency of dosing at Thorneythwaite was so great that seasonal differences were generally obliterated but at Hendy, where the frequency of dosing was much less, the average intensity of infection in both sheep and cattle was greatest during the late winter and spring of 1974. The overall mean e.p.g. for sheep (22.09) at Hendy was about 13 times greater than that estimated for cattle (1.69) on the same farm. To some extent at least this was due merely to the fact that cattle produce about 15 times more faeces than sheep each day and so the concentration of fluke eggs in the droppings was much less. The remainder of the difference may have been due to the rejection of many

of the adult flukes that is typical of bovine fascioliasis (Kendall and Parfitt, 1975) or else simply a reflection of the different management procedures that applied to sheep and cattle at Hendy.

In order to estimate the actual intensity of infection in terms of the number of mature flukes in each host rather than the relative intensity of infection that is indicated by the geometric mean e.p.g. then the relationship between the fluke burden and e.p.g. counts needs to be numerically expressed. Given the assumption that the concentration of fluke eggs in the faeces is proportional to the number of adult flukes in the liver of the host then the results presented in subsection(viii) of this chapter suggest that mature F. hepatica were distributed in a logarithmic way among individuals in the host populations. Thus the geometric mean e.p.g. engenders a misleading view of the actual disease status of the farm stock on Hendy and Thorneythwaite. Individual egg counts should be used.

Honer (1971) has reviewed some of the literature concerned with the relationship between egg counts and the intensity of infection. He quotes suggestions by Taylor (1964) that 1 e.p.g. of cattle faeces and 25 e.p.g. of sheep faeces represent one adult F. hepatica. Boray (1969) reports that the average e.p.g. per fluke varied between 12 and 33 in sheep infections depending upon the intensity of infection. These results are complicated by various findings. First there is evidence of a diurnal fluctuation of egg output in cattle, though apparently not in sheep. Second, there is an inverse relationship

between the density of flukes in the liver and the rate of egg production per fluke and third, the daily egg output per fluke can double from 9.3 e.p.g. in the 13th week post infection to 20.2 e.p.g. in the 27th week post infection (sheep). (See Boray, 1969, for a brief review). In addition, the reported variation in daily egg production of an individual fluke is often as high as three times the mean (Happich and Boray, 1969b). However, the relationship reported in Appendix I, that in sheep 1 fluke is represented by an egg count of 12 e.p.g. (which is consistent with the results reported in the literature), will be used here for the sake of simplicity without further consideration of these complicating features.

There were no losses from acute fascioliasis reported on either farm but one case of chronic ovine fascioliasis was diagnosed during the summer of 1974 at Hendy. (C. M. Edwards, pers.comm.) Soulsby (1965) suggested that a faecal egg count in sheep of between 200-500 e.p.g. indicated disease. Boray (1967) and Happich and Boray (1969b) suggested that fluke densities in excess of 250 per liver produce serious chronic fascioliasis with a much reduced life expectancy while sub-clinical chronic fascioliasis is produced by up to 100 flukes per liver. Inspection of the raw data revealed 12 individual fluke egg counts at Hendy and 1 at Thorneythwaite which exceeded 100 e.p.g. In 2 cases at Hendy egg counts approached 200 in April and May 1974 and two other cases in the same months on the same farm exceeded 300 e.p.g. A detailed analysis follows.

Hendy (Sheep)

Date	e.p.g.	Estimated fluke burden	Probable disease status
Dec. 1973	155	13	Sub clinical chronic fascioliasis
Apr 1974	143	12	"
	199	16	"
	457	37	"
May 1974	193	16	"
	315	25	"
	156	13	"
June 1974	146	12	"
Aug 1974	128	10	"
Oct 1974	135	11	"
	168	14	"
Nov 1974	134	11	"
Thorneythwaite (Sheep)			
Apr 1975	113	9	"

Most of the Welsh cases can be confidently assigned to the "summer infection" of 1973-74 and it is to be noted that the only case at Thorneythwaite occurred during the period when dosing had ceased.

(iii) The Course of the Infection 1973-1975 (Site D and Site B compared)

The prevalence of fascioliasis and the intensity of the infection in the primary hosts was greatest at Hendy during the winter of 1973-74. It has been argued that the density of metacercariae on the pasture was

probably much less in the following winter of 1974-1975. At Thorneythwaite, the ewes were so frequently dosed that seasonal variations in prevalence and intensity were not observed.

It was not possible to ascribe the infection of the primary hosts to particular habitats but details concerning the management of the stock throughout the year could be used to identify the approximate source of metacercariae and also provided information about the length of time for which habitats were exposed to infected animals. At Hendy, for example the field system which contained site D was continuously grazed by stock infected with F. hepatica except for an interval of only a few weeks in February and March 1975. Site B, at Thorneythwaite, on the other hand was exposed to infected ewes on only two occasions in the year: about four weeks in April and May, and up to six weeks in November and December. Much of the infection at this farm was probably acquired from habitats on the fell side; the establishment of an additional infection picked up from site B itself depended upon precisely when the sheep were dosed. In 1973, for example, a flukicide was applied in mid November before the ewes were exposed to the habitat whereas in 1974 the sheep were dosed at the end of December after they had been exposed to the habitat. The situation at Hendy was rather simpler. Any infection that the sheep may have picked up between August and December from site D and other habitats in that field system would have been reduced by the application of a flukicide in the beginning of January and the bulk of any subsequent reinfection was acquired from habitats in fields 100 and 99 (See Figure 2.2.)

The daily input of fluke eggs to sites D and B is compared with redial abundance and the microclimate of the habitats in the composite Figures 6.15 and 6.16. With a few exceptions the estimated rates ranged between 0–400 eggs per day. By assuming that each estimate was representative of the whole month for which it was made it was possible to calculate the cumulative input for certain periods. In 1974, the total numbers of eggs deposited on site D, Hendy, by sheep and cattle respectively were of the order 112,000 and 33,500. Between February and May alone in that year over 60,000 eggs were deposited in site D resulting in an accumulated density of around 15,000 eggs per m^2. The equivalent figure for 1975 was only 300 eggs per m^2 reflecting not only the reduction in prevalence and intensity of infection but the interval during which the field was empty of stock. At site B, Thorneythwaite, in 1974 the spring accumulation of eggs was of the order 1000 eggs per m. It was not possible to make an equivalent estimate for 1975 because of the uncertainty regarding alterations in the management practices after the farm had changed hands. Locally, of course, the actual densities of fluke eggs would have been far in excess of these figures. The stock move over the habitats in a non-random manner, dependent upon the accessibility and abundance of suitable grazing, so that the faeces are more likely to be deposited in some ares of habitat than others.

The fate of the fluke eggs can be inferred from an examination of Figures 6.15a and 6.16b. Once they have been washed clean of faecal material the eggs remain viable provided that they are covered by a surface film of water (Rowcliffe and Ollerenshaw, 1960). Such a film is likely to persist provided that the soil surface is at least "wet"

Fig. 6·15 Site D, Hendy

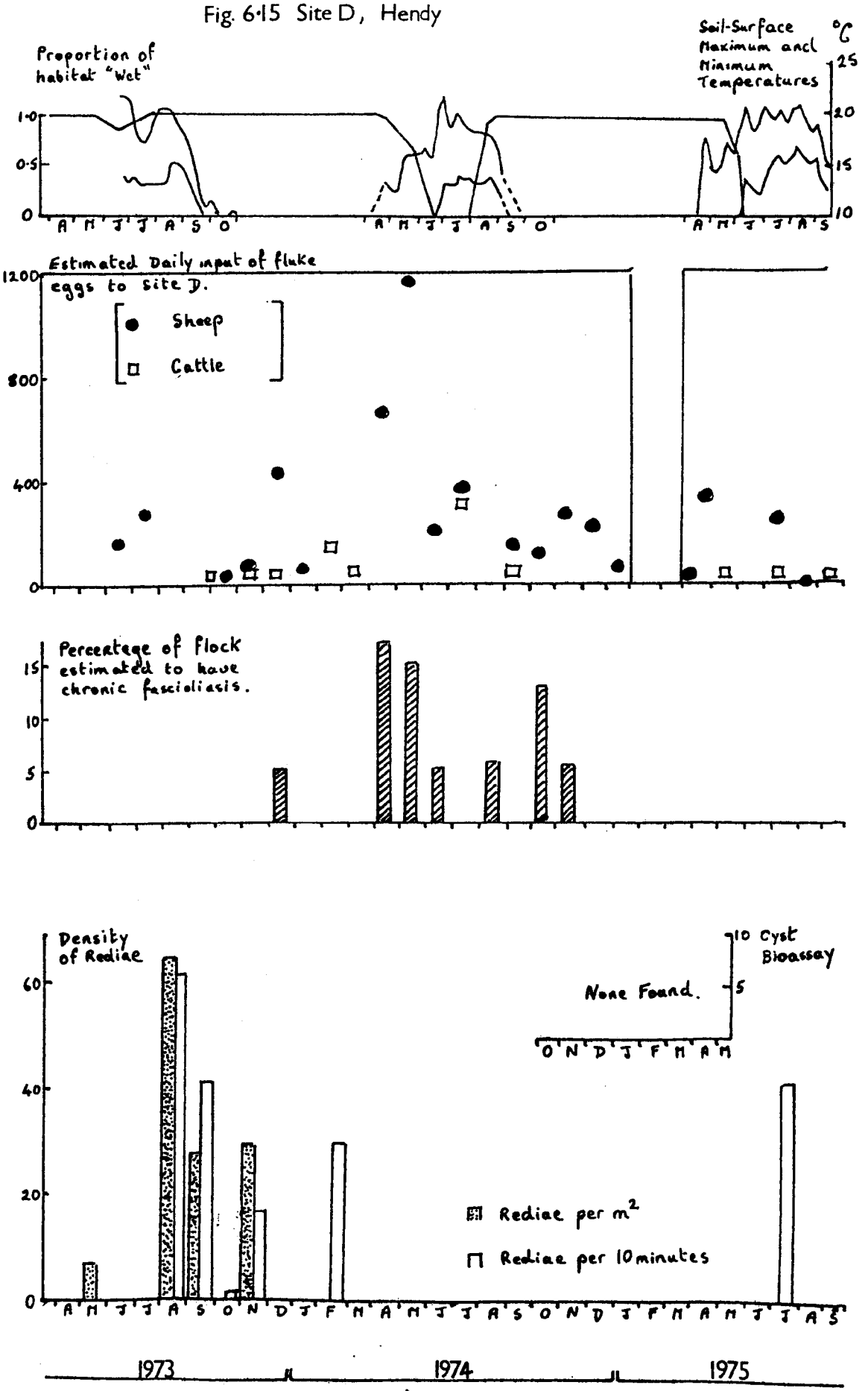
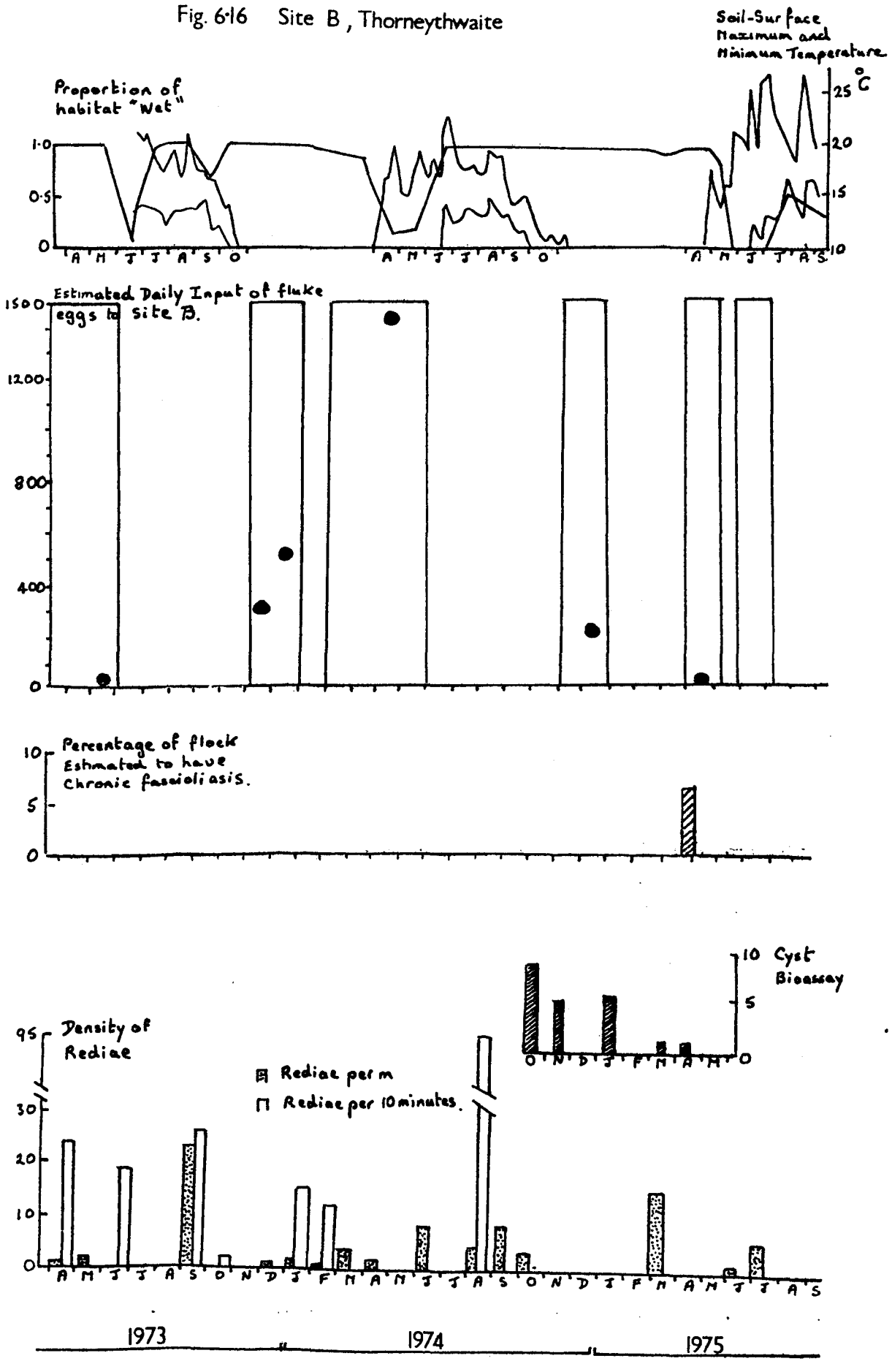


Fig. 6·16 Site B, Thorneythwaite



and then the main limitation to further development is the ambient temperature. Development is negligible below 10°C and under field conditions a minimum of three weeks is required for the miracidia to hatch out, though the average period of development is probably twice that. (Rowcliffe and Ollerenshaw, 1960). Taking site D first: there was only a small proportion of the habitat that did not provide suitable conditions of soil surface moisture at any time between April and October 1973. There was a substantial increase in the number of infected snails found in August of that year which implied that numbers of miracidia had hatched out in July. Suitable surface moisture conditions had prevailed since the wintertime and assuming that mean surface temperatures first exceeded 10°C some time in April then there had been probably 8-10 weeks in which the fluke eggs could develop. In 1974 the whole area of the habitat was rated as "damp" or "dry" on four successive occasions between the 2nd June and the 30th July. Such conditions were assumed to be inimical to the survival of the fluke egg whether in a faecal pat or free of faecal material (Rowcliffe and Ollerenshaw, 1960). This left only two months on either side of the drought when development was possible. The habitat was already drying out in April and the weekly mean soil surface temperatures did not exceed 12°C until the third week in May. (Development takes 40 days at 15°C). It is unlikely that development under these conditions would have proceeded to hatching before the onset of the drought in June. The daily input of eggs onto the habitat was in fact greatest in May, just before the drought, so it is probable that these eggs perished in the

faeces. The return of "wet" conditions in August coincided with well below average air temperatures and mean soil-surface temperatures that were 2-3°C lower than over the equivalent period in 1973. The opportunity for complete development of the eggs was consequently rather small. In addition, the density of the intermediate hosts was very low indeed. Eventually, only one infected snail was found and there was no evidence of metacercariae in herbage samples taken from the site throughout the winter of 1974-1975. The situation was very similar in 1975 except that the drought had not ended even by September when observation ceased.

