STUDIES ON THE INTERACTIONS BETWEEN LARVAL STAGES OF DIGENETIC FLUKES AND THEIR MOLLUSCAN HOSTS.

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SUMMARY

Snails of the species Thais (Nucella) lapillus (L) were collected from Scarborough South Bay, and Robin Hoods Bay, North Yorkshire. The presence of the rediae of Parorchis acanthus NICOLL (Digenea: PHILOPHTHALMIDAE) in T. lapillus individuals was previously associated with abnormal shell growth by Feare (1970a). His work has been extended to provide more conclusive evidence of parasitic gigantism in T. lapillus infested with P. acanthus.

The energy increment and soft tissue mass increase associated with shell growth has been calculated for a sample of infested T. lapillus individuals.

As reported by Cooley (1958) and Feare (1969) infestation with P. acanthus rediae progressively destroys the host gonad. The resultant reproductive saving was estimated for non-infested male and female T. lapillus from Robin Hoods Bay in 1981 and the energy values obtained were compared with estimates of the average energy loss from infested T. lapillus as a result of cercarial production and redial growth.

The proportion of the whole body dry mass of infested T. lapillus individuals contributed by the redial population was generally similar to the gonadal proportion of non-infested females, but did not follow the same seasonal cycle.

The digestive gland of infested dogwhelks was proportionally reduced from that of non-infested females in August only.

The growth of redial populations within the hosts through the summer is suggested as a possible cause of host

gigantism.

The relative advantages to the parasite of selecting female hosts are also discussed.

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CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

The dogwhelk Thais (Nucella) lapillus (L) is a neogastropod prosobranch that inhabits the littoral zone of rocky shores on both sides of the Atlantic Ocean. The northern limit of the species range tends to correspond to the -1°C winter isotherm, except in North America where it may extend further northwards until it is in areas of winter icing of the shore, and the southern limit corresponds to the 19°C summer isotherm (Moore 1936).

T.lapillus is a carnivorous species, as are its relatives the drills of North America and the Pacific, and it feeds principally on the two main sedentary, intertidal, rock space-competing invertebrates - barnacles (species of Balanus and Chthamalus) and mussels (Mytilus edulis) (Moore 1936, 1938). The frequency of contact with these species is probably the main factor for their importance in the diet of T. lapillus, as dogwhelks have been found to attack, with varying degrees of success, most shelled species of particular size range (Largen,1967, Morgan,1972). Moore (1938a) correlated the cessation of growth by adult T. lapillus (also noted by the same author in 1936), with the attainment of sexual maturity, at around 2.5 to 3 years of age. Coinciding with this was the formation of a single row of white projections just inside the shell lip, called aperture teeth and an associated thickening of the shell lip. Cowell and Crothers (1970) found that on exposed shores many individuals had more aperture teeth rows than the single row reported by Moore (1938a) for sheltered shore

dogwhelks. Bryan (1969), Cowell and Crothers (1970) and Feare (1969) all experimentally induced growth cessation and teeth row formation in immature Thais lapillus individuals by subjecting them to an extended period (at least 3 months) of starvation. The normal number of teeth rows for adult Thais lapillus on the exposed North Cheek of Robin Hoods Bay, North Yorkshire, was found by Feare (1970a) to be 2. The latter author suggested that the first row of teeth would be produced when the 18 month old immature dogwhelks formed their first winter aggregation - a self-imposed period of starvation. During the following summer the immature snails would recommence growth for the final time, and then re-enter aggregations as sexually mature adults and form a final row of teeth.

Aggregating in rocky clefts through the cold, stormy winter months is a defensive behavioural trait of T. lapillus (Feare,1971). The low shore temperatures experienced during winter were shown by the latter author not to impair the ability of dogwhelks to remain adhered to the shore substrate, but if they are dislodged they are unable to regain their foothold and may be swept away. While on the exposed shore at Robin Hoods Bay it appears necessary for 2-year-old immature dogwhelks to aggregate on more sheltered shores, this requirement is outweighed by the rewards of a prolonged feeding period. The above behaviour results in the higher incidence of animals with multiple teeth rows on exposed shores compared with sheltered shores reported by Cowell and Crothers (1970).

Aggregations take on a reproductive function in the spring as first described by Moore (1938a). Following copulation the females remain in aggregation to lay egg capsules (Moore 1938b), while the males disperse onto the shore to feed (Feare,1969). This pattern of reproductive behaviour localises the gene flow between Thais lapillus populations resulting in considerable variation between populations. The visible variation of shell form has been the subject of a number of studies on T. lapillus (for example Colton 1922, and Moore, 1936), and the effects of interspecific interactions on shell morphology illustrated recently by Vermeij (1982). Two different but interbreeding genotypes of T. lapillus were discovered by Staiger (1954,1957) with functional phenotypic differences as described by Kitching et al. (1966). The phenotype best adapted to exposed shores has a relatively large shell aperture - enabling individuals to adhere firmly to the shore - and a relatively thin shell. The phenotypic adaptations for sheltered conditions are a small shell aperture and a thick shell as a defence against crabs. The presence of the penis in male T. lapillus can be used as reliable evidence for sexing normal dogwhelks, but, as reported by Blaber (1970), reproductively spent female dogwhelks may also possess a small penis-like structure. The presence of the redial stages of the digenetic trematode Parorchis acanthus NICOLL (considered to be a member of the PHILOPHTHALMIDAE (Joose,1899) by Yamaguti (1975)) in T. lapillus individuals is associated with the destruction of the host gonads (Cooley, 1958, Feare, 1969). Infested dogwhelks collected during the present study were found usually to possess a small penis-like structure. The

observation noted above by Blaber (1970) on spent females and the report on Koie (1969) on the regression of the penis of trematode infested male Buccinium undatum made the possession of a small penis-like structure by infested Thais lapillus individuals an unreliable character for sexing. However the sex ratio of infested T.haemastoma individuals with gonad tissue remaining was found by Cooley (1962) to be 1:1, suggesting that there was no sexual discrimination by the infecting rediae. The same author reported that the gonads of infested T. lapillus individuals are ingested by Parorchis acanthus rediae, in a similar manner to that described for redial infestations of Littorina littorea by Rees (1934).

P.acanthus (Nicoll 1906) was initially thought to have a North American relative, P. avitus (Linton 1914), which was very similar in adult appearance except for different sucker diameter ratios and the relative extent of uterine convolution (Linton 1928). A list of the gull species which are either naturally infected or have been artificially infected in the laboratory, with P. acanthus and P. avitus was given by Cooley (1958). The redial stages of both of these so-called species occur in carnivorous neogastropods. "Cercaria purpura" of Lebour (1907) in the dogwhelk T.lapillus in Europe was later demonstrated by Lebour (1914) to be the larval stage of P.acanthus, while "Cercaria sensifera" from the drill Urosalpinex cinereus in North America was reported by Stunkard and Shaw (1931) to be the larval stage of P.avitus. Lebour and Elmhurst (1922) showed that the life cycle of P. acanthus involved the mussel,

Mytilus edulis, as a second intermediate host in which the metacercaria of Parorchis acanthus became encysted in tissues of the mantle and foot. Later, Stunkard and Cable (1932) found that P. avitus cercariae would encyst on almost any surface. Encysted metacercariae were found not only on the shell but also on the surfaces of soft body tissues such as the mantle and foot of M.edulis. Cable and Martin (1935) subsequently reduced P. avitus to synonymity with P. acanthus, as Nicoll had suggested to Linton in a personal communication, according to Stunkard and Cable (1932).

Rees, in a series of papers, studied a number of aspects of the morphology and biology of P.acanthus including the anatomy and encystment of the cercariae (1937), the germ cell cycle (1939, 1940),factors affecting the release of cercariae (1948), the light microscope structure of the rediae (1966) and the metacercarial cyst walls and cercariae (1967), and the ultrastructure of the rediae and cercariae (1971, 1980, 1981).

As a result of a report by Butler (1953) of heavily infested drills suffering considerable gonad damage, Cooley (1958) investigated the possibility of controlling Thais haemastoma populations by introducing P.acanthus infestations. Cooley reported that between 70% and 90% of infested T. haemastoma "liver" comprised P. Acanthus rediae but concluded (1962) that P. acanthus was not a successful biological control agent for T. haemastoma because he could find "no effective means of spreading the parasite in increased numbers".

Despite the nature of the apparent damage to the host, Feare (1970a) reported that Thais lapillus infested with Parorchis acanthus appeared to show signs of continued growth, which as described above, is not the case for non-infested individuals. The increased numbers of teeth rows in a sample of 30 infested T. lapillus compared with parasite-free individuals, together with an increase in the incidence of infestation in larger dogwhelks, provided the evidence for Feare's observation. It was decided by the present author to investigate further the occurrence of parasite-induced gigantism in T. lapillus on the North Yorkshire coast, with particular reference to the alteration in life style and energy partitioning of infested dogwhelks.

The two sample sites used throughout the study were selected because the local levels of P. acanthus infestation in the T. lapillus population made it possible to obtain sufficient numbers of infested animals from the field for the investigation. The sample sites were Robin Hoods Bay, North Yorkshire,(in particular the scar leading down to the sea directly in front of what was formerly the Wellcome Marine Laboratory) and Scarborough South Bay (where most animals were collected from just south, and to the seaward side, of the council swimming pool). Both non-infested and infested T. lapillus were collected from similar shore levels, around the mid-range of the T. lapillus distribution (that is, a little below mean tide level - Colman 1933, Lewis 1964).

CHAPTER TWO

INVESTIGATIONS INTO ABNORMAL GROWTH OF *THAIS LAPILLUS* ADULTS INFESTED WITH *PARORCHIS ACANTHUS* REDIAE

INTRODUCTION

The first published record of larval trematode infestations being linked with abnormal growth of their gastropod hosts was that of Wesenberg-Lund (1934). He noted that all uncharacteristically large *Lymnaea auricularia* were infested, and he suggested that this was the result of increased feeding activity by the infested individuals which was over-compensating for the energy drain associated with the presence of the parasite.

Two years later the results of a study by Rothschild on *Hydrobia* (formerly *Peringua*) *ulvae* at Plymouth, confirmed the observation that larger hosts tend to be infested. At least 6 species of trematode were found by Rothschild (1936) to infest *H. ulvae*, but she grouped them into 4 categories depending on their life cycle pattern. Two of these groups were of species with gonad-seeking sporocysts; in one case (*Cercaria oocysta*) the cercariae encysted within the host gonads while in the other (*C. ubiquita*) the cercariae were released in the usual manner. These two were the most commonly found infestations at all times of year. Rothschild suggested that this could be due to the other host species in the cycle also being present all year round. Alternatively it is possible that the parasites' use of the gonad as the infestation site causes minimal disruption to the vital processes of the host and the infestation can thus

persist longer, producing more cercariae, which in one case await, in encysted form, the ingestion of the snail by the next host in the life cycle. The longevity of the infestations would increase the frequency with which it was encountered in the snail population.

As a result of his study on the Trematode parasites of Littorina littorea, Rees (1936) concluded that there was no evidence of enhanced growth in infested L. littorea. Both Cercaria hismathla secunda and C. lophocerca with large rediae (which feed partially by direct ingestion of gonad and digestive gland), appeared to cause less pathological damage to their host than species like C. emasculans, with small sporocysts, and hence the former persist and increase in incidence,in large old hosts.

Rothschild and Rothschild (1939) published the results of a laboratory study of growth rates of infested and non-infested Hydrobia ulvae in a range of conditions and concluded that infested animals had increased growth rates. Two years later Rothschild (1941a) confirmed this result in the field by monitoring the growth of H. ulvae in a pool on the saltings associated with the River Tamar in Cornwall. She concluded that the infested animals grew faster since they were found more commonly in the larger size categories for each year class. Rothschild reported that while non-infested individuals only grew up to a shell length of 6.7mm, infested animals of the same age could be as large as 10mm. It is a common property of growth simulating models that old animals have low growth rates. Thus, slow growth coupled with reduced numbers of old, large, H. ulvae

individuals must decrease the accuracy of ageing larger snails, and so Rothschild may have erroneously included animals of different ages in the same size/year class. The shallow pool Rothschild used in her study was chosen, in part, because of the high level of infestation of Hydrobia ulvae. In these conditions it might be expected that virtually all the older snails would have acquired a trematode infestation. Associated with the increased size of infested H. ulvae Rothschild noted that the shell was characteristically ballooned and she suggested that this was the result of increased pressure within the visceral whorls caused by the presence of the parasites.

Lysaght (1941) observed increasing infestation levels with increase in size of Littorina neritoides on Plymouth breakwater. Larger snails were more frequently female, and so the most common sporocyst infestation (Cercaria B) tended to be more often found in females. The presence of the encysted metacercariae of another unnamed trematode species (Metacercaria A) was more frequent in males than females. Interaction between the parasite species seems likely. Lysaght commented on the very slow growth rate of animals above 6.0mm in size, and said that there was insufficient evidence that the increased incidence of sporocyst infestation in larger snails was the result of enhanced growth rather than increased age.

In a paper published at the same time as that of Lysaght,Rothschild (1941b) attempted to provide evidence of increased growth rates in infested L. neritoides using the same data as Lysaght. Rothschild assumed equal chances of

parasitisation with either the encysted metacercariae or the sporocyst infestation for all Littorina neritoides individuals on Plymouth breakwater throughout their life span. From this theoretical stand point, the prevalence of infestation in snails of particular size classes can be used as an indicator of relative age, with the incidence of infestation increasing exponentially with the age of the host. A growth curve was obtained by plotting host shell length (mm) against a time factor calculated from the distribution of the parasite, 2 - Log U, where U was the "percentage of specimens of snail free from infection". Cercaria B had a rapid rise up to 6mm followed by a very shallow incline upwards to 8mm shell length. The growth curve that was obtained by the same method for Metacercaria A had a comparatively constant rate of increase up to the maximum shell length of 8mm, with only very little decrease in incline.

In her discussion of the results Rothschild comments that if the size-to-age curve calculated from the distribution of cercaria B were true, it would take an individual L. neritoides "7 1/2 times as long to grow from a shell length of 6mm to 8 1/2mm as it takes to reach 6mm". Rothschild found on the other hand, "nothing remarkable" about the size-to-age curve calculated from the distribution of Metacercaria A, given that "the habitat involved is peculiarly favourable to growth". By considering the size-to-age curve calculated from the distribution of Cecaria B to be unnaturally flattened for larger animals, Rothschild suggests that the presence of Cercaria B causes

the host to increase in size more rapidly than if not infested, and thus a higher incidence of infestation occurs in larger animals, so a greater period of time is calculated between the larger size classes, using an exponential increase in infestation probabilities as a time scale.

I consider that the curve calculated from the distribution of Metacercaria A represents an incorrect time scale against which to judge the effects of Cercaria B on growth of Littorina neritoides. The exponential increase in infestation of Metacercaria A is only a measure of presence, or absence of the parasite. Older animals may well have been invaded by Metacercaria A on more than one occasion. (Lysaght (1941) found considerable variation in the numbers of metacercarial cysts present in infested snails). This would mean that the growth curve calculated from the increase in presence of the infestation in larger animals rises too sharply, because some of the larger snails have been invaded by metacercariae on more than one occasion. This problem is less likely to occur in infestations of Cercaria B as Rothschild explains by including a discussion of the work of Winfield (1932) and Nolf and Cort (1933), on the resistance of gastropods to subsequent, concurrent invasions by larval trematodes of the same species as the established infestation.

Although Rothschild did not consider the growth curve calculated for L. neritoides from the distribution of Cercaria B to be correct it is in fact supported by Lysaght's (1941) published annual growth rates, obtained by releasing marked L. neritoides on the breakwater, and then

collecting as many as could be found a year later. From the growth rates obtained by Lysaght it can be deduced that it would take a Littorina neritoides individual over 26 years to grow from 6.9mm to 8.5mm shell height, assuming that there was no decline in growth rate during the 26 year period. (Only one of the snails retrieved in the relatively large size category of 6.8 - 6.9mm was not infested, so the growth rate obtained can be assumed to be that for infested snails). In her discussion Rothschild (1941b) suggested three other possible errors, but considered that none of them were relevant. The reported immunity of immature snails to infestation (Kemp and Gravely (1919) and Manson-Bahr and Fairley (1920)) was in Rothschild's opinion due to the authors not considering the time factor involved. The possibility that infestation with Cercaria B was lethal to small L. neritoides was dismissed on the grounds that some individuals producing cercariae, yet less than 2mm in length, had been found on the breakwater. The likelihood of cercariae of Metacercaria A being less infective to L. neritoides already infested with Cercaria B was discounted due to the moderately high percentage of snails with both parasites.

The criticisms that can be made against Rothschild's work with L. neritoides, as outlined above, undermine the evidence she produced in support of enhanced growth rates in infested L. neritoides. The very low growth rates observed by Lysaght (1941) are very interesting. The largest snail found by Lysaght was 10.4mm high, and so presumably was of some considerable age. Extension of the normal lifespan as a

result of infestation could provide a possible mechanism for gigantism, but this could take many years study to determine. A similar extended growth period argument could fit the field data for Hydrobia ulvae collected by Rothschild (1941a), where, as suggested above, mistakes in the allocation to year classes of large, scarce, animals could have caused Rothschild to have deduced incorrect enhanced growth rates.

The increased incidence of a larval trematode in larger individuals of its marine gastropod host species was shown by Crewe (1951) for Cercariae patellae infestations in Patella vulgata and P. depressa. Despite finding one hundred percent of the largest sized snails to be infested Crewe felt that there was no clear evidence for gigantism in infested limpets.

The incidence of infestation of Lymnaea truncatula with Fasciola hepatica was noted by Kendall and Ollerenshaw (1963) to be reduced in host populations of small individuals, as compared with populations of large L. truncatula. This is not evidence for gigantism, but is another very interesting correlation between host size and infestation. Croll (1966) suggested that the mucous trails of snails will increase with size and may be responsible for attracting greater numbers of miracidia to the snails. The attraction of miracidia to snail slime was first seen when Schistosoma japonicum miracidia were observed by Faust (1924) to attempt to penetrate the mucous trail of a host snail. Thus, in a population of small L. truncatula the F. hepatica miracidia may be less successful at locating hosts.

Alternatively, the populations of small Lymnaea truncatula in the study of Kendall and Ollerenshaw (1963) may be stunted as a result of starvation so that the further energy drain and digestive gland damage resulting from infestation with Fasciola hepatica becomes lethal.

Studies by Chernin (1960) and Pan (1965) demonstrated an initial growth rate increase in young Biomphalaria glabrata infested with Schistosoma mansoni, lasting for 5 or 6 weeks. After this period the growth of infested snails reduced so that there was ultimately no difference in size between infested and non-infested individuals (Chernin 1960), or the non-infested individuals were larger (Pan 1965). Infestations in mature B. glabrata did not produce a growth rate increase at any time according to Pan (1965). S. japonicum has been reported to retard the growth of Oncomelania quadrasi (Pesigan et al.,1958) and O. nosophora (Moose 1963).

Zischke and Zischke (1965) and Zischke (1967), found that Stagnicola (= Lymnaea) palustris infested with Echinostoma revolutum exhibited reduced growth in comparison to non-infested snails. Zischke (1967) found that the quickest growing infested individuals tended to harbour the largest numbers of rediae. Zischke interpreted this result as increased production by the parasite in response to increased availability of nutrients in the host, although the results are also open to the interpretation that more heavily infested snails have to produce more shell to accommodate the increased load of parasite and host

materials.

Following a review of literature on growth effects in gastropods associated with the presence of larval trematodes, Cheng (1971) introduced original results of an investigation into shell deposition in parasitised snails. His results are in agreement with earlier findings of Etges (1961a,b) that Helisoma anceps individuals infested with rediae of Cercaria reynoldsi and sporocysts of Diplostomum scheuringi are heavier than non-infested snails, and some tend to have thicker shells. Having been critical of earlier studies on gigantism because of their dependence upon infested snails found in the field, and therefore of indeterminate age and length and varying level of infestation, Cheng (1971) collected his own experimental Nitocris dilatatus subjects, both non-infested individuals as well as those infested with Acanthatrium anaplocami from a local "creek". He assumed that, as they were from the same population, if they were "essentially" the same size they would be also of the same age. Cheng also used laboratory reared Physa sayii subjects, some infested with Echinostoma revolutum.

The snails were weighed whole then their shells removed, dried and weighed. The parasites were dissected out of the soft tissues of the infested snails, and then the parasite and snail tissue fresh masses were determined. The fresh masses of non-infested snail soft tissues were determined directly after the removal of their shells. In both snail species the total fresh weights of infested animals were greater than those of non-infested individuals. The fresh

soft body tissue weights of infested snails were slightly, but not significantly, greater than those of non-infested snails of the same species although the shells of infested animals were significantly ($p \leqslant 0.05$) heavier than those of non-infested animals.

In conclusion Cheng wrote "....although the gross weights of both species of parasitized snails are greater than those of their non- parasitized counter parts, the mean weights of the soft tissues of both categories of snails of both species are not significantly different. This could only mean that the greater total weights are due to heavier shells. This as indicated in tables 1 and 4 is the case". A footnote relating to this quotation is an amendment by Cheng, as a result of this study, to his joint report in 1966 in which he could find no statistical difference between shell weights of infested and non-infested Nitocris dilatatus individuals (Cheng et al., 1966).

