STUDIES ON THE ECOLOGY OF *LYMNAEA TOMENTOSA* PFEIFFER 1855 AND *L. COLUMELLA* SAY 1817 (MOLLUSCA : GASTROPODA), INTERMEDIATE HOSTS OF THE COMMON LIVER FLUKE *FASCIOLA HEPATICA* LINNAEUS 1758 IN NEW ZEALAND

A thesis presented in partial
fulfilment of the requirements for the degree
of Doctor of Philosophy in
Veterinary Science at
Massey University

ROBERT ERIC HARRIS

December 1974

ABSTRACT

Studies on the ecology of Lymnaea tomentosa and L. columella were carried out to provide a basis for further studies on the ecology of Fasciola hepatica infections in New Zealand.

Intermediate hosts of F. hepatica in New Zealand are the native Australasian species L. tomentosa, an introduced American snail L. columella, and the European host L. truncatula. The literature on the systematics, geographical distribution, general biology and ecology of these snails, together with their relationships with F. hepatica, is reviewed.

Field observations on snail habitats indicated that L. columella occupied ponds and spring fed marshes which remained wet even in dry seasons. L. tomentosa was found in similar marsh habitats. Stability appeared to be an important quality of the habitats of both species; factors unfavourable to the snails included rapidly flowing water, marked seasonal fluctuations in water level, and shade from tall vegetation. The calcium content of water in habitats ranged from 4 to 70 ppm. Snails were more often found on flocculent than on firm mud and this preference was more marked in the case of L. tomentosa.

Snail population dynamics were examined on one pond habitat and three marsh habitats of L. columella and a marsh habitat of L. tomentosa. Age structure and density of populations fluctuated widely between and within each of the five years of the study. These changes were greater in populations of L. columella and not directly related to rainfall or temperature variations. Field and laboratory evidence showed that both species could breed throughout the year even when temperatures were as low as 5°C but populations were much larger in summer and early autumn.

Studies of the fecundity of the two snail species and the relationship between temperature and rate of development of eggs showed that whilst L. columella has a higher adult mortality rate it also has a much greater reproductive potential than L. tomentosa. Eggs of L. columella develop over a wider range of temperatures, hatching up to 34.5°C whereas temperatures above 30°C are lethal to eggs of L. tomentosa. Eggs of both species underwent some development at temperatures between 5 and 10°C but the proportion that hatched at low temperatures was very small, particularly in the case of L. tomentosa.

Both species showed similar responses to desiccation on filter paper at 16.5°C and 80 to 90% relative humidity; survival time was closely related to shell length, with large snails considerably more resistant than smaller specimens. When snails were subjected to desiccation on mud results were much less predictable and the mean shell length of survivors was often less than that of snails which died.

A biometric analysis of shell shape showed distinct differences between large specimens of L. tomentosa and L. columella but shell dimension ratios were not diagnostic in specimens under 4 mm in length. Examination of a pond and a marsh population of L. columella showed no differences in shell shape attributable to habitats.

Over a 5 year period there was no detectable relationship between the intensity of F. hepatica transmission from a L. columella habitat and snail population density. The only correlation between F. hepatica transmission and rainfall was a possible inverse relationship between uptake in tracer sheep and December/January (mid-summer) rainfall. There was no evidence of any overwintering infection of F. hepatica infection in L. columella. Uptake of flukes by tracer sheep was almost totally confined to the period between mid-summer and mid-winter. Both experimental and circumstantial evidence indicated that pugging by cattle rendered marsh habitats more suitable for snails, although exclusion of cattle from the experimental area coincided with an increase in the uptake of F. hepatica by tracer sheep.

PREFACE

Fasciola hepatica Linnaeus 1758, the common liver fluke, is a common parasite of grazing animals in many parts of the world; its wide range of mammalian hosts includes man. It is of considerable economic importance due to its own pathogenicity and as a precursor of "black disease" in association with the bacterium *Clostridium novyi*. The liver fluke is also of great biological and pedagogic interest as a model of a parasite with a succession of free-living and parasitic stages, as testified by the space devoted to descriptions of its life-cycle in textbooks of zoology and parasitology.

There has been a recent revival of interest in fascioliasis in New Zealand due to an increase in the prevalence and distribution of the disease. The aim of the present study of the ecology of two of the intermediate hosts of *F. hepatica*, *Lymnaea tomentosa* Pfeiffer 1855 and *L. columella* Say 1817, is to provide some basis for further studies on the ecology, epidemiology and control of fascioliasis in New Zealand.

Whenever possible the information obtained in this study was quantified and analysed statistically. The maximum level of probability that a result could have arisen by chance regarded as "statistically significant" is .05. The use of one, two or three asterisks after the result of an analysis implies a probability of less than .05, .01 and .001 respectively, and is equivalent to the terms $p < .05$, $p < .01$ and $p < .001$.

ACKNOWLEDGEMENTS

Many people helped both directly and indirectly in the production of this thesis. Among the latter, who will not receive individual mention, were those friends whose casual enquiries acted as a stimulus to greater effort. More direct assistance was given by the following people:

My supervisors, Dr. W.A.G. Charleston of the Veterinary Faculty of this university and Dr. L.K. Whitten from the Veterinary Research Division of the Ministry of Agriculture and Fisheries were unfailingly patient and helpful. Dr. Charleston, being on hand, bore the greater burden but Dr. Whitten gave much useful advice, usually at short notice and often at inconvenient times. Professor R.N. Munford of the Veterinary Faculty, Mr. G.C. Arnold of the Mathematics Department, and Mr. C.S. Clarke of the Department of Food Technology all assisted with advice on statistical analyses but since they did not act as proof readers any remaining errors are my own.

I had some stimulating discussions with Mr. N.B. Pullan in the early stages of the study, often during the course of a visit to a snail habitat one of us thought would be of interest to the other. Dr. M.J. Winterbourn of the Zoology Department, Canterbury University, identified the Physa found on the Pohangina farm. Others to whom my thanks are due include Mr. D.K. Signal, Pohangina, and Mr. J.M. Ryder, Tokomaru, the farmers on whose property the snail population studies were conducted; both were totally co-operative at all times. The same is true of the management and staff of the C.W.S. Ltd. Meat Export Works, Longburn and the veterinary and lay staff of the Meat Division of the Ministry of Agriculture and Fisheries. Other Massey University staff whose help was invaluable were Mr. T.G. Law of the central photographic unit, the university printer Mr. P.J. Herbert, Mrs. L.C. Battye of the library, and the sheep farm supervisor Mr. P.H. Whitehead. I also acknowledge gratefully the grant from the Veterinary Faculty research fund which paid for sheep, fencing materials and other equipment.

I am especially grateful to a most efficient and enthusiastic typist, Mrs. Joy Pearce. Almost none of the problems alleged to be inevitable in producing type drafts of PhD theses arose, and most of the minor errors discovered in the final draft were due to my inadequate editing at an earlier stage.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION AND LITERATURE SURVEY

> ..."the ecologist is something of a chartered libertine. He roams at will over the legitimate preserves of the plant and animal biologist, the taxonomist, the physiologist, the behaviourist, the meteorologist, the geologist, the physicist, the chemist and even the sociologist ...."
>
> (Macfadyean, 1957).

1.1 SYSTEMATICS OF THE NEW ZEALAND LYMNAEIDAE

The phylum Mollusca is divisible into six or seven classes, since some authorities consider the Aplacophora and Polyplacophora to be sub-classes of the Amphineura, while others regard them as having the status of classes. In either taxonomic system the Gastropoda are a major class with three sub-classes: the Prosobranchia, the Opisthobranchia, and the Pulmonata.

The outstanding feature of the pulmonate snails and slugs is their conversion of the mantle cavity into an air-breathing organ with a highly vascularised "lung" on its roof. The Pulmonata comprise two orders: the terrestrial Stylommatophora are snails and slugs with eyes at the tip of one pair of tentacles, while the aquatic Basommatophora have their eyes at the bases of their tentacles.

Terrestrial molluscs are believed to have made the adaptation to life on land by first invading estuaries and later evolving through freshwater forms. Lack of oxygen in estuarine waters probably led to aerial respiration (Carter, 1931) and another necessary adaptation was internal fertilisation. By contrast, the freshwater pulmonates are believed to be making their way back to water from terrestrial habitats, the most primitive being the exclusively air-breathing amphibious forms (Hunter, 1964). *Lymnaea* is a freshwater genus of Basommatophora whose species range from truly aquatic forms such as *Lymnaea stagnalis* which can breathe either air or water, to air-breathing amphibious snails like *L. truncatula* (see section 1.3.3).

Until Hubendick's (1951) comprehensive revision of the genus the taxonomy of the Lymnaeidae was in some disorder. Many taxonomists had erected species solely on the basis of shell morphology, a hazardous practice in a genus with variable intra-specific shell-shapes and much overlapping between species. Hubendick used a combination of shell and

soft-part morphology, and his publication is the standard reference work for the Lymnaeidae. More recently chromosome studies, chromatography of body mucus, and examinations of haemolymph antigens have been applied to taxonomic problems in snails. Results have sometimes appeared to create new confusion rather than elucidate the species and generic relationships being investigated. For example Michelson (1966) on serological grounds suggests that the Lymnaeidae may contain more than the one genus Lymnaea but Hubendick (loc. cit. 1951) asserts that the genus as a whole has great morphological uniformity. Further reasons for caution are the fact that paper chromatography patterns of body mucus show seasonal changes (Wright, 1959a) and are altered when snails become parasitised (Sudds, 1965), perhaps due to utilisation of some proteins by the parasites (Gress and Cheng, 1973).

Climo and Pullan (1972) have brought up to date the taxonomy of the New Zealand Lymnaeidae, describing four species: Lymnaea stagnalis Linnaeus, L. truncatula Muller, L. tomentosa Pfeiffer, and L. columella Say.

1.1.1 Some morphological features of the New Zealand Lymnaeidae

Full descriptions are given in Hubendick (1951) and only the main distinguishing features are mentioned in the following sections. Except where otherwise stated, descriptions are based on Hubendick's publication (Figs. 1.1; 1.2).

Shells

The shells, in order of maximum length as reported by various authors are:

L. stagnalis up to 52 mm (Baker, 1911);

L. columella up to 21.5 mm (Baker, 1911);

L. tomentosa up to 16 mm (Boray and McMichael, 1961);

L. truncatula up to 13 mm (Kendall, 1953).

In all of them the shell spirals to the right.

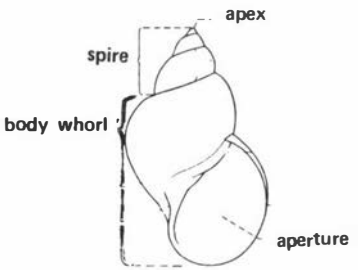


Fig. 1.1 Terminology used in describing shells (after Hubendick, 1951).

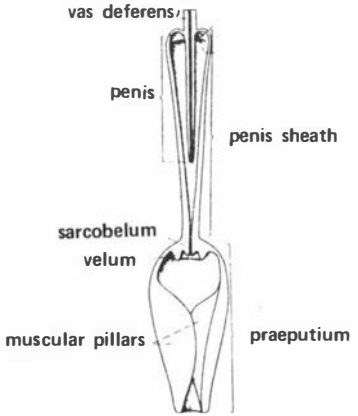


Fig. 1.2 Terminology used in describing distal genitalia (after Hubendick, 1951).

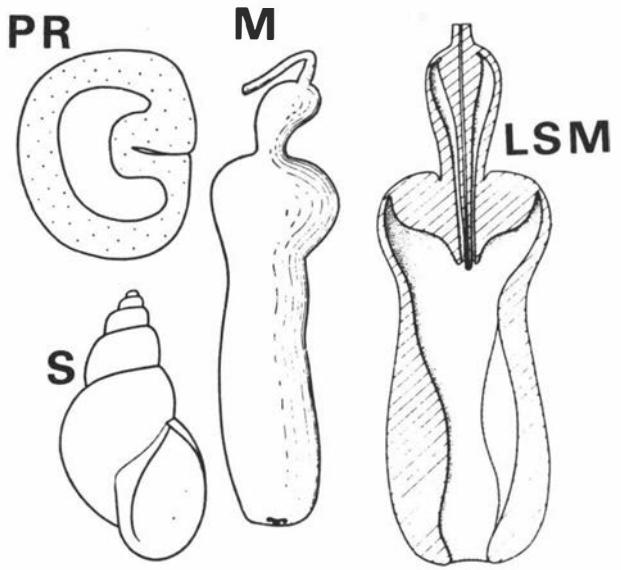


Fig. 1.3 *Lymnaea truncatula* (from Hubendick, 1951).

KEY to Figs. 1.3, 1.4 and 1.5.

PR, distally sectioned prostate; m, male copulatory organs;

S, shell; L S M, longitudinal section of M;

FM, distal portion of female genitalia.

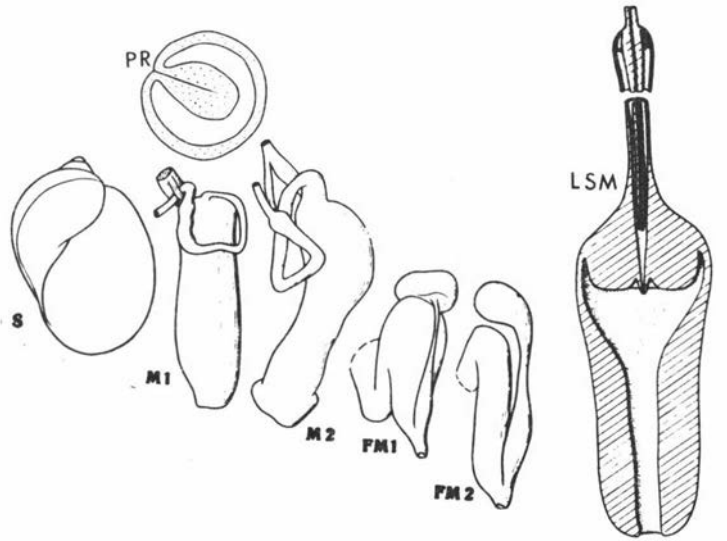


Fig. 1.4 *Lymnaea tomentosa* (from Hubendick, 1951).

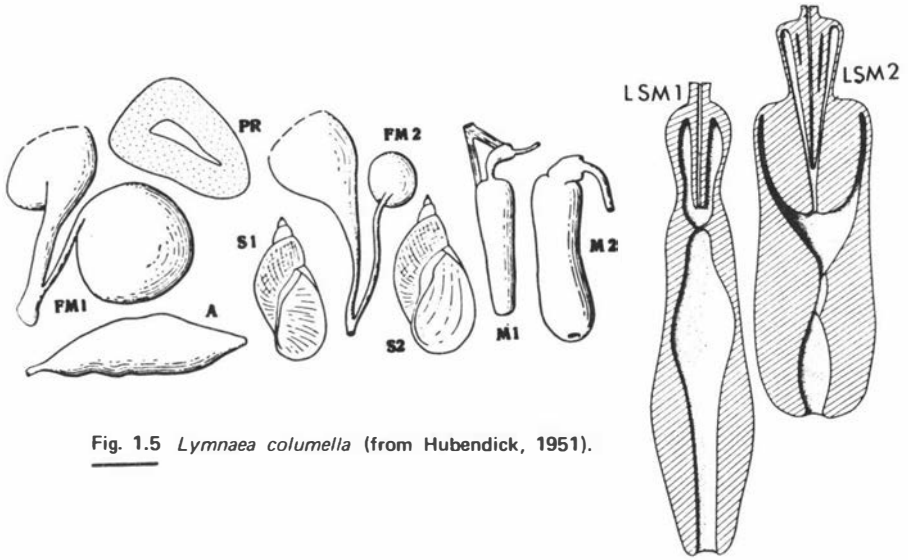


Fig. 1.5 *Lymnaea columella* (from Hubendick, 1951).

Apart from its generally greater size L. stagnalis is easily distinguishable by its greater number of whorls, four to six according to Hubendick (1951) but Climo and Pullan (1972) describe five to eight. It has a much more pronounced spire than the other New Zealand lymnaeids, although Hubendick (loc. cit.) warns that the spire is too variable a feature to be diagnostic between species in the genus.

The other species normally have four shell whorls. The shell of L. truncatula is pyramidal with a relatively short aperture, about one third of its length (Fig. 1.3). The shell is usually smooth, without spiral striations, and the body whorl bulges less than that of L. tomentosa or L. columella.

The thin, fragile shell of L. tomentosa has a short spire merging gradually into the large, ovate body whorl (Fig. 1.4). There is a comparatively coarse microsculpture parallel to the peristome. The aperture is wide and the shell is globose when compared with L. columella in which the aperture is relatively narrow and whose shape is more succiniform (Climo and Pullan, 1972).

The shell of L. columella is also thin and fragile and has a large body whorl (Fig. 1.5). As well as its succiniform shape a useful distinguishing feature is a characteristic microsculpture of fine spiral striae which cross the main growth lines (Climo and Pullan, loc. cit.). According to Hubendick (1951) the species shows the usual lymnaeid variability in shell proportions, the form of the aperture, whorls and spire, but Climo and Pullan (1972) report relatively little variation in New Zealand specimens.

Soft parts

For taxonomic purposes the only soft parts of diagnostic significance are the distal genitalia (Fig. 1.2).

The penis sheath of Lymnaea stagnalis is relatively small compared to the large, muscular praeputium. The sarcobelum and velum are usually small, and the distal end of the penis is rather bulbous.

L. columella is characterised by a short penis and penis sheath, a large sarcobelum in some specimens, and a slender and unfolded prostate (Fig. 1.5). In L. tomentosa the sarcobelum and velum are of similar length, but the velum is very thick. The prostate has a large inward

fold (Fig. 1.4). The penis sheath of L. truncatula is shorter than the praeputium, and the prostate has only one small inward fold (Fig. 1.3).

In New Zealand the lymnaeids most likely to be confused with one another are small specimens of L. columella and L. tomentosa. Climo and Pullan (1972) have given a useful comparative description, noting that the darker colour of L. columella can be lost under some laboratory conditions.

1.2 GEOGRAPHICAL DISTRIBUTION OF THE SNAIL HOSTS OF FASCIOLA HEPATICA

The temperature optima and tolerance limits of the free-living stages of Fasciola hepatica are such that it is best adapted to temperate zones, and its usual intermediate host over most of its distribution outside America and Australasia is Lymnaea truncatula. The descriptions of the distribution of the following lymnaeid species are based on Hubendick (1951) unless otherwise stated.

1.2.1 Lymnaea truncatula

This is the recognised snail host throughout most of Europe (Fig. 1.6). It also occurs in Iceland, the Faeroes, Madeira, north-west Africa and Egypt. Its range extends across central Asia into Alaska, and its distribution in Africa appears to reflect the migration path of birds. In North America L. truncatula occurs in southern Alaska and in isolated pockets elsewhere. It is closely related to L. humilis which is found over most of the United States and southern Canada, and probably also to L. cubensis which has a circum-Caribbean distribution.

In New Zealand L. truncatula is restricted to a few parts of inland Nelson and Marlborough (Pullan et al., 1972), a surprisingly limited distribution in view of its introduction some time last century. Although it has a limited capacity for active migration and shows great site fidelity (Heppleston, 1972) there must have been many opportunities for passive migration to new habitats through the agency of birds or grazing mammals. Previously known as Lymnaea tenella (Hutton) or L. alfredi (Suter), its true identity has only recently been established (Climo and Pullan, 1972) although it had been suspected (Hubendick, 1951).

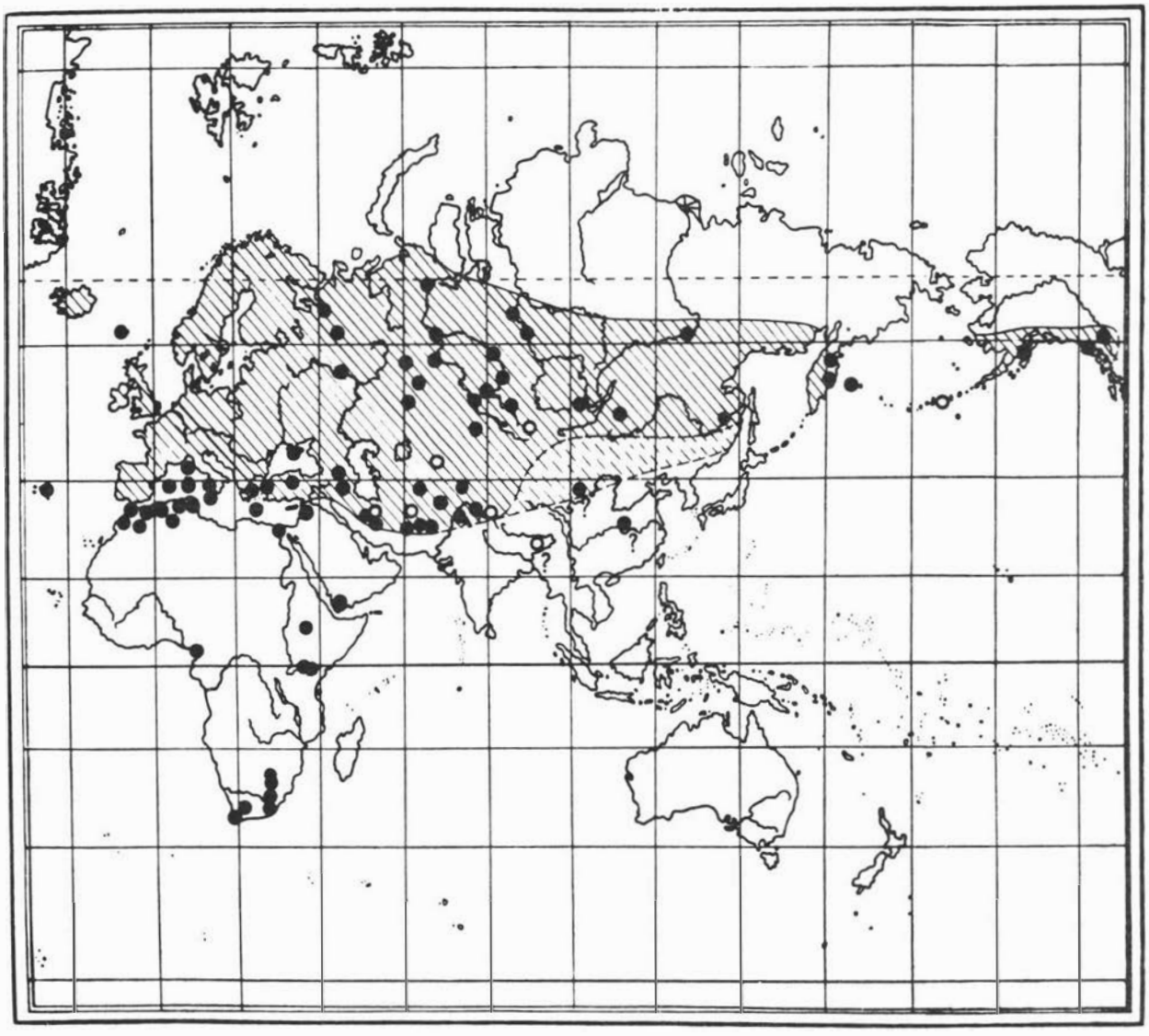


Fig. 1.6 Geographical distribution of *L. truncatula* (from Hubendick, 1951).

● precisely reported localities

○ approximately reported localities

common occurrence

probable occurrence

1.2.2 Lymnaea tomentosa

L. tomentosa is confined to south-eastern Australia and both islands of New Zealand, with a few isolated loci in northern Australia; it is also widely distributed in highland areas of New Guinea from 2,500 to 7,000 feet above sea-level (Boray, 1969). L. tasmanica (Tennison-Woods) is now included in the species and other synonyms were L. brazieri (Smith), L. launcestonensis (Tennison-Woods) and L. sub-aquatilis (Tate) (Boray and McMichael, 1961).

In New Zealand L. tomentosa occurs mainly in the traditional, long established foci of fascioliasis (Pullan and Whitten, 1972) and sub-fossil deposits show that it predates man (Dell, 1956).

1.2.3 Lymnaea columella

Baker (1911) describes the distribution of L. columella as occupying the drainage areas of the St. Lawrence and the Mississippi as well as various other Atlantic rivers, and extending just over the Canadian border. Other "races" of L. columella are said to be found in Mexico, Central America and Cuba. The species can be found fairly commonly in European hothouses, and is con-specific with the South American L. peregrina (Meeuse and Hubert, 1949; Hubendick, 1951). Although it was first reported in hothouses in California in 1923 (Gregg, 1923) it does not seem to have been reported from the field until Barber (1962) collected specimens for a laboratory study. There must be some doubt as to whether Barber's specimens were really L. columella or at any rate a typical strain, because they were rather refractory to F. hepatica infection and all cercariae encysted in the tissues of the snail without emerging to the exterior.

L. columella has extended its range beyond the American continent becoming established in Hawaii (Alicata, 1953), South Africa (van Eden and Brown, 1966) and New Zealand (Pullan, 1969). Its failure to become established outside hothouses in Europe is a little surprising; it is unlikely to be due to the harshness of the European climate since its American range includes Quebec, Manitoba, Nova Scotia, Central and South America. It must have had many opportunities to extend its range into Europe since its discovery in a Copenhagen glasshouse in 1927 (Schlesch, 1930), particularly since Schlesch recommended encouraging

the species as a means of keeping down algae in aquaria.

In New Zealand L. columella occurs mainly in the North Island, and it can be found more often than L. tomentosa in North Auckland and in the western half of the North Island. In the South Island L. columella has been found in only two areas: parts of Nelson province, and a small area just south of Timaru (Pullan et al., 1972). The method by which L. columella reached New Zealand is not known, but it seems to have been introduced to Europe along with aquatic plants delivered to botanical gardens and many aquatic plants are known to have been imported both legally and illegally into New Zealand. Once inside a country dispersal, although possible in many ways, is thought most likely to occur through the agency of aquatic birds (Rees, 1965).

1.2.4 Other snail hosts of Fasciola hepatica

F. hepatica occurs in parts of the world where none of the aforementioned snail hosts can be found. In natural outbreaks a lymnaeid snail is always involved, particularly the more amphibious species. Pantelouris (1965) has listed reports of other lymnaeid hosts and the countries in which they are responsible for F. hepatica infection. L. gedrosiana, regarded by Hubendick (1951) as a transitional form between the races auricularia and rufescens of L. auricularia, is said to act as a host for F. hepatica in Iran (Arfaa et al., 1969). Galba occulta is described as a new host in Poland (Furmaga, 1968) but since its main distinguishing feature is greater output of cercariae it is probably a variant of L. truncatula.

1.3 GENERAL BIOLOGY OF THE SNAIL HOSTS OF FASCIOLA HEPATICA

1.3.1 Growth

Growth rates in snails depend upon age and environmental conditions, so laboratory and field observations on growth are applicable only to the conditions pertaining at the time. Only such relatively simple growth parameters as increases in shell-length with age have been studied in the snail hosts of F. hepatica.

Lymnaea truncatula

The early workers with L. truncatula had trouble rearing snails in the laboratory, and growth rates were slower than those achieved later. From data published by Walton and Jones (1926) snails at unspecified laboratory temperatures in late spring and summer appeared to take at least 70 days to reach maturity at a shell length of 4.0 to 4.5 mm. Roberts (1950) found that from hatching to a shell length of 2.5 mm took 21-35 days at a mean 17°C and growth to 4.5 mm needed a total of 76-94 days. At an incubator temperature of 25°C the times were 14-19 days and 53-62 days respectively.

In a laboratory where snails commonly grew to 12 or 13 mm, a size rarely encountered in the field, L. truncatula reached 4.8 mm, 4.3 mm and 5.0 mm in 23 days (Kendall, 1953). Optimum temperatures were between 18°C and 21°C. The growth curve of well fed snails was steepest until the onset of sexual maturity when the shell length was about 4.5 mm, thereafter it levelled off but there was still some growth as long as 300 days after hatching.

Lymnaea tomentosa

At 25°C L. tomentosa reached sexual maturity 26 days after hatching, when the mean shell length of eight snails was 6 mm (Lynch, 1963). There was a slackening in growth rate at 5-7 mm shell length.

Lymnaea columella

Colton (1908) reported attainment of sexual maturity after two months at favourable temperatures, when the shell length was 7 or 8 mm. In a more detailed study, Baily (1931) fitted logistic curves to growth data from L. columella but did not quote laboratory temperatures. His snails began egg laying at 50 days when their shell length was approximately 10 mm (estimated by extrapolating the shell lengths from the aperture lengths given by Baily, by multiplying aperture lengths by 1.4). Inspection of Baily's logistic curves shows a rapid decline in growth rate when the shell length is about 11 mm. This parallels observations on L. truncatula (Kendall, 1953) and

L. tomentosa (Lynch, 1963) whose growth rate declines soon after the onset of sexual maturity.

Puerto Rican L. columella attained sexual maturity 21 to 23 days after hatching when laboratory temperatures were 26 to 28°C. Their shell-length at maturity was said to be only 5 to 6 mm (Leon-Dancel, 1970) but they were kept in groups of 10 or 20 so egg-laying may have been initiated by one or two larger snails.

1.3.2 Circulatory System

The Gastropoda have an open circulatory system. The head, foot and viscera are supplied directly with blood from closed arteries. Blood seeps from the arteries into pseudovascular spaces in connective tissue, and is returned to central spaces known as the cephalo-pedal and visceral sinuses, both of which empty into a subrenal sinus. From the subrenal sinus blood is distributed in varying proportions through the respiratory organs and kidney before returning to the auricle. In Pulmonata the heart consists of two chambers, the auricle and the ventricle.

Besides its normal functions the blood in connective tissues serves as a fluid skeleton, giving temporary rigidity to parts of the body when pressure is raised by muscle contraction. The only gastropods with erythrocytes are the Planorbidae, whose respiratory pigment is haemoglobin. In the rest, including the Lymnaeidae, the respiratory pigment is copper-based haemocyanin which is dissolved in plasma (Gardiner, 1972 p. 395). The blood cells in these haemocyanin based circulatory systems are amoebocytes, phagocytes which bear similarities to at least some of the leucocytes of vertebrates. F. hepatica infections cause an intense amoebocytic response in heavily parasitised snails (Barber, 1962, p. 46).

1.3.3 Respiration

In pulmonate gastropods the mantle cavity has been converted into a heavily vascularised air-breathing organ. The greater part of the mantle is fused to the dorsal surface of the body, converting the mantle into a sac which communicates with the exterior through a pore known as the pneumostome. Respiration in lymnaeid snails may involve

one or more of the following mechanisms:

1. Air breathing by inhalation and exhalation through the pneumostome is most important in amphibious species like L. truncatula (Purchon, 1968, p. 29).

2. Cutaneous respiration, by diffusion of oxygen and carbon dioxide through the body surface, is the most important method of respiration in more aquatic species such as L. stagnalis. When water temperature rises in summer oxygen tension falls and cutaneous respiration becomes more difficult, so trips to the surface for pulmonary respiration become necessary (Ghiretti, 1966, p. 193).

3. Some of the more aquatic lymnaeids fill the mantle with water, converting it into a water lung so that the pulmonary sac functions as a gill (Hunter, 1953).

4. At other times the pulmonary sac may contain a bubble with about 84% nitrogen into and out of which respiratory gases diffuse from the surrounding water (Hunter, 1968).

1.3.4 Feeding and Digestion

The most primitive gastropods are herbivores, feeding on detritus and algae which are at first subjected to extracellular digestion by amylases. More advanced herbivores consume larger pieces of plant material with the aid of extracellular cellulases. Most lymnaeidae are in the second category and some are supplementary carrion feeders. Digestion is largely extracellular, the products of digestion being absorbed in the digestive diverticula (Purchon, 1968, p. 210).

The alimentary tract of a typical lymnaeid snail consists of a mouth, buccal cavity with radula and odontophore, a tubular oesophagus, a stomach and an intestine (Figs. 1.7; 1.8). There are two glandular areas: salivary glands which open into the buccal cavity, and mid-gut glands or digestive diverticula. The stomach has become modified to form a muscular gizzard and the mid-gut glands are often referred to as the liver or hepato-pancreas.

Digestion

The chitinous radula, bearing numerous teeth, lies over the muscular, tongue-like odontophore (Fig. 1.7). The radula is used to rasp and scrape food so that it may pass into the buccal cavity to be mixed with mucus which contains amylase and a tryptic enzyme (Carriker, 1946; Hyman, 1967, p. 250).

Food then passes down the oesophagus into the gizzard which must contain grains of soil or sand if the digestion system is to function properly (Colton, 1908). The finely ground food then passes through ciliated ducts to the hepato-pancreas for intracellular digestion (McDonald, 1969) or absorption after extracellular digestion. Electron microscopy has shown that there is not such a clear-cut distinction between these processes as was once thought (Owen, 1966).

The digestive diverticular or hepato-pancreas consists typically of numerous blind-ended tubules which communicate with the stomach by a series of branched ducts. The organ secretes digestive enzymes and acts as an organ of absorption, phagocytosis, food storage and excretion.

The two main mechanisms responsible for passage of food through the digestive system are ciliary action and muscular contractions.

1.3.5 Reproduction

Fertilisation

The lymnaeid snails are simultaneous hermaphrodites (Fig. 1.9). Self fertilisation seems to be the rule in L. columella (Colton, 1912) and L. truncatula (Walton and Jones, 1926) and is common in L. tomentosa although chain-copulation will occur at suitable temperatures (Boray, 1964b).

When hermaphrodite snails reared in isolation produce eggs it is difficult to say whether fertilisation occurs as both ova and spermatozoa travel down the genital duct, or as a result of self-copulation. Self-copulation is anatomically feasible in L. columella and has been observed (Colton, 1918) but has not been reported in L. tomentosa or L. truncatula.

Fig. 1.7 Diagrammatic longitudinal section of gastropod head (modified from Owen, 1966 after Runham, 1963).

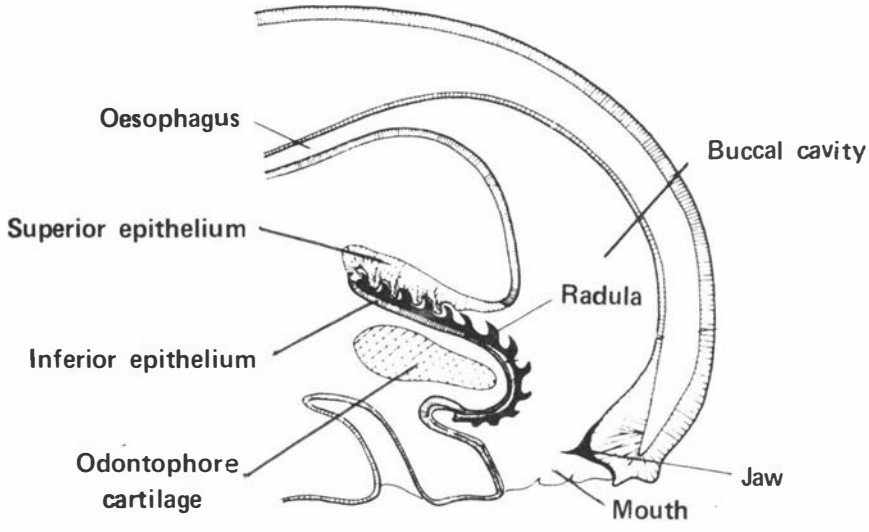


Fig. 1.8

Diagram showing the main features of the alimentary canal of basommatophoran pulmonates (from Owen, 1966).
C, caecum; C U cuticular plates; G G, gastric gizzard; I, intestine; O, oesophagus; S G, salivary glands.

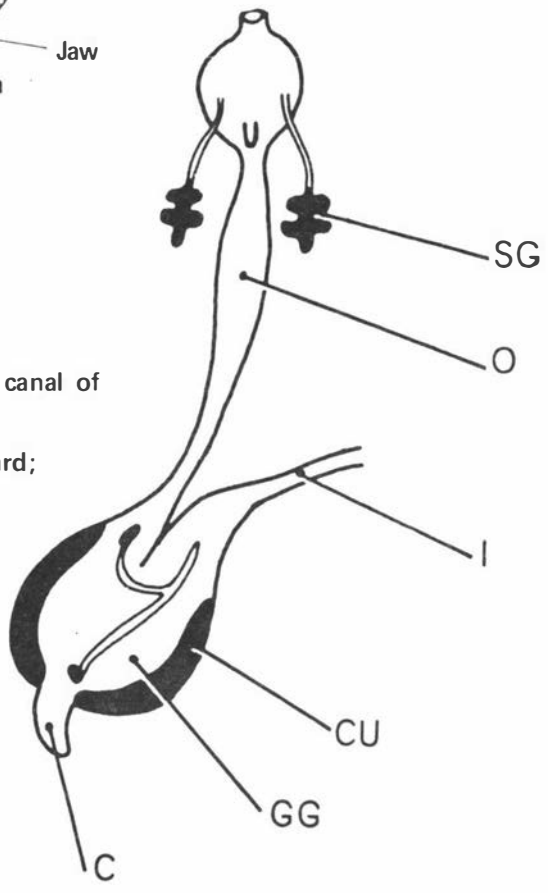
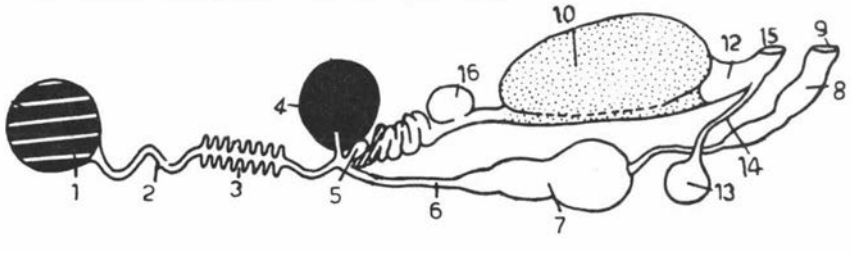


Fig. 1.9 Diagram of the reproductive system of *Lymnaea peregra* (from Hyman, 1967 after Duncan, 1960).
1, ovotestis; 2, hermaphroditic duct; 3, seminal vesicles; 4, albumen gland; 5, fertilisation pouch; 6, sperm duct; 7, prostate; 8, penial complex; 9, male gonopore; 10, oothecal gland; 12, vagina; 13, spermatheca; 14, duct of 13; 15, female gonopore; 16, muciparous gland.



Oviposition

Lymnaeid snails lay their eggs in a gelatinous capsule attached to plants, stones, or other objects. After a period of development which varies with temperature the young snails gnaw their way out.

1.4 ECOLOGY OF THE SNAIL HOSTS OF FASCIOLA HEPATICA

1.4.1 Habitats

The freshwater Basommatophora are in the process of evolving back to aquatic habitats from land. Even among the Lymnaeidae they vary in their degree of adaptation to aquatic environments. Those which act as intermediate hosts of Fasciola hepatica are generally amphibious, spending a good deal of time out of water on mud slopes browsing on algae.

Hydrography

Lymnaea truncatula is a truly amphibious snail which utilises the interface between water and land, being particularly adapted to the mud patches resulting from poaching by grazing animals. In dry seasons the snail is active only in reservoir habitats, permanently wet areas such as ponds, ditches, and spring-fed marshy areas. Rapidly flowing water is unsuitable. Surprisingly, L. truncatula is usually present in only small numbers in these apparently more favourable reservoir habitats and most snails are quite small (Taylor, 1964). Its preference for marginal habitats is such that it can be reared more easily on mud slopes in laboratories than in conventional aquaria (Taylor and Mozley, 1948). L. truncatula is very sensitive to water-flow, being absent from streams but present in small backwaters away from the main flow (Heppleston, 1972). In permanent water it is replaced by other species of snails (Ollerenshaw, 1959).

When rainfall is greater than evapotranspiration, extension habitats become available. These are temporarily muddy areas on which surface water accumulates, and they are responsible for a rapid increase in snail numbers and extension into a greater proportion of the area

grazed by farm stock. The mud of extension habitats is colonised by the micro-algae which are the essential food of L. truncatula.

Springs that provide sufficient water for wide, swampy areas of slow-moving, shallow water are ideal conditions for L. tomentosa (Macfarlane, 1937; Boray, 1964a; Pullan et al., 1972). Dams that provide drinking water for stock, irrigation channels, swampy edges of lakes, and small shallow streams with flow-rates not greater than 15 cms/sec. are also suitable. Boray observes that permanent habitats often have small numbers of snails compared to some temporary habitats in wet seasons, but are important as snail reservoirs in dry seasons. This observation parallels that made by Taylor (1964) for L. truncatula. In fact L. tomentosa may disappear from ponds if they fail to dry out for a little while in summer (Lynch, 1965), which indicates that they are less adapted to a totally aquatic life than the true pond snails. The reason for this phenomenon is not clear, but colonisation of the mud slopes by coarse vegetation in permanent habitats cuts out light and reduces the amount of micro-algae available to the snails.

Although it seems more adapted to shallow, marginal habitats L. tomentosa spends more time on the bottom than on the surface or out of the water (Climo and Pullan, 1972). It prefers shallow tanks with mud slopes but it can complete its life cycle in conventional aquaria so it seems to be at a slightly more advanced stage of evolution back to freshwater habitats than is L. truncatula.

Information on the natural habitats of L. columella is scarce. Baker (1911) says that it is "an inhabitant of ponds and streams where the water is more or less stagnant", presumably meaning that it prefers loci away from the main flow of water. Baker also mentions "shallow bays and small ponds or creeks". Spring-fed marshes with exposed mud are suitable as are dams and small ponds with weed floating on the surface. The latter are unsuitable for L. tomentosa, which prefers to feed on benthic algae in clear, sunlit water, but L. columella can utilise these habitats because of its preference for the surface and edges of ponds (Pullan et al., 1972).

The literature contains no direct reference to the permanence of L. columella habitats, but Pullan et al. (loc. cit.) report that

L. stagnalis was found in association with L. columella, but not with L. tomentosa or L. truncatula. This suggests that L. columella may occupy larger and more permanent bodies of water than either of the other two snail hosts of Fasciola hepatica.

Shade

As mentioned above, surface pond weed renders a habitat unsuitable for L. tomentosa. Heavy shading by trees or tall vegetation prevents growth of micro-algae and is not found in habitats of L. truncatula (Taylor, 1964), L. tomentosa (Macfarlane, 1937; Boray 1964a) or L. columella (Pullan et al., 1972).

Substratum and Vegetation

L. truncatula is found more often on firm clay and flocculent mud is unsuitable (Walton, 1918; Peters, 1938b), but it may be found on almost any soil type if there is sufficient soil moisture (Over, 1967). Peaty soils constitute unsatisfactory habitats for L. truncatula, perhaps due to low soil pH, but small numbers will live in permanent spring-fed areas on sour hill soils (Taylor, 1964). The snails will live on quite acid mud so it is probably the physical quality of peat that is unsuitable rather than its low pH (Heppleston, 1972), or the algae favoured by the snail may not grow on peat.

Certain plant associations are suggestive of L. truncatula habitats in Europe since the nature of the vegetation depends on the water table over a period rather than at a given point in time (Over, 1962). Other plants indicate unsuitable habitats; for example sphagnum moss indicates a degree of soil acidity which cannot be tolerated by L. truncatula (Taylor, 1964).

According to Macfarlane (1937) only fine, silty loams form a suitable substratum for L. tomentosa, and it is usually associated with a short, green rush Juncus lampocarpus and he found suitable habitats in Hawke's Bay on limestone, sandstone or mudstone where there was a covering of fine alluvium. He considered peat, volcanic ash and bush soils unsuitable, but Brunsdon (1967) believed there was little correlation between the geographical distribution of L. tomentosa and soil-type.

Australian habitats are mainly podzols or podzolic soils with

a muddy, alluvial bottom, but turbidity is harmful to the snails (Boray, 1964a). The fine, silty, alluvial bottom described by these authors seems significantly different from the firm, clay substratum preferred by L. truncatula but the three host species may all be found in one locality in New Zealand (Pullan et al., 1972).

There seem to be no specific references to the substratum of L. columella habitats, but these snails, unlike L. tomentosa, are able to utilise habitats covered with the floating pond weeds Azolla rubra and Lemna minor (Pullan et al., 1972).

pH

pH readings of natural waters are notoriously unreliable; they differ before and after reaching equilibrium with air (Boycott, 1936) so the high degrees of accuracy achieved with pH meters are probably spurious and indicator papers graded in steps of 0.5 are sufficient to detect general trends.

L. truncatula may be found in waters ranging from pH 5.5 to 8.6 but it is most common in neutral or near neutral habitats (Walton and Wright, 1926; Peters, 1938b; Heppleston, 1972). L. tomentosa also tolerates a wide range of pH values, but seems to have a preference for the pH 6.0 to 7.0 zone (Ross and McKay, 1929; Boray, 1964a). L. columella may have slightly narrower tolerance limits, but the preferred zone appears to be similar (Pullan et al., 1972). Most authors agree that pH is rarely a factor limiting the distribution of these three species.

Salinity

By far the most important element of freshwater is calcium. It is a necessary component of shells, it clears the water, and it allows a richer growth of vegetation (Boycott, 1936). All three functions should improve lymnaeid habitats since the algae have broadly similar mineral requirements to higher plants (Round, 1965).

The calcium content of water from habitats of Lymnaea truncatula L. tomentosa and L. columella does not appear to have been examined, but total salinity in Australian habitats of L. tomentosa was usually 25 to 160 ppm, ranging up to 940 ppm. Boycott describes the requirements of some of the British Lymnaeidae as:

soft water	moderate calcium	hard water
Lymnaea peregra	L. auricularia	no Lymnaeidae
L. palustris	L. stagnalis	
L. glabra		

Unfortunately he does not include consideration of L. truncatula in his paper on the grounds that it is not truly aquatic.

While snails with a high calcium requirement cannot survive in soft water habitats those from soft water can be reared in hard water in the laboratory (Boycott, loc. cit.). Their absence from most hard-water habitats is probably due to increased competition from the wider range of species they are likely to encounter.

1.4.2 Food

The amphibious nature of the intermediate hosts of F. hepatica is considered to be evidence of their primitive status, being less adapted to aquatic habitats than such species as L. stagnalis. Further evidence may be their microphagous feeding habitats (see section 1.3.4).

The preferred food of L. truncatula is the algae that grow on mud surfaces (Walton, 1918) but they will eat oatmeal and dead leaves of higher plants (Kendall, 1953). There is no mention in the literature of L. truncatula feeding on animal matter, although some other lymnaeids are carrion feeders and some are even said to feed on living animal material. Baker (1911, p. 42) collected some early references to carnivorous behaviour in lymnaeids. They seem to indicate that it is the more aquatic species such as L. stagnalis and L. palustris which include animal tissues in their diet; these are also the species considered to be most advanced in evolutionary development. Lymnaeids that feed on animal tissues are able to locate them in a diffusion gradient by chemoreception, but are unable to do so with vegetable matter (Bovbjerg, 1968).

The food requirements of L. tomentosa appear to be similar to those of L. truncatula but it can utilise cellulose (Boray, 1964a).

There are no references to animal food in the literature but Boray (pers. comm.) has seen snails apparently feeding on dead moths.

The normal food of L. columella is algae but adults can consume some higher plant material (Colton, 1908). Colton emphasised the importance of soil or sand in the gizzard for all lymnaeids. L. columella has been maintained in the laboratory with micro-algae (Colton, 1908), dried poplar leaves (Colton and Pennypacker, 1934), fresh lettuce (Baily, 1931; Krull, 1933a; Winsor and Winsor, 1935; Forbes, 1946) or calcium alginate (De Witt and Sloan, 1958; Barber, 1962). Barber found that newly hatched snails survived only when green algae were available, but they could be transferred to calcium alginate after three weeks.

1.4.3 Effects of temperature

The temperature tolerance range of a species is generally much wider than the range at which it operates most efficiently, its optimum, which is usually near the top end of the tolerance zone. For example the egg of the liver fluke Fasciola hepatica can survive at temperatures from 2°C (Krull, 1934) to something in the region of 37°C, although mortality increases with exposure to the top end of this range. Development begins at 9.5 ± .5°C and accelerates to reach its maximum rate at 30°C; above 30°C temperatures inhibit development until at 37°C there is 100% mortality after 24 days (Rowcliffe and Ollerenshaw, 1960). Of course organisms may survive short periods of exposure to temperatures outside the normal tolerance range and there may be many interactions between such factors as humidity, light, atmospheric pressure or dissolved salts (Allee et al., 1949).

Lymnaea truncatula is active at temperatures as low as 1.5°C, and begins egg laying at 10-11°C. The optimum range appears to be 18-21°C; above 20°C mortality increases and 25°C seems to be the upper tolerance limit (Kendall, 1953). L. tomentosa can survive for three years at 2-5°C and for at least six weeks at 36°C, but its optimum appears to be about 26°C (Boray, 1964a). Oviposition has been observed between 15°C (Boray, 1964b) and 30°C (Lynch, 1963) and copulation occurs only at 16°C or above (Boray, 1964b). No data on temperature tolerance limits, optima, or oviposition have been published for L. columella;

most experimental work seems to have been conducted at room temperatures.

The rate of development of the eggs of L. truncatula at different temperatures as reported by Roberts (1950) and Kendall (1953) is shown in Table 1.1. No upper or lower tolerance or developmental limits are given by either author. Boray (1963a) provided fuller and more precise data on the hatching rates of the eggs of L. tomentosa and these are included in Table 1.1 for comparison. The tolerance range for L. tomentosa eggs is 5-31 °C; most rapid hatching takes place at 25-26 °C and development slows down as temperatures approach the upper tolerance limit. No data are available for the eggs of L. columella.

TABLE 1.1 Reported oviposition to hatching times for the eggs of L. truncatula and L. tomentosa

Temperature (°C)	Days to hatching		Source of data
	L. truncatula	L. tomentosa	
2		Failed to hatch	Boray, 1963a
5		56 - 60	" "
7 - 8		47 - 50	" "
10 - 11	32		Kendall, 1953
11	29		" "
16 - 17		17 - 19	Boray, 1963a
18 - 19		14 - 15	" "
20 - 21		9 - 14	" "
16 - 21	12 - 13		Kendall, 1953
17	17 - 22		Roberts, 1950
21 - 30	11 - 12		Kendall, 1953
23 - 24		7 - 10	Boray, 1963
25	8 - 12		Roberts, 1950
25 - 26		5 - 8	Boray, 1963a
27 - 28		7 - 9	" "
30 - 31		9 - 10	" "

L. tomentosa is clearly adapted to higher environmental temperatures than L. truncatula, as might be expected from their geographical distributions. Its upper tolerance limit may prevent establishment of L. truncatula in Australia and may at least partially explain its limited distribution within New Zealand.

1.4.4 Aestivation

The hydrography of the habitats of the snail hosts of Fasciola hepatica is such that they have had to adapt to some degree of desiccation. The nature of this adaptation has been studied with a view to understanding more fully the epidemiology of fascioliasis.

Kendall (1949a) found that newly hatched L. truncatula could survive drying on mud in his laboratory for over 60 days, while some adults recovered after a year. Aestivation did not appear to increase the differential mortality usually seen between snails infected with F. hepatica and uninfected controls, and the parasite resumed its normal rate of development in recovered snails. Many snails burrowed under the mud surface, appearing again when they were re-hydrated.