Site B at Thorneythwaite offered a striking contrast. The spring drought of 1974 was certainly more severe here than in 1973 but it occurred much earlier than at Hendy and by the 17th of June the habitat was rated as "wet" again over the whole of its area. There remained nearly three months of optimum surface moisture conditions before the weekly mean soil-surface temperatures subsided once more below 10°C. This may have been sufficient for some of the newly acquired infections to complete their development within the snail before October when the first samples of herbage were tested for metacercariae but it is more likely that most of the cysts originated from mature infections that had overwintered from 1973. Nevertheless, maximum soil surface temperatures in excess of 10°C were recorded not infrequently until mid December so the possibility that a few of the infections acquired in 1974 matured and shed their cercariae in the same year must be considered. (Release of cercariae is inhibited below 9°C, Kendall and McCullough, 1951).

The greatest density of infected L. truncatula was found on both farms in the summer of 1973 though one month later at Thorneythwaite than Hendy. This may have been a consequence of the rather more severe spring drought and generally lower surface temperatures experienced at the Cumbrian site.

CHAPTER 7. THE CONTROL AND REGULATION OF F. HEPATICA POPULATIONS

1. INTRODUCTION

In an ecological context the word stability has been used in at least two senses (Anderson 1974). It may refer to the ability of a species to resist environmental change and is measured by the length of time for which a population has persisted in a given area or else it suggests a population's capacity to return to an equilibrium state after the number of individuals has been altered by variations in factors extrinsic to the system. The fluke populations of North Wales, for example, are certainly stable at least in the first sense since fascioliasis has been recorded in the ewes on Welsh hillsides for as long as 10 centuries (Crossland, in press). However, not much is known about the stability of fluke populations in the second sense, that is to say about the factors which control and regulate the numbers of individuals.

A regulated population is one which tends to an equilibrium density following any departure from this level. The processes which bring about this regulation are necessarily dependent upon the density of individuals in the population but need also to result in the mortality of a greater proportional number of individuals as the population density increases if regulation is to occur (Solomon, 1969; Varley et al., 1973). Density-dependent mortality may arise as the result of competition, predation or parasitism but the interaction must be carefully examined in each case to establish whether or not the criteria for regulation are

fulfilled. Factors that affect a population in a way that is unrelated to its density are called density-independent. Such factors cannot regulate the density of a population but may be a significant cause of population change and are held to determine or control population density. The operation of these factors may bring about perturbations in population density which are either away from or towards the equilibrium level that is set in the first instance by the carrying capacity of the environment but any apparently stabilising influence they may have is entirely fortuitous. It is a logical requirement that regulation proper is brought about by density-dependent processes (Lack, 1966).

Much of the research into fascioliasis has been concerned with those factors which control rather than regulate parasite numbers. The stability of the system and the mechanisms which might promote it have received scant attention. These are by no means trivial considerations. It may be, for example, that intermediate host parasite systems like Fasciolas are inherently unstable (Kennedy, 1974) in which case attempts to create fluke free areas in regions where fascioliasis was otherwise endemic would not be unreasonable. In any event, the feasibility of fluke-eradication programmes will be decided by the power of the regulatory processes that operate at low parasite densities. Even if it proves impossible to eradicate fascioliasis altogether then a more precise understanding of how the equilibrium level is determined and maintained would be of material use in bringing about a more permanent reduction in the prevalence of the disease.

In the introduction to this thesis I wrote that one of its aims was to describe the system that the research group at York was attempting to model. Like others before me I found that I was concerned principally with those factors which controlled rather than regulated parasite numbers but the description towards which I was working would not have been complete had I made no reference at all to the latter. In describing the system I have drawn as much upon the literature as upon my own field experience. It was simpler to consider the primary and intermediate host systems separately and I have postulated a descriptive model for each. (Figures 7.1 and 7.2). The models constitute a series of hypotheses about the way in which the number of parasites might be controlled and represent a summary of my understanding of the epidemiology of fascioliasis. The thick lines in the figures represent the flow of actual quantities, the thin lines the flow of information. The curves are approximate indications of the relationship between the variables. In one case the shape of the curve is frankly speculative (?) but in all others the form of the relationship has been inferred from the literature.

2. PROCESSES WHICH ARE POTENTIALLY REGULATORY

(i) The Mortality of Heavily Infected Host Individuals

Regulation occurs if the chances of an infected host dying are proportionately greater at high rather than low parasitic densities. The efficiency of this form of regulation is thought to increase as the

Fig. 7·1 Intermediate Host—Parasite System. (On a Single Snail Habitat).

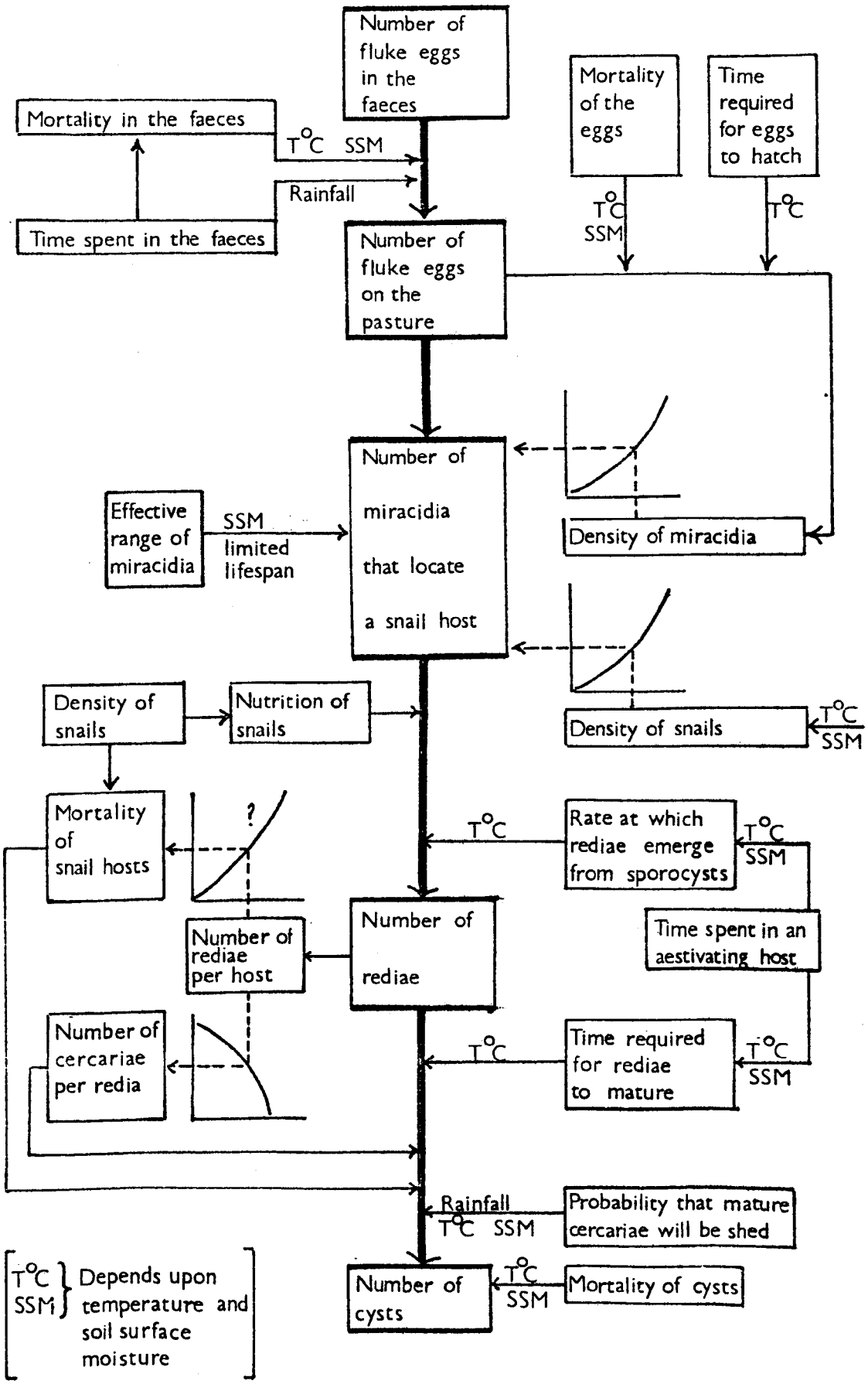
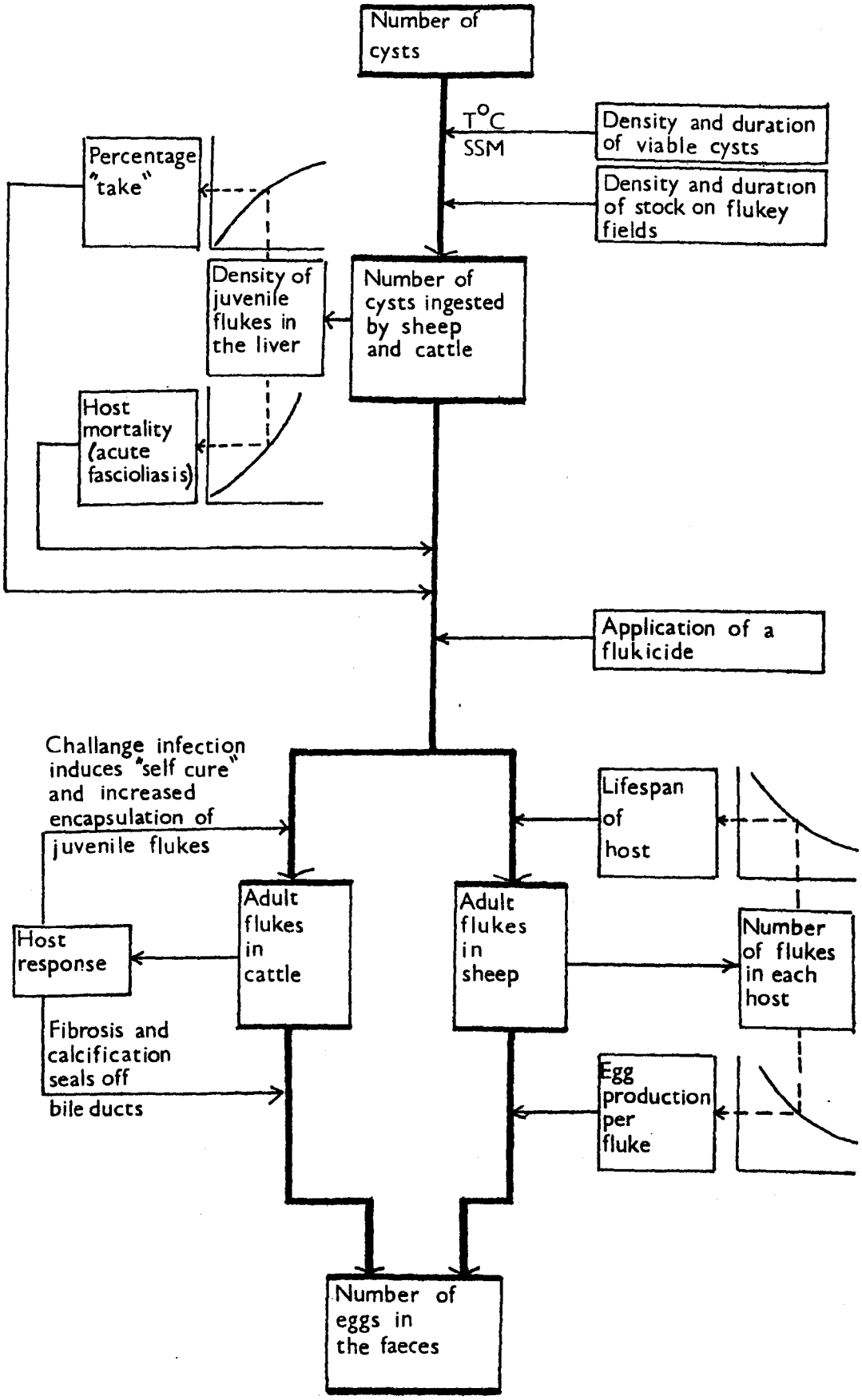


Fig. 7·2 Primary Host—Parasite System



parasite population becomes over dispersed (Bradley, 1972, 1974; Crofton, 1971a). The mortality of a large proportion of the parasite population is then brought about by the death of a relatively small number of hosts. The sheep and young cattle which die each year of acute fascioliasis must account for large numbers of juvenile flukes. During epidemic years huge numbers of stock die before the parasites have even matured as a result of acute disease. Even in chronic infection where the flukes have had time to mature the life span of the host depends upon its parasite burden (Happich and Boray 1966) and so the potentially large egg output from heavily infected animals is curtailed by their early death.

Host mortality is likely to be greater when the host population is additionally stressed. The ability of stock to cope with the debilitating effect of fascioliasis is much less when there is a reduction in the amount of protein included in the diet (Crossland, in press) and some tentative evidence was presented earlier that infected L. truncatula may be at a competitive disadvantage at high snail densities. Certainly, under drought conditions in the field, the mortality of infected snails is higher than that of uninfected ones. In normal laboratory cultures of snails there appears to be no difference in mortality rates, the populations are unstressed, but parasitic castration has been shown to occur in the infected snails (Nice, pers.comm.) There exists the possibility that by reducing the egg output of the snail population high densities of parasites in one year might lessen the chances of miracidial transmission in the next by reducing the density of the host population.

(ii) The Induction of an Immune Response in Vertebrate Hosts

The ability to respond to foreign antigens is a characteristic of vertebrates but it has proved impossible to demonstrate the existence of a specific response to infection by F. hepatica in sheep. Cattle on the other hand are resistant to invasion. In primary infections the inhibition of migrating flukes appears to depend upon the density of the parasites in the liver exceeding a particular threshold and it has been suggested that the occurrence of a 'self-cure' seen after subsequent challenge infections indicates the presence of an acquired immune mechanism. It is thought that the cirrhosis which occurs as a result of the primary infection provides a barrier to further infections and when combined with an immune response results in an increased likelihood that migrating flukes will be inhibited in the parenchyma of the liver at much lower densities than is seen during primary infections. (Ross, 1966, 1967). In addition the calcification of bile ducts which is characteristic of prolonged bovine fascioliasis and which depends upon the parasite burden eventually seals off the route by which the fluke eggs leave the liver and enter the alimentary canal of the host.

(iii) Intra-specific Competition within the Hosts

Boray (1969) found that the establishment and development of F. hepatica in sheep was severely affected by overcrowding. The percentage of flukes that became established in the bile ducts was lower with very heavy infections of metacercariae (see also Sinclair, 1962) and the prepatent period was longer. This may be due to the mutual interference experienced by large numbers of flukes migrating

simultaneously through the liver parenchyma. Boray (1969) also reported that the number of eggs per fluke declined as the fluke burden increased. The precise reason for this in sheep is not clear.

So far as the infection within the intermediate host is concerned, Kendall (1949) has shown that the number of rediae within each snail depends upon the nutrition of the host and that in turn the numbers of mature cercariae present in each redia is inversely related to the density of redia. Nice (pers.comm.) has found that the cercarial output from infected snails is not increased if the snails are infected with several miracidia rather than just one.

(iv) Inter-specific Competition within the Intermediate Host

Apart from F. hepatica the most frequently encountered trematode parasite of L. truncatula is Cercaria cambrensis I. Between 1973 and 1975 nearly 150 infected snails were found on the habitats at Hendy and Thorneythwaite. In none of these did F. hepatica and C. cambrensis I occur together. Rees (1932) dissected over 5,000 L. truncatula and found F. hepatica and C. cambrensis I together in the snail only once. This suggests but does not prove competition between these two species for snail hosts. It might merely reflect the very low probability of any miracidium infecting a snail.

(v) The Regulation of F. hepatica Populations in the Field

If the density of parasites is to be regulated by any of the processes outlined above then there must be a greater proportional increase in the adverse affect as the density of the parasites increases.

This is easily demonstrated where the effect has been measured in terms of the performance per individual parasite (e.g. the egg production per fluke or the number of cercariae produced by each redia) but is less obvious in those cases where the mortality of the hosts is dependent upon the parasite burden. The overdispersed nature of the parasite population on both Hendy and Thorneythwaite has been referred to several times in this thesis and it is indeed likely that given such a situation there would have been a proportional increase in host mortality as the numbers of parasites increased. Even so the regulatory nature of such processes in the field remains speculative in this case rather than proven.