In his discussion of the results Cheng (1971) appears to have overlooked the fact that he removed all the parasites from the infested snails before weighing their soft tissues. If the mean weights of the two parasite species (supplied by Cheng in tables 1 and 4) are added to the relevant infested snail mean soft tissue weights, the resulting weights of the shell contents, (including parasites), are significantly ($p \leqslant 0.01$) greater than those of non-infested snails. (The variance for the adjusted mean infested snail soft body mass was calculated from the sum of the mean snail tissue mass and mean parasite mass standard deviations.) In my opinion it is incorrect not to consider the infested snails to be

producing shell to accommodate both snail and parasite tissues. New shell is laid down at the mantle edge, and so if the invasion of the viscera by larval trematodes means the upper whorls of the shell have to accommodate more tissue (snail and parasite) the snail will lay down new shell at the exposed mantle edge.

It would appear that Cheng was incorrect to conclude that the heavier total masses of infested snails "could only mean that the greater weights are due to heavier shells", and also in error to amend his earlier report (1966) that there is no difference in infested and uninfested Nitocris dilatatus shell masses.

The slightly, but insignificantly greater masses of the fresh soft body tissues of infested snails may well have been reduced due to evaporation of water from the tissues during the time taken to remove the parasite tissue from the snail viscera. In contrast, the fresh masses of the soft tissues of noninfested snails were determined immediately after removal from their shells. Had the tissues been treated similarly, the mean mass of infested snail tissue may have been significantly heavier than that for non-infested snails.

The most conclusive laboratory investigation into enhanced growth in trematode infested gastropods is the study of McClelland and Bourns (1969). Lymnaea stagnalis individuals infested with Tricholbilharzia ocellata when 10 weeks old were found by McClelland and Bourns to be significantly larger than the non-infested controls when 16 weeks old. At

25 weeks old the former had an average shell length of approximately 44mm compared with 35mm in control snails. The fecundity of infested snails was considerably reduced and the authors suggested that their abnormal growth rate was a manifestation of this energy saving. As well as showing faster growth, infested Lymnaea stagnalis were found by McClelland Bourns to have an increased life span under laboratory conditions. Wright (1966) cited a personal communication from Bourns that, marked, trematode infested L. stagnalis released in autumn and retrieved in spring of the following year had grown significantly less than non-infested controls treated in the same way. The laboratory results obtained later by McClelland and Bourns (1969) for L. stagnalis infested with Tricholbilharzia ocellata are contradictory to this earlier study. The earlier result may have been influenced by the adverse conditions of winter to which the infested animals were more susceptible or the reduction in reproductive effort in the non-infested snails during the winter could have saved them some energy for growth.

Feare (1970a) reported that Thais lapillus individuals infested with Parorchis acanthus were castrated, and that the infestation incidence was greater in larger host size classes. Ballooning of the last formed but one shell whorl was also linked with the presence of P. acanthus in T. lapillus in Feare's investigation. Thirty infested T. lapillus inspected by Feare (1970a) had more horizontal rows of shell 'teeth' inside the aperture, than would have been expected for non-infested animals. Teeth row formation, and

associated shell lip thickening were reported to coincide with the cessation of growth on reaching maturity in Thais lapillus by Moore (1938a). Moore reported that adult T. lapillus only ever had one row of teeth, and thus concluded that growth totally ceased in mature animals. Bryan (1969) agreed that this was true for T.lapillus on most normal shores, and demontrated that starvation was the causal factor of teeth row deposition and growth cessation. Feare (1970a) and Cowell and Crothers (1970) also reported that teeth rows were the product of a prolonged period of starvation. Feare (1970a) suggested that the period of starvation responsible for the normal production of aperture teeth in T. lapillus was the first winter aggregation endured by adults or 2 year old immature individuals on exposed shores. Feare's study was on an exposed shore population of dogwhelks, and the normal number of teeth rows in adult T. lapillus in the population he studied was 2, as the 2 year old immatures grow in the summer after depositing their first teeth row. Cowell and Crothers (1970) explained the incidence of multiple teeth rows by suggesting that immature T. lapillus were swept off exposed shores, and had to endure a period of starvation before re-locating a food source. Feare's (1970a) explanation seems to be the most appropriate application of information on the biology of T. lapillus.

The fact that T.lapillus individuals with Parorchis acanthus infestations were larger and had more teeth rows in Feare's (1970a) investigation, indicated to him both that they continued to grow after normal individuals had ceased, and

also started to reproduce.

Recently, Lauckner (1980), working with Littorina littorea, found the trematode species Cryptocotyle lingua and Hismathla elongata, to be more common in larger individuals of L. littorea, in agreement with Rees (1936) and Robson and Williams (1970). Lauckner also stated that the larger individuals of any given year class were more likely to have a redial infestation of C. lingua or H. elongata than smaller animals of the same age. This observation is similar to that of Rothschild (1941a) for infested Hydrobia ulvae. Instead of interpreting his findings as evidence of increased growth rates in infested Littorina littorea, as Rothschild (1941a) did for H. ulvae, Lauckner (1980) suggested that infective miracidia are preferentially attracted to littorinids with gonads and, as reported by Williams (1964), larger winkles mature earlier than smaller specimens. On the other hand, Hayes (1927, 1929), Green and Green (1932) and Moore (1937) found no evidence of shell deformity or larger host size associated with the presence of trematode infestations in L. littorea.

Although Lauckner 1980, accepted the evidence of Feare (1969, 1970a) that parasite induced growth occurs in Thais lapillus, it is clear to the present author that the investigation by Feare was much less extensive than that of Lauckner, where the latter author reached different conclusions from superficially similar observations.

In the light of conflicting evidence from field studies on the question of gigantism in gastropod molluscs associated

with infestation by larval trematodes it appeared desirable to investigate the subject in as much detail as possible. The Thais lapillus - Parorchis acanthus association was selected for study both because of the large volume of information already published and also because of the ready availability of material at Robin Hoods Bay and Scarborough.

Methods Employed for the Assessment of Shell and Body Growth of Infested and Non-Infested Thais lapillus Adults.

T. lapillus used for shell measurements were obtained from Scarborough South Bay and Robin Hoods Bay, North Yorkshire.

All shell measurements were made using vernier calipers to the nearest 0.1mm. The dimensions selected for measurement are as follows. The length of the shell was taken as the distance from the top of the spire to the distal end of the syphonal canal. The shell height was taken as the greatest distance from the lower to the upper surface of the shell, measured with the lower jaw of the calipers resting along the syphonal canal and the other jaw on the upper surface of the shell. The width of the shell was taken as the widest point across the last-formed whorl with the shell resting aperture - downwards on a flat surface.

The Whorl Width Ratio

W.W.R. = width of second last whorl/width of last formed whorl

was calculated by taking the widest width measurement as above and then taking the widest width of the second to last whorl by the same method.

The shell aperture lip thickness was taken as the thickness of the shell measured 1mm in from the lip edge. The shell aperture lip length was taken as the distance measured from the extreme edge of the shell to the distal edge of the last formed row of teeth. Plate 2.1 shows the internal view of a Thais lapillus shell aperture, showing the teeth rows.

To measure the distance between successive teeth rows a low-speed dentist's drill and cutting wheel was used. The shell lip length was measured as before, except that the measurement was taken to the proximal edge of the last-formed teeth row. The lip and teeth row were then removed with the cutting tool and the measurement taken as before. This procedure was repeated until all the teeth rows had been removed. After the removal of each row of teeth the maximum shell height and width were again measured, as previously described, in order to elucidate any relationship between lip length and shell size at different stages of growth. Shell length was considered an unreliable measurement because of the effects of cutting on the shape of the syphonal canal. (See Plates 2.3-2.9)

To ensure that all the teeth rows were noted in individuals that were not cut with the dentists cutting wheel, the number of teeth rows was determined by cracking the shell behind the last-formed whorl, and removing the snails head and foot out of the hole thus created leaving the last-formed whorl and aperture intact, so that the teeth rows could be easily seen and counted.

The following methods were employed for the determination of changes in the dry mass of the soft body tissues of infested and non-infested Thais lapillus subjects. Animals were killed by immersion in boiling water for 30 seconds before the shell was measured and cracked open, and the body divided into a number of components for other investigations. Their total dry weights were determined by drying the different tissue components on separate aluminium pans under vacuum and then weighing to the nearest 0.01mg on a Metler HL52 balance. The total dry body mass is the sum of all the component masses. Annual body growth increments were calculated for infested T. lapillus used in the shell growth investigation as described above. The dry body mass for a particular shell size (taken as shell height + shell width) was predicted from a Log shell (height + width) to Log dry body mass regression calculated for a sample of infested T. lapillus collected from Scarborough South Bay in June 1981 and thus the changes in body mass as teeth rows were added could be estimated. The body mass increment was converted into an estimate of net energy increment using the ash percentages of tissue dry mass and tissue caloric values for June infested T. lapillus given below (Chapter 3).

STATISTICAL METHODS

All the data were statistically analysed with reference to the following authors; Student t-tests were applied as described by Bailey (1981), and Student Newman Keuls tests applied after one way analysis of variance as described by Parker (1979). One and Two way ANOVA were either applied as described by Parker,or using computer programmes written by

Mr. R. Willows and Mr. J. Rosewell (personal communication) for a Commadore Pet microcomputer, using statistical methods as described by Sokal and Rohlf (1981). Regression analyses and correlation coefficients were calculated as described by Parker.

Evidence of Shell Growth

Shell length (mm) frequency distributions obtained for infested and non- infested mature Thais lapillus collected at Scarborough South Bay, and Robin Hoods Bay during the period of study are shown in Fig. 2.1. The existence of small numbers of abnormally large infested animals from both collection sites is graphically demonstrated by the histograms.

The distributions of teeth rows among mature infested and non-infested Thais lapillus collected from Scarborough South Bay and Robin Hoods Bay are shown graphically in Fig.2.2. It can be seen that at both collection sites a single row of teeth was the most common occurrence for non-infested animals. Only infested individuals had more than 5 teeth rows, although a very few non-infested snails had 5 teeth rows. Similar frequency histograms constructed for collections of dogwhelks from sheltered (Dale Fort) and exposed (West Dale) shores where no infested animals were found, are shown in Fig.2.3. It can be seen that no individuals were found with as many as 5 teeth rows.

The frequency distributions of shell lip thickness (mm) for infested and non-infested Thais lapillus adults collected from Robin Hoods Bay are shown in Fig. 2.4. It can be seen

from the histogram that the majority of the individuals with thin shell lips are infested. The result of a Student t-test on the two mean lip thicknesses is shown in Table 2.1. The value for t is significant to $p \leq 0.001$ for 533 degrees of freedom, so the mean infested shell lip thickness is significantly thinner than the non-infested mean.

The shell lip length (mm) frequency distribution for infested and non-infested T. lapillus adults collected from Robin Hoods Bay during February, March and May of 1982 are shown in the lower histogram of Fig. 2.5. The upper histogram shows the size frequency distributions for the non-infested and infested animals collected. It should be noted that not one infested or non-infested individual had a shell lip length greater than 4.5mm. The shell lip length (mm) frequency distribution for infested and non-infested T. lapillus adults collected from Robin Hoods Bay during July, August and October are shown in Fig. 2.6. The upper histogram shows the size frequencies of the infested and non-infested T. lapillus collections, for comparison with the previous figure (2.5) of shell lip length frequencies for the earlir samples. It can be seen in the lower histogram of Fig. 2.6. that in later months of the year a number of infested animals were found with lip lengths exceeding 5mm.

The frequency histograms for the Whorl Width Ratios of infested and non-infested adult Thais lapillus collected from Scarborough South Bay are shown in Fig. 2.7. The histogram demonstrates that the majority of the animals with small Whorl Width Ratios (relatively wide last-but-one

whorls) are infested. The result of a Student t-test on the infested and non-infested means is shown in Table 2.2. The value for t obtained is significant to $p \leq 0.001$ for 456 degrees of freedom, so the infested mean Whorl Width Ratio can be considered significantly reduced from the normal condition.

The correlation between Whorl Width Ratio and the number of teeth rows for infested T. lapillus collected from Scarborough South Beach, and Robin Hoods Bay is shown in Fig. 1.8. The sample correlation coefficient (r) was calculated to be 0.660, which means that 43.6% ($r^2 \times 100$) of the variation in Whorl Width Ratio can be explained by variation in teeth row numbers. The value for r is significant to $p \leq 0.001$ for 56 degrees of freedom.

The changing shape of the infested T. lapillus shell (number 2 from Plate 2.2) resulting from the successive removal of aperture teeth rows is illustrated in Plates 2.3-2.9. The shape of the shell can be seen to change from the large ballooned shape characteristic of infestation to the more usual shape for dogwhelks. The transition in the shape of the shell can be traced from top left to bottom right on Plate 2.2 which shows a collection of infested animals from Scarborough South Beach.

Annual Changes in Body Dry Mass (mg)

Tables 2.3-2.7 contain the regression slopes (b) and intercepts (a) for the Log dry body mass (mg) against Log shell length (mm) regressions calculated for non-infested male and female Thais lapillus in February 1982 (no infested

animals were found when sampling), non-infested male and female and infested T. lapillus in March, 1982, non-infested female and infested animals in July, and August 1982 (the male T. lapillus relationship was not significant in these months) and non-infested male and female and infested T. lapillus in October 1982.

The results of analysis of covariance (ANCOVA) of the Log dry body mass (mg) against Log shell length (mm) regressions for male and female T. lapillus collected in February 1982 are shown in Table 2.8. For both regressions a significant proportion of variance on the dependent variable is explained by variance in the independent variable. F is significant to $p \leq 0.001$ for females, and significant to $p \leq 0.01$ for males.

The between sex variation in Log dry mass is very significantly greater than the variation within sexes ($p \leq 0.001$) showing that the sex mean dry body masses are different. A common slope calculated from all the data, then fitted through the individual sample means explains a significant ($p \leq 0.001$) amount of the Log dry mass variation. The slopes calculated for each sex are not significantly different from the pooled slope, when compared with the error deviations from the slopes.

The total sums of squares is the sum of the between and within sex sums of squares. The same value can also be obtained if one line is plotted through all the data, the total variation now being the sum of the variation explained by the regression, and the deviations from the common slope.

The deviations can be further split into two sources. There are the variations from the slope, as calculated for fitting a common slope through each of the different means, and the variations associated with the sexes having different intercepts with the y axis (or adjusted means). The ratio of the latter to the former is in this case highly significant ($p \leqslant 0.001$), so it can be concluded that the intercepts are different for male and females.

A summary of the above is that the non-infested male and female Thais lapillus collected in February 1982 have Log dry body mass (mg) against Log shell length (mm) regression slopes that are not significantly different from a common pooled slope, but the intercepts on the y axis are significantly different, so the groups are considered separately from each other. The slopes are graphically displayed in Fig. 2.9. The non-infested female animals can be seen to be significantly heavier (dry mass) than non-infested male snails of the same size.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for male, female and infested T. lapillus collected in March 1982 are shown in Table 2.9. The non-infested male and female regressions are both highly significant ($p \leqslant 0.001$), but the infested regression is less so ($p \leqslant 0.025$). The difference between mean dry weight values for each group is significantly greater than the difference within group ($p \leqslant 0.025$). The common slope fitted through the different group means is highly significant ($p \leqslant 0.001$), and the individual group slopes are not significantly different from it ($p \geqslant 0.1$).

The intercept values for each group are responsible for significantly more of the error variation for a pooled line than the errors around the common slope, ($P \leq 0.001$), so the three groups can be considered different from each other. The slopes are graphically displayed on Fig. 2.9.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for non-infested female and infested Thais lapillus collected in July 1982 are shown in Table 2.10. The female regression line is highly significant ($P \leq 0.001$), the infested regression a little less so ($P \leq 0.005$), and the males show no relationship between shell length and dry body mass, and so are not included in the ANCOVA.

The difference between mean dry mass values for infested and female samples is significantly greater than the difference within the groups ($P \leq 0.005$). The two lines of common slope fitted through the data are highly significant ($P \leq 0.001$), and the individual group slopes are not significantly different from it. ($P \geq 0.1$). The intercept values for each sample are responsible for significantly more of the error variation around a single pooled line, than the errors around the two lines of common slope ($P \leq 0.001$), so the two groups can be considered separately from each other. The regression slopes are graphically displayed in Fig. 2.9.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for non-infested female and infested Thais lapillus collected in August 1982 are shown in Table 2.11. The female regression line is most

significant ($p \leq 0.005$), the infested line is just significant ($p \leq 0.05$), and the males show no significant relationship between shell length and dry body mass, and so are not included in the ANCOVA. The difference between the mean dry weight values for infested and female samples is significantly greater than difference within samples ($p < 0.001$). The two lines of common slope plotted through the two data groups are highly significant ($p \leq 0.001$), and the individual group slopes are not significantly different from the pooled slope ($p \geq 0.1$). The intercept values for each sample are responsible for significantly more of the error variation around a single pooled line than the errors around the two lines of common slope ($p \leq 0.001$), so the two can be considered separately from each other. The regression slopes are graphically displayed in Fig. 2.10.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for non-infested male and female and Infested T. lapillus collected in October 1982, are shown in Table 2.13. The male regression line is significant for this month ($p \leq 0.005$).The female regression line is highly significant ($p \leq 0.001$), and so is the infested line ($p \leq 0.001$). The difference between the mean dry mass values for male, female and infested samples is significantly greater than the difference within the samples. ($p \leq 0.001$). The three lines of common slope plotted through the three data groups are highly significant ($p \leq 0.001$), and the individual group slopes are not significantly different from the pooled slope ($p \geq 0.1$). The intercept values for each sample are responsible for

significantly more of the error variation around a single pooled line than the errors around the three lines of common slope ($p \leq 0.001$), so the three groups should be considered separately from each other. The regression slopes are graphically displayed in Fig. 2.10.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for infested Thais lapillus collected in March, July, August, and October 1982 are shown in Table 2.13. The March regression line is significant, with a probability ≤ 0.025. The July regression line is significant, with a probability < 0.005. The August regression line is least significant with a probability ≤ 0.05, and the October regression line most significant with a probability ≤ 0.001. The difference between the group mean dry masses for March, July, August and October samples is significantly greater than the differences within samples ($p \leq 0.001$). The four lines with a common slope plotted separately through the four data groups explain a significant proportion of the dry mass variation ($p \leq 0.001$). The individual group slopes are not significantly different from the pooled slope ($p \geq 0.1$). The intercept values for each sample are responsible for a significantly larger proportion of the error variation around a single pooled line drawn through all the data points than the errors around the four lines with the common slope ($p \leq 0.001$). Thus the four samples should be considered separately from each other. The regression slopes are graphically displayed in Fig. 2.11.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for non-infested male Thais lapillus collected in February, March and October are shown in Table 2.14. July and August samples showed no significant relationship between shell length and dry body mass. The February regression line is significant, with a probability $\leq$ 0.01. The March regression line is more significant, with a probability $\leq$ 0.001, but the October regression line is slightly less significant having a probability $\leq$ 0.005.

The difference between the group mean dry masses for February, March and October samples is significantly greater than the differences within samples ($P \leq 0.01$). The three lines with a common slope plotted separately through the three data groups explain a significant proportion of the dry mass variation ($p \leq 0.001$). The individual group slopes are however just significantly different from the pooled slope ($p \leq 0.025$), and so the three samples are considered separately from each other. The regression slopes are graphically displayed in Fig. 2.11.

The results of ANCOVA of the regressions of Log dry body mass (mg) against Log shell length (mm) for non-infested female dogwhelks collected in February, March, July, August, and October 1982, are shown in Table 2.15. The February, regression line is significant, with a probability $\leq$ 0.01. The March regression line is significant, with a probability $\leq$ 0.001 and the July regression line is similarly significant. The August regression line is significant with a probability $\leq$ 0.005. The October regression line is

significant with a probability $\leq$ =0.001. The difference between the group mean dry masses, for February, March, July, August and October samples is significantly greater than the differences within the samples ($P \leq 0.001$). The five lines with a common slope plotted through the different sample means explain a very significant proportion of the variation in body dry mass ($P \leq 0.001$). The individual group slopes are not significantly different from the pooled lines ($P \geq 0.1$). The intercept values for each sample are responsible for a significantly larger proportion of the error variation around a single pooled line drawn through all the data points than the errors around the five lines drawn with the same common slope ($P \leq 0.001$). Thus the five samples should be considered separately from one another. The regression slopes are graphically displayed in Fig. 2.11.

Estimate of Net Energy Investment in Soft Body Tissue Growth.

A summary of the measurements made on infested Thais lapillus collected in April 1981 from Scarborough South Beach is shown in Table 2.16. In the first column (from the left) of Table 2.16. is the (height + width) (mm) of the shell after it has had the distance in the second column removed from its aperture lip, before which its measurements were as recorded in the third column. The data is arranged as shown so that the increment from one teeth row to the next can be clearly demonstrated. The increase in dry mass is calculated from the Log dry body mass (mg) against Log shell (height + width)(mm) regression shown in Fig. 2.12, and Table 2.18.

The June regression is more significant than the regression for the April sample actually cut down (Fig. 2.12, and Table 2.17), having a probability $\leq$ 0.001 as opposed to the April value, $p \leq 0.05$.

The calculation of the estimate of the net energy investment into growth is shown in Table 2.19. The final value has been shown +-the 95% confidence limits for shell growth converted into dry mass and finally energy increment. Other errors have been ignored, eg. variation around the June Log dry body mass against Log shell(height + width) regression, and variation around the mean tissue caloric value, because the magnitude of the increment error greatly limits the accuracy of the estimate.