L. tomentosa can survive for several months in dry mud in the field. In laboratory experiments 30% of snails survived on the dry surface for 100 days and a few survived under the mud for 332 days at 18-24°C and 55-75% R.H. (Boray, 1963a). Well-fed L. tomentosa burrow into the mud prior to aestivation, but poorly nourished snails often aestivate without burrowing (Lynch, 1966). Lynch's snails burrowed only when soil moisture content was between 33 and 40% and high atmospheric humidity decreased the proportion of burrowing snails.

No information on the response of L. columella to desiccation is available, but some points of general interest emerge from work on other species, mainly vectors of schistosomiasis. There is evidence that snails infected with trematodes do not survive aestivation as well as controls (Cridland, 1957) and small specimens of L. natalensis can survive desiccation on mud for longer than adults, apparently by being more able to burrow or enter cracks in the soil (Cridland, 1957; 1967; Shiff, 1960). The main factors influencing resistance to desiccation

when this is not complicated by behavioural factors are size of snails, exposure time, saturation deficit and temperature with several interactions between these (Sturrock, 1970).

Comparison of results of experiments on responses to desiccation on mud is difficult if the experiments have been conducted in different laboratories. Even experiments carried out by the same worker are liable to give extremely variable results as exemplified in the report from Shiff (1960). The saturation deficit of a soil sample depends not only on its moisture content but also on its porosity, and there may be gradients within a single aquarium or other experimental area. Snails may behave differently when placed on semi-dry mud than when surface water is allowed to evaporate and there must be many other unconsidered variable factors.

1.4.5 Light

The influence of light is often difficult to separate from other ecological factors, for instance the harmful effect of shade on lymnaeid habitats (section 1.4.1.) is almost certainly due to its inhibiting effect on algal growth rather than any direct effect upon the snails. Depression of algal growth may have been responsible for an assertion that darkness reduces growth rate and egg production in L. columella (Colton, 1908).

Some more recent work suggests that not only are more eggs laid at night regardless of light and dark sequences in the laboratory (Duncan, 1959) but at a given temperature egg-production is inversely proportional to day-length (De Witt, 1967; Joy, 1971). De Witt's snails, Physa pomilia and Lymnaea columella, laid many more eggs in total darkness using an experimental method which carefully excluded all traces of light, but quite different results were obtained by van der Steen (1967) whose L. stagnalis laid fewer eggs in total darkness. Van der Steen found that anything less than total absence of light had no influence on egg production and he suggested that such total darkness occurs rarely if ever, in nature.

The evidence for a direct effect of light upon the biology of lymnaeid snails is equivocal and if such an effect exists it is likely to be overcome by more powerful influences such as temperature, food and moisture.

1.4.6 Behaviour

While all three species occupy shallow freshwater habitats L. truncatula spends a lot of time out of the water, L. tomentosa mainly occupies the bottom of shallow pools such as cattle footprints, and L. columella often floats on the surface or browses on pond weed (see section 1.4.1). However their behaviour overlaps considerably with similarities being at least as obvious as differences.

In laboratory aquaria all three species will climb out of water when conditions are unfavourable but L. columella is particularly prone to this type of behaviour (Meeuse and Hubert, 1949). The tendency to migrate from unfavourable conditions favours active dispersal of snails within a habitat or to an adjacent one.

L. truncatula shows great site fidelity; although it can move at a sustained rate of 33 cm per hour in the laboratory, 60-70% of marked snails in a permanent habitat were still within 60 cm of the place of release 21 days later, and in a temporary habitat the percentage was 33 (Heppleston, 1972). L. tomentosa migrates further but recovery rates of marked snails were too low to allow Boray (1969) to evaluate results. In a further experiment using a filtered artificial channel with a current velocity of 15-18 cm/sec. snails migrated upstream 150 metres in three months, the last 100 metres being covered in two weeks when temperatures were higher.

The dispersal rate of L. columella does not appear to have been studied.

1.4.7 Intra-specific relationships

Low population density

There are hazards to a species when its numbers become too small or too great within a habitat or a region. The problems associated with underpopulation have received much less attention from ecologists than the crowding phenomenon. Andrewartha and Birch (1954) cite examples of species such as elephants, reindeer, sheep tick, and tse-tse flies which will die out if their population in an area falls below a critical level; others, including many invertebrates, have a

reduced capacity for population increase.

The hazards of underpopulation are mainly:

1. The difficulty experienced by dioecious species in finding a mate.
2. An increased death-rate in sparse populations due to predation by non-specific predators.

The first hazard does not apply to self-fertilising hermaphrodites like the Lymnaeidae, although the rate of population increase may be a little less in isolated snails than in pairs for both L. columella (De Witt and Sloan, 1958) and L. tomentosa (Boray, 1964b). The second hazard has not been investigated, but it does not seem to have hindered the establishment of L. columella in new geographical locations.

High population density

The harmful effects of crowding are better known. The phenomenon is more easily studied in the laboratory and egg-laying is a convenient indicator, so most studies have been concerned with the growth and egg-production of laboratory snails at different population densities.

In experiments where sufficient food was available, results of experiments on freshwater snails may be summarised as follows:

1. In a given volume of water, increasing the number of snails will result in reduced growth rates and fecundity (Winsor and Winsor, 1935; Chernin and Michelson, 1957a; Wright, 1960; Ritchie et al., 1963; van der Schalie and Davis, 1965; Mooij-Vogelaar et al., 1970).
2. With a constant volume of water per snail, smaller groups grow faster and lay more eggs than larger groups (Chernin and Michelson, 1957b).
3. With a constant number of snails per group, reducing the volume of water has little effect upon growth rate or fecundity (Chernin and Michelson, 1957b; Wright, 1960).
4. Flowing water gives improved growth-rates and fecundity compared to snails in stagnant water (Ritchie et al., 1963; van der Steen, 1967).

If surface area of mud is substituted for water volume as a measure of density, L. tomentosa reacts to population density in similar ways, except that with groups of a constant number reduction of the area per snail is deleterious (Lynch, 1963). Experiments with L. truncatula on mud slopes show reduction in growth rate, fecundity, and production of Fasciola hepatica cercariae per snail as numbers increase in a constant area (Kendall, 1949b).

Interpretation of these results varies; some authors believe a toxin or a build-up of excretory products may be responsible (Wright, 1960), and it has been shown that a toxin from L. pereger will kill fish if it is sufficiently concentrated (Macan, 1963). However since L. tomentosa performed just as well when water came from other snail tanks as when it was fresh Lynch (1963) rejected this theory, at any rate under his experimental conditions, but others including Wright (1960) found that water from crowded aquaria was harmful to other snails.

Other possibilities include some sort of physical interaction between snails (Chernin and Michelson, 1957a) as occurs in vertebrates with increases in group size. In spite of the presence of excess food in aquaria Lynch (1963) considers that a relative shortage of food can result from snail behaviour since they spend a lot of time on the side-walls of aquaria and egg-production can be correlated with the length of these sides.

Some authors believe shortage of food limits snail productivity in natural habitats (Kendall, 1949b; Taylor, 1964; Eisenberg, 1966; 1970), and Eisenberg's experiments with caged groups of snails in natural habitats show that this is true under at least some field conditions.

The fact that snails grow and reproduce more efficiently in small groups, even when the volume of water per snail remains constant, can be interpreted in several ways. The small groups will have a greater length of container wall per snail, and they also have fewer physical contacts with other snails. Physical contact irritates snails, causing them to spend more time withdrawn into their shells (Lo, 1967).

1.4.8 Inter-specific relationships

Plants

With the exception of the micro-algae on which they feed and

tall plants which shade the habitat, reducing algal growth, the relationship between the lymnaeid hosts of F. hepatica and plant life is one of mutual sharing of the habitat - a mere accidental association (see sections 1.4.1, 1.4.2 and 1.4.5). A number of species of algae which are unsuitable as food can harm L. tomentosa either directly by eroding the shell or inhibiting embryonic development, or indirectly by overgrowing the habitat (Boray, 1964a).

Micro-organisms

Snails, like other species, are susceptible to bacterial, viral and fungal infections, but the only record of a specific infection of the three species in question is an account of a fungal infection of the egg-masses and in some cases the body cavity of L. tomentosa by an aquatic Phycomycetes. Experimental infections in the field failed to produce any significant effect upon the snail population (Boray, 1964a). Iron bacteria, whose presence is characterised by a rusty brown precipitate on mud and snail shells in natural habitats, are said to indicate suitable habitats (Boray, loc. cit.). Other bacteria are responsible for a surface scum which causes a high mortality in young L. columella (Colton and Pennypacker, 1934) and L. tomentosa (Lynch, 1963) in laboratory aquaria.

Metazoan parasites

The snails can act as hosts for trematodes other than F. hepatica. Kendall (1954; 1965) asserts that the trematodes Fasciola gigantica and F. hepatica are separated ecologically by the different requirements of their snail hosts, those of F. hepatica being amphibious mud dwellers while those which transmit F. gigantica are true pond snails. However L. truncatula, L. tomentosa and L. columella are all capable of producing cercariae of F. gigantica (Alicata, 1953; Boray, 1966). Fascioloides magna is endemic in European deer, using L. truncatula as its intermediate host (Erhardova, 1961) and L. columella is fully susceptible to laboratory infections (Krull, 1933b). There appear to be no references to experimental or natural infection of L. tomentosa with F. magna.

The molluscan hosts of F. hepatica can also carry trematodes

of frogs, water-fowl and other vertebrates including man. L. columella seems to transmit a more varied selection of trematodes than the others, perhaps because of its distribution through both temperate and tropical zones. It is an intermediate host for the frog lung-fluke Cercaria pricei (Rothschild, 1940) a trematode of newts, Plagitura salamandra (Owen, 1946) the frog trematodes Gorgoderina attenuata and Gorgodera amplicava (Goodchild, 1948), Paramphistonum cervi and P. microbothrium from sheep and goats (Soulsby, 1965, p. 594) and a human schistosome, Heterobilharzia americana (Malek, 1967).

Predators

Michelson's (1957) review of the literature contains many reports of predation and parasitism, but he points out that most of them are casual observations whose significance has not been tested by experiment.

Larvae of sciomyzid flies will kill snails and they have been investigated as a possible means of biological control (Berg, 1964). Several species are known to kill snails in Australia. They may be responsible for the high mortality seen in L. tomentosa in spring (Lynch, 1965) but they do not seem to have any effect upon the epidemiology of fascioliasis (Boray, 1964a). Boray (1969) subsequently found no evidence that sciomyzid larvae exerted any significant influence on snail numbers. Even if they did reduce snail populations a few well-fed snails in a suitable habitat, as could occur after the population decline noted by Lynch, can produce as many F. hepatica metacercariae as large, overcrowded populations (Kendall, 1949b; Boray, 1963a) and sciomyzid larvae show no particular preference for either infected or uninfected snails (Fontana, 1972).

Although most species are filter-feeders ostracods will sometimes attack snail egg-masses and young snails and their foraging in and around the aperture of adult snails is intensely irritating, inhibiting the normal feeding behaviour of the snails (Lo, 1967).

Ducks and other water-fowl will eat snails but their effect upon snail populations has not been studied empirically. Turbellaria may have a disastrous effect upon laboratory snail colonies by preying upon newly hatched snails (Wright, 1960) but whether they have a significant effect upon natural populations is not known.

Symbionts

The oligochaete Chaetogaster limnaei is frequently associated with freshwater snails. There are two subspecies; Ch. l. limnaei is usually regarded as a commensal, inhabiting the outer surface of the shell, but Ch. l. vaghini is a parasite of the kidney (Buse, 1971). Ch. limnaei will protect Lymnaea natalensis from Fasciola gigantica miracidia by ingesting up to 15 per worm, and with up to 50 worms per snail the protection is of a high order (Khalil, 1961). Ch. limnaei is also commonly found with L. tomentosa, and there may be up to 500 worms per snail. Their part in the epidemiology of F. hepatica is unknown, but they will ingest miracidia (Boray, 1964a).

In an investigation into the relationships between Biomphalaria glabrata, Schistosoma mansoni, and various aquatic invertebrates Chernin and Perlstein (1971) found that mosquito larvae could ingest miracidia and planarian excretions were also lethal to miracidia. The toxicity of the excretions to miracidia varies with planarian species (Glaudel and Etges, 1973) but the importance of these inter-relationships in the field is not known.

1.4.9 Life Cycles in the Field

In most parts of Britain L. truncatula seems to complete two or three generations per year (Walton and Jones, 1926; Ross and Morphy, 1970) but in the north of Scotland only one is normal, with two in exceptional conditions (Heppleston, 1972). In temporary habitats the life cycle may be interrupted by periods of aestivation.

Growth and development is more rapid in summer but mortality is higher, particularly in very young snails and those which are laying eggs. Snails are less numerous in winter but part of the reason is their tendency to sink below the mud surface in cold weather (Heppleston, loc. cit.).

No information is available on the number of generations of L. tomentosa or L. columella occurring annually in natural habitats. L. tomentosa copulates at any time between September and May in response to environmental changes or population depletion in its New South Wales habitats (Boray, 1964b). L. tomentosa numbers may undergo a marked seasonal decline in spring, attributed by Lynch (1965) to predation by sciomyzid larvae. The seasonal decline was observed by Boray (1969, p.106)

in November 1962 but not in the spring of 1963, and he considers climatic factors to be mainly responsible for population fluctuations. I have found no published information on seasonal population fluctuations of L. columella.

1.5 THE RELATIONSHIP BETWEEN FASCIOLA HEPATICA AND ITS SNAIL HOSTS

Throughout its distribution Fasciola hepatica infects amphibious lymnaeids like Lymnaea truncatula. The most important ecological requirement of F. hepatica within its zone of temperature tolerance is water, so it must use a freshwater snail. Truly aquatic snails would limit its opportunities for infecting grazing animals and its infectivity to various amphibious lymnaeids is a good example of the evolution of an ecological adaptation.

Some other lymnaeid snails not normally responsible for transmission of the parasite can be infected with F. hepatica only when they are very young and they usually reject the parasites before cercariae are released (Kendall, 1950; Boray, 1966).

1.5.1 Miracidia and Snails

Life-span of miracidia

Miracidia of F. hepatica which are to complete their life-cycle must locate a suitable snail in a relatively short time. They survive for up to 3 days at 8 to 11 °C (Ross and McKay, 1929); at 19 to 21 °C all are dead within 24 hours (Yasuraoka, 1953) and at 27 °C they live for only 5½ hours (Tagle, 1944).

While temperature is the most important factor determining the life-span of trematode miracidia it is also affected by the pH, salinity and oxygen content of water and their infectivity declines with time (Wright, 1956). Miracidia of F. hepatica die rapidly in distilled water but will survive salinity up to 13‰ so long as the egg has been incubated under the same conditions (Styczyncka-Jurewicz, 1966).

Behaviour of miracidia

F. hepatica miracidia are strongly phototropic, negatively

geotropic and positively rheotaxic (Yasuraoka, 1953), behaviour patterns which take them nearer to their semi-aquatic hosts. In the opinion of most authorities they also respond chemotactically to the presence of their snail hosts. Wright (1959b) attributes the doubt as to the occurrence of chemotaxis expressed by some workers to their taxonomic problems with closely related snails and failure to distinguish between phases (ii) and (iii) of host location which he described as:

(i) Attempts to find the host environment e.g. negative geotropism and positive phototaxis

(ii) Random movements within that environment

(iii) Chemotaxis over short distances - not in straight lines as in visual location, but over a twisting track to distinguish the direction of the concentration gradient.

Trematode miracidia will respond actively to various snail tissues and also to filtered water from containers in which snails have been confined overnight (Wright, 1966; Chernin and Perlstein, 1969; Shiff and Kriel, 1970). The first successful attempt to resolve quantitatively the question of miracidial chemotaxis was made with miradidia of Schistosoma mansoni and the snail Australorbis glabratus (Etges and Decker, 1963). The experiment was criticised by Smyth (1966) on the grounds that amino-acids released by the cracked snails may have attracted miracidia in a non-specific manner. However, the original paper records a slight repulsion of miracidia by other species of cracked snails, probably due to turbidity.

When the miracidium of F. hepatica succeeds in attaching itself to its lymnaeid host it digests the snail epithelium, penetrating the tissues in about 30 minutes (Dawes, 1959). The infectivity of schistosome miracidia depends upon temperature, pH, salinity, turbidity and oxygen tension (Wright, 1956; Chu et al., 1966; Upatham, 1972). The infectivity of F. hepatica in water with different physical and chemical properties does not seem to have been investigated.

Susceptibility of snails to trematode infection

The other half of the host-parasite interface, the snail, varies in its susceptibility to infection. There may be a temporary acquired

immunity whereby snails are refractory to further infection from the same species of trematode (Winfield, 1933; Wright, 1956), or a type of interference phenomenon in which infection with one species will block infection by another (Basch et al., 1969). There are probably several mechanisms involved but in one the rediae of the dominant species prey upon the larval stages of the other (Lie et al., 1968; Basch, 1970). There is circumstantial evidence that a high rate of infection with echinostome larvae will block the infection of snails in natural habitats with Schistosoma haematobium (Gilles et al., 1973) or Fasciola hepatica (Gordon and Boray, 1970).

Reports on infection of the normal hosts of F. hepatica with varying numbers of miracidia differ in their assessment of snail infectivity. Roberts (1950) found large specimens of L. truncatula more resistant to miracidial penetration, but infected all of 98 snails measuring 2.5 mm by exposing them to 5 miracidia. Penetration was achieved in from 1 to 20 minutes. Kendall (1949b) could infect about 30% of L. truncatula with a single miracidium but 100% became infected when exposed to large numbers.

Krull (1941) found that L. columella of any age could easily be infected with a single miracidium, but Barber (1962) could only infect 63 out of 176 snails of the same species using from 1 to 300 miracidia.

1.5.2 The intra-molluscan stages of Fasciola hepatica

The miracidium sheds its outer epithelium during penetration to become a sporocyst. The rate of development from sporocyst through one or two generations of rediae to cercariae, which are released through a rupture in the body wall, depends mainly on temperature. In L. truncatula 10°C is the lowest temperature at which any appreciable development takes place; the rate of development accelerates to a maximum at 27°C when cercariae may be released in as little as 21 days and above this temperature snail mortality increases rapidly (Kendall and McCullough, 1951; Kendall, 1965). In L. tomentosa the total intra-molluscan stages occupy 56 to 86 days at 15°C, 29 to 39 days at 25°C, 24 to 28 days at both 30°C and 35°C (Boray, 1963a). Metacercariae produced by snails which have been maintained at 35°C are not infective

to mice or rabbits (Lynch, 1963).

There is less information on the rate of development in L. columella; Krull (1934) reports cercarial release after 38 days in young L. columella and 66 days in mature snails, and Leon-Dancel (1970) gives a figure of 57 to 60 days. In both cases the snails were maintained at varying laboratory temperatures.

Snail aestivation almost halts development of the intra-molluscan stages and reduces cercarial output, probably due to under-nutrition, but development resumes after re-hydration (Kendall, 1949a). The nutrition of the snail also affects the rate of development of its parasites but a more marked effect is upon the total production of cercariae (Kendall, 1949b; Kendall and Ollerenshaw, 1963).

Multiple infections with several miracidia do not necessarily result in greater production of cercariae. Infection with a single miracidium resulted in production of from 14 to 613 cercariae from L. trancatula (Kendall, 1949b). Kendall found that multiple infections produced more rediae but fewer cercariae per redia, with a maximum of 795 cercariae from a single snail. The most spectacular record of cercarial production in a single snail is 3,390 resulting from a multiple infection in L. tomentosa (Boray, 1963a).

In natural populations most cercariae are found in mature snails (Walton, 1918; Ollerenshaw, 1959; 1971b) which is hardly surprising since development from sporocyst to cercariae occupies a large proportion of the growth period of adequately fed snails (see section 1.3.1). Release of cercariae may be stimulated by sprinkling or immersing mud-reared L. truncatula with water, and it seems likely that showers of rain will act in the same way in natural habitats (Walton, 1918). The important factor is a stimulus to the snail by a change in the environment rather than the specific nature of the stimulus; for example merely changing snails from one container to another will increase muscular activity accelerating the rate of cercarial release (Kendall, 1965). Cercariae are not released from L. truncatula below 9°C (Kendall and McCullough, 1951); minimum temperatures for release from L. tomentosa or L. columella are not known.

Infection with F. hepatica reduces the life expectancy of snails (Walton, 1918), particularly in multiple infections (Leon-Dancel,

1970). In spite of the pathogenicity of the intra-molluscan stages the hazards to the parasite are less than those to the host because the snail usually survives until cercariae are being released (Kendall, 1965).

1.6 THE EPIDEMIOLOGY OF *FASCIOLA HEPATICA*

1.6.1 Prevalence

Fasciola hepatica infection is a serious economic problem in many temperate zones and in some cooler upland areas of semi-tropical countries. Pantelouris (1965) cites abattoir surveys of infected and condemned livers ranging from over 60% in cattle in Northern Ireland and Japan to 2% in South African sheep, and the prevalence in smaller areas can be even higher (Ross, 1966). In most reports the prevalence is lower in sheep. There are three possible reasons for this:

1. Sheep tend to be grazed on hill farms or on the drier parts of lowland farms.
2. When sheep and cattle graze marshy paddocks the cattle are likely to spend more time in the wetter areas.
3. Cattle consume more pasture per head than sheep.

In New Zealand the overall prevalence of *F. hepatica* infections is about 6% for cattle and 3% for sheep (Pullan and McNab, 1972), but it is much higher in some areas and is a serious problem on individual farms. Successive descriptions of the fascioliasis situation in New Zealand have shown an expanding distribution, notably to North Auckland and the west coast of the North Island (Macfarlane, 1937; Whitten, 1945; Brunsdon, 1967; Pullan and Whitten, 1972).

The pattern of infection in grazing animals depends upon climatic conditions and the ecological characteristics of the intermediate host. In most epidemiological investigations the snail host has been *L. truncatula* and a good deal of work has been done on the epidemiology of fascioliasis in Australia involving *L. tomentosa*. In New Zealand a little is known of the epidemiology of fascioliasis when the intermediate host is *L. tomentosa* but almost nothing is known about the

pattern of infection due to transmission by L. columella, either in New Zealand or elsewhere.

1.6.2 The epidemiology of Fasciola hepatica infections transmitted by Lymnaea truncatula

F. hepatica eggs hatch at not less than 10°C. They need high environmental oxygen and at least a surface film of moisture throughout their development to hatching, which takes at least three weeks in optimum field conditions. In practice this means they must be washed out of faeces in a damp environment such as a snail habitat. Their protracted developmental period at low temperatures is associated with a high mortality and only those eggs deposited from late spring onwards are of epidemiological importance (Rowcliffe and Ollerenshaw, 1960). Eggs deposited after mid-August in Britain give rise to overwintering infections since temperatures fall below 10°C before development in the snail is completed (Ollerenshaw, 1971b).

Factors influencing development of the intra-molluscan stages have been described in section 1.5.2. In L. truncatula habitats the intra-molluscan stages which come to a halt during the winter resume development in spring when temperatures rise above 10°C. Winter snail mortality is such that overwintering infections are of relatively minor clinical importance in most areas, but may be more important in Mediterranean countries where winters are milder and summers are drier (Ollerenshaw, 1966a). In southern Texas, which also has hot, dry summers and mild, wet winters grazing animals only become infected in winter and spring (Olsen, 1945). The snail host in Olsen's investigation was Lymnaea bulimoides.

The summer infection arising from fluke eggs deposited in late spring and early summer takes about 8 weeks to mature in L. truncatula in optimum British conditions even though as few as 3 weeks may be necessary in the laboratory (see section 1.5.2). The earliest time at which metacercariae from summer infections are available to grazing animals, allowing 3 weeks for the miracidium to hatch and 8 weeks intra-molluscan development, is about mid-August - six weeks after mid-summer (Ollerenshaw, 1959). Metacercariae may be deposited until temperatures fall below 10°C. They survive for several months at winter temperatures if relative humidity remains high or they are immersed in water, but

their infectivity decreases with time (Ollerenshaw, 1959; Kakatcheva-Avramavo, 1963; Chowaniec, 1967). Hay or silage is unlikely to contain significant numbers of infective metacercariae (Boray and Enigk, 1964; Wikerhauser, 1966).

In northern Europe metacercariae from summer infections in L. truncatula are likely to be available to grazing animals from mid-August until the following spring (Ollerenshaw, 1971a). Peak infection rates usually occur in early autumn but this depends on fluctuations in annual rainfall (Over and Jansen, 1966; Ross, 1967a; 1967b; 1970; Armour et al., 1968; 1970; Reid et al., 1970; Ross and Morphy, 1970; Ollerenshaw, 1971a). This dependence of F. hepatica infections from L. truncatula habitats upon rainfall had been shown to be highly predictable even before any concentrated effort had been made to study the epidemiology of the disease in natural habitats (Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1966b). It is due to a greater proportion of the total grazing area becoming infected from temporary or extension habitats of L. truncatula in wet seasons (Taylor, 1964; Kobulej, 1968).

1.6.3 The epidemiology of Fasciola hepatica infections transmitted by Lymnaea tomentosa

In Australia where the only intermediate host is L. tomentosa, sheep are the main source of pasture contamination by F. hepatica eggs, producing about ten times as many per head as cattle. Other species such as rabbits are of only minor importance (Boray, 1969). The epidemiology of fascicliasis in Australia takes a number of different forms depending upon climatic conditions and grazing management. These can be summarised, after Boray (1969) as:

Type A

This is the pattern of infection found in the central and southern Tablelands of New South Wales. Rainfall averages 650 mm and is distributed irregularly but approximately evenly throughout the year. Type A conditions represent about 75% of the endemic areas of Australia and are not very different from New Zealand conditions.

Snails are most numerous in summer and metacercariae may be available in significant numbers from October or November until May. Peak levels vary in occurrence depending upon rainfall, the presence of

other infected sheep, and whether sheep are obliged to graze in marshy areas to obtain enough grass. The October and November pasture infections are a result of overwintering snail infections with larval development halted for about five winter months.

Type B

In the "mediterranean" type of climate found in much of Victoria and South Australia winters are mild and damp while summers are hot and dry. Snail numbers fall during summer droughts (Lynch, 1963) but metacercariae are available from September to May with peak levels in the earlier part of this period due to overwintering infections. Low temperatures halt larval development in snails during the winter.

Type C

Rainfall is similar in amount to types A and B, but is distributed mainly in spring, summer and early autumn. Upland areas of northern New South Wales and southern Queensland are the geographical locations of this epidemiological zone, in which there is little or no larval development in L. tomentosa from May to September.

Overwintering infections seem to be less important here, with the bulk of pasture infection available between January and April.

Type D

Some irrigation areas of New South Wales and Victoria are a good example of the spread of fascioliasis by agricultural practices to areas where it would not otherwise occur. The annual rainfall of about 430 mm which falls mainly in winter and early spring is supplemented at approximately 10 day intervals by irrigation between September and April. Conditions would otherwise cause aestivation and eventual disappearance of L. tomentosa. Up to 43% of snails were found to be infected in one study, compared to the usual 1-2% in type A districts (Boray, et al., 1969).

Only poorly drained pastures are a source of danger in

irrigation areas, but on such pastures metacercariae are available throughout the year. The level of infection depends largely on the degree of pasture contamination by infected sheep.

Type E

In coastal regions of northern New South Wales and southern Queensland 1000 mm of rain falls mainly in summer and temperatures allow year-round development of F. hepatica.

Type F

Higher parts of the Snowy mountains and Tasmania resemble central Europe with larval development in L. tomentosa occurring for only 3 or 4 months per year.

New Zealand

The only epidemiological investigation using tracer sheep indicates that overwintering infections can be important, with metacercariae becoming available from October, but a more consistent feature of the epidemiological pattern is the January to June infection (Pullan and Mansfield, 1972). The New Zealand climate and the pattern of infection involving transmission by L. tomentosa seems to be similar to type A areas of Australia.

1.6.4 The epidemiology of Fasciola hepatica infections transmitted by Lymnaea columella

I have been unable to find any account of the epidemiology of fascioliasis in which the intermediate host was L. columella.

2. HABITATS

2.1 TERMINOLOGY

In this study the term habitat means a place characterised by a certain uniformity of physiography, vegetation, or some other arbitrarily defined character or characters; it describes the animal's location. Macrohabitats are the general areas occupied by snails, such as ponds, streams or marshes. The favoured areas within macrohabitats, the microhabitats, can be defined more precisely and may be patches of bare mud or the water-filled depressions left by hoofmarks.

In describing relationships with other snail species I have been guided by the terminology of Ponder (1964) and Winterbourn (1973).

2.2 MACROHABITATS

Prior to more detailed examinations of individual habitats, preliminary observations were made on about one hundred likely snail habitats on farms in the Manawatu, in Taranaki and on one Hawke's Bay property. Most were on farms on which fascioliasis was endemic, and there were usually several snail habitats on each farm. After some initial impressions were formed as a result of these preliminary observations, the characteristics of nine Lymnaea columella, two L. tomentosa, and five other snail habitats were examined in more detail and compared with one another.

The habitats of L. columella were almost exclusively divisible into either marshes or ponds. The spring-fed marsh habitats remained wet over at least some of their surfaces at all times. The ponds were small dams for watering farm stock. A typical pond habitat of L. columella is shown in Plate 2.1.

All of the L. tomentosa habitats were spring-fed marshes impossible to distinguish from typical L. columella habitats except that two of the former contained more pasture species and were less clearly separated from normal grazing areas. The marsh habitat of L. tomentosa shown in Plate 2.2 was indistinguishable from L. columella habitats on the same farm.

Plate 2.1 Pond habitat of *L. columella.*

Plate 2.2 Marsh habitat of *L. tomentosa.*

Fluctuations in water level

Marsh habitats which dried out for a period in summer over their entire surface were apparently unsuitable for L. columella or L. tomentosa, but often contained the operculate prosobranch snail Potamopyrgus antipodarum. Many marshes whose surface area varied seasonally could support lymnaeids as long as a moist focus remained in dry periods. Other apparently similar marshes nearby often contained no lymnaeid snails, and one marsh habitat of L. columella which was highly productive in 1970-71 produced only one recoverable snail in 1971-72 and none thereafter.

Of the four pond habitats of L. columella from which data were collected, none normally had an annual fluctuation in depth greater than one metre, but two of them dried out briefly in dry summers. Ponds whose annual fluctuation in depth was much greater than one metre were not usually suitable habitats.

Source of water

Only spring-fed marshes appeared to be suitable habitats for L. columella and L. tomentosa. Wet areas resulting from surface run-off in periods when rainfall exceeded evapo-transpiration did not appear to be suitable. Lateral extensions of slow-moving creeks supported either species during periods of population expansion but these areas did not regularly contain lymnaeids, presumably because their water levels fluctuated too much. In low density populations of either species the snails were mainly found near the sources of underground seepages.

Flow rate

Rapidly flowing streams did not contain L. columella or L. tomentosa except for rare individuals being carried downstream. Small slow-flowing creeks were populated by either species, but most snails were located away from the main flow. Over the greater part of the marsh habitats of both species the flow was imperceptible and could be deduced only from the fact that the habitat was spring-fed.

When ponds were fed by trickles of water from springs or

small creeks L. columella populations were distributed mainly around the entrances and exits, particularly when these had gently sloping banks.

Slope

Drainage ditches with steeply sloping banks have too variable a depth and flow rate of water for L. columella or L. tomentosa; they were usually inhabited by Potamopyrous antipodarum and/or Physa sp..(1) In ponds there was a marked preference for the more gently sloping parts of the bank. Steeply sloping marsh habitats were quite suitable as long as there were flat pockets which collected and held water from underground seepage.

Altitude

Both species were found from sea level to 350 metres above. There was no evidence that within these limits altitude was of any direct significance in the ecology of either L. tomentosa or L. columella.

Vegetation

The study did not include a comprehensive survey of plant species, but there did not seem to be any obvious differences in vegetation between the marsh habitats of L. columella and L. tomentosa, or between some of the lymnaeid and non-lymnaeid habitats. Marshes contained various species of Juncus (rushes) Lemna minor (duckweed) and Nasturtium spp. (watercress). The pond habitats of L. columella were usually covered to varying degrees by floating weeds such as Lemna minor and the aquatic fern Azolla rubra. Ponds with no weeds did not appear to be suitable habitats but the converse was not true; many heavily weeded ponds contained no lymnaeids.

(1) The identity of this snail is in some doubt. Dr. Winterbourn of the Zoology Department, Canterbury University, has confirmed that it is a species of Physa.

Substratum

The type and consistency of soil in lymnaeid habitats varied, but marsh habitats always had some flocculent mud resulting from underground seepage. The soils of L. columella habitats included clays, sand, loams, and volcanic ash. One L. columella habitat in North Taranaki was a spring-fed marsh by a beach, just above the high-water mark, with snails basking on the iron-sand of the seaward edge. Laboratory snails of both species were maintained successfully on whatever soil was available, including subsoil from a building site.

Oxygen content of water

The dissolved oxygen content of eight habitats was measured with an electronic oxygen analyser. At temperatures between 16°C and 18.5°C the percentage saturation ranged from 80% to 105% (Table 2.1); in the latter case air-bubbles were forming on submerged weeds.

Calcium and Magnesium

The calcium and magnesium contents of eleven water samples from different kinds of habitat were measured with the aid of an atomic absorption flame spectrophotometer. The great range of values (Table 2.1) particularly the very low values in habitats 20, 21 and 22, indicate that L. columella and L. tomentosa can utilise habitats whose water has a very low calcium and magnesium content.

pH

The pH values of water from sixteen habitats were estimated with pH indicator papers (Rota brand, M & B Ltd.) graded at intervals of 0.5. The range of values (Table 2.1) was well within that reported by other authors (section 1.4.1) and did not differ between lymnaeid and non-lymnaeid habitats.

TABLE 2.1 Chemical values of water from some selected habitats of *L. tomentosa* and *L. columella*

Habitat number	Snail Species	Type of habitat	Location	Dissolved oxygen content of water (% saturation)	Calcium (ppm)	Magnesium (ppm)	pH
2	*Physa* sp.; *L. columella*	pond	Rongotea	95% at 17.5°C	28.0	11.2	7.0
7	Physa spp.; *P. antipodarum*; *L. columella*	pond	Pohangina	105% at 18.5°C	70.0	3.0	6.0
8	*P. antipodarum*; *Physa* spp.; *L. columella*	marsh	Pohangina	80% at 18.5°C	67.0	9.5	6.0
14	*P. antipodarum*; *L. columella*	pond	Pohangina	88% at 17.5°C	47.0	4.5	6.0
22	*L. columella*	marsh	Tokomaru		4.0	0.60	6.0
20	*L. tomentosa* *P. antipodarum*	marsh	Tokomaru		11.0	1.05	6.0
21	*L. tomentosa*	marsh	Tokomaru		8.0	1.10	6.0

Shade

Lymnaeids were absent from habitats or parts of habitats shaded by trees or other tall vegetation. If such areas were otherwise suitable for snails they were usually populated by the ubiquitous Potamopyrgus antipodarum.

Associations with other snail species

In most marsh habitats of L. columella and L. tomentosa the lymnaeids were outnumbered by the prosobranch snail Potamopyrgus antipodarum. This was particularly true of marginal habitats or less suitable areas within habitats where water flow was too swift or the water level too variable. In a small proportion of marsh habitats in the Manawatu Basin a pulmonate Physa sp. was also present, usually in parts of the habitat with deeper water.

Pond habitats of L. columella were often shared by Physa sp., Potamopyrgus antipodarum and Gyraulus corinna. L. columella aggregated mainly on the surface and along the shore, sometimes on the mud surface. Physa shared this part of the habitat to some extent, but was also found in deeper water and further from the shore. P. antipodarum inhabited the bottom of the pond, mainly near the shore, and G. corinna was found attached to the stems and leaves of plants in deeper water. Random sweeps with a hand net usually produced vastly greater numbers of species other than L. columella.

2.3 MICROHABITATS

2.3.1 Sampling methods

Three sizes of microhabitat were examined in marshes. The first was a one metre square sample area used in the snail population studies of 1970-71 (Chapter 3). Another was one ninth of the square metre, the sampling area of the 1971-72 population studies. The third was a ten centimetre square which was part of a thirty centimetre square grid. Grids used in studying the three sizes of microhabitat are shown in Plates 2.3 and 2.4. The one metre square, one ninth of a square metre and ten centimetre square areas will be referred to as type (a),

Plate 2.3 Grids used for sampling microhabitats

Plate 2.4 Two specimens of *L. columella* in a 10 cm^2 quadrat

(b) and (c) microhabitats respectively in the subsequent text.

Microhabitats of types (a) and (b) were randomly selected during population studies conducted in four marsh habitats (see Chapter 3), but type (c) were selected arbitrarily. In this case when a snail or group of snails was located the thirty centimetre square grid was placed over them. Squares 1 to 9 were regarded as separate samples; they were compared for physical characteristics and the presence or absence of snails.

The following features of all samples were recorded on a standard record sheet (Appendix 2.1):

(i) Consistency of mud

A ruler was dropped on its end from a height of five centimetres onto the softest part of each sample area. The depth to which it sank was taken as an index of flocculence; for analytical purposes all samples in which the ruler sank for one centimetre or more were regarded as flocculent and the rest were classified as having firm mud.

(ii) Amount of visible mud

Some sampling areas were so overgrown with grass or weeds that the soil surface was not visible while others contained expanses of bare mud. The percentage of the surface area seen to be bare mud was estimated and the microhabitat was then placed in one of three categories: (a) no visible mud (b) up to 25% visible mud or (c) more than 25% visible mud.

(iii) Water surface

In some sample areas the water surface had an oily film. The presence or absence of this oily film was recorded for each sample; even a small patch in one corner of a square was regarded as a positive sample.

(iv) Amount of water

Samples of quadrat size (a) and (b) one metre square and one ninth of a square metre respectively, were taken during random sampling for the population studies described in Chapter 3. Some of

them had no free surface water but were muddy enough to be regarded as potential snail habitats. If they were too dry to constitute part of the marsh they were not used in either the population study or the investigation of microhabitats. The percentage of the surface under water was estimated for each sample, which was then classified as (a) no surface water, (b) up to 25% surface water, or (c) over 25% of the sample area under water.

(v) Slope

A subjective judgement was made regarding the steepness of each sample area, dividing samples into (a) flat, (b) gently sloping, or (c) steep. A steep sample was anything considered to have a slope greater than 30°.

(vi) Vegetation

In each sample the presence or absence of Lemna minor (duckweed), Nasturtium spp (watercress) or Juncus spp (rushes) was noted. Since virtually all samples contained some species of rush this factor was dropped from the analyses.

(vii) Water flow

Flow rates are rarely measurable in Lymnaea habitats, particularly where snails are located. In some cases a slow trickle could be induced where the observer's footprints allowed surface water to drain from the sample area. Flow rates were classified as (a) zero, (b) induced by the observer, or (c) positive, i.e. discernible as a trickle of water flowing into and out of the sample area without it having been disturbed.

(viii) Other snails

Both Physa sp. and Potamopyrgus antipodarum were sometimes found in the same sample area as L. columella, and P. antipodarum was occasionally associated with L. tomentosa. The presence or absence of these snail species in each sample was recorded.

2.3.2 Analytical method

Data collected on forms shown in Appendix 2.1 were analysed by means of a series of Chi-square tests. Each sample was regarded as positive or negative for the presence of snails; any number of snails constituted a positive sample. Each habitat factor was considered in turn for its positive or negative association with the species of Lymnaea occupying the macrohabitat.

Microhabitats of types (a) and (b) were combined in order to have enough data for analysis, and the ten centimetre squares of type (c) were considered separately. A total of 489 microhabitats was examined.

2.3.3 Results (Table 2.2)

(i) Consistency of mud

Microhabitats with flocculent mud were more likely to contain L. tomentosa than those with firm mud. Tendencies for similar results from L. columella microhabitats were not statistically significant.

(ii) Amount of visible mud

Microhabitats with more than 25% of visible mud were more likely to contain snails than those with less than this amount of bare mud surface, but the results were statistically significant only in the type (c) microhabitats of L. columella and the larger microhabitats of L. tomentosa.

(iii) Amount of water

The greater the proportion of the habitat surface under water the more likely it was to contain snails of either species.

(iv) Water surface

There was no evidence of a preference for a clear or oily water in any type of microhabitat occupied by either species.

Table 2.2 ASSOCIATIONS BETWEEN *Lymnaea tomentosa* OR *Lymnaea columella* AND SOME HABITAT FACTORS

Microhabitat Factors		*Lymnaea tomentosa**				*Lymnaea columella**			
		Microhabitats type (a) & (b)		Microhabitats type (c)		Microhabitats type (a) & (b)		Microhabitats type (c)	
		*L. tomentosa**	None	*L. tomentosa**	None	*L. columella**	None	*L. columella**	None
MUD	Firm	26	36	8	46	36	83	26	22
	Flocculent	44	18	16	20	29	46	22	11
AMOUNT OF VISIBLE MUD	0	1	15	0	1	12	34	2	4
	0 - 25%	19	19	0	11	32	71	2	8
	25%	50	20	24	54	21	24	44	21
WATER	Clear	57	43	18	58	56	102	43	28
	Oily	13	11	5	7	9	27	5	5
	0	3	12	2	16	1	12	6	13
	0 - 25%	29	20	7	24	33	78	17	9
	25%	38	22	15	26	31	39	25	11
SLOPE	1	18	29	10	18	39	52	29	16
	2	45	24	14	46	25	66	19	15
	3	7	1	0	2	1	11	0	2
VEGETATION	*Lemna minor*	31	15	10	18	45	66	32	25
	No Duckweed	39	39	14	48	20	63	16	8
	Nasturtium spp.	12	2	0	9	30	37	0	1
	No.Cress	58	52	24	57	35	92	48	32
FLOW	-	25	32	18	56	41	99	34	26
	Induced	22	10	0	0	18	27	7	4
	+	23	12	6	10	6	3	7	3
OTHER SNAILS	*Potamopyrgus antipodarum**	22	2	1	3	15	14	1	0
	No *P. antipodarum*	48	52	23	63	50	115	47	33
	Physa					12	3	10	0
	No *Physa*					53	126	38	33

*Only the presence or absence of snails is considered.
A positive sample contains one or more snails.

(v) Slope

The evidence for a relationship between steepness of the habitat and the presence of snails is contradictory. In three cases the flatter habitats were more likely to contain snails, although the result was statistically significant only in the larger microhabitats of L. columella. However the larger microhabitats of L. tomentosa showed the opposite trend, with steeper habitats yielding more positive samples.

(vi) Vegetation

There was a tendency for the presence of Lemna minor (duckweed) to indicate suitable habitats, but the association was statistically significant only in the case of the larger microhabitats of L. columella. Nasturtium spp (watercress) indicated suitable microhabitats of types (a) and (b); in the smaller microhabitats there was a tendency for negative associations but there were too few samples containing watercress for statistically significant results.

(vii) Water flow

Microhabitats with a visible flow of water were more likely to contain snails but this tendency was significant at the .05 level of probability only in the larger microhabitats of both species.

(viii) Other snails

In the larger microhabitats there were positive associations between Potamopyrgus antipodarum and the two Lymnaea species. There were too few Potamopyrgus recoveries from the smaller microhabitats to provide meaningful results.

Physa sp. was only recovered from L. columella habitats. In microhabitats of all sizes the two species were positively associated.

2.4 DISCUSSION

The results recorded in this chapter should be interpreted with caution for several reasons:

1. Since the data were not derived from random samples of New Zealand habitats on randomly selected sampling dates, they should be regarded only as being indicative of conditions in the selected habitats on the chosen dates.

2. An association between a habitat factor and a snail species does not necessarily imply that conditions are favourable to the snail because of that factor; it may only mean that they share certain ecological requirements.

If these reservations are borne in mind speculation on the findings in both macro and microhabitats may be a useful aid to understanding the ecology of L. tomentosa and L. columella.

(i) Slope, vegetation and fluctuations in water level

Marked fluctuations in the depth of ponds are usually associated with steep banks and a lack of vegetation. All of these factors seem to indicate a degree of unsuitability for L. columella but the precise reasons can only be a subject for conjecture. Algal growth is greater in shallow water near gently sloping banks and floating pond-weeds allow a safe "anchorage" for this surface-dwelling snail.

The associations with Lemna minor and Nasturtium spp. in microhabitats may indicate some useful property of the weeds such as oxygenation of the water, or they may merely indicate that the ecological requirements of the snails and the plants are broadly similar. The tendency for negative associations in the smaller microhabitats is interesting; the snails may prefer not to be too closely associated with these plants even though their presence in the general vicinity indicates suitable hydrological conditions.

In marsh habitats the degree of slope does not appear to matter so long as there are enough suitable microhabitats such as water-filled hoofmarks. The equivocal nature of the results of the association analysis is probably due to extraneous factors; for

example the square metre microhabitats of L. tomentosa were all from one macrohabitat and the steepest part of it seemed to coincide with the most constant source of underground water.

L. columella was sometimes found in ponds which dried out for a short period in dry summers, but never in marshes without some underground water even in the driest periods. Population density was usually much greater in ponds and snails usually grew to a larger size than those from marshes (see Chapter 3). L. columella seems therefore to be a pond snail which can occupy some stable marsh habitats. In this it differs from L. tomentosa which seems to be better adapted to marsh habitats than to permanent ponds (section 1.4.1).

(ii) Source of water

Marsh habitats were all spring fed. Consequences of underground seepage as a water source include:

1. less likelihood of the habitat drying out in summer;
2. in most parts of the habitat water flows very slowly and less erratically than water from surface run off;
3. there is a renewal of water from a relatively pure source, even when the turnover of water is imperceptible.

(iii) Flow rate

The negative association between rapidly flowing water and the snail hosts of Fasciola hepatica may be due to the scouring of micro algae upon which the snails feed. Where flow rates were variable, as in steep sided drainage ditches which dried out in summer but carried rapidly moving water in wet weather, lymnaeids were almost never found. Stability appeared to be an important characteristic of the habitats of both species.

In microhabitats the "positive flow" factor investigated by the Chi-square analysis was usually a mere trickle, not sufficient to be unsuitable for the snails. Even so, the statistically significant associations between positive or induced flow and snails were found in the larger microhabitats of both species, not the ten centimetre square plots. In the larger microhabitats a visible flow through one section usually indicated that there was enough water for a snail habitat, but

the lymnaeid snails were often found away from the main flow. In the smaller microhabitats this meant that they were just as likely to be found in an adjacent quadrat as the one with the visible flow of water.

(iv) Consistency of mud

If snails appear to prefer firm mud to a loose, flocculent substratum it is difficult to be sure that such a preference is real and not just a sampling phenomenon, since snails will be more easily seen on and recovered from firm mud. The preference of L. tomentosa for flocculent mud, revealed in the association analysis, is therefore almost certainly even greater than the data suggest because many more snails may have been overlooked in or under the flocculent mud than on the firmer surfaces.

It is difficult to understand why flocculent mud should be desirable since a firmer substratum should be more suitable for the growth of microalgae. On the other hand flocculence is usually due to underground water making its way to the surface, and underground seepages are an essential feature of the marsh habitats of both species. The greater association between L. tomentosa and flocculent mud may indicate a greater dependence upon underground water than is the case with L. columella, but this needs confirmation in other habitats of both species.

(v) Amount of visible mud

Microhabitats with a lot of visible mud were more likely to contain either L. tomentosa or L. columella than those overgrown with grass or weeds, or with only a small amount of bare mud. However it must be conceded that snails are easier to see on a bare mud surface than under vegetation, so results could be biased in favour of muddy habitats. A more clear-cut result in favour of mud surfaces may be seen in Chapter 3.

(vi) Altitude

Most marsh habitats were found in hilly areas, but this was due to the presence of spring fed marshes and dams in gullies on hill farms. It seems likely that low temperatures would limit

distribution at very high altitudes before reduced oxygen tension or barometric pressure could exert any significant effect, but this question was not investigated.

(vii) Oxygen content of water

L. tomentosa and L. columella occupy shallow water in marshes or around the more gently sloping banks of small ponds. Such water will usually contain saturation or near-saturation levels of oxygen, and the snails can come to the surface to breathe air if the oxygen content of the water is inadequate. The surface films which sometimes block the pneumostomes of young snails in laboratory aquaria are less common in outdoor habitats, being dispersed by the action of wind and rain. Oxygen is therefore unlikely to be a limiting factor in the habitats of L. tomentosa or L. columella.

(viii) Calcium and magnesium

Both L. tomentosa and L. columella have relatively light, brittle shells which may account for their ability to utilise habitats with very low calcium and magnesium levels. However, without a detailed knowledge of their food requirements and the mineral content thereof, it is impossible to say to what extent their mineral requirements are supplemented by their diets.

In the very soft water of habitat 22 (Table 2.1) L. columella shells were particularly fragile, especially in early spring, and some shells had a trumpet-shaped end due to incomplete calcification of the matrix at its growing margin. This effect could be reproduced in the laboratory by maintaining L. columella in de-ionised water without calcium supplements.

Macan (1963) states that natural freshwaters have a range of 1-100 ppm dissolved calcium. The calcium content of habitat no. 7, 70 ppm, supports Boycott's (1936) observation that freshwater snails which occupy softwater habitats may also inhabit sites with much higher calcium contents if given the opportunity to do so.

(ix) Associations with other snail species

Although Physa sp., Potamopyrgus antipodarum and Lymnaea columella inhabit their own preferred parts of pond habitats, these overlap to the extent that a given volume of water will often contain all three species. The greater the volume of water in question the greater the chances of it containing more than one species of snail.

The same principle applies to marsh habitats; the marsh as a whole may contain several snail species but as the sample area decreases there is less likelihood of it containing more than one snail species. Thus in the association analysis combining microhabitats (a) and (b) there were strong positive associations between the lymnaeids and P. antipodarum but these were not seen in the smaller type (c), microhabitats. The preferred microhabitats of L. tomentosa and L. columella appear to be areas not much more than ten centimetres square; the larger microhabitats often have enough variation within them for microhabitats of more than one species if the sample as a whole is suitable for freshwater snails.

The association between Physa sp. and L. columella appeared to be more intimate than that between either lymnaeid and P. antipodarum because it occurred in microhabitats of all sizes.

2.5 CONCLUSIONS

This chapter contains evidence that typical macrohabitats of Lymnaea columella are:

1. small ponds whose depth does not fluctuate markedly, and
2. spring-fed marshes which remain wet throughout the year.

L. tomentosa was found in marsh habitats which were not readily distinguishable from those of L. columella. Both snails could utilise habitats with very soft water.

Typical microhabitats of L. columella are:

1. by the gently sloping margin of a small pond, near its inlet or outlet, adherring to floating Lemna minor or Azolla rubra, or

2. a muddy, water-filled depression with a flocculent substratum through which flows an often imperceptible trickle of underground water. The microhabitat contains or is not far from marsh vegetation such as rushes, duckweed or cress. Other freshwater snails in the macrohabitat may share the microhabitat but are more likely to occupy others nearby.