3. THE PROCESSES WHICH CONTROL RATHER THAN REGULATE PARASITE NUMBERS

The microclimate of the snail habitats controls rather than regulates the number of parasites for its influence varies independently of the parasite density and depends in a general sense upon the fluctuations in local weather conditions. Soil surface temperature is a rate limiting factor and determines the proportion of parasites in any particular stage that completes development before the chances of progressing to the next stage are terminated by the death of the host or an adverse change in soil surface moisture. High temperatures in conjunction with low levels of soil surface moisture may also cause the death of those parasite stages that occur on the herbage and increase the probability that the development of the sporocysts and

rediae within the snails continues at a much reduced rate due to the aestivation of the intermediate hosts. Soil surface moisture is a permissive factor. When the soil surface dries out the development of the parasite within the snail is reduced whatever the temperature since the host ceases to feed or may be curtailed altogether if the snail succumbs to dessication. The survival of the fluke eggs, the invasion of the snails by miracidia, the shedding of cercariae and the subsequent survival of the metacercariae are all contingent upon the presence of adequate soil moisture conditions.

At Hendy and Thorneythwaite the most significant feature of the habitat microclimate appeared to be the drier period that occurred during the spring and early summer of each year and here, at least, an assumption essential to the fluke forecasting system described by Ollerenshaw and Rowlands (1959) has been confirmed by the events on the habitats observed in this study. The fluke forecast is based upon the cumulative difference between rainfall and evapotranspiration for May, June and July since a forecast issued later than mid August could not be acted upon. Comparison of site D, Hendy, and site B, Thorneythwaite, demonstrated the qualitative relationship that exists between the soil-surface moisture of the habitats during the spring and early summer and the subsequent variations in abundance of the flukes and snails. The duration, intensity and timing of the dry period were all of significance. The spring and early summer of 1974 nicely illusrates the case. There was a severe reduction in the density of snails and rediae at site D during 1974 but the populations at site B were relatively

unchanged. There was no difference in either the duration or intensity (proportion of habitat affected) of the drought on the two habitats but at site B optimal moisture conditions were re-established two months earlier leaving at least three months when soil-surface temperatures were suitable for the development of both the fluke and snail.

Variations in the microclimate of the habitats between May and July affect the transmission of the parasite between primary and intermediate host. Ollerenshaw clearly believes that the mortality of the fluke eggs which occurs during this period is the key factor controlling the number of metacercariae that eventually appear on the pasture but his ecological arguments in support of this claim are ill founded. (See Chapter 1). The later stages of the transmission sequence are also susceptible to adverse changes in soil-surface microclimate. The miracidial invasion of L. truncatula, for example, is a particularly fragile process. The miracidia are very short lived (24 hours or so), their effective range appears to be limited by the extent of standing water that covers the habitat and the probability of locating a host is a function of the relative density of hosts and parasites. (Wilson and Taylor, 1978.)

An intermediate host-parasite system in which the number of parasites is determined entirely by variations in transmission is likely to be very unstable (Bradley, 1974) Local extinctions will be the rule. The parasite within the snail is most likely to persist in those areas that support habitats of different kinds which exhibit a

varied response to adverse changes in local weather conditions. The extent to which this description applies to the F. hepatica/L. truncatula system is unknown; potentially regulatory processes which might stablise the system have been identified in the laboratory (e.g. competitive interaction between the rediae) but whether or not these actually operate in the field remains to be seen.

At the primary host level the principal controlling factor is the application of a flukicide. At the moment this is carried out on a routine basis with the minimum of reference to the actual density of parasites within the hosts and so may be included as a controlling rather than regulatory influence.

4. THE FOCAL NATURE OF FASCIOLIASIS

So far I have paid no attention to the focal nature of fasciolliasis, indeed the descriptive models presented in Figures 7.1 and 7.2 have been so constructed as to ignore the discontinuous distribution of the snail habitats. Even on individual farms there was no reason to assume that the patterns of population change would be similar and the marked differences in soil-surface moisture conditions that prevailed on sites subjected to the same microclimatic conditions only served to reinforce this view. The transmission of F. hepatica from intermediate to primary host and vice-versa is surely sensitive to what Bradley (1972) calls focus geometry. Successful transmission depends upon the spatial and temporal coincidence of hosts and infective parasite stages. This in turn depends upon the number, size and distrubtion of the

habitats, the management of farm stock and the development time of the parasites within the hosts and upon the herbage. These parameters will themselves vary from year to year being affected to differing degrees by fluctuations in the local weather conditions and changes in land use (some of the largest L. truncatula habitats were lost to this study within the first few months because the sites were ploughed up!) The fluid interplay between stock movement and the changing focus geometry of the snail habitats presents a descriptive problem of considerable complexity. In principle then, a simulation model of the parasite-host system for a single snail habitat would appear to be a more tractable proposition than any attempt to model the total situation on a farm which may contain more than thirty different sites.

CHAPTER 8. SUMMARY

So far as I am aware the work reported in this thesis represents the first sustained attempt to compare and contrast the microclimate of particular L. truncatula habitats over a succession of seasons and to interpret the population dynamics of F. hepatica and its intermediate host directly in terms of the soil-surface moisture conditions and soil-surface temperatures experienced in the field. As such I have depended greatly upon the field work of others who have studied these organisms in relation to more easily measured climatic parameters such as rainfall and air temperature and also upon the laboratory work of those who have demonstrated the extent to which the viability and development of the fluke and snail are related to temperature in particular. In addition I know of no other study in which all of the accessible stages of the fluke and snail life cycles were monitored simultaneously on individual habitats.

I conclude with a summary of those areas in which I believe that this thesis made an original contribution to the study of the epidemiology of fascioliasis and to the study of the biology of L. truncatula in particular.

1. A general qualitative relationship was demonstrated between the temperature at the soil surface of the habitats of L. truncatula and the air temperature measured at a height of 1m. During the months between April and October the monthly mean temperature at the soil-surface was

always higher than the corresponding monthly mean air temperature; for the remainder of the year the monthly mean temperatures as the soil surface were lower than the corresponding air temperatures. The difference was sometimes as much as $4^{o}C$. The daily temperature range at the soil surface was always less than that experienced at screen height particularly during the autumn and winter. Temperature maxima on the habitats exceeded the corresponding maximum air temperatures only in the spring and early summer when the moisture content of the pastures had fallen below field capacity.

The relationship between soil-surface and air temperatures was interpreted in terms of the shelter provided by the vegetation covering the habitats and the effect of variations in water content on the thermal conductivity of the soil. Since these parameters varied from habitat to habitat it was concluded that a general quantitative relationship between the temperature at screen height and the temperature at the soil surface could not be established.

2. A visual method for the estimation of soil surface moisture was described. The method used a four point scale ranging from "dry" to "standing water". The categories used constituted an ordinal rather than interval scale with respect to soil moisture but were deliberately chosen so as to correspond with conditions for which the responses of the snail and fluke had already been established. The method is rapid, requires no instrumentation and may be used to estimate the total area of habitat over which a particular moisture condition prevails. It had

the additional advantage that it dealt directly with the moisture regime actually experienced by the organisms in the habitat.

3. Though all of the habitats studied were wetter than the surrounding pasture and generally characterised by helophytic plant species of the Agropyro-Rumicon crispi alliance there was otherwise considerable heterogeneity in size, aspect, drainage characteristics, mean depth of water and the density of the plant cover. At Thorneythwaite the soil on the lower slopes of the fell was found to be a stagnopodzol. Such a soil normally drains well and so the habitats here depended upon a very high rate of direct precipitation and the continuous overflow from fell streams. At Hendy, and on the valley bottom at Thorneythwaite the soils showed considerable gleying. Though the annual rainfall was one third of that at Thorneythwaite the poor drainage characteristics of the soil were sufficient to maintain a large number of habitats.

The differences between one site and the next rendered futile all attempts to establish a precise quantitative relationship between the soil surface moisture of the habitats and some areal average like percentage soil water, pF, or soil moisture deficit though it was suggested that this last parameter was the most convenient and useful general measure of soil moisture so far as the epidemiology of fascioliasis is concerned.

4. The intermediate host-parasite systems on two contrasting sites were described in relation to variations in the microclimate of the habitats. The most significant annual feature of the microclimate

appeared to be the period of drought that occurred in the spring and early summer. The duration, intensity, and timing of the drought were all important. It was argued that in terms of its effect on both the parasite and intermediate host populations the soil-surface temperature of the habitat could not be regarded as a conservative factor from year to year.

5. (a) An overdispersed distribution was characteristic of both the snail and parasite populations.

   (b) The mean daily input of fluke eggs onto the habitats was calculated to be between 0 and 400 eggs per day though some estimates were in excess of 1000 eggs per day.

   (c) The anatomy and histology of the reproductive system of L. truncatula was described together with an account of the previously unrecorded copulatory activity of the snails. The rate of oviposition was found to be related to the ambient temperature and egg masses were more often laid in shaded situations where the probability of dessication was least. (See Appendices IV and V).

6. Various control and regulatory mechanisms were postulated for the entire parasite-host system and expressed in diagrammatic form as descriptive models. The intermediate host-parasite system and primary host-parasite system were treated separately. The models constituted a series of hypotheses concerning the way in which the

incidence of fascioliasis might be determined by factors extrinsic to and intrinsic to the system and were intended to provide a coherent framework for further research.

APPENDIX I

THE SEDIMENTATION METHOD OF EXTRACTING THE EGGS OF F. HEPATICA FROM FAECES

1. INTRODUCTION

The procedure for estimating the number of fluke eggs per gramme of faeces was described in Chapter 6. The method was a modification of one described by Happich and Boray (1969). The principal differences were these:

(a) The sedimentation columns comprised "quick-fit" air condensers and detachable stopcocks rather than the test tubes used by Happich and Boray. If large numbers of samples are to be handled in a reasonable time without contamination of one sample by another then the entire apparatus must lend itself to quick and thorough washing. The sedimentation columns were easily dismantled and, being then open at both ends, were easily washed through by a rapid jet of water.

(b) It was not always convenient to count the number of fluke eggs in the sediment immediately after extraction as did Happich and Boray and so the extracts were collected, fixed, and stored on filter papers. They were kept like this for periods up to 6 months without noticeable deterioration and consequently the egg counts were delayed until a convenient time or even repeated if necessary.

(c) The method of collecting the extracts on filter paper required that the amount of faecal material finally present in the sediment

was not enough to obscure the fluke eggs. It was found that the two periods of sedimentation suggested by Happich and Boray were not enough and so the samples were sedimented once more.

The modifications rendered this method sufficiently different from the original to warrant a recalibration. This is described below together with a comparison of the method with a standard flotation technique.

2. MATERIALS AND METHOD

(i) Calibration

Sheep faeces were collected from farms around York in early July, 1975. Taking into account the location of the farms in a relatively "fluke-free" area and the date of the collections it was assumed that the samples would be uncontaminated by the eggs of F. hepatica. The samples were weighed and homogenised as described in Chapter 6.

Mature eggs dissected out of living flukes were kept under distilled water in a plugged conical flask at $4^{o}C$. They were transferred in batches as required to a small circular counting tray. The exact number of eggs in each batch was ascertained, the count being checked three times, and the eggs were washed into the screw top flasks containing the homogenised faecal samples. The counting tray was then re-examined under a stereomicroscope to ensure that no eggs had been left behind. Finally the flasks were thoroughly

shaken and the eggs extracted and counted by the sedimentation method.

The procedure was randomised throughout: the batches of eggs were assigned to particular flasks at random and the samples were extracted and counted in random order. In addition, the flasks were labelled by a third party so that the actual number of eggs present remained unknown to the operator at both the extraction and counting stages. There were 63 samples in all, 8 were blanks containing no fluke eggs and the rest contained between 4 and 342 eggs per g of faeces.

The filter paper preparations were stored in closed petri dishes and the counts were repeated after an interval of two months in order to provide an estimate of the count-recount reliability of the method.

(ii) Comparison of the Sedimentation Method with a Standard Flotation Technique

A widely used technique for the enumeration of helminth eggs in faeces is by flotation in a zinc sulphate solution. (Tech. Bull. No. 18, pp 2-9, Min.Agr.Fish.and Food). The method is generally held to recover about five-sixths of all the eggs present. (Parfitt, 1958). This method was compared with the sedimentation method described in Chapter

About 200 recently encysted metacercariae of F. hepatica were divided equally between two gelatin capsules. The cysts in each capsule were suspended in a little distilled water. Each of two hoggets was induced to swallow one capsule and received a similarly

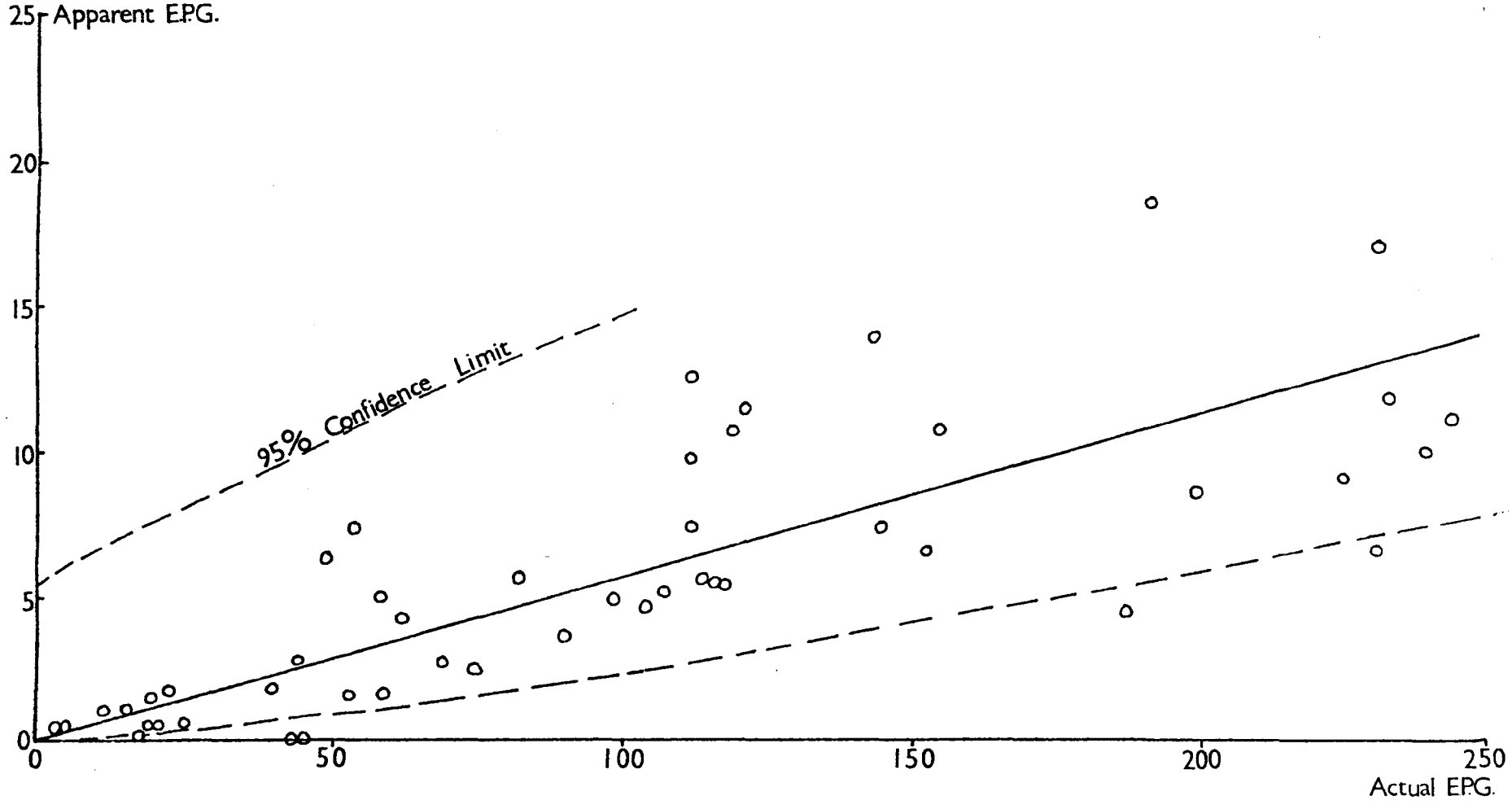
prepared batch of metacercariae 14 days later. After 8 months, faecal samples were taken from the floor of the compound in which the sheep were maintained. Each sample was divided in half. The eggs from one half were extracted by the flotation technique and the eggs from the other half were extracted by the sedimentation method. A week after the collection of the samples the sheep were slaughtered and a count was made of the number of flukes contained in their livers.