Plate 2.1

Appearance of multiple rows of aperture teeth on the interior of the shell of an infested Thais lapillus host.

Plate 1

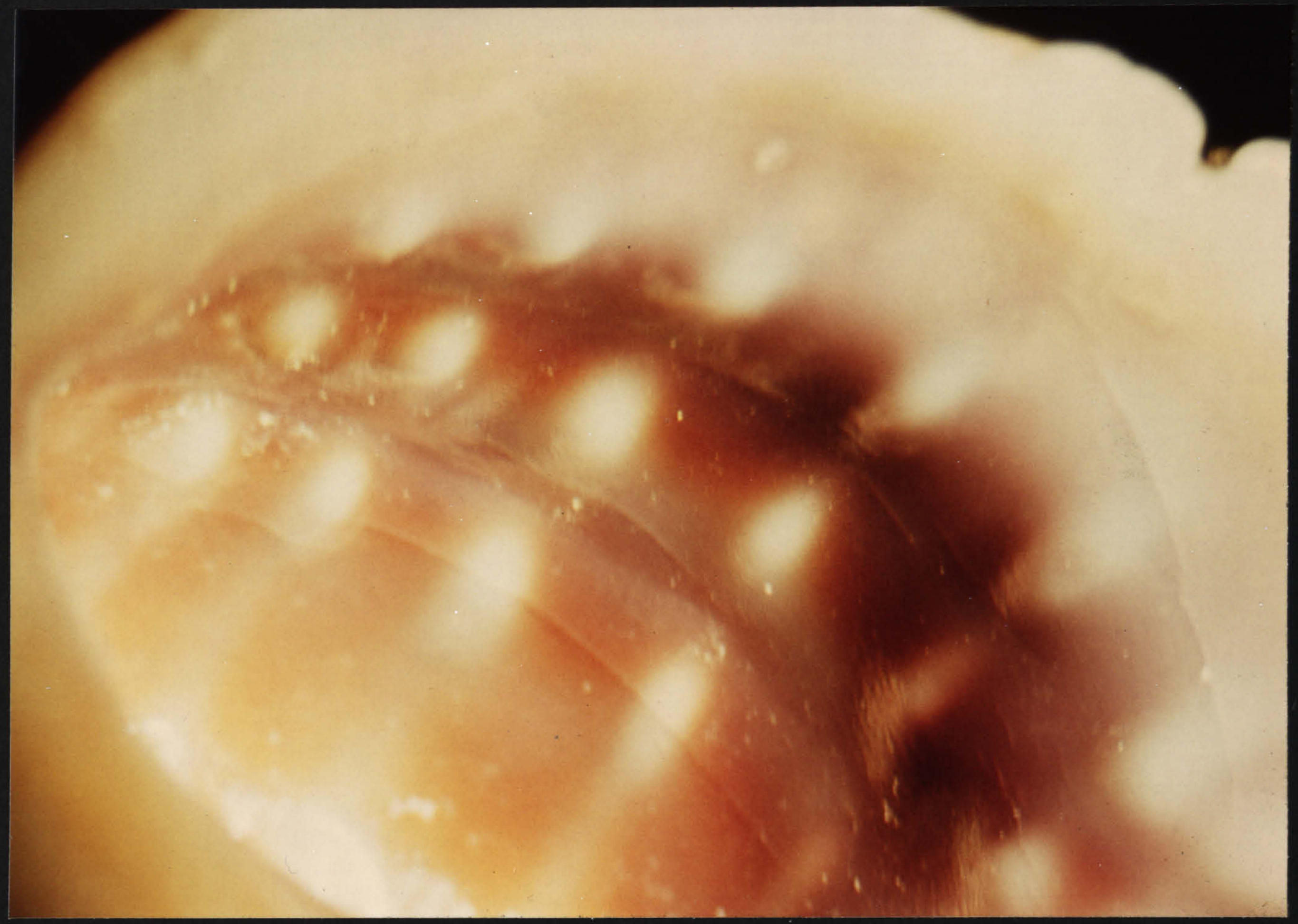


Plate 2.2

A collection of Thais lapillus adults from Scarborough South Bay which demonstrates a range of shell deformities associated with the presence of Parorchis acanthus infestations.

Individuals 1-3 demonstrate the shell deformities only found in infested animals whereas animals 20-23 are normal in appearance.

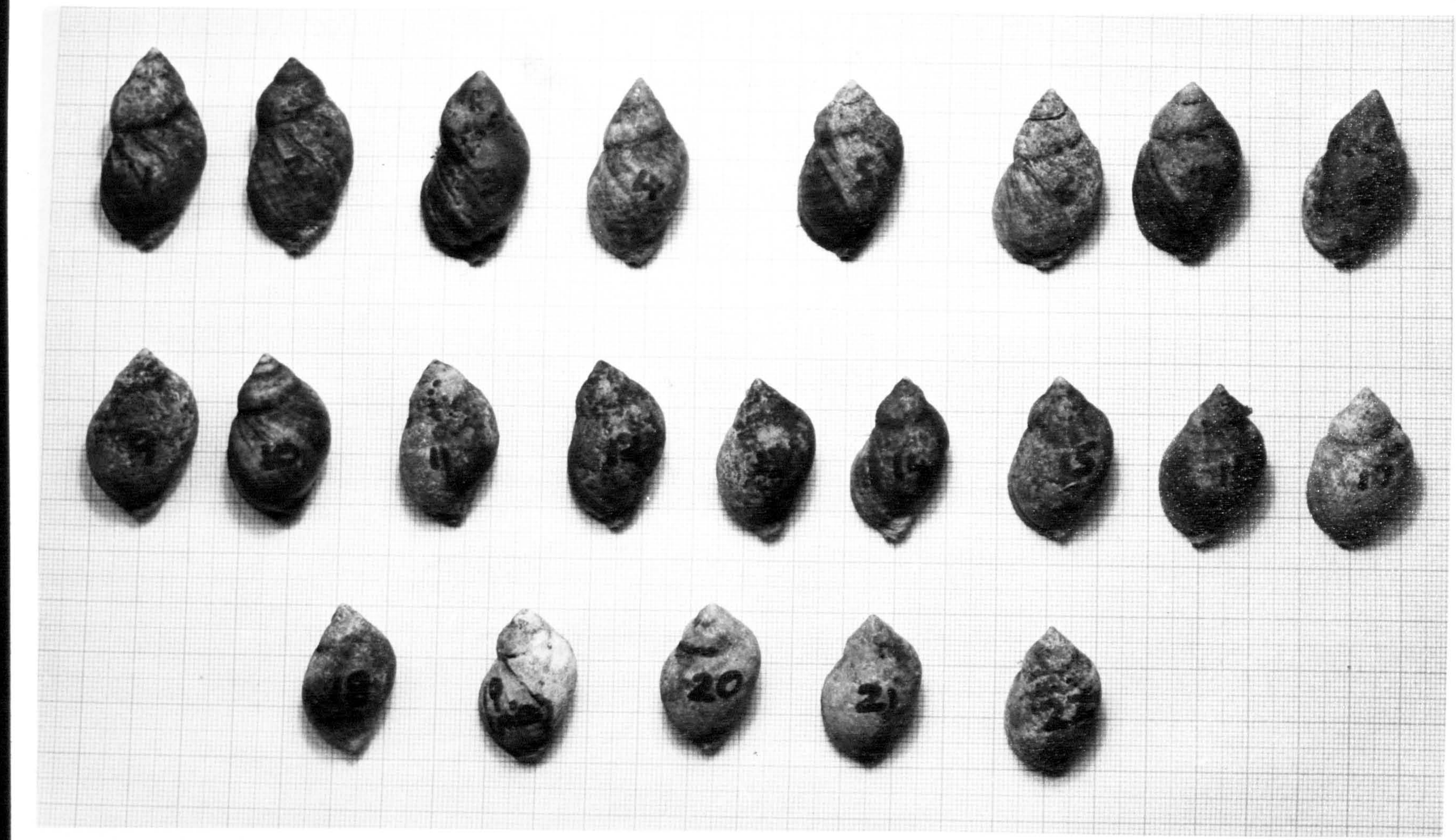


Plate 2

Plates 2.3 - 2.9

This series of plates illustrates the changes in shell shape resulting from the removal of successive rows of teeth from the shell aperture of an individual, *Thais lapillus* infested with *Parorchis acanthus*.

Plate 3

Plate 4

Plate 5

Plate 6

Plate 7

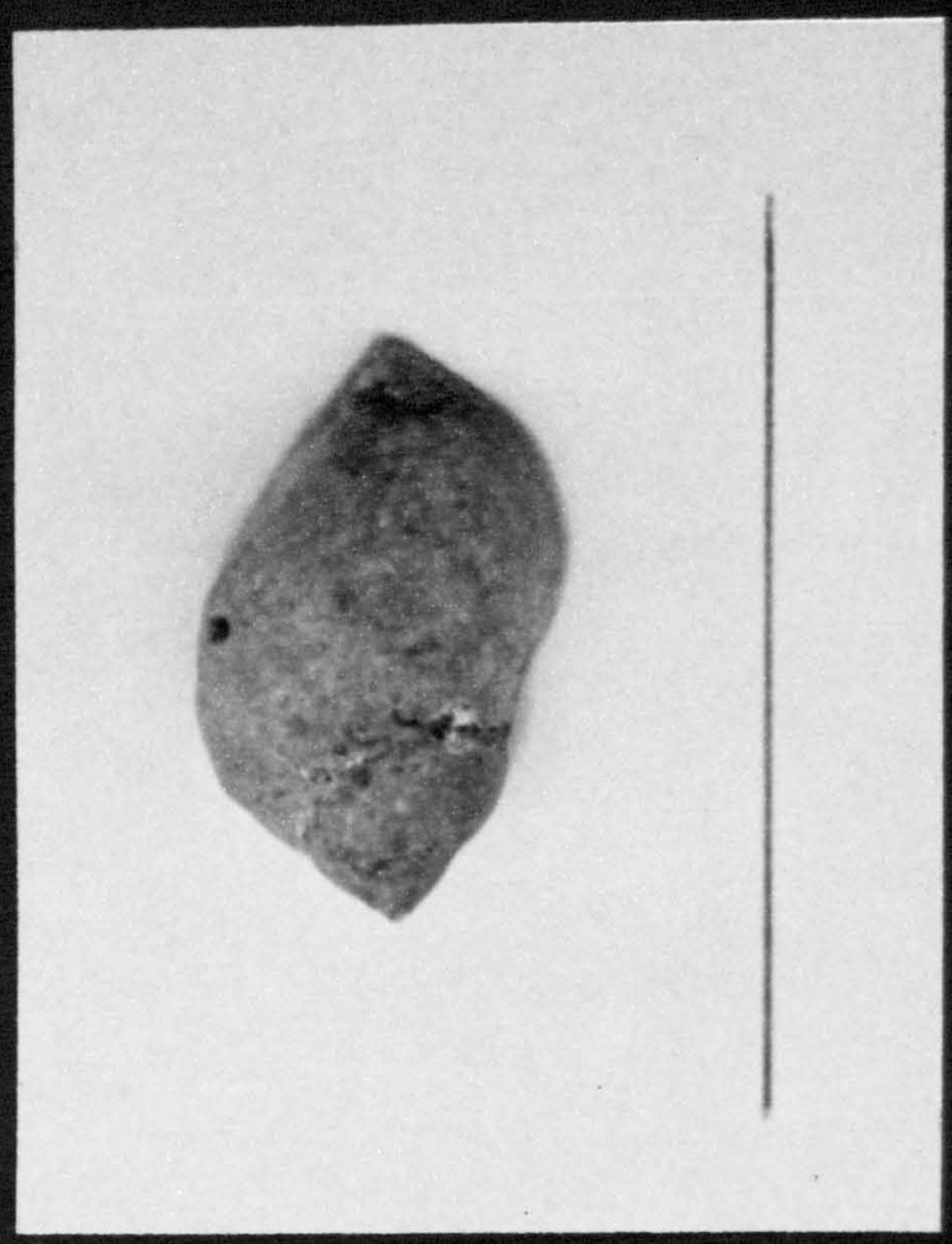


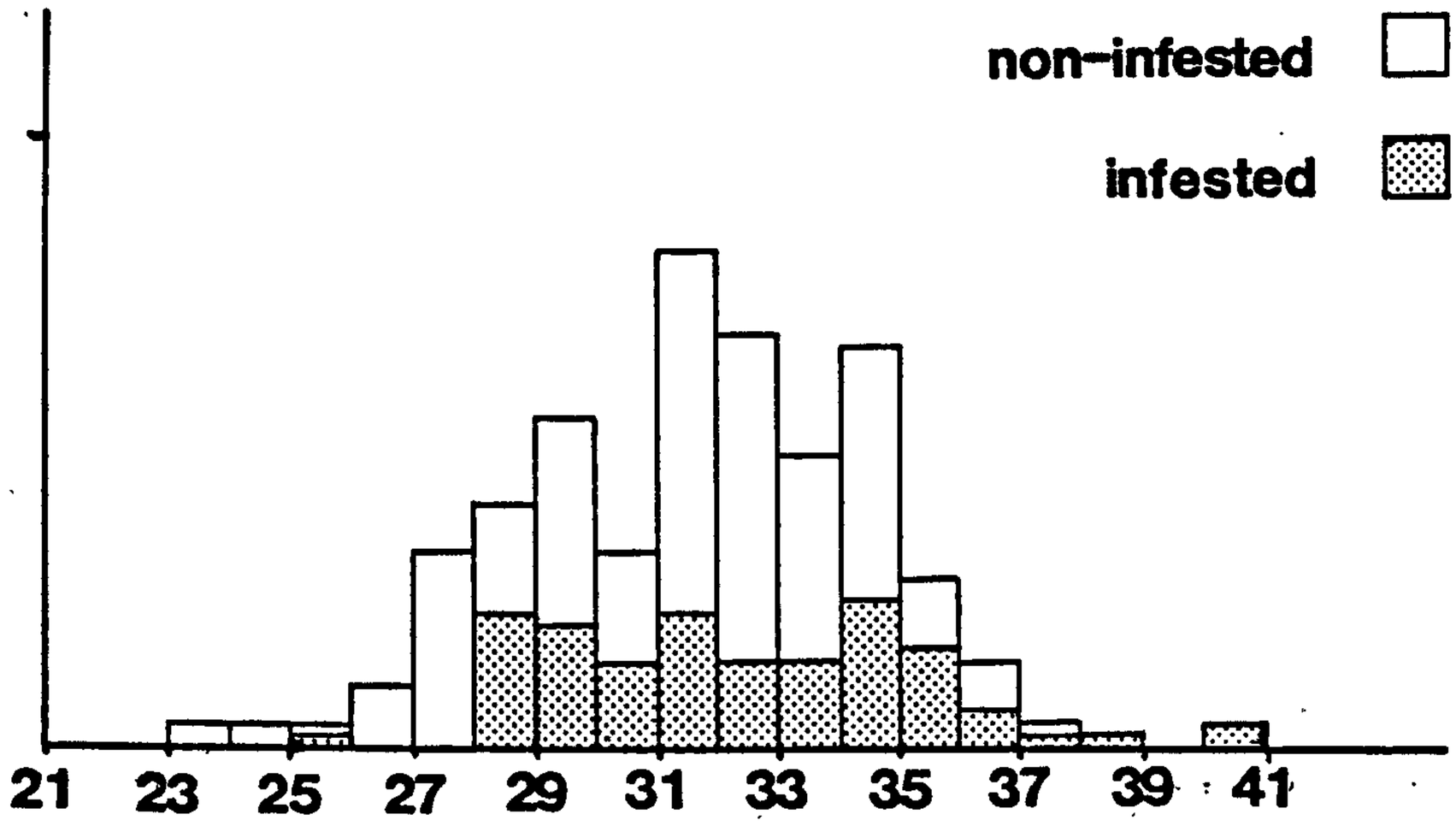
Plate 8

Plate 9

Fig. 2.1

Shell length frequency distributions for infested and non-infested *Thais lapillus* from Scarborough South Bay and Robin Hoods Bay.

SCARBOROUGH SOUTH BAY.



ROBIN HOOD'S BAY.

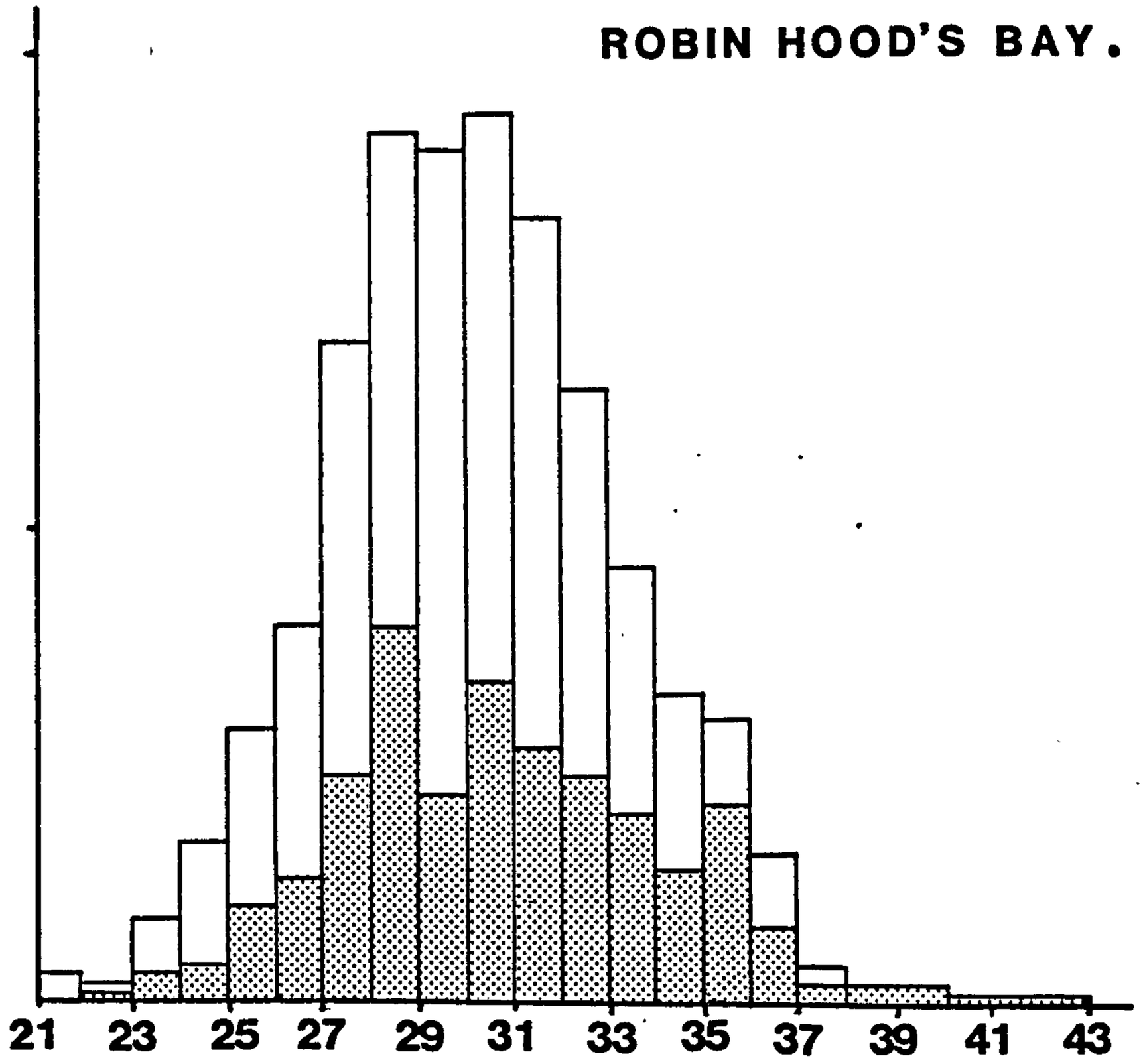


Fig. 2.2

Frequency distribution of numbers of shell teeth rows for infested and non-infested *Thais lapillus* from Scarborough South Bay and Robin Hoods Bay.