The microhabitats of L. tomentosa are not readily distinguishable from the marsh microhabitats of L. columella. The association between L. tomentosa and flocculent mud may be more pronounced.

3. POPULATION STUDIES

3.1 HABITATS (Table 3.1)

Snail populations under investigation were from two Manawatu farms, one of them in the Pohangina Valley 40 km north-east of Palmerston North and the other in the foothills of the Tararua ranges near Tokomaru, some 30 km south of the city. The Pohangina and Tokomaru habitats were respectively 300 and 350 m above sea level. Soil in the Pohangina habitats was a Raumai sandy loam while the Tokomaru habitats contained the soil known as Tokomaru silt loam. *L. columella* was found on both farms but *L. tomentosa* was present only on the Tokomaru property.

Snail populations were observed in five habitats whose main features are described below.

1. A pond on the Pohangina farm (Plate 2.1)

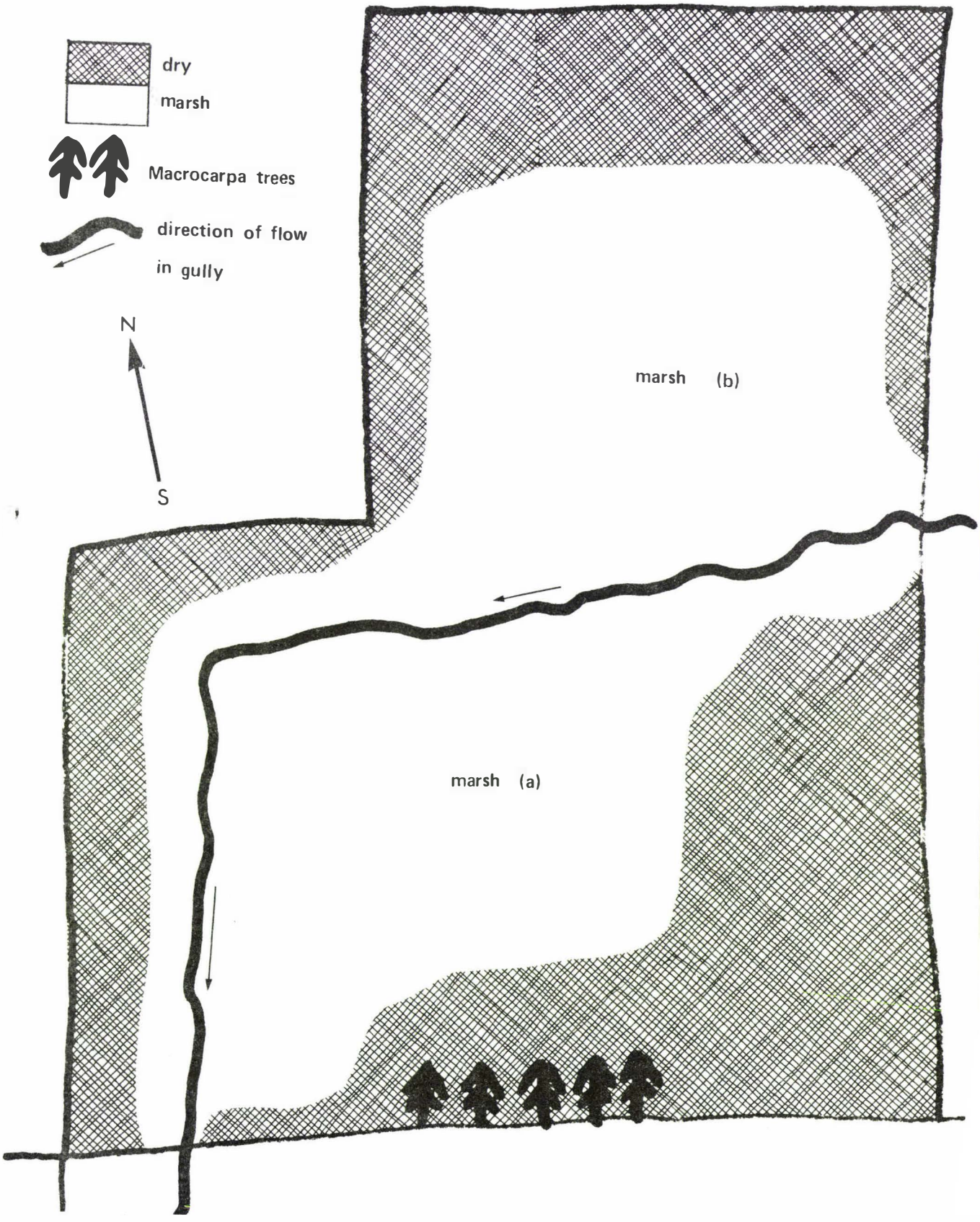
The pond had been created by damming a small gully fed by underground springs and surface water. It was approximately circular with a surface area of about 100 m^2, and its depth varied from 2.5 m in winter to 1 to 1.5 m in late summer. The banks sloped steeply on two thirds of its perimeter. Water pH and calcium and magnesium concentrations tested by the methods described in Chapter 2, were 6.0, 70 ppm and 3 ppm respectively.

As well as *L. columella* the pond contained an unidentified species of *Physa* (see footnote in section 2.2), *Potamopyrgus antipodarum*, and *Gyraulus corinna*, the last-named being retrieved only from the deeper parts of the pond. *P. antipodarum* appeared sporadically and was confined mainly to the bottom. The main interspecies interface with *L. columella* was its relationship with *Physa* sp.. *L. columella* usually inhabited the surface and edges sharing these locations to a varying degree with *Physa* sp., but the latter became numerically dominant in deeper water and below the surface.

2. A marsh on the Pohangina farm (Fig. 3.1)

An area of 0.3 hectares of this marsh habitat was fenced off for an epidemiological study described in Chapter 4. The experimental

Fig. 3.1 MARSH HABITAT no. 2.



area was divided almost equally by a small, slow flowing stream which often stopped flowing in dry weather. The southern half of the habitat, later referred to as 2(a) had more surface water in dry seasons than the northern half, 2(b). The marshy portions of the experimental plot sloped gently upwards from the stream to the steeper dry areas around the perimeter. Underground seepage kept most of the marsh damp even in periods of drought. The pH of the water was 6.0 and it contained 76 ppm calcium and 9.5 ppm magnesium.

The habitat was populated by L. columella, P. antipodarum and Physa sp.. P. antipodarum was distributed irregularly throughout the habitat, mainly in running water, but Physa sp. was confined to a relatively small area near a source of spring water at the northern end.

3. A marsh habitat on the Tokomaru farm (Plate 2.2)

This marsh was a long, narrow strip only 8 m across at its widest point, with two more irregular tributaries or sub-habitats entering it on its northern side. It ran from east to west and in July 1971 its first 70 m was fenced off, along with some dry grazing, to provide the main experimental area. It contained L. tomentosa and P. antipodarum; the former were more numerous out of the main stream and near the sources of spring water, but P. antipodarum was more often found where there was a noticeable flow of water. This habitat, like the marsh habitats of L. columella, was kept moist at all times by underground spring water. Water pH, calcium and magnesium values were 6.0, 11.0 ppm and 1.05 ppm respectively.

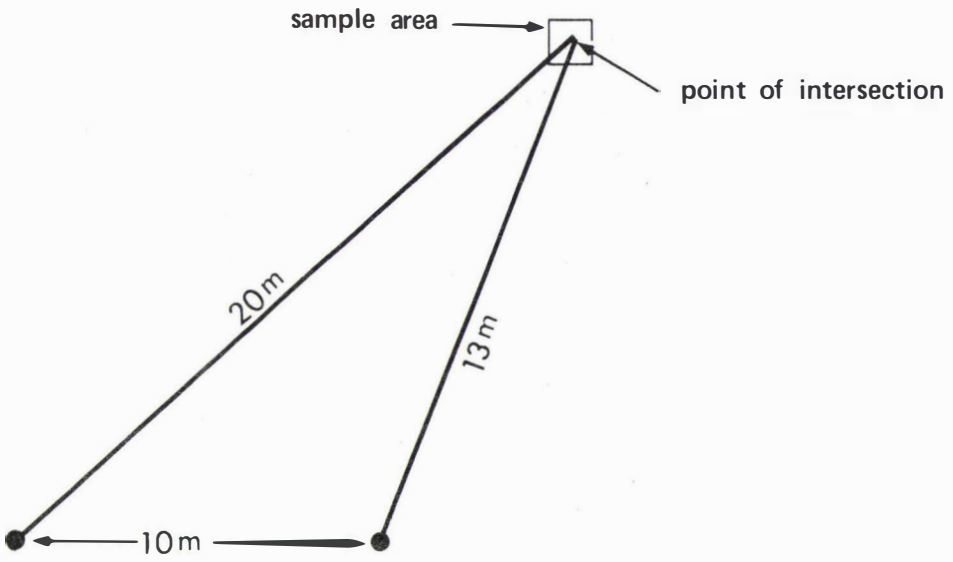


Fig. 3.2 An example of the selection of one sample point in habitat no. 2 by the random co-ordinate method. The random number sequence in this instance was 2013.

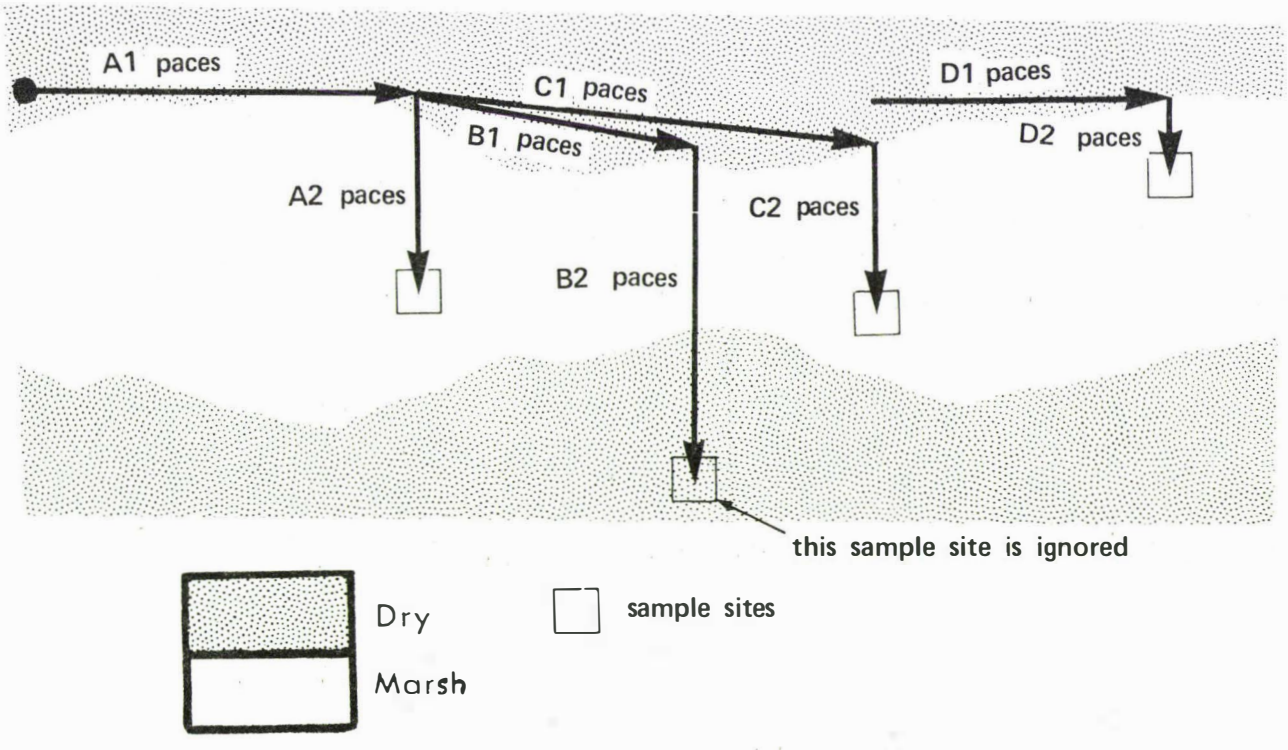


Fig. 3.3 An example of the selection of sample points in habitat no. 3. A, B, C and D are random numbers from 1 to 9.

4. A marsh habitat on the Tokomaru farm

This lay on the other side of a ridge from no. 3, so that its main flow was from west to east. It was up to 9 m wide and 110 m long, opening into a slow flowing stream which drained the gullies from a large part of the farm. The habitat was very similar to no. 3 but it supported large numbers of L. columella during 1970-71, the first year in which it was searched. The pH of the water was again 6.0, but habitat no. 4 had an even lower calcium content of 4.0 ppm with 0.6 ppm magnesium.

Adjacent habitats which were physically and chemically indistinguishable from no. 4 contained a few L. tomentosa, but no L. columella.

5. A marsh habitat on the Tokomaru farm

Another marsh, physically similar to numbers 3 and 4, ran from west to east. It was first examined on 10.11.72 when it and an adjacent habitat contained large numbers of L. columella. On this and a few other occasions a few L. tomentosa were found at the western extremity near the source of spring water at the origin of the marsh. The water in habitat no. 5 was not tested for pH or minerals.

TABLE 3.1 Habitats whose Lymnaea populations were studied

habitat number	type	location	lymnaeids	other snails	sampling period
1	pond	Pohangina	L. columella	Physa sp., P. antipodarum Gyraulus corinna	4/11/69 - 26/6/74
2	marsh	Pohangina	L. columella	Physa sp. P. antipodarum	4/11/69 - 26/6/74
3	marsh	Tokomaru	L. tomentosa	P. antipodarum	21/11/70 - 27/5/74
4	marsh	Tokomaru	L. columella	P. antipodarum	31/1/71 - 12/4/71
5	marsh	Tokomaru	L. columella	P. antipodarum	10/11/72 - 27/5/74

3.2 METHODS

3.2.1 Sampling

(a) Pond sampling

The perimeter of the pond was divided into eight equal sections and an equal time was devoted to searching each. On the first five occasions there were 8 x 15 minute searches, but thereafter 8 x 5 minutes were considered adequate. Population estimates were made equivalent to one another by dividing the numbers of snails recovered on each of the first five occasions by three to approximate the results expected from 8 x 5 minute searches.

Collections were made by hand during visual searches. Preliminary attempts at net-sweeping were unsatisfactory because of the large numbers of other snails and dense weed in summer. The tendency for L. columella to inhabit the surface and edges of the pond favoured the simple visual search.

(b) Marsh sampling

The irregularly shaped habitat no. 2 was sampled by

the random co-ordinate method during the first two years of the study. In each half of the habitat two posts 10 m apart served as anchors for 30 m tapes which were drawn out to lengths selected from a table of random numbers. The intersection of the two tapes served as the centre of the sample area which was one metre square (Fig. 3.2, Plate 2.3). Snails were collected and counted after a visual search of each sample site, and were taken to the laboratory to be measured and examined for parasites. From eighteen to thirty-six samples were taken on each sampling date.

The long, narrow shape of habitat no. 3 allowed a more convenient form of random sampling. Beginning at the water source and using a random number sequence from one to nine the sampler paced a number of steps along the bank, then another number of steps into the habitat at right angles to the bank. The starting point for selecting the next sample site was the point on the bank adjacent to the previous one, and so on until the end of the fenced off portion of the habitat was reached (Fig. 3.3). The method allowed ten to sixteen samples on each date.

With either of the above methods if the randomly selected sampling area was outside the habitat it was ignored and a new site was chosen from the same starting point.

In 1971-72, the third year of the study at Pohangina and the second year at Tokomaru, a fixed quadrat design was employed in marsh habitats 2 and 3. Ten blocks, each consisting of 2 x 1 m^2 plots, were chosen arbitrarily as being good snail habitats. One of the plots in each block was dug over and trampled to simulate pugging by cattle while the other was used as a control (Fig. 3.4; Plate 3.1). The initial decision as to which plot was to be trampled or left intact was made by tossing a coin. These treatments remained constant until the end of the experiment in June, 1974, the pugged area being trampled after each snail collection, and samples being drawn repeatedly from the same sites. The blocks were unchanged during the entire three year period, and when the initial decision was made as to which plots were to be treated and which were to act as controls these also remained unaltered.

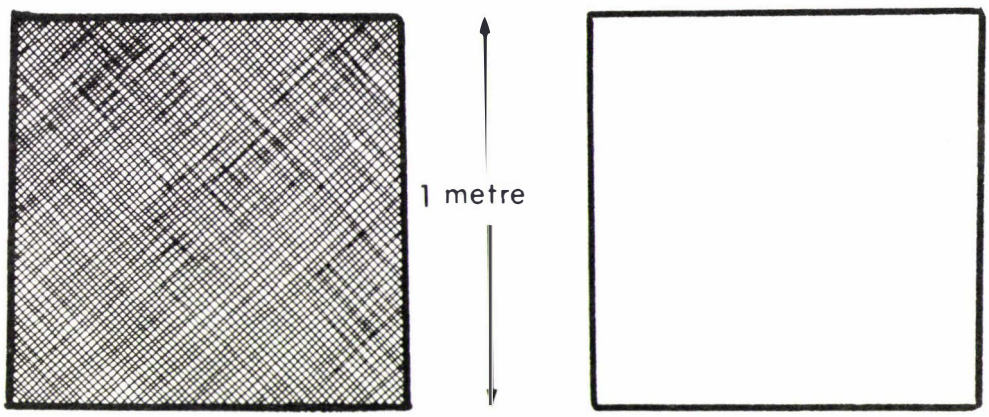


Fig. 3.4 Fixed quadrat sampling method used in habitats 2 and 3 after July 1971. treatment (pugging) and control plots (one square metre)

Plate 3.1 A square metre quadrat dug over and tramped to simulate pugging by cattle.

In 1971-72 one ninth of each square metre plot was chosen with the aid of a table of random numbers as the sample area at each visit, using the smaller of the two grids shown in Plate 2.3. Snails were removed for measuring (and examination for F. hepatica infection in the case of habitat no. 2). From September 1972 onwards when snail populations were smaller the whole square metre was the sample area, but snails were measured to the nearest mm with a ruler and returned immediately to the sample site. Whichever sample area was used, results were always expressed as snails/m^2.

Because the snail sampling methods in marsh habitats 2 and 3 differed in the first two years (random co-ordinates) from that used in the third, fourth and fifth (fixed quadrats), population density can only properly be estimated during the first two years. The third, fourth and fifth years give an exaggerated estimate of population density in the habitats as a whole because the blocks were selected for suitability as snail habitats. However comparisons between the first two years and between the third, fourth and fifth years are valid.

At the beginning of the study cattle were brought into the habitats periodically to control surplus grass growth. They were excluded from habitats 2 and 3 in July 1971, but sheep were grazed as before. In July 1972 sheep were also excluded from habitat no. 3 so that the owner could plant conifers in the drier parts.

The sampling method in habitats 4 and 5 was a thirty minute search from one end of the habitat to the other. When snails were located a small area was searched thoroughly in an attempt to remove snails of all sizes before moving on to another part of the habitat. Groups of snails were located by scanning, but when a snail or group of snails was found their immediate vicinity was searched much more thoroughly.

Ideally all habitats would have been sampled regularly, perhaps monthly, throughout the period of study, but this was not logistically possible. During the first four years samples were taken less frequently, with most being drawn in summer when populations were larger. In 1973-74 habitats 1,2,3 and 5 were sampled at approximately monthly intervals in an attempt to follow more closely their population dynamics within a single year (Figs. 3.5; 3.6; 3.7; 3.8; 3.9; 3.10).

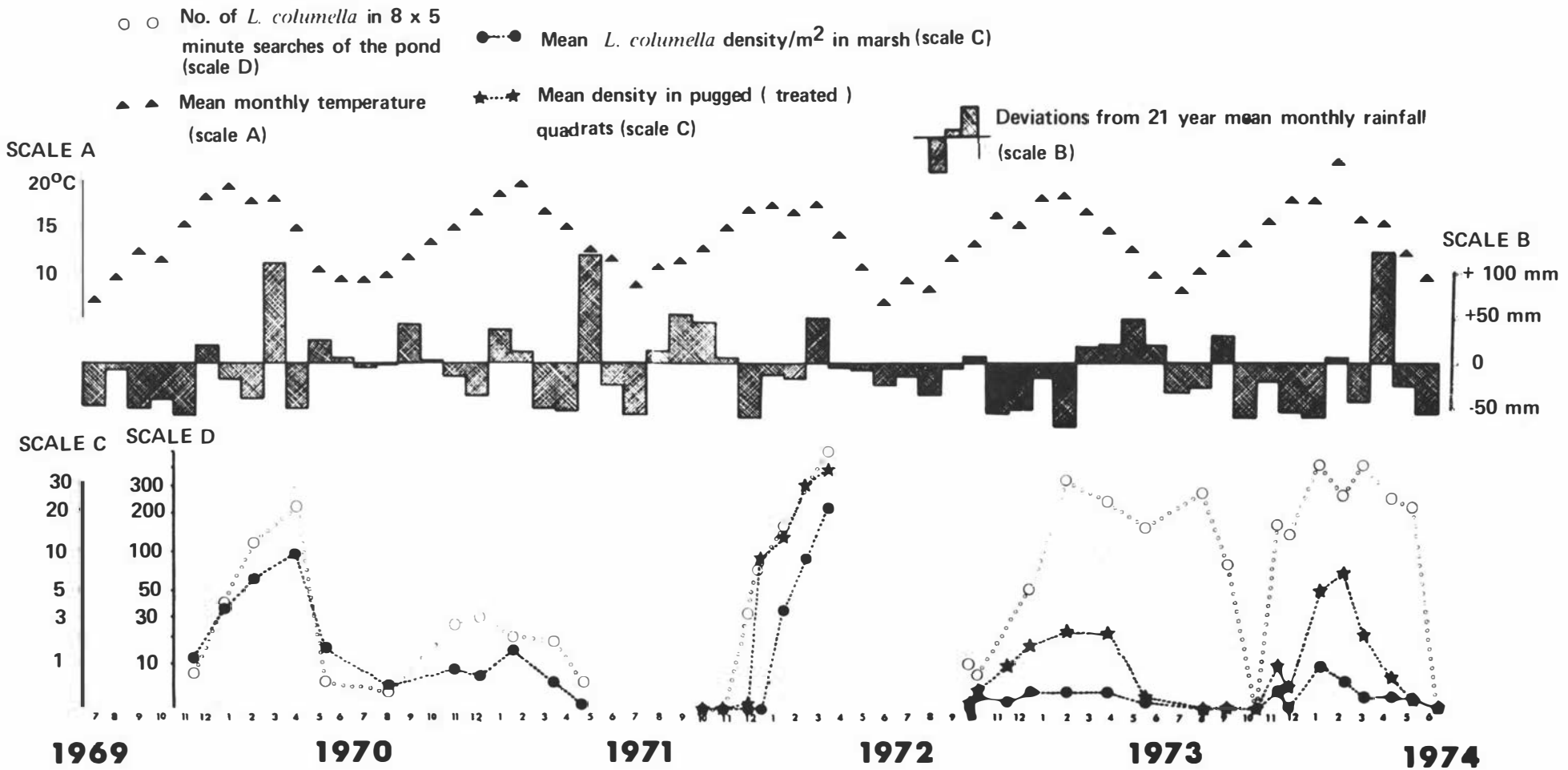


Fig. 3.5 *L. columella* populations in pond and marsh habitats at Pohangina, mean monthly temperatures, and deviations from a 21 year mean monthly rainfall

3.2.2 Temperature and Rainfall

No continuous temperature readings could be taken from the habitats because of their distance from the laboratory and from the farmhouses. Mean monthly temperatures (mean daily maximum + mean daily minimum)/2 from the D.S.I.R. "Grasslands" recording station in Palmerston North, 34 m above sea level, were used to estimate temperature variations within and between years.

Rainfall readings were from the meteorological recording stations at Pohangina Domain, 5 km from habitats 1 and 2 and 274 m above sea level, and Tiritea, 11 km north-east of Tokomaru and 131 m above sea level.

3.3 RESULTS

3.3.1 Population density

L. columella

The pond habitat appeared to be the most consistently favourable for L. columella. On most occasions large numbers of snails were recovered and many were larger than any recovered from the marshes. In all L. columella populations there were marked seasonal fluctuations. A decline to very low levels in winter and spring preceded a rapid re-population in summer, but details of the seasonal pattern varied from year to year (Fig. 3.5).

There were also marked differences between populations in different years. The first year (1969-70) yielded many more snails than the second (1970-71) in both pond and marsh habitats 1 and 2. In 1969-70 the maximum number of snails recovered in 8 x 5 minute searches of the pond was 210 in April but in the following year the largest collection was 29 in December, the usual population increase in late summer failing to eventuate (Fig. 3.5). The mean population density in marsh habitat no. 2 reached $9.88/m^2$ in April 1970 but never exceeded $1.40/m^2$ in 1970-71.

The third year produced large numbers of snails in habitats 1 and 2, but in the fourth and fifth years the marsh population declined. This decline in the marsh population of L. columella during 1972-73-74 did not occur in the pond (Fig. 3.5). Thus pond and marsh populations

of L. columella on the Pohangina farm, when graphed on appropriate scales, followed one another quite closely during the first three years but not in the following two.

The character of the marsh habitat no. 2 had begun to change after exclusion of cattle in the winter of 1971; a year later it was heavily overgrown with rushes and other tall vegetation and there was much less bare flocculent mud visible than when its surface was periodically churned by the actions of grazing cattle. This circumstantial evidence that cattle render marsh habitats more suitable for Lymnaea species was tested experimentally during the last three years of investigation with artificially pugged and control plots (see section 3.3.3).

On the Tokomaru farm the marsh habitat no. 4 yielded up to 114 L. columella during thirty minute searches in the 1970-71 season but only a single specimen was recovered from it in the following year, after which it produced no more. A new L. columella habitat, marsh no. 5, was discovered on 10/11/72. Population density is recorded in Fig. 3.6.

L. tomentosa

The density of L. tomentosa populations in habitat no. 3 followed the same general trends as the L. columella populations i.e. there were few snails in winter and early spring but numbers generally increased in summer (Fig. 3.6;) Inside the fenced-off portion of the habitat the random samples of 1970-71 showed mean densities ranging from 2.38 snails/m^2 in November to 9.29/m^2 in February. Following the exclusion of cattle in the winter of 1971 mean L. tomentosa densities fell, ranging from a maximum of 1.55/m^2 in January to zero in March and May of the 1971-72 season. When sheep were excluded to allow tree-planting in the following winter, 1972, snail numbers fell still further. In fact the maximum recorded mean density during the last two years of the study was 0.05 snails/m^2. As in L. columella habitat no. 2, these declines in snail numbers were parallelled by increasing vegetation cover and a reduction in the amount of flocculent mud in the habitat.

When numbers in the enclosed part of the habitat no. 3 fell to such low levels that they served no useful purpose, thirty minute collections from outside the wire were used as an alternative estimate of snail density (Fig. 3.6; and to measure the shell length distribution of field populations (Fig. 3.10). The fluctuations in the size of thirty minute collections of L. tomentosa from habitat 3 and L. columella from habitat 5 did not follow each other very closely, although both populations were greater in summer than in winter.

3.3.2 Shell lengths and population structure

L. columella

The largest specimens of L. columella were always found in the pond, particularly during the first three years (Fig. 3.7). Marsh specimens rarely reached shell lengths of 10 mm (Figs. 3.8; 3.9) and yet field specimens below 9 mm were sexually immature (Chapter 6.3.2).

The size, and hence age, distribution of different collections varied irregularly. There appeared to be constant increments to the populations with irregular periods of high mortality which sometimes had a selective effect upon different age groups. The dice-grams of the pond populations (Fig. 3.7) showed relatively constant shell length distributions in the first, fourth and fifth years but in the second and third years there was evidence of the maturation of relatively discrete populations. In November and December 1970 the snails appeared to need little more than one month to reach maturity but in the 1971-72 season their growth rate seemed to be slower as shown by the progressively maturing population during December, January and February. In this third year there must have been a high mortality rate in young snails or a failure of eggs to develop - at any rate there were no immature snails in the April 1972 collection (Fig. 3.7).

The shell length distributions of marsh populations of L. columella (Figs 3.8; 3.9) were often based on very small samples so their reliability as indicators of population structure is suspect. Like the pond populations, no clear or consistent pattern emerged. In some years there were many more mature snails; for example in the first three years the pond produced a much greater percentage of mature L. columella than years four and five, while in marsh habitat no. 2 this trend was reversed (Figs. 3.7; 3.8; 3.11).

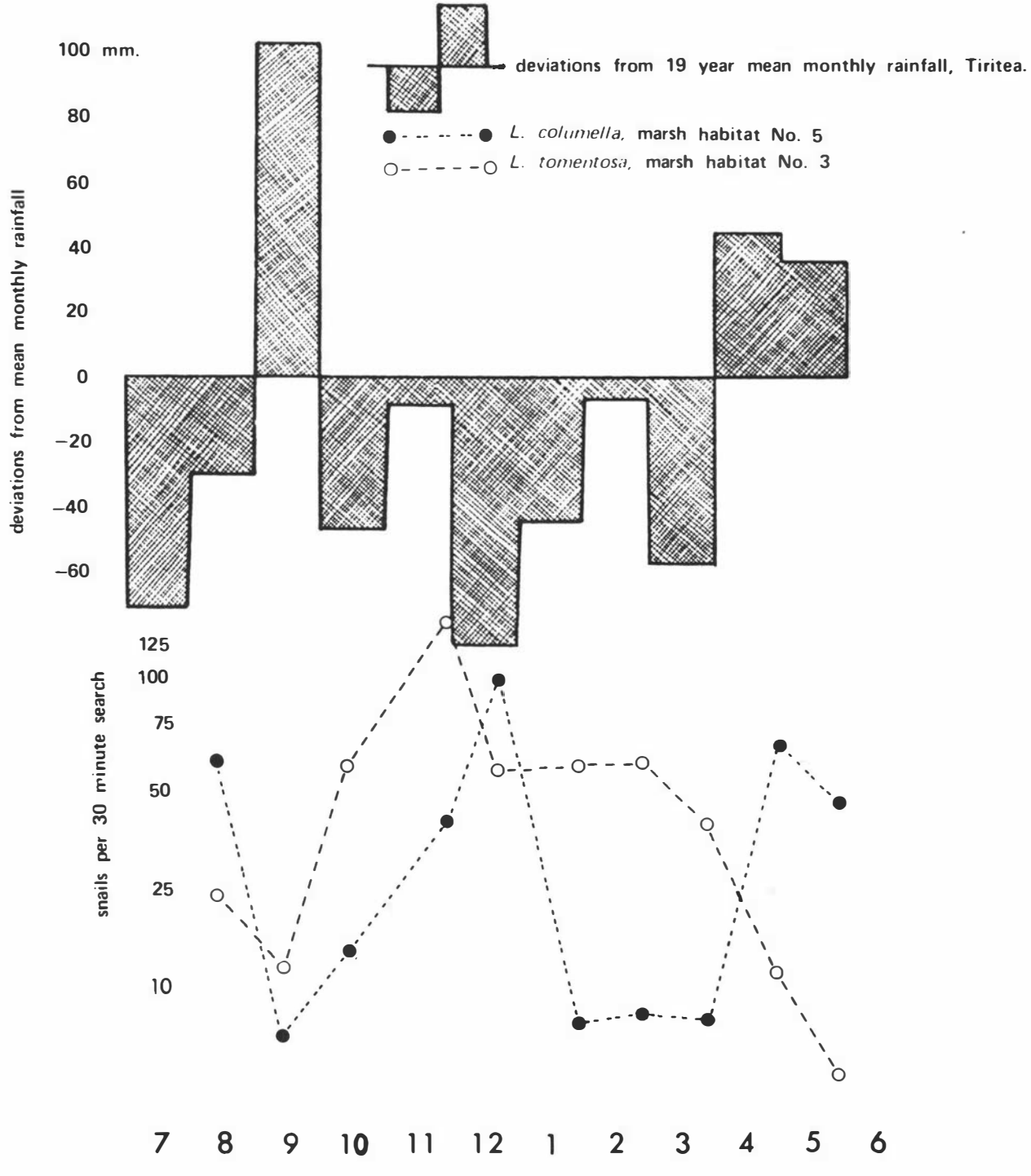


Figure 3.6 Population density estimated by 30 minute searches in two Tokomaru marsh habitats during 1973–74.

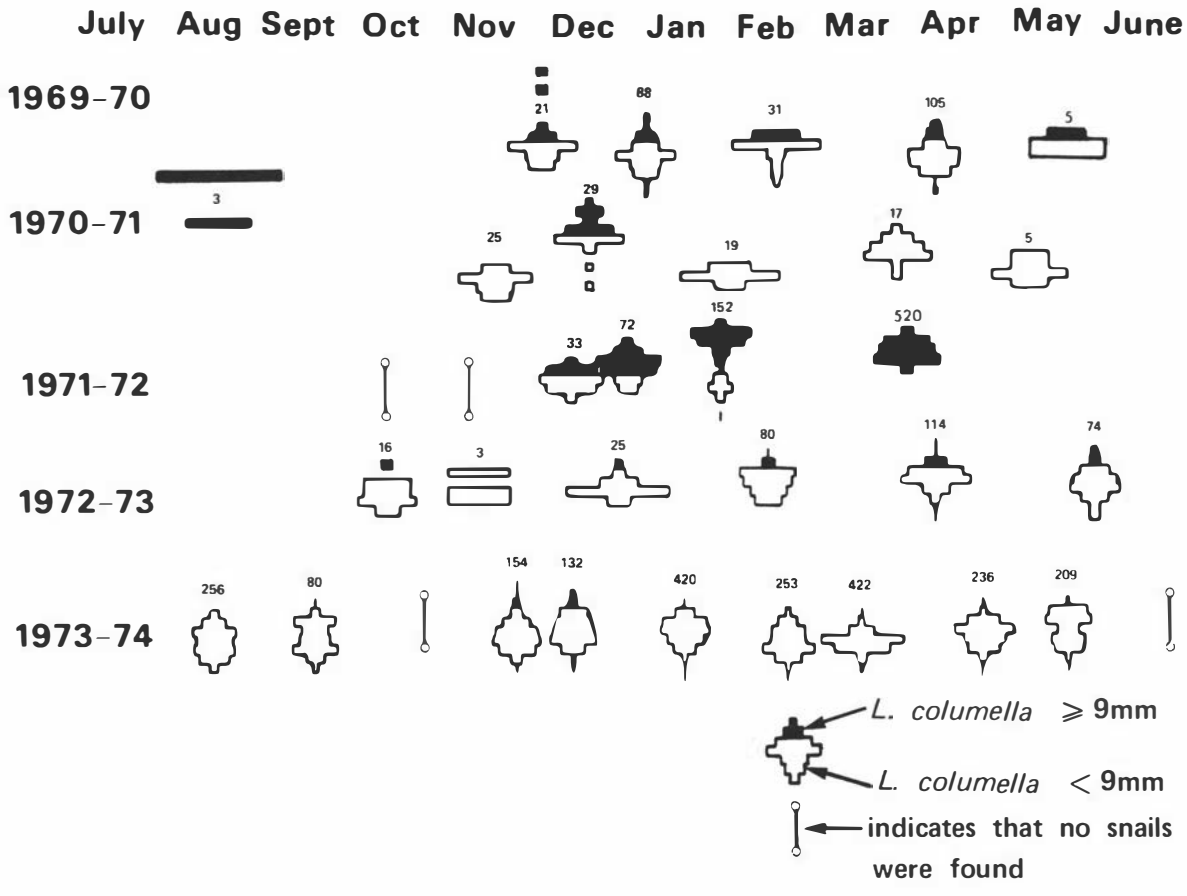


FIGURE 3.7: Field populations of *L. columella.* Pond habitat.

In Figs. 3.7 to 3.10 the shape of the population pyramid indicates the percentage of snails in each shell length class at intervals of 1 mm.

Numbers above population pyramids indicate numbers of snails measured.

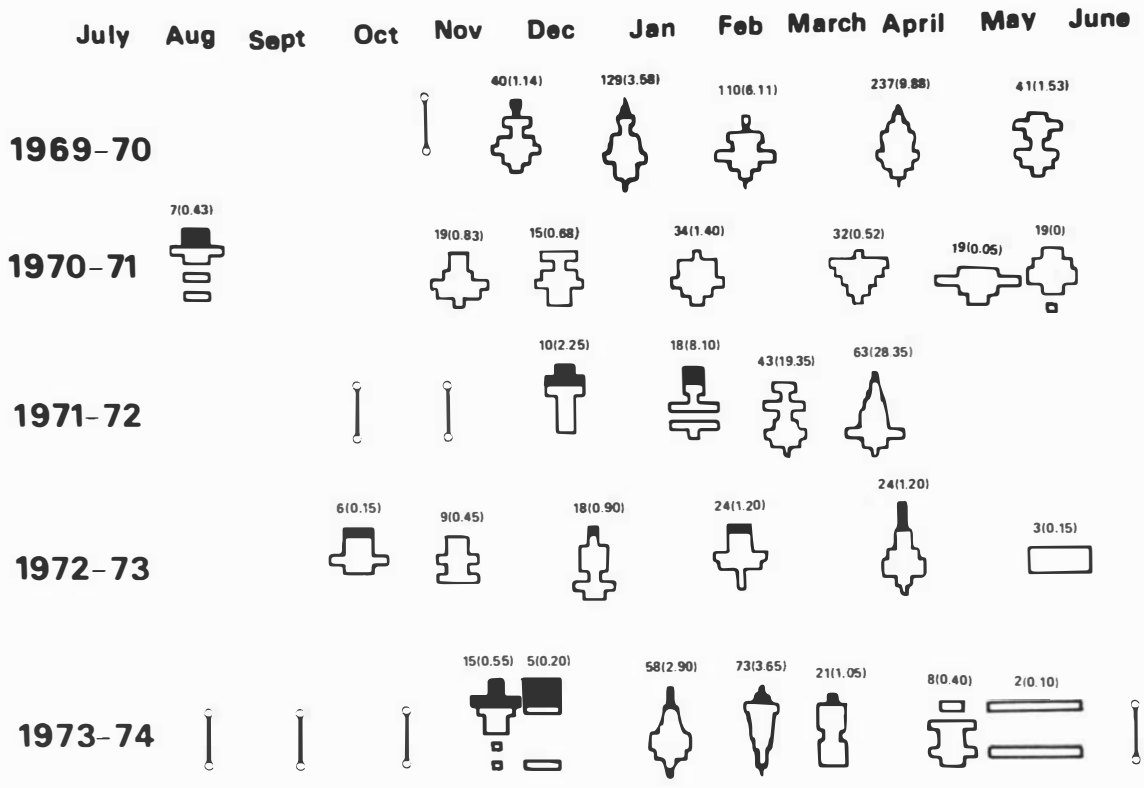


FIGURE 3.8: Field populations of *L. columella*. Marsh habitat No. 2
KEY: as for Fig. 3.7 except that numbers in brackets show population density/m^2 in the experimental area

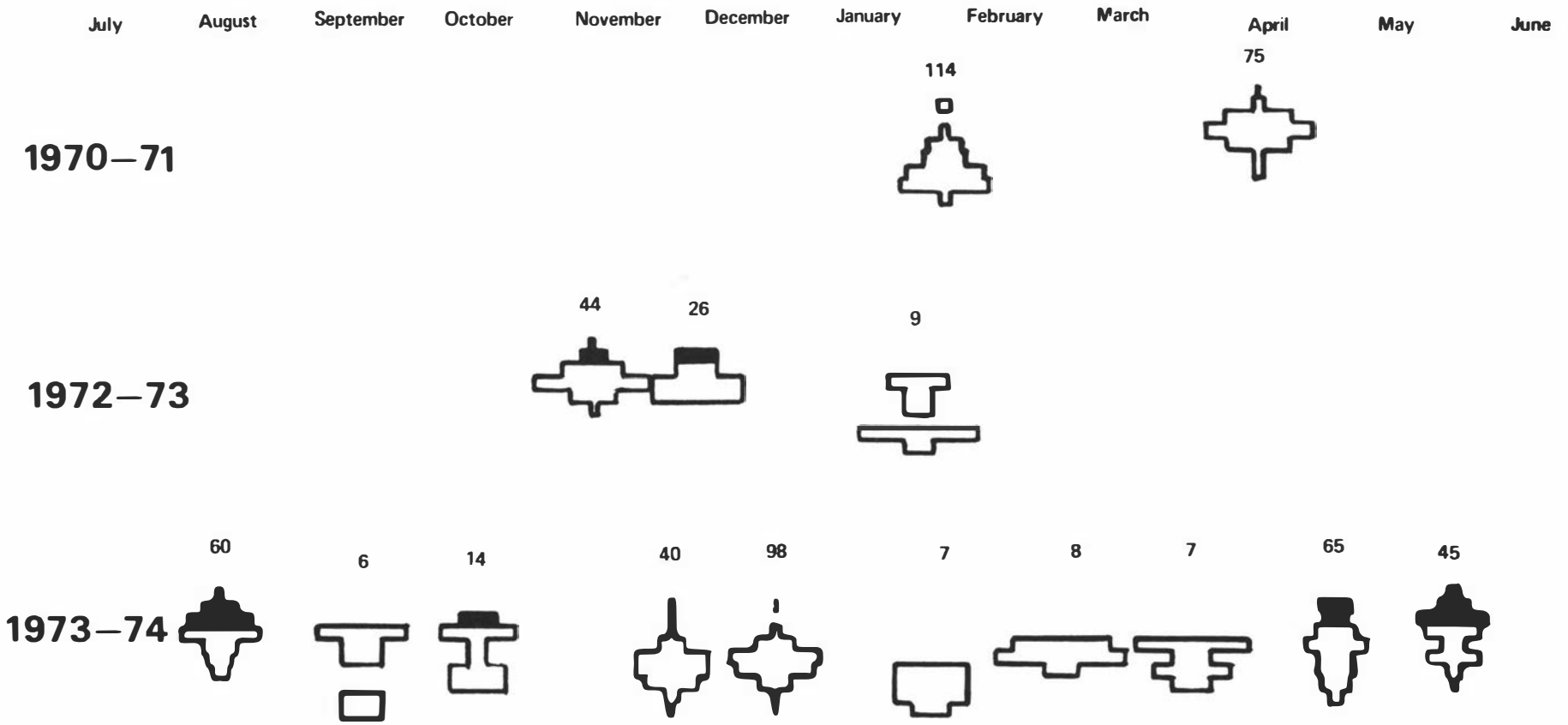


FIGURE 3.9: Field populations of *L. columella*. Marsh habitats, Tokomaru.
KEY: as for Fig. 3.7.

Except on a few occasions there was no evidence of the maturation of discrete populations so it was not possible to estimate from field samples the number of generations per year. There appeared to be almost constant increments of young snails to the population with the numbers and sizes of snails in populations influenced by intermittent and irregular periods of high and low mortality (Figs. 3.7; 3.8).

A number of L. columella as small as 2 mm were found in August and September collections (Figs. 3.7; 3.9), indicating that breeding takes place in winter as well as summer. Under these conditions L. columella does not depend on snails overwintering until spring temperatures rise high enough for oviposition; in the laboratory snails will produce eggs at 5°C and perhaps even at 2°C (section 6.3.7) while development of the embryos begins at 5°C (section 7.2.2).

L. tomentosa

Laboratory studies (section 6.3.2) showed that shells of sexually mature L. tomentosa are normally at least 5 mm long. There was usually a higher percentage of potentially mature L. tomentosa in field populations than in marsh populations of L. columella (Figs. 3.10; 3.11). As with L. columella there appeared to be constant increments of young snails in the population with little evidence of the maturation of discrete populations. The percentage of immature snails was usually greatest in summer (Fig. 3.10), but snails only 2 mm long were found in September. This observation and laboratory experiments showing that oviposition will take place at 5°C suggest that all year round breeding also occurs in L. tomentosa in the Manawatu.

3.3.3 The effects of pugging

The reduction in snail numbers following the exclusion of cattle from marsh habitats has been mentioned above. The effect was immediate in the case of L. tomentosa in habitat no. 3, but was delayed for about a year in L. columella habitat no. 2. On several occasions there were large numbers of lymnaeid snails outside the fenced-off experimental areas, giving rise to the suspicion that poaching by

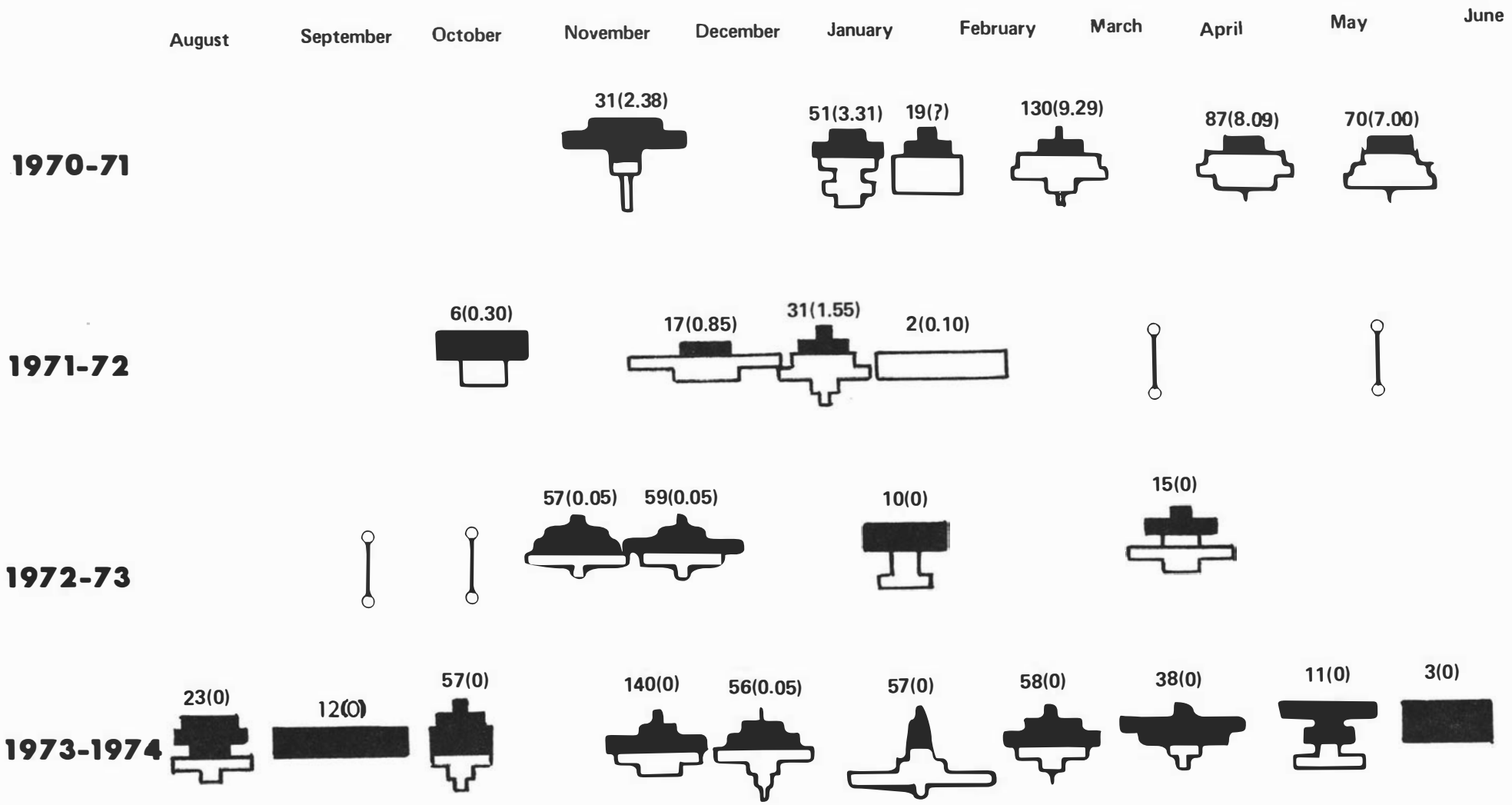


Fig. 3.10 Field populations of *L. tomentosa*. Marsh habitat no. 3, Tokomaru. KEY: as for Fig. 3.7 except that filled squares indicate snails ≥ 5mm and numbers in brackets show population density/m^2 in the experimental area.