3. RESULTS

(i) Calibration

The egg counts (eggs per g of faeces) are presented in Table No eggs were found in any of the blank samples, a finding which was consistent with the assumption that the faeces were originally free of fluke eggs. There were zero counts in only 2 of the 55 faecal samples that had been mixed with eggs. The results are presented as a scatter diagram in Figure 1. The regression line was fitted by a weighted regression analysis in which it was assumed that the variance of the dependent variable (number of eggs per g found) increased linearly with x (number of eggs per g present) and that the line passed through the origin. The null hypothesis of zero intercept was confirmed by the method suggested by Snedecar and Cochran (1972, p.167), i.e. it was assumed that the model for the regression line was:

Fig. I.1 Calibration Curve : Sedimentation Method of Fluke Egg Extraction



$$y = \alpha + \beta (x - \bar{x}) + \varepsilon \qquad (1)$$

If the line passes through the origin then $\alpha - \beta\bar{x} = 0$. This is estimated by $\bar{y} - b\bar{x}$. In this case the condition was satisfied ($t = 0.518$, 53 d.f.). Having confirmed the zero intercept the model for the regression was now assumed to be:

$$y = \beta x + \varepsilon \qquad (2)$$

where $\varepsilon \propto x$.

It was found that

$$y = 0.0559x \qquad (3)$$

where y = number of eggs per g found

and x = number of eggs per g present.

which approximates to

$$y = 0.06x \qquad (4)$$

The use of equation (4) to estimate the number of eggs present in the faecal samples involved the prediction of x from the regression of y upon x and so required the calculation of <u>inverse</u> confidence limits for x. It was found that these limits are given by

$$x \pm \frac{t.Sy}{b} \qquad \text{(Snedecar and Cochran 1967, p.160)}$$

where Sy = standard error of a predicted value of y

b = regression coefficient.

This expression holds provided that the parameter c=t.Sb/b remains small. (Sb = standard deviation of regression coefficient). In this case c = 0.01.

The variance of a predicted value of y (Sy^2) for model (2) is:

$$Sy^2 = \frac{Syx^2}{\Sigma x} \cdot x^2 + Syx^2 \cdot x$$

where Syx^2 is the error variance ($Syx^2 = \left[\Sigma(y^2/x) - (\Sigma y)^2/\Sigma x\right]/(n-1)$)

Hence

$$Sy = Syx \sqrt{\frac{x+x^2}{\Sigma x}}$$

and the confidence limits for x are given by

$$x \pm t. \frac{Syx}{b} \sqrt{x + \frac{x^2}{\Sigma x}}$$

The 95% confidence limits are plotted in Figure

The count-recount reliability of the method was estimated by the Spearman rank order correlation coefficient. The value of the coefficient was 0.92 ($t = 17.09$, 53 d.f. p 0.001).

(ii) Comparison of the Sedimentation Method with a Standard Flotation Technique

The mean e.p.g. count obtained by the standard flotation technique was 128.14 $\pm$ 35.41, the mean e.p.g. count obtained by the sedimentation method was 32.7 $\pm$ 2.56 ($t = 3.257$, d.f. 15 $p < 0.01$). It appears that the flotation technique is 4 times more sensitive than the method used throughout this study. However, these figures represent the mean e.p.g. for each method before the application of the calibration coefficient appropriate to each. The full results are given below.

Method	Mean e.p.g	Estimated e.p.g.	Actual number of Liver Flukes
Flotation	128.14	154	54 and 38
Sedimentation	32.7	545	

The flotation method is held to recover about five-sixths of all the fluke eggs that are present. In our hands at least this was not the case. Although, in later work on faecal samples containing known numbers of fluke eggs a maximum recovery rate of 80% was achieved the efficiency of the procedure depended very much upon the nature of the foodstuff available to the laboratory animals. Highest efficiencies were obtained with animals fed on commercial sheep pellets. (Wilson and Denison, pers. comm.) The sheep in this particular case had been grass fed just prior to sampling.

Based upon the results presented above it seemed reasonable to assume that to a first approximation 1 adult fluke was represented by an egg count of 12 e.p.g.

APPENDIX II

THE MEASUREMENT OF TEMPERATURE

1. THERMISTORS AS TEMPERATURE SENSORS

Electrical resistance thermometers (thermistors) were well suited to the remote recording of temperature that was required in this study. However, the accuracy with which an average value of the temperature of the air or soil surface, representative of any appreciable area around the thermistor, could be determined was limited by the variations of temperature that occur over short distances and short intervals of time. These variations may exceed $2^{o}C$ over as small a period of time as 10 minutes (Meteorological Office, Handbook of Meteorological Instruments, Part I). The problem was compounded by the relatively small lag coefficient of the thermistor (an index of the rate at which the temperature of the thermistor approaches that of the medium by which it is surrounded).

In the recording system described in Chapter 3 not only was each cycle of recordings complete within 60 secs but the temperature measurements were replicated by thermistors that were spread over the area of the habitat. A frequent repetition of readings throughout the day (hourly, on the hour) enabled a reasonable estimate of mean daily temperature to be calculated by a summation of the readings from each cycle. (Nevertheless, readings were infrequent enough to render negligible any errors caused by the self heating of the resistance element during its operation).

2. THE LABORATORY TREATMENT OF THE TEMPERATURE RECORDS

The paper charts that were removed from the Grant Temperature Recorders in the field were returned to the laboratory for processing. At the beginning of each chart there were two calibration marks corresponding to 15°C and 27°C respectively. At the beginning of each month the recorders were calibrated so that these two marks coincided exactly with the appropriate divisions on the paper charts. Each hourly cycle that followed consisted of 9 temperature measurements, 7 at the soil surface and 2 at a height of one metre. At the end of the chart there were two more calibration marks: these indicated the extent to which the calibration had altered over the preceding month. All of the measurements were transcribed onto computer punched paper tape using a plotter that incorporated a sliding pointer and a digital volt meter (D.V.M.). The computer print-out obtained from the punched paper tape was checked for transcription errors and the calibration readings at the beginning and end of the record were compared.

It was found that the 15°C calibration mark at the end of the record was always identical to that at the beginning, however, the 27°C mark deviated from its original position on several occasions. Field trials demonstrated that there was a slight daily variation in the position of the calibration mark corresponding to 27°C throughout the whole month. This was approximately $\pm$ 0.25°C and corresponded to changes in the temperature of the Grant recorder itself. Laboratory tests had shown that the performance of the

Nickel-Cadmium cell was unaffected by the sorts of temperature change experienced in the field. However, the partial failure of a poorly charged cell caused a much larger and always negative deviation of the calibration mark from its original position. This deviation generally occurred first at the end of the third week and increased in an approximately linear fashion for the remaining few days of the record.

When the deviation was at least $1^{o}C$ from the original position those temperatures in excess of $15^{o}C$ were adjusted so as to take this deviation into account.

It was assumed:

(a) That the deviation increased linearly from midnight of day 21 of the record.

(b) That the effect of this deviation on the value of the recorded temperatures in each hourly cycle increased linearly between $15^{o}C$ and $27^{o}C$ actual temperature.

Figure II.1 shows the relationship between actual and recorded temperatures during the last hourly cycle of a record. The dotted line indicates the relationship when the deviation is zero.

T_1 = Actual Temperature

T_2 = Recorded Temperature

T_3 = Recorded value for the calibration mark corresponding to $27^{o}C$.

When $T_3 \leqslant 26^{o}C$ the following correction was applied:

Let $T_1 - T_2 = d_1$

$27 - T_3 = d_2$

It follows from Figure II.1 that:

$$\frac{d_1}{T_2} = \frac{d_2}{T_3}$$

i.e. $$d = \frac{d_2 \cdot T_2}{T_3}$$

Dividing each side by the number of hours (H) elapsed between midnight of day 21 and the final cycle gives:

$$\frac{d_1}{H} = \frac{d_2 \cdot T_2}{H \cdot T_3}$$

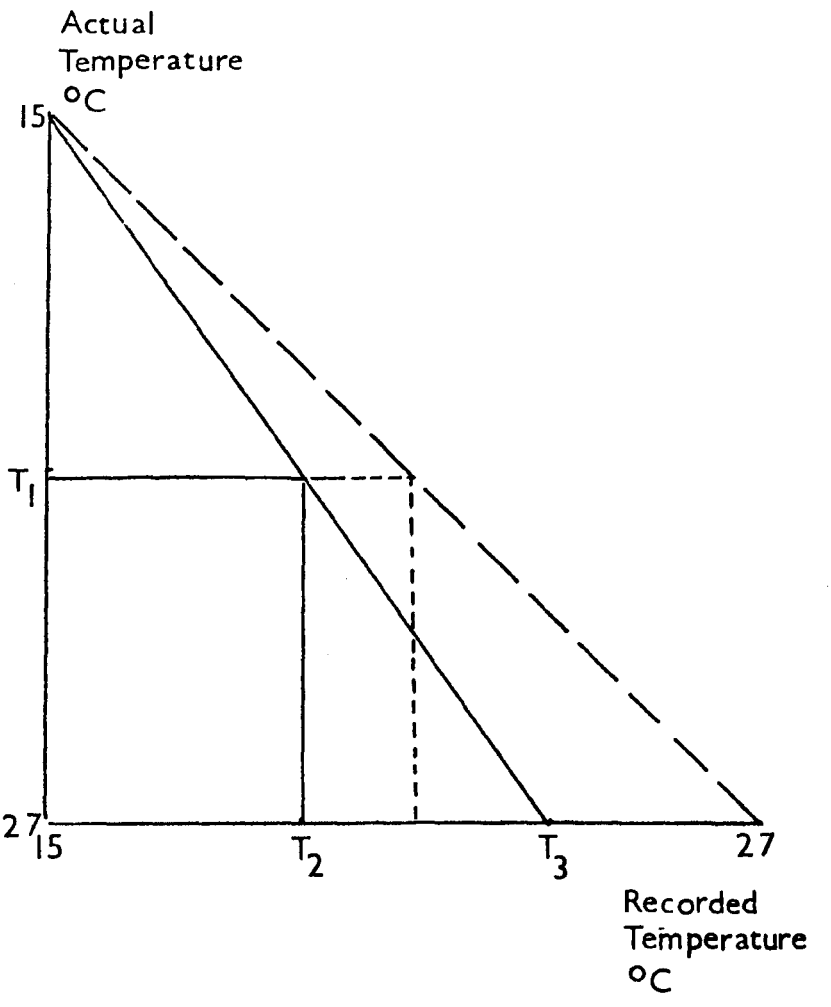
where d_1/H is the rate of change of the deviation corresponding to T_2. The value of the actual temperature (T_1) corresponding to the value of the recorded temperature (T_2) in any particular cycle is given by:

$$T_1 = T_2 + \left(\frac{d_2 \cdot T_2}{H \cdot T_3}\right) \cdot h$$

where h = the number of hours elapsed between midnight of day 21 and the cycle in question.

After correction and adjustment the temperature records were stored on computer disc pending analysis.

Fig II.1.



The Relationship between Actual and Recorded Temperatures during the Last Hourly Cycle of the Recording.

3. THE EFFICIENCY OF THE RADIATION SCREEN

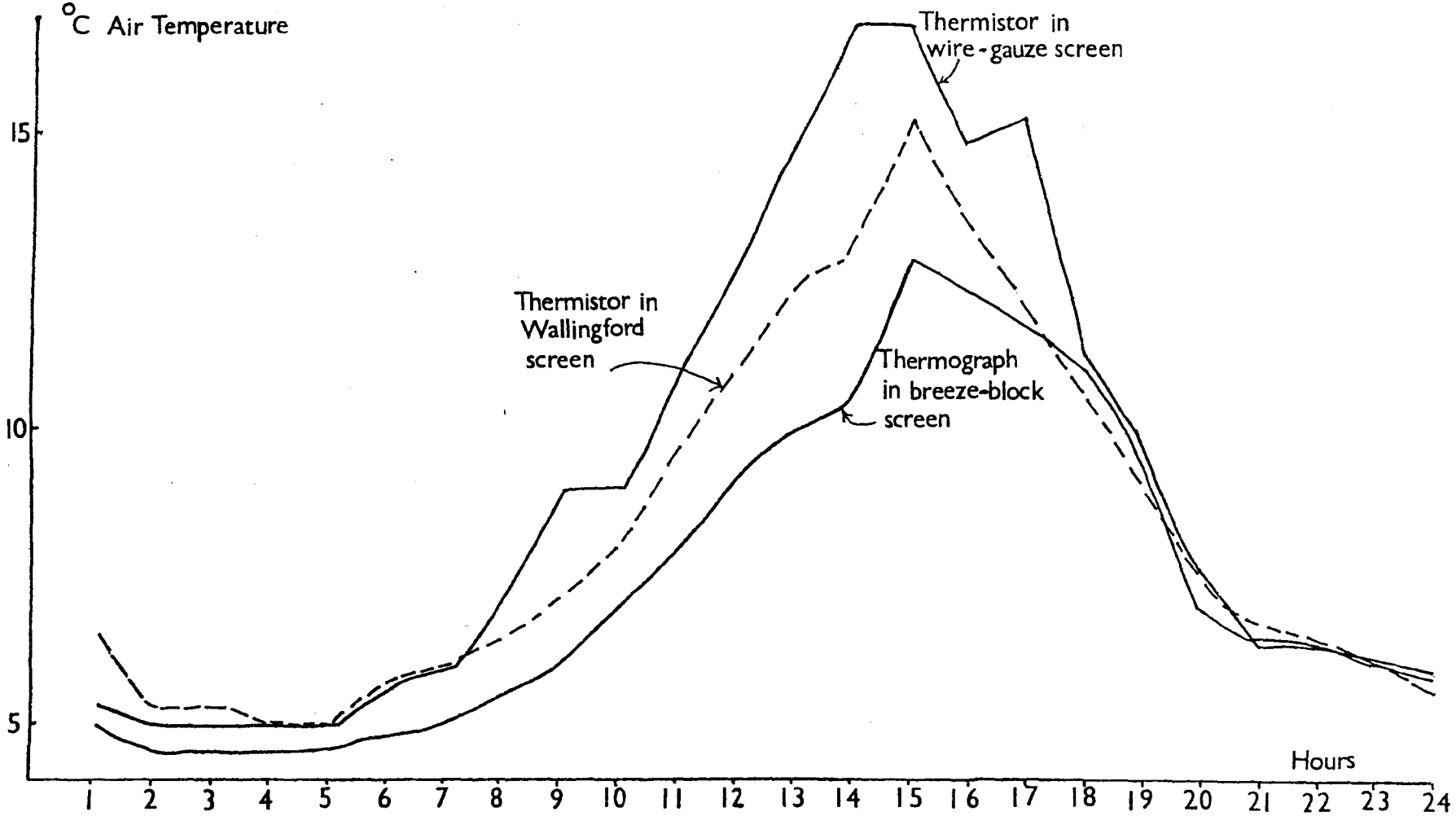
The construction of the wire gauze radiation screens has been described in Chapter 3. Long (1957) described a very similar radiation screen which comprised a hemi-cylinder of white-painted copper gauze. He suggested that this design was the best compromise between the need for adequate ventilation of the thermometers and the provision of adequate screening from sources of radiation. Calibration in full sunlight against an Assmann psychrometer showed that Long's shield was adequate except when the sun shone into the end of the unit or when the ventilation was less than 1 m.sec.

An opportunity arose to test the efficiency of the gauze screens that had been incorporated into the recording system used on the snail habitats when the research team was loaned two radiation screens of different design by the Hydrological laboratory at Wallingford, Oxon. These latter screens sandwiched the thermistors between two large white-painted metal plates and were used in a series of roof top experiments adjacent to the laboratory. Accordingly, an additional thermistor was set up within two metres of the thermistors in the Wallingford screen and at the same height. This thermistor was covered with a gauze screen identical to those used in the field. Readings were taken hourly, on the hour, over several weeks. A representative series of readings for a bright summer day (May 1975) is given in Figure II.2. Between the hours of 7 a.m. and 8. p.m. the thermistor in the Wallingford screen registered a temperature that was consistently lower than that recorded by the thermistor

under the gauze screen. The maximum difference ($4^{o}C$) occurred at 2 p.m. Such a consistent discrepancy cannot be accounted for in terms of short term variations over short distances, but it is not clear either that the difference was due to the effects of direct radiation from the sun. Both screens were shaded for some of the time by features of the laboratory building to the north of the roof top experiments. It may be that the irregularities in the record are due to the shading of one screen but not the other as the instant of measurement. In addition, the thermistors in the Wallingford screen were shielded from above and below, whereas the thermistor in the gauze screen was shielded only laterally and from above. It is possible then that the higher temperatures recorded by this thermistor were the result of its exposure to radiation reflected up from the flat concrete tiles of the roof.