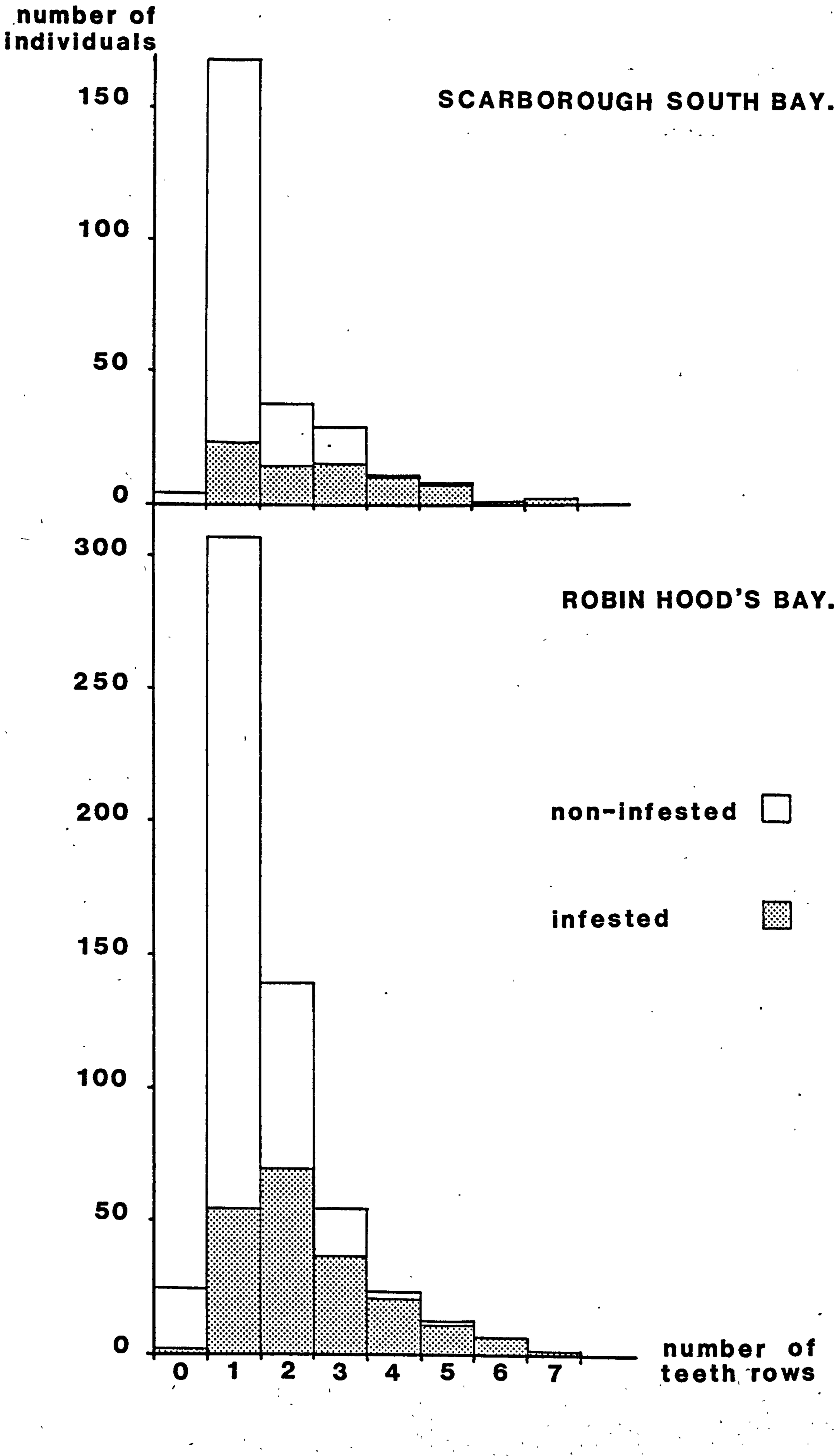
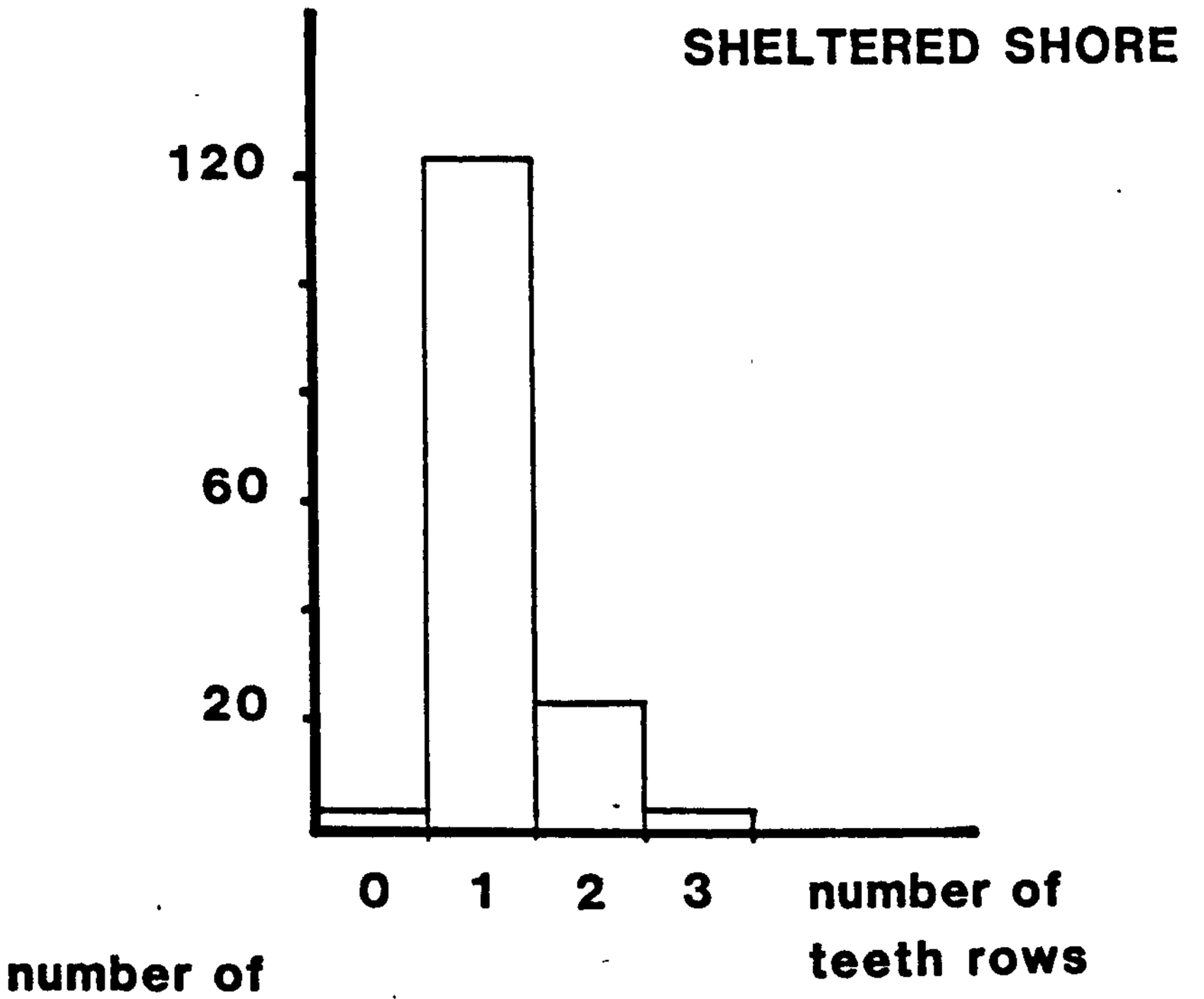

number of individuals
150
100
50
0
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7
number of teeth rows
SCARBOROUGH SOUTH BAY.
ROBIN HOOD'S BAY.
non-infested
infested

Fig. 2.3

Frequency distributions of numbers of shell teeth rows for non-infested Thais lapillus collected from exposed (West Dale) and sheltered (Dale Fort) shores where no infested T. lapillus were found.


number of individuals
SHELTERED SHORE
120
60
20
0
1
2
3
number of teeth rows

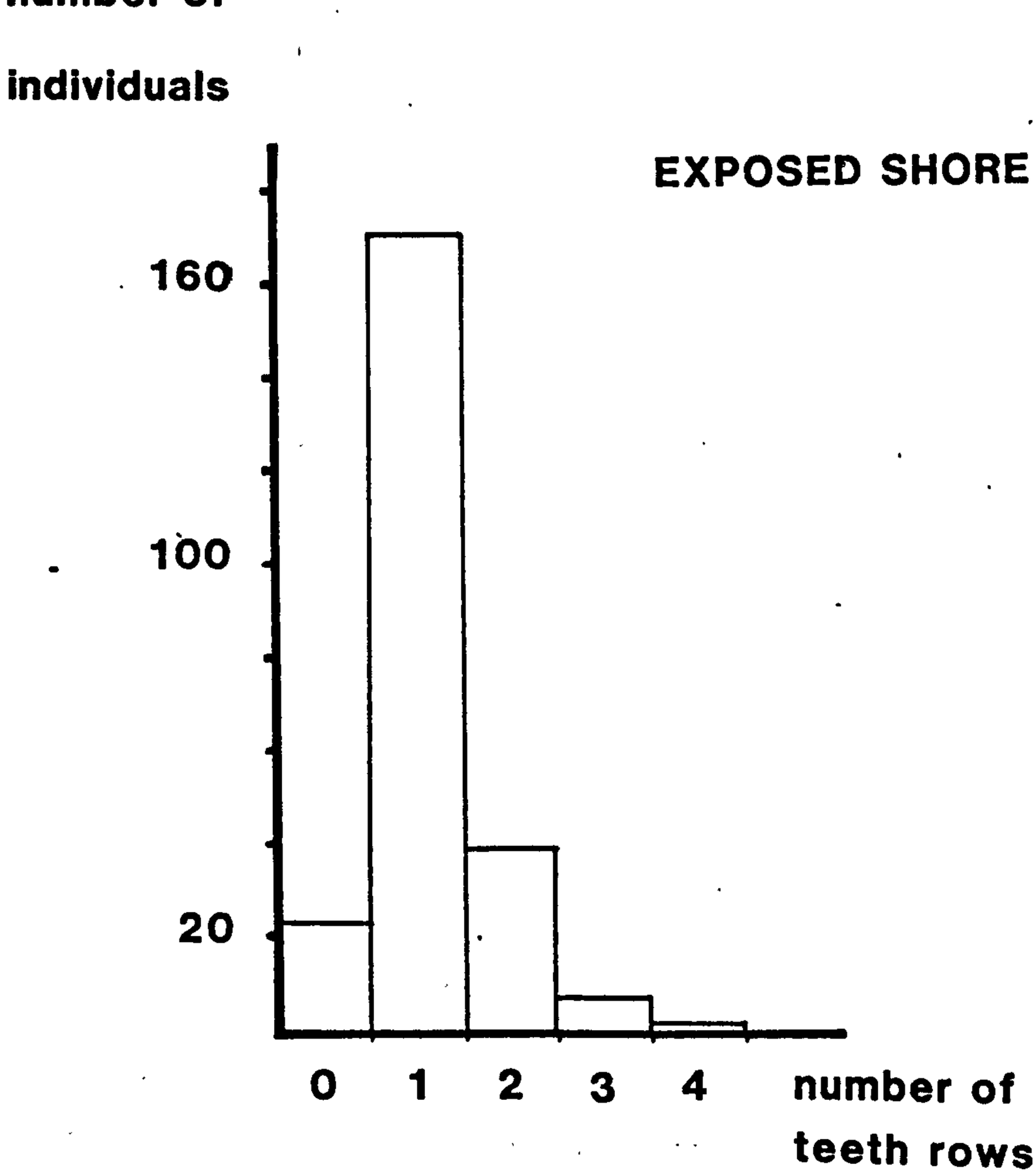

number of individuals
EXPOSED SHORE
160
100
20
0
1
2
3
4
number of teeth rows

Fig. 2.4

Shell lip thickness frequency distributions for infested and non-infested Thais lapillus from Robin Hoods Bay.

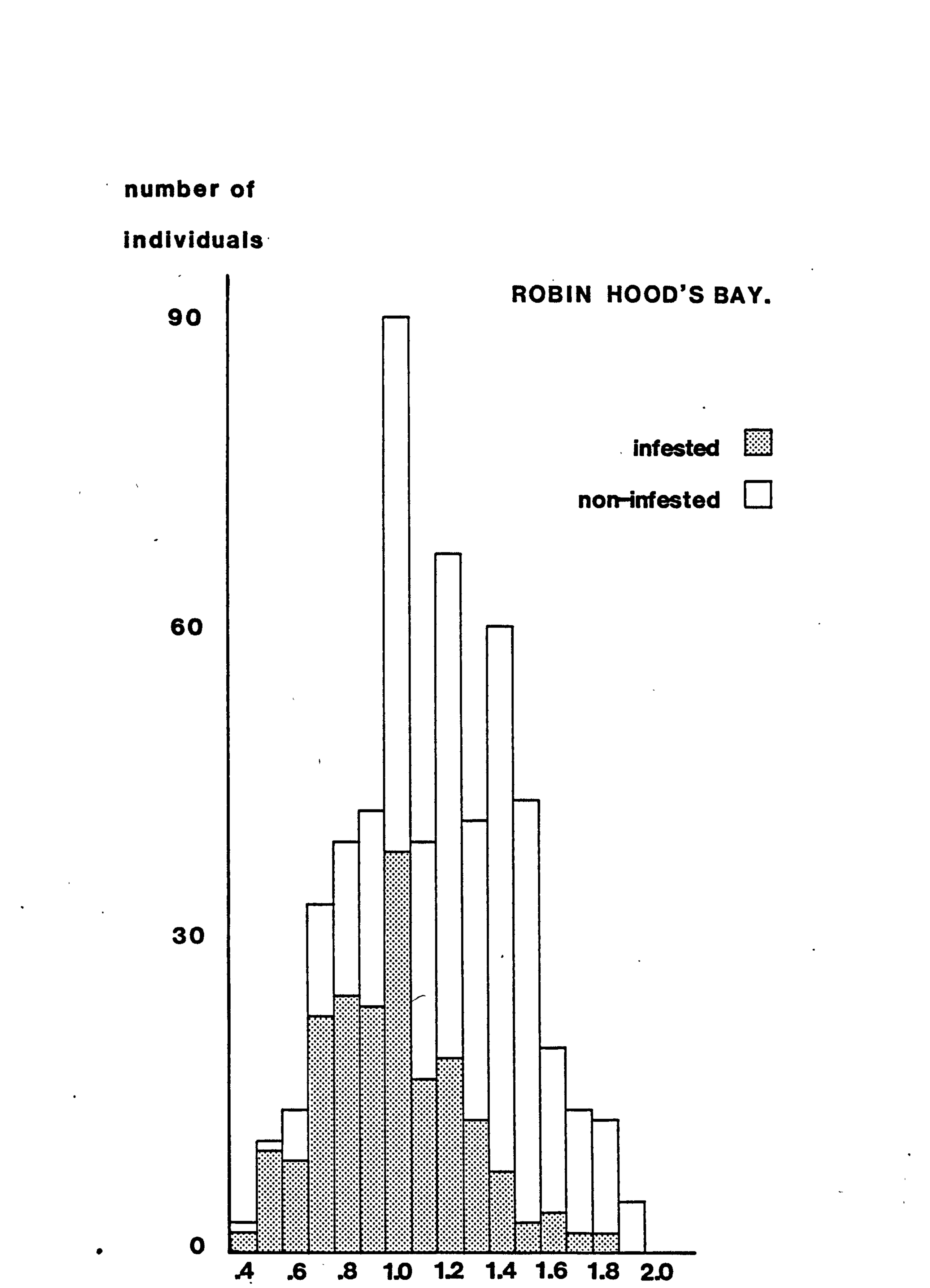
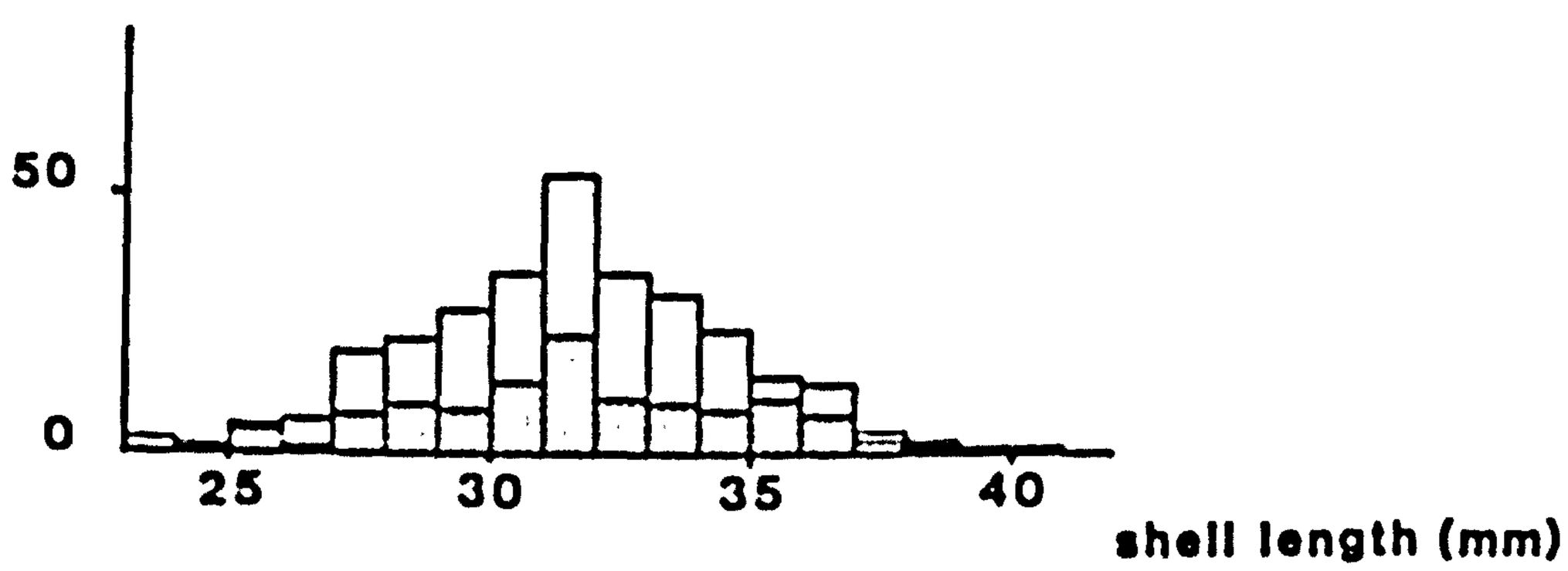

ROBIN HOOD'S BAY.
infested
non-infested
number of individuals
90
60
30
0
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
shell lip thickness (mm)

Fig. 2.5

Spring shell lip length frequency distributions for infested and non-infested Thais lapillus from Robin Hoods Bay, 1982, with the sample shell length frequency distributions above.

FEBRUARY MARCH MAY



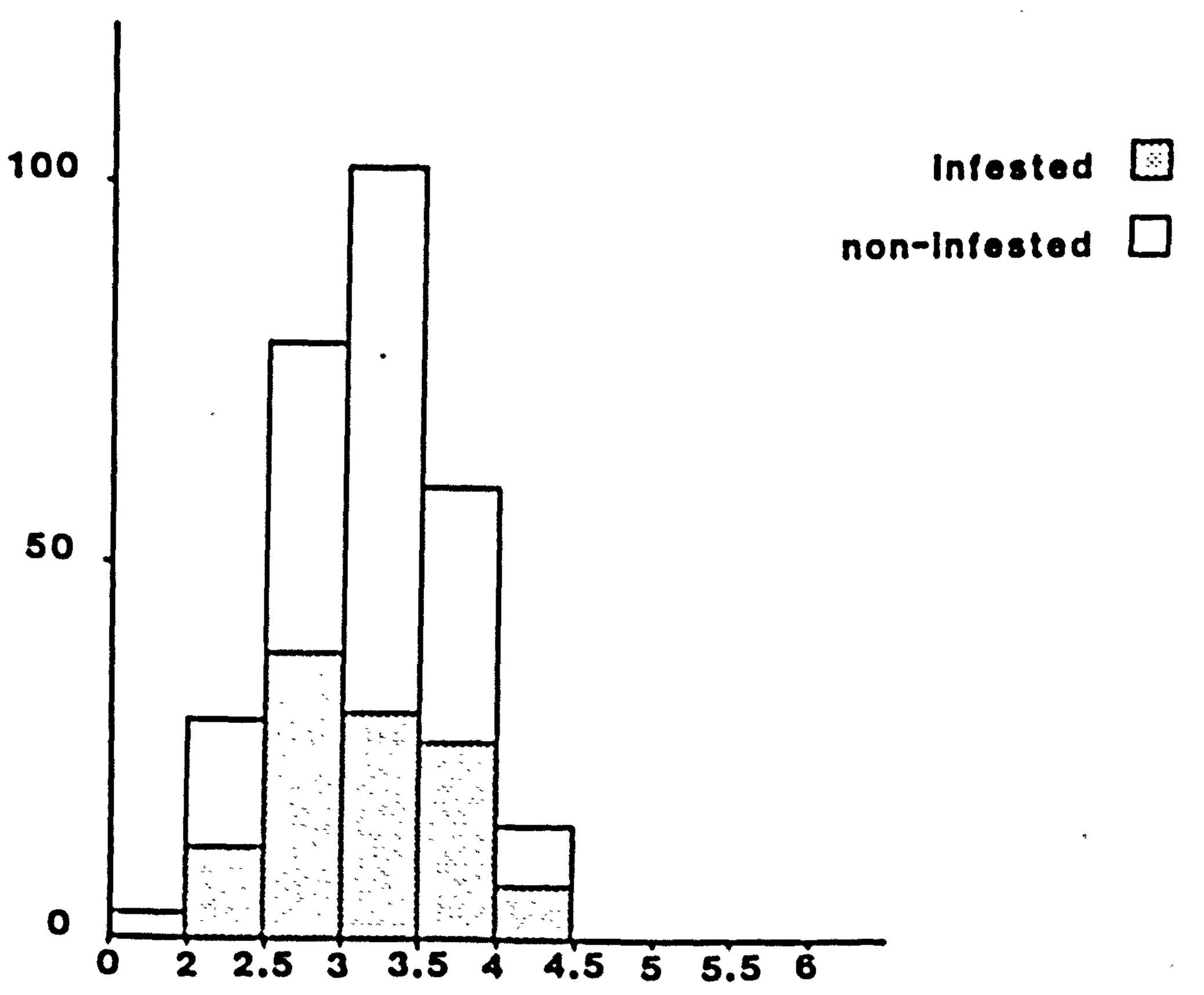


Fig. 2.6

Summer and autumn shell lip length frequency distributions for infested and non-infested *Thais lapillus* from Robin Hood's Bay, 1982, with the sample shell length frequency distributions above.