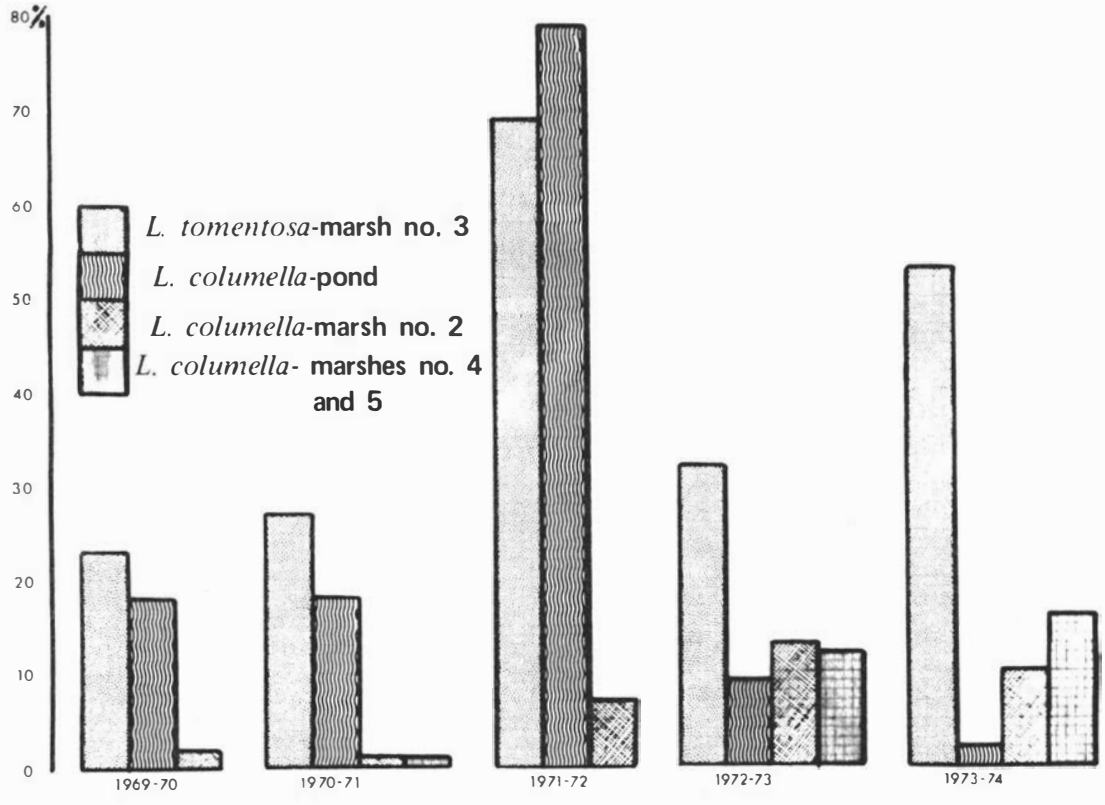


Fig. 3.11 Percentage of mature snails in different habitats

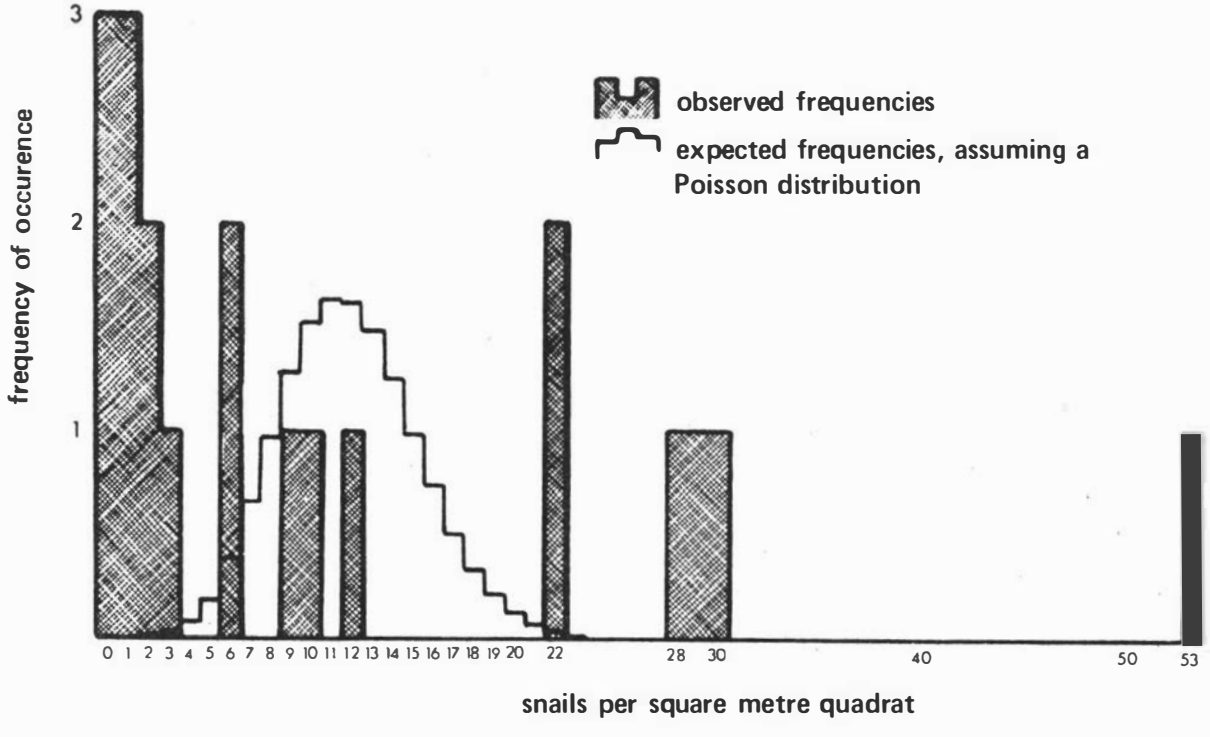


Fig. 3.12 Observed and expected frequencies of *L. columella* in square metre plots on marsh no. 2 sampled on 11/4/70.

cattle may render habitats more suitable for these species. The change from random sampling to repeated sampling from poached and control quadrats after July 1971 was an attempt to test this hypothesis experimentally.

Results (Table 3.2a) indicate that plots which were trampled to simulate the effect of pugging by cattle were much more likely to yield one or more lymnaeids than untreated areas. The total number of snails collected from pugged habitats was also significantly greater (Table 3.2b).

3.3.4 Temperature and Rainfall

The mean monthly temperatures recorded at Palmerston North did not vary sufficiently from one year to another to account for fluctuations in snail populations (Table 3.5); nor were the regular seasonal temperature changes closely related to increases and decreases in snail numbers other than that snail populations were usually larger in summer (Fig. 3.5).

Rainfall varied considerably between years, both in its total amount and in its pattern of distribution. Nevertheless there was no clear pattern between rainfall and snail populations (Fig. 3.5; Table 3.6). For example the driest years were the first, fourth and fifth while the largest populations of L. columella in the pond (the only habitat where sampling methods were unchanged throughout the study) occur in the first, third, fourth and fifth years. The same lack of correlation between rainfall and snail numbers was seen in marsh no. 2, bearing in mind that comparisons are strictly valid only between years one and two and between the last three years because of the change in sampling methods after the second year. It is more difficult to compare different years in habitats 3, 4 and 5. In habitat 3 populations of L. tomentosa almost disappeared within the fenced-off portion after the exclusion of cattle and later sheep.

TABLE 3.2a Numbers of positive samples (one or more snails) in pugged and control quadrats from habitats no. 2 and 3

	poached	controls	totals	
1971-72				
L. columella +	26	12	38	$\chi_1^2 = 6.508$ *
L. columella -	34	48	82	
Total samples	60	60	120	
1972-73				
L. columella +	27	7	34	$\chi_1^2 = 31.815$ ***
L. columella -	33	53	86	
Total samples	60	60	120	
1973-74				
L. columella +	34	11	45	$\chi_1^2 = 17.209$ ***
L. columella -	26	49	75	
Total samples	60	60	120	
Pooled results				
L. columella +	87	30	117	$\chi_1^2 = 39.709$ ***
L. columella -	93	150	243	
Total samples	180	180	360	
1971-72(1)				
L. tomentosa +	18	3	21	$\chi_1^2 = 11.333$ ***
L. tomentosa -	42	57	99	
Total samples	60	60	120	

(1) There were too few *L. tomentosa* in the experimental area during 1972-73 to allow valid comparisons between treated and experimental plots.

TABLE 3.2b Numbers of lymnaeid snails collected from habitats 2 and 3, pugged and control quadrats

	pugged	control	total	
1971-72 L. columella	98	36	134	χ_1^2 = 27.769 ***
1972-73 L. columella	93	13	106	χ_1^2 = 58.877 ***
1973-74 L. columella	156	21	177	χ_1^2 = 101.446 ***
Pooled results L. columella	347	70	417	χ_1^2 = 182.676 ***
1971-72(1) L. tomentosa	53	4	57	χ_1^2 = 40.421 ***

(1) There were too few L. tomentosa in the experimental area during 1972-73 to allow valid comparisons between treated and experimental plots.

TABLE 3.3 Monthly rainfall (mm) Pohangina Domain

Year	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Total	Deviation from 21 year mean
1969-70	60	97	29	60	31	133	41	37	190	35	137	120	970	- 175
70-71	102	102	132	102	75	78	121	89	31	34	229	89	1184	+ 39
71-72	50	117	131	143	95	55	71	58	138	81	105	90	1134	- 11
72-73	93	69	73	106	34	63	68	7	99	106	160	133	1011	- 134
73-74	74	77	109	40	66	60	24	82	38	206	88	58	922	- 223
21 year mean	107	104	79	99	89	114	84	76	81	86	112	114	1145	

TABLE 3.4 Monthly rainfall (mm) Tiritea

Year	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Total	Deviation from 19 year mean
1970-71	119	76	219	153	54	87	197	105	50	32	157	181	1430	+ 110
71-72	85	147	122	162	75	94	78	82	160	89	166	56	1316	- 4
72-73	147	137	70	98	51	54	74	42	122	98	178	107	1178	- 142
73-74	55	87	207	66	85	45	56	83	44	137	166	53	1084	- 236
19 year mean	126	117	105	113	94	128	101	90	102	93	131	120	1320	

TABLE 3.5 Mean monthly temperatures (°C) recorded at D.S.I.R., Palmerston North

	1969	1970	1971	1972	1973	1974	maximum difference
Jan.		19.4	18.6	17.3	18.0	17.7	2.1
Feb.		17.8	19.5	16.7	18.4	22.1	5.4
March		18.0	16.7	17.5	16.7	15.5	2.5
April		14.7	14.9	14.1	14.4	15.1	1.0
May		10.2	12.4	10.5	12.3	12.0	2.2
June		9.2	11.4	6.8	9.5	9.2	4.6
July	6.9	9.1	8.5	9.0	7.8		2.2
Aug.	9.3	9.6	10.5	8.1	9.9		2.4
Sept.	12.2	11.5	11.0	11.5	11.9		1.2
Oct.	11.3	13.3	12.5	13.1	13.0		2.0
Nov.	15.3	14.8	14.7	16.1	15.3		1.4
Dec.	18.1	16.5	16.9	15.1	17.7		2.6

TABLE 3.6 Annual rainfall and *Lymnaea* population density

★ cattle excluded ☆ sheep excluded

	Year	1969-70	1970-71	1971-72	1972-73	1973-74
	Pohangina Domain:					
	Rainfall deviations from 21 year mean (mm)	- 175	+ 39	- 11	- 134	- 223
L. columella	maximum collection from pond (8 x 5 min searches)	210	29	520	320	422
L. columella	maximum density/m^2 in marsh no. 2	9.88	1.40 ★	28.35	1.20	3.65
L. columella	maximum number in 30 minute collections from marshes 4 and 5		114		44	98
L. tomentosa	maximum density/m^2 in marsh no. 3		9.29 ★	1.55 ☆	0.05	0.05
L. tomentosa	maximum number in 30 minute collections from marsh no. 3 (outside fenced off portion)					140

3.4 DISCUSSION

3.4.1 Sampling methods

(a) Pond

In early visits to the Pohangina habitats various other sampling methods were investigated. Net sweeping was unsatisfactory because the few L. columella recovered from below the surfaces of the pond were usually greatly outnumbered by Physa sp. and Potamopyrgus antipodarum and their recovery from pond weed and mud was extremely tedious. A cylindrical sampling device resembling a core sampler was designed and used to sample a constant area of the bottom because of the surprising lack of L. columella on some sampling dates. The sampler was intended to test whether the snails had migrated from the surface to the bottom. However on no occasion were any recovered from the bottom unless they were also present on or near the surface, and even then very few were recovered from other levels.

(b) Marshes

The distribution of snails in marshes was heavily clumped, with more very small and very large recoveries than would have occurred in randomly distributed populations. An example of this departure from the theoretical Poisson distribution is shown in Fig. 3.12. This aggregation of snails into some parts of the habitat is only to be expected as some locations are more favourable than others, an assumption which was the basis of the study on microhabitats described in Chapter 2.

Visual searches are obviously less efficient at recovering small snails than large specimens, particularly small L. tomentosa whose shell colouring blends with the mud background. Timed searches are also less likely to recover small snails than quadrat sampling methods. To minimise the error, when carrying out timed searches all snails were recovered from a given square metre before moving on to the next area to be searched. Hepplestone (1972) compared results from timed searches with quadrat sampling. He found that 10% of snails in the smaller size classes were missed in the timed searches, but concluded that where a single observer makes thorough searches per unit of time useful and consistent results can be obtained.

The only way of obtaining a true estimate of snail population density over a whole marsh habitat is a method whereby all parts have an equal likelihood of being selected. The random co-ordinate method (Fig. 3.2) used in marsh habitat no. 2 for the first two years will achieve this but in a marsh with clumps of rushes and other tall vegetation it requires two operators. A sufficiently large number of samples must be taken to allow extrapolation from the samples to the habitat as a whole; with only a few samples the number of snails recovered is less likely to reflect the situation over the whole marsh, particularly if population density varies markedly between different parts of the habitat. In long narrow habitats the sampling method first used in marsh no. 3 (Fig. 3.3) also achieves total randomisation and is a great deal more convenient.

Fixed quadrat sampling, as used in habitats 2 and 3 for the last three years of the study, will only give information about the plots that are being repeatedly sampled. On the other hand the information obtained on one sampling date can be compared directly with that on another date, no matter how few plots are involved, rather than by inference as is the case with random methods.

3.4.2 Snail populations

There were no obvious reasons for the considerable differences in snail populations between years (Fig. 3.5; Table 3.6). The maximum collection of L. columella from 8 x 5 minute searches of the pond was 520 in the third year but never exceeded 29 in the second. In marsh habitat no. 2 there were up to 7 x as many L. columella in the first year as in the most productive collection from the second; in the third year the population density on one occasion reached $28.35/m^2$, more than 23 x greater than at any time during the fourth year.

Valid comparisons between L. tomentosa populations in different years were not possible. Random sampling was confined to one year and the change to fixed quadrat sampling coincided with the exclusion of cattle from the experimental area and the virtual disappearance of L. tomentosa.

These differences between snail populations in different years did not appear to be related to temperature or rainfall. Temperatures at Palmerston North varied very little between years (Table 3.5) and

while temperatures at the snail habitats were probably lower because of the altitude there is no reason to believe that they did not reflect the same general pattern as the rest of the Manawatu district.

Since snails much larger than 10 mm in length were found only in the pond (Figs. 3.7; 3.8; 3.9) it seems likely that it was a more favourable habitat for L. columella than any of the marshes. The frequently high population density adds to this hypothesis. L. columella in the marshes, and in some seasons in the pond, evidently did not survive long after sexual maturity had been attained since there were very few mature snails in field collections (Fig. 3.11).

Field specimens of L. columella brought into the laboratory have a high mortality rate, particularly in adult snails. The higher mortality rate of adult snails was confirmed in a laboratory experiment in which the mean shell length of field specimens of L. columella seventy-two hours after transfer to the laboratory was 6.33 mm for living and 8.61 mm for dead snails. An analysis of variance confirmed that the mean shell lengths of living and dead snails were significantly different ($p < .01$). This higher mortality rate in larger snails could account for their failure to survive long after oviposition in habitats which are only marginally suitable for them.

The growth pattern of the 1971-72 pond population seems to indicate that snails will grow to a larger size in the absence of younger specimens. It would be interesting to test in the laboratory whether adults and young snails have any selective inhibitory effect upon one another in dense populations and whether such an effect, if it exists, operates in one or both directions. The inhibition of growth and reproduction in overcrowded colonies is well known (Chapter 1.4.7) but there seem to be no references to selective inhibition of one age group by another although some pulmonate snails are cannibalistic (Purchon, 1968).

The greater proportion of adult snails in L. tomentosa collections from marsh habitat no. 3 indicates that the mortality rate of adults was lower than in marsh populations of L. columella. Conversely the turnover rate of L. columella populations must have been higher, with greater increments of young snails. These observations are supported by laboratory data showing a higher mortality rate in adult L. columella than in L. tomentosa but a much higher reproductive

capacity in L. columella (Chapters 5 and 6).

Since field censuses of both species failed to show sequential development of discrete populations to maturity, direct estimation of the life span of field specimens was not possible. The pond populations of L. columella in 1970-71 and 1971-72 (Fig. 3.5) give some indications that development to maturity may occupy two to three months in summer so there are probably at least three overlapping generations per year. It is not possible to make any direct inferences about the annual number of generations of L. tomentosa (Fig. 3.8) but their life-cycle in the laboratory occupies approximately the same time as that of L. columella.

There were sharp fluctuations in the population densities of L. columella and L. tomentosa with periodic disappearance from habitats which yielded large numbers on other dates. This indicates that some caution is needed when investigating snail habitats in connection with outbreaks of fascioliasis. A scarcity or even an absence of lymnaeids on a given date does not necessarily preclude the possibility that the habitat may act as a focus of Fasciola hepatica infection. On the basis of the evidence presented in this chapter the best time to look for either L. columella or L. tomentosa seems to be between January and April, while negative results may be obtained from known habitats on some occasions in winter and spring. The zero population densities did not occur every year or at the same time each year, and were not clearly linked to temperature or rainfall distribution.

3.4.3 The effects of cattle upon marsh habitats

Although the pugged plots produced many more snails than the control areas, the inference that pugging creates more favourable snail habitats should be made with the following reservations:

1. digging over and tramping a square metre plot may produce different microhabitats from those created by cattle. The great weight and narrow hooves of cattle penetrate more deeply, tearing the roots of plants, covering the surface vegetation with silt and interspersing areas of mud with clumps of vegetation,
2. snails are more easily seen on bare mud surfaces than under vegetation, so the numbers of snails on the control plots may well have been underestimated,

3. if pugging by cattle does significantly increase the suitability of the habitat for snails, excluding them will reduce snail numbers in the habitat as a whole. However this should not prevent differences from appearing between pugged and control plots.

In spite of these reservations the numerical evidence in fabour of pugging is impressive (Tables 3.2a; 3.2b) and there is further circumstantial evidence to support the hypothesis. In the first three years the shape of the population graphs for habitats 1 and 2 is very similar. Thereafter relatively few snails were recovered from the marsh, whose physical characteristics had altered after exclusion of cattle, but there were many more snails on the other side of the fence i.e. on the side still grazed by cattle. An even greater and more immediate drop in snail numbers followed exclusion of cattle from L. tomentosa habitat no. 3, again with normal recoveries from the portion of the habitat still available to cattle. There seems therefore no doubt that cattle alter the physical characteristics of marsh habitats so as to render them more favourable habitats for L. columella and L. tomentosa. The implications of this for the epidemiology of Fasciola hepatica will be examined in Chapter 4.

4. THE EPIDEMIOLOGY OF *FASCIOLA HEPATICA* INFECTIONS IN SHEEP GRAZING A MARSH HABITAT OF *LYMNAEA COLUMELLA*

4.1 INTRODUCTION

Little has been published on the epidemiology of *Fasciola hepatica* infections in New Zealand other than some general accounts (Macfarlane, 1937; Whitten, 1945; Brunsdon, 1967) and one experimental investigation of a *L. tomentosa* habitat on a Hawke's Bay farm (Pullan and Mansfield, 1972). There appear to be no published accounts of the epidemiology of *F. hepatica* infections involving *L. columella* either in New Zealand or in any other part of the world in which it is recognised as an intermediate host of the trematode.

There are special difficulties inherent in an epidemiological investigation of fascioliasis in New Zealand:

1. There are three snail hosts; the epidemiological pattern associated with any one of them would not necessarily apply to infections carried by the others.

2. Acute fascioliasis is almost unknown in New Zealand. In countries such as Britain where acute disease is common, a considerable amount of information on the seasonal pattern of infection can be obtained through examination of the necropsy records of the Ministry of Agriculture and Fisheries Veterinary Investigation Centres or their equivalent. The severity of outbreaks and variations in the rate of infection in different years can also be estimated from these records. Records of deaths due to chronic fascioliasis which may be available to a limited extent in New Zealand provide no indication of the date at which infection was acquired. Surveys estimating the numbers of infected livers going through meat works assist in establishing the relative levels of infection in different years, and whether or not the prevalence is increasing. In a survey of this nature in this country livers were examined over four months, July to October, 1969. Complete randomisation was impossible for logistic reasons, but an overall prevalence of 6% in cattle and 3% in sheep was estimated (Pullan and McNab, 1972).

3. The variation in climate from North Auckland to South Otago means that trials with tracer sheep, the most reliable method of

investigation, need to be repeated over a range of climatic conditions and with different snail hosts.

This chapter contains an account of one epidemiological investigation using tracer sheep on a Manawatu farm where the intermediate host was L. columella.

4.2 METHODS

The L. columella habitats studied were:

1. The pond described in Chapter 3 and illustrated in Plate 2.1.
2. The marsh known as "L. columella habitat no. 2", described in Chapter 3.

An area of 0.15 hectares was fenced off around the pond. A cull ewe known to be excreting Fasciola eggs remained on the plot, as a permanent source of contamination. Pairs of fluke-free weaned lambs or hoggets were introduced at four to six week intervals from 18/11/69 until 3/2/71. After grazing on the experimental area each pair of tracer lambs was grazed on University land which contained no snail habitats. They remained on this fluke-free grazing for at least eight weeks to allow flukes to mature before the sheep were slaughtered.

The second experimental plot, 0.3 hectares in area, was approximately 60% marsh and 40% dry grazing. Two ewes known to be excreting fluke eggs remained in the enclosure. Groups of four fluke-free lambs or hoggetts were introduced in series from 18/11/69 until 3/2/71 when the group size was increased to six. The period for which each group of tracer animals grazed the experimental area varied from sixteen to eighty-eight days; groups were changed more frequently during the transmission period than at other times. Subsequent management of these tracer sheep prior to slaughter was as described for those from the pond habitat.

When sheep were slaughtered the flukes were first removed from the gall bladder and common bile duct. The liver was then cut into 0.5 cm slices to facilitate recovery of flukes from the smaller bile ducts. Since the sheep were on the infected habitat for periods

ranging from sixteen to eighty-eight days the results were standardised by expressing them as uptake of flukes per sheep per day (Fig. 4.1).

Reference has been made in Chapter 3 to the methods used in estimating snail population density in the marsh habitat. During the first two seasons the random sampling method gave a valid estimate of L. columella population density in the habitat as a whole. The fixed quadrat method used in the following three seasons gave a valid comparison between years but almost certainly overestimated the true population density in the rest of the habitat, particularly if pugged quadrats are included in the sample. Pugged habitats represented only ten square metres which were physically very different from the rest of the habitat so they have been excluded from the estimates of snail density during the last three seasons (Fig. 4.1).

Until July 1971 surplus grass growth was controlled by cattle and additional sheep. After this date these were excluded as the grazing was adequately controlled by the infected and tracer sheep. Throughout the investigation snail populations were monitored as described in Chapter 3. Pond snails were examined for infection up to 3/2/71, the date on which tracer sheep were removed from the enclosure. Snails from the marsh were checked for infection until 31/3/72, after which date they were measured and immediately returned to the habitat.

Temperature and rainfall records referred to in this chapter are from the D.S.I.R. in Palmerston North and Pohangina Domain, and are those used in Chapter 3.

4.3 RESULTS

The experimental area could not be visited daily. Because of this seven tracer sheep on the marsh habitat which died during the five year period were not necropsied. These never numbered more than two in one season.

No Fasciola were recovered from any of the sheep grazing the area around the pond in spite of an overall F. hepatica infection rate of 2.6% in the snails. Only one infected snail was found in 790 examined from the marsh habitat although tracer sheep became infected each year. The pattern of Fasciola uptake is shown in Fig. 4.1.

The numbers of flukes recovered from the sheep in the first two years were extremely small; much larger numbers were acquired in the third, fourth and fifth years (Fig. 4.1; Table 4.1).

In spite of the large difference between the first two and last three years the seasonal pattern of uptake was broadly similar with the majority of the infection being picked up between January and May. On no occasion was there evidence of a "spring" infection associated with cercarial release following overwintering of infection in snails. It is impossible to say with certainty whether infection in a group of tracer sheep was acquired mainly at the beginning or end of their exposure period or accumulated steadily throughout it. For this reason the precise limits of the transmission period cannot be defined but it seems probable that transmission of no more than one or two flukes occurred before January or after July.

No Fasciola could have been picked up before the eighteenth of February, third of February, or fifth of January in the first three years respectively. A single parasite was picked up before the twelfth of January in the fourth year; in the fifth year 0.64 flukes per sheep per day were acquired between 12/12/73 and 20/1/74 but in view of the pattern of the previous years it seems likely that all or most of them were picked up in January. The end of the transmission period was almost as clearly defined as the beginning. A single Fasciola was acquired after 25/7/70, none after 13/7/71 and two after 4/7/72. In the fourth year 0.23 flukes per sheep per day were acquired between the twenty-first of June and the tenth of August, but only one was picked up after the latter date. The investigation was terminated on 28/6/74 so the total uptake of Fasciola in the fifth year has almost certainly been slightly underestimated.

Snail population density in the marsh habitat is discussed in Chapter 3. The pattern of snail population density is shown in Fig. 4.1. Comparisons of the numbers of Fasciola acquired each year and the percentages of mature L. columella ($\geqslant$ 9 mm in shell length) are shown in Table 4.1 and Fig. 4.2. There was no observable relationship between the numbers of snails in the habitat and the numbers of flukes picked up by tracer sheep. However, the years in which the greatest numbers of Fasciola were transmitted, the third, fourth and fifth, were also the years with the greatest percentages of mature L. columella.

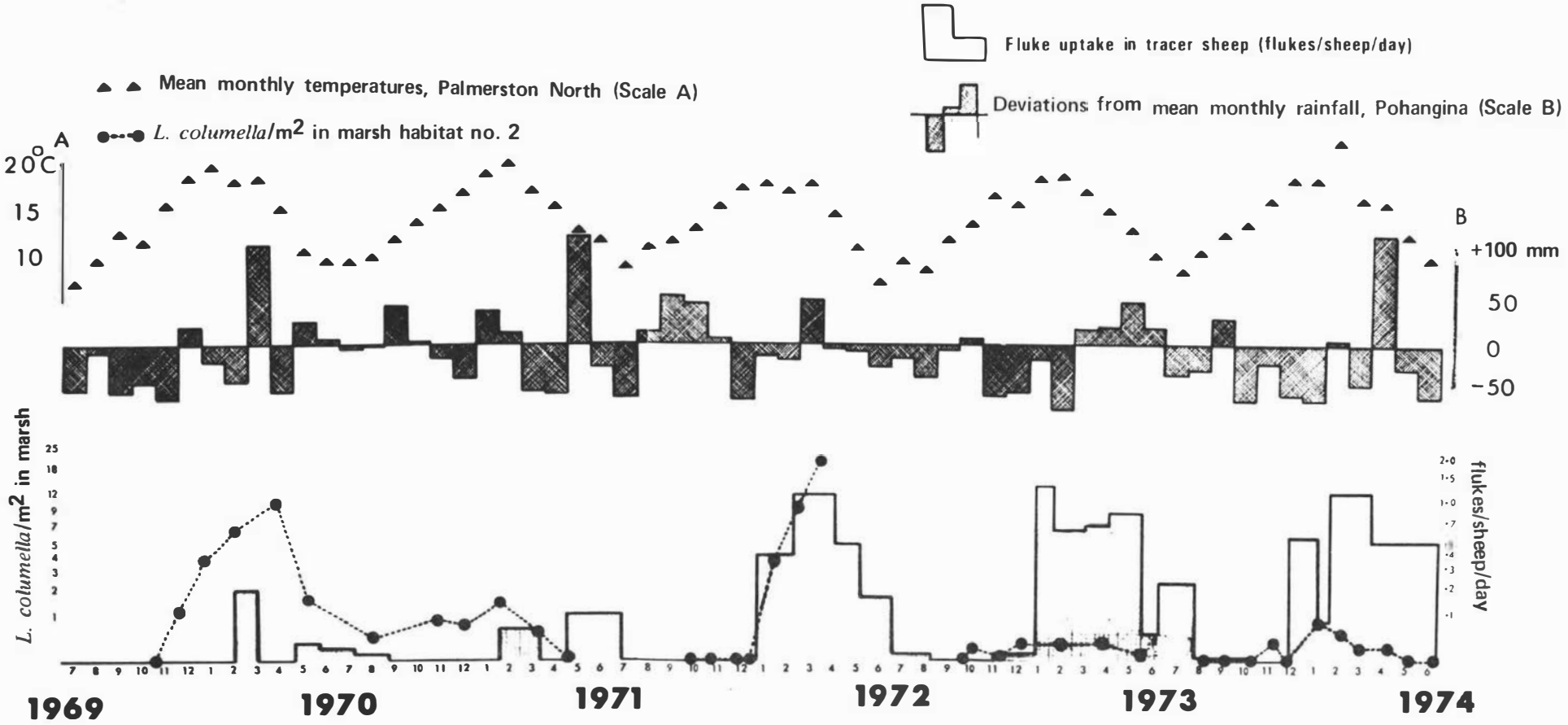
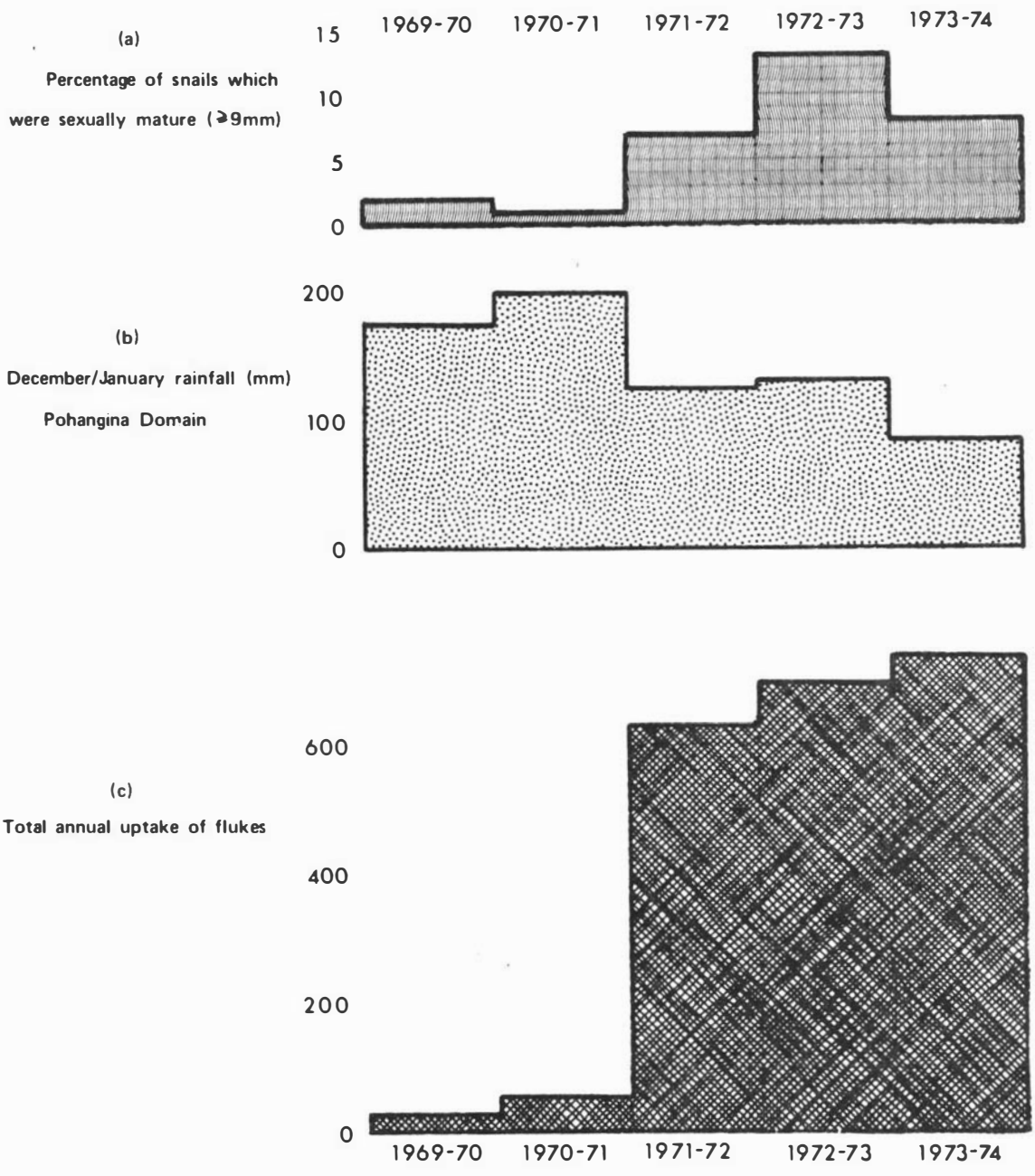


Fig. 4.1 Uptake of F. hepatica in tracer sheep in relation to temperature, rainfall and snail populations.

Fig 4.2 The relationship between annual uptake of flukes, December/January rainfall, and the percentage of *L. columella* which were sexually mature.



There were only small differences in mean monthly temperatures between successive years (Table 3.5). Much wider variations in rainfall occurred, both in total amount and in the pattern of distribution (Table 3.3). The first, fourth and fifth years were much drier than average whilst the second and third deviated little from the twenty one year mean.

A search was made for patterns in rainfall distribution which correlated with the rate of infection in tracer sheep. A number of these are shown in Table 4.2. The only consistent feature found was that those years with the lowest December/January rainfall were those in which Fasciola transmission was greatest (Fig. 4.2).

TABLE 4.1 Percentages of L. columella ≥ 9 mm in shell length in pooled annual collections from the marsh habitat, and the uptake of flukes in tracer sheep

Season	No. of flukes transmitted	Range of flukes in infected sheep	Percentage of mature snails
1969-70	30	1 - 9	2
70-71	58	1 - 39	1
71-72	626	1 - 150	7
72-73	697	1 - 77	13
73-74	738	1 - 124	8

TABLE 4.2 Deviations from a 21 year mean rainfall (mm) in Pohangina Domain

Season	Dec.	Nov-Dec.	Oct. Nov. Dec.	Dec.-Jan.	Jan. Feb. Mar.	Jan.- May	Nov.- May
1969-70	+19	- 39	- 78	- 24	+ 27	+ 1	- 38
70-71	-36	- 50	- 47	+ 1	+ 0	+ 65	+ 15
71-72	-59	- 53	- 9	- 72	+ 26	+ 14	- 39
72-73	-51	-106	- 99	- 67	- 67	+ 1	-105
73-74	-54	- 77	-136	-114	- 97	- 1	- 78

4.4 DISCUSSION

The total lack of transmission of Fasciola from the pond habitat over two seasons, although many infected snails were present, suggests that such habitats are of little importance in the epidemiology of fascioliasis. Similar observations have been made by Pullan (pers. comm.). In the marsh habitat, however, transmission of Fasciola occurred in all of the five years.

There are many uncontrollable variables in an investigation of this nature and two require particular mention. The first concerns the number of Fasciola eggs deposited on the area. It is obviously not possible to maintain a constant level of infection and egg output in animals grazing an infected area. Nor is it possible to estimate the numbers of eggs put out by infected animals over a period of time with any accuracy. The faeces of the contaminator animals were examined for Fasciola eggs at least once a year but quantitative estimates of the numbers of eggs contained in the faeces were not made. Almost certainly the egg output of the infected animals increased in successive years. At the time, because of the impossibility of measuring egg output over periods of time, it was felt that quantitative estimates of eggs per gm faeces would serve no useful purpose. In retrospect, however, it might have been profitable to make periodic counts to provide an indication of the order of change in contamination rate of the habitat with time.

One of the permanent contaminator sheep died two weeks after the end of the experiment; the author was not notified and the sheep was not necropsied. The other infected sheep was killed and found to be harbouring 235 mature F. hepatica indicating a potentially high output of fluke eggs. It is unlikely that either of the sheep carried such a high burden in previous years; they would have been unlikely to survive to the end of the experiment. As sheep do not lose their previous infections as may cattle when subjected to challenge infections (Boray, 1967; Ross, 1967c; 1968) it is to be expected that the permanent animals acquired progressively more Fasciola and excreted progressively larger numbers of eggs. However if the rate of contamination of the habitat had been a major factor in determining the rate of transmission of Fasciola, a step by step linear or exponential increase in transmission would be expected rather than an abrupt change

such as occurred between the second and third years (Fig. 4.1).

The second uncontrollable variable relates to the distribution of Fasciola eggs on the trial area. The rate of contamination of areas which were snail habitat would depend very largely on the grazing behaviour of infected animals. This would be expected to vary within and between seasons according to the amount of grazing available; in wet years the sheep would be less likely to graze the marshy area. Certainly it cannot be assumed that the distribution of faeces would be random or in any set pattern. The distribution of snails within the habitat is also markedly aggregated i.e. non-random.

These factors must affect the transmission of Fasciola and complicate the interpretation of seasonal and annual differences in transmission rate. Theoretically, where investigations of this nature are to continue for several years, it would be desirable to apply to the habitat by hand known numbers of viable Fasciola eggs in a set pattern at regular intervals. The practical difficulties of such an undertaking would be considerable.

Although there were considerable quantitative differences in fluke transmission from the marsh in different years the seasonal pattern of infection was similar. The transmission period was relatively long, from mid-summer to mid-winter. The absence of an overwintering infection which would have resulted in metacercariae on pasture in the spring may be due to the fact that extremely small numbers of snails survived the winter and early spring. A steep decline in snail numbers occurred each year in winter or early spring and it might be expected that infected snails would be more likely to die than uninfected ones. However the possibility remains that overwintering of infection in L. columella could occur in other localities. Overwintering infection in L. tomentosa in Hawke's Bay has been described (Pullan and Mansfield, 1972).

The considerable differences in snail populations in different years were not related to the level of Fasciola uptake. In the first two years in which there were similarly small numbers of flukes in tracer animals, there were 7-10 times as many snails/m^2 in the first year as in the second. When the third, fourth and fifth seasons are compared, there were in the largest collection from untreated (control) quadrats in the third year, 66 times as many snails as in the fourth and 25 times as many as in the fifth. Fluke transmission was high and

similar in the third, fourth and fifth years; in the fourth year this high level of transmission occurred when the mean snail densities on the selected good habitat areas were never more than $0.3/m^2$. It was clear, furthermore, that after cattle were excluded from the area in 1971, the proportion of it suitable for snails decreased; this might have been expected to reduce Fasciola transmission though its actual effects cannot be measured.

The absence of a direct relationship between snail numbers and Fasciola transmission is similar to the situation with L. truncatula in Britain (Kendall, 1949b; Kendall and Oberenshaw, 1963; Ollerenshaw, 1959) and with L. tomentosa in Australia (Boray, 1963a).

The infection rate of the snails in the marsh was evidently very low even in the seasons when relatively high levels of infection of sheep occurred. This observation is comparable with that of Barber (1962) who, working in California, examined 1810 L. columella in late summer and found none infected, whilst almost 100% of cattle grazing the area were infected with F. hepatica. This suggests that the few snails that are infected produce large numbers of cercariae.

In the last three seasons when transmission of Fasciola was greatest, there were considerably larger proportions of mature snails in the samples than in the first two years (Table 4.1; Fig. 4.2). It has been found in Britain that the larger specimens of L. truncatula are mainly responsible for transmitting F. hepatica (Walton, 1918; Ollerenshaw, 1959; 1971b). Similarly, only the largest specimens of Bulinus nasutus and Lymnaea natalensis caillaudi contained cercariae of Schistosoma haematobium and Fasciola gigantica respectively (Cridland, 1957).

The implication that the larger L. columella were primarily involved in F. hepatica transmission in the present experiment could not be verified because of the low infection rate of the snails. However, by analogy with the work with L. truncatula which demonstrated that better fed snails grew faster and produced more cercariae (Kendall, 1949b) it seems probable that larger L. columella are also better hosts for Fasciola. The last three seasons apparently provided better conditions for snail growth and/or survival of mature snails than the first two, but the way in which the seasons were better or worse is not clear.

It might be expected that variations in Fasciola uptake would be reflected in yearly variations in weather conditions, particularly in terms of temperature and rainfall. Although temperatures could not be recorded on the study area, beyond being slightly lower because of altitude, they would be expected to parallel those recorded 40 km away. The differences between years were small and there is nothing to suggest a relationship between these differences and parasite transmission.

Rainfall records from a point at approximately the same altitude and only 5 km away and in the same valley as the trial area would not be expected to differ appreciably from those that would have been obtained on the farm. In Europe the transmission rate of F. hepatica is closely linked with rainfall; the intermediate host there, L. truncatula, is highly adapted to temporary habitats which comprise a larger proportion of grazing areas in wet seasons (Ollerenshaw, 1959; Taylor, 1964; Kobulej, 1968). The relationship with rainfall over the summer is so close that forecasts of the likely incidence of fascioliasis can be made with some confidence (Ollerenshaw and Rowlands, 1959; Ollerenshaw, 1966b; Ross, 1970).

There are a priori reasons for suspecting that the transmission of Fasciola by L. columella and L. tomentosa, both of which inhabit spring fed marshes rather than temporarily wet areas, might not be as closely linked to rainfall. Areas suitable for snails expand comparatively little in seasons that are wetter than usual; furthermore, animals may be less dependent on these wet areas for grazing in wetter years. Conversely spring fed snail habitats contract relatively little in seasons that are drier than usual unless the springs themselves dry up.

Comparison of the observed transmission rate with rainfall over the whole year or subdivisions of it before and during the main period of fluke transmission, showed no indication of a direct relationship between the two. The only consistent finding was an apparently inverse relationship between intensity of transmission and the combined December/January rainfall. This is particularly interesting as Ross (1970) found that the June rainfall i.e. two months before the main transmission period in Northern Ireland, is of critical importance to the level of infection transmitted by L. truncatula: there the relationship is a

direct one whilst here it appears to be inverse.

This could be taken to indicate that in dry seasons the sheep were forced to graze more in the wetter areas. Certainly mid-summer rainfall must influence grass growth at this time. However, it must be pointed out that there was no such inverse relationship between rainfall over the whole transmission period and Fasciola uptake. The observed relationship could be entirely fortuitous and the matter needs further investigation. It should also be borne in mind that the animals were confined to a relatively small area comprising 60% marsh - a situation unlikely to occur on most farms. This must have affected the animal's ability to avoid grazing the marsh to an extent that would not occur under normal farm conditions.

There is another factor of unknown influence, alluded to briefly before, namely the effect of the exclusion of cattle from the area. The upsurge in fluke transmission coincided with the exclusion of cattle after the winter of 1971. However, there seems no reason to postulate any cause and effect relationship unless the cattle selectively and almost exclusively grazed the wetter areas in the first two years, allowing the sheep to remain on the drier areas. This seems extremely unlikely; further studies using Fasciola-free cattle and sheep together would be needed to fully elucidate the point. In fact it appeared that cattle made the area more suitable for snails.

It is clear that the observed variations in Fasciola uptake between years are not to be explained simply in terms of the data recorded. In all probability complex interactions of various factors determined the rate of Fasciola transmission in this experiment and it seems likely that the situation would be no less complex under normal farm conditions. This is an interesting contrast to the situation with L. truncatula in Britain where one factor - rainfall - is of such overriding importance that it can be used to forecast the level of Fasciola transmission. Beyond the possible inverse relationship of December/January rainfall and transmission rate, there are no indications from the results of the experiment described here, that forecasting the incidence of fascioliasis in New Zealand will be possible.

5. REARING SNAILS IN THE LABORATORY

5.1 INTRODUCTION

A major problem confronting workers interested in the relationships between flukes and snails is the determination of optimum laboratory conditions for the snails. While this has been achieved to the satisfaction of researchers in many laboratories throughout the world, the steps along the way have rarely been published.

In this laboratory snails were usually maintained in aquaria with slowly flowing water in conditions which more closely resembled those described by Boray (1963b) than the recommended methods for laboratory culture of Lymnaea truncatula (Taylor and Mozley, 1948; Taylor, 1964). High populations densities could not be maintained for long without high mortality rates, particularly in the case of L. columella which also showed a marked tendency to migrate from the aquaria under unfavourable conditions. At first the tapwater was suspected of being responsible for the periodic population declines and supplies of stream water appeared to give better results for a time but a more systematic approach to the problem was clearly indicated.

Determination of optimum foods, substrates, rates of water exchange and the interactions between these factors would necessitate an enormous experiment with very large numbers of snails, so large as to be impractical. This chapter contains accounts of some smaller experiments to determine the relative values of some simple foods and substrates on growth, reproduction and mortality in L. tomentosa and L. columella.

5.2 EXPERIMENTS

In experiments described in this chapter two kinds of container were used. One was a glass jar whose inside diameter and depth were each 8.5 cms; the other was a large glass petri dish of inside diameter 14 cms and 2 cms depth. These are referred to as jars and petri dishes in the subsequent text. Both were covered to prevent snails from escaping.

Jars contained soil from a L. columella habitat spread to a depth of 2 cm. Petri dishes had smaller amounts of soil from the same

source, usually enough to cover one third of the bottom. The soil was baked at 45°C for twelve hours to eliminate contamination by other snails and invertebrates such as ostracods and water beetles. In some experiments water was drawn from a cattle trough with a rich algal growth. This was used fresh, or after incubation at 45°C for 12 hours. Water from this source is referred to as "fresh algal soup" or "cooked algal soup". Otherwise tapwater or de-ionised water was used.

Food supplements which were examined included:

1 wheat germ(1)
2 fish food(2)
3 lettuce, boiled for two minutes and cooled
4 wheat germ as in 1, but boiled for two minutes before use

The above foods are described as wheat germ, fish food, lettuce, or cooked wheat germ; use of these names implies any treatment referred to above.

All experiments were set out in the laboratory as complete random blocks. Snails were allotted to treatments and replicates with the aid of a table of random numbers. Except for experiment 5.3 which was conducted at 22°C, all experiments were carried out at room temperatures.

EXPERIMENT 5.1 Competition between L. tomentosa and L. columella

Method

Experimental conditions were similar for all snails which were field specimens brought into the laboratory two days earlier. Soil, Lemna minor (duckweed) and tapwater were added to ten jars and five petri dishes one month before the experiment began to allow algal and higher plant growth in the containers. The snails were given wheat germ and fish food twice weekly, care being taken to avoid excess food which would have fouled the water. Water was replaced only on one occasion, on the eighteenth day, and was otherwise replenished at approximately weekly intervals with de-ionised water to prevent increasing salinity due to evaporation.

(1) Betta B Wheat germ (Sanitarium Health Food Co.)
(2) Dandy goldfish food (Dandy Pet Products, Auckland)

One L. tomentosa and one L. columella were put in each container at the beginning of the experiment which ran for ninety six days at room temperatures varying from 15 to 28°C. Shell lengths of L. tomentosa ranged from 5.0 to 7.7 mm and of L. columella between 9.0 and 10.8 mm at the beginning of the experiment. Previous experience had shown that too frequent disturbance of jars with soil and growing plants destroyed their biotic equilibrium, killing the plants and rendering the water so turbid that the jars were no longer suitable for rearing L. tomentosa or L. columella. For this reason the first thorough search of the containers was made on day eighteen and, thereafter, at approximately weekly intervals, when a record was made of:

1. the presence or absence of the original snails,
2. whether or not egg masses were visible,
3. the presence of unidentifiable juvenile snails, and
4. the presence of young snails identifiable as L. tomentosa or L. columella.

Results (Appendix 5.1)

Mortality

Some snails which died could not be found among the mud and weeds at the bottom of the jars, but in a few instances live snails were also difficult to find.

L. columella had a high initial mortality rate when transferred to the laboratory. All five in the petri dishes had died by the time of the first thorough examination on day eighteen; three of the ten L. columella in the jars had died, five could not be found and only two were seen to be alive. This was the situation until the third examination on day thirty-five, when one of the two live L. columella had died and the other was not visible. Since it was never found later but was replaced by developing juveniles it may be assumed that none of the field specimens of L. columella survived as long as thirty five days, and most died soon after transfer to the laboratory.

All L. tomentosa were alive on day eighteen (one was not seen but proved to be alive later), and the first death was recorded on day twenty-six in a jar whose water had become cloudy and rust-coloured.

One other L. tomentosa disappeared from a jar at this time and was not re-sighted, so it was considered dead also. These were the only mortalities observed in L. tomentosa until the sixth examination on day seventy, when one snail in a petri dish had died and one other could not be found in a jar and did not reappear. Nevertheless eleven of the original fifteen L. tomentosa were still alive on day seventy while all fifteen L. columella had died by the thirty fifth day of the experiment.

Reproduction

In spite of their earlier and heavier mortality, reproductive rates of L. columella were much higher than those of L. tomentosa. There were juvenile L. columella in all of the jars and two of the petri dishes, but L. tomentosa offspring were seen in only one jar and two petri dishes. The jar in which young L. tomentosa were found was the one with the lowest population density, a total of six snails (Appendix 5.1). Immature L. columella were present in three jars and two dishes at least seventy eight days after the death of the parent snails and without the appearance of an intervening adult generation. This could have been due to unintentional transfer of eggs or very small snails from one container to another, although it seems unlikely because each jar and dish had its own wooden spatula which was used to manipulate snails only in that container. The slow growth rate described below seems a more probable explanation.

Growth

The growth rate of all snails was unusally slow. Only in one jar did second generation L. columella reach 9 mm in length, the normal minimum length associated with maturity (Chapter 6). None of the new generation of L. tomentosa reached 5 mm in length, the equivalent size for this species.

EXPERIMENT 5.2 Effects of water and food supplements upon the mortality, growth and ovipostion of L. columella

Method

Soil and jars resembled those of the previous experiment.

Three feeding regimes and three types of water were tested as follows:

A fresh algal soup
B cooked algal soup
C tapwater

1 fish food
2 wheat germ
3 no supplementary food

The experimental design, using twenty seven jars, was three replicates of a complete random block of the form

	A	B	C
1	A1	B1	C1
2	A2	B2	C2
3	A3	B3	C3

Each jar contained a single laboratory reared L. columella. Their shell lengths were more variable than was desirable, ranging from 2.5 to 9.3 mm, because there were not enough snails available within a narrower range of sizes. The duration of the experiment was twenty three days. Observations were made on the mortality, growth and oviposition of the snails during the experiment.

Results

Mortality

Eight of the twenty seven L. columella died during the course of the experiment. These numbers were too small to be a reliable guide to the effects on mortality of the factors tested, but unfed snails and those maintained in tapwater seemed least likely to survive (Table 5.1).

Oviposition

The number of eggs and capsules produced during experiment

5.2 are shown in Table 5.1. Since eight of the twenty-seven snails died during the experiment and not all of the remainder were sexually mature, no statistical analysis was attempted. Fish food or wheat germ clearly stimulated egg production, but the apparent advantage gained from maintenance in fresh algal soup rather than cooked algal soup or tapwater should be regarded with caution because of the variability in size of snails at the beginning of the experiment.

TABLE 5.1 Summary of results of experiment 5.2 (shell lengths of *L. columella* expressed in mm)

		A Fresh Algal soup	B Cooked Algal soup	C Tapwater	Totals
1. fish food	died	0	0	1	1
	mean length at start (mm)	4.93	4.83	4.90	4.89
	mean length at end (mm)	10.53	9.30	7.50	9.11
	mean gain in shell length (mm)	5.60	4.47	2.60	4.22
	eggs	559	350	201	1,110
	capsules	37	20	13	70
	eggs/capsule	15.11	17.50	15.46	15.86
2. wheat germ	died	0	1	1	2
	mean length at start	6.80	5.70	6.95	6.48
	mean length at end	11.40	9.80	9.95	10.38
	mean gain in shell length	4.60	4.10	3.00	3.90
	eggs	944	150	264	1,358
	capsules	58	9	21	88
	eggs/capsule	16.28	16.67	12.57	15.43
3. no supplement	died	1	2	2	5
	mean length at start	5.75	9.30	7.60	7.55
	mean length at end	6.00	9.40	7.70	7.70
	mean gain in shell length	0.25	0.10	0.10	0.15
	eggs	20	30	0	50
	capsules	2	4	0	6
	eggs/capsule	10.00	7.50	0	8.33
4. TOTALS	died	1	3	5	8
	mean length at start	5.83	6.61	6.48	6.31
	mean length at end	9.31	9.50	8.38	9.06
	mean gain in shell length	3.48	2.89	1.90	2.75
	eggs	1,523	530	465	2,518
	capsules	97	33	34	164
	eggs/capsule	15.70	16.06	13.68	15.35

Growth

There were unequal numbers of survivors in the various treatment groups in experiment 5.2, so the replicates were pooled to give a mean growth rate for each treatment. A two factor analysis of variance showed that L. columella fed on fish food or wheat germ grew significantly faster than unfed controls, but there were no significant differences in growth rate attributable to the type of water in which they were maintained (Tables 5.1; 5.2).