The results of the rest were equivocal, but it is worth mentioning two things: first, that in the field the screens were placed over the far less reflective surface of cut pasture, and second, that Long (loc.cit) reported in conclusion that he and other workers found this design of screen was at least as good as any other except under arid tropical conditions.

Fig. II. 2.


°C Air Temperature
15
10
5
Thermistor in wire-gauze screen
Thermistor in Wallingford screen
Thermograph in breeze-block screen
Hours
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

APPENDIX III

THE GROWTH OF L. TRUNCATULA POPULATIONS ON EXPERIMENTAL PLOTS

1. INTRODUCTION

Experience with small laboratory cultures clearly indicated that when the mud slopes upon which the snails were grown were allowed to dry to a "damp" condition most of the snails were found to be withdrawn into their shells. The experiments of Kendall (1949) demonstrated that if the cultures were allowed to dry any further than this then the snails quickly died if their shell apertures were prised away from the mud surface. The soil-surface moisture ratings of "damp" and "dry" were plainly sub-optimal for L. truncatula.

As part of a series of experiments on populations of snails maintained in experimental plots on the roof of the laboratories at York it was decided to investigate whether it was worth distinguishing between the soil-surface moisture ratings of "wet" and "standing water."

2. MATERIALS AND METHOD

16 experimental plots were constructed. Each plot consisted of a flat area of chalk soil (60 x 40 x 5 cm) contained within an open-topped "box" of polythene sheet whose sides were supported by building blocks. The soil was collected from a nearby chalk down. It was autoclaved and mixed with water to a plastic consistency. The soil surface in each plot was worked until it was flat and smooth. A small quantity of liquid fertiliser was added to each plot.

A suspension of the blue green algae, Oscillatoria sp., was poured over each plot a week before the snails were introduced and thereafter at weekly intervals.

The plots were arranged in three rows across the laboratory roof. Half the plots were kept submerged under about 5cm of water (tap water which had been left to stand for 48 hours) and the other half were maintained in a "wet" condition by daily watering. These treatments were assigned to the plots at random.

A week after the plots had been seeded with Oscillatoria sp. 16 batches of 30 snails were counted out. The snails were selected so that in each batch there were 15 snails in the 3.00-3.99mm size-class and 15 snails in the 4.00-4.99mm size-class. The shell of each snail was marked on the spire and body whorl with red nail varnish and the batches were assigned at random to the plots. These snails constituted the initial breeding stock (i.b.s.) The following observations were then made.

(a) The number of egg masses in each plot were counted weekly. Only those masses which had been laid on the polythene sides of the plots were counted.

(b) At fortnightly intervals the i.b.s. snails were removed, counted and accurately measured. Empty shells were recorded and discarded before returning the snails to the plots.

(c) As the egg masses hatched out the progeny were also removed and counted at fortnightly intervals. Snails were assigned to particular size classes by means of a notched template. Mature daughter snails were easily distinguished from the ibs snails since they were unmarked.

Regular observations were made between the 5th July and the 9th October 1973.

3. RESULTS

Since each plot was regularly seeded with blue-green algae the amount of food available was assumed to be always in excess. It was relatively easy to maintain the "standing water" condition but the "wet" plots very frequently underwent a "wet / damp / wet" cycle with a 24 hour period particularly on bright, warm days. However, the time spent in the "damp" phase was rarely more than three or four hours. It was often necessary to water the "wet" plots twice a day.

All stages of the life cycle of L. truncatula were more easily found on the "wet" plots than on the "standing water" plots where the mere activity of searching made the water turbid.

(i) The Mortality of the Initial Breeding Stock (Fig III.1)

The i.b.s. snails were removed from the plots on the 11th September. The numbers of i.b.s. snails had fallen on both sets of plots. With the exceptions of the first and last sampling dates the mean number of i.b.s. snails on the "wet" plots was significantly smaller than on the "standing water" plots ($p < 0.05$ on each occasion). The number of empty shells found on the "wet"plots was also significantly higher than the number of empty shells found on the "standing water" plots. (Means: 5.13, 2.13; $t = 2.27$, $p < 0.05$). It appeared that, initially at least, the mortality rate of the initial breeding stock was greater on the "wet" plots.

(ii) The Number of Egg Masses (Figure III.2)

The relative density of egg masses was consistently less in the "wet" plots.

(iii) The Daughter Snails (Figure III.3)

The mean density of daughter snails was often higher on the "wet" plots but the difference was significant on only one occasion. (11th September, immature snails, $t = 2.59$, $p < 0.05$). The direction of the difference was attributable to the particular search difficulty encountered on the "standing water" plots.

Fig. III.1

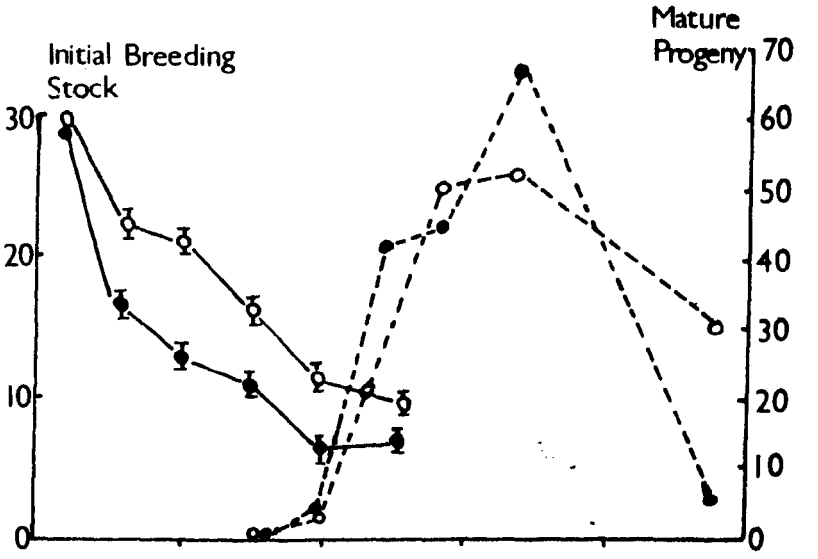


Fig. III.2

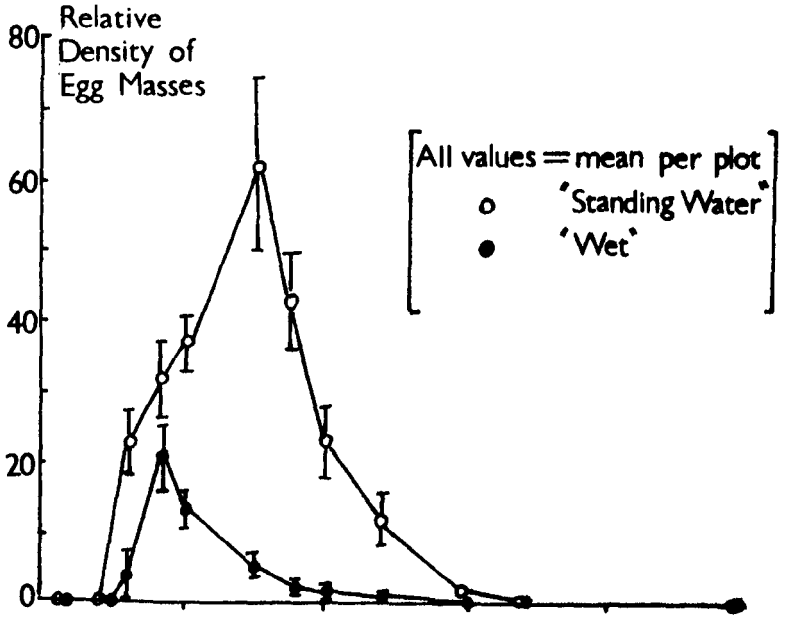
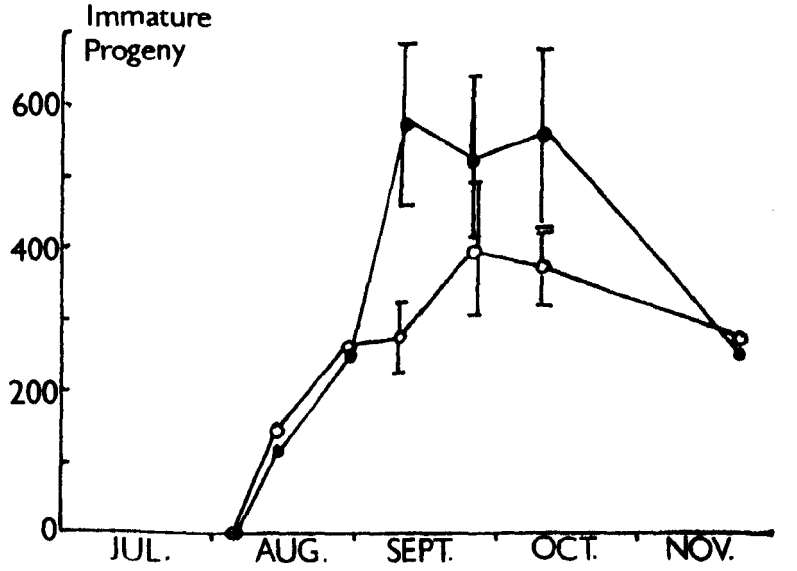


Fig. III.3



4. DISCUSSION

It seems that snails maintained under "wet" conditions experience a significantly greater mortality rate and lay fewer egg masses per snail than snails maintained under "standing water" conditions. It was felt that any difference that there might have been between the density of the progeny on the "wet" and "standing water" plots was probably obscured by the turbidity of the water on the latter.

As a result of this experiment it was deemed reasonable to distinguish between "wet" and "standing water" conditions in the field.

APPENDIX IV

THE REPRODUCTIVE ANATOMY AND COPULATORY BEHAVIOUR OF LYMNAEA TRUNCATULA

SYNOPSIS

The hermaphrodite reproductive system of Lymnaea truncatula is described and compared with the same system in other Lymnaeidae. Morphologically and histologically similar, it differs mainly in the form of the ovotestis and in the occurrence of a proximal inflation of the vas deferens, the proximal sac. Copulation follows the usual Lymnaeid pattern in which one partner acts the male role and the other acts the female role. The snails appear to mate relatively infrequently even at high population densities. The significance of copulation is discussed.

1. INTRODUCTION

Lymnaea truncatula is a member of one of the four families of freshwater Basommatophora which together comprise the group Hygrophila. It has long been a subject of study as the intermediate host of the Liver Fluke, Fasciola hepatica, even so, comparatively little is known about its reproductive biology.

There have been a number of investigations into the anatomy of the Lymnaeid reproductive system. For example, Crabb (1927, a, b) Holm (1946) and Bretschneider (1948 a, b) have described in detail the histology and morphology of the genital tract of Lymnaea stagnalis and Duncan (1960) has provided an account of the same system in Lymnaea peregra. The most detailed description of the sexual

anatomy of Lymnaea truncatula is that of Roskowski (1922), however, the account is incomplete and poorly figured.

Various aspects of the reproductive life history of Lymnaea truncatula have been examined by Walton (1918), Walton and Jones (1926) and Kendal (1953) but there apprears to have been no previous account of its copulatory behaviour. Walton stated that neither in the field nor in the laboratory was Lymnaea truncatula ever observed to copulate, although carefully watched. Walton and Jones confirmed that this had remained true even after much further work and observation. Kendal isolated individual Lymnaea truncatula soon after hatching and found that these produced normal eggs upon reaching maturity. He also noted that the snails kept in his laboratory colonies were never seen to mate.

2. MATERIALS AND METHODS

Only mature Lymnaea truncatula of 4mm shell length and over were used for dissection. The snails were obtained from routinely maintained laboratory colonies or else collected from field sites at Caernarvon (Gwynedd) and Borrowdale (Cumbria). They were dissected in a variety of aqueous media: aerated tap water, 30 per cent alcohol, or a 0.47 per cent saline solution istonic with the snails' Haemolymph (Pullin 1971). For histological work the entire genital tract was removed and fixed in Bouin's solution. Sections were cut at 6μ and 8$\mu$and stained with Erlich's Haematoxylim and Eosin. The results obtained in this way were compared with teased preparations of living tissues which were examined either unstained or having been treated

with a dilute solution of Methylene Blue.

Since there is considerable confusion in the nomenclature used to describe the organs of the pulmonate reproductive system, especially those of the female tract, the standardised terminology suggested by Duncan (1958, 1960) will be followed throughout.

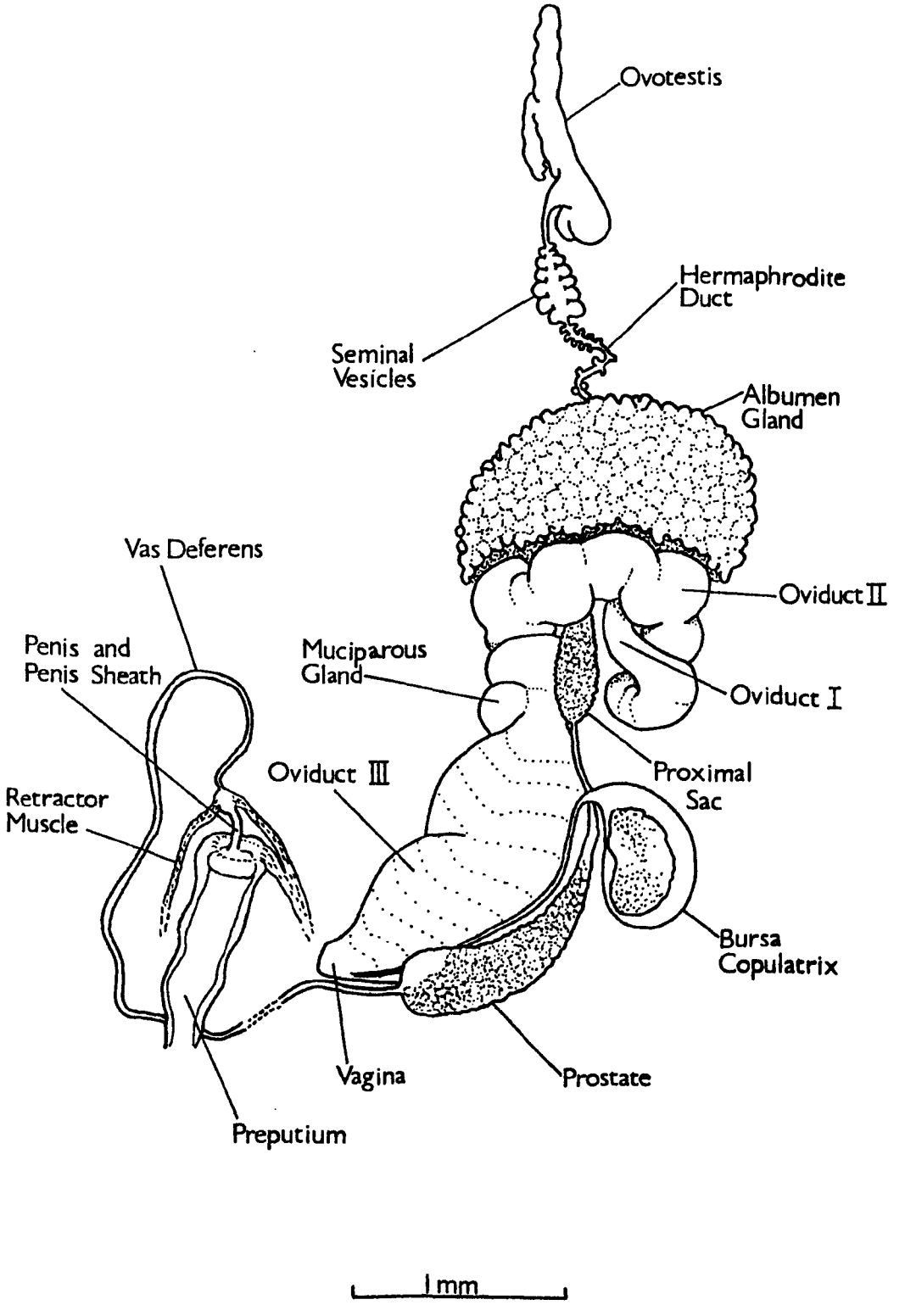
With one exception, the description of copulatory behaviour derives from observations made on snails in laboratory colonies.