number of individuals

JULY AUGUST OCTOBER

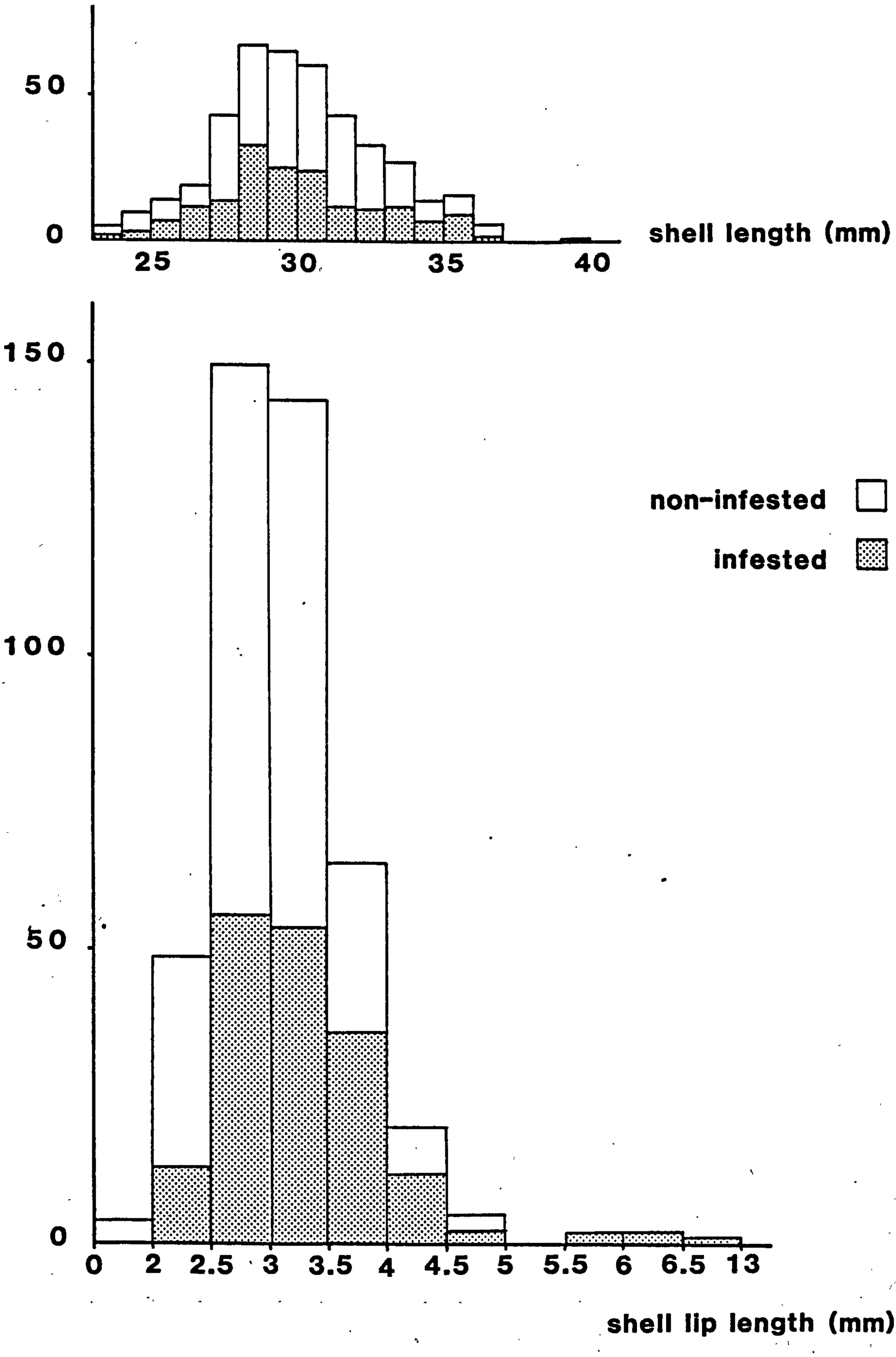


Fig. 2.7

Frequency distributions of whorl width ratios for non-infested and infested Thais lapillus from Scarborough South Bay.

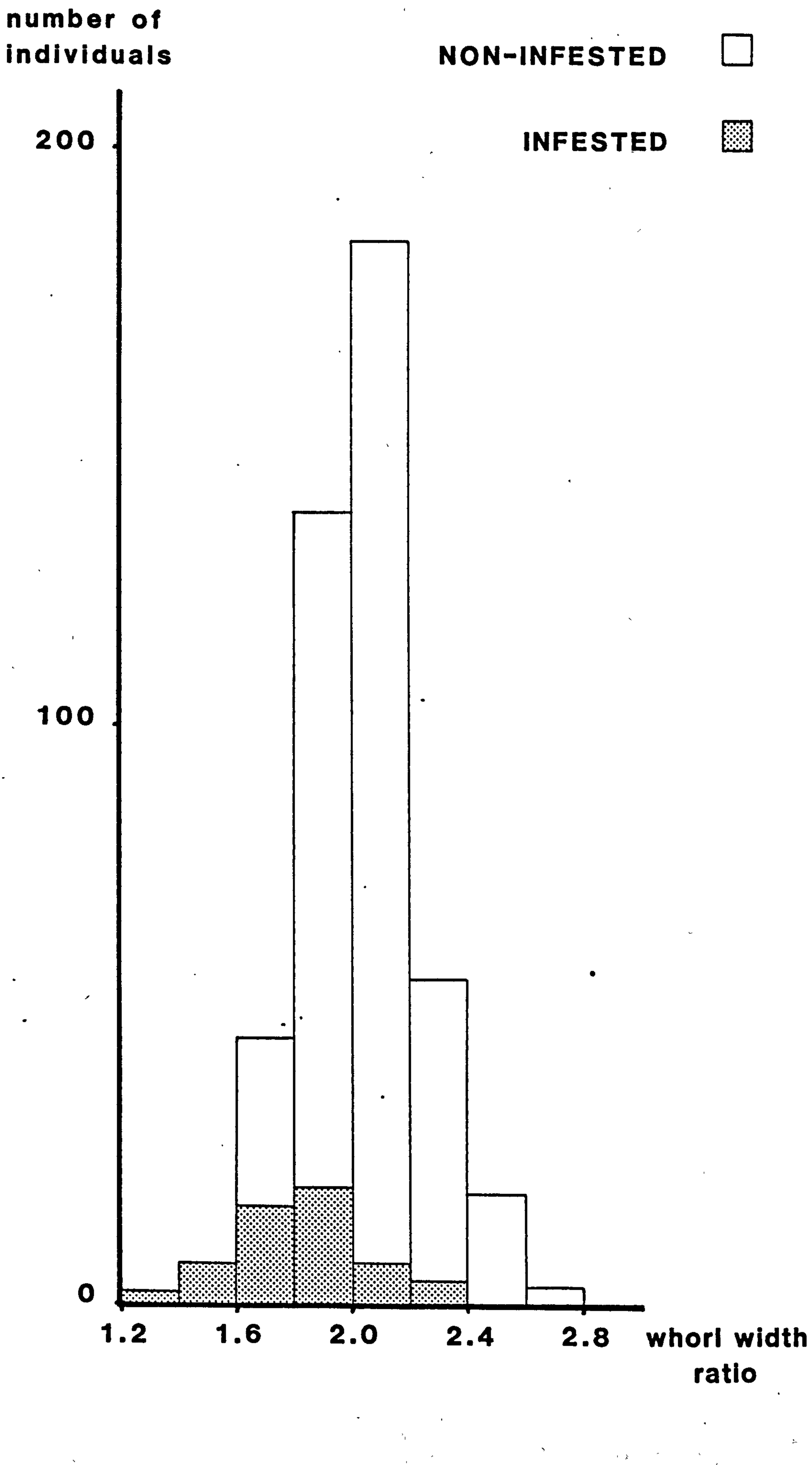

number of individuals
NON-INFESTED
INFESTED
200
100
0
1.2
1.6
2.0
2.4
2.8
whorl width ratio

Fig. 2.8

Correlation between the shell whorl width ratio and the number of aperture teeth rows for infested Thais lapillus from Scarborough and Robin Hoods Bay.

($r = 0.66$ $n = 58$ $p \leqslant 0.001$).

⊙ = 2 DATUM POINTS

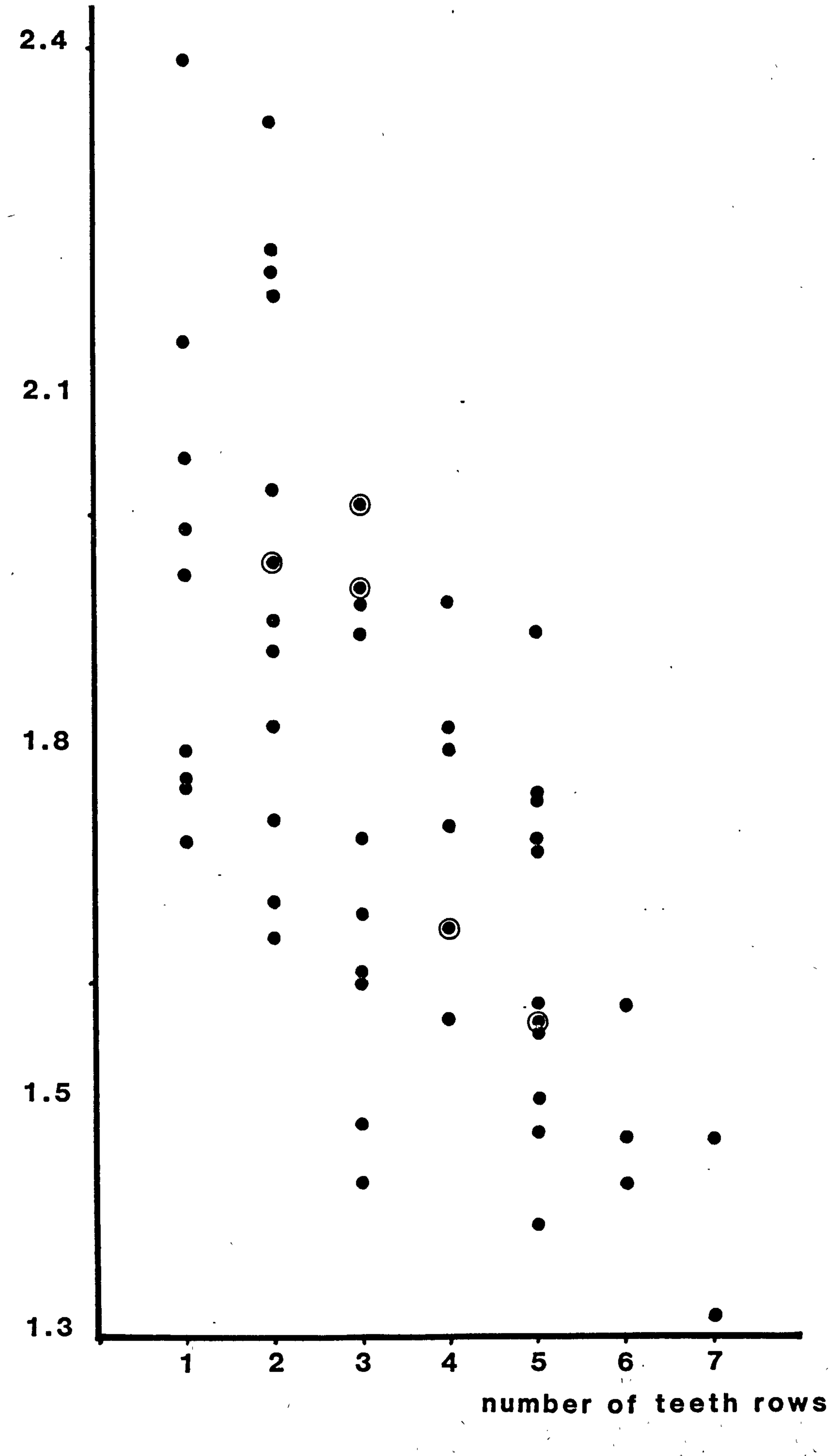

whorl width ratio
2.4
2.1
1.8
1.5
1.3
1
2
3
4
5
6
7
number of teeth rows

Fig. 2.9

Log dry body mass versus Log shell length regressions for infested and non-infested male and female Thais lapillus collected in February, March and July, 1982.

Key:- 1 = individuals infested with Parorchis acanthus

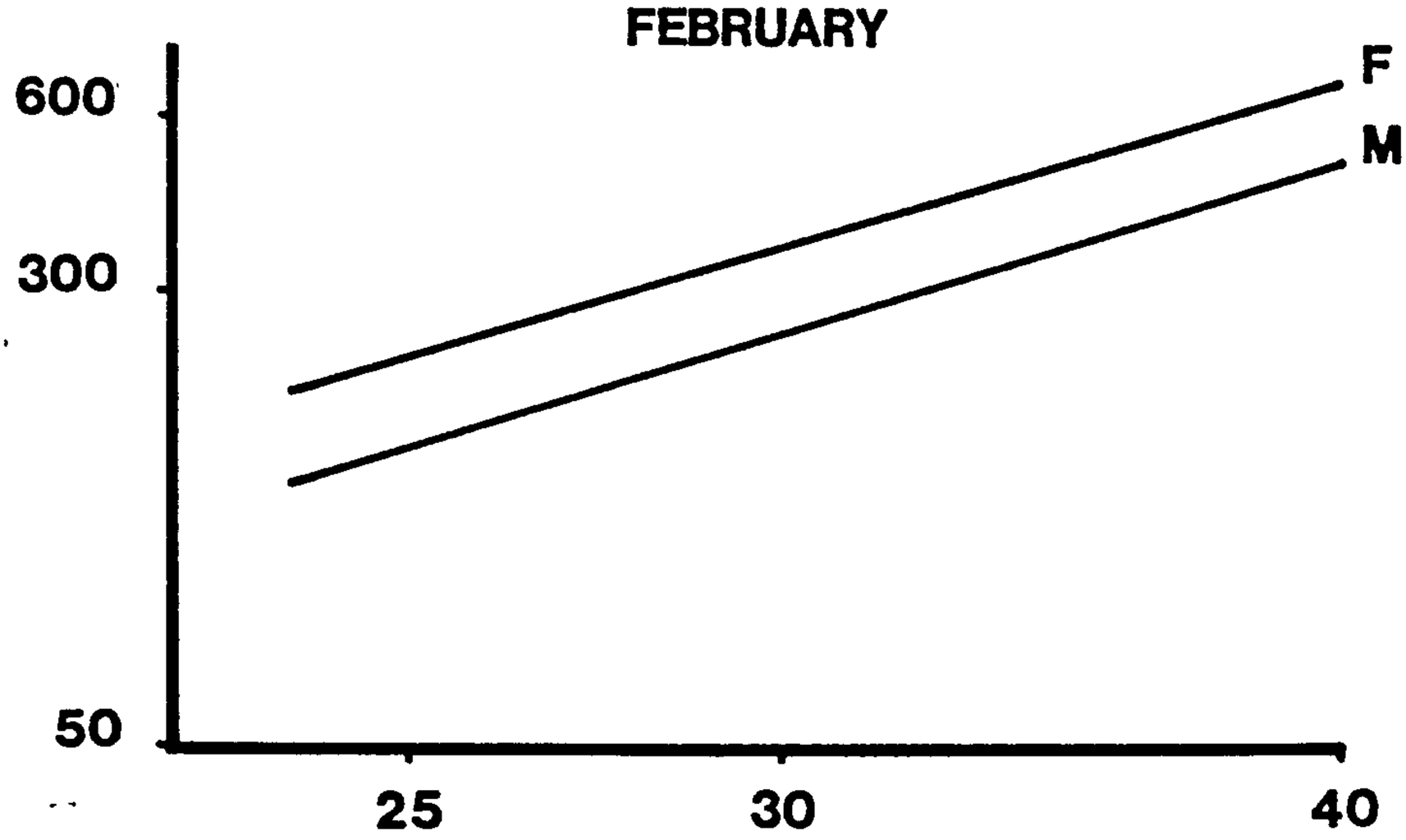
M = non-infested Male

F = non-infested Female

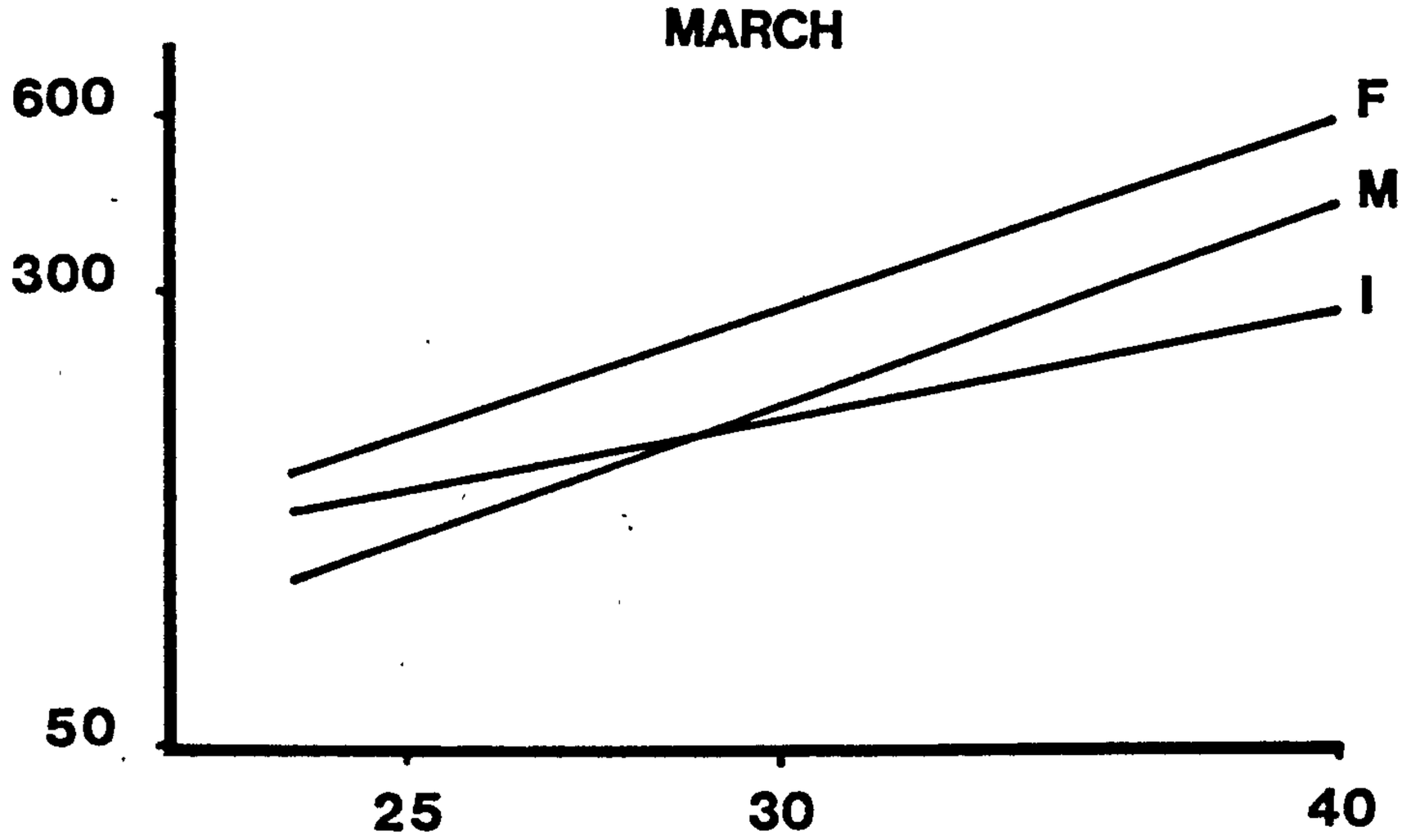
(Regression equations and F values are shown in Tables 2.3, 2.4 and 2.5).

log dry body mass (mg)

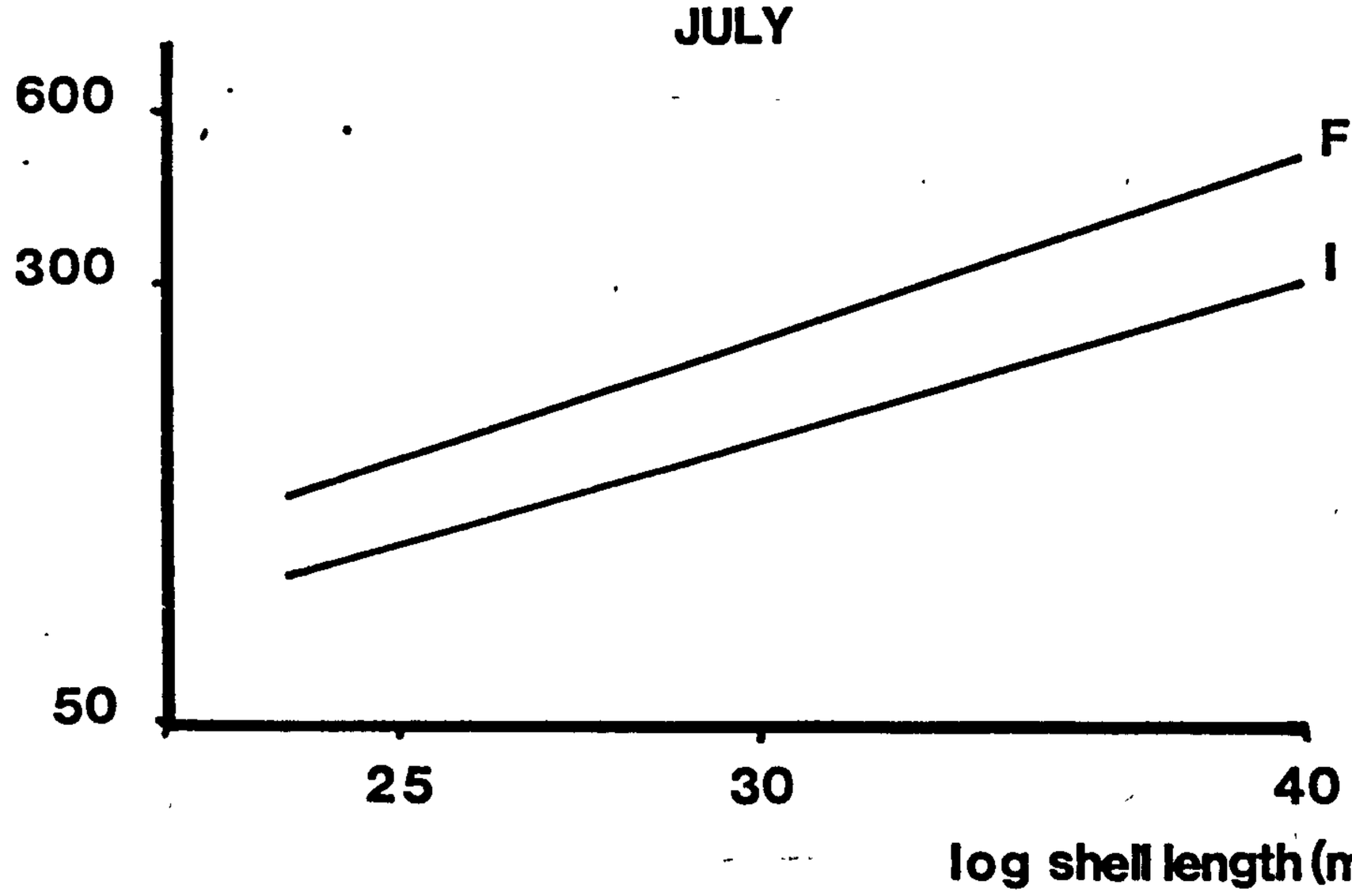
FEBRUARY



MARCH



JULY



log shell length (mm)

Fig. 2.10

Log dry body mass versus Log shell length regressions for infested and non-infested male and female Thais lapillus collected in August and October 1982.