TABLE 5.2 Effects of food and water upon mean growth rates (expressed as mm/day x 100) of L. columella during experiment 5.2

KEY A fresh algal soup — 1 fish food
B cooked algal soup — 2 wheat germ
C tapwater — 3 no supplementary food

	A	B	C	TOTALS
1	5.60	4.47	2.60	12.67
2	4.60	4.10	3.00	11.70
3	0.25	0.10	0.10	0.45
TOTALS	10.45	8.67	5.70	

Source of variation	d.f.	S.S.	M.S.	F
water	2	3.84	1.920	3.657
food	2	30.76	15.380	29.295 **
error	4	2.10	.525	
total	8	36.80		

EXPERIMENT 5.3 Comparison of the effects of water and food supplements upon the growth and mortality of *L. tomentosa* and *L. columella*

Method

Three types of water and three feeding regimes were employed using the same design as in experiment 5.2. There were five replicates with nine jars in each. One replicate had three snails of each species per jar; two others had six *L. tomentosa* and the remaining two had six *L. columella* in each jar. All snails were laboratory reared. They measured from 0.9 to 2 mm (*L. columella*) and from 0.6 to 1.5 mm (*L. tomentosa*). For subsequent calculations of increases in shell length the starting points were assumed to be 1.5 and 1 mm respectively, since identification of individual snails was not possible.

The experiment ran for thirty four days in a permanently lit thermostatically controlled laboratory at 22°C. Observations were made on the relative growth rates of *L. tomentosa* and *L. columella* and on the effects of food and water on the growth rates of both species. In order to overcome the problem of unequal numbers of snails in the jars at the end of the experiment a single measure, the mean growth rate was used for each jar.

Results

Mortality

Deaths in experiments 5.2 and 5.3 whose treatments were similar, were pooled to give sufficient data for analysis. Unfed snails had a higher mortality rate than those given fish food or wheat germ (Table 5.3).

TABLE 5.3 Deaths of both species in experiments 5.2 and 5.3, pooled samples

	fresh algal soup	cooked algal soup	tapwater	TOTALS
fish food	7	9	9	25
wheat germ	6	12	10	28
no food	14	18	14	46
TOTALS	27	39	33	

Water $\chi^2_2 = 1.84$

Food $\chi^2_2 = 7.04$ *

Interaction $\chi^2_4 = 0.32$

Growth

A striking feature of the growth rates of both species was the variability of results within and between treatments and replicates. Nevertheless it was clear that L. columella was the more rapidly growing snail and its overall mean growth rate was approximately $1\frac{1}{2}$ x that of L. tomentosa (Table 5.4). This does not necessarily imply earlier attainment of maturity since L. columella is a larger snail. Some of the recently hatched (about one week old) snails of both species in experiment 5.3 were sexually mature in one month at 22°C.

TABLE 5.4 Mean increases in shell length (mm/day x 100)

Experiment 5.3 (22°C)

Replicates	1	2	3	4	5	MEAN
L. tomentosa	4.80		6.88		6.60	6.09
L. columella	11.13	11.20		7.53		9.95

Experiment 5.5 (room temperature)

Replicates	1	2	3	MEAN
L. tomentosa	10.77	12.33	14.16	12.42
L. columella	21.80	15.43	20.32	19.18

The beneficial effects of supplementary feeding on L. columella growth rates, already noted in experiment 5.2, were confirmed in experiment 5.3, but neither fish food nor wheat germ significiantly increased the growth rate of L. tomentosa in this experiment (Tables 5.5; 5.6). Neither species grew well in fresh algal soup. L. tomentosa grew best in tapwater and cooked algal soup seemed equally suitable for L. columella.

TABLE 5.5 Effects of food and water upon mean growth rates (expressed as mm/day x 100) of L. columella during experiment 5.3

KEY A fresh algal soup 1 fish food
B cooked algal soup 2 wheat germ
C tapwater 3 no supplementary food

	A	B	C	TOTALS
1	6.96	18.73	11.91	103.12
	7.20	17.67	16.50	
	2.06	9.42	12.67	
	16.22	45.82	41.08	
2	10.39	14.61	9.22	109.73
	5.61	17.33	17.33	
	7.11	12.74	15.39	
	23.11	44.68	41.94	
3	2.94	9.26	16.18	55.92
	5.07	10.00	4.11	
	0.53	4.41	3.42	
	8.54	23.67	23.71	
TOTALS	47.87	114.17	106.73	

Source of variation	d.f.	S.S.	M.S.	F
food	2	191.37	95.69	8.99**
water	2	293.16	146.58	13.78***
interaction	4	18.29	4.57	
error	18	191.60	10.64	
total	26	773.93		

TABLE 5.6 Effects of food and water upon mean growth rates (expressed as mm/day x 100) of *L. tomentosa* during experiment 5.3

KEY
A fresh algal soup
B cooked algal soup
C tapwater
1 fish food
2 wheat germ
3 no supplementary food

	A	B	C	TOTALS
1	2.79 6.17 6.48	7.65 9.33 4.97	7.54(1) 11.50 6.90	63.33
	15.55	21.95	25.94	
2	4.12 5.17 4.83	2.35 3.60 8.56	4.71 9.89 10.92	54.15
	14.12	14.51	25.52	
3	1.86 5.00 4.94	5.29 5.44 5.52	9.41 5.78 6.83	50.07
	11.80	16.25	22.02	
TOTALS	41.36	52.71	73.48	

Source of variation	d.f.	S.S.	M.S.	F
food	2	6.50	3.25	
water	2	49.48	24.74	5.96*
interaction	4	7.93	1.98	
error	18	74.65	4.15	
total	26	162.06		

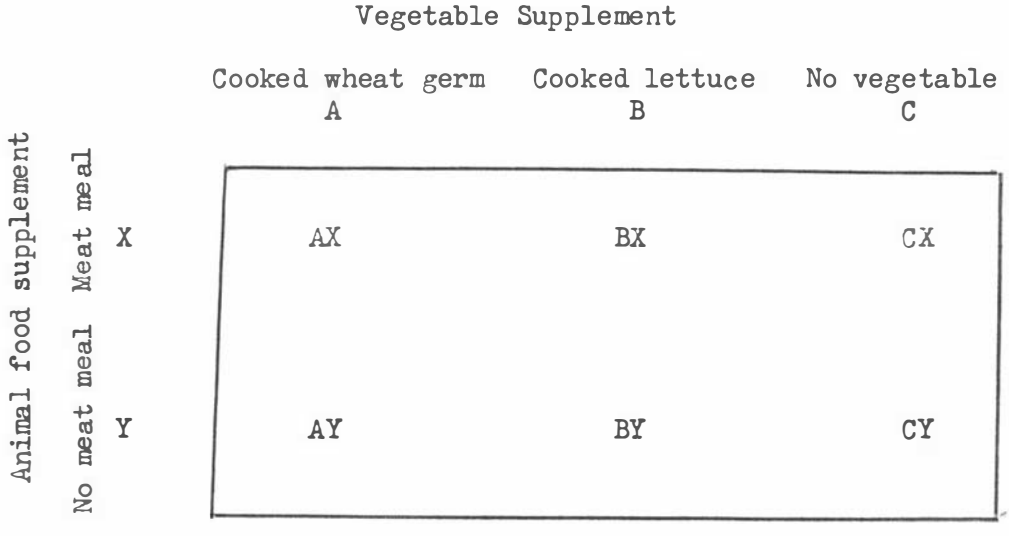
(1) Value interpolated by the method recommended by Sokal and Rohlf (1969a p.338).

EXPERIMENT 5.4 Effects of food supplements upon the growth and mortality of *L. tomentosa*

Method

L. tomentosa collected from a marsh habitat the previous day were allotted in groups of three to petri dishes, each of which contained a little soil and dried grass which had been incubated for 24 hours at 50°C. Feeding and water changes were carried out at one to two day intervals throughout the experiment which ran for 25 days. All snails were at least 5.0 mm long at the start.

The experiment was designed to test the effects of cooked wheat germ, lettuce and meat meal. There were three replicates of the form:



The criteria used in assessing the effects of treatments were: the numbers of egg capsules produced, the numbers of young snails in dishes at the end, and the numbers of the original snails that died. Egg capsules were allowed to remain in the dishes so that the number of young snails in each treatment could be assessed. Due to the ease with which *L. tomentosa* capsules become detached from their substratum a few may have been lost before their final count. However there is no reason to believe that such losses, if they occurred, were other than random so that they might have contributed to the experimental error but are unlikely to have biassed the results in favour of any particular treatment.

Results

Mortality

Ten of the fifty four L. tomentosa in experiment 5.4 died before the end of the experiment. Five of these were in the group receiving no food (Table 5.7). Although these numbers were too small to assess their statistical significance the tendency for unfed snails to have a higher mortality, as observed in experiments 5.2 and 5.3 was continued.

TABLE 5.7 Deaths of L. tomentosa during experiment 5.4

KEY A cooked wheat germ
B cooked lettuce
C no vegetable supplement
X meat meal
Y no meat meal

Treatment	AX	AY	BX	BY	CX	CY
Deaths	1	2	0	0	2	5

Reproduction

Since the egg capsules were not removed from the place where they were deposited it was often difficult to be certain how many eggs some of them contained. The criterion for reproductive efficiency was therefore taken to be capsule production with no account being taken of total egg production. The effects of the treatments on capsule production were assessed by means of a two factor analysis of variance (Table 5.8).

L. tomentosa produced more capsules when given cooked wheat germ or lettuce than when neither food was supplied. The addition of meat meal to the diet did not significantly improve capsule production.

The numbers of young snails in dishes at the end of the experiment should theoretically resemble a Poisson rather than a normal distribution. Accordingly the analysis of variance was carried out after conversion of the raw data to $\sqrt{X+1}$, where X = the number of snails in a dish. Because of the negative results and consequent lack of variance in the dishes receiving no vegetable

supplement this group was omitted from the analysis (Table 5.9).

Snails in dishes supplied with cooked wheat germ or lettuce were apparently more likely to produce live offspring than those receiving no vegetable supplement, but the significance of this result could not be tested statistically for the reasons mentioned above. Inspection of the data in Table 5.9 suggests that cooked wheat germ and meat meal were less suitable for the production and survival of young snails than cooked lettuce. This result is in accord with subjective impressions gained from non-experimental situations in the laboratory, but in this experiment it did not reach the .05 level of statistical significance (Table 5.9).

TABLE 5.8 The effects of meat and vegetable supplements on the production of egg capsules by L. tomentosa in experiment 5.4

	meat meal	no meat meal	X
cooked	7	5	
wheat	20	11	75
germ	14	18	
	41	34	
cooked	9	4	
lettuce	6	18	74
	19	18	
	34	40	
no	2	1	
vegetable	3	2	15
supplement	6	1	
	11	4	
X	86	78	

Source of variation	d.f.	S.S.	MS	F
vegetable	2	393.45	196.725	5.87*
meat meal	1	3.56	3.56	
interaction	2	18.75	9.375	
error	12	402.02	33.502	
total	17	817.18		

TABLE 5.9 The effects of meat and vegetable supplements on the number of young snails produced by L. tomentosa in experiment 5.4

KEY X = no. of young snails per dish

	meat meal		no meat meal		Totals	
	X	$\sqrt{X+1}$	X	$\sqrt{X+1}$	X	$\sqrt{X+1}$
cooked wheat germ	0	1.00	0	1.00		
	2	1.73	12	3.61	25	12.34
	8	3.00	3	2.00		
	10	5.73	15	6.61		
cooked lettuce	1	1.41	6	2.65		
	1	1.41	14	3.87	89	19.40
	3	2.00	64	8.06		
	5	4.82	84	14.58		
no vegetable supplement	0		0			
	0		0		0	
	0		0			
	0		0			
Totals	15		99		114	

Analysis of the above data after conversion to $\sqrt{X+1}$, and leaving out groups receiving no vegetable supplement

Source of variation	d.f.	S.S.	M.S.	F
vegetable	1	4.1536	4.1536	1.521 (n.s)
meat meal	1	9.4341	9.4341	3.454 (n.s)
interaction	1	6.5713	6.5713	2.406 (n.s)
error	8	21.8529	2.7316	
total	11	42.0119		

EXPERIMENT 5.5 Effects of food supplements upon growth, mortality and reproduction of *L. tomentosa* and *L. columella*

Method

Factors examined in this experiment were:

A lettuce

B no vegetable supplement

X cooked meat (approximately 2 g cooked mutton per dish)

Y no animal protein supplement

There were three replicates with one recently hatched snail of each species per petri dish. Feeding and water changes were carried out twice weekly for the first fifty-one days, but the dishes were left undisturbed between days fifty-one and sixty-four when numbers of each species were finally assessed. Shell lengths of the original snails were recorded on days 0, 11, 25, 33, 43 and 51 and growth was expressed as mm/day x 100, since only four of the original twenty-four snails, two of each species, were alive at the end. The presence or absence of second generation snails was also noted.

Mortality rates of the two species, and the effects of treatments on mortality, were assessed by noting the last date on which each snail was seen to be alive. Survivors to the end of the experiment were credited with fifty-one days. The effects of treatments and species were calculated in a two factor analysis of variance (Table 5.10).

Growth rates in different treatments were compared in a two factor analysis of variance (Table 5.11). One snail of each species disappeared in the early stages of the experiment before an adequate estimate of their growth rates could be established, so one result was removed from each of the other treatments with the aid of a table of random numbers, giving two growth rates per treatment for the analysis.

Results

Mortality

Although *L. tomentosa* survived far longer than *L. columella*

the difference in means was not significant at the .05 level of probability (Table 5.10), nor did any of the treatments influence survival times.

TABLE 5.10 Survival times (days) of immature L. tomentosa and L. columella in experiment 5.5

KEY A lettuce X meat
B no lettuce Y no meat

Species	Treatments AX	AY	BX	BY	Totals
L. columella	51	28	51	25	
	20	17	7	43	331
	18	19	20	32	
	89	64	78	100	
L. tomentosa	51	35	7	47	
	34	41	51	43	457
	30	43	30	45	
	115	119	88	135	
Totals	204	183	166	235	

Source of variation	d.f.	S.S.	M.S.	F
species	1	661	661	3.305(n.s.)
treatments	3	441	147	
interaction	3	177	59	
error	16	3,200	200	
total	23	4,479		

Growth

Growth rates within treatments were highly variable and no differences were attributable to any of the treatments (Table 5.11). The mean growth rate of L. columella was again approximately $1\frac{1}{2}$ x that of L. tomentosa. Snails of both species reached shell lengths normally indicative of sexual maturity (5.0 mm for L. tomentosa and 9.0 mm for L. columella) in less than twenty-five days, i.e. approximately one month after hatching.

TABLE 5.11 Growth rates of L. columella and L. tomentosa in experiment 5.5 expressed as mm/day x 100. (Random selection of two values to give equal cell sizes)

KEY A lettuce X meat
B no lettuce Y no meat

	L. columella			L. tomentosa		
	A	B	TOTAL	A	B	TOTAL
	19.61	23.53		12.75	10.78	
X	14.50	15.71	73.35	17.50	20.40	61.43
	34.11	39.24		30.25	31.18	
	14.29	21.60		8.86	10.70	
Y	25.00	17.50	78.39	16.28	6.74	42.58
	39.29	39.10		25.14	17.44	
TOTAL	73.40	78.34		55.39	48.62	

Source of variation	d.f.	S.S.	M.S.	
treatments	3	9.76	3.25	
within	4	109.38	27.35	(L. columella)
total	7	119.14		
treatments	3	59.45	19.82	
within	4	92.93	23.23	(L. tomentosa)
total	7	152.38		

Reproduction

No records of oviposition were kept because snails matured at different times giving unequal starting dates but there were large numbers (ca. 50) of young snails in some dishes at the end of the experiment while others had none. All of the young snails appeared to be L. columella; although some were too small to be positively identified there were no recognisable L. tomentosa. Dishes which had not been provided with any supplementary food appeared to be more suitable for the development of young snails (Table 5.12).

TABLE 5.12 Second generation snails at the end of experiment 5.5

KEY A lettuce X meat
B no lettuce Y no meat

\+ many young L. columella in one replicate of the treatment

++ many young L. columella in two replicates, etc.

(no second generation L. tomentosa appeared)

AX	AY	BX	BY
+		+	++

5.3 SUMMARY OF RESULTS

1. Growth rates were highly variable between and within experiments, but when the two species were maintained in identical conditions the mean growth rate of L. columella was consistently about $1\frac{1}{2}$ x that of L. tomentosa (Tables 5.4, 5.11). Both species reached sexual maturity at about the same time, less than one month after hatching in favourable conditions.

2. The reproductive capacity of L. columella as measured by the number of young snails hatched was much greater than that of L. tomentosa (Table 5.12; Appendix 5.1).

3. Mortality rates of mature L. columella were higher than those of mature L. tomentosa (appendix 5.1) but the mean survival times of recently hatched snails of both species were not significantly different (Table 5.10).

4. L. columella given fish food or wheat germ grew faster, laid more egg capsules and eggs, and had a lower mortality rate than unfed controls (Tables 5.1; 5.2; 5.3; 5.5).

5. L. tomentosa given fish food or wheat germ also had a reduced mortality rate but neither fish food, wheat germ, boiled lettuce nor cooked meat improved their growth rates over unfed controls (Tables 5.6; 5.11).

Boiled lettuce and wheat germ increased the output of egg capsules from L. tomentosa, but meat meal did not (Table 5.8).

6. Supplementary foods appeared to increase the mortality rate of recently hatched snails in some circumstances (Table 5.12) but not in others (Table 5.10).

7. Fresh algal soup appeared to stimulate growth and egg production of L. columella during experiment 5.2, but because of the variability in the size of snails the effect on oviposition could not be assessed statistically and the effect on growth rate did not reach the .05 level of statistical significance (Tables 5.1; 5.2). In experiments 5.3 and 5.4 the situation was reversed; cooked algal soup and tapwater produced better growth rates in both species than fresh algal soup (Tables 5.5; 5.6).

5.4 DISCUSSION

Population density of L. columella in natural habitats, as described in Chapter 3, fluctuated more rapidly than was the case with L. tomentosa. Results quoted in this chapter and in Chapter 6 offer some possible explanations:

(i) L. columella produced more eggs than L. tomentosa and a greater proportion of these hatched. In environments favourable to both species the innate capacity for rate of increase of L. columella populations seems therefore to be much greater.

(ii) the mortality rate of mature L. columella was higher, particularly in field specimens brought into the laboratory. L. columella may therefore be more susceptible to adverse environmental changes.

Although adult snails generally responded favourably to supplementary foods with increased fecundity and reduced mortality rates, hatching juveniles did not. The supplementary foods, particularly wheat germ, encouraged bacterial and fungal contamination with formation of surface films on the water. Frequent water replacements were partially successful in keeping the water clean but very young snails had difficulty in breaking through the surface film with their pneumostomes. As a consequence many appeared to suffocate while others left the water and died on the walls of the containers. Partially cooked lettuce was the cleanest and most satisfactory food for very young

snails but even lettuce was best excluded until the snails were two or three millimetres in length.

L. columella was more responsive to the availability of supplementary food with an improved growth rate which could not be demonstrated in L. tomentosa. Without more detailed knowledge of the preferred foods of these species this result should be interpreted with caution; L. columella may be more responsive than L. tomentosa to supplementary feeding, or the supplementary foods offered, fish food and wheat germ in the growth trial, may have been more suitable for L. columella. It should also be remembered that both species had micro-algae growing in their dishes, which are generally regarded as the preferred food of the lymnaeid hosts of Fasciola hepatica (Colton, 1908; Walton, 1918; Boray, 1964a), particularly for recently hatched snails (Barber 1962; Boray, 1963b).

The variable responses to water from different sources should not be surprising; the water from a cattle trough referred to as "fresh algal soup" must have been a highly variable commodity. It may have contained, for example, biotic factors such as vitamins and it certainly contained many contaminants. Ostracods were commonly present and these filter-feeding arthropods appeared to cause great annoyance to snails when they entered their shell apertures and came in contact with their soft parts. An investigation of the relationship between ostracods and the snail hosts of Fasciola hepatica might prove interesting since different snail species are said to respond differently (Lo, 1967).

It might also be useful to repeat some of these, or similar experiments in darkened incubators where the effects of the feed factors would not be disguised or diluted by algal growth in the containers.

Mortality rates of adult L. columella were higher than those of adult L. tomentosa, particularly in animals brought into the laboratory from natural habitats. For example in experiment 5.1 thirteen of the fifteen L. columella died within the first eighteen days and only one survived the ninety-six days duration of the experiment. Of the fifteen L. tomentosa in the same experiment none died within the first eighteen days and five were still alive at the end.

The mortality rates of recently hatched snails in experiment

5.4 did not show significant differences between species. Because mature L. columella have a higher mortality rate and also a greater reproductive capacity than L. tomentosa it follows that their turnover rate in natural and laboratory populations is likely to be more rapid.

6. OVIPOSITION

6.1 INTRODUCTION

The literature relating to factors affecting oviposition in snails has been reviewed in Chapter 1. The experiments described in this chapter compare the fecundity of Lymnaea columella and L. tomentosa and investigate the influence of some environmental factors upon the fecundity of these species.

6.2 METHODS

Snails were maintained in large glass petri dishes 14 cm in diameter and 2 cm deep. Soil from a L. columella habitat was baked at 45°C for twelve hours to eliminate contamination by other invertebrates and a few grams of this soil were added to each dish. The dishes were filled to a depth of 1.5 cm with tapwater which was changed two or three times per week. At each water change the snails were provided with a small amount of a commercial fish food.(1) Egg capsules were removed and eggs were counted, usually at twenty-four hour intervals.

Experiment 6.1

L. tomentosa ranging in shell length from 3.3 to 7.8 mm were collected from a marsh habitat. On the following day, day 0 of the experiment, they were placed singly in petri dishes numbered 1 to 35. The dishes were then allocated to places on a bench with the aid of a table of random numbers. The permanently lit, thermostatically controlled laboratory was maintained at 24°C and egg masses were removed daily.

On day 14 survivors were transferred to another permanently lit, thermostatically controlled laboratory at 12°C. Water in the dishes was replaced from a container in the laboratory to ensure maintenance of a constant temperature. On day 28 the survivors were returned to 24°C for a further thirteen days.

(1) Dandy goldfish food; Dandy Pet Products, Auckland.

Experiment 6.2

L. columella collected from a pond habitat were placed singly in petri dishes. Fourteen snails whose shell lengths ranged from 7.0 to 10.3 mm were maintained at 12°C and twenty-five with shell lengths from 7.4 to 10.2 mm were kept at 24°C.

The experiment was abandoned after seven days because too few snails laid eggs for any worthwhile analyses other than consideration of the minimum shell length compatible with sexual maturity.

Experiment 6.3

Forty L. columella from the same pond were maintained singly in petri dishes at room temperature for thirteen days. Twenty-four of these snails produced egg capsules during this period of acclimatisation to the laboratory and these snails were transferred to the thermostatically controlled laboratory at 24°C. Their shell lengths ranged from 11.3 to 13.9 mm at this time. The shell lengths of survivors were recorded at the end of the experiment thirteen days later.

Experiment 6.4

Thirty L. tomentosa and twenty-three L. columella were maintained at a density of two or three snails per petri dish for several days to ensure that eggs were being laid. They were then transferred to a permanently lit laboratory which was thermostatically controlled at 13°C. Egg capsules were removed and counted at three day intervals.

Experiment 6.5

Twenty-seven L. tomentosa and nine L. columella whose shell lengths were not less than 5 mm and 9 mm respectively, were collected from marsh habitats the day before the experiment began. They were maintained in petri dishes at a density of three snails of one species per dish in a permanently lit laboratory at 22°C for eight days, and then transferred to another laboratory at 10°C for a further eight days.

Experiment 6.6

Four L. tomentosa and three L. columella which had been acclimatised to the laboratory and were all known to produce eggs were maintained singly in petri dishes. They were placed in an unlit incubator at a series of descending and ascending temperatures which were all below ambient. Water changes were made from cooled containers to avoid temporary rises in temperature. Eggs were removed and counted periodically to ascertain the effect of each temperature upon oviposition.

6.3 RESULTS

6.3.1 Comparative fecundity of L. tomentosa and L. columella

Fecundity of L. columella was always much greater than that of L. tomentosa under comparable laboratory conditions. L. columella usually produced more capsules and the capsules always contained many more eggs (Table 6.1).

TABLE 6.1 Fecundity of mature snails under different experimental conditions

KEY (1) one snail per dish (2) two or three snails per dish (3) three snails per dish

Experiment		6.1(1)	6.3(1)	6.4(1)	6.5(2)					6.6(3)			
Temperature		24°C	24°C	13°C	22°C	10°C	2°C	5°C (1st period)	5°C 2nd	7°C	10°C	11°C	14°C
L. tomentosa	capsules/snail/day x 100	72		16	16	8	0	9	0	14	11	9	33
	eggs/snail/day x 100	765		190	187	94	0	94	0	107	33	121	78
	eggs per capsule	10		12	12	11				8			
L. columella	capsules/snail/day x 100		86	40	17	13	4	17	6	9	17	21	33
	eggs/snail/day x 100		2955	817	521	201	115	383	200	237	458	545	611
	eggs per capsule		34	20	31	16				27			

6.3.2 Shell length and fecundity

The smallest egg laying specimen of L. tomentosa measured 5 mm. Laboratory and field specimens of L. columella did not normally produce eggs until their shell length was at least 9 mm.

The snails in experiment 6.1 were divided into three size categories with five snails in each group to compare capsule and egg production. Only snails known to produce eggs were used in the comparisons which were by analyses of variance as shown in Tables 6.2 to 6.5.

TABLE 6.2 Comparison of fecundity of three size groups of snails from experiment 6.1 (L. tomentosa) for the first period of 13 days at 24°C

Capsule production per snail			Egg production per snail		
Group 1 (6.8-7.2mm)	Group 2 (6.4-6.7mm)	Group 3 (5.1-6.1mm)	Group 1 (6.8-7.2mm)	Group 2 (6.4-6.7mm)	Group 3 (5.1-6.1mm)
9	10	10	72	104	79
12	12	10	124	99	87
7	12	10	148	88	120
8	7	9	169	78	73
8	9	8	97	62	91
44	50	47	610	431	450

There were no significant differences in capsule production between the three groups, nor did the greater egg production in group 1 reach the .05 level of significance (Table 6.3) but it did suggest that there were larger numbers of eggs per capsule in this group.

The relationship between shell length and eggs per capsule was tested by a two level nested analysis of variance (Tables 6.4 and 6.5). The greater numbers of eggs per capsule in the largest size group was not significant at the .05 level, but there were very highly significant differences between individual snails within groups. The percentage of variation in eggs per capsule attributable to size groups was only 16% of the total variation, but that due to individual snail differences was 41% of the total. The estimations of variance components were by

the method described by Sokal and Rohlf (1969a, p. 277 et. seq.).

TABLE 6.3 Analysis of variance of differences in egg production between the three size groups in Table 6.1 (*L. tomentosa*)

Source of variation	d.f.	S.S.	M.S.	$F_{2,12}$
Between groups	2	3866.8	1933.4	2.739 (n.s.)
Within groups	12	8470.8	705.9	
Total	14	12337.6		

TABLE 6.4 Differences in Eggs per capsule between the three size groups of *L. tomentosa* in experiment 6.1

	size group 1				
snail size (mm)	7.2	6.8	7.0	7.2	7.0
eggs	72	124	148	169	97
capsules	9	12	7	8	8
eggs/capsule	8.00	10.33	21.14	21.13	
	size group 2				
snail size (mm)	6.6	6.6	6.4	6.4	6.7
eggs	104	99	88	78	62
capsules	10	12	12	7	9
eggs/capsule	10.40	8.25	7.33	11.14	6.89
	size group 3				
snail size (mm)	5.9	5.9	6.1	5.1	6.1
eggs	79	87	120	73	91
capsules	10	10	10	9	8
eggs/capsule	7.90	8.70	12.00	8.11	11.38

TABLE 6.5 Two level nested analysis of variance of results shown in Table 6.4

Source of variation	d.f.	S.S.	M.S.	F.
Size groups	2	714.02	357.01	2.702 (n.s.)
Snails within groups	12	1540.35	128.36	9.732 ***
Within snails	126	1662.10	13.19	
Total	140	3916.47		

The general nature of the relationship between shell length of L. tomentosa and fecundity is shown in Fig. 6.1 and Table 6.6 where shell length is plotted against the number of capsules, eggs, and eggs per capsule for snails in experiment 6.1 over the first period at 24°C. There was a weak positive regression of egg production and its logarithmic and square root transformations on shell length and the logarithm of shell length, but in no case were results significant at the .05 level. None of the transformations gave more significant results than the raw data.

TABLE 6.6 Regression of egg production for the first period of experiment 6.1 on shell length of L. tomentosa

Source of variation	d.f.	S.S.	M.S.	F.
Explained	1	2519.22	2519.22	3.34 (n.s.)
Unexplained	13	9818.38	755.26	
Total	14	12337.60		

There was no clear relationship between shell length of L. columella and fecundity as measured by capsule production, egg production, or capsule size over the range of shell lengths in experiment 6.3 (Fig. 6.2).

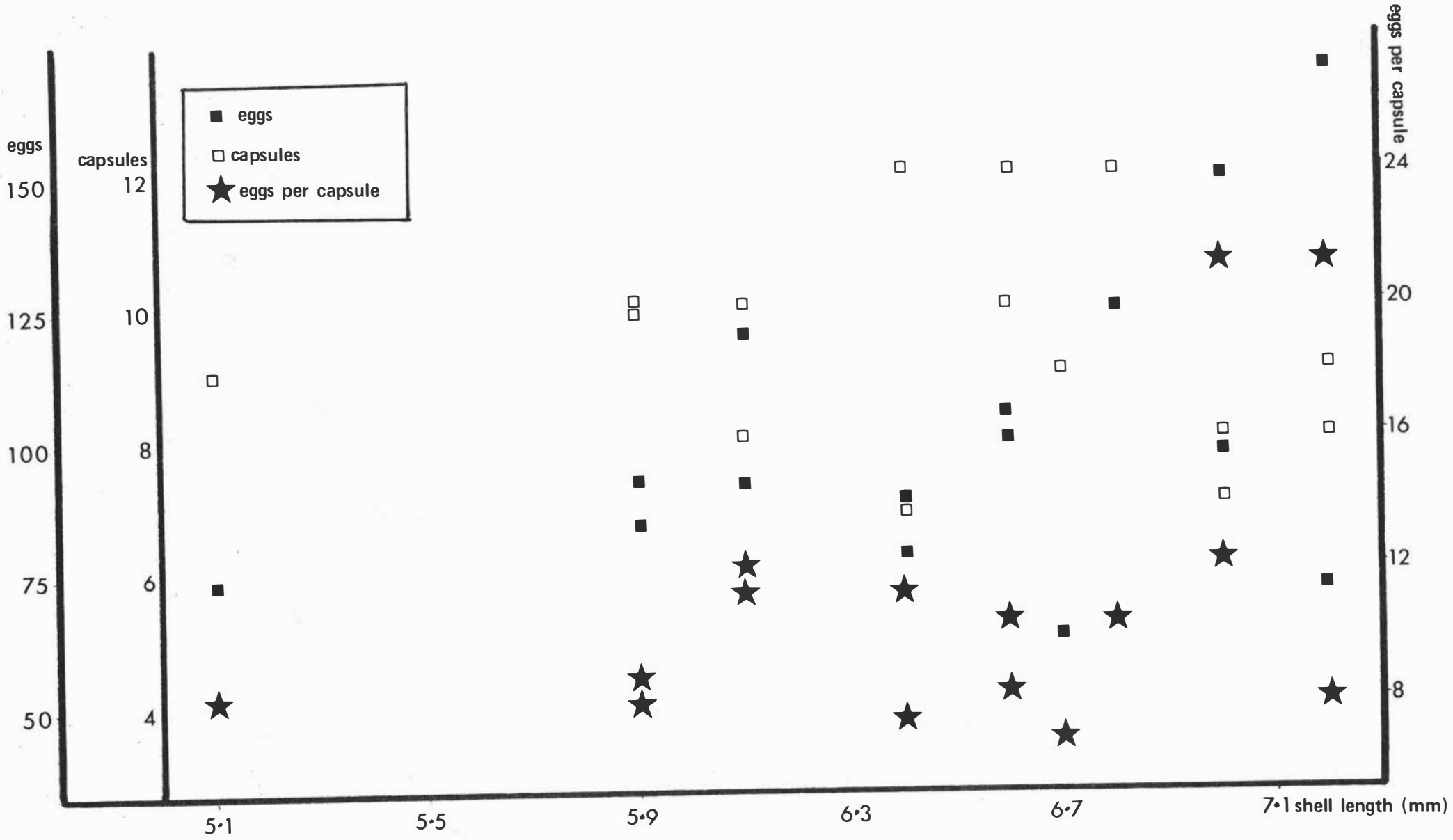


Fig. 6.1 The relationship between shell length and fecundity of *L. tomentosa* during experiment 6.1

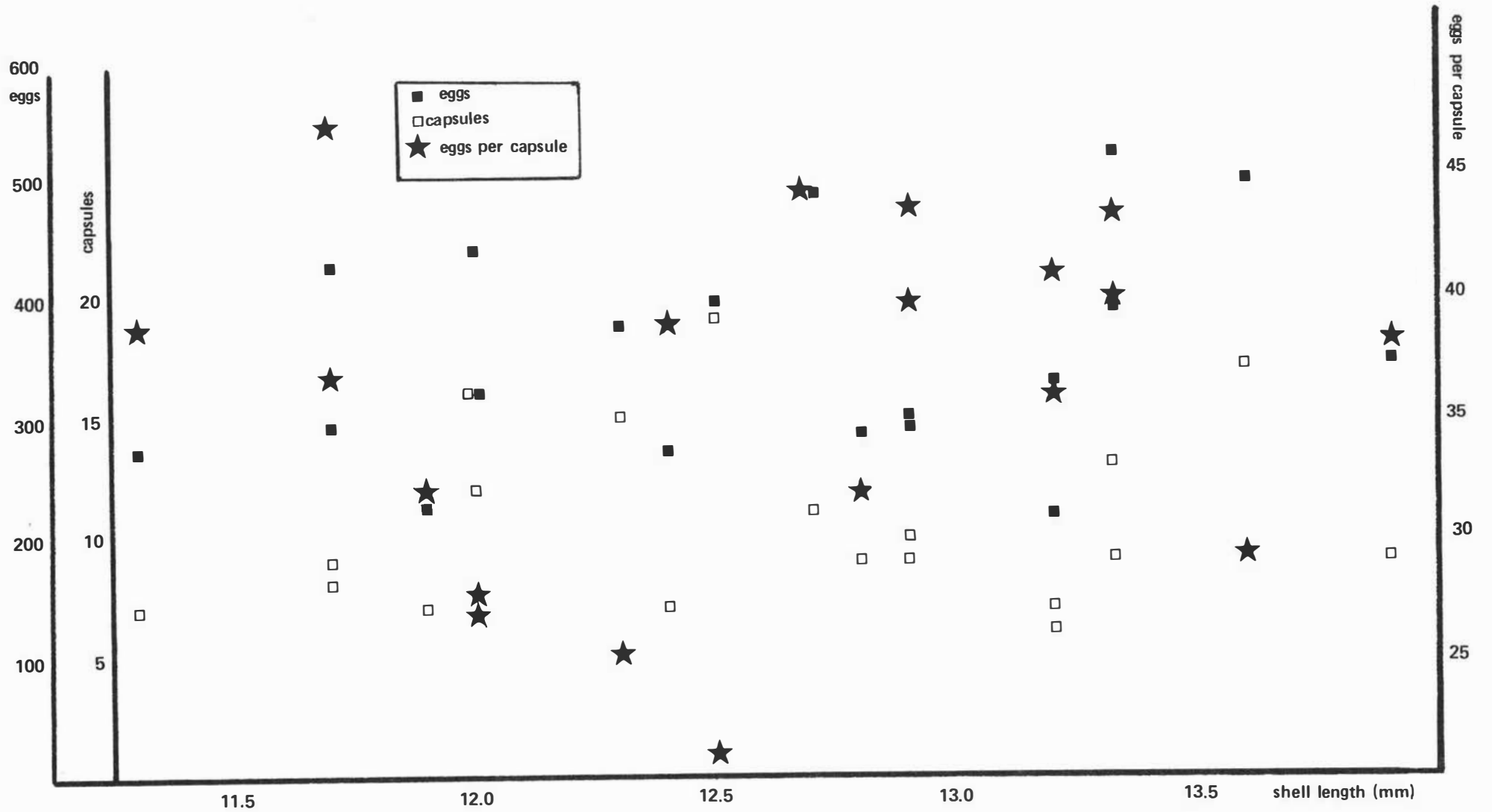


Fig. 6.2 The relationship between shell length and fecundity of *L. columella* during experiment 6.3

6.3.3 Growth rate and fecundity

During the thirteen days of experiment 6.3 the growth of *L. columella* varied from 0.3 mm to 2.7 mm but the increase in shell length was not related to egg production (Fig. 6.3). There was an inverse linear regression of capsule production on growth (Fig. 6.4) but this did not quite reach the .05 level of significance (Table 6.7).

TABLE 6.7 Linear regression of capsule production on the increase in shell length of *L. columella* during experiment 6.3

Source of variation	d.f.	S.S.	M.S.	F.
Explained	1	52.000	52.000	4.23 (n.s.)
Unexplained	17	208.737	12.279	($F_{1,17(.05)}$ = 4.45)
Total	18	260.737		

However, the more rapidly growing snails tended to produce larger egg capsules, and the regression of eggs per capsule on growth was statistically significant (Fig. 6.5, Table 6.8).

TABLE 6.8 Regression of eggs per capsule on increase in shell length of *L. columella* during experiment 6.3

Source of variation	d.f.	S.S.	M.S.	F.
Explained	1	321.63	321.63	8.42 **
Unexplained	17	649.39	38.20	
Total	18	971.03		

6.3.4 Eggs per capsule and the stage of oviposition

(a) Survivors v. snails that died

A number of *L. tomentosa* died before the end of experiment 6.1 and their eggs per capsule were compared with those of snails that survived and laid eggs throughout the duration of the experiment. Only oviposition data from the first period at 24°C were

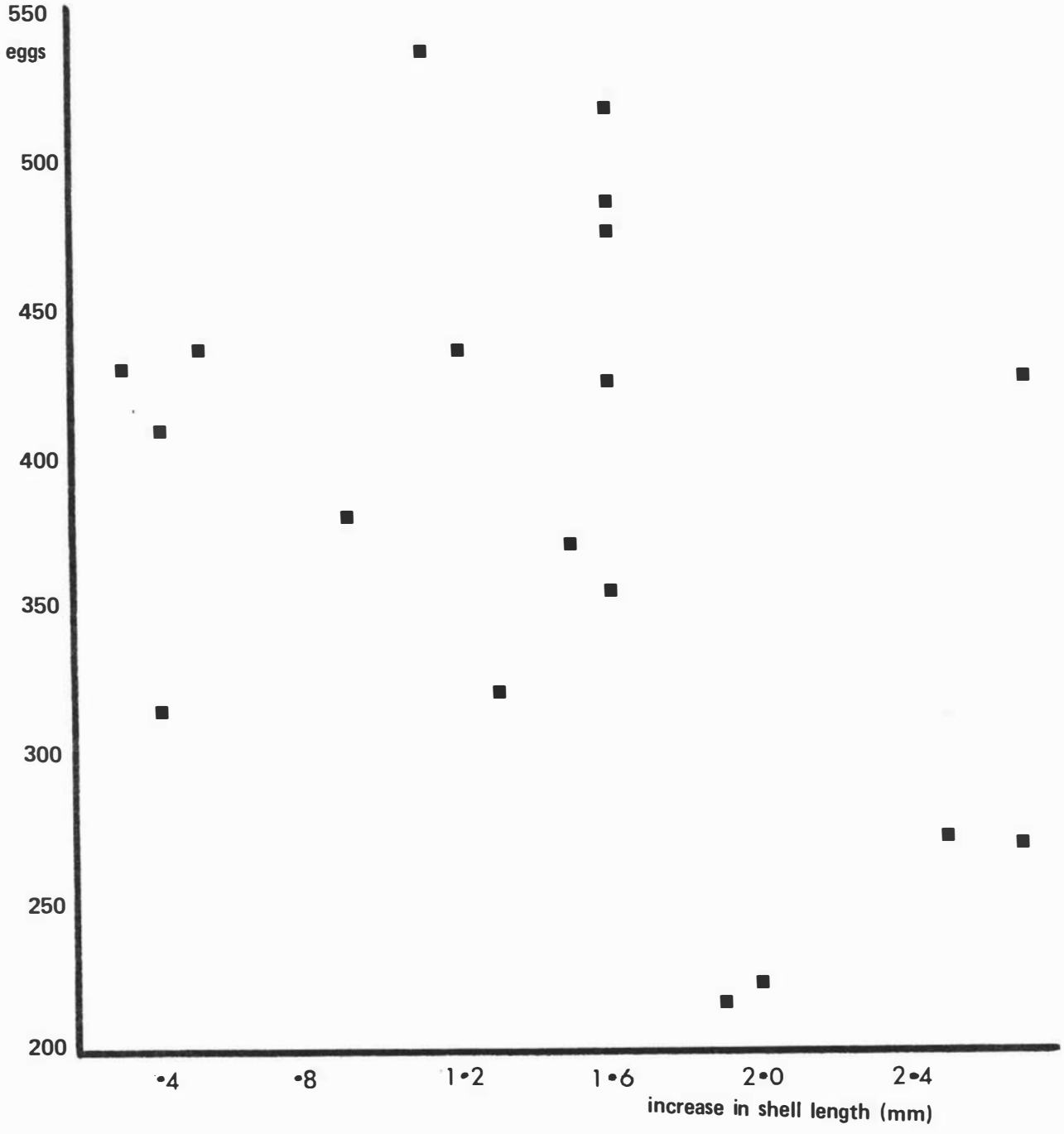


Fig. 6.3 The relationship between egg production by *L. columella* and increases in shell length during experiment 6.3

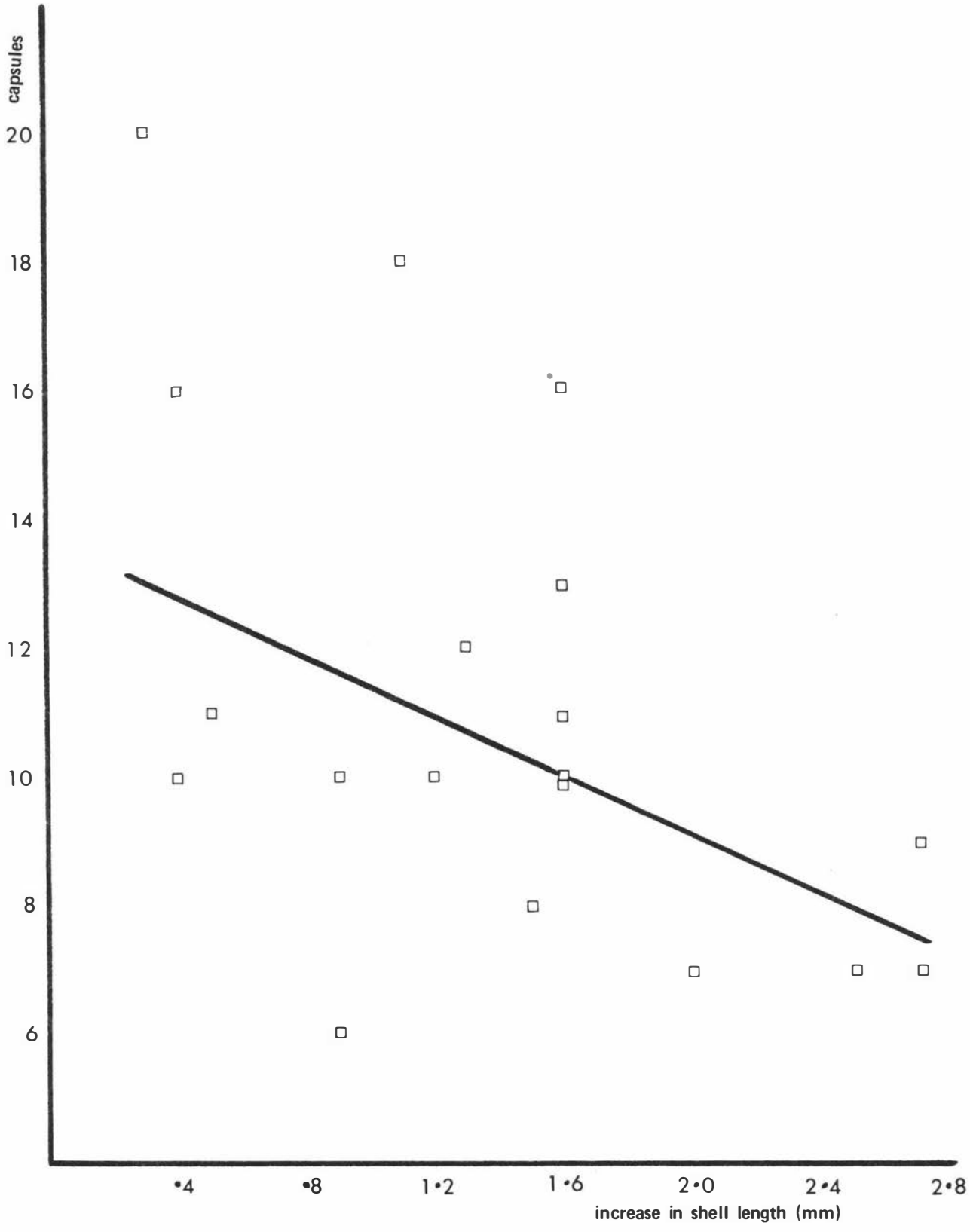


Fig. 6.4 The relationship between capsule production by *L. columella* and increases in shell length during experiment 6.3

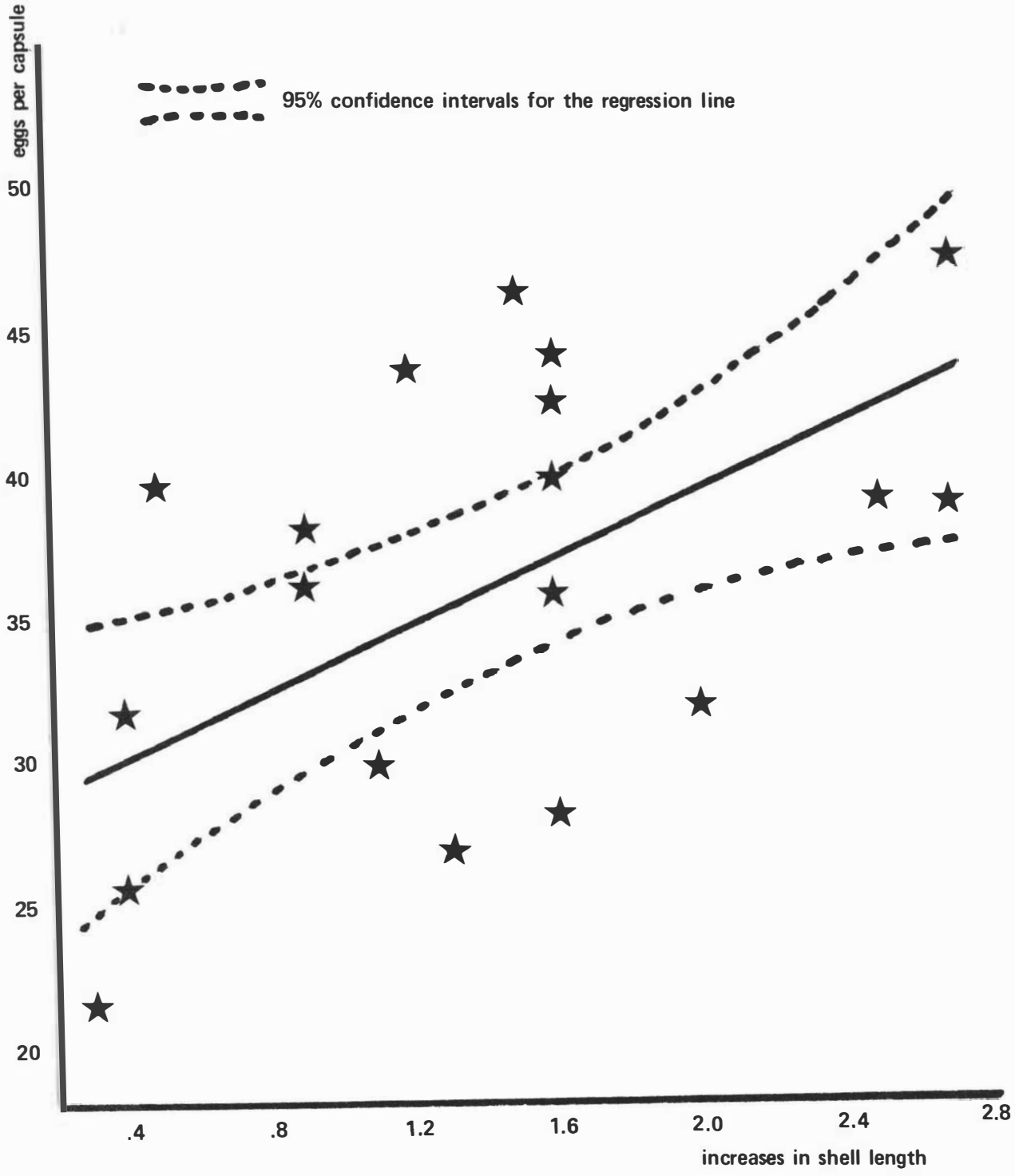


Fig. 6.5 The relationship between eggs per capsule produced by *L. columella* and increases in shell length during experiment 6.3

used because capsules produced during the second period at 24°C contained fewer eggs and results from the period at 12°C were lost. Thus snails that died during the period at 12°C or the second period at 24°C were included in the "snails that died" group, but data for analysis were taken only from the first period at 24°C in all cases.

A nested analysis of variance showed no significant difference in eggs per capsule between the group of snails that died and the group that survived, but once again there were very highly significant ($P < .001$) differences between snails within the groups (Table 6.9).

TABLE 6.9 Differences in eggs per capsule produced by L. tomentosa during the first period at 24°C in experiment 6.1 comparing (a) snails which died at some stage of the experiment with (b) snails which survived

Source of variation	d.f.	S.S.	M.S.	F
Groups	1	270.09	270.09	n.s.
Snails within groups	15	2005.48	133.69	10.26***
Within snails	134	1746.10	13.03	
Total	150	4021.67		

(b) Snails that continued to lay eggs v. snails that ceased oviposition

Of the five L. tomentosa that survived to the end of experiment 6.1 three stopped egg production for five or more consecutive days during the second period at 24°C. Eggs per capsule from the six that produced eggs continuously were compared with the numbers of eggs per capsule from the three that did not. The two-level nested analysis of variance was performed with data from the second period at 24°C.

The snails whose egg production was coming to an end had fewer eggs per capsule than those that continued laying throughout the experiment. The mean numbers of eggs per capsule in the two groups, 3.32 and 9.95 were significantly different in spite of the small numbers in the groups (Table 6.10). There were the usual very highly significant differences between snails within groups.