3. THE ANATOMY OF THE REPRODUCTIVE SYSTEM

(i) General Anatomy

The reproductive system of the Lymnaeidae comprises three main divisions (1) the ovotestis and hermaphrodite duct, (2) the female genital tract and (3) the male genital tract. (Figure 1) Both ova and sperm are produced in the ovotestis and pass down the hermaphrodite duct to the point where the male and female systems separate. The site of fertilisation is uncertain. The oviduct may be divided into three distinct glandular regions responsible for laying down the successive membranes and layers of mucus which, enclosing the eggs, constitute the egg mass. There is a short muscular vagina which opens to theexterior on the right side of the animal near the pneumontome. Two accessory glands are associated with the female system, the albumen gland situated at the origin of the oviduct, and the bursa copulatrix, a blind diverticulam, whose duct opens into the vagina. Sperms are carried to the penial complex

Fig. IV.1 Reproductive System of L.truncatula viewed from the front and left of the animal.



in the vas deferens, receiving the secretions of the prostate gland along the way. The male aperture is situated just beneath the rear edge of the right tentacle.

(ii) The Ovotestis and Hermaphrodite Duct

In contrast to the complexly branched acinous ovotestis of Lymnaea stagnalis (Crabb 1927, a; Holm, 1946), the ovotestis of Lymnaea truncatula is a bulbous, transparent, Y-shaped organ. It lies partially imbedded in the columellar surface of the digestive gland and has a superficially fusi form appearance because its ventral arm is tucked back along the main stem. A taut strip of connective tissue passes from the tip of the dorsal arm to the apex of the digestive gland. Distally the organ terminates in two rounded lobules. In fixed preparations the lumen is seen to be filled with male and female gametes in all stages of development, together with a variety of accessory cells. The tracts of cilia, which extend along the columellar side of the organ into the two arms and which convey the mature yellow ova and the mass of sperm to the hermaphrodite duct, are clearly seen in preparations of living material. The ciliation converges on a funnel which opens near the two distal lobules and narrows to join the convoluted hermaphrodite duct.

Near the ovotestis the lumen of the hermaphrodite duct is wide and confluent with the spacious lumen of each of the numerous large diverticula, (the seminal vesicles), which characterise this

portion of the duct. Distally the diverticula become smaller and less frequent and the lumen of the duct becomes constricted. Throughout, the duct is lined by low cuboidal ciliated epithelium and surrounded by a very thin layer of connective tissue. The seminal vesicles are lined with similar but unciliated cells.

Only a very few of the comma-headed sperm which pack the lumen of the duct and diverticula can be seen to move, and these so ineffectually that the cilia of the hermaphrodite duct must be entirely responsible for the passage of sperm to the vas deferens.

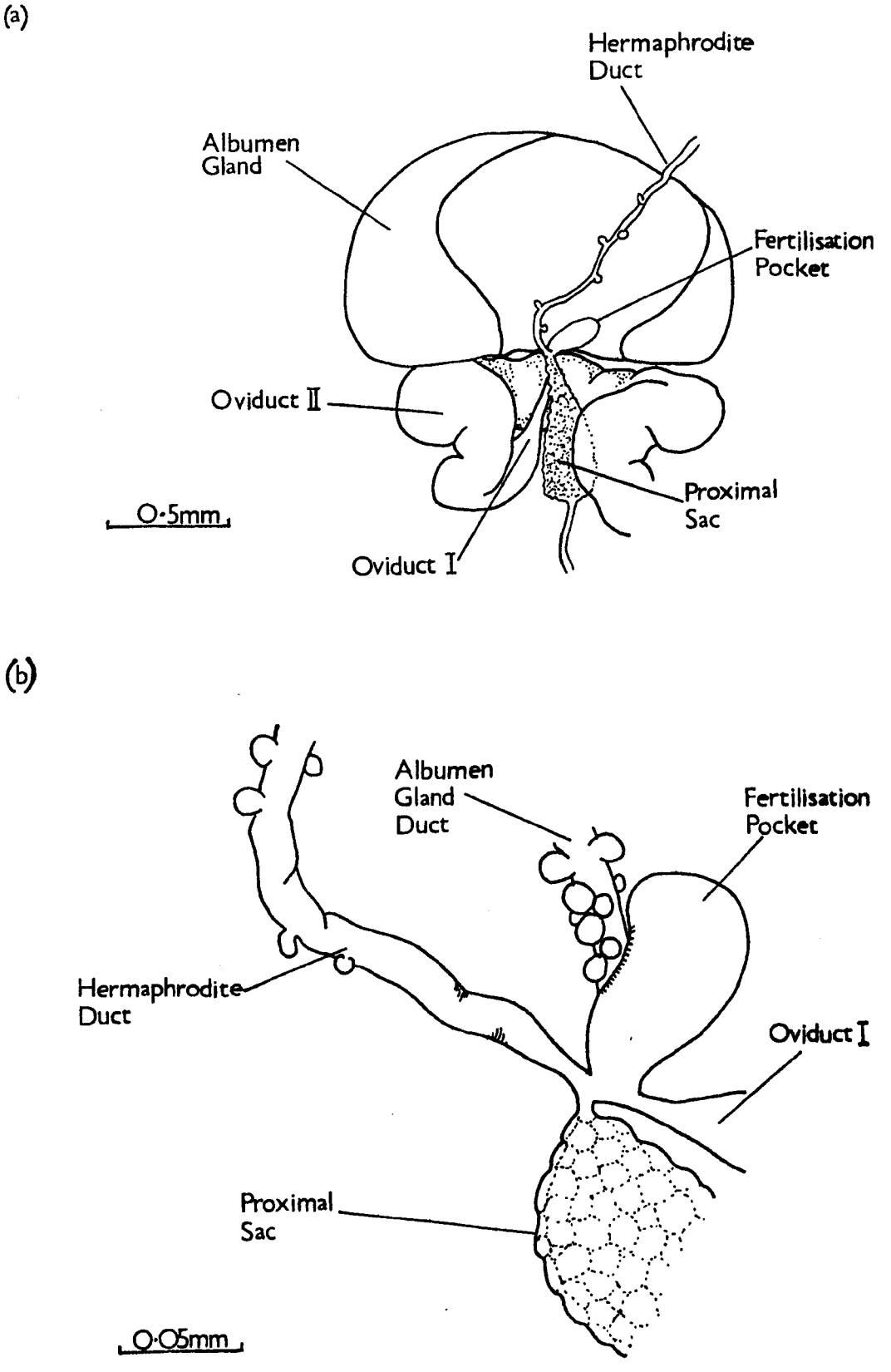
(iii) The Carrefour

The duct of the albumen gland opens into the oviduct just after the separation of the male and female systems. The base of the duct is almost completely enfolded by the base of a translucent digitate vescicle, the fertilisation pocket, which also opens into the oviduct. This vesicle is lined by columnar ciliated epithelium with basal nuclei and invested with a thin connective tissue sheath. (Figure IV·2.a, IV 2.b.)

(iv) The Female System

The peach coloured albumen gland is a branched tubular gland whose numerous secretory follicles are circular or hexagonal in cross section. Very often, several of these follicles are grossly distended. Holm (1946) associated this distension in Lymnaea stagnalis with the onset of egg mass formation. Duncan (1958) suggested that a galactose polymer, galactogen, is secreted by the cells of the

Fig. IV·2 The Carrefour



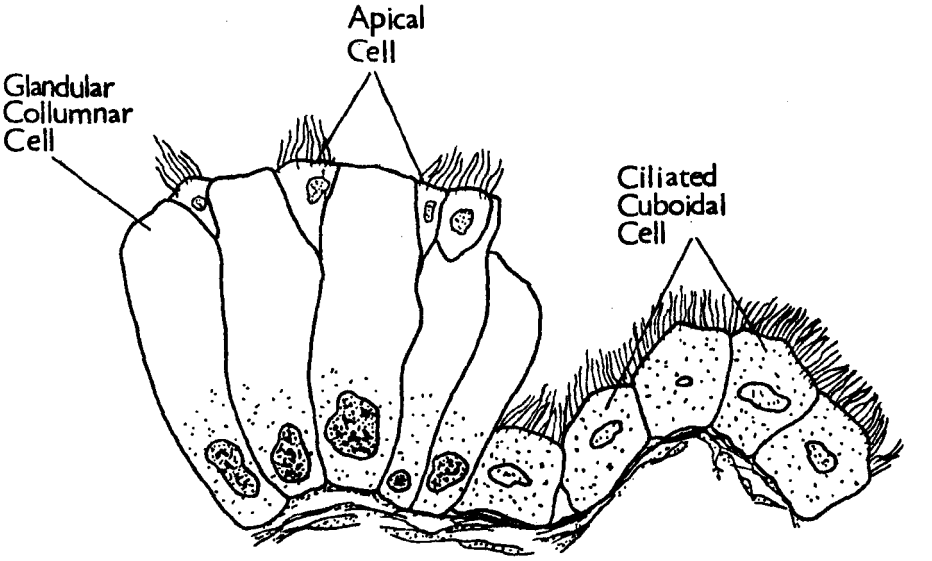
albumen gland in all four families of the Hygrophila and provides the eggs with a nutritive investment before they pass down the oviduct.

The large lumen of each follicle is lined by twenty or more darkly staining cuboidal cells with large central or basal nuclei. As the lumen reduces to a duct these cells become ciliated. The extensive ciliation and limited capacity for muscular movement of the main duct of the albumen gland is plainly visible in living material.

Oviduct I is a short narrow duct which extends forward from the carrefour and loops downwards to join oviduct II. Proximally it is lined with ciliated cuboidal epithelium whose cytoplasm stains a deep purple with Haemoloxylin and Eosin. Distally the duct becomes wider, the epithelium becomes rather more folded, and the cells more columnar. The nuclei are basal throughout. The transition from Oviduct I to Oviduct II is abrupt. The lumen widens considerably and the lining epithelium is thrown into a series of transverse folds. The cytoplasm of the cells takes up very little stain and the pattern of small ciliated tetrahedral cells interspersed among the apices of tall columnar cells is one which is repeated throughout the rest of the female system and in the prostate gland as well. (Figure 8) Oviduct II loops around the longitudinal axis of the system and a broad tract of ciliated cuboidal cells runs along the whole inner edge of the loop.

At the junction of oviducts II and III there is a blind, pouch-like diverticulum, the muciparous gland. A triple fold of epithelium

Fig. V-3

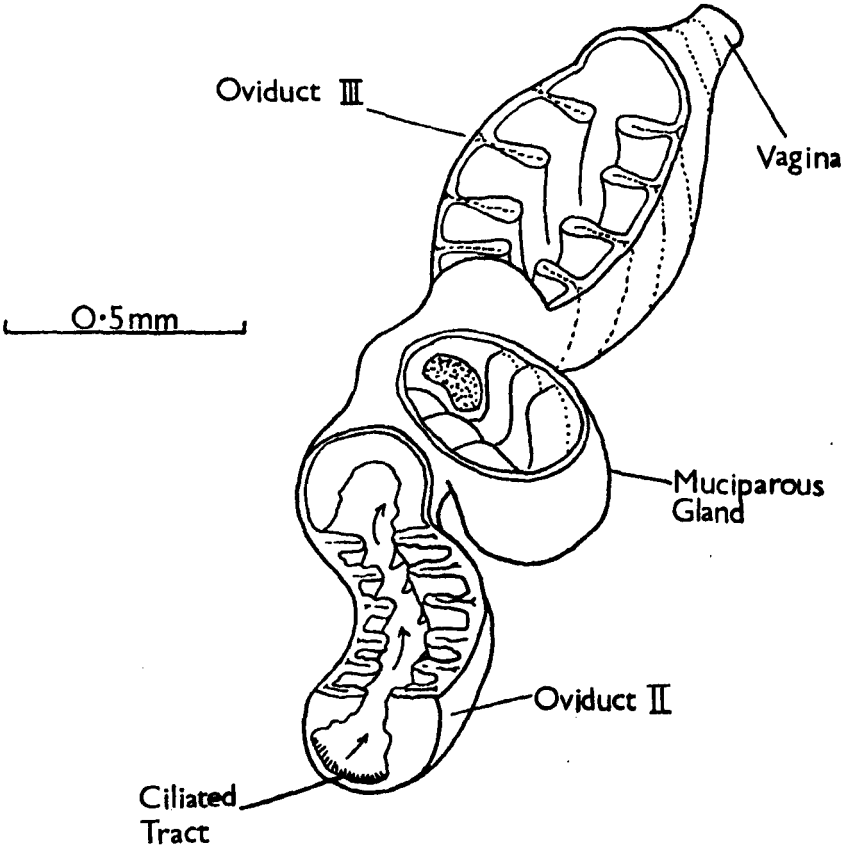

Apical
Cell
Glandular
Collumnar
Cell
Ciliated
Cuboidal
Cell
20 µm

arises from the base of the gland and, except centrally where the height of the fold is considerably reduced, occupies two-thirds of the lumen. The cells are similar in appearance to those of oviduct II except that their outlines are extremely faint.

The swollen cylinder of oviduct III, also termed the oothecal gland, runs anteriorly towards the bucchal mass before bending right and narrowing to join the short muscular vagina. Yellow diagonal striae represent the bases of the internal folds of glandular epithelium which arise on each side of the oothecal gland and meet dorsally and ventrally. The columnar cells in the folds are predominantly kite shaped, otherwise the typical histological pattern of the oviduct prevails. However, the characteristically pale nuclei of the small apical cells are difficult to identify here and the extensive tracts of cilia are adequately seen only in preparation of living tissue. The epithelium rests upon an indistinct basement membrane and the whole gland is bounded by a loose connective tissue covering. Oviduct III is typically yellow. (Figure 4)

Cuboidal cells line the flattened lumen of the vagina and the thick connected tissue matrix which surrounds them has within it several layers of longitudinal and circular muscle fibres. The duct of the bursa copulatrix arises ventrolaterlly at the junction of the vagina and oothecal gland. Its thin wall consists of unciliated cuboidal epithelium and a sparse complement of muscle

Fig IV·2


Oviduct III
Vagina
0·5mm
Muciparous Gland
Oviduct II
Ciliated Tract

fibres is embedded within the connective tissue sheath surrounding its distal end. The duct runs for most of its length within the larger connective tissue envelope of the oothecal gland. The sac of the bursa is reflected anteriorly and is characterised by an orange or red secretion which partially or completely fills the lumen. Its epithelium consists of irregular columnar cells. These rest upon a pronounced basement membrane and the whole is surrounded by a layer of connective tissue.

(v) The male system

Immediately as the male and female systems separate there is a flattened oval inflation of the vas deferens. Its epithelium consists of cuboidal cells whose cytoplasm stains scarcely at all and whose nuclei are basal and prominent. Examination of living tissue reveals that the epithelium is feebly ciliated. No comparable structure has been described in Lymnaea peregra although it may be equivalent to the flattened upper portion of the prostate in Lymnaea stagnalis. At about the region of the muciporous gland this proximal sac narrows to a fine ciliated duct which continues anteriorly to a second inflation of the vas deferens, the prostate.

Running vertro-laterally along the left side of the oothecal gland, the prostate consists of a flattened proximal portion and a more bulbous distal portion. Anteriorly and ventrally a quadrant of translucent tissue contrasts with the opaque milky white of the remainder of the gland. Histologically there appears to be no basis for this differentiation, the epithelium consisting throughout of

tall columnar cells with prominent basal nuclei and a granular cytoplasm which stains more intensely towards the basement membrane. The characteristic ciliated cells with their smaller pale nuclei are packed between the apices of the columnar cells.

The prostate narrows abruptly to the thick walled, muscular vas deferens, which travels in the musculature of the body wall from the female aperture to the base of the right tentacle. Leaving the body wall it loops extravagantly around the penial complex before becoming confluent with the penis sheath. The living vas deferens undulates continuously, this muscular activity, however appears to have not much influence on the diameter of the lumen of the duct. The lining of ciliated epithelium is best appreciated in living material in which the vigorous ciliary activity is obvious.

The ciliated penis, a modified portion of the vas deferens, is enclosed by the muscular penis sheath. It opens into the preputium, a tall more or less cylindrical organ, which opens directly to the exterior at the male aperture. Circular and longitudinal muscles are irregularly arranged throughout the length of the preputium. The point of entrance of the penis is encircled by a thick band of muscle. Observations suggest that the concave lip of the everted preputium and this muscular ring beneath it act together as a sucker during copulation. Proximally the preputium is lined with extensive tracts of vigorous cilia, distally the ciliation is sparse and feeble.