Key:- 1 = individuals infested with Parorchis acanthus

M = non-infested males

F = non-infested females.

(Regression equations and F values are shown in Tables 2.6 and 2.7).

log dry body mass (mg)

AUGUST

600

300

50

F

I

25

30

40

OCTOBER

600

300

50

F

I

M

25

30

40

log shell length (mm)

Fig. 2.11

Log dry body mass versus Log shell length regressions for infested and non-infested male and female Thais lapillus collected in 1982.

Key:- F = February

M = May

J = July

A = August

O = October

(Regression equations and F values are shown in Tables 2.3, 2.4, 2.5, 2.6, and 2.7).

log dry body mass (mg)

INFESTED

600
300
50

O
J
M
A

25 30 40

MALE

600
300
50

F
O
M

25 30 40

FEMALE

600
300
50

F
O
M
J
A

25 30 40

log shell length (mm)

Fig. 2.12

Log dry body mass versus Log shell (height + width) regressions for infested Thais lapillus collected from Scarborough South Beach in April and June 1981.

(Regression equations and F values are shown in Tables 2.17 and 2.18).

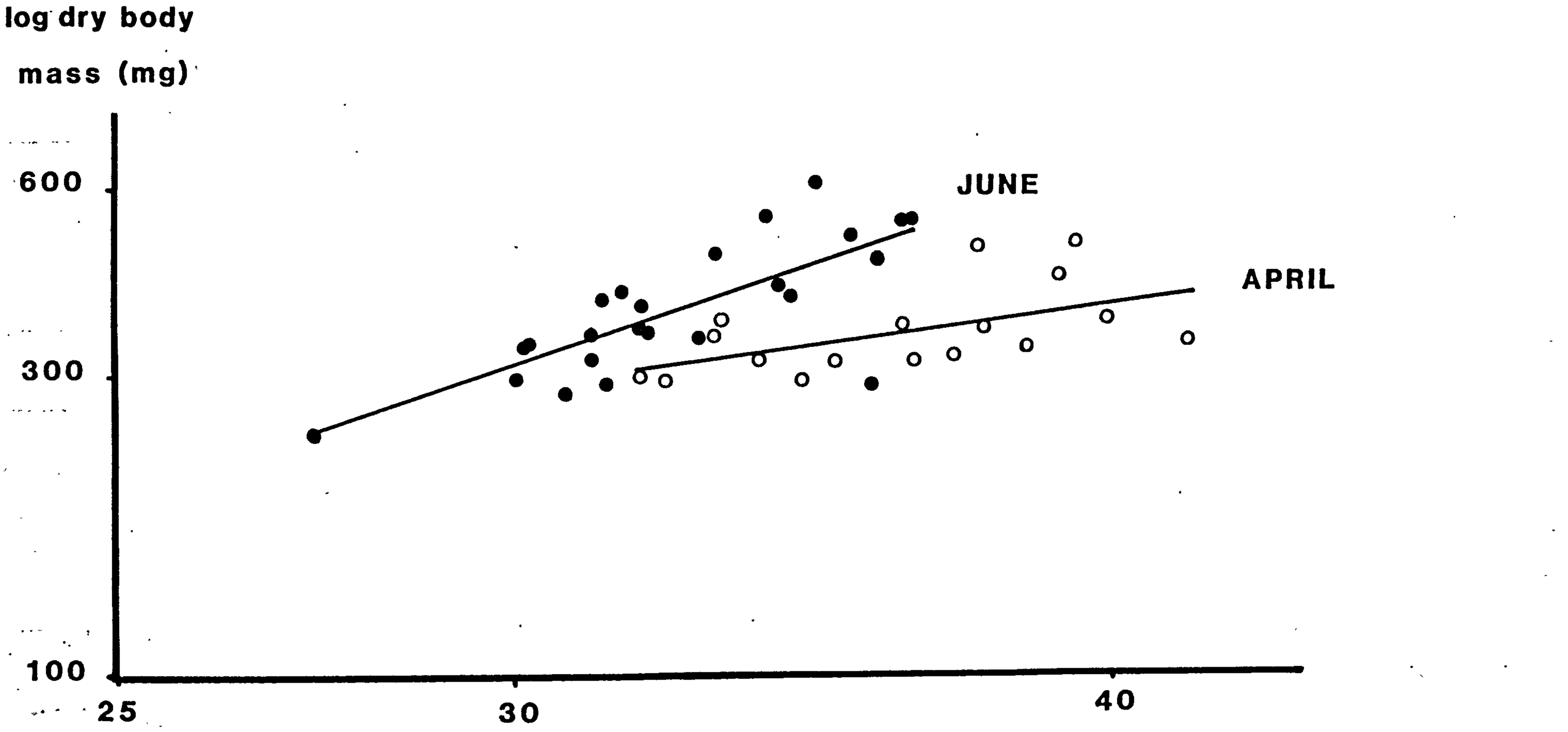

log dry body
mass (mg)
600
300
100
25
30
40
JUNE
APRIL
log shell height + width (mm)

TABLE 2.1. t-TEST ON SHELL LIP THICKNESS MEANS (mm) OBTAINED FOR NON-INFESTED AND INFESTED THAIS LAPILLUS.

	N	$\Sigma(x)$	$\Sigma(x^2)$	$\bar{x}$	t
NON-INFESTED	335	544.81	415.50	1.24	8.67
INFESTED	200	209.30	196.80	0.98	

TABLE 2.2. t-TEST ON WHORL WIDTH RATIO MEANS OBTAINED FOR NON-INFESTED AND INFESTED T. LAPILLUS COLLECTED FROM SCARBOROUGH.

	N	$\Sigma(x)$	$\Sigma(x^2)$	$\bar{x}$	t
NON-INFESTED	399	1705.23	821.39	2.059	8.60
INFESTED	59	107.68	199.47	1.825	

REGRESSION EQUATIONS FOR LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR MALE, FEMALE AND INFESTED THAIS LAPILLUS COLLECTED IN 1982.

TABLE 2.3.

FEBRUARY

	MALE	FEMALE	INFESTED
b.	2.456 se=0.353 with 30df	2.349 se=0.777 with 25df	
a.	0.059	0.119	

TABLE 2.4.

MARCH

	MALE	FEMALE	INFESTED
b.	2.763 se=0.123 with 19df	2.641 se=0.377 with 13df	1.470 se=0.285 with 14df
a.	0.016	0.035	1.216

TABLE 2.5.

JULY

	MALE	FEMALE	INFESTED
b.		2.605 se=0.547 with 25df	2.265 se=0.465 with 19df
	Not significant		
a.		0.033	0.071

TABLE 2.6.

AUGUST

	MALE	FEMALE	INFESTED
b.		1.592 se=0.229 with 25df	1.044 se=0.223 with 37df
	Not significant		
a.		0.129	6.152

TABLE 2.7.

OCTOBER

	MALE	FEMALE	INFESTED
b.	1.282 se=0.172 with 31df	2.009 se=0.062 with 44df	1.904 se=0.106 with 70df
a.	3.412	0.389	0.412

TABLE 2.8.ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR NON-INFESTED MALE AND FEMALE THAIS LAPILLUS COLLECTED IN FEBRUARY 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.1794	0.1794	48.3011
Error (deviations from slope)	30	0.1114	0.0037	
MALE group total	31	0.2908		
Due to FEMALE regression	1	0.1031	0.1031	9.1515
Error (deviations from slope)	25	0.2815	0.0113	
FEMALE group total	26	0.3846		
Between groups	1	0.4668	0.4668	39.3887
Within groups	57	0.6755	0.0119	
Common slope within groups	1	0.2824	0.2824	40.2220
Error (deviations from common slope)	56	0.3931	0.0069	
Difference between slopes	1	0.0013	0.0013	0.0182
Error (deviations from slope within each group)	55	0.3930	0.0072	
Total (groups + within)	58	1.1422		
Common slope within study	1	0.4639	0.4639	
Error (deviations from common slope)	57	0.6783	0.0119	
Among intercepts	1	0.2852	0.2852	40.6337
Error (deviations from common slope)	56	0.3931	0.0070	

TABLE 2.9.ANALYSIS OF COVARIANCE (ANCOVA) OF REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR NON-INFESTED MALE AND FEMALE AND INFESTED THAIS LAPILLUS COLLECTED IN MARCH 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.2238	0.2238	46.7200
Error (deviations from slope)	19	0.0910	0.0048	
MALE total	20	0.3148		
Due to FEMALE regression	1	0.1714	0.1714	18.5273
Error (deviations from slope)	13	0.1203	0.0093	
FEMALE total	14	0.2916		
Due to INFESTED regression	1	0.0878	0.0878	7.5737
Error (deviations from slope)	14	0.1624	0.0116	
INFESTED total	15	0.2502		
Between groups	2	0.2344	0.1172	6.7027
Within groups	49	0.8566	0.0175	
Common slope within groups	1	0.4473	0.4473	52.4560
Error (deviations from common slope)	48	0.4093	0.0085	
Difference between slopes	2	0.0357	0.0178	2.1969
Error (deviations from slope within each group)	46	0.3736	0.0081	
Total (groups + within)	51	1.0909		
Common slope within study	1	0.3077	0.3077	
Error (deviations from common slope)	50	0.7832	0.0157	
Among intercepts	2	0.3739	0.1870	21.9258
Error (deviations from common slope)	48	0.4093	0.0085	

TABLE 2.10.ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR NON-INFESTED MALE AND FEMALE AND INFESTED THAIS LAPILLUS COLLECTED IN JULY 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.0354	0.0354	3.9360*
Error (deviations from slope)	19	0.1707	0.0090	
MALE total	20	0.2061		
Due to FEMALE regression	1	0.1893	0.1893	22.6568
Error (deviations from slope)	14	0.1170	0.0084	
FEMALE total	15	0.3063		
Due to INFESTED regression	1	0.1316	0.1316	11.0342
Error (deviations from slope)	19	0.2267	0.0119	
INFESTED total	20	0.3883		
Between groups	1	0.2429	0.2429	12.7925
Within groups	35	0.6646	0.0100	
Common slope within groups	1	0.3194	0.3194	31.4600
Error (deviations from common slope)	34	0.3452	0.0102	
Difference between slopes	1	0.0016	0.0016	0.1491
Error (deviations from slope within each group)	33	0.3437	0.0104	
Total (groups + within)	36	0.9076		
Common slope within study	1	0.2572	0.2572	
Error (deviations from common slope)	35	0.6504	0.0186	
Among intercepts	1	0.3052	0.3052	30.0568
Error (deviations from common slope)	34	0.3452	0.0102	

* not significant - not included in comparison.

TABLE 2.11. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR FEMALE AND INFESTED THAIS LAPILLUS COLLECTED IN AUGUST 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.0072	0.0072	0.0432*
Error (deviations from slope)	43	0.7714	0.01719	
MALE total	44	0.7786		
Due to FEMALE regression	1	0.1222	0.1222	11.0801
Error (deviations from slope)	25	0.2758	0.0110	
FEMALE total	26	0.3980		
Due to INFESTED regression	1	0.0590	0.0590	4.8858
Error (deviations from slope)	37	0.4468	0.0121	
INFESTED toal	38	0.5058		
Between groups	1	0.1721	0.1721	12.1861
Within groups	64	0.9038	0.0141	
Common slope within groups	1	0.1736	0.1736	14.9748
Error (deviations from common slope)	63	0.7302	0.0116	
Difference between slopes	1	0.0077	0.0077	0.6564
Error (deviations from slope within each group)	62	0.7225	0.0011	
Total (groups + within)	65	1.0758		
Common slope within study	1	0.0431	0.0431	
Error (deviation from common slope)	64	1.0327	0.0161	
Among intercepts	1	0.3025	0.3025	26.1025
Error (deviation from common slope)	63	0.7302	0.0116	

* Not significant - not included in analysis.

TABLE 2.12. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR MALE, FEMALE AND INFESTED THAIS LAPILLUS COLLECTED IN OCTOBER 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.0840	0.0840	9.5604
Error (deviations from slope)	31	0.2725	0.0088	
MALE total	32	0.3568		
Due to FEMALE regression	1	0.3090	0.3090	65.2274
Error (deviations from slope)	44	0.2084	0.0047	
FEMALE total	45	0.5174		
Due to INFESTED regression	1	0.3163	0.3163	34.3630
Error (deviations from slope)	70	0.6444	0.0092	
INFESTED total	71	0.9608		
Between groups	2	0.4983	0.2492	20.0985
Within groups	148	1.8347	0.1240	
Common slope within groups	1	0.6914	0.6914	88.8904
Error (deviations from common slope)	147	1.1433	0.0078	
Difference between slopes	2	0.0180	0.0090	1.1587
Error (deviations from slope within each group)	145	1.1254	0.0078	
Total (groups + within)	150	2.3330		
Common slope within study	1	0.7090	0.7090	
Error (deviation from common slope)	149	1.6240	0.0109	
Among intercepts	2	0.4807	0.2403	30.9006
Error (deviations from common slope)	147	1.1433	0.0078	

TABLE 2.13. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR INFESTED THAIS LAPILLUS COLLECTED IN MARCH, JULY, AUGUST AND OCTOBER 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MARCH regressionn	1	0.0878	0.0878	7.5737
Error (deviations from slope)	14	0.1624	0.0116	
MARCH total	15	0.2502		
Due to JULY regression	1	0.1316	1.1316	11.0342
Error (deviations from slope)	19	0.2267	0.0119	
JULY total	20	0.3883		
Due to AUGUST regression	1	0.0590	0.0590	4.8858
Error (deviations from slope)	37	0.4468	0.0121	
AUGUST total	38	0.5058		
Due to OCTOBER regression	1	0.3163	0.3163	34.3630
Error (deviations from slope)	70	0.6444	0.0092	
OCTOBER total	71	0.9608		
Between groups	3	0.3929	0.1310	9.0890
Within groups	144	2.0750	0.0144	
Common slope within groups	1	0.5583	0.5583	52.6365
Error (deviations from common slope)	1143	1.5167	0.0106	
Difference between slopes	3	0.0365	0.0122	1.1512
Error (deviations from slope within each group)	140	1.4802	0.0106	
Total (groups + within)	147	2.4680		
Common slope within study	1	0.1583	0.1583	
Error (deviations from common slope)	146	2.3097	0.0140	
Among intercepts	3	0.7930	0.2643	24.9227
Error (deviations from common slope)	143	1.5167	0.0106	

TABLE 2.14. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR NON-INFESTED MALE THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH, JULY, AUGUST AND OCTOBER 1982.

SOURCE OF VARIATION	.F.	S.S.	M.S.	F
Due to FEBRUARY regression	1	0.1794	1.1794	48.3011
Error (deviations from slope)	30	0.1114	0.0037	
FEBRUARY total	31	0.2908		
Due to MARCH regression	1	0.2238	0.2238	46.7200
Error (deviations from slope)	19	0.0910	0.0048	
MARCH total	20	0.3148		
Due to JULY regression	1	0.0354	0.0354	3.9360
Error (deviations from slope)	19	0.1707	0.0090	*
JULY total	20	0.2061		
Due to AUGUST regression	1	0.0072	0.0072	0.4032
Error (deviations from slope)	43	0.7714	0.0179	*
AUGUST total	44	0.7786		
Due to OCTOBER regression	1	0.0840	0.0840	9.5604
Error (deviations from slope)	31	0.2725	0.0088	
OCTOBER total	32	0.3566		
Between groups	2	0.1254	0.0627	5.4068
Within groups	83	0.9622	0.0116	
Common slope within groups	1	0.4377	0.4377	68.4230
Error (deviations from common slope)	82	0.5245	0.0064	
Difference between slopes	2	0.0496	0.0248	4.1742
Error (deviations from slope within each group)	80	0.4749	0.0059	
Total (groups + within)	85	1.0875		
Common slope within study	1	0.3384	0.3384	
Error (deviations from common slope)	84	0.7491	0.0089	
Among intercepts	2	0.2246	1.1123	17.5577
Error (deviations from common slope)	82	0.5245	0.0064	

* Not significant - not included in analysis

TABLE 2.15. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG DRY BODY MASS (mg) AGAINST LOG SHELL LENGTH (mm) FOR NON-INFESTED FEMALE THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH, JULY, AUGUST AND OCTOBER 1982.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to FEBRUARY regression	1	0.1031	0.1031	9.1515
Error (deviations from slope)	25	0.2815	0.0113	
FEBRUARY total	26	0.3846		
Due to MARCH regression	1	0.1714	0.1714	18.5273
Error (deviations from slope)	13	0.1203	0.0093	
MARCH total	14	0.2916		
Due to JULY regression	1	0.1893	0.1893	22.6568
Error (deviations from slope)	14	0.1170	0.0084	
JULY total	15	0.3063		
Due to AUGUST regression	1	0.1222	0.1222	11.0801
Error (deviations from slope)	25	0.2758	0.0110	
AUGUST total	26	0.3980		
Due to OCTOBER regression	1	0.3090	0.3090	65.2274
Error (deviations from slope)	44	0.2084	0.0047	
OCTOBER total	45	0.5174		
Between groups	4	0.6316	0.1579	10.4824
Within groups	126	1.8979	0.0151	
Common slope within groups	1	0.8664	0.8664	104.9857
Error (deviations from common slope)	125	1.0315	0.0083	
Difference between slopes	4	0.0286	0.0072	0.8621
Error (deviations from slope within each group)	121	1.0030	0.0083	
Total (groups + within)	130	2.5295		
Common slope within study	1	0.9956	0.9956	
Error (deviations from common slope)	129	1.5339	0.0119	
Among intercepts	4	0.5024	0.1256	15.2191
Error (deviations from common slope)	125	1.0315	0.0083	

TABLE 2.16. CONVERSION OF SHELL GROWTH INCREMENTS INTO TISSUE DRY MASS (DM).