TABLE 6.10 Differences in eggs per capsule produced by L. tomentosa during the second period at 24 C in experiment 6.1 comparing (a) snails that stopped laying for at least five consecutive days and (b) snails that produced eggs throughout the experiment

Source of variation	d.f.	S.S.	M.S.	F
Groups	1	949.44	949.44	5.78* ($F_{1,7(.05)}$ = 5.59)
Snails within groups	7	1149.44	164.21	6.98*** ($F_{6,60(.001)}$ = 4.37)
Within snails	114	2683.41	23.54	
Total	122			

6.3.5 Effects of water changes upon fecundity

L. tomentosa

In experiment 6.1 the water in the petri dishes was replaced on days zero, three, six, eight, ten and thirteen. Snails were fed on these occasions also but there was always a little food left over at the subsequent water change. Data before day four were excluded from all analyses because some capsules deposited before day three may have been overlooked on the first two days. On and after day three the dishes were emptied daily for removing and counting capsules, but except on days three, six, eight, ten and thirteen the same water was returned.

Capsules, eggs and eggs per capsule were compared in the groups (a) days on which water had been replaced 24 hours earlier, and (b) days on which fresh water had not been added to the dishes for 48 or 72 hours. Results are summarised in Table 6.11. There were no statistically significant differences in egg production, capsule production, or eggs per capsule from L. tomentosa which could be attributed to water changes.

TABLE 6.11 Fecundity of L. tomentosa in experiment 6.1

Capsules		Eggs	
24 hours after water change	>24 hours after water change	24 hours after water change	>24 hours after water change
14	14	142	125
9	10	84	81
18	13	163	145
	13	186	147
	15		190
	14		125
n 4	6	4	6
58	79	575	813

	24 hours after water change	>24 hours after water change
mean daily capsule production	14.50	13.17
mean daily egg production	143.75	135.50
mean number of eggs per capsule	9.91	10.29

L. columella

Rather different results were obtained with L. columella in experiment 6.3. In this experiment water was replaced on days zero, one, four, six, seven, nine and eleven. Data from the first two days were excluded from the analyses because oviposition rates were lower on these days until the snails became acclimatised to the experimental conditions.

The fecundity of L. columella was significantly greater on days following water replacement than on other days, as measured by production of eggs and capsules, but the numbers of eggs per capsule

were unaffected by water changes (Tables 6.12; 6.13; 6.14).

TABLE 6.12 Fecundity of L. columella in experiment 6.3

Capsules		Eggs	
24 hours after water change	>24 hours after water change	24 hours after water change	>24 hours after water change
16	13	596	494
20	11	768	410
24	20	781	697
22	16	672	497
33	13	1159	390
	12		400
n 5	6	5	6
115	85	3976	2888
		24 hours after water change	24 hours after water change
mean daily capsule production		23.00	14.17
mean daily egg production		795.20	481.33
mean number of eggs per capsule		33.70	33.98

TABLE 6.13 Analysis of variance of capsule production by L. columella in experiment 6.3 to test the effect of water changes

Source of variation	d.f.	S.S.	M.S.	F
Water changes	1	213	213.00	8.92*
Days within groups	9	215	23.89	
Total	10	428		

TABLE 6.14 Analysis of variance of egg production by L. columella in experiment 6.3 to test the effect of water changes

Source of variation	d.f.	S.S.	M.S.	F
Water changes	1	268.670	268.670	9.48*
Days within groups	9	255.114	28.346	
Total	10	523.784		

An estimated 59% of the total variation in capsule production is due to differences between treatment groups (water changes). The remainder is attributable to differences between days within treatment groups, these differences being due to differences between snails and random variation, factors which could not be separated. Of the total variation in egg counts 61% is attributable to differences between treatment groups.

The estimations of variance components were by the method described in Sokal and Rohlf (1969a, p. 211).

6.3.6 The relationship between capsule production and egg production

A number of environmental and biological factors appeared to influence the number of eggs per capsule. Thus L. tomentosa near the end of their laying periods had fewer eggs per capsule than those which were still actively producing eggs (Table 6.10). Larger L. tomentosa tended to have more eggs per capsule than smaller snails but this result was not statistically significant (Tables 6.2; 6.3). Rapidly growing L. columella laid more eggs per capsule than snails whose growth was slower (Fig. 6.5; Table 6.8). The number of eggs per capsule also varied at different temperatures (Table 6.1). In all experiments there were marked differences in the mean numbers of eggs per capsule produced by different snails within each treatment.

Correlations between numbers of capsules and numbers of eggs recovered at each collection

In spite of all these factors influencing the number of eggs per capsule there were high correlations between the total number of capsules produced by a group of snails and their total egg production at a given collection (Table 6.15). Correlations were usually higher for data from one experiment; pooled data often produced lower correlation coefficients, presumably because of the mean number of eggs per capsule tended to vary with experimental conditions.

TABLE 6.15 Correlations between total capsule production by groups of snails and their total egg production at each collection date

Experiment		r
6.1 (L. tomentosa)	(a) 1st period at 24°C	0.88
	(b) 2nd period at 24°C	0.77
	(c) pooled data from both periods	0.83
6.4 and 6.5 (L. tomentosa)	Pooled data	0.91
Pooled data from all experiments on L. tomentosa		0.83
6.3 (L. columella) at 24°C		0.98
6.4 and 6.5 (L. columella)	Pooled data	0.97
Pooled data from all experiments on L. columella		0.84

Correlations between numbers of capsules and numbers of eggs from individual snails within an experiment

The total numbers of capsules and eggs produced by individual snails over the duration of an experiment did not show such high correlations because of variations in the numbers of eggs per capsule produced by different snails (Table 6.16).

TABLE 6.16 Examples of some correlations between total capsule production and egg production by individual snails

Experiment		r
6.1 (L. tomentosa)	(a) 1st period at 24°C	0.63
	(b) 2nd period at 24°C	0.81
	(c) pooled data from both periods	0.70
6.3 (L. columella)		0.69

6.3.7 Oviposition at low temperatures

Both species were capable of oviposition at 5°C and one L. columella produced a single capsule at 2°C. In all cases total production dropped with temperature (Table 6.1). When temperatures were altered in experiment 6.6 the immediate effect seemed to be cessation of oviposition until the snails became acclimatised to their new conditions.

6.3.8 Summary of results

1. L. columella had a much higher reproductive capacity than L. tomentosa with many more eggs per capsule.
2. There was a tendency, not significant at the .05 level of probability, for larger specimens of L. tomentosa to lay more eggs. More rapidly growing L. columella produced more eggs per capsule than specimens which grew more slowly.
3. L. tomentosa nearing the end of the oviposition period produced fewer eggs per capsule; too few L. columella ceased oviposition for this phenomenon to be examined in that species.
4. The fecundity of L. columella was enhanced by frequent water replacement, but the fecundity of L. tomentosa was not noticeably affected.
5. There were high correlations between the numbers of capsules produced by groups of snails at one collection and their egg production at the same collection. These correlations were usually lower when individual snails were compared over a period of time, or when results from experiments carried out under different conditions were pooled.
6. L. columella and L. tomentosa produced eggs at temperatures as low as 2°C and 5°C respectively.

6.4 DISCUSSION

6.4.1 Relative fecundity of L. tomentosa and L. columella

At the range of temperatures and conditions tested the fecundity of L. columella was clearly greater than that of L. tomentosa

(Table 6.1). Whether this greater reproductive potential really represents a greater capacity for population increase depends upon such factors as differential mortality and rates of development of eggs and snails. Mortality rates in the field and in the laboratory are investigated in Chapters 3 and 5, and the rates of development of eggs are reported in Chapter 7.

6.4.2 Shell length and fecundity

Shell length is regarded as a useful indicator of sexual maturity in snails, perhaps more reliable than chronological age (van der Schalie and Davis, 1965). Field specimens of L. tomentosa did not produce eggs if their shell length was less than 5 mm while for L. columella the critical size was 9 mm. Nevertheless slow growing underfed laboratory specimens of L. columella whose development from hatching to maturity took several months occasionally produced eggs at shell lengths as low as 7 mm. Variable rearing conditions may account for the variety of shell lengths reported as being indicative of sexual maturity in L. columella (see section 1.3.1).

Baily (1931) reported a positive relationship between shell length and fecundity as measured by capsule production, total egg production, and the number of eggs per capsule produced by L. columella. Examination of his data shows that he is referring to the size of snails at death or at the end of the period of oviposition, so his correlations between shell length and total production of eggs and capsules are hardly surprising; the larger snails have been producing eggs over a longer period of time. When shell length at death is related to egg and capsule production per day x 100 from three days before the first oviposition to death i.e. the oviposition rate, not quantity, the correlations are less apparent (Fig. 6.6). The oviposition period was considered to have begun three days before production of the first egg capsule because there are often gaps between periods of oviposition. To count only the days from production of the first capsule would therefore exaggerate the oviposition rate of snails which produced eggs for only a few days before death.

In experiments 6.1 and 6.3 the tendency for larger snails to produce more eggs per unit of time and to have larger capsules could not be demonstrated at the .05 level of statistical significance because

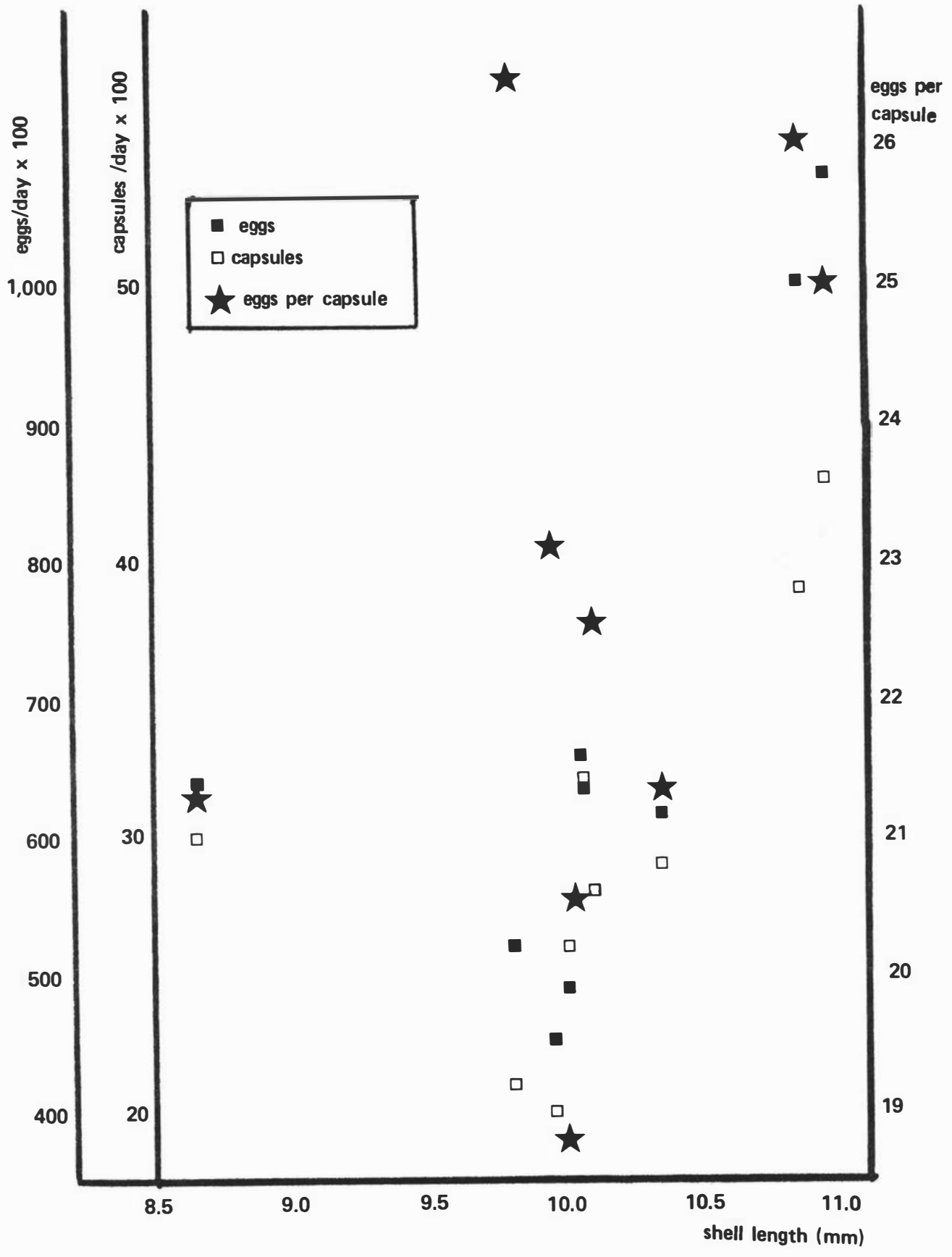


Fig. 6.6 The relationship between shell length at the end of oviposition and the fecundity of *L. columella* (Data from Baily, 1931)

of the variations between snails of similar size. Such a demonstration would require a large number of mature snails over a wide range of shell lengths.

6.4.3 Factors influencing the number of eggs per capsule

Rates of growth of L. tomentosa during experiment 6.1 were not recorded. Live specimens of L. tomentosa are more difficult to measure accurately because of parallax errors due to their globose shape, and their relatively slow growth rate increases the potential error.

The larger capsules produced by the more rapidly growing specimens of L. columella during experiment 6.3 suggests that there may be a link between the number of eggs per capsule and the general health and well-being of snails. The investigation into the capsule size of L. tomentosa that died or ceased oviposition was an attempt to test this possibility. In this series of experiments too few L. columella died or ceased oviposition to allow a similar investigation in this species. Results for L. tomentosa indicate that the number of eggs per capsule is not a good predictor of the snail's impending death, at any rate not for more than a few days. The last few capsules laid before oviposition ceases, however, tend to be much smaller than normal. This reduction in capsule size near the end of the oviposition period has been noted in other species (De Witt, 1954; De Wit, 1955).

Since all snails had produced eggs before the experiment began it was impossible to tell whether the first few capsules were smaller than average, as has been reported for Physa fontinalis (De Wit, 1955). However raw data from Baily (1931) can be used for comparing the numbers of eggs per capsule from L. columella at the beginning and end of the oviposition period with that at mid oviposition.

Baily gave only total daily capsule and egg production for each snail, so where more than one capsule was laid by a snail in one day the number of eggs in each capsule cannot be derived from the data. Columns 2 and 3 in Table 6.17 show the mean numbers of eggs in the first four and last four capsules from each snail where possible; in a few cases it was necessary to average three or five for the reason given above.

TABLE 6.17 Mean eggs per capsule produced by *L. columella*
Data from Baily (1931)

1	2	3	4
Snail	1st 4 capsules(1)	Last 4 capsules(1)	Remaining capsules
4	27.25	14.60	27.15
7	15.75	7.00	22.50
8	32.25	11.00	23.00
9	17.50	8.20	24.88
11	34.25	16.80	28.35
14	25.75	11.50	28.80
16	22.67	15.00	23.00
s^2	48.49	13.25	7.25
mean	25.06	12.01	25.38

(1) See text of section 6.4.3

F max test of variances (Sokal and Rohlf, 1969b) $\frac{48.49}{7.25} = 6.69$ (n.s.)

Two-factor analysis of variance of capsule sizes

Source of variation	d.f.	S.S.	M.S.	F
Snails	6	265.83	44.31	3.59*
Stages of oviposition	2	814.36	407.18	32.98***
Error	12	148.15	12.35	
Total	20	1228.34		

Analysis of Baily's data shows that the mean number of eggs per capsule at the beginning of oviposition was more variable than those from the main period, but the differences between variances were not significant at the .05 level when subjected to the F max test (Sokal and Rohlf, 1969b). A two-factor analysis of variance was then carried out, testing the mean numbers of eggs per capsule from (a) the beginning, middle and end of the oviposition period and (b) between snails. Results in Table 6.17 show differences between snails ($p < .05$)

and very highly significant ($p < .001$) differences in eggs per capsule between the small capsules laid near the end of the oviposition period and those laid earlier.

It is interesting to see a much lower level of significance than usual for differences between snails in an analysis which simultaneously takes account of differences due to the period of oviposition. Some of the differences in eggs per capsule between snails in experiments 6.1 to 6.5 may have been because snails were at different stages of their oviposition periods. However Baily's snails came from a single egg capsule, perhaps reducing any genetic component of differences in eggs per capsule.

6.4.4 Water changes and fecundity

Under similar experimental conditions water changes appeared to stimulate both egg and capsule production in L. columella but not in L. tomentosa. This is interesting in view of the generally greater mortality of L. columella both in the laboratory and in field populations (see Chapters 3 and 5), and suggests that L. columella may be more sensitive to the effects of stagnant water.

6.4.5 The relationship between capsule production and egg production

Capsule production and egg production are believed to operate by separate biological mechanisms even though there is a high correlation between the two (Forbes, 1946; van der Steen, 1967; 1970). Forbes' suggestion that in large numbers of snails egg production may be estimated from capsule production without the tedious practice of counting eggs seems a little dangerous because the number of eggs per capsule within a given species varies in accounts from different authors e.g. Forbes found more eggs per capsule in L. columella than reported by Baily (1931).

Where periodic collections are made from a number of snails within one experiment there are usually high correlations between capsule production and egg counts (Table 6.15) because differences between snails tend to be averaged out in such collections. It would be more dangerous to attempt to estimate the fecundity of individual snails from their capsule production (Table 6.16).

6.4.6 The significance of oviposition at low temperatures

There are no records of oviposition by either L. columella or L. tomentosa at low temperatures other than Boray's (1946b) observation that L. tomentosa will lay eggs at 15°C. L. truncatula, whose temperature tolerance zone is below the upper limits of these two species (see section 1.4.3) is said to begin oviposition at 10°-11°C (Kendall, 1953), although Taylor (1964) more cautiously states that below 9°C egg production by L. truncatula is negligible. Oviposition by L. columella and L. tomentosa at 2°C and 5°C respectively was therefore surprising. It is unlikely to have been due to a carry over effect of their oviposition at higher temperatures; the immediate effect of transfer to a low temperature was partial or complete suppression of oviposition for a few days until the snails became acclimatised.

Experiment 6.6 was conducted upon snails which were already actively producing eggs at room temperature. Its results do not prove that snails reared at temperatures at or below, say, 10°C will begin oviposition at that temperature without being "triggered" by exposure to some higher temperature. However, even if this were necessary, it is probable that diurnal temperature variation and mean monthly temperatures in most parts of New Zealand are high enough to allow oviposition by both L. columella and L. tomentosa throughout the year. Field evidence for this is examined in Chapter 3.

7. THE EFFECTS OF TEMPERATURE AND DESICCATION UPON THE DEVELOPMENT OF THE EGGS OF LYMNAEA TOMENTOSA AND L. COLUMELLA AND OBSERVATIONS ON THE RESPONSE OF THE SNAILS TO HEAT STRESS

7.1 INTRODUCTION

L. columella egg capsules are firm, transparent, gelatinous, banana-shaped envelopes. They are flattened on one side, by which they adhere firmly to the glass wall or floor of the container in which they have been laid. They may also be found adhering to sticks, stones, or even the shells of other snails. The capsules contain two, occasionally three, parallel rows of ovoid eggs which measure approximately 0.8 x 0.6 mm. The capsules are commonly 10 to 15 mm long and 2.5 to 3.5 mm wide.

The egg capsules of L. tomentosa are much softer in consistency and less regular in shape. Their round eggs, approximately 0.9 mm in diameter, are arranged in no particular pattern and a typical capsule measures approximately 8 mm long, 4 mm wide and 3 mm deep. L. tomentosa egg capsules are much less firmly attached to the substratum than those of L. columella.

Some effects of temperature and desiccation upon the egg capsules of L. tomentosa and L. columella are described in this chapter. Observations on the responses of the snails to heat stress are also included. These observations were made in order to investigate whether the differences observed in the effects of temperature upon the egg capsules of the two species were reflected in the resistance of the snails to heat stress.

7.2 EXPERIMENTS

7.2.1 Comparison of the oviposition to hatching times of eggs from different snails of the same species

Method

The possibility that the progeny of different snails might have different hatching times was investigated by incubating at 20°C a number of egg capsules from each of twenty-one L. columella. The snails

were collected from a cattle trough near the laboratory and were the progeny of snails from a single pond habitat. The numbers of capsules from each snail ranged from six to fourteen and their hatching times were compared in a simple analysis of variance. Hatching was considered to have occurred when the first egg in any capsule hatched. The rationale for this is explained in the next section (7.2.2). Capsules were examined daily at approximately the same time each day.

Results

The mean oviposition to hatching times at 20°C for groups of egg capsules from twenty-one L. columella snails ranged from 12.88 to 14.36. Analysis of variance (Table 7.1) showed no significant differences between these means.

TABLE 7.1 Comparison of the oviposition to hatching time of egg capsules from twenty-one L. columella at 20°C

Source of variation	d.f.	S.S.	M.S.	
Between snails	20	27.44	1.3720	n.s.
Within snails	181	270.74	1.4958	
Total	201	298.18		

7.2.2 The effect of temperature upon the rate of development from oviposition to hatching

Method

Snails of both species were maintained singly or in small groups in petri dishes 14 cm in diameter and 2 cm deep. In many cases the snails were involved in other experiments but their egg capsules were removed daily after removal of all water from the dish. This precaution was particularly necessary for L. tomentosa whose transparent egg capsules have a refractive index similar to that of water, so that they may be overlooked if all water is not carefully decanted.

For each temperature being investigated the egg capsules were removed at the same time each day. One to three capsules were immersed in tap water in petri dishes and held at a constant temperature until

hatching occurred. The endpoint of development was taken as rupture of the first egg in each capsule even if the hatched snail had not escaped from the outer confines of the capsule. This gave more consistent hatching times than, for example, waiting until 50% of the eggs had hatched. This is illustrated by the analysis shown in Table 7.2 in which it can be seen that the standard deviations of development times for 50% of eggs were usually larger than those relating to hatching of the first eggs. Indeed many L. tomentosa and a few L. columella capsules failed to achieve a 50% hatching rate.

TABLE 7.2 Means and standard deviations of oviposition to hatching times for L. columella as assessed by (a) hatching of the first egg in each capsule, and (b) hatching of 50% of the eggs in each capsule

KEY H_1 = days to hatching of first egg in each capsule
H_{50} = days to hatching of 50% of eggs in each capsule
$\bar{X}$ = mean number of days to hatching
s = standard deviation

	22°C		27°C		28°C		29°C	
	H_1	H_{50}	H_1	H_{50}	H_1	H_{50}	H_1	H_{50}
$\bar{X}$	10.9	11.7	7.7	8.4	7.7	8.0	7.6	8.6
s	.79	.96	.67	.75	.76	.58	.57	.72

At least five egg capsules were incubated at each temperature investigated so that the mean number of days to hatching and 95% confidence intervals for that mean could be calculated. Numbers of capsules used and hatching times are shown in Appendix 7.1. Temperatures investigated were 4°, 5°, 8°C, the range between 10° and 34.5°C shown in Appendix 7.1 and 36° and 37°C

A regression line was calculated to show the relationship between temperature and oviposition to hatching time. The method of least squares was employed in a computer programme to fit a polynominal equation with temperature as the fixed variable X and the log transformation of oviposition to hatching time as the ~~in~~dependent variable Y

(Goulden, 1952). The log transformation of Y was empirically chosen to remove a relationship between the means and variances of observed oviposition to hatching times; with raw data longer development times were associated with larger variances (Appendix 7.1).

Results

Results are summarised in Appendix 7.1 and Fig. 7.1. The embryos of neither species underwent any visible development at 4°C or below. Some development occurred at 5°C in both species, but the embryos failed to survive to hatching. Mean hatching times between 5°C and 10°C could not be ascertained because of high mortality rates in the developing embryos on the two occasions when an incubator was available for a sufficiently long time. Temperatures above 30°C were lethal to developing L. tomentosa embryos; L. columella hatched at up to 34.5°C but at 36°C none survived.

Between these extremes the relationship between Y (the number of days to hatching) and X (temperature) was clearly non-linear (Fig. 7.1) even when the Y axis was converted to logarithms (Fig. 7.2). In an attempt to find a more accurate expression of the relationship between oviposition to hatching times and temperature a stepwise multiple regression of the form $\log Y = a + b, X + b_2X^2 + b_3X^3 \ldots$ was fitted to individual observations on hatching time. Statistically significant "improvement" of the fit was achieved up to the fourth power of X in both species, at which point 97% and 96% of the variances of individual observations on hatching times for L. tomentosa and L. columella respectively were explained by their regression formulae (Table 7.3). Between 15.5°C and 22°C the regression of log development time on temperature approaches linearity; above and below these limits the relationship is curvilinear (Fig. 7.2). As can be seen from Fig. 7.1 at most temperatures the development times for eggs of L. columella were significantly shorter than those for the eggs of L. tomentosa.

TABLE 7.3 Stepwise multiple regression of days to hatching of egg capsules on temperature. Log $Y = a + b_1X + b_2X^2 + b_3X^3 + b_4X^4$ where Y = days to hatching, a = constant, b = regression coefficient, X = temperature, t = value of Student's t test, p = probability that such a value would arise by chance, and r = the correlation coefficient

Y	X	b	standard error	t	p
		L. tomentosa (r^2 = .97)			
log Y	a	10.25	.697	14.719	<.0001
log Y	X	-1.639	.148	11.075	<.0001
log Y	X^2	.1133	.0114	9.949	<.0001
log Y	X^3	- .003546	.000378	9.378	<.0001
log Y	X^4	.00004164	.00000457	9.097	<.0001
		L. columella (r^2 = .96)			
log Y	a	5.896	.288	20.468	<.0001
log Y	X	- .7891	.0592	13.338	<.0001
log Y	X^2	.05113	.00430	11.899	<.0001
log Y	X^3	- .001511	.000132	11.415	<.0001
log Y	X^4	.00001653	.00000147	11.226	<.0001

7.2.3 Viability of the eggs of *L. tomentosa* and *L. columella* after holding at 4°C

Method

During investigation of the rate of development to hatching, the embryos of *L. tomentosa* were found to have a much higher mortality rate than those of *L. columella*. In order to compare their survival potential at low temperatures the eggs of both species were held in a refrigerator at 4°C for varying periods, then returned to room temperatures (ca. 20°C) or an incubator at 25° to 27°C to ascertain the numbers which hatched. In order to ascertain whether hatching would take place the capsules returned to room temperatures were held

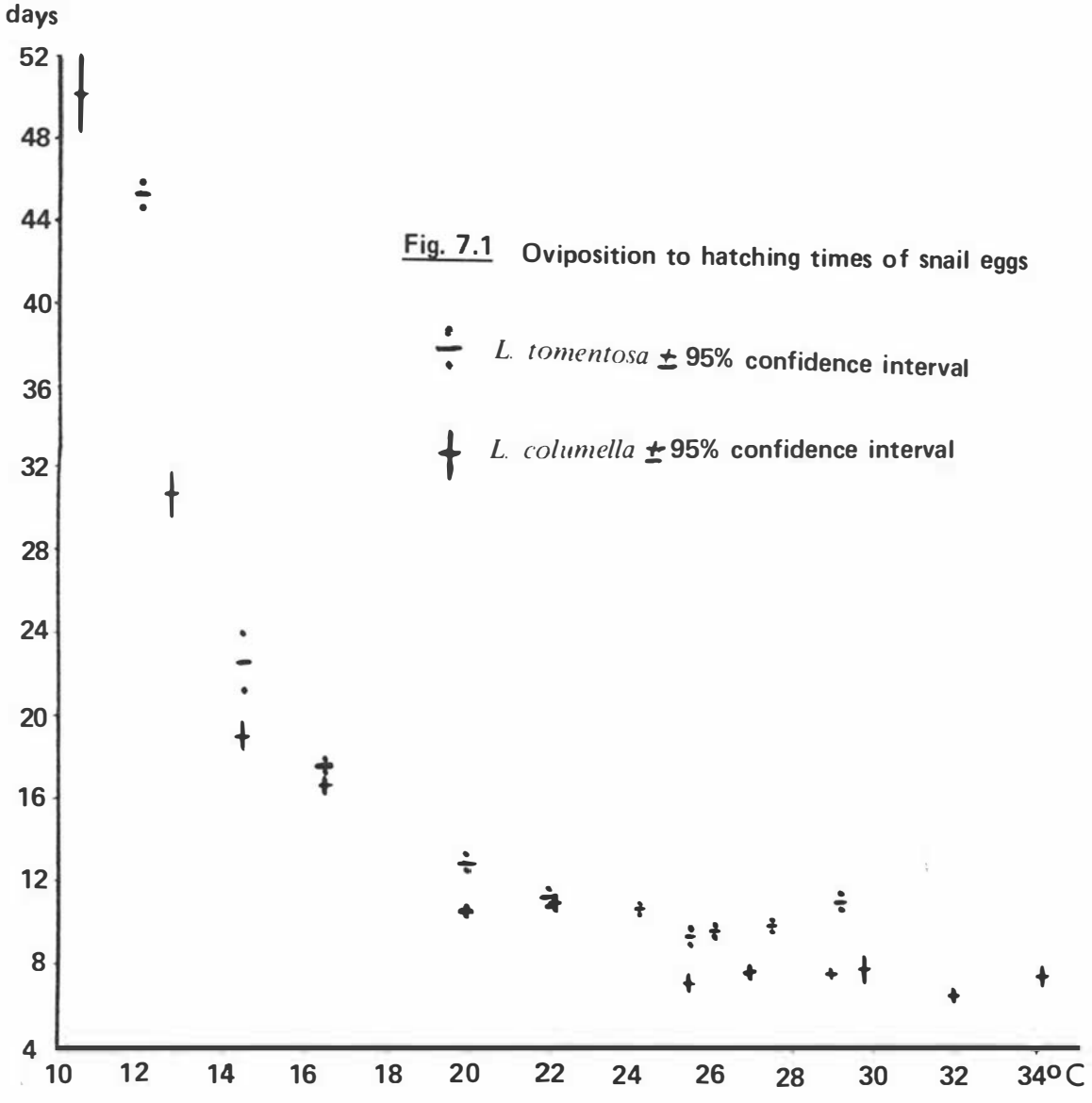


Fig. 7.1 Oviposition to hatching times of snail eggs

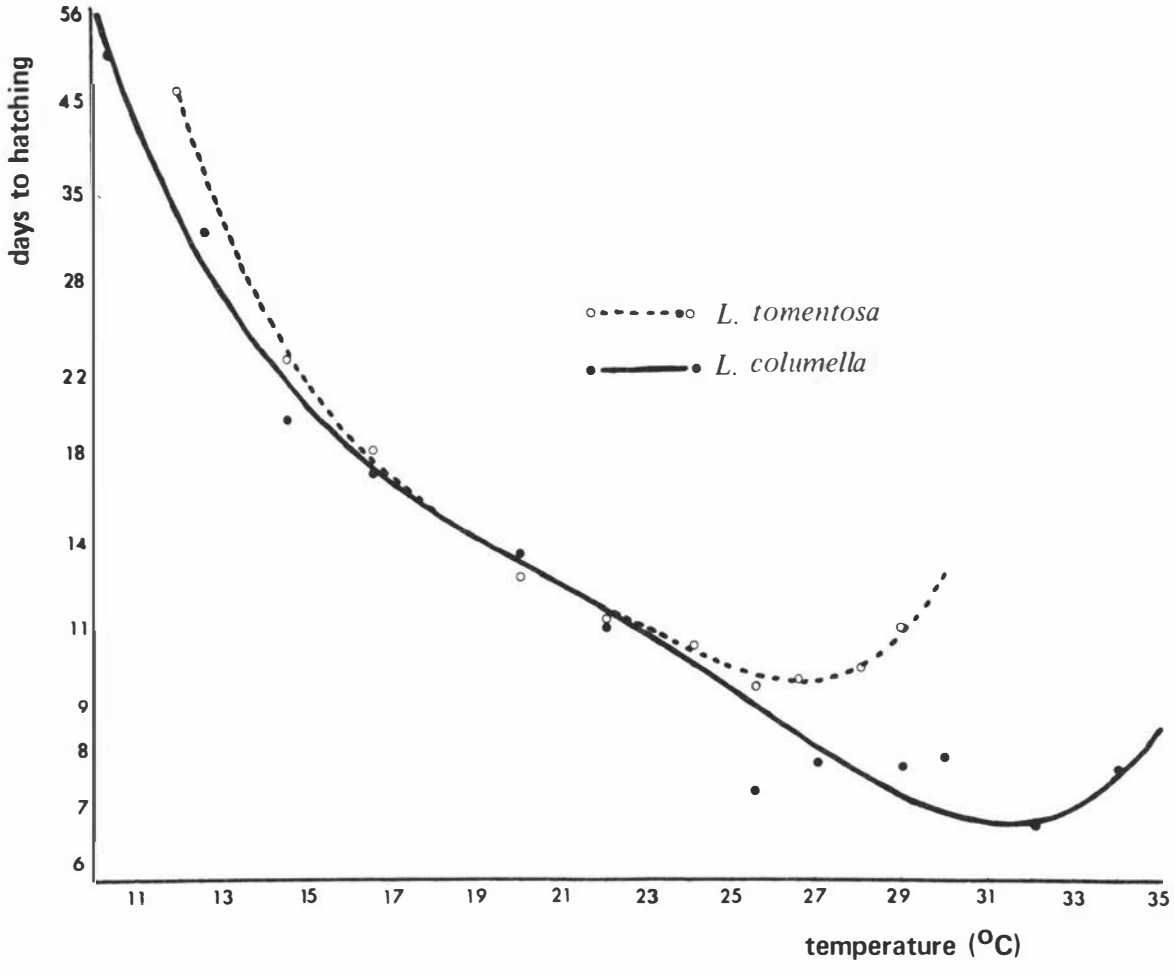


Fig. 7.2 The relationship between mean oviposition to hatching times and temperatures at which egg capsules of *L. tomentosa* and *L. columella* were incubated

for at least twenty-one days before being discarded; those incubated at 25-27°C were considered to be infertile if they had not begun to hatch by the fourteenth day.

Some of the egg capsules were put in the refrigerator at 4°C within twenty-four hours of oviposition. Another group was allowed to develop until moving embryos could be seen before being incubated at 4°C. Embryos could be seen moving when eggs were approximately halfway between oviposition and hatching, that is, at a temperature which allowed hatching in ten days, motile embryos could be seen on or about the fifth day. The periods of storage at 4°C and the numbers of egg capsules involved in each experiment are shown in Table 7.4.

Results

Egg capsules of L. columella had a higher hatching rate than those of L. tomentosa after storage for varying periods at 4°C (Tables 7.4; 7.5). Eggs which had undergone partial development at room temperatures retained their viability for longer periods at 4°C than those which were subjected to this temperature within twenty-four hours of oviposition. Although in all cases more L. columella capsules hatched than L. tomentosa, the number of capsules hatching decreased with increased periods of exposure at 4°C. The lethal effect of prolonged exposure at 4°C was less marked in the case of L. columella, but although egg capsules appeared less likely to hatch the longer they were maintained at this temperature the result was not statistically significant with the number of capsules in the experiment (Table 7.5d).

TABLE 7.4 Viability of the eggs of L. tomentosa and L. columella after storage at 4°C

KEY + = At least one egg in the capsule hatched
- = No eggs in the capsule hatched
I = Capsules stored at 4°C within 24 hours of oviposition
II = Capsules stored at 4°C after partial development of embryos

Days at 4°C	L. tomentosa I		L. columella I		L. tomentosa II		L. columella II	
	+	-	+	-	+	-	+	-
1-10	13	43	27	5	4	1	6	0
11-20	6	72	16	4	0	4	6	0
21-30	0	3	11	2	3	1	2	1
31	0	29	0	5	2	8	5	0

TABLE 7.5 Statistical evaluation of some of the results shown in Table 7.4. Only data from capsules transferred to 4°C within 24 hours of oviposition have been analysed

KEY + At least one egg in the capsule hatched
- No eggs in the capsule hatched
I Capsules stored at 4°C within 24 hours of oviposition
II Capsules stored at 4°C after partial development of embryos

Hatching rate of capsules held at 4°C for

(a) 1-20 days

	L. tomentosa	L. columella	Totals
+	19	43	62
-	115	9	124
Totals	134	52	186

(b) >20 days

	L. tomentosa	L. columella	Totals
+	0	11	11
-	32	7	39
Totals	32	18	50

$\chi^2 = 76.33^{***}$ $\chi^2 = 21.31^{***}$

i.e. the hatching rate of L. columella capsules is significantly greater ($p < .001$) than that of L. tomentosa capsules after holding at 4°C for all of the holding periods tested.

(c) L. tomentosa capsules held for increasing periods at 4°C

	1-10 days	11-20 days	> 20 days	Totals
+	13	6	0	19
-	43	72	32	147
Totals	56	78	32	166

$\chi^2 = 10.42^{**}$ i.e. the hatching rate of L. tomentosa capsules decreased significantly ($p < .01$) with increasing periods of exposure at 4°C.

(d) L. columella capsules held for increasing periods at 4°C

	1-10 days	11-20 days	>20 days	Totals
+	27	16	11	54
-	5	4	7	16
Totals	32	20	18	70

$\chi_2^2 = 2.39$ (χ_2^2 (.05) = 3.841) i.e. although there was a tendency for the hatching rate of L. columella capsules to decrease with increasing periods of exposure at 4°C this was not significant at the .05 level of probability in this experiment.

7.2.4 The relative susceptibility of the eggs of L. tomentosa and L. columella to desiccation

Method

Egg capsules less than forty-eight hours old were dried on filter paper. They were immediately placed on other pieces of filter paper in uncovered petri dishes floating on water in an incubator at 16.5°C and a relative humidity of 80-90%. Egg capsules were removed periodically and tested for viability by adding water to the petri dishes and incubating them at room temperature for twenty-one days or in an incubator at 25°-27°C for fourteen days. The periods for which eggs were desiccated and the numbers of eggs involved are shown in Table 7.6.

Results

The longest periods of survival for capsules dried on filter paper and incubated at 16.5°C and a relative humidity of 80-90% were two hours for L. columella and one and a half hours for L. tomentosa (Table 7.6).

TABLE 7.6 Resistance of egg capsules to desiccation at 16.5°C and 80-90% relative humidity

KEY N = number of eggs in capsule
D = number showing signs of development
H = number hatched

L. columella				L. tomentosa			
Time (hours)	N	D	H	Time (hours)	N	D	H
½	23	23	23	½	12	12	12
1	7	7	7	½	2	0	0
1½	9	5	5	1	21	19	19
1¾	11	0	0	1	6	0	0
2	22	22	22	1	2	0	0
2	6	0	0	1	14	13	13
2½	23	0	0	1½	3	3	0
Capsules of both species were exposed for longer periods at half hour steps but none survived to hatching, or showed any signs of development				1½	16	11	11
				1½	2	0	0
				1½	2	0	0
				2	5	0	0
				2	8	0	0
				2	5	0	0
				2	3	0	0
				2	10	0	0

7.2.5 The resistance of *L. tomentosa* and *L. columella* to heat stress

Method

The materials used in the investigation were:

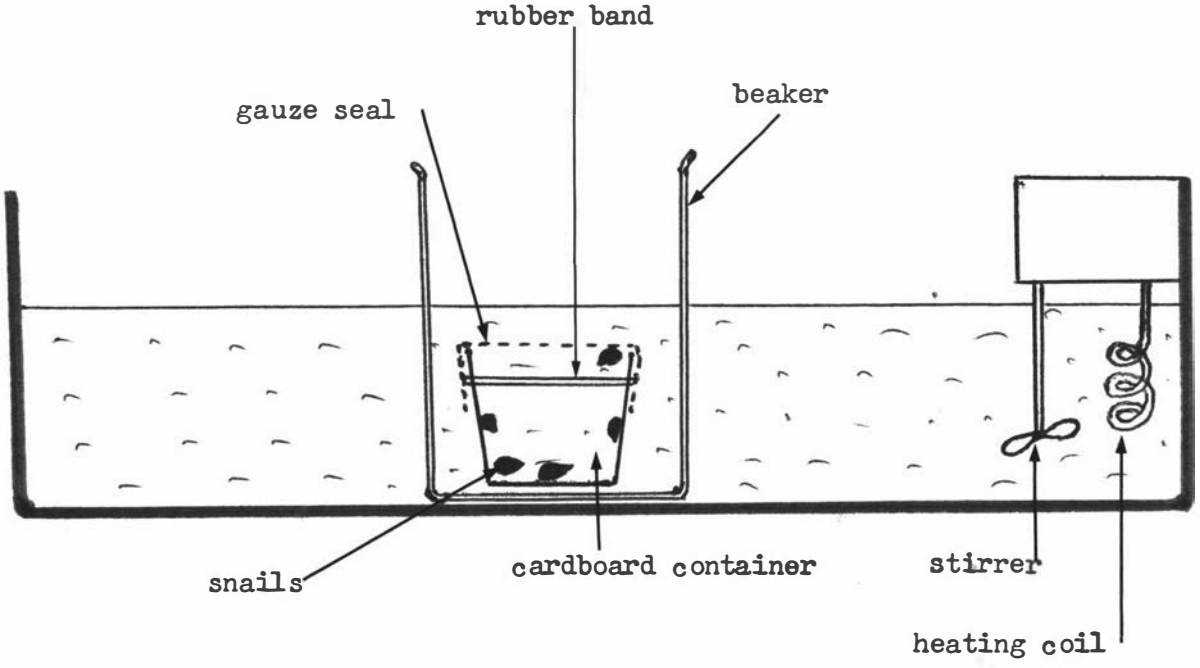
(i) a waxed cardboard container of 150 ml capacity

(ii) a 1000 ml pyrex beaker, and

(iii) a thermostatically controlled water bath.

Groups of laboratory adapted snails comprising a range of sizes were placed in the cardboard containers which were sealed with cotton gauze covers held in place by rubber bands (Fig. 7.3). The container was then submerged in the glass beaker in tapwater which had been allowed to come into thermal equilibrium with the water in the bath. The tapwater in the beaker was not allowed to come in contact with the water in the bath in case contamination from a copper heating coil should prove harmful to the snails.

Fig. 7.3 Water bath in which the heat stress experiment was conducted



Groups of L. tomentosa were submitted to temperatures of 39, 40, 41, 42 and 43.5°C and L. columella to temperatures of 37, 39, 40.5, 41.5 and 42°C for a standard period of 30 minutes.

At the end of the treatment period snails were placed in water at room temperature (18 to 21°C). Three hours later they were examined for signs of life. Snails which survived were not used again.

Results

Detailed results appear in Appendix 7.2. L. columella tolerated exposure for thirty minutes at 40.5°C but 41.5°C and 42°C were lethal to most snails in this time. L. tomentosa had a slightly higher temperature tolerance with most snails surviving thirty minutes at 42°C but unable to withstand 43.5°C. The lethal limit was approached quickly in both species; the lowest temperature which killed a proportion of the snails was only 1 or 2°C below temperatures lethal to all of them. Winterbourn (1969), working with Potamopyrgus antipodarum, found a similarly rapid onset of temperatures which inactivated 100% of his experimental snails.

When only some snails were killed by a given treatment, those which survived tended to be the largest.

7.3 DISCUSSION

A small error is inherent in the method of estimating the ovipostion to hatching times. Capsules collected at twenty-four hour intervals will have spent up to twenty-four hours at room temperature before being transferred to the incubator. If oviposition is randomly distributed in time then each estimated development time will underestimate the true development time by a factor equivalent to twelve hours at room temperature. When the incubator temperature is below ambient the true development time will be underestimated by more than half a day; when it is above ambient temperature the underestimation will be smaller. If oviposition times are not random then the situation is more complex, and there is some evidence that oviposition occurs mainly during the hours of darkness (Steen, 1967). Since the main purpose of this

investigation was a comparison of the incubation times of the eggs of L. tomentosa and L. columella the error described above can be ignored.

As shown in Chapters 5 and 6 L. columella produced more eggs than L. tomentosa. Laboratory observations also indicated that a greater proportion of them was likely to hatch. During periods of observation at 10°C and 22°C, the only temperatures at which records of the total numbers of eggs hatching were kept, at least one egg hatched in 17 out of 22 L. columella capsules and a total of 342 out of 455 eggs hatched. During the same period eggs hatched in only 14 out of 48 L. tomentosa capsules and the total number of eggs hatching was 109 out of 547. The relatively poor hatching rate of L. tomentosa capsules after holding at 4°C may have been merely a reflection of their higher mortality during prolonged development rather than any specific harmful effect of low temperature. The capsules of L. tomentosa were more susceptible to fungal infections but many which failed to hatch had no macroscopic lesions of any kind. Further advantages to the eggs of L. columella are their slightly shorter incubation period and the greater range of temperature over which development takes place, particularly their longer period of rapid development between 25°C and 34.5°C.

The observed relationship between log Y (i.e. the log transformation of oviposition to hatching time) and X (temperature) departed from a linear model of the type $\log Y = a + bX$ (Fig. 7.2). At temperatures below 15°C the oviposition to hatching times were longer than those predicted by the linear model, particularly in the case of L. tomentosa, perhaps due to some harmful effect of low temperatures on the embryos. A similar effect was seen at the upper end of the range of temperatures at which development takes place, and the effect upon L. tomentosa was again greater. In fact when only temperatures between 25 and 34°C were considered a linear regression analysis showed no significant change in the rate of development for L. columella but there was a statistically significant ($p < .05$) tendency for development times of L. tomentosa capsules to increase as their upper lethal limit was approached (Table 7.7). Since the egg capsules of L. columella are firmer in texture, are less prone to fungal infections and have a consistently higher hatching percentage than those of L. tomentosa, it

is scarcely surprising that the harmful effect of extremes of temperature shown in Fig. 7.2 should be more marked in the case of L. tomentosa.

TABLE 7.7 The relationship between the log of days to hatching and temperatures above 25° C for egg capsules of L. tomentosa and L. columella

KEY b = regression coefficient
L_1 and L_2 = lower and upper 95% confidence limits for b

Species	b	L_1	L_2
L. tomentosa	1.20	0.45	1.95
L. columella	-0.16	-0.99	0.67

The stepwise multiple regression $\log Y = a + b_1X + b_2X^2 + b_3X^3 + b_4X^4$ was still adding a statistically significant "improvement" to the fit of the regression lines to the observed data for both species at the fourth power of X but there seemed no biological justification for going beyond this point. To do so may have produced a line even closer to the experimental mean oviposition to hatching times but the shape of the line would have been impossible to explain on biological grounds.

Direct comparisons with the hatching times reported by other authors cannot be made since their results sometimes refer to a range of hatching times, presumably for individual capsules, and their definitions of hatching may differ from that used in this study. Oviposition to hatching times reported for L. truncatula and L. tomentosa are shown in Table 1.1. There appear to be no such data published for L. columella but Boray's (1963a) results for L. tomentosa appear to be similar to those reported in this study (Appendix 7.1). The method described here, estimation of the mean hatching times for groups of capsules at different temperatures, allows more accurate definition of the effect of temperature upon hatching times. Furthermore two species may be compared with a high degree of precision by observing the altitude and shape of their regressions of temperature upon hatching times.

The egg capsules of L. tomentosa and L. columella were highly susceptible to desiccation. This is also true of other freshwater snails such as Australorbis glabratus (Chernin and Adler, 1967), Ross and McKay (1929) found that eggs of L. tomentosa survived out of water for up to $6\frac{1}{2}$ hours at 24°C and an unspecified relative humidity, but in the experiment described here the filter paper probably drew more water from the capsules. There appear to be no published observations on the effects of desiccation upon the eggs of L. truncatula or L. columella. The stage which survives desiccation in drying habitats is unlikely to be the egg; on the basis of evidence presented in Section 1.4.4 and Chapter 9 it is most probably the immature snail.

In the heat stress experiment on snails a treatment period of only thirty minutes was selected to reduce the risk of oxygen deprivation interfering with the results. Laboratory investigations had previously shown that both species were apparently unaffected by total immersion for at least 72 hours at 16.5°C and at least 24 hours at 25°C.

L. tomentosa's slightly higher resistance to heat stress is a surprisingly different result from that obtained by incubating egg capsules at high temperatures; egg capsules of L. columella were much more resistant to high temperatures than those of L. tomentosa. On the other hand these results are consistent with the observation that under most circumstances adult L. tomentosa have lower mortality rates than adult L. columella (Chapters 3 and 5).

8. THE EFFECTS UPON LYMNAEA TOMENTOSA AND L. COLUMELLA OF DESICCATION IN AIR

8.1 INTRODUCTION

Most investigations into the responses of snails to desiccation have been concerned with their survival in drying habitats or laboratory simulations of field conditions (see section 1.4.4). The results of such experiments are complicated by behavioural factors such as snail burrowing and experimental variables like gradients of soil porosity. The investigation described in this chapter was designed:

1. to see if there was any difference in the capacity of the two species to resist desiccation when behavioural responses were eliminated and the environment was identical for both, and
2. to establish the relationship between snail size and resistance to desiccation under controlled conditions.

8.2 METHOD

The external surfaces of snails of both species were dried with filter paper. The snails were then held at 16.5 ± .5°C in an incubator for various periods, during which they remained on a piece of filter paper in a covered petri dish floating on water. The incubator contained several open trays of water and its relative humidity varied between 80 and 90% (Fig. 8.1).

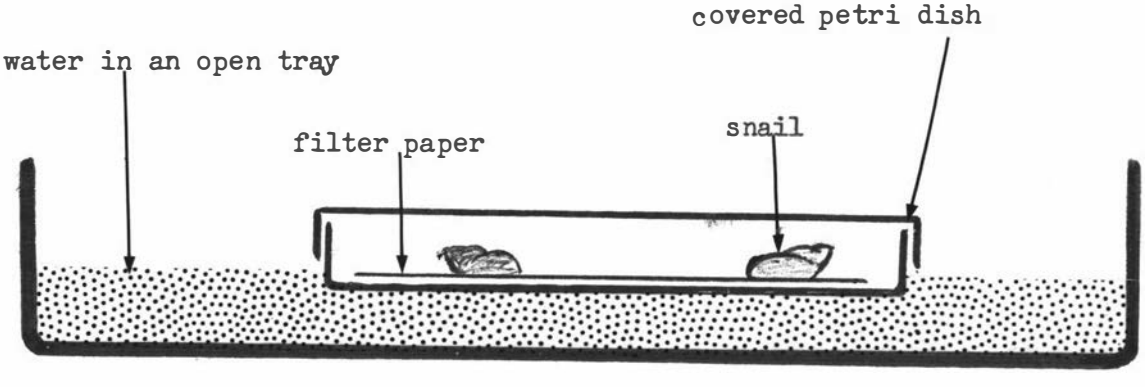


Fig. 8.1 Snails being desiccated on filter paper in an incubator

After a predetermined period ranging from one to fifty-seven hours each petri dish was removed and filled with water. Snails were examined for signs of life under a dissection microscope and those showing no activity within twenty-four hours were assumed to be dead. Although almost all survivors showed signs of life within three hours, the twenty-four hour period was chosen since some snails were removed from the incubator in the late evening. When there were no obvious signs of life, tapwater at approximately 40°C was added to the petri dish. If a snail responded with sufficient muscle movement to change its position or orientation it was considered to be alive.