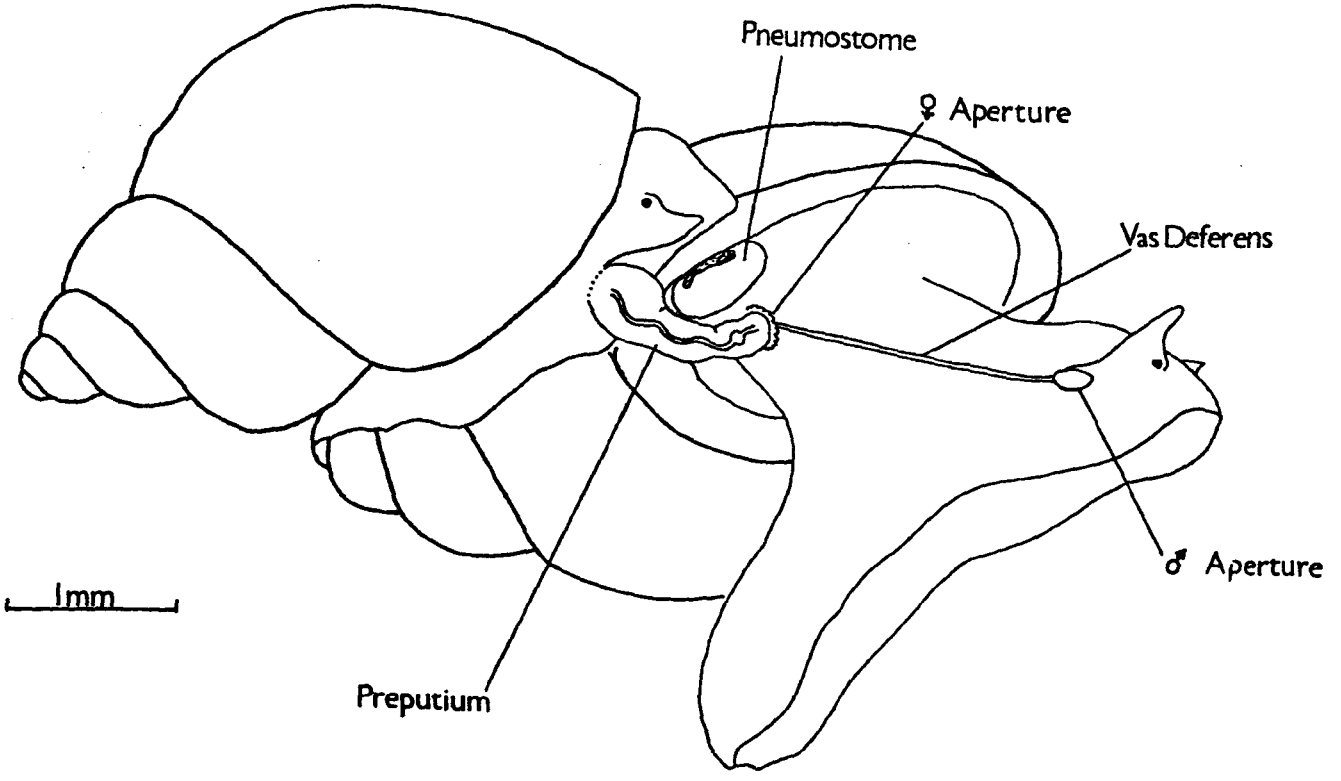
A broad retractor muscle passes from its insertion beneath the vagina to the proximal end of the preputium. Along its way a narrow muscle band branches off and extends to the bulbous origin of the penis sheath. The penis sheath receives a second retractor muscle which runs down the length of the preputium to its insertion in the body wall. The retractor muscles are speckled with a black pigment.

4. COPULATION

As is usual in the Lymnaeidae, reciprocal copulation does not occur. Although hermaphrodite one partner acts in the male role and the other in the female role. Chain copulation, described in Lymnaea stagnalis by Barraud (1957) and Lymnaea tomentosa by Boray (1964) has not been observed here in Lymnaea truncatula.

Provided that there is sufficient moisture for the snails to remain active, copulation occurs whether the individuals are submerged or not. The "male" mounts the shell of the "female" and may be carried along by an active "female" for some time before it finally takes up the characteristic right side position. (Figure 5). The preputium may be rapidly everted to its maximum length of 2mm or so, or more usually everted gradually and in stages. It manifests an extremely versatile repertoire of movements and continually probes the shell and head-foot of the "female". Eventually its tip becomes firmly affixed to the surrounding lips of the female aperture. By this stage all locomotion has ceased and the shell of both partners is drawn closely

Fig. IV•5 Copulation in L.truncatula



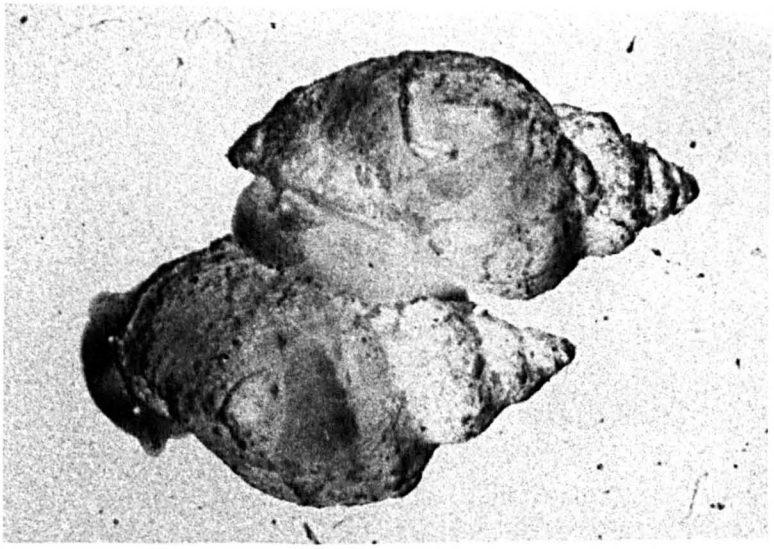


Plate 7. Copulation in L. truncatula
(dorsal view)

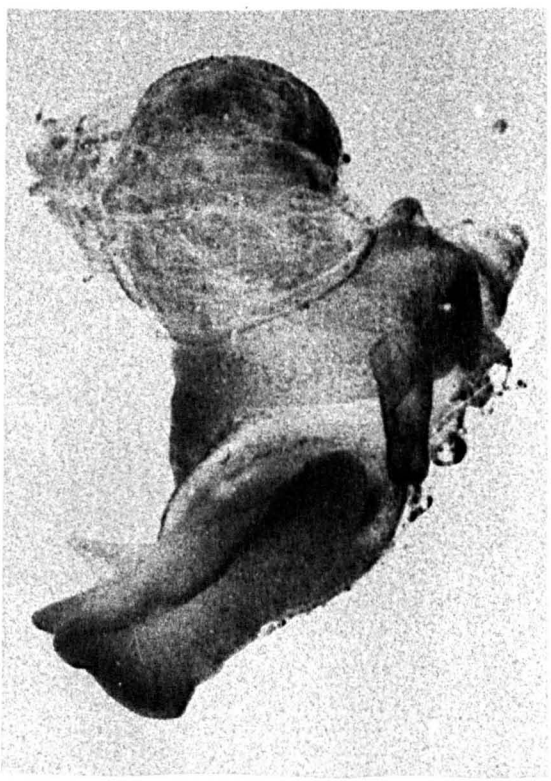


Plate 8. Copulation in L.truncatula
(ventral view - exploratory behaviour)

down over the foot. (~~Figure 6~~). It is not uncommon to find that the tip of the preputium has attached itself to the male aperture of the female partner, or, even on occasion, to the folds of the muscular foot. The snails may separate after a few minutes or remain coupled for over half an hour. Throughout this time, the undulations and sinous coilings of the vas deferens are visible through the transparent wall of the preputium. Ejaculation has not been observed.

5. DISCUSSION

With the exception of the ovotestis and the proximal sac, the reproductive system of L. truncatula is similar in most aspects of morphology and histology to the reproductive systems of L. stagnalis and L. peregra. Its copulatory behaviour is also similar to that of other Lymnaeids. However, in all of the freshwater Basommatophora there is considerable dispute with regard to the significance of copulation. Opinion is divided as to whether cross fertilisation or self fertilisation is the rule, or even whether cross fertilisation is possible. Duncan (1958) has summarised the relevant arguments. So far as the Lymnaeidae are concerned the following points have been established:

1. Copulation has been described in all species investigated. (Boycott, Diver et al. 1931, Barraud 1957, Duncan 1958, Boray 1964).
2. Cross fertilisation has been demonstrated genetically in L. peregra (Boycott, Diver et al. 1931) and L. tomentosa

(Boray 1964) and shown to be usual.

3. Isolated L. truncatula, L. tomentosa, L. columella and L. stagnalis produce fertilse eggs (Kendall 1953, Boray 1964, Colton and Pennypacker 1934, Crabb 1927a)

In the particular case of L. truncatula it has long been assumed that copulation did not occur. The laboratory colonies upon which the foregoing observations were made are maintained at a density of about 800 snails per m^2, and at a temperature of 20^oC. The density of snails in these colonies is high, though not excessive when compared with the computed densities of the local aggregations of snails encountered in the field. Even so, copulation appears to occur relatively infrequently. Each dish contains about 100 individuals and it is rare to find more than one or two copulating pairs per dish. However, it is not uncommon for experienced collectors to find pairs of firmly adhering snails in the field. Moreover, the first copulatory pair noticed in this study was observed on an experimental plot with a snail density of less than 50 per m^2.

It appears that L. truncatula is largely self-fertilising, an obvious advantage in a species whose mobility is low and whose dispersal is determined by the very heterogeneous moisture conditions that prevail at the soil surface of most habitats. Presumably the low copulation rate is just sufficient to maintain the genetic vigour of the species (heterosis - see Sinnot, Dunn and Dobzhansky, 1958) though, of course, cross fertilisation has yet to be experimentally demonstrated in these snails.

6. SUMMARY

The reproductive system of the hermaphrodite snail, L. truncatula is similar to the reproductive systems of L. stagnalis and L. peregra. The arrangement of ducts at the bifurcation of the male and female systems is typical of the Lymnaeidae (Duncan 1960). The oviduct is divisible into three distinct regions on morphological and histological grounds. Nevertheless, a characteristic epithelium prevails. Small ciliated cells with pale nuclei are dispersed between the apices of tall unciliated columnar cells with larger and more densely staining nuclei.

Two inflations of the vas deferens characterise the male system. The first, for which the term proximal sac has been tentatively suggested, and which appears to be peculiar to L. truncatula, occurs immediately after the division of male and female systems. The second, the prostate gland, runs along most of the length of Oviduct III. Tall columnar cells interspersed with small apical cells, occur here also.

With the exception of the bursa copulatrix and its duct, extensive ciliary tracts pervade the whole system, and muscle fibres are present in the duct of the albumen gland, and in the walls of the vagina, vas deferens and the whole penial complex.

The ovotestis does not conform to the Lymnaeid pattern. It is a bulbous, Y-shaped organ with a single large lumen.

The copulatory behaviour of L. truncatula is similar to that of other Lymnaeids. It is suggested that copulation occurs infrequently even at high population densities and that self fertilisation is the

rule. It has yet to be demonstrated that cross fertilisation occurs in L. truncatula.

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APPENDIX V OVIPOSITION IN L. TRUNCATULA

1. MATERIALS AND METHOD

(i) The Oviposition Site

In the field the egg masses of L. truncatula are most often found on the undersurface of submerged leaves. Observations on populations of snails maintained in plots near the laboratory suggested that egg masses were more likely to be found on the north and shaded sides of the plot. Accordingly, an experiment was carried out to test the hypothesis that more egg masses are laid in shaded sites than unshaded sites.

A group of 36 mature snails was divided at random into 3 equal parts; an analysis of variance showed that there was no significant difference between the mean lengths of snails in each of the 3 batches. Each batch of snails was maintained for 12 days on a standard Oscillatoria culture. The crystallising dishes which contained the cultures were half covered (top and sides) with black polythene. The dishes were oriented at random in a constant temperature room (20^{0} C).

At the end of the experiment the snails were removed from the cultures and their shell length was measured. The number of egg masses found on the dark side and light side of each dish was counted. The number of eggs in each egg mass was found by dissection.

(ii) Competition for Oviposition Sites

Casual observation suggested that a larger number of egg masses could be recovered from laboratory cultures of L. truncatula if the masses were removed daily rather than weekly. Coupled with the

observation that there seemed to be preferred oviposition sites it was decided to investigate the possibility that there might be competition for suitable places to lay egg masses.

A group of 72 snails, the progeny of snails collected from habitats on Thorneythwaite farm, was divided at random into 6 equal parts. All of the snails were between 4.0-5.5mm in length; an analysis of variance showed that there was no significant difference between the mean lengths of snails in each of the 6 batches. Each batch of snails was placed in a crystallising dish containing a standard Oscillatoria culture. The dishes were placed outside the laboratory in a small group and shaded from direct sunlight. The snails were left undisturbed for two days to acclimatise to the change in temperature regime and then the dishes were cleared of egg masses.

Three dishes were chosen at random. The egg masses were removed from these dishes every 24 hours for the next 6 days. At the end of this period the snails were measured once more and the egg masses in the undisturbed dishes were removed and counted. The Oscillatoria cultures were replaced and the experiment repeated over the next 8 days. This time the egg masses were removed every 24 hours from the dishes containing those snails which had previously been undisturbed. On completion all of the dishes were cleared of egg masses and the snails were measured for the last time. Throughout the whole experiment the air temperature was continuously recorded by a shielded thermograph.

(iii) Copulation as a Stimulus to Oviposition

It has been thought that copulation in the Lymnaeacea may serve as a stimulus to oviposition. For example, Duncan (1958) suggested that this was the case for Physa fontinalis and Boray (1964) noted that L. tomentosa which were kept in pairs produced the first egg mass earlier than single snails reared in isolation.

The following experiment was carried out to see if there were any grounds for the belief that a similar mechanism existed in L. truncatula.

Standard Oscillatoria cultures were made up in 100 petri dishes. Within 24 hours of their hatching, 150 L. truncatula were distributed between the dishes. Single snails were placed in 50 of the dishes and pairs of snails were put into the remainder. Each dish was examined daily and the culture renewed as necessary. The date was noted when the first egg mass was found and the length of either the single snail or each of the pair of snails was measured.

2. RESULTS

(i) The Oviposition Site

In this and the following experiment, in order to ensure that the results from each culture of snails were comparable, a check was made of the mean growth of each population. Analyses of variance of the mean length of snails before and after the present experiment showed that there was no significant difference between any of the three cultures (~~Table ——~~). The total numbers of egg masses and eggs

found in the shaded and unshaded portions of the cultures are presented in Table V1 . In both cases there were significantly more found in the shaded portions ($p < 0.05$ and $p < 0.01$ respectively). More than two-thirds of all the eggs had been laid in parts of the dishes that received least light.

(ii) Competition for Oviposition Sites

The snails were measured on three occasions. On each occasion there was no significant difference between any of the 6 cultures. ~~(Table~~

The results in Table V.2 and Table V.3 are presented in terms of the number of eggs or egg masses estimated to have been laid each day. There was no significant difference between the number of egg masses laid daily on any of the dishes nor was there any significant difference between the number of eggs laid daily on any of the dishes. There was, however, a significant difference in both cases between the number laid during the first and second period of the experiment. For example, twice as many egg masses were laid during the first 6 days as were laid during the last 8. This was attributed to the higher air temperatures that prevailed during the first period. (First 6 days: mean max. 17.58 $\pm$ 1.38°C, mean min. 9.75 $\pm$ 2.10°C; last 8 days: mean max. 15.63 $\pm$ 2.09°C, mean min. 7.13 $\pm$ 1.99°C). The number of egg masses laid each day is compared with the variation in air temperature in Figure V.1.

Although a cursory examination of the data indicated that there was an increase in the number of eggs found in each egg mass when the number of egg masses laid each day declined, as it did in the second

TABLE V. 1.

	(a) Egg Masses			(b) Eggs	
	Shaded Portion	Unshaded Portion		Shaded Portion	Unshaded Portion
Dish 1	62	20	Dish 1	376	117
Dish 2	38	17	Dish 2	279	142
Dish 3	50	18	Dish 3	364	147
Total	150	55	Total	1010	406
	F=	20·56		F=	44·15

TABLE V.2.

Mean Number of Egg Masses Laid Daily in Each Dish.

	Removed Daily	Removed Weekly	Block Totals
Week 1	11·67, 7·00, 11·33	10·33, 10·33, 8·83	59·49
Week 2	4·76, 5·13, 4·88	3·13, 6·38, 5·63	29·90
Treatment Totals	44·76	44·63	

ANOVAR.

Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F
Treatments	0·0014	1	0·0014	0·0006
Blocks	72·9640	1	72·9640	27·90
Interaction	0·0660	1	0·0660	0·0252
Error	20·9186	8	2·1492	
Total	93·95	11		

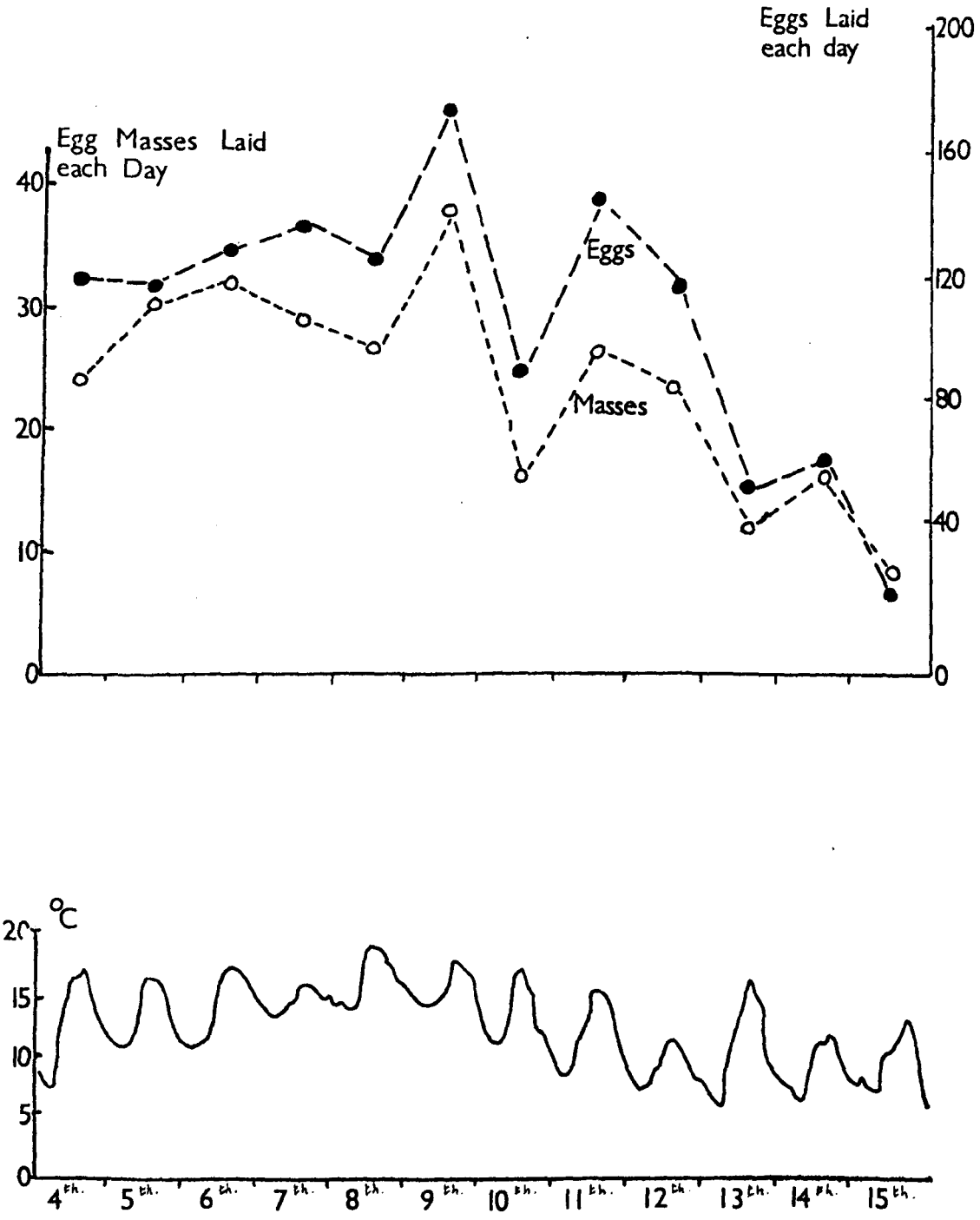
TABLE V.3.

Mean Number of Eggs Laid Daily in Each Dish.

	Removed Daily	Removed Weekly	Block Totals
Week 1	67·33, 43·83, 66·67	64·33, 67·83, 56·66	354·65
Week 2	32·26, 36·75, 37·50	26·62, 37·62, 34·62	209·36
Treatment Totals	287·33	276·68	

ANOVAR.

Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F
Treatments	9·4518	1	9·4619	0·17
Blocks	1759·0986	1	1759·0986	31·19
Interaction	13·2900	1	13·2900	0·24
Error	461·1700	8	56·4000	
Total	2233·0053	11		


Eggs Laid each day
Egg Masses Laid each Day
Eggs
Masses
0
10
20
30
40
0
40
80
120
160
200
°C
0
5
10
15
20
4th.
5th.
6th.
7th.
8th.
9th.
10th.
11th.
12th.
13th.
14th.
15th.
Days in April

period, the increase was not significant ~~(Table ——)~~.

Overall the mean number of eggs per egg mass was 6.30 ± 0.14 (95% confidence limits; range 2-13; 604 egg masses were examined). The overall mean number of egg masses estimated to have been laid by each snail every day was 0.70 ± 0.18 (95% confidence limits; overall mean max. air temperature 16.46 ± 1.40°C, mean min. 8.25 ± 1.59°C; food unlimiting.)

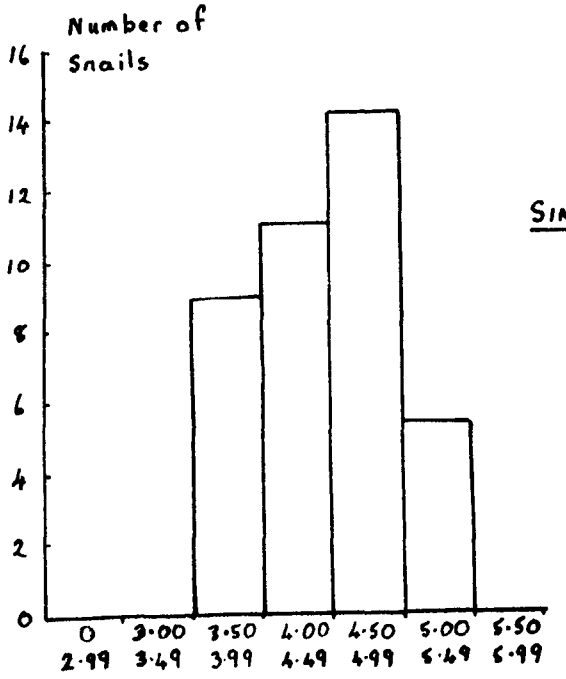
(iii) Copulation as a Stimulus to Oviposition

39 of the 50 snails kept in isolation and 19 pairs of the original 50 pairs of snails remained alive to lay one egg mass. The results for each series are plotted as histograms in Figure V.2. The first egg masses were laid 22 days after hatching. Over a quarter of the egg masses were laid by snails that were between 3.50-3.99mm in length. The snails that were reared in isolation attained maturity at a mean shell length of 4.74 ± 0.68mm (95% confidence limits; range 3.55-5.46mm).

In order to compare the pairs of snails with the singletons it was necessary to group the latter into simulated pairs. This was done using random number tables. The results for each "pair" thus created were inspected and the earliest date upon which one of the "pair" laid an egg mass was used in the subsequent analysis. This process mimicked the experimental procedure for real pairs but precluded the possibility that the two snails had copulated.

The number of days that elapsed before the first egg mass was laid by the real and simulated pairs of snails is given in Table V.4. The distributions of both sets of data are markedly skewed and so a

Fig V.2. Size at which First Egg Mass was Laid.



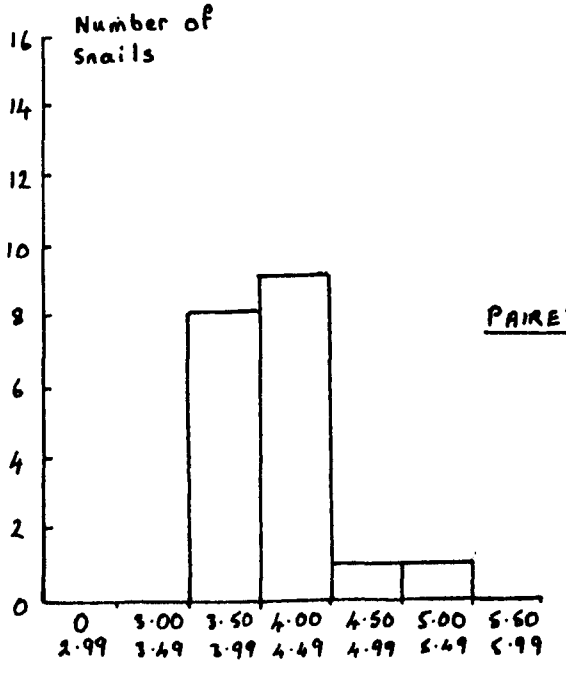


TABLE V.4.

The Number of Days After Hatching that the First Egg Mass was Laid.

Actual Pairs of Snails	Simulated Pairs of Snails
23, 25, 25, 25, 27, 27, 27, 28, 29.	22, 22, 27, 27, 27, 27.
31, 32, 33, 34.	32, 32, 32, 32, 33, 34, 34, 36.
40, 48.	41, 41, 41.
60, 61, 62	60, 65

logarithmic transform was applied. There was no significant difference between the respective means ($t = 1.46$; $df = 35$).

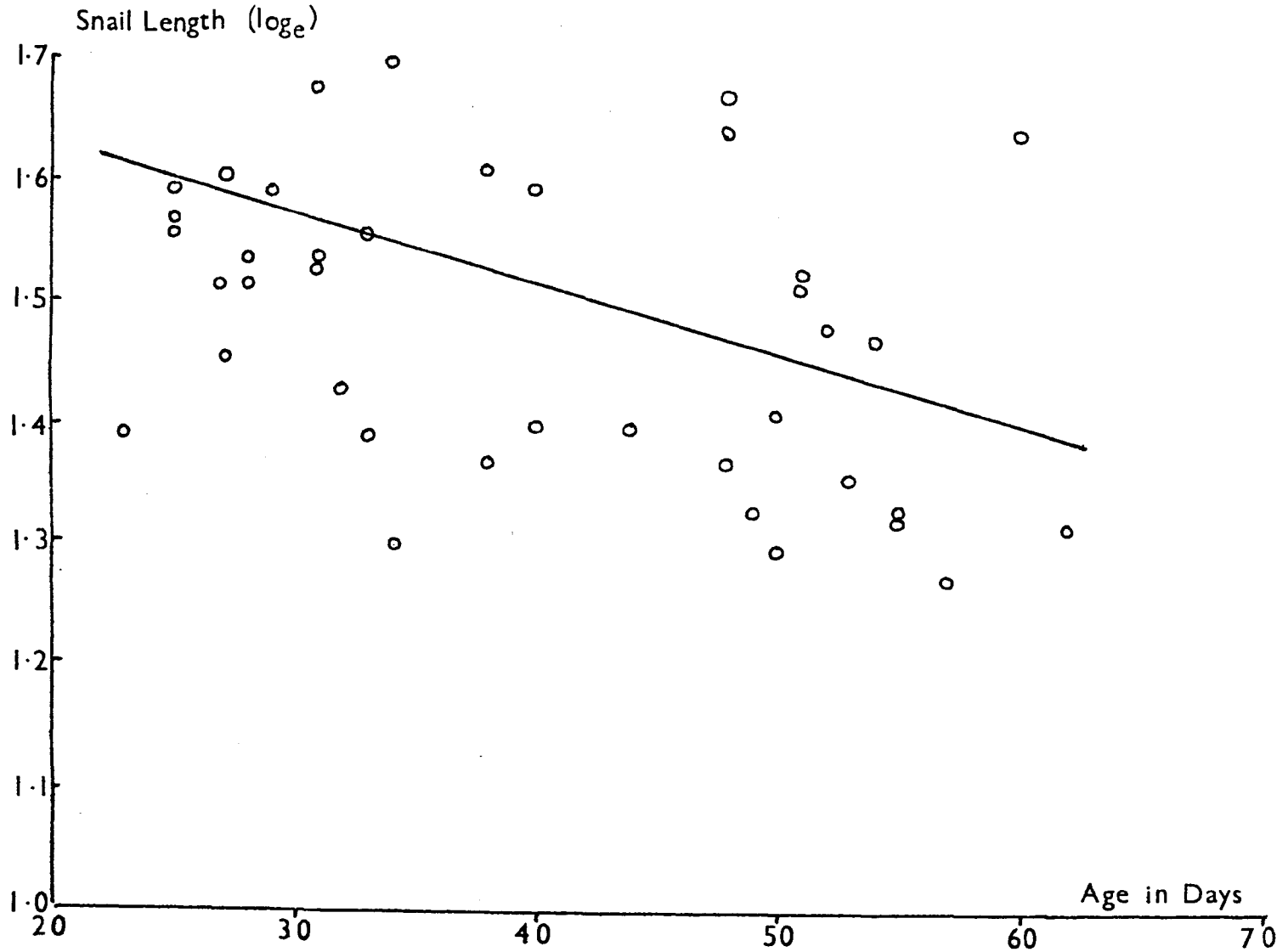
Casual inspection of the raw data for the singleton snails suggested that there was an inverse relationship between the age of the snail and its size at the time that the first egg mass was laid. The age in days of each snail is plotted against the natural logarithm of its size in Figure V.3. Despite the large scatter of points the linear regression was found to be significant ($0.05 > p > 0.01$; $df = 37$).

DISCUSSION

The evidence that larger numbers of egg masses were laid in shaded regions of the cultures was unequivocal and entirely consistent with anecdotal reports from field studies. Egg masses deposited in such situations would be protected from dessication but it is not clear whether the experimental result arose because the probability of oviposition is greatest when the snails enter a region of low light intensity or because the snails tend to spend more time in shaded areas anyway. There was no evidence of competition for oviposition sites, indeed, in some respects it may be of advantage to crowd egg masses together (as frequently happens) since this may retard the dessication of those masses near the centre of the aggregation. The general relationship between rate of oviposition and mean weekly temperature is consistent with observations in the laboratory of oviposition under varied fixed temperatures (Nice, pers.comm.)

Reports that copulation serves as a stimulus to oviposition could

Fig. I.3


Snail Length (log$_e$)
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
20
30
40
50
60
70
Age in Days

not be confirmed. The experimental basis for such conclusions has not been fully described in the literature and it is possible that Boray (1964) for example, failed to group the single snails in simulated pairs before comparison with the real pairs. In the present case this would have resulted in a spurious confirmation of the hypothesis.

The size of L. truncatula (expressed as shell length) is more an index of its nutritional state that its age since the snails aestivate for irregular periods throughout their life span. It is usual practice, therefore, to assign the snails to size classes for life table purposes or before the presentation of more general data. Implicit in this procedure is the assumption that the size of the snails is indicative of their physiological state; in other words particular rates of mortality or fecundity can be ascribed to all of the snails in a discrete size class irrespective of their actual ages. Within limits then the physiological state of a snail in a particular size class is assumed to be independent of the rate of growth of that snail.

A physiological event of particular significance is the attainment of maturity. The size at which L. truncatula is regarded as mature has been variously given as 4.0–4.5mm (Walton and Norman Jones 1926), 4.5mm (Kendall, 1953) and 3.0mm (Bruce et al., 1973). Not all of these authorities used the same index of maturity: Walton and Norman Jones (1926) and Kendall (1953) recorded the size at which the first egg mass was laid whereas Bruce et al. (1973) examined the gonads in dissection. Nevertheless, even when a single index is used the

range is still considerable. In the present study, for example, the first egg mass was laid by snails varying between 3.5mm and 5.5mm in length. Furthermore, the size at which a particular snail laid its first egg mass was inversely related to its age. The slower the rate of growth the smaller the size at which the snail laid its first egg mass. This result is counter to the usual assumption that the physiological state of a snail in a particular size class is independent of its rate of growth.

Snail length is an imperfect indicator of physiological state. The variance of the mean qualities ascribed to snails in a particular size class is due not only to the usual individual variations but also to the nutritional and microclimatic history of the population in question. This is not, however, an argument for abandoning the size class method especially as no other convenient physiological index exists. In any case, the total variance can sometimes be calculated from laboratory data and the extent of the uncertainty made plain.

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