Initial HT.+ WID. (mm)	Teeth Gap (mm)	Final HT.+ WID. (mm)	Increase (mm)	Increase DM (mg)	Number of animal
31.3	4.1	32.0	0.7	20.40	
32.0	4.5	33.1	1.1	33.55	1
33.1	4.5	35.5	2.4	79.67	
35.5	3.9	36.6	1.1	39.56	
30.1	5.0	31.5	1.4	39.05	
31.5	4.9	32.9	1.4	41.96	2
32.9	5.9	35.4	2.5	82.40	
35.4	4.5	37.2	1.8	65.47	
31.7	2.8	33.2	1.5	45.52	
33.2	4.8	34.0	0.8	25.68	3
34.0	4.6	34.8	0.8	26.68	
32.2	3.6	33.5	1.3	40.24	
33.5	1.9	34.5	1.0	32.72	4
34.5	2.2	34.9	0.4	13.53	
32.5	3.6	33.6	1.1	34.38	
33.6	3.1	35.2	1.6	53.35	5
35.2	5.1	35.6	0.4	13.97	
30.1	5.0	31.1	1.0	27.60	6
31.1	5.5	33.4	2.3	69.11	
33.3	1.8	33.8	0.5	16.01	7
31.8	1.5	32.4	0.6	17.89	
32.4	1.6	33.1	0.7	21.56	8
33.1	1.8	33.4	0.3	9.47	
31.3	3.7	32.6	1.3	38.47	9
32.6	2.2	34.3	1.7	54.18	
31.2	2.6	32.0	0.8	23.26	10
25.7	3.0	28.1	2.5	53.81	
28.1	4.0	28.7	0.6	14.68	11
28.7	3.6	29.3	0.6	15.18	
30.4	2.5	31.6	1.2	33.82	12
27.8	2.9	28.7	0.9	21.83	
28.7	2.9	29.6	0.9	22.97	13
29.6	3.1	30.8	1.2	32.43	
$\bar{x}$ 31.76				$\bar{x}$ 35.17	+-6.80 95%

REGRESSION ANALYSIS FOR LOG DRY BODY MASS (mg) AGAINST LOG SHELL (HEIGHT + WIDTH) (mm) FOR INFESTED THAIS LAPILLUS COLLECTED IN APRIL AND JUNE 1981.

TABLE 2.17.

APRIL

b = 1.073 se = 0.201 with 15 degrees of freedom.
a = 0.872

Source of variation	SS	DF	MS	F
TREATMENTS	0.020	1	0.020	5.738
ERROR	0.053	15	3.5x10-3	
TOTAL	0.073	16		

TABLE 2.18.

JUNE

b = 2.615 se = 0.248 with 22 degrees of freedom.
a = 0.042

Source of variation	SS	DF	MS	F
TREATMENTS	0.140	1	0.140	27.549
ERROR	0.112	22	5.1x10-3	
TOTAL	0.252	23		

TABLE 2.19. BODY GROWTH INCREMENTS BETWEEN APERTURE TEETH ROWS FOR INFESTED THAIS LAPILLUS FROM SCARBOROUGH SOUTH BEACH.

Mean total dry mass	355.36	
Mean dry mass increment	35.17+-6.80 (mg)	(Table 2.16)
*June tissue ash percentages	1-(15.69+11.87)/2x100	(Table 3.61)
Ash free increment dry mass	30.32+-5.86mg	
*June tissue caloric values	(25.60+25.33)/2 Jmg-1	(Table 3.66)
Mean energy increment	741.78+-143.44 (J)	

* Ash percentages of dry tissue mass, and tissue caloric values for infested T. lapillus collected in June have been adjusted as if the growth increment was equally divided between foot and infested viscera tissue components.

DISCUSSION

In concurrence with Feare (1970a), larger Thais lapillus were found to be more commonly infested than smaller individuals (Fig. 2.1). T. lapillus above 38mm shell length from both Scarborough South Bay and Robin Hoods Bay were few in number, but always infested. All the snails used in the size frequency histograms were adult individuals. Moore (1938a), Feare (1970a) and Cowell and Crothers (1970), all agree that growth after reaching maturity is minimal or non-existent for T.lapillus. The fact that adult animals can vary from 21mm to 37mm at Robin Hoods Bay could be a reason why relatively few infested animals appear as giants. The age of adult dogwhelks cannot be determined beyond saying that they are more, or less, than three years old (Moore (1938b), Feare (1970a),and Coombs (1973), so the larger animals are not older animals which have grown for a longer period as is the case with Hydrobia ulvae (Rothschild, 1936, 1941a), Littorina neritoides (Lysaght, 1941, Rothschild, 1941b), Patella vulgata and P. depressa (Crewe 1951), and Littorina littorea (Lauckner 1980). The increased numbers of infested T. lapillus in the larger size classes can only be indicative either of continued adult growth as a result of infestation, or of selection of larger hosts by Parorchis acanthus miracidia,as suggested for other species by Croll (1966) and Lauckner (1980).

The number of teeth rows in adult T. lapillus individuals from Scarborough South Bay, and Robin Hoods Bay (Fig. 2.2) differs from that reported for collections of the same species from Robin Hoods Bay by Feare (1970a) who found that

2 rows was the normal condition, compared with 1 row found in this study. Feare suggested that a row was formed in the winter by immature individuals at 2 years of age after which they resumed growth before forming their second adult teeth row. This difference in teeth row number is probably a consequence of Feare studying a more exposed part of Robin Hoods Bay than the present author since Cowell and Crothers (1970) found some correlation between the presence of extra rows of teeth and increasing shore exposure. Feare (1969) included a photograph comparing infested and non-infested Thais lapillus to demonstrate giantism. The large infested snails illustrated could not - in the opinion of the present author - have been taken from the same exposed location as the small non-infested individuals, since these snails were of the exposed strain, and the large infested snails of the sheltered shore type (Staiger 1954). Even normal sized sheltered strain T. lapillus individuals would have difficulty adhering to an exposed shore, according to Kitching, et al. (1966).

Infested T. lapillus have been found with up to 7 rows of teeth (Fig. 2.2) at both Scarborough South Bay, and Robin Hoods Bay. The significance of increased number of teeth rows is that it suggests, not increasing age, but the occurrence of repeated periods of growth between teeth row deposition by infested T. lapillus, not experienced by non-infested individuals. The deposition of teeth rows was correlated by Feare (1970a) with the starvation incurred during winter aggregation. This explanation suggests that T. lapillus individuals with 7 rows of teeth could have been

infested for around 6 years. The observation of Rothschild (1942) that an infestation of Cryptocotyle lingua lasted for 7 years in Littorina littorea as well as the finding of Donges (1970) that Echinostoma revolutum can produce up to 104 generations of rediae, make the 6 year presence of Parorchis acanthus in Thais lapillus a plausible explanation. The occurrence of a very few non-infested individuals with up to five teeth rows on both shores studied, might be indicative of lost infestations.

The teeth row frequency distributions in Fig. 2.3. confirm the observation of Cowell and Crothers (1970) that the incidence of multiple teeth rows is higher on exposed shores than sheltered shores, although a single row of of teeth is still the most common category here. The sample of T.lapillus taken from the sheltered shore (Dale Fort) in Fig. 2.3., contained no P. acanthus infestations, and no individuals with more than three rows of teeth. This provides some evidence that the presence of P. acanthus infestation on a shore may be associated with an increase in the number of teeth rows in non-infested T. lapillus which have recovered from infestations.

Thickening of the shell lip in mature T. lapillus which have ceased growth was noted by Moore (1938a), Feare (1970a), Cowell and Crothers (1970) and Coombs (1973). An increased number of teeth rows in infested T. lapillus individuals has been interpreted as indicative of continued growth by Feare (1970a), and in this study. The data contained in Fig. 2.4. and Table 2.1. support this hypothesis by demonstrating that infested snails have significantly thinner shell lips than

non-infested dogwhelks. The few non-infested individuals that have a shell lip thinner than 0.6mm were young adults collected in August 1982. Infested snails will tend to be older individuals, because of the likelihood of exposure to infestation increasing with time, hence lip thinning as a result of infestation may be more marked than it appears.

The data for aperture lip lengths of Thais lapillus individuals collected in spring and combined summer and autumn (1982) samples shown in Figures 2.5 and 2.6 respectively show that a few individuals had lip lengths greater than 4.5mm in autumn, but non had in spring. Only infested animals had lip lengths greater than 5mm which indicates that at least some infested T. lapillus individuals have growth increments after the summer feeding period. The association of ballooning of the second to last whorl with infestation is shown in Fig. 2.7, and Table 2.2.

The Whorl Width Ratio, a quantification of shell ballooning is then correlated with the number of teeth rows in Fig. 2.8. These observations, and the Plates 2.2 to 2.9. provide evidence that whorl ballooning is not the consequence of parasite pressure deforming the whorls in T. lapillus, as suggested by Rothschild (1936) to account for the shell deformity found in infested Hydrobia ulvae. Feare (1970a) reported the discovery of an infested T. lapillus which had 8 rows of teeth and a larger-than-normal number of body whorls. The photographs of samples of infested and non-infested H. ulvae provided by Rothschild (1936) to illustrate the nature of shell deformation associated with infestation also show infested animals with increased

numbers of shell whorls.

It can be suggested that the effect of increased pressure upon the viscera of the host snails caused by the presence of larval trematodes may be to cause slight extrusion of the head, foot and mantle beyond the normal limits of the shell, causing the addition of new shell material by the extended mantle edge. This process would result in the re-orientation of the shell so that what was formally part of the widest last-formed whorl would become incorporated into the second last whorl, thus increasing its width.

The regression lines for Log dry body mass against Log shell length shown in Fig.2.9 and 2.10, and based on the data in Tables 2.3. to 2.12 show that at all times of year the intercepts of the regression lines calculated for infested individuals are significantly greater than the non-infested male and female intercepts. The biological significance of this result is not at all clear. It may be that the smaller infested individuals are comparatively heavy because of the redial population they contain, or alternatively the calculated high intercept values could be the result of the shallow increase in body mass with size exhibited by the larger infested subjects.

The seasonal change in dry body mass demonstrated by the significant differences between the intercepts of Log dry body mass versus Log shell length regression lines of similar slopes for non-infested male and female and infested individuals collected in different months during 1982 (Fig. 2.11., Tables 2.3-2.7 and 2.13-2.15) is most marked between

the low March value and high October value for infested snails. The infested animals appear to gradually increase their body mass through summer, but the regression slopes do change significantly during the course of the year. The non-infested female animals, on the other hand, can be clearly seen to lose weight during the February to July egg producing season, and then to regain the early weight loss in October, after the summer feeding period. The pattern for the non-infested male animals is difficult to discuss because of the limited number of positive correlations obtained.

August and October samples of infested Thais lapillus contained individuals with longer shell lips than had been found in similar samples taken earlier in the year (Fig. 2.13 and 2.14). Perhaps, on the one hand, infested animals can obtain sufficient food during active feeding periods so as to be able to not only repay the weight loss incurred during winter aggregation, but grow a little as well, but on the other hand, during the winter starvation period infested animals may lose more weight than similar non-infested snails. This hypothesis is in agreement with the findings of Bourns, (as cited by Wright (1966)), that infested Lymnaea stagnalis grew more slowly than non-infested snails during the winter, but when good conditions were provided the infested Lymnaea stagnalis grew faster than non-infested controls (McClelland and Bourns, 1969). The calculations of mass and energy increment for a sample of infested T. lapillus from Scarborough South Bay (Table 2.19) predict that the average annual mass increment is roughly 10% of the

mean of the calculated body masses. To achieve this amount of growth the mean lip length growth from one year to the next need only be 3.5mm. The large size of Thais lapillus individuals, both infested and non-infested, at Scarborough South Bay where the subjects for the growth increment study were collected, suggests that the conditions there are very favourable for dogwhelks. The mean energy increment calculated for parasite induced growth will be compared below (Chapter 4) with the reproductive saving resulting from parasitic castration, and the costs of cercarial and redial production.

In conclusion, an attempt has been made to prove the existence of parasite-enhanced growth in field populations of T. lapillus, as first suggested by Feare (1970a). The more frequent presence of the infestations in larger individuals is not by itself a conclusive indication of enhanced growth (Rees, 1936, Crewe, 1951, Lauckner, 1980, among others), but the numerous observations that non-infested T. lapillus normally stop growing on reaching sexual maturity (Moore, 1938a, Feare, 1970a, Cowell and Crothers, 1970, Coombs, 1973), suggest that the distribution of infestations cannot be explained by the greater age of the larger individuals. The presence of multiple teeth rows in infested animals is indicative of successive summers growth in between starvation induced growth checks in winter (Feare, 1970a). The thin (and at times abnormally long) shell lips found in some infested individuals also indicate the occurrence of active growth by infested dogwhelks (Moore, 1938a, Feare, 1970a, Coombs, 1973). The existence of

ballooned second to last whorls in infested individuals has been correlated with shell deposition at the aperture edge, rather than direct deformation of the shell whorl, as suggested by Rothschild (1936), by both physical removal of teeth rows and the positive correlation between degree of whorl ballooning (small W.W.R.) and the number of aperture teeth rows in infested Thais lapillus.

Infested dogwhelks at all times of year have a lower dry body mass than females of the same shell length, but in October when male dogwhelks have a significant relationship between shell length and dry body mass, they are similar in mass to the infested snails. Thus, although not as heavy as females, the infested T.lapillus do not produce a disproportionately large shell, as suggested for other parasitised gastropod species (Etges 1961a,b, Cheng 1971). There is a notable increase in the dry body mass of infested T.lapillus between March and October (1982). The increase suggests, perhaps, that although considerable weight is lost through the winter starvation period as a result of the redial infestation it is compensated for during the active feeding periods of the summer.

CHAPTER THREE

SEASONAL CHANGES IN BODY COMPONENTS OF INFESTED AND NON-INFESTED *THAIS LAPILLUS* HOSTS.

INTRODUCTION

The useful comparative technique of dividing the body of a mollusc into a number of components, and comparing their relative masses was described by Giese (1969). Giese used the black abalone, *Haliotis cracherodii,* and the keyhole limpet, *Megathura crenulata,* as examples of gastropods. The shell and soft body percentages of the total fresh mass of the snails was determined and then the soft body was divided into "parts which can be conveniently separated from one another". Unfortunately, as illustrated by Giese, in practice the components into which individuals of different molluscan species can be divided are often slightly different, rendering detailed interspecific comparisons difficult to make.

Giese compared both the fresh tissue masses and the dry masses of the various components, and thus obtained the water component of the tissues. In the present study the component comparisons have been confined to the soft body tissues of infested and non-infested *T. lapillus.* Component dry weights were used in this investigation, as in that of Lambert and Dehnel (1974) on *T. lamellosa,* because of the greater accuracy of dry masses as opposed to fresh masses.

In conjunction with the gravimetric investigation of component masses, Giese (1969) described annual changes in the relative levels of biochemical constituents of the different components. The gastropod soft body tissue was assayed for its relative levels of water, protein, lipid and carbohydrate, from which glycogen was treated separately.

A number of studies have been conducted into the annual fluctuations of relative component masses, or change in component indexes (the proportions of unit mass, or percentages of total body mass, contributed by each component) and biochemical constituents of Thaisid snails. The seasonal fluctuations in the organ component indices of Thais lamellosa were investigated by Stickle (1973) and Lambert and Dehnel (1974), and those of T.haemastoma were investigated by Belisle and Stickle (1978). The results of the three studies were broadly similar, although Lambert and Dehnel (1974) used different components to the other two studies. For example Stickle (1973) divided up each T. lamellosa individual into four components; shell, body water, visceral mass (dry mass) and foot (dry mass). The shell contributed the greatest proportion of the weight of the animal, being as much as 88% of the mass of the whole snail. The fluctuations in shell proportion (82% to 88%) were mirror images of the fluctuations of the visceral mass and body water proportions. In both sexes the foot dry mass remained an almost constant percentage all year, at less than 1%. For both male and female snails the visceral mass was at its peak percentage of the total body mass in October, declining to a fairly constant low level between

April and June, thus reflecting the importance of reproduction on the organ component indices.

The inclusion of the relatively massive shell and body water content as separate body components in the above studies makes the visceral dry mass and foot dry mass components proportionally small; this may to some extent mask the significance of their fluctuations. For the present study it was decided to compare the dry masses of four components which together comprised the whole of the soft body mass, as described below. The body water and shell were not included. The main aim of the study was to discover how the relative proportions of parasite and infested digestive gland fluctuated throughout the year in the castrated, infested hosts in comparison with the change in gonad and digestive gland percentages of total body dry mass in non-infested dogwhelks. The seasonal changes in the proportional contributions of different organs and organ complexes to the total soft body mass of Thaisid snails are related to the reproductive cycle and, to a lesser extent, winter starvation periods.

Stickle and Duerr (1970) investigated the nutrient components of Thais lamellosa for starved and non-starved control individuals. After 53 days of starvation the soft tissue mass of experimental animals was lower than that of non-starved as was the total lipid %, total polysaccharide % and total nitrogen % decreased per unit dry mass of soft tissues. The decrease in the total lipid percentage from 20.2% to 14% led the authors to the suggestion that "the metabolism of the carnivorous prosobranch T.lamellosa is

lipid orientated". In a later study on the effects of starvation of male and female Thais lamellosa immediately after spawning, Stickle (1971) decided that protein was the most important respiratory substrate for this species, and not lipid as previously suggested. It should be noted, though, that Stickle found the lipid level of male and female T. lamellosa viscera just after spawning to be only 6.2% and 9.9% respectively. These snails were frozen immediately after spawning and used as controls for the experimental animals starved for 91 days. In the earlier study, Stickle and Duerr (1970) had found that lipid is metabolised when it is available. Giese (1969) considered that a total lipid level of 5% of tissue dry weight could be obtained solely as a result of structural lipid content of cell membranes. Although the diethyl ether lipid extraction technique employed by Stickle (1971) does not remove phospholipids from cell membranes, the lipid levels of the post-reproductive animals prior to starvation may be so low that the preferential utilisation of protein as a metabolic substrate might be expected. The low initial lipid level may also be responsible for the small but significant increase in lipid content observed by Stickle in starved animals.

The presence of lipid in the egg capsules of T.lapillus was demonstrated by Bayne (1968), presumably as a nutrient supply for the eggs. Stickle (1975) also found a similar high lipid proportion in the egg capsules of T.lamellosa and he calculated that female T. lamellosa invest more lipid into their egg capsules than they lose from the digestive gland during the non-feeding winter aggregation. The

conclusion drawn from observations on lipid partitioning is that, at least in Thais lamellosa, lipid serves as a reproductive energy source rather than an overwintering energy source. Although Stickle has demonstrated the reproductive expenditure of lipids by female T. lamellosa, the role it plays in male snails is not clear.

Stickle and Bayne (1982) found that starved T. lapillus had oxygen to nitrogen ratios which suggested that they were catabolising proteins as respiratory substrates rather than lipid. The authors felt that as the diet of the dogwhelk is mainly protein (as described for example by Bayne and Scullard 1978), it is logical that this should be the case. It should also be added that in the absence of specialised storage organs, comparable to those of vertebrates (Giese, 1969), the breakdown of the proteinaceous digestive gland, as observed in Littorina saxatilis by James (1965), among others, would result in the catabolism of protein for respiration during starvation. The increased amounts of lipid present in starved T. lamellosa (Stickle 1971), may be caused by the autolysis of the digestive gland cells, which temporarily supplies the starved snails with enough haemolymph nutrients to anabolise lipid, thus increasing the very small amounts present.

Lipid has been described as the over-wintering respiratory substrate for other littoral gastropods such as Patella vulgata (Blackmore 1969), and Littorina littorea (Williams 1970). The latter species shows peak lipid levels in October and lowest levels between February and June on the North Yorkshire coast (Williams 1970). It was noted by the same

author that individuals of both sexes had a slight but significant rise in lipid levels in February. This suggests that a rise in lipid levels following a period of starvation would apear to be a response not confined to Thais lamellosa (Stickle, 1971). It was suggested by Williams (1970) that lipids may also be used for gamete production. He also interpreted Blackmore's (1969) observation, that despite similar growth patterns in mature and immature Patella vulgata, only mature individuals demonstrate seasonal fluctuations in lipid and carbohydrate levels, as being indicative of a reproductive, rather than maintenance, allocation of the nutrients.

Lipid levels, as percentages of tissue dry mass, in the visceral masses of male and female T. lamellosa from Alaska were found by Stickle (1975) to vary from 23.3% in November to 9.3% in May, for males, and 25% in December to 14.9% in May, for females. The lipid levels reported for T. lamellosa from Vancouver by Lambert and Dehnel (1974) were also higher than those reported by Stickle (1975) for T. lamellosa from Washington, with the visceral lipid % of tissue dry mass varying in a similar manner for both male and female snails, from 38.5% in December to 20.0% in April. (This finding is, in part, due to the chloroform-methanol lipid extraction technique used by Lambert and Dehnel (1974) extracting structural lipids, which the diethyl ether technique employed by Stickle (1975) - as described by Stickle and Duerr (1970) - does not).

An investigation into the effects of larval trematode infestation on the fatty acid composition of the

neogastropod Nassarius obsoletus led Lunetta and Vernberg (1971) to suggest that the presence of trematode larvae caused a disturbance of the long chain (C_{20}+) fatty acid metabolism of the host. They suggested that there could be a link between this metabolic effect of parasitisation and a previously noted host intolerance of extreme temperatures, with reference to their own unpublished observations that lipid levels increased inversely with temperature in shore crabs, and the finding of Farkas and Herodek (1964), that planktonic copepods increase their amounts of C_{20} and C_{22} fatty acids with declining environmental temperatures. The function of long chain fatty acids in cold temperature survival of copepods was suggested, by Farkas and Herodek (1964), to be the maintenance of a low melting point for extracellular fluids. Lunetta and Vernberg (1971), intimating that lipids may have a similar cold adaption function in gastropods, referred to the previous studies of Vernberg and Vernberg (1963, 1967) and Vernberg (1969) where parasitised Nassarius obsoletus were found to be not as resistant to thermal stress as non-parasitised individuals.