To achieve as much randomisation as possible, snails from each laboratory aquarium were allocated to several treatment times, and within a given treatment time snails were taken from as many aquaria as possible. The whole investigation involved 1,114 L. columella and 420 L. tomentosa. Shell lengths of all snails were measured at the end of each treatment period. For the purposes of analysis the snails were placed in 1 mm size classes ranging from 2.0 to 13.9 mm (i.e. the first size class comprised snails from 2.0 to 2.9 mm in length) in the case of L. columella and 2.0 to 6.9 mm for L. tomentosa. The numbers of snails in each class are shown in appendix 8.1.

From the survival data the exposure time that would be fatal to 50% of snails (LD_{50}) was calculated by probit analysis (Finney, 1971) for each size group. These calculations were performed on an IBM 1620 computor, using a programme already in use. When all the LD_{50}s had been calculated their relationship with the shell length of the snails was investigated by the least squares method of linear regression, using raw data or various transformations as shown:

1. shell length and LD_{50} (Fig. 8.2)
2. shell length and log LD_{50}
3. $\sqrt{\text{shell length}}$ and LD_{50}
4. $(\text{shell length})^2$ and LD_{50}
5. log shell length and log LD_{50} (Fig. 8.3).

These calculations were performed on data from L. columella since the range of shell lengths for this species was greater than that for L. tomentosa, allowing more classes for calculation of the analyses.

The lower limit of each shell length class was used in analysing all data.

The relationship between shell length and log LD_{50} was clearly non-linear. In all the other cases the square of the correlation coefficient, r^2, was used to test goodness of fit. Relationship number five, the regression of log LD_{50} on log shell length, was then examined for L. tomentosa and the regression lines for the two species were compared in an analysis of covariance (Snedecor and Cochran, 1967).

8.3 RESULTS

Larger snails resisted desiccation for longer periods than smaller specimens. The relationships between resistance to desiccation and shell length are shown in Table 8.1, Figs. 8.2; 8.3, and Appendix 8.1.

The relationship between LD_{50} and shell length of L. columella appeared to be linear (Fig. 8.2; Table 8.1). The equation for the linear regression of LD_{50} on shell length calculated by the least squares method whose general formula is

$$Y = bX+a$$

was $LD_{50} = 4.107X - 8.614$ ($p < .001$).
Variances, however, increased markedly with shell length and since one of the assumptions of the least squares analysis is that variances are homogeneous the method was invalid for raw data. Transformation of LD_{50}s to square roots or base 10 logarithms gave homogenous variances in all shell length classes as shown by the 95% confidence intervals in Appendix 8.1.

The regression equations for some transformations of LD_{50} and shell length are shown in Table 8.1. The best equation to describe the relationship between LD_{50} and shell length was that for the total log transformation although some of the others gave an almost equally close fit (Table 8.1).

TABLE 8.1 Relationships between shell length and LD_{50} in *L. columella* and *L. tomentosa*

KEY

LD_{50} = estimated time in hours required to kill 50% of snails, calculated by probit analysis from raw data

log LD_{50} = LD_{50} calculated from base 10 logarithms of treatment times

$\sqrt{LD_{50}}$ = square root of LD_{50} calculated from raw data

r2 = the square of the correlation coefficient, used as a test for goodness of fit

a = the intercept of the regression line on the Y axis

b = the regression coefficient, i.e. the slope of the regression line

P = the probability that similar results could have occurred randomly

				L. columella		
X	Y	a	b	regression equation	r2	P
shell length	LD_{50}	-8.614	4.107	LD_{50} = 4.107X-8.614	.9806	<.001
shell length	log LD_{50}	non-linear				
$\sqrt{\text{shell length}}$	LD_{50}	0.8220	0.478	LD_{50} = 0.4784X+.8220	.9858	<.001
$(\text{shell length})^2$	LD_{50}	3.92	0.268	LD_{50} = .268X+3.92	.9790	<.001
log shell length	log LD_{50}	-0.1546	1.654	log LD_{50} = 1.654-.1546	.9966	<.001
				L. tomentosa		
X	Y	a	b	regression equation	r2	P
log shell length	log LD_{50}	-.0358	1.525	log LD_{50} = 1.525X-.0358	.9863	<.001

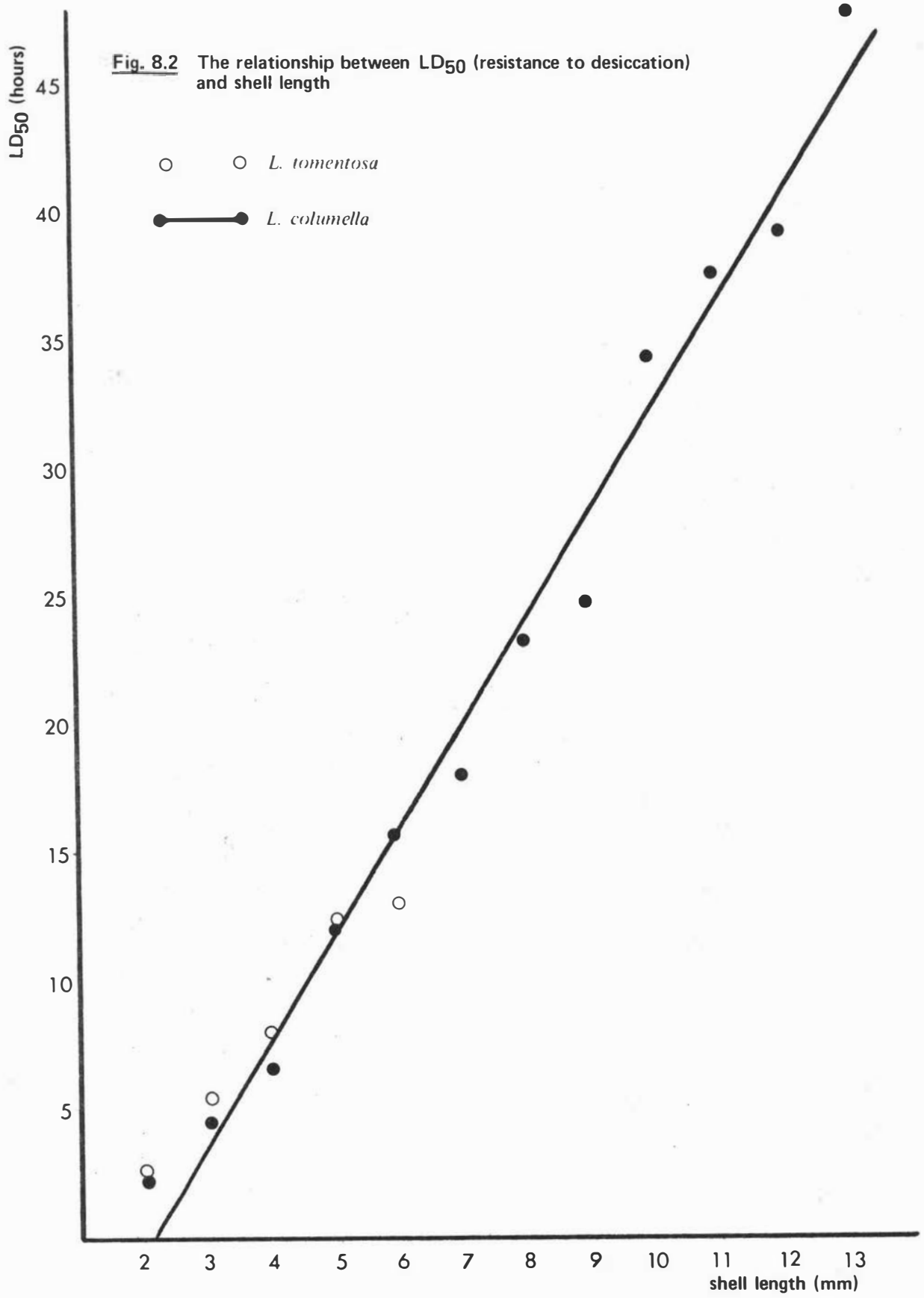


Fig. 8.2 The relationship between LD_{50} (resistance to desiccation) and shell length

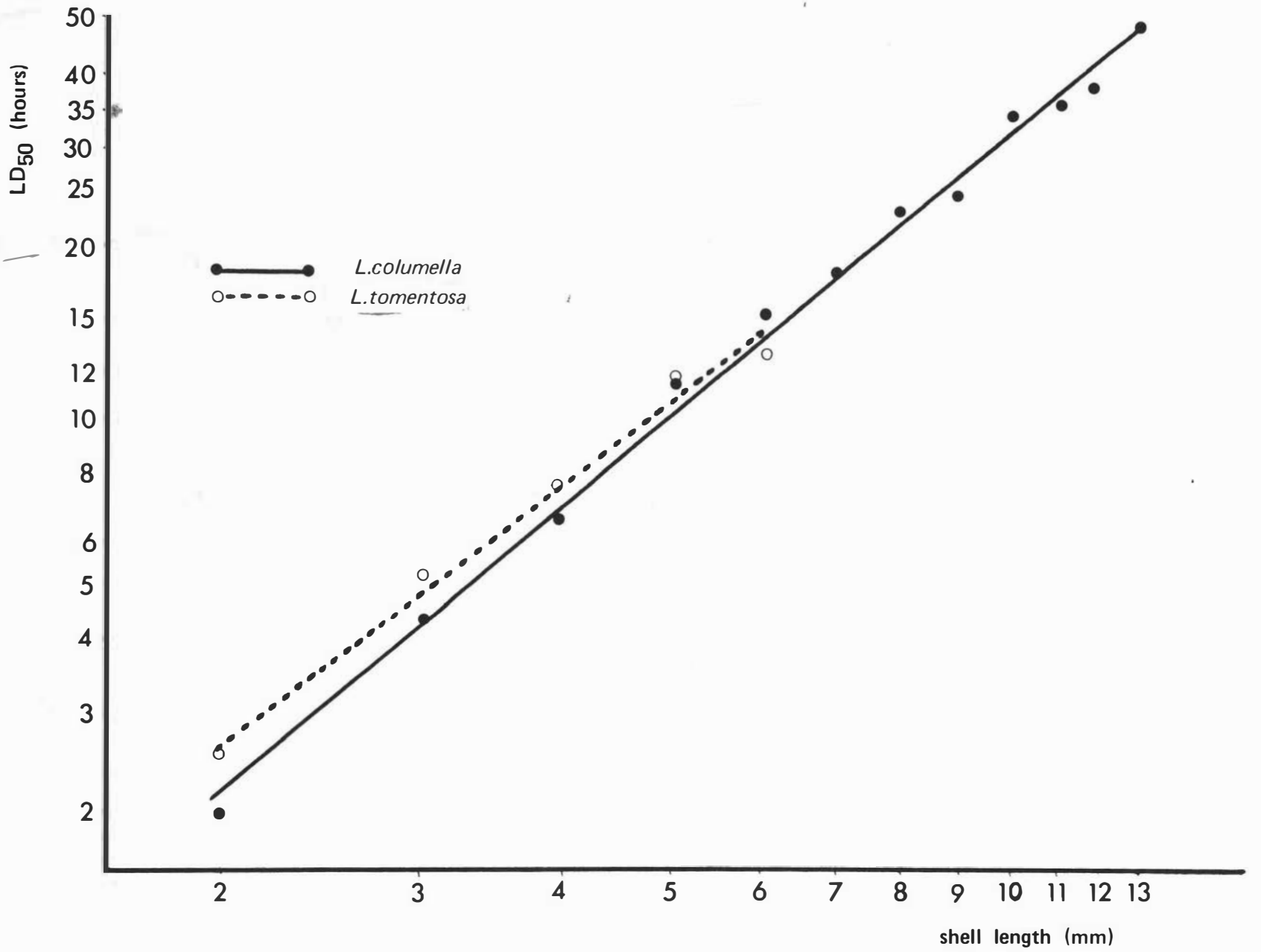

LD50 (hours)
50
40
35
30
25
20
15
12
10
8
6
5
4
3
2
L.columella
L.tomentosa
2
3
4
5
6
7
8
9
10
11
12
13
shell length (mm)

TABLE 8.2 Comparison of the regression lines for log LD_{50} on log shell length in *L. columella* and *L. tomentosa* by analysis of covariance

Source of variation	d.f.	x^2	xy	y^2	b	deviations from regression d.f.	S.S.	M.S.
Within								
L. tomentosa	4	.1420	.2167	.3353	1.5254	3	.0046	.0015
L. columella	11	.7112	1.1760	1.9581	1.6535	10	.0135	.0014
						13	.0181	.0014
Pooled, W	15	.8532	1.3927	2.2934	1.6323	14	.0201	.0015
		difference between slopes				1	.0020	.0020
Between, B	1	.2114	.3101	.4548				
W+B	16	1.0646	1.7028	2.7483		15	.0247	
		between adjusted means				1	.0046	.0046

Comparison of slopes: $F_{1,13} = .0020/.0014 = 1.429$ (n.s.)

Comparison of elevations: $F_{1,14} = .0046/.0015 = 3.08$ (n.s.)

Since total log transformation gave the best linear regression of LD_{50} on shell length for *L. columella* a similar calculation was performed for *L. tomentosa*. *L. tomentosa*'s smaller range of shell lengths gave only five points from which to calculate a regression line but the fit was again very good. Inspection of the regression lines for the two species (Fig. 8.3) shows that their resistance to desiccation in air was similar for snails of comparable size. An analysis of covariance confirmed this similarity (Table 8.2).

8.4 DISCUSSION

When formulating a hypothesis it is necessary to begin with a number of assumptions even if some of these must later be abandoned. The assumptions which form the background to this chapter are:

1. The water content of snail tissues remains constant throughout the body and does not alter with age.

2. Some percentage loss of water is tolerated up to an end point which remains constant for snails of all ages.

3. Although a small amount of water is lost through the shell wall, in non-operculate snails the greatest loss is through the aperture (Cameron, 1970).

4. If shell shape remains constant in snails of different size and age, the rate of water loss from the surface (aperture + shell surface) remains constant also. Evidence that the shell shape of L. columella remains relatively constant is presented in Chapter 10.

There is a three dimensional increase in body volume with each unit increase in shell length, while body surface and aperture area are increasing in only two dimensions. If X = shell length, then with increases in shell length the surface from which water is lost increases as a proportion of X^2 but body volume, and hence the volume of body water available to be lost, increases proportionally to X^3.

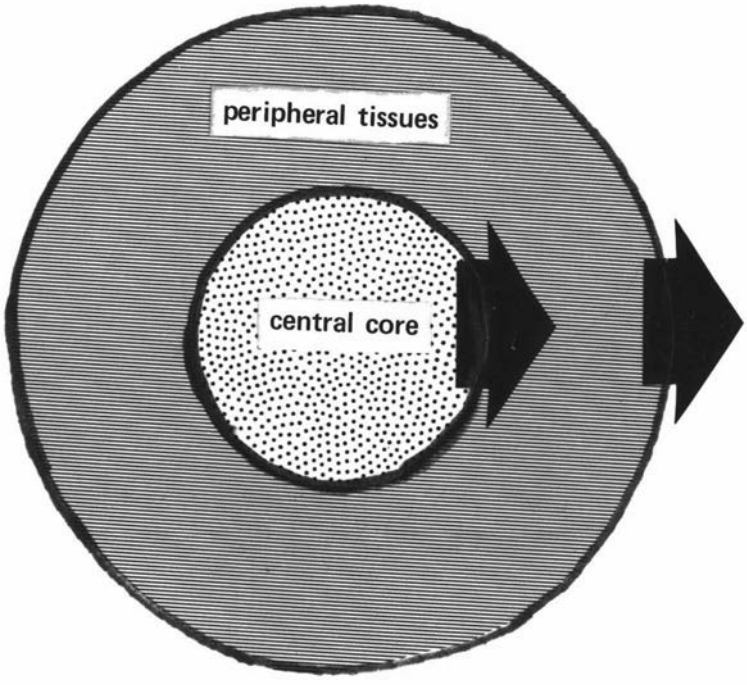
If X and B are unknown constants and Y = LD_{50} (time), then a snail must lose αX^3 water to die, but the time in which it does so is βYX^2 if water is lost at a constant rate per unit of surface area. (If the ratio of aperture area to shell surface remains constant this will be equivalent to losing water at some intermediate constant rate over the whole body surface).

$$\text{Therefore } \beta YX^2 = \alpha X^3$$

$$\text{so } Y = \frac{\alpha X}{\beta} \text{ (equation 8.1)}$$

This implies a linear relationship between Y and X and as may be seen from Table 8.1 and Fig. 8.2 there is a good linear approximation from raw data. However the arrangement of points above the regression line at the extremities and below the line near the middle suggests a mildly exponential relationship. This is confirmed by the better fitting log transformation (Table 8.1; Fig. 8.3) which also renders the variances homogenous (appendix 8.1). This means that the larger snails survive longer than might be expected from the proposed linear model. A possible explanation may be as follows:

Fig. 8.4 A diagrammatic representation of the way in which water may be lost from snail tissues



Water is initially lost from the body at a given rate. This rate might be expected to slow down as the surface dries out and water has to diffuse from deeper parts of the snail's body (Fig. 8.4). In larger snails water would have further to diffuse.

Alternatively, assumption 1 may be wrong. The peripheral tissues consist of the tough outer epithelial layer of the head-foot which protects the more delicate structures beneath. When the snail retracts into its shell, blood from the sinuses in the head-foot returns to the deeper tissues. Therefore even in non-operculate snails the peripheral zone forms a protective layer across which water from the central core diffuses relatively slowly. The absolute thickness of the peripheral tissues will obviously be greater in large snails so the rate of water loss will be less than from smaller specimens.

Either of these hypotheses would explain an increase in the

LD_{50} of larger snails beyond the time predicted by a linear model, and would account for the better fitting regression line from data transformed to logarithms. A new model may be derived from the data shown in Table 8.1 and represented in Fig. 8.3. The equation for the regression line when both axes have been converted to base logarithms is:

$$\log Y = a + b \log X \quad \text{(equation 8.2)}.$$

Since the intercept on the Y axis is on a logarithm scale "a" is really the logarithm of a number "A". Equation 8.2 is then equivalent to:

$$\log Y = \log A + b \log X$$
$$= \log (AX^b)$$
$$\text{i.e. } Y = AX^b \quad \text{(equation 8.3)}.$$

Equation 8.3 may be solved using data from Table 8.1. If Y(c) and Y(t) are predicted LD_{50}s for L. columella and L. tomentosa, A(c) and A(t) are the antilogarithms of the intercepts "a" on the Y axis, and b(c) and b(t) are the regression coefficients for the two species, then for L. columella

$$Y(c) = A(c)X^{b(c)}$$
$$= (\text{antilog } a(c))(X^{1.654})$$
$$\text{i.e. } Y(c) = .7004\ X^{1.654} \quad \text{(equation 8.4)}$$

and for L. tomentosa

$$Y(t) = A(t)\ X^{b(t)}$$
$$= (\text{antilog } a(t))\ (X^{1.525})$$
$$\text{i.e. } Y(t) = 9208\ X^{1.525} \quad \text{(equation 8.5)}.$$

This means that for any known shell length we can predict an exposure time at 16.5°C and 80-90% relative humidity which will be fatal to 50% of either species by applying equation 8.4 or 8.5 to the known data. X represents the lower limits of class sizes, so if we take as an example L. columella in the size class 4.0 to 4.9 mm their LD_{50} can be calculated from equation 8.4 as follows:

$$Y(c) = (.7004)\ (4^{1.654})$$
$$= (.7004)\ (9.907)$$
$$= 6.94 \text{ hours},$$

which compares with an observed LD_{50} of 6.62 hours for this class.

9. DESICCATION ON MUD

9.1 INTRODUCTION

The innate capacity for resistance to desiccation appears to be similar in L. columella and L. tomentosa of comparable size (Chapter 8). This does not necessarily mean that their survival rates in drying habitats are similar, since survival on drying mud depends to a large extent upon behavioural adaptations which differ between species (section 1.4.4). The purpose of the experiments recorded in this chapter was an assessment of the relative abilities of the two species to withstand desiccation on mud. All snails used in these experiments had been laboratory adapted or laboratory reared, since the mortality rate in field specimens of L. columella brought into the laboratory was particularly high during the first week in their new environment (Chapter 5).

9.2 METHODS

For all experiments mud from a L. columella habitat was incubated at 40°C for twelve hours to kill stray snails. It was then vigorously stirred to fracture any snail shells it may have contained.

Experiment 9.1

Mud, prepared as described above, was spread to a depth of 6 cm in two glass aquaria whose internal dimensions were 35.5 x 21.5 x 12 cm. Three rows of five plastic flower-pots with internal diameters of 5.2 cm were placed in each aquarium (Fig. 9.1a). Mud was placed in the outer rows of pots to the same depth as in the aquarium outside the pots (Fig. 9.1b). The centre row contained 1 cm of tapwater which was replenished every three to four days to maintain some soil moisture throughout the experiment.

Snails were randomly allocated to pots in the following way:

1. Ten groups of five snails of each species were chosen arbitrarily to include a range of sizes.

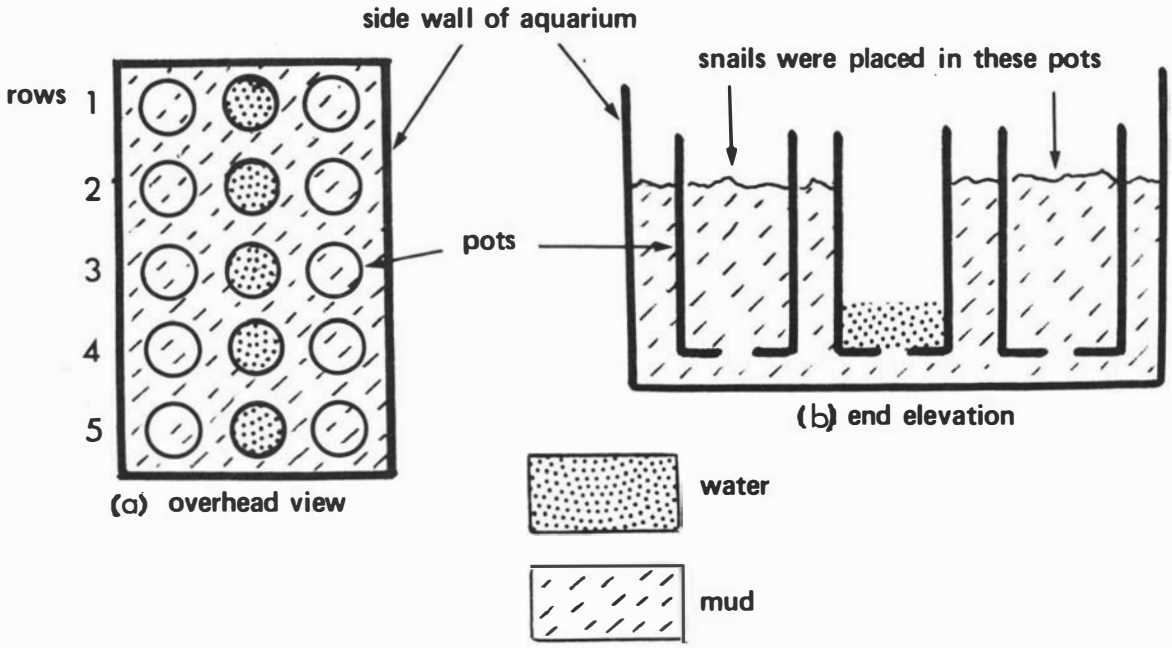


Fig. 9.1 Diagrammatic representation of the aquaria used in experiment 9.1

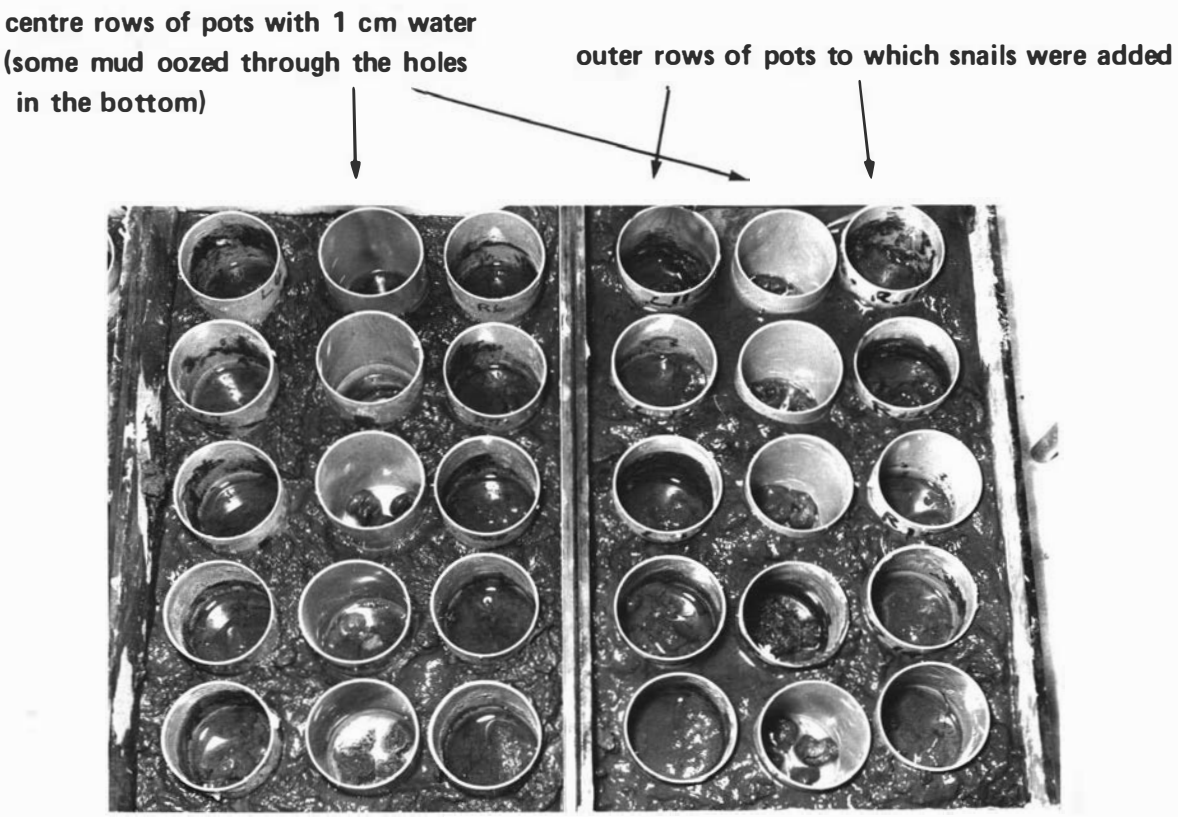


Plate 9.1 Pots used in experiment 9.2 with gauze covers removed

2. One group of each species was assigned to one of the ten rows of pots using a table of random numbers.

3. The allocation of these L. tomentosa and L. columella groups to the right or left pot in the row was decided on the toss of a coin.

In Experiment 9.1 the snails were kept in an incubator at 16.5°C. Relative humidity, recorded by a hair operated hygrograph, was 80-90% for the first seven days, but it must have dropped in the later stages because the soil surface dried and cracked. Unfortunately no monitoring device was available in the latter period.

Randomly selected groups of snails were removed at various times from twenty-eight to forty-nine days after the experiment began. Their mud filled pots were placed in glass jars filled with water to a depth of 10 cm. Live and dead snails were collected over a three hour period. At the end of the experiment the aquaria were flooded with tapwater for twenty-four hours in an attempt to find some of the snails which may have escaped from their pots into the mud outside.

The size ranges of snails at the start of experiment 9.1 were:

L. tomentosa - 2.4 to 7.0 mm
L. columella - 2.5 to 14.3 mm.

Experiment 9.2

The method employed was similar to that for experiment 9.1 except that there were three aquaria and the depth of mud in the pots was approximately 4.5 cm i.e. about 1.5 cm below the level of the mud outside (Fig. 9.2; Plate 9.1). Each pot was covered with a piece of cotton gauze held in place with a rubber band in order to prevent snails escaping from the pots.

There were unequal numbers of the two species in this experiment so individual snails were randomly allocated to pots until there were four to each pot. The sizes and species in each pot were thus decided randomly, but when there were four snails in a pot it was removed from the possible choices for the next snail.

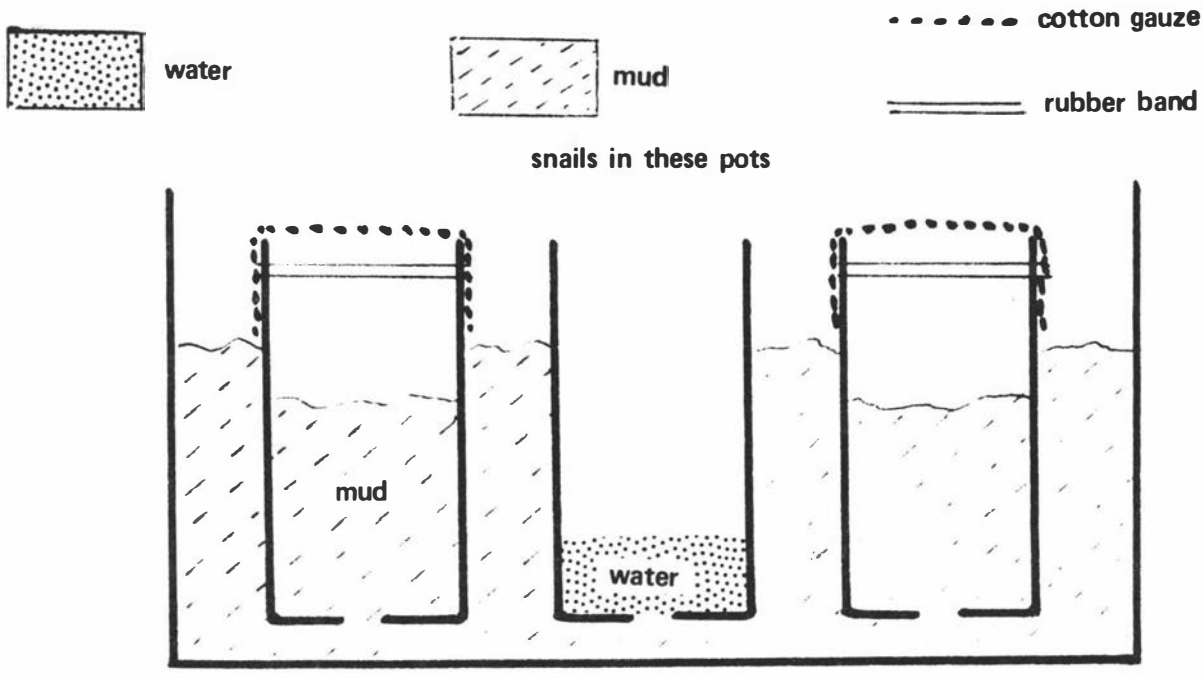


Fig. 9.2 End elevation of an aquarium from experiment 9.2

(a) aquaria 1 and 4
snails placed on mud surface

(b) aquaria 2 and 3
snails in water

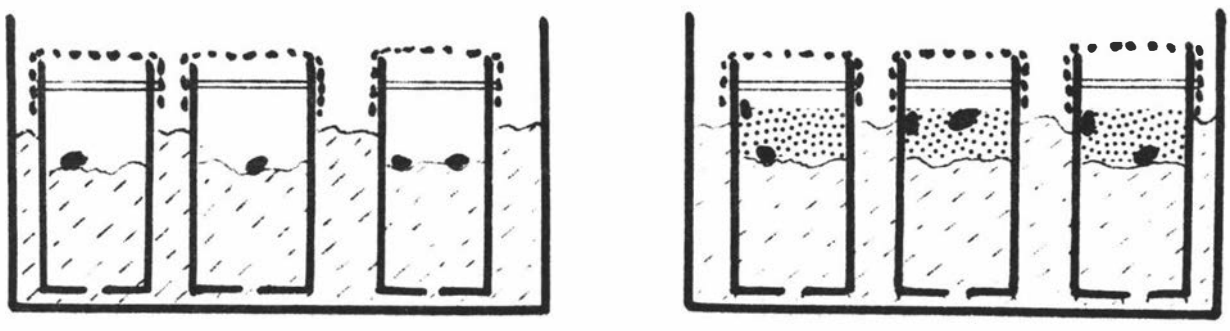


Fig. 9.3 End elevation of aquaria in experiment 9.3 showing the two treatments

In experiment 9.2 snails were kept in a thermostatically controlled room at 22°C. Relative humidity varied between 59 and 97% due to evaporation from other open aquaria in the room, and the mud surface never dried or cracked as in experiment 9.1. After thirty-nine days visible snails were removed and the mud in the pots and aquaria was flooded for twenty-four hours in an attempt to recover live snails which may have burrowed below the surface.

Experiments 9.2 and 9.3 included consideration of the position of snails at the end of the experiment i.e. whether they were found on the mud surface, on the side of the pot, had escaped from it and were recovered from outside, or were lost. Size ranges at the start of experiment 9.2 were:

L. tomentosa - 3.1 to 7.4 mm
L. columella - 3.2 to 10.1 mm.

Experiment 9.3

In the four aquaria used in this experiment the depth of mud was as in experiment 9.2 i.e. 6 cm outside the pots and approximately 4.5 cm inside them. However this time the middle row contained mud and was used in the same way as the rest. This gave a total of sixty pots, to each of which was randomly allocated one L. tomentosa and one L. columella.

In two of the aquaria, replicates one and four, snails were placed directly on wet mud. In the other two the pots were three-quarters filled with water which subsided slowly over a few days, partly by evaporation and partly by diffusion into the mud outside (Fig. 9.3). Selection of aquaria for treatments was as for random block experiments i.e. having decided that aquaria one and four would constitute one block while two and three would be the other, the treatments were decided on the toss of a coin.

In experiment 9.3 the aquaria were kept in a room whose temperature varied from 15 to 28°C and relative humidity fluctuated between 34 and 94%. Temperature and relative humidity were recorded by a hair operated thermo-hygrograph. The range of shell lengths of snails at the start of the experiment was:

L. tomentosa - 3.4 to 7.8 mm
L. columella - 3.0 to 12.8 mm.

After thirty-five days experiment 9.3 was terminated by removing visible snails and flooding the pots as in experiments 9.1 and 9.2.

9.3 RESULTS

Results are summarised in Appendices 9.1, 9.2 and 9.3.

Survival

The survival rate of L. tomentosa recovered at the end of experiment 9.1 was significantly higher than that of L. columella (Table 9.1) but there were no significant differences in survival rates between the species in the other two experiments (Tables 9.2; 9.3). This was true whether snails remained on mud or climbed onto the sides of the pots during experiment 9.2 (Table 9.9; 9.11). There was a surprising tendency, not quite significant at the .05 level of probability, for L. columella to survive better on the side walls of the pots during experiment 9.3 than on the mud surface (Table 9.7) and to be more likely to survive in this location than L. tomentosa (Table 9.12). There was a converse tendency in experiment 9.3 for L. tomentosa to survive better on mud than on the side walls (Table 9.8) and to be more likely to survive on mud than L. columella (Table 9.10). However differences did not reach the .05 level of significance and there was no evidence of any of these trends in experiment 9.2 (Tables 9.5; 9.6; 9.9; 9.11). In experiment 9.2 snails survived equally well on mud or on the sides of the pots and there were no differences in survival rates between the species in either location.

Experiment 9.3 afforded an opportunity to test whether the snails were more likely to survive when placed directly on a mud surface as in aquaria one and four, or if subjected to the receding water level of aquaria two and three. When placed directly onto mud the survival rates of L. tomentosa and L. columella were almost identical (Table 9.13); L. tomentosa tended to survive better than L. columella when subjected to a receding water level although this result was not statistically significant (Table 9.14). L. columella had a significantly higher survival rate when placed on mud than

when water was allowed to recede (Table 9.15) but the proportions of L. tomentosa surviving in the two locations were almost identical (Table 9.16).

Shell length clearly did not play the same role in determining the ability of snails to survive on mud which it did when they were dried in air (see Chapter 8). L. tomentosa survivors in experiment 9.1 were a little smaller than the snails that died, the mean shell lengths of the two groups, 4.58 mm and 5.53 mm, being significantly different at the .05 level of probability (Fig. 9.4; Appendix 9.1). Too few L. columella survived to allow meaningful comparisons between mean shell lengths of live and dead snails. In neither species were there any obvious differences in the sizes of live and dead snails at the end of experiment 9.2 (Appendix 9.2). The analysis of variance could not be used on the results of experiment 9.3 for L. columella because the variances were heterogeneous (Appendix 9.3). Although the mean shell length of L. columella survivors, 7.53 mm, was less than that of snails that died, 8.38 mm, there was sufficient overlapping of their 95% confidence intervals (Fig. 9.4) to show the difference was of no statistical significance. However the small difference between the mean shell lengths of L. tomentosa survivors and dead snails in experiment 9.3, 4.91 mm compared to 5.78 mm, was significant at the .001 level of probability (Fig. 9.4).

Behaviour

L. columella exhibited a marked tendency to migrate from the pots during experiment 9.1 and had to be replaced on the mud surface several times daily in the early stages. Many were returned from outside the pots and may have been returned to ones other than those from which they escaped. Only two escaped from the confines of the aquaria and these were assigned to the "lost" category (Table 9.1). Twenty other L. columella could not be located at the end of the experiment, a surprisingly high proportion of the original fifty, including some quite large snails which should have been easy to recover. Only eight of the fifty L. tomentosa could not be recovered, and relatively few had to be returned to the mud surface in the early stages of the experiment.

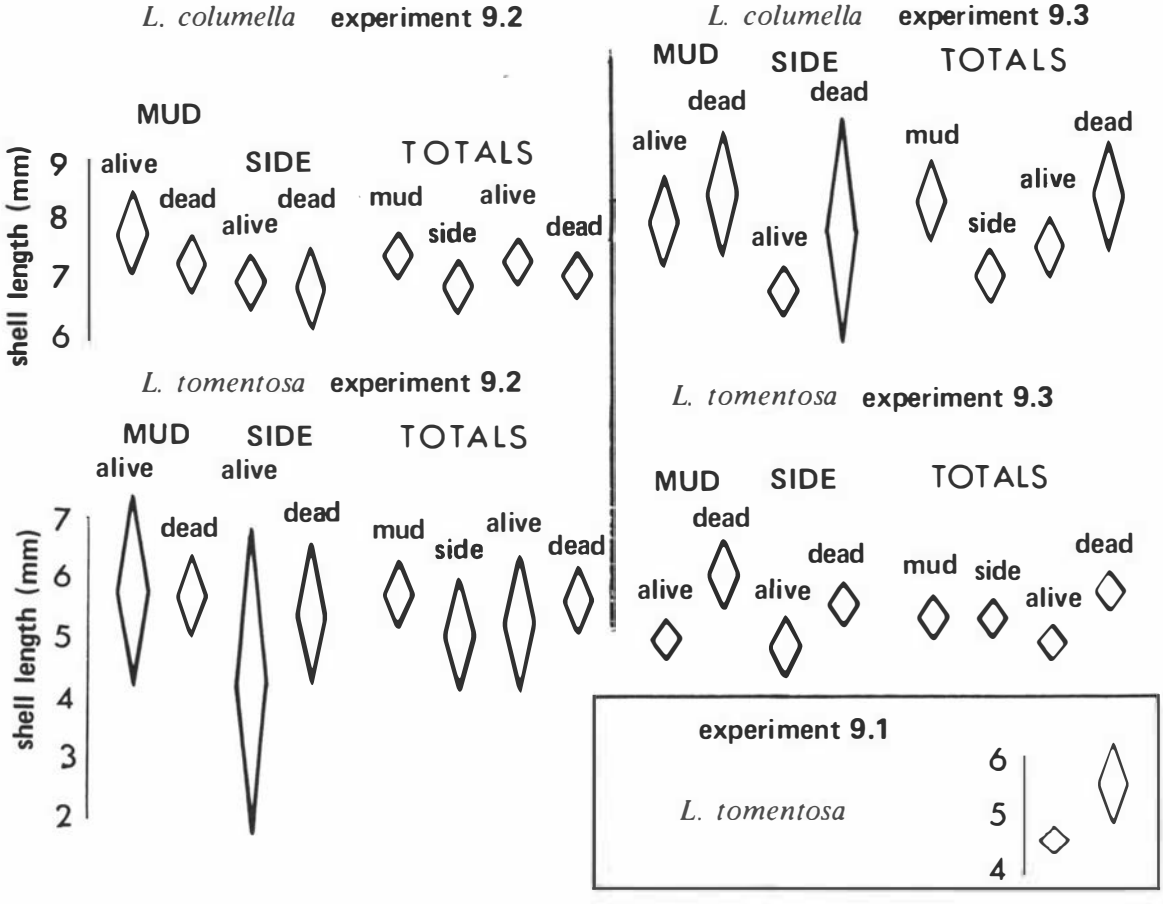


Fig. 9.4 Means and 95% confidence limits of shell lengths for groups of *L. tomentosa* and *L. columella* exposed to desiccation on mud

At the end of experiment 9.2 eight of the original seventy-seven L. columella were not recovered from their pots in spite of the gauze covering but all of the thirty-two L. tomentosa were recovered. In experiment 9.3 the numbers not recovered from the pots were identical, nine out of sixty snails of each species.

In experiment 9.2 L. tomentosa tended to remain on mud while almost half of the L. columella moved to the sides of the pots (Table 9.17). This trend was reversed in experiment 9.3 with L. columella being the more likely to remain on mud (Tables 9.18; 9.19; 9.20). Neither of these results showed statistically significant behavioural differences between L. tomentosa and L. columella in experiments 9.2 and 9.3.

There was a marked tendency for smaller L. columella to leave the mud surface and attach themselves to the sides of the pots, particularly in experiment 9.3 (Fig. 9.4). A similar tendency for L. tomentosa in experiment 9.2 was not statistically significant and in experiment 9.3 the mean shell lengths of L. tomentosa on mud and on the sides of the pots were almost identical. In experiment 9.3 the snails remaining on mud varied in size much more than the more homogenous groups on the sides of the pots. This applied particularly to L. columella whose variances for the two locations, when subjected to an F max. test (Sokal and Rohlf, 1969b) gave

$$F \text{ max. } 2,10 = \frac{4.708}{.545} = 8.64\ **$$

The two variances for L. tomentosa did not differ significantly, and there was no evidence of this phenomenon in either species in experiment 9.2 (Appendices 9.2; 9.3).

TABLE 9.1 Survival rates of snails recovered at the end of experiment 9.1

	alive	dead	totals	
L. tomentosa	30	12	42	
L. columella	2	26	28	
totals	32	38	70	$\chi^2 = 26.568^{***}$

TABLE 9.2 Survival rates of snails recovered at the end of experiment 9.2

	alive	dead	totals	
L. tomentosa	24	53	77	
L. columella	8	24	32	$\chi^2 = 0.170$ (n.s.)
totals	32	77	109	

TABLE 9.3 Survival rates of snails recovered at the end of experiment 9.3

	alive	dead	totals	
L. tomentosa	24	27	51	
L. columella	27	24	51	$\chi^2 = 0.157$ (n.s.)
totals	51	51	102	

TABLE 9.4 Survival rates of snails recovered at the end of experiment 9.2 with regard to the location of the snails

L. columella

mud	side	outside pots	lost
40 (1)	38	2	6

alive	dead	alive	dead
10	29	14	24

L. tomentosa

mud	side	outside pots	lost
23 (1)	10	0	0

alive	dead	alive	dead
5	17	3	7

(1) one snail was lost before being tested for signs of life

TABLE 9.5 Survival of L. columella with regard to its location at the end of experiment 9.2

	alive	dead	totals	
mud	10	29	39	
side	14	24	38	$\chi^2 = 0.70$ (n.s.)
totals	24	53	77	

TABLE 9.6 Survival of L. tomentosa with regard to its location at the end of experiment 9.2

	alive	dead	totals	
mud	5	17	22	
side	3	7	10	$\chi^2 = 0.00$ (n.s.)
totals	8	24	32	

TABLE 9.7 Survival of L. columella with regard to its location at the end of experiment 9.3

	alive	dead	totals	
mud	15	24	39	$\chi^2 = 3.681$ (n.s.)
side	9	3	12	(χ^2 (.05) = 3.841)
totals	24	27	51	

TABLE 9.8 Survival of L. tomentosa with regard to its location at the end of experiment 9.3

	alive	dead	totals	
mud	20	11	31	$\chi^2 = 3.173$ (n.s.)
side	7	13	20	(χ^2 (.05) = 3.841)
totals	27	24	51	

TABLE 9.9 A comparison of survival rates among snails located on mud at the end of experiment 9.2

	alive	dead	totals	
L. tomentosa	5	17	22	χ^2 = 0.00 (n.s.)
L. columella	10	29	39	
totals	15	46	61	

TABLE 9.10 A comparison of survival rates among snails located on mud at the end of experiment 9.3

	alive	dead	totals	
L. tomentosa	20	11	31	χ^2 = 3.706 (n.s.)
L. columella	15	24	39	(χ^2(.05) = 3.841)
totals	35	35	70	

TABLE 9.11 A comparison of survival rates among snails located on the sides of the pots at the end of experiment 9.2

	alive	dead	totals	
L. tomentosa	3	7	10	
L. columella	14	24	38	χ^2 = 0.00 (n.s.)
totals	17	31	48	

TABLE 9.12 A comparison of survival rates among snails located on the sides of the pots at the end of experiment 9.3

	alive	dead	totals	
L. tomentosa	7	13	20	χ^2 = 3.334 (n.s.)
L. columella	9	3	12	
totals	16	16	32	

TABLE 9.13 Survival of snails placed directly on the mud surface of aquaria one and four in experiment 9.3

	alive	dead	totals	
L. tomentosa	16	12	28	χ^2 = 0.00 (n.s.)
L. columella	18	12	30	
totals	34	24	58	

TABLE 9.14 Survival of snails in pots whose water was allowed to recede gradually i.e. aquaria two and three of experiment 9.3

	alive	dead	totals	
L. tomentosa	16	13	29	χ^2 = 1.670 (n.s.)
L. columella	8	15	23	
totals	24	28	52	

TABLE 9.15 Survival of L. columella (a) on mud and (b) in pots with a receding water line (experiment 9.3)

	alive	dead	totals	
(a) mud	18	6	24	χ^2 = 6.077 *
(b) receding water	8	15	23	
totals	26	21	47	

TABLE 9.16 Survival of L. tomentosa (a) on mud and (b) in pots with a receding water line (experiment 9.3)

	alive	dead	totals	
(a) mud	16	12	28	χ^2 = 0.00 (n.s.)
(b) receding water	16	13	29	
totals	32	25	57	

TABLE 9.17 Location of snails recovered at the end of experiment 9.2

	mud	side	totals	
L. tomentosa	23	10	33	$\chi^2 = 2.50$ (n.s.)
L. columella	40	38	78	
totals	63	48	111	

TABLE 9.18 Location of snails recovered at the end of experiment 9.3 - pooled results

	mud	side	totals	
L. tomentosa	31	20	51	$\chi^2 = 2.231$ (n.s.)
L. columella	39	12	51	
totals	70	32	102	

TABLE 9.19 Location of snails recovered at the end of experiment 9.3(a); snails placed on mud surfaces of pots in aquaria one and four

	mud	side	totals	
L. tomentosa	17	11	28	
L. columella	22	7	29	$\chi^2 = 1.200$ (n.s.)
totals	39	18	57	

TABLE 9.20 Location of snails recovered at the end of experiment 9.3(b); snails placed in pots with receding water lines

	mud	side	totals	
L. tomentosa	14	9	23	
L. columella	17	5	22	$\chi^2 = 0.720$ (n.s.)
totals	31	14	45	

9.4 DISCUSSION

The main purpose of these experiments was an attempt to compare the responses of L. tomentosa and L. columella under similar conditions rather than to obtain an absolute measure of the resistance of either species under specified conditions. This was the reason for attempting to confine the snails to the flower pots to which they had been randomly allocated; conditions were as similar as possible for both species even though such factors as soil porosity and moisture were not measured. The results nevertheless suggest that under some experimental conditions about 50% of L. tomentosa and L. columella will survive desiccation on mud for a period of approximately one month. Whether some of the snails that survived the initial desiccation would have been capable of surviving for very much longer periods was not investigated.

The large number of L. columella lost during experiment 9.1 made interpretation difficult in spite of the apparently clear-cut result. The significantly smaller mean length of the missing snails may have meant that the smaller snails were more likely to migrate as in experiments 9.2 and 9.3, or merely that they were more difficult to find. Nevertheless the disappearance of some specimens up to 13 mm in length was somewhat of a mystery since they should have been relatively easy to locate even outside the pots or in some part of the incubator. If they burrowed below the surface of the mud the twenty-four hour flooding at the end of the experiment should have allowed survivors an opportunity to return to the surface since virtually all desiccated snails that revive after re-hydration will do so within a maximum period of three hours (see Chapter 8). However some live snails that burrowed could have been trapped in the firm mud even after the aquarium was flooded, and dead snails below the surface would not have been recovered.

Ideally the mud would have been sieved under running water to search the deeper layers for missing snails. This was not practicable in this series of experiments because the dry soil set very firmly and the roots of vegetation acted as a reinforcing material. Attempts at sieving had to be accompanied by efforts to break up the larger lumps manually, a procedure which almost certainly destroyed any shells that may have been present. Another point is that at least twenty-eight days elapsed before examination of the pots and the remains of snails dying at an early stage would probably not be recoverable since both

species have thin, fragile shells.

The heterogeneous variances between the groups of L. columella on mud and on the sides of the pots in experiment 9.3 meant that an analysis of variance on raw data was not a strictly valid method of comparing mean shell lengths. Nevertheless consideration of the results, a larger variance and greater mean shell length in the group remaining on mud, gives some insight into the behaviour of L. columella in this experiment. Some of the smaller snails migrated to the sides of the pots and some remained on the mud surface but almost all the larger snails remained on the mud. This explains the smaller variance and mean shell length of the group on the sides of the pots. Only twelve L. columella were found on the walls while thirty-nine remained on mud in experiment 9.3. In experiment 9.2 where almost equal numbers of L. columella were present in both locations the differences between the two groups were less obvious.

The different behaviour of snails in each experiment may have been due to variations in relative humidity. Lynch (1966) found that L. tomentosa would burrow into mud only when soil moisture was between 33 and 40% and relative humidity was low. Some L. columella that were not recovered may have migrated, since their inclination to leave unfavourable habitats seems to be greater than that of most species (see section 1.4.6) and has often been observed during their routine maintenance in this laboratory.

It was surprising that snails that remained on the mud surface (or returned to it, since this could also have been responsible for their location on mud at the end of the experiments) did not have a much lower mortality rate than those on the sides of the pots. The traces of mud on the sides of the pots may have been sufficient to block their apertures and reduce dehydration since snails in both situations survived very much longer than those dried in an environment where they were denied access to mud (Chapter 8).

In most cases smaller snails were more likely to survive (Fig. 9.1), a reversal of the results obtained when snails are dried in air. The better survival rate of smaller snails is in accordance with results obtained by some other authors working with lymnaeid snails (Cridland, 1957; 1967; Shiff, 1960) but at variance with one observation on L. truncatula (Kendall, 1949a).