Thus, the increased levels of lipids in Thaisids and other littoral gastropods during winter aggregations, and in more northerly populations, may be a low temperature acclimation response.

The presence of larval trematodes does not always cause a decrease in the lipid present in the infested snail host. Brand and Files (1947) found that Australorbis (=Biomphalaria) glabrata individuals infested with Schistosoma mansoni sporocysts contained the same quantity

of lipid as non-infested snails. An earlier investigation by Hurst (1927) indicated that there was a slight increase in fats in individuals of the pulmonate species Physa occidentalis infested with Echinostoma revolutum. A similar result was obtained by Cheng and Snyder (1962) when they investigated the effects of the larval stages of Glypthelmins pennsylvaniensis on the lipids of the pulmonate host snail Helisoma trivolvis. The latter authors found increased quantities of cytoplasmic neutral fat in infested hosts, much of which appeared to be catabolised to fatty acids in H. trivolvis individuals supporting prolonged, heavy infestations.

To summarise the above; the role of lipids in the metabolism of Thaisids is thought to be an energy source for reproduction (Stickle, 1971, 1973, 1975, Stickle and Bayne, 1982) rather than solely maintenance (Stickle and Duerr, 1970). The increased lipid content of Thais lamellosa collected from more northerly shores (Lambert and Dehnel, 1974, and Stickle, 1975) may also suggest a low temperature survival role for lipids, as discussed by Lunetta and Vernberg (1971). The effects of infestation by larval trematodes on the lipid content of their gastropod hosts are not uniform (Cheng and Snyder, 1962, and Lunetta and Vernberg, 1971). Since in T.lapillus infestations with the rediae of Parorchis acanthus are associated with gonad destruction (Feare 1970a), it was decided to compare the lipid levels of non-infested and infested dogwhelks shortly before they entered winter aggregations.

The effects of parasitism and seasonal starvation and reproduction on the total organic constituent levels of dogwhelk tissues were investigated using microbomb calorimetery (Phillipson 1964), rather than assaying the protein carbohydrate, and lipid levels individually. This facilitated a more rapid determination of the effect of parasitism on the total energy content of the infested host tissue than the latter procedure, together with the comparison of parasite and host caloric values.

Comparisons Between Tissue Component Proportions of the Soft Body Dry Masses of Infested and Non-Infested Thais lapillus Hosts.

METHOD

Infested and non-infested T.lapillus were sampled from Robin Hoods Bay and Scarborough South Bay at regular intervals throughout the year. They were killed by immersion in boiling water for 30 seconds; this also denatured the tissue proteins and firmed the texture of the tissue. The shell measurements were made, and the shell removed, as described previously. The non-infested snails were then sexed, as described in Chapter 2,and divided into four components. The visceral whorls posterior to the kidney were removed and then, due to the action of the boiling water they could be further separated into gonad and digestive gland, which had been found impossible by Stickle (1973) and Belisle and Stickle (1978) for thawed, previously frozen, T. lamellosa and T. haemastoma. The distinctive colours of the two organs - the digestive gland always being brown, the gonad varying

from brick red (in males) to light orange - aided the precision of their separation. The head, foot and collumellar muscle were separated from the remaining body organs.

Each of the four body components was then placed on an aluminium foil weighing pan of known mass and dried under vacuum at 60°c for 24 hours before being weighed to the nearest 0.00001g on a Metler HL 52 balance. Infested Thais lapillus were treated in a similar manner to non-infested snails except that in infested individuals the viscera were divided into digestive gland and parasite components by the removal of individual rediae under a binocular microscope at x10 magnification, with Number 7 watchmakers forceps. All the rediae were placed on a pre-weighed aluminium foil planchet. The two components, digestive gland and parasite, were then dried under vaccuum as described above.

RESULTS

The pooled data for organ component dry mass percentages of whole body dry mass of infested and non-infested male and female snails collected in February, March and April are depicated in Fig. 3.1. The condensed data for non-infested male and female and infested foot component percentages are in Table 3.1. The result of a one way analysis of variance (Table 3.2) indicates that there is a highly significant difference between the foot dry mass proportion of the whole body dry mass ($P \leq 0.01$) of non-infested male and female and infested individuals. Table 3.3 shows the results of a GT2 test for significant differences between paired means

indicating that non-infested female and male, and infested dogwhelk foot percentages of dry mass are significantly different from each other, in ascending order.

The summarised results for mantle dry mass percentages of whole body dry mass contained in Table 3.4 have a significant value of F (p less than 0.01) when treated with one way analysis of variance (Table 3.5). The result of a GT2 test (Table 3.6) shows that non-infested female and male and infested Thais lapillus each have different mantle percentages of dry mass, in descending order.

The digestive gland dry mass percentages of whole body dry mass are summarised in Table 3.7. The results of a one way analysis of variance in Table 3.8 indicate that there is a significant difference between the different means, ($p \leq 0.01$). Table 3.9 contains the result of a GT2 test, showing that non-infested male and female digestive gland percentages are significantly different, but that infested animals occupy an intermediate position, not significantly different from either of the other two.

The summarised results for gonad, or parasite dry mass percentage of whole body dry mass are contained in Table 2.10. The one way of analysis (Table 2.11) shows that the classes are significantly different from one another ($p \leq 0.01$). A GT2 test on the class means (Table 2.12) shows that non-infested female gonad is a significantly higher percentage of whole body dry mass than non-infested male gonad, or Parorchis acanthus redial population, which are not significantly different from each other.

The pooled data for organ component dry mass percentages of whole body dry mass of infested and non-infested male and female snails collected in June and July are depicted in Fig. 3.2. Comparatively few infested snails were collected in these months. They have not been included in Fig. 3.2 because the limited amount of data does not benefit from graphical analysis. However, the data from the infested animals are included in Tables 3.13, 3.16, 3.19 and 3.22. The foot component dry mass percentages are condensed into Table 3.13. The one way analysis of variance (Table 3.14) has a significant value for $F(p \leq 0.01)$. The GT2 test (Table 3.15) shows that infested snail and non-infested male foot percentages are similar, but significantly elevated from the non-infested female values.

The summarised data of mantle dry mass percentages of whole body dry mass (Table 3.16), when treated with a one way analysis of variance (Table 3.17), has a significant value for $F(p \leq 0.01)$. The result of a GT2 test (Table 3.18) is that the non-infested female mantle mean dry mass percentage is significantly elevated above the similar non-infested male and infested mean percentages.

The summarised data of arcsine transformed digestive gland dry mass percentages of whole body dry mass (Table 3.19), when treated with a one way analysis of variance (Table 3.20), have a significant value for $F(p \leq 0.01)$. The result of a GT2 test (Table 3.21) is that non-infested female and infested snails have similar mean percentages of digestive gland that are both significantly lower than the mean

non-infested male percentage.

The summarised results for arcsine transformed gonad, or parasite, dry mass percentages of whole body dry mass (Table 3.22), when treated with a one way analysis of variance (Table 3.23), have a significant value for $P \leq 0.01$). The result of a GT2 test (Table 3.24) is that non-infested female Thais lapillus have a significantly greater percentage of their whole body dry mass contributed by gonad than is the case with non-infested male animals. The redial percentage of the dry mass of infested animals is similar to the contribution of the gonad in non-infested males.

The data for organ component dry mass percentages of whole body dry mass of infested and non-infested male and female snails collected in August are depicted in Fig. 3.3. The summarised results for foot dry mass percentages of whole body dry mass (Table 3.25), when treated with a one way analysis of variance (Table 3.26) have a significant value for $F(P \leq 0.01)$. The result of a GT2 test (Table 3.27) is that infested and non-infested male and female foot percentages are all significantly different in descending order.

The summarised data for mantle dry mass percentages of whole body dry mass (Table 3.28), when treated with a one way analysis of variance (Table 3.29) have a significant value for $F(P \leq 0.01)$. The result of a GT2 test (Table 3.30) is that non-infested female mean mantle percentage is significantly greater than the infested neuter and non-infested male means, which are similar.

The summarised arcsine transformed results for digestive gland dry mass percentages of whole body dry mass (Table 3.31), when treated with a one way analysis of variance (Table 3.32) have a significant value for $F(p \leq 0.01)$. The result of a GT2 test (Table 3.33) is that non-infested male and female and infested digestive gland mean dry mass percentages are all significantly different in that descending order.

The summarised arcsine transformed results for gonad, or parasite, percentages of whole body dry mass (Table 3.34), when treated with a one way analysis of variance (Table 3.35), have a significant value for $F(p \leq 0.01)$. The result of a GT2 test (Table 3.36) is that the infested animals have a significantly greater percentage of their dry body mass contributed by parasite than either non-infested females or males have by their gonads.

The data for organ component dry mass percentages of whole body dry mass of infested and non-infested male and female snails collected in October are depicted in Fig. 3.4. The summarised results for foot dry mass percentages of whole body dry mass (Table 3.37), when treated with a one way analysis of variance (Table 3.38), have a significant value for $F(p \leq 0.01)$. The result of a GT2 test (Table 3.39) is that infested individuals and non-infested males have similar mean foot dry mass percentages of whole body dry mass, both being greater than the non-infested female mean.

The summarised data for mantle dry mass percentage of whole body dry mass (Table 3.40), when treated with a one way

analysis of variance (Table 3.41), have a significant F value ($P \leq 0.01$) which the GT2 test result (Table 3.42) shows to be caused by the non-infested female mean percentage being significantly higher than the similar non-infested male and infested neuter values.

The summarised data for digestive gland dry mass percentages of whole body dry mass (Table 3.43), when treated with a one way analysis of variance (Table 3.44), have a significant F value ($p \leq 0.01$). The result of a GT2 test (Table 3.45) is that infested animals have a significantly smaller mean percentage than non-infested males, with non-infested females occupying an intermediate position.

The summarised arcsine transformed results for Thais lapillus gonad, or Parorchis acanthus redial population dry mass percentages of whole body dry mass (Table 3.46), when treated with a one way analysis of variance (Table 3.47), have a significant value for F($p \leq 0.01$). The result of a GT2 test (Table 3.48) is that the redial population dry mass percentage of whole body dry mass is not quite significantly greater than non-infested female gonad percentage, but they are both significantly greater than non-infested male gonad percentage.

The data for seasonal differences in parasite dry mass percentages of the host's total dry body mass are summarised in Table 3.49. The results of a one way analysis of variance on the data (Table 3.50), indicates a significant difference between the seasonal means ($P \leq 0.01$). The GT2 test for differences between means of unequal sample sizes, in Table

3.5l, is that the mean dry mass percentage for August is significantly elevated above those of July and March (which are not significantly different from each other), but not significantly different from the intermediary October dry mass percentage of infestation, which is also similar to the lower July and March dry mass percentages.

Comparisons Between Tissue Caloric Values for Infested and Non-Infested Thais lapillus Hosts.

METHOD

The caloric value of dry tissue components were determined using a Phillipson Microbomb Calorimeter and the methods described by Phillipson (1964). The Benzoic acid calibration regression for the June and April investigations explained 99.5% of the variation in corrected peak value. The October caloric values were determined similarly, but a different Benzoic acid calibration had to be calculated as a different bomb was used. The regression for this bomb explained 94% of the variation in corrected peak value.

The ash content of the tissue components was determined by heating dry tissue samples of known weight on pre-weighed aluminium pans in a muffle furnace at 500 c for 4 hours. The inorganic ash remains were then re-weighed, and the ash percentage for the tissue calculated.

The tissue caloric values were calculated using the calibration curves and corrected peak values by the following equations:-

APRIL/JUNE (ORIGINAL BOMB)

$$CV = (CP - 0.09) / 0.15$$

OCTOBER (NEW BOMB)

$$CV = (CP - 4.04) / 0.10$$

Where:-

CV = Caloric Value ($J.mg^{-1}$)

CP = Corrected Trace Peak

0.09 = Intercept on Y Axis } From corrected trace peak against
} caloric value regression calculated
0.15 = Regression Slope } for the original bomb.

4.04 = Intercept on Y Axis } From corrected trace peak against
} caloric value regression calculated
0.10 = Regression Slope } for the new bomb.

RESULTS

April

A summary of the April ash percentage of dry tissue mass for non-infested male and female Thais lapillus foot and viscera components is shown in Table (3.52). The two way analysis of

variance result shown in Table (3.53) indicates that there is no significant difference between foot and viscera ash percentages, but that there is a significant difference (F is significant with a probability $\leq$ 0.01) between those for male and female T. lapillus means shown in Table (3.54).

The tissue caloric values ($J.mg^{-1}$ Ash Free Dry Mass) were calculated using these pooled ash percentage values and are summarised in Table (3.55). A two way analysis of variance indicates that the interaction term is significant, with a probability $\leq$ 0.05, showing that caloric value differences between male and female foot and viscera do not follow a similar pattern Table (3.56).

The same summarised caloric values are shown in Table (3.57) and the result of a one way of analysis of variance in Table (3.58). The F value obtained is significant to $P \leq 0.01$. In Table 3.59 is the result of a Student Newman Keuls test for significant differences between mean values which is that male foot, male viscera and female foot all have similar caloric values ($J.mg^{-1}$), but that female viscera has a significantly higher caloric value ($J.mg^{-1}$), so the pooled energy values shown in Table 3.60 can be calculated.

June

A summary of the data for June ash percentages of dry tissue mass for non-infested male and female and infested Thais lapillus foot and viscera components are shown in Table 3.61. The result of a two way analysis of variance on these data (Table 3.62) is that there is no significant difference between foot and visceral components of either infested or

non-infested snails, but that there is a very significant difference between non-infested males and females and infested animals ($P \leq 0.01$). Table 3.63 shows the summarised ash percentage data for non-infested males and females and infested animals, with foot and visceral components pooled, with data for Parorchis acanthus rediae removed from infested snails. The resultant value for F calculated from a one-way analysis of variance on the above data is significant ($P \leq 0.01$) (Table 3.64). The result of the GT2 test for significant differences between paired means of unequal samples (Table 3.65) is that the variance arises because the ash percentages of non-infested female T. lapillus and P.acanthus rediae are similar to each other, and significantly greater than the two similar ash percentages of non-infested male and infested T. lapillus tissues.

Using the pooled ash percentages calculated from Table 3.65, the tissue caloric values ($J.mg^{-1}$ ash free dry mass) summarised in Table 3.66 were calculated. The result of a two way analysis of variance in Table 3.67 is that non-infested males and females and infested snails have similar tissue caloric values, but that there is a significant difference ($P \leq 0.01$) between foot and visceral components. The result of a t-test on the caloric values for the removed rediae and pooled non-infested and infested visceral values (Table 3.68) is that the difference between sample means is not significant ($p > 0.05$).

October

A summary of the data for October ash percentages of tissue dry mass for non-infested males and females and infested Thais lapillus foot and viscera components is shown in Table 3.69. The result of the two way analysis of variance, in Table 3.70, is that the interaction term is significant ($P \leq 0.05$), because the ash percentage differences between non-infested males and females and infested foot and viscera do not follow a similar pattern. One way analysis of variance on the non-infested male and female and infested foot component ash percentages (Tables 3.71 and 3.72) indicates a significant difference between them (at a probability level $\leq$ 0.01). Application of a Student Newman Keuls (SNK) test for significantly different means (Table 3.73) results in the pooled non-infested foot ash percentage, and the separate infested value in Table 3.74.

Similar treatment of the viscera ash percentages (Table 3.75) leads to a significant value for F ($P \leq 0.01$) (Table 3.76), and the SNK test (Table 3.77.) results in the pooled non-infested female and infested visceral ash percentage mean, and the higher non-infested male value in Table 3.78.

Using the calculated ash percentages, the summarised tissue caloric value ($J.mg^{-1}$) in Table 3.79 was calculated. A two way analysis of variance (Table 3.80) indicates that there is a highly significant difference ($P \leq 0.01$) between foot and visceral components, but that non-infested males, females and infested snails have similar tissue caloric values.

One way analysis of variance on the summarised visceral caloric values and the redial caloric values (Tables 3.81 and 3.82) indicates a slightly significant difference between them ($p \leq 0.05$), but the SNK test (Table 3.83) is not sufficiently subtle to show a significant difference between means, so a pooled caloric value for parasite and viscera tissues is shown in Table 3.84.

Seasonal Changes in Caloric Values

Tables 3.85 and 3.86 show that there is a significant elevation in tissue caloric values for infested Thais lapillus in October compared with June for foot (significant to $p \leq 0.001$) and visceral tissues (significant to $p \leq 0.01$) respectively,

When the summarised data for non-infested male foot component caloric values ($J.mg^{-1}$ ash free dry mass) for April, June and October samples, summarised in Table 3.87, are treated with a one way analysis of variance (Table 3.88) there is a significant difference between them ($p \leq 0.01$). A GT2 test for differences between paired means of unequal sample sizes (shown in Table 3.89) shows that October caloric values are significantly elevated above those for April and that June caloric values are intermediate, not being significantly different from either of the other two values.

The summarised seasonal caloric values for non-infested male viscera are contained in Table 3.90. The result of a one way analysis of variance in Table 3.91 is that there is a

significant difference between caloric values at different times of year ($p \leq 0.01$). A GT2 test on the means (Table 3.92) reveals that the April visceral caloric value for non-infested male Thais lapillus is significantly lower than those for October and June, which are not significantly different from each other.

Non-infested female foot caloric values for April, June and October are summarised in Table 3.93, and as a result of a one way analysis variance (Table 3.94) they can be shown to be significantly different ($p \leq 0.01$). The GT2 test for significant differences between paired means result is shown in Table 3.95, indicating that the caloric values for non-infested female T. lapillus in October are significantly elevated above those for June and April, which are not significantly different from each other.

Non-infested female visceral caloric values for the same periods are summarised in Table 3.96. One way analysis of variance on the data, results in a significant variance ratio ($p \leq 0.01$) (Table 3.97), meaning there is a difference between the mean caloric values. The result of a GT2 test for significant differences between paired means is shown in Table 3.98. It indicates that the mean caloric value ($J.mg^{-1}$ ash free dry mass) for females collected in October is significantly elevated above June and April samples, which are similar to each other.

The mean tissue caloric value of Parorchis acanthus rediae collected in October is shown (Table 3.99) to be significantly elevated ($p \leq 0.02$) above the mean value for

rediae collected in June.

Comparisons Between Fresh Tissue Lipid Content and Lipid Percentage of Dry Mass of Infested and Non-Infested Thais lapillus Hosts

METHOD

T.lapillus collected in October, 1982, from Robin Hoods Bay, North Yorkshire, were removed alive from their shells, checked for the presence of P. acanthus rediae and the non-infested animals sexed. The animals were then dissected into two components - the 'Viscera' component, which consisted of the digestive gland and gonad, and the 'Foot' component, which included everything left after removal of the viscera. Infested viscera were not separated into parasite and digestive gland fractions. The components were pooled into groups of 10, wrapped in pre-weighed silver foil, and the fresh tissue weight determined. Component samples not being used immediately were kept at 2 °C while the first sample was treated. Animals awaiting dissection were maintaned at a constant 5 °C. Lipid was extracted from the homogenised tissue using Analar chloroform and methanol as described by Bligh and Dyer (1959). The dry weight to wet weight ratios for the different tissue components indicated that the water content of the dogwhelk tissue is less than 80% by mass, so a small amount of distilled water was added to each sample to increase the water content to the level recommended by Bligh and Dyer.

The chloroform/lipid fraction was extracted from the homogenate by centrifugation in an IEC spinette centrifuge

rather than by the filtration method described by Bligh and Dyer. The chloroform/lipid fraction was extracted from beneath the homogenised tissue using a hypodermic needle and glass syringe, and passed through a Whatman No.1 filter into a measuring cylinder. Virtually all the initial chloroform volume could be reclaimed by this method, so the total lipid content of the sample was calculated by drawing off 1ml of the chloroform/lipid fraction, and pipetting it into a pre-weighed planchet which was then placed in a drying oven at 40 °C. After the evaporation of the chloroform the planchet with lipid residue was re-weighed and the result multiplied by the initial volume of chloroform in ml.

To convert lipid content (mg lipid per g fresh tissue mass) to a percentage of tissue dry mass (mg) it was necessary to calculate the fresh mass/dry mass ratio for foot and visceral components for male female and infested animals. The followng procedure was adopted.

Fresh animals were removed from their shells and sexed. The digestive gland and gonad, or parasite material, (labelled 'viscera') was separated by dissection from the rest of the animal (labelled 'foot'). The two tissue components were placed immediately into separate pieces of pre-weighed aluminium foil which were sealed to prevent evaporation. Each component was weighed on a Metler P162 top pan balance to the nearest 0.01 g. The aluminium foil was then opened slightly so that the tissue was no longer totally sealed, and the tissue dried for 48 hours under a vacuum at 60 °C. The period of drying was this long because of the foil.The dry tissue was then weighed to the nearest 0.0001g on a

Torbal balance. The dry mass of a tissue sample was calculated from the line obtained by this method for the corresponding tissue component.

RESULTS

The results for the lipid content of fresh foot and visceral tissues obtained (with 95% confidence limits) are shown in Fig.(3.5). A significant interaction term obtained when treating the fresh tissue lipid content (mg lipid per g fresh tissue) with a two way analysis of variance (Tables 3.100,3.101) indicates that there are different patterns of lipid distribution in the foot and visceral components of non-infested male and female and infested snails. One way analysis of variance in the data for foot components (Table 3.102, 3.103) followed by SNK multiple range test (Table 3.104) showed