The variability of results reported in this chapter suggests that reports of experiments in which snails are subjected to desiccation on mud should be treated with some caution. There are clearly many variable factors involved. Some of them, such as soil moisture gradients in the immediate vicinity of the snails at different stages of the experiment, may not be amenable to measurement. Even when it is known, soil moisture is not an adequate measure of its properties as a desiccant unless its porosity is also known, and other important factors may as yet be unsuspected.

10. SHELL SHAPE

10.1 INTRODUCTION

There are quite marked variations in shell shape within species of Lymnaea, so that shell shape is not a reliable diagnostic aid in species identification (Hubendick, 1951). Hubendick attributed the great variability within species, coupled with a relatively constant shell shape within populations, mainly to "the general occurrence of self-fertilisation" and the consequent tendency to establish clones. This variability between different populations of one species occurs in L. tomentosa (Boray and McMichael, 1961), L. truncatula (Peters 1938a) and L. columella (Hubendick, 1951) although there seems to be little variation between New Zealand populations of L. columella (Climo and Pullan, 1972).

Where differences between populations are gross they may be obvious enough for a general description to suffice, but less marked differences need a more objective treatment. Shells should preferably be measured by one person using a single method and statistical analysis ought to take account of:

1. the sizes of the shells, and
2. the dates on which they were collected and measured.

If there are changes in shell shape associated with size, these could cause apparent differences between populations composed of snails of different sizes. The dates on which collections were made could be important if there are seasonal changes in shape, and there could be unintentional changes in the method of measuring on different dates.

This chapter contains a description of changes in shell shape associated with changes in size in two field populations of L. columella and a laboratory colony of L. tomentosa derived from a single field population.

10.2 METHODS

The two L. columella populations occupied a pond and a spring fed marsh only 400 m apart on the Pohangina farm. The habitats were the

pond and the marsh referred to in Chapter 3. Five pond and six marsh collections were made between January 1970 and March 1971. Since the snails were to be examined later for Fasciola hepatica infection, a procedure which involved crushing the shells, all L. columella measurements were of live snails, 183 from the pond and 310 from the marsh.

The L. tomentosa shells were from a laboratory population derived originally from the marsh described as habitat no. 3 in Chapter 3. Shells were collected over a period of six months from aquaria or from laboratory experiments. (Snails were not normally returned to aquaria at the end of experiments in case their treatment interfered with responses in subsequent experiments). Empty L. tomentosa shells were measured, the shells being held by a thin film of petroleum jelly on a glass slide. The total number of L. tomentosa shells measured was seventy-seven.

A dissection microscope with a micrometer eyepiece was used for all snail measurements, care being taken to keep both extremities in focus to avoid parallax errors (Fig. 10.1). The shell dimensions used were those shown in Fig. 10.2. A programme for an IBM 1620 computer was prepared by the author. It calculated the following proportions (expressed as percentages) and output data in a suitable form for subsequent statistical analysis:

PA = FG/AB = altitude of the main body whorl as a percentage of shell length

PB = BC/AB = aperture length as a percentage of shell length

PC = DE/AB = aperture width as a percentage of shell length

PD = BC/DE = aperture length as a percentage of shell width.

Snails were grouped into the following shell length categories in order to examine the way in which shell shape altered with size:

1. 0 - 1.9 mm
2. 2.0 - 3.9 mm
3. 4.0 - 5.9 mm
4. 6.0 - 7.9 mm
5. 8.0 - 9.9 mm
6. $\geq$ 10 mm.

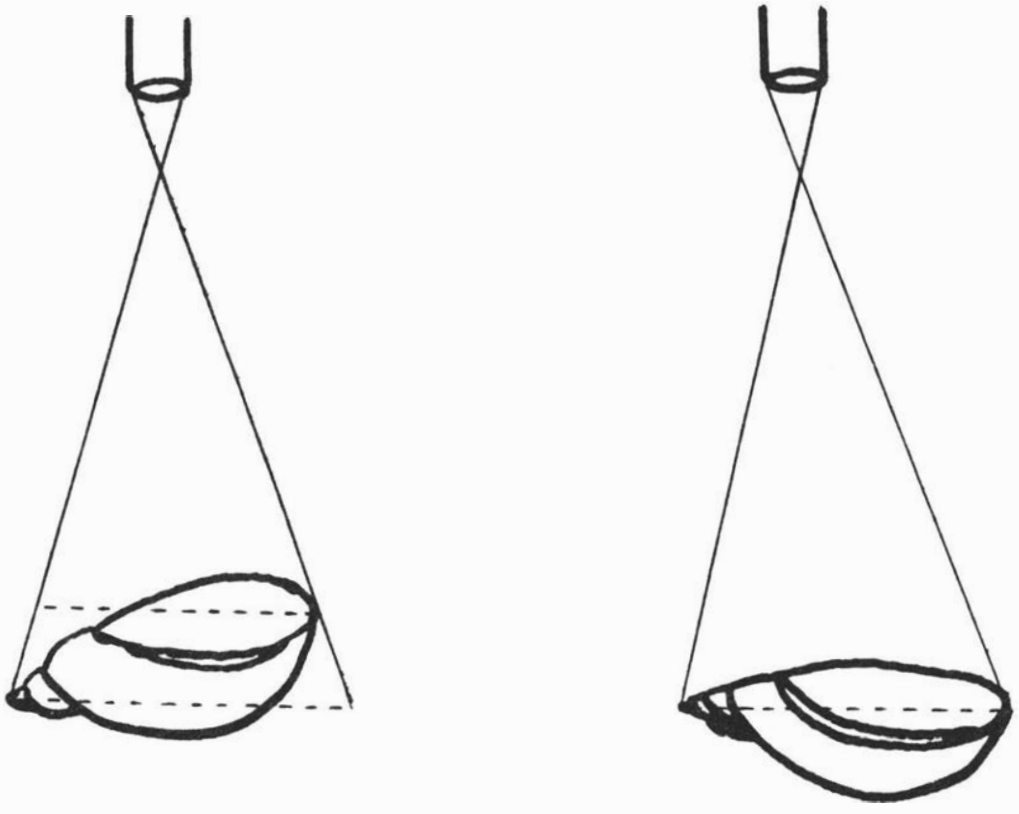


Figure 10.1 Parallax errors arise if both extremities of the object are not kept in focus

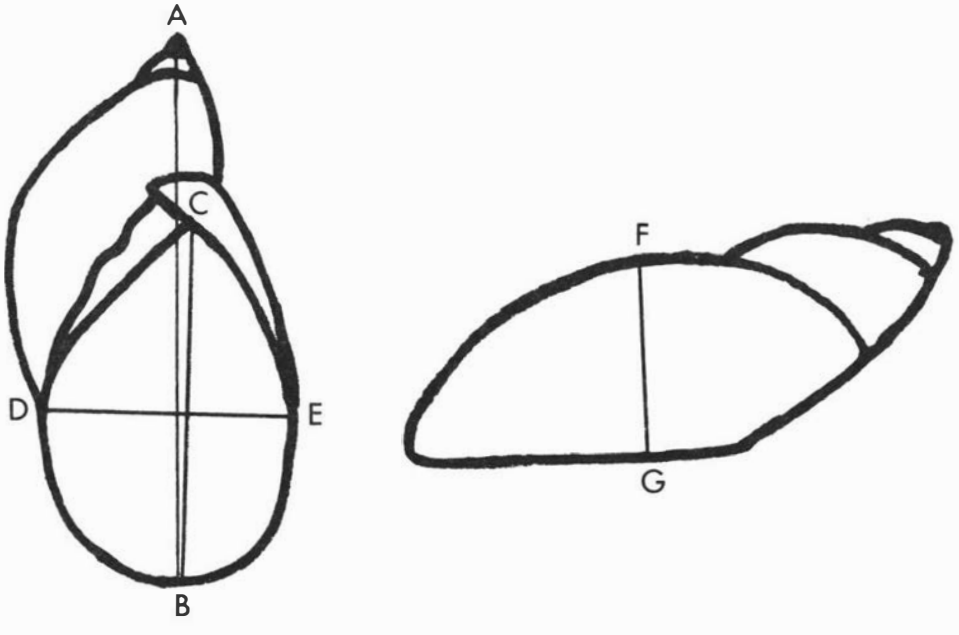
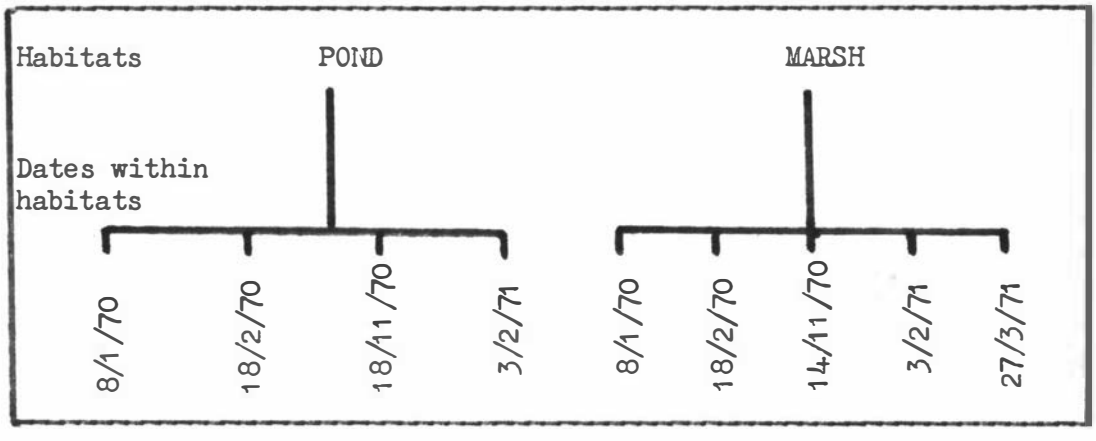


Figure 10.2 AB = shell length BC = aperture length DE = aperture width
FG = altitude of the main body whorl
Shell shape parameters referred to in the text
PA = FG/AB PC = DE/AB PB = BC/AB PD = BC/DE

Only data from L. columella were in a form which could be used to investigate the effects of habitats and collection dates on shell shape, the samples being drawn from two habitats on a number of dates. The data were subjected to two level nested analyses of variance in which habitats were the main factor with collection dates as the second level "within habitat" factor (Table 10.1).

TABLE 10.1 Design of the two level nested analysis of variance used to investigate the importance of habitats and dates of collection as factors influencing the shell shape of L. columella



Analyses were carried out for each of the four proportions being investigated. Since PB (Fig. 10.2) was constant throughout all shell sizes (Fig. 10.3) a pooled collection of shells of all sizes was used as the sample. However PA, PC and PD varied with shell length so only measurements from class three, snails measuring 4.0 to 5.9 mm, were used in the analyses. This was the only class with enough snails in collections from both habitats.

10.3 RESULTS

10.3.1 Comparison of L. tomentosa and L. columella

When pooled samples of L. columella were compared with L. tomentosa none of the four ratios could be used as a diagnostic aid in separating smaller specimens of the two species (Fig. 10.3). Larger shells clearly differed, but these are easily distinguished visually without resort to measurement. In snails of similar shell length the aperture of large L. tomentosa is clearly longer (Fig. 10.3b)

and wider (Fig. 10.3c) than that of L. columella. Since L. tomentosa appears more globose than L. columella it was surprising that they did not differ more markedly with regard to PA, altitude of the main body whorl as a percentage of total shell length, but in all except the largest specimens there was some overlapping between species (Fig. 10.3a).

10.3.2 Changes in shell shape associated with size

PA, shell altitude as a percentage of shell length

L. columella became flatter with increases in shell length; the altitude of the main body whorl was a smaller percentage of total shell length in larger specimens (Fig. 10.3a; Table 10.2). There was a similar but less marked tendency in L. tomentosa but the trend was reversed in the four largest specimens which comprised the 8.0 - 9.9 mm group. The differences in PA between L. tomentosa of different shell lengths just failed to reach the .05 level of statistical significance.

PB, aperture length as a percentage of shell length

The length of the aperture of L. columella remained a constant percentage of the total shell length in all size classes (Fig. 10.3b; Table 10.2). A similar finding was reported by Colton (1912). By contrast, the aperture of the larger L. tomentosa became relatively longer and wider with increases in shell length until it extended to approximately 80% of the shell length of the largest specimens (Fig. 10.3b and c; Appendix 10.1).

PC, aperture width as a percentage of shell length

There was a slight, but statistically significant, tendency for the aperture of L. columella to become relatively narrower with increases in shell length (Fig. 10.3c; Table 10.2). As noted previously, the aperture of L. tomentosa became relatively wider. Snails of both species had similar aperture widths in the smaller size classes but their aperture width to shell length ratios became increasingly divergent with increases in overall length.

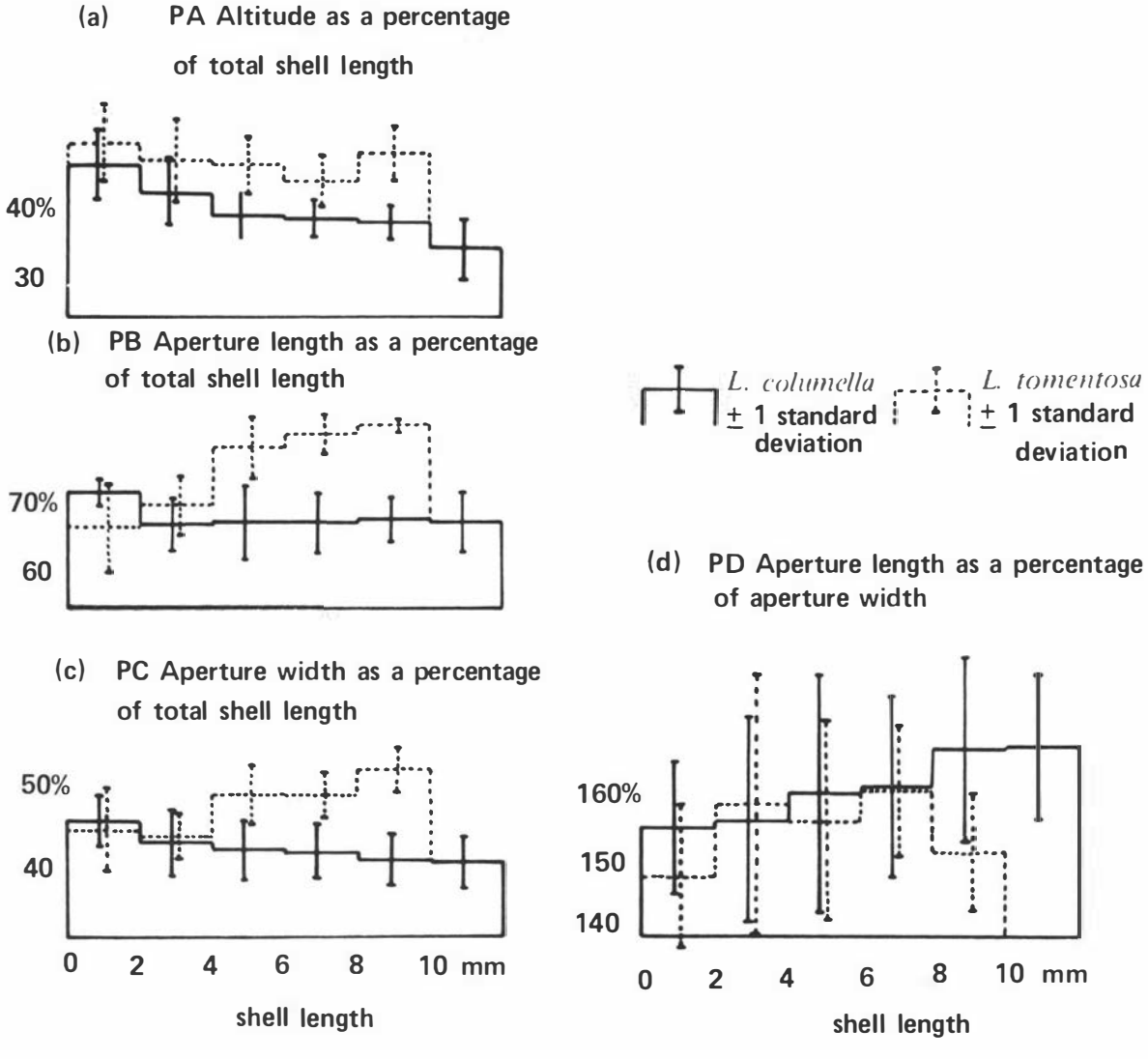


Fig.10.3 Changes in various shell dimension ratios in groups of snails of different shell length

KEY (See Fig. 10.2)

PA = altitude as a percentage of total shell length
PB = aperture length as a percentage of total shell length
PC = aperture width as a percentage of total shell length
PD = aperture length as a percentage of aperture width

TABLE 10.2 Analyses of variance to determine whether some shell proportions change significantly between snails in different shell length groups

KEY PA = shell altitude as a percentage of total shell length
PB = aperture length as a percentage of total shell length
PC = aperture width as a percentage of total shell length
PD = aperture length as a percentage of aperture width

shell ratio	source of variation	d.f.	S.S.	M.S.	F 5,487
			L. columella		
PA	groups	5	1303.18	260.64	23.682 ***
	within	487	5359.75	11.01	
	total	492	6662.93		
PB	groups	5	95.82	19.16	1.053 (n.s.)
	within	487	8866.98	18.21	
	total	492	8962.80		
PC	groups	5	319.84	63.97	4.636 ***
	within	487	6719.21	13.80	
	total	492	7039.05		
PD	groups	5	4501.61	900.32	4.526 ***
	within	487	96870.39	198.91	
	total	492	101372.00		
shell ratio	source of variation	d.f.	S.S.	M.S.	F 4,72
			L. tomentosa		
PA	groups	4	153.19	38.30	2.214 (n.s.)
	within	72	1245.65	17.30	($F_{4,70(.05)}$ = 2.50)
	total	76	1398.83		
PB	groups	4	1238.00	309.50	20.521 ***
	within	72	1085.91	15.08	
	total	76	2323.91		
PC	groups	4	438.28	109.57	7.768 ***
	within	72	1015.54	14.11	
	total	76	1453.82		
PD	groups	4	695.80	173.95	1.126 (n.s.)
	within	72	11125.50	154.52	
	total	76	12517.10		

PD, aperture length as a percentage of aperture width

Since the aperture of L. columella formed a constant percentage of its total shell length but became relatively narrower with increases in shell length, one would expect the aperture length to width ratio to increase. This in fact occurred, as may be seen in Fig. 10.3d. The aperture length to width ratio of L. tomentosa did not alter in any predictable manner, a result compatible with the observation that both the relative length and width of the aperture increase in larger shells.

10.3.3 Changes in the shell shape of L. columella associated with habitats and collection dates

There were no significant differences in shell shape between snails from the pond and the marsh, as measured by any of the four chosen ratios (Table 10.3). The most variable ratio, PA, differed between habitats to a degree which was almost significant at the .05 level of probability, but this was almost certainly due to random error as illustrated in Fig. 10.4. The value of PA decreased almost linearly in samples from the marsh and in the pooled sample of all L. columella, but in pond samples the value of PA was less than expected in class 3, shells 4.0 to 5.9 mm. Class 3 was the group selected for analysis and the histograms of Fig. 10.4 suggest that its value was probably less than would be found on re-sampling.

There were, however, highly significant differences in the shape of shells collected and measured on different dates. Although in probability terms the variation between collection dates was highly significant, the actual amount of variation was small. This is shown in Table 10.4, where the standard deviations from the pooled samples of L. columella are no greater than those from the L. tomentosa sample which was measured on a single date.

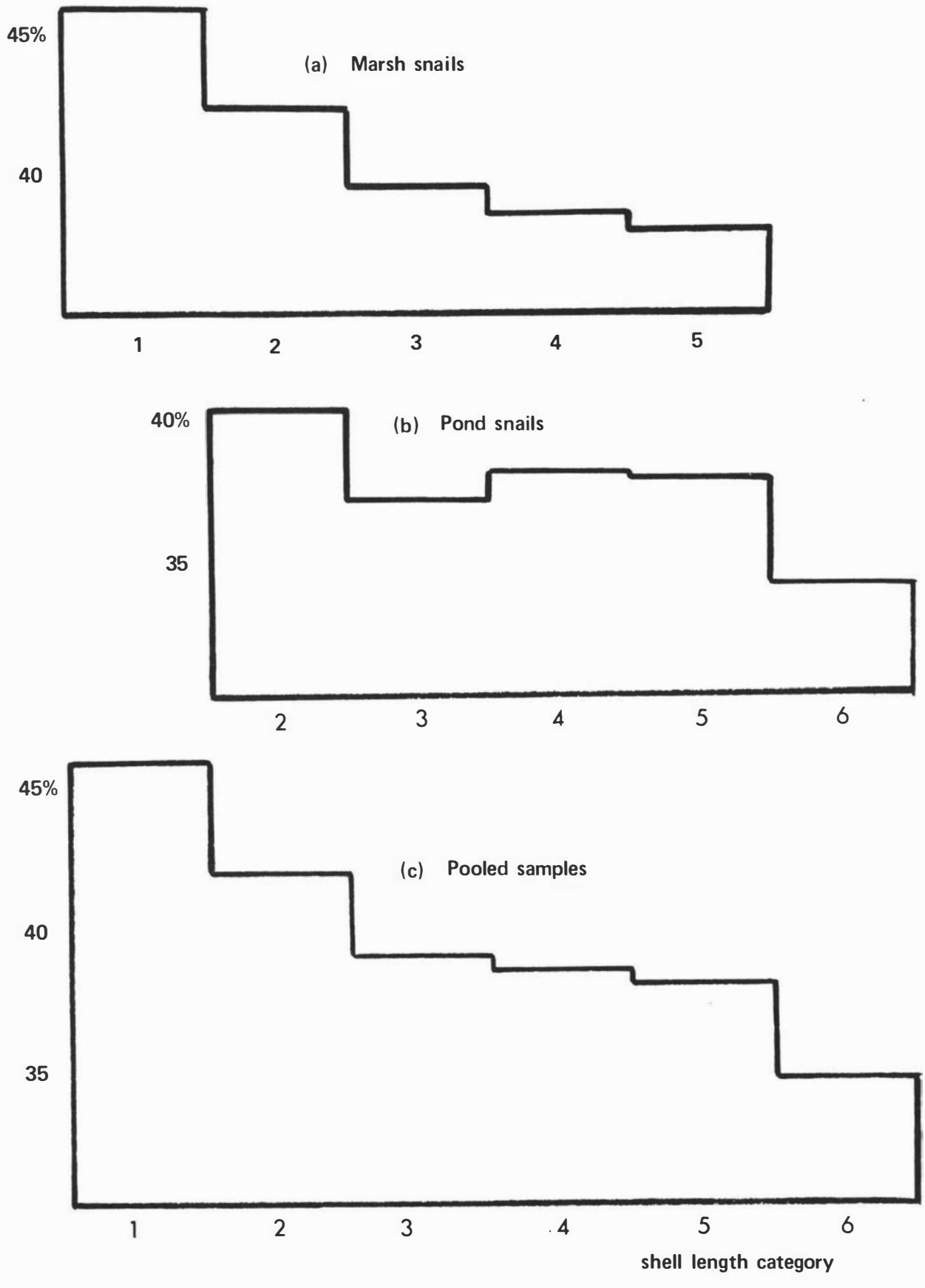


Fig. 10.4 Changes in PA associated with shell length in *L. columella*
(PA is expressed as height/shell length x 100)

shell length categories:
1 = 0-1.9 mm
2 = 2.0 - 3.9 mm
3 = 4.0 - 5.9 mm
4 = 6.0 - 7.9 mm
5 = 8.0 - 9.9 mm
6 = ≥ 10 mm

TABLE 10.3 Results of two level nested analyses of variance to determine whether the shell shape of *L. columella* differed between habitats and collection dates

proportion	shell lengths	source of variation	d.f.	M.S.	F
PA	4.0 to 5.9 mm	habitats	1	206.409	5.254 (n.s.)
		collections	7	37.598	4.502 ***
		residual	169	8.351	
		total	177		($F_{1,7(.05)}$= 5.59)
PB	all sizes	habitats	1	14.170	0.807 (n.s.)
		collections	8	52.414	2.986 **
		residual	480	17.555	
		total	489		
PC	4.0 to 5.9 mm	habitats	1	2.991	0.029 (n.s.)
		collections	7	42.374	4.156 ***
		residual	169	10.196	
		total	177		
PD	4.0 to 5.9 mm	habitats	1	26.068	0.103 (n.s.)
		collections	7	448.032	1.775 (n.s.)
		residual	169	252.342	
		total	177		($F_{7,150(.05)}$= 2.07)

TABLE 10.4 Variability of shell shape in L. tomentosa and L. columella

KEY $\bar{X}$ = mean value
s = standard deviation
n = number of shells measured

shell length	L. columella (pooled samples) n		PA	PB	PC	PD	L. tomentosa n		PA	PB	PC	PD
<2.0 mm	4	$\bar{X}$	45.82	70.86	45.90	154.86	4	$\bar{X}$	48.78	65.93	44.68	148.21
		s	4.84	1.80	3.40	9.11		s	5.26	6.30	5.79	10.81
2.0-	91	$\bar{X}$	41.90	66.53	42.99	155.92	11	$\bar{X}$	46.30	68.97	43.88	158.18
3.9 mm		s	4.67	3.66	4.42	14.07		s	5.77	4.20	3.20	17.71
4.0-	180	$\bar{X}$	38.94	66.77	42.01	159.72	34	$\bar{X}$	45.74	76.90	49.66	155.71
5.9 mm		s	3.30	5.03	3.39	16.03		s	3.94	4.32	4.18	13.76
6.0-	137	$\bar{X}$	38.41	66.76	41.80	160.52	24	$\bar{X}$	43.60	78.84	49.45	159.88
7.9 mm		s	2.44	4.10	3.77	12.36		s	3.45	2.75	3.02	9.09
8.0-	70	$\bar{X}$	37.91	67.08	40.65	165.89	4	$\bar{X}$	47.43	80.10	52.97	151.55
9.9 mm		s	2.35	3.10	3.44	12.69		s	3.75	0.86	3.03	7.97
10.0 mm	11	$\bar{X}$	34.49	66.72	40.31	166.05	0	$\bar{X}$				
		s	4.31	4.13	3.42	9.83		s				
Total	493	$\bar{X}$	39.15	66.80	41.93	160.22	77	$\bar{X}$	45.40	75.97	48.68	156.76
		s	3.68	4.27	3.78	14.35		s	4.29	5.53	4.37	12.83

10.4 DISCUSSION

Where differences in shell shape are visually obvious an illustrated description of the differences may suffice, as in Boray and McMichael's (1961) paper on the taxonomy of L. tomentosa. Hubendick (1951) graphed various indices of shell shape to illustrate changes associated with increases in the length of L. peregra and differences between populations, but his approach was descriptive rather than analytical. He could presumably have tested his results by some form of statistical analysis but he was dealing with a very large number of populations, some of which contained only a few snails whose mean shell lengths varied from 7.3 to 25.9 mm.

Where there are adequate numbers of snails of comparable size the analytical methods described in this chapter provide an objective method of obtaining:

1. a quantitative description of the shell shape of a snail population, including changes of shape associated with shell length, and
2. a comparison of one species with another, or different populations of the same species.

The simple analysis of variance was used to compare the shell shape of groups with different shell lengths. They could also have been compared by regression analysis, but this would have required an assumption that the changes in shape occurred linearly or exponentially. However with smaller numbers of snails a regression analysis may be a more satisfactory way of expressing the changes associated with shell length, since the analysis of variance requires an adequate number in each group.

The two level nested analysis of variance was chosen to test the effects of habitats and collection dates on shell shape because there were different numbers of collection dates for each habitat, and the collection dates were regarded as the equivalent of a random sample of all possible collection dates.

The results show that the mean shell length of a population may influence shell shapes, and this should be borne in mind when comparing populations with different mean shell lengths. Another important source of difference is the date of collection or measurement. This may be due to technical error, or to seasonal changes in shell

shape, since variations in the rate of growth may alter shell morphology (Boray and McMichael, 1961). Whether one or both of these factors were responsible in the present study could not be determined because shells were crushed after measurement so that the snails could be examined for F. hepatica infection. If they had been re-measured on other dates the source of the variation might have been determined.

The relatively homogenous shell shape of New Zealand populations of L. columella as reported by Climo and Pullan (1972) is interesting in view of its relatively recent introduction to this country (Pullan, 1969) and the fact that it is a self fertilising hermaphrodite (Colton, 1912) which has not been seen to copulate during the five years in which it has been reared in this laboratory. If one can assume that New Zealand populations of L. columella originated from a few clones, or even a single clone, introduced within the last forty odd years then their homogeneity suggests that the hereditary component of shell shape may be more important than environmental conditioning in this species. The similarity of shells from the marsh and pond habitats supports this view. Quite different results were obtained by Boray and McMichael (1961), who took extreme forms of L. tomentosa from different parts of Australia; after several generations of separate development under controlled conditions these ended up as a single "laboratory" type. It would be interesting to rear extreme forms of L. columella from different parts of North America to see whether a similar phenomenon occurred since North American populations are sufficiently variable to have been regarded at one time as separate subspecies (Baker, 1911).

11. GENERAL DISCUSSION

Fasciola hepatica must have been introduced into New Zealand early in the settlement of the country. Here it found an indigenous lymnaeid host through which its life cycle could be continued. By early in this century the main areas of the country in which *Fasciola* was established were known (Brunsdon, 1967). Judging from the distribution of these areas it seems likely that *Fasciola* was introduced on a number of separate occasions. For many years the situation appeared relatively stable and there was little evidence of spread from the traditional areas even though the intermediate host *Lymnaea tomentosa* is, though often in small numbers, widely distributed throughout the country (Pullan *et al.*, 1972). The change in the distribution of *Fasciola* infected properties that occurred in the last twenty years or so appears to have continued with little comment until Brunsdon (1967) drew attention to it in his review.

The limited distribution and economic importance of *F. hepatica* provided little stimulus for study on the epidemiology of infection or on the ecology of its intermediate hosts. With the realisation that a situation which had been regarded as relatively stable was no longer so, considerably more interest was aroused. When Pullan (1969) pointed out the existence in New Zealand of an exotic snail host whose presence had been unsuspected up to that time, and which appeared to be involved in the transmission of *F. hepatica* in "new" areas (Pullan and Whitten, 1972), the need to study the fluke and its snail hosts became more urgent.

At this stage little was known of the epidemiology of *F. hepatica* in the "traditional" areas and almost nothing about it in the "new" ones. There was evidence that *L. columella* was successfully colonising large areas of freshwater habitat (Pullan *et al.*, 1972) but little information on its ecological requirements, its potential for breeding and further spread, its ability to compete with indigenous snails or on its efficiency in transmitting *F. hepatica*.

The study described in this thesis was carried out to provide some basic information on the biology and ecology of the exotic snail *L. columella* and to compare it with its indigenous rival. The occurrence of an infected property within working distance of the laboratory afforded an opportunity to combine laboratory and field studies on the ecology of the snails with an epidemiological study of the transmission

of F. hepatica on a property colonised by L. columella.

The first stage of the study was an attempt to define typical habitats of L. columella and L. tomentosa. Approximately one hundred habitats of these and other freshwater snails in the Manawatu, Taranaki and Hawke's Bay were visited. Too few of these were inhabited by L. tomentosa to allow definition of unsuitable conditions for that species, although it did not appear in ponds whereas L. columella was a frequent occupier of these habitats. There is evidence in the literature that while L. tomentosa may be found in ponds (Boray, 1964a; Pullan et al., 1972) large, permanent bodies of water are not particularly suitable habitats (Lynch, 1965).

The calcium content of water, pH or soil type did not appear to be limiting factors in determining the habitat of either species. Physical characteristics were more important; rapidly flowing water, marked seasonal fluctuations in depth, and shade from tall vegetation all rendered habitats unsuitable. Hydrological stability was particularly important in marsh habitats; all of those examined were fed by underground springs that kept them moist over at least part of their area at all times.

The apparent similarity of the marsh habitats of L. columella and L. tomentosa suggests that these snails may be competitors in such situations. No certain evidence for this was obtained from field data as snails were rarely found in numbers that seemed likely to tax food or other resources. The fact that L. columella often grew to a larger size in ponds than in marshes suggests that it is primarily a pond snail, perhaps having evolved further than L. tomentosa back towards a truly aquatic existence. It is clear, however, that L. columella is well able to colonise marginally suitable marsh habitats contiguous with or adjacent to its reservoir pond habitats. The greater reproductive capacity of L. columella apparent from field and laboratory studies, would give it a considerable advantage over L. tomentosa as an opportunist in such circumstances.

After the general characteristics of the habitats were defined a logical next step was an attempt to discover the essential features of microhabitats within the major habitats. It was clear from observation of ponds that L. columella was distributed mainly on the surface and near the edges, particularly along the more gently sloping banks.

A more objective approach was made to the study of marsh

microhabitats because of their greater complexity and their importance in the epidemiology of F. hepatica infections. Application of the methods described in Chapter 2 showed that the marsh microhabitats of both species were very similar, perhaps identical. Nevertheless in marshes occupied by both species they each seemed to have their own preferred areas, as in marsh no. 5 referred to in Chapter 3, so there may well be subtle differences undetected by the methods chosen. The fact that L. columella appears to have become established in the northern and western parts of the North Island in habitats previously unoccupied by L. tomentosa suggests that the range of conditions tolerated by L. columella may be greater.

Whether the marsh microhabitats of L. columella wholly overlap those of L. tomentosa is a question of major importance when predicting the future distribution of the two species. If L. columella is a more efficient occupier of all the preferred microhabitats of L. tomentosa then the latter is likely to be totally ousted eventually. However if there are some microhabitats more suited to L. tomentosa than to L. columella then the indigenous snail will never be totally replaced. Prediction of the outcome from the results of the present study would obviously be premature, although L. columella appears to have the advantages of a higher reproductive rate and perhaps a greater range of habitat types.

The temperature tolerance range of a species will clearly influence its ability to colonise new areas. Laboratory data indicated that L. columella eggs have a wider temperature tolerance than those of L. tomentosa. Both species underwent some development above 5°C but at high temperatures L. columella had a clear advantage; its eggs developed more rapidly and their upper lethal limit was something in excess of 34.5°C compared to 30°C for L. tomentosa. The geographical distribution of L. columella from Central America to Manitoba suggests that it has considerable potential for spread beyond its present limited distribution in the South Island. Its adaptability to a wide range of physical and chemical conditions and its rapid spread in the North Island (Pullan, 1969) and in Southern Africa (van Eeden and Brown, 1966) suggest that this could happen relatively quickly. On the other hand its failure to colonise outdoor habitats in Northern Europe in spite of its presence in hothouses since 1927 (Schlesch, 1930) is puzzling. It may be a more efficient

competitor with native species at high temperatures, or there may be "races" with different ecological requirements within the species. Continued monitoring of the distribution of L. columella and L. tomentosa in New Zealand should prove interesting.

The potential further spread of L. columella, either by ousting L. tomentosa or by occupying a vacant ecological niche, has important implications regarding the distribution and epidemiology of F. hepatica infections. If L. columella ousts L. tomentosa from some of its established habitats the effect upon the transmission of F. hepatica will depend upon the relative efficiency of the two species as intermediate hosts. There have been no attempts to ascertain whether L. columella or L. tomentosa habitats are potentially more dangerous as foci of F. hepatica infection. Such an investigation would be extremely difficult: no two habitats are alike and it would probably be impossible to separate "differences between habitats" from "differences between snails" in any manageable experimental design. Extrapolation from cercarial output in the laboratory could also be hazardous; it may simply reflect the suitability of the experimental conditions for one species or the other. Nevertheless it would provide some necessary basic information, particularly if conducted over a range of conditions.

The seasonal pattern of transmission is easier to establish but so far there have been only two epidemiological studies using tracer sheep. One study on a L. tomentosa habitat established that cercarial release in spring and early summer due to overwintering infection in snails was less important than the infection arising in late summer and autumn from snails that had been infected by miracidia in spring (Pullan and Mansfield, 1972). The other study of a L. columella habitat, reported in Chapter 4, failed to establish any evidence of an overwintering infection, probably due to a high turnover rate in the snails. Whether these results represent seasonal patterns of infection from other marsh habitats of the two snail species requires further investigation. The milder winters in North Auckland, for example, may allow overwintering infections in L. columella and an even longer period of transmission from the main summer infection in the snails.

If L. columella continues to move into marsh habitats at present

unoccupied by either species the expansion of the total acreage at risk from F. hepatica infection is likely to continue. Movements of stock from endemic areas to farms with marsh habitats of L. columella or L. tomentosa are an obvious risk, but rabbits, goats, deer and even opossums can also be infected. At present there are farms with both L. tomentosa and L. columella habitats but no Fasciola infection, like the Tokomaru farm on which some of the snail population studies were conducted. Their continued freedom from infection depends on the absence of infected farm stock or feral animals, and the risk from the latter clearly depends upon the distance from the nearest endemic area. These distances appear likely in at least some cases to decrease.

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APPENDICES

Name (1-10) & Signal, Pohangina Habitat (11-14) 8(b) Date (15-23) 31/3/72

Sample No. (24-28)	Visible Mud				Surface Water					Slope			Vegetation			Flow			Snails							Remarks
	floc	0	0-25	25	clear	oily	0	0-25	25	1	2	3	duckweed	cress	rushes	-	induced	+	L.col.	L.tom.	Pot.	Physa	mean	infected		
6T-9	1			+	+				+	+			+		+		+		0							
6U-5	0		+		+			+		+			+	+	+	+			0		5					
7T-9	0		+		+				+	+			+	+			+		5			4				
7U-1	0		+		+			+		+				+	+	+			8		2					
8T-4	1			+	+				+	+				+	+		+		1		1					
8U-3	0	+			+		+			+				+	+	+			0							
9T-4	0			+	+				+		+		+		+			+	0							
9U-1	0		+			+		+				+	grass			+			0							
10T-4	2			+	+				+	+			+		+	+			6							
10U-3	0		+		+			+		+				+	+		+		5		10					

APPENDIX 2.1. A completed data sheet used for collecting information on microhabitats

APPENDIX 5.1 Raw data from experiment 5.1

Key - original snail died before 2/9/73
+ original snail still alive at end of experiment on 19/11/73
$\bar{x}$ mean shell length

Snails found in jars at end of experiment (19/11/73)
Measurements in mm

Jar	Shell length at beginning of experiment		original *L. columella* still alive on	original *L. tomentosa* still alive on	*L. columella* egg masses first seen	*L. tomentosa* egg masses first seen	*L. columella* juveniles first seen	*L. tomentosa* juveniles first seen	unidentified juveniles first seen	*L. columella*	*L. tomentosa*	Unidentified
	L.col.	*L.tom.*										
1	10.6	7.5	-	24/10	2/9	2/9	19/11		2/9	3.3		27 snails $\bar{x}$ = 1.8; range 1.0 to 3.2
2	10.8	7.1	10/9	2/9			10/9			9.9, 9.3, 9.1, 9.2, 2.4, 2.6		2.6, 2.5, 2.6, 2.8, 2.6
3	10.0	7.7	-	24/10			19/11			3.2, 3.2, 3.0		17 snails $\bar{x}$ = 2.5; range 1.5 to 4.1
4	9.5	5.8	-	6/11		24/10	10/9		2/9	15 snails $\bar{x}$ = 3.5; range 2.4 to 6.7		23 snails $\bar{x}$ = 2.4; range 1.8 to 2.9
5	9.5	6.4	-	24/10		19/9	19/11			3.6, 3.4, 4.0		2.6, 2.4
6	9.2	7.3	10/9	16/10		3/10	3/10			14 snails $\bar{x}$ = 3.6; range 2.9 to 4.5		3.9
7	10.1	6.6	-	2/9	pot removed from experiment							
8	9.3	6.7	-	+		2/9	19/11	19/11		6.4, 4.8	3.6, 7.3, 3.9	2.1
9	9.9	6.3	-	24/10			19/11			3.2		2.0, 1.8, 2.4
10	9.1	7.5	-	24/10		2/9	6/11			3.8, 3.2, 3.5		1.0, 1.4, 3.5, 3.0, 3.4
11	10.1	5.5	-	+		18/9					7.1	
12	9.6	6.4	-	16/10		2/9		16/10			2.4, 2.5, 3.0, 2.4	1.9
13	9.1	5.0	-	+	2/9		19/11	16/10		2.5, 2.2	5.8, 2.2	10 snails $\bar{x}$ = 1.9; range 1.4 to 2.3
14	9.0	6.3	-	+							7.0	
15	9.0	5.0	-	+			19/11			2.3, 2.1	5.1	10 snails $\bar{x}$ = 1.8; range 1.3 to 2.3
										52	12	105

APPENDIX 7.1 The rate of development of the eggs of L. tomentosa and L. columella to hatching at a range of temperatures

KEY T = temperature (^{o}C); n = number of capsules observed; $\bar{X}$ = mean number of days to hatching; S^2 = variance of individual capsules; $\bar{X} \pm L.05$ = 95% confidence interval for the mean $\bar{X}$

L. tomentosa					L. columella				
T	n	$\bar{X}$	S^2	$\bar{X} \pm L.05$	T	n	$\bar{X}$	S^2	$\bar{X} \pm L.05$
12	16	45.25	1.80	44.54-45.96	10-11	19	50.21	13.76	48.42-52.00
14-15	5	22.60	1.30	21.18-24.02	12.5-13	19	30.79	4.84	29.73-31.85
16.5	33	17.67	0.77	17.36-17.98	14-15	14	19.07	1.14	18.45-19.69
19.5-20.5	18	12.89	0.46	12.55-13.23	16.5	16	16.69	0.74	16.23-17.15
22	16	11.25	0.74	10.79-11.71	19.5-20.5	202	13.54	1.49	13.37-13.71
24-24.5	36	10.75	0.36	10.55-10.95	22	20	10.90	0.62	10.53-11.27
25-26	8	9.38	0.28	8.94- 9.82	25-26	7	7.14	0.17	6.73- 7.55
25-27.5	29	9.62	0.31	9.41- 9.83	27	20	7.65	0.45	7.34- 7.96
27-28	30	9.87	0.53	9.60-10.14	28-31.5	7	7.71	0.58	7.01- 8.41
29-29.5	25	11.04	1.21	10.59-11.49	29	31	7.55	0.32	7.34- 7.76
					32	15	6.47	0.28	6.18- 6.76
					34-34.5	8	7.38	0.28	6.94- 7.82

APPENDIX 7.2 The resistance of L. tomentosa and L. columella to heat stress for 30 minutes

temperature	shell lengths of survivors (mm)	shell lengths of snails which died (mm)
	L. tomentosa	
39°C	5.2, 2.6, 4.0, 2.4, 6.0	
40°C	2.5, 3.8, 4.1, 5.0, 5.5	
41°C	7.0, 6.3, 4.7, 3.4, 3.6	2.1
42°C (first batch)	5.8, 4.4, 3.2, 4.5	5.8
42°C (second batch)	6.2, 6.0, 2.4, 1.6, 3.5	
43.5°C		6.2, 6.0, 4.9, 4.6, 2.5, 2.4
	L. columella	
37°C	12 snails ranging from 1.5 to 11.2 mm	
39°C	23 snails ranging from 1.9 to 9.8 mm	
40.5°C	15 snails ranging from 2.2 to 11.5 mm	
41.5°C	6.0, 6.2, 7.3, 7.0, 9.4	15 snails ranging from 2.1 to 6.0 mm
42°C (first batch)		28 snails ranging from 2.5 to 10.3 mm
42°C (second batch)	8.7, 8.0, 6.9	5.1, 4.6, 4.4, 2.7

APPENDIX 8.1 Response of snails to desiccation at 16.5°C and relative humidity 80-90%

KEY Size = shell length in millimetres

n = number of snails tested

LD_{50} = LD_{50} in hours calculated from raw data

L = 95% confidence interval for LD_{50} (upper to lower limit)

Log L = 95% confidence interval for LD_{50} calculated from log doses

L. columella

Size	n	LD_{50}	L	Log L
2.0- 2.9	63	2.36	1.16	.24
3.0- 3.9	78	4.51	.89	.09
4.0- 4.9	125	6.62	4.06	.18
5.0- 5.9	140	12.02	2.49	.10
6.0- 6.9	126	15.77	3.08	.09
7.0- 7.9	92	18.04	2.91	.07
8.0- 8.9	55	23.38	4.64	.08
9.0- 9.9	71	24.85	4.86	.11
10.0-10.9	88	34.41	5.86	.07
11.0-11.9	81	36.53	5.72	.07
12.0-12.9	97	39.19	7.27	.08
13.0-13.9	98	48.60	10.38	.11

L. tomentosa

Size	n	LD_{50}	L	Log L
2.0- 2.9	67	2.68	.95	.16
3.0- 3.9	66	5.51	1.99	.14
4.0- 4.9	114	7.95	2.03	.11
5.0- 5.9	124	12.07	2.22	.09
6.0- 6.9	49	13.08	4.14	.12

APPENDIX 9.1 Summary of results of experiment 9.1

KEY $\bar{X}$ = mean shell length (mm)

S^2 = variance of shell lengths

$S_{\bar{x}}$ = standard deviation of the mean shell length

L_1 and L_2 = lower and upper limits of the 95% confidence interval for the mean shell length

period of desiccation (days)	*L. columella*				*L. tomentosa*			
	alive	dead	lost	total	alive	dead	lost	total
28	1	2	2	5	4	1	1	5
36	1	4	5	10	9	0	0	10
42	0	8	12	20	9	6	5	20
49	0	12	3	15	8	5	2	15
total	2	26	22	50	30	12	8	50
$\bar{X}$	10.45	9.88	6.96		4.58	5.53	5.39	
S^2	.12	10.77	12.94		1.59	1.14	1.93	
$S_{\bar{x}}$	.25	.64	.77		.23	.31	.49	
L_1	7.34	8.56	5.36		4.11	4.85	4.23	
L_2	13.56	11.20	8.56		5.05	6.21	6.55	

APPENDIX 9.2 Summary of results of experiment 9.2

KEY as for appendix 9.1

L. columella

	mud		side		totals			
	alive 1	dead 2	alive 3	dead 4	alive 1+3	dead 2+4	mud 1+2	side 3+4
snails	10	29	14	24	24	53	39	38
$\bar{X}$	7.78	7.25	6.94	6.84	7.29	7.06	7.38	6.88
S^2	.96	1.64	.71	2.76	.94	2.13	1.49	1.96
$S_{\bar{x}}$	.31	.24	.22	.34	.20	.20	.20	.23
L_1	7.08	6.76	6.46	6.14	6.88	6.66	6.98	6.42
L_2	8.48	7.74	7.42	7.54	7.70	7.46	7.78	7.34

L. tomentosa

	mud		side		totals			
	alive 1	dead 2	alive 3	dead 4	alive 1+3	dead 2+4	mud 1+2	side 3+4
snails	5	17	3	7	8	24	22	10
$\bar{X}$	5.78	5.70	4.27	5.39	5.21	5.61	5.72	5.05
S^2	1.66	1.85	1.08	1.69	1.88	1.74	1.72	1.66
$S_{\bar{x}}$	.58	.33	.60	.49	.48	.27	.28	.41
L_1	4.17	5.00	1.69	4.19	4.07	5.05	5.14	4.12
L_2	7.39	6.40	6.85	6.59	6.35	6.17	6.30	5.98

APPENDIX 9.3 Summary of results of experiment 9.3

KEY as for appendix 9.1

L. columella

	mud		side		totals			
	alive 1	dead 2	alive 3	dead 4	alive 1+3	dead 2+4	mud 1+2	side 3+4
snails	15	24	9	3	24	27	39	12
$\bar{X}$	7.97	8.45	6.79	7.80	7.53	8.38	8.27	7.04
S^2	2.04	6.44	.32	.57	1.69	5.79	4.71	.55
$S_{\bar{x}}$	.37	.52	.19	.44	.27	.46	.35	.21
L_1	7.18	7.38	6.36	5.92	6.98	7.43	7.57	6.57
L_2	8.76	9.52	7.22	9.68	8.08	9.33	8.97	7.51

L. tomentosa

	mud		side		totals			
	alive 1	dead 2	alive 3	dead 4	alive 1+3	dead 2+4	mud 1+2	side 3+4
snails	20	11	7	13	27	24	31	20
$\bar{X}$	4.95	6.02	4.83	5.57	4.91	5.78	5.32	5.31
S^2	.63	.83	.31	.38	.54	.61	.95	.47
$S_{\bar{x}}$	.18	.28	.21	.17	.14	.16	.18	.15
L_1	4.58	5.41	4.32	5.20	4.62	5.45	4.96	4.99
L_2	5.32	6.63	5.34	5.94	5.20	6.11	5.68	5.63

APPENDIX 10.1 Shell shape of L. tomentosa and L. columella.
Means and standard deviations of some shell ratios

KEY PA = altitude of the main body whorl expressed as a percentage of the total shell length
PB = aperture length as a percentage of total shell length
PC = aperture width as a percentage of total shell length
PD = aperture length as a percentage of aperture width
N = number of shells measured

shell length	N	PA	PB	PC	PD
		L. columella - marsh			
2.0 mm	4	45.82 ±4.84	70.86 ±1.80	45.90 ±3.40	154.86 ±9.11
2.0-3.9 mm	75	42.24 ±4.68	66.78 ±3.74	43.20 ±4.42	155.69 ±13.63
4.0-5.9 mm	134	39.59 ±2.90	66.93 ±5.54	42.04 ±3.51	160.03 ±17.09
6.0-7.9 mm	76	38.58 ±2.63	66.47 ±4.47	41.69 ±3.69	160.23 ±13.03
8.0-9.9 mm	20	37.99 ±2.15	67.70 ±2.73	39.70 ±3.12	171.37 ±12.56
10.0 mm	1				
		L. columella - pond			
2.0 mm	0				
2.0-3.9 mm	16	40.33 ±4.37	65.35 ±3.14	40.01 ±4.44	157.01 ±16.40
4.0-5.9 mm	46	37.05 ±3.68	66.28 ±3.14	41.92 ±3.02	158.81 ±12.53
6.0-7.9 mm	61	38.19 ±2.20	67.13 ±3.60	41.94 ±3.90	160.90 ±11.58
8.0-9.9 mm	50	37.88 ±2.45	66.83 +3.22	41.03 +3.52	163.71 ±12.19
10.0 mm	10	34.04 ±4.26	66.59 ±4.33	40.24 ±3.60	166.07 ±10.36
		L. tomentosa			
2.0 mm	4	48.78 ±5.26	65.93 ±6.30	44.68 ±5.79	148.21 ±10.81
2.0-3.9 mm	11	46.30 ±5.78	68.97 ±4.20	43.88 ±3.20	158.18 ±17.71
4.0-5.9 mm	34	45.74 ±3.94	76.90 ±4.32	49.66 ±4.18	155.71 ±13.76
6.0-7.9 mm	24	43.60 ±3.45	78.84 ±2.75	49.45 ±3.02	159.88 ±9.09
8.0 mm	4	47.43 ±3.75	80.10 ±0.86	52.97 ±3.03	151.55 ±7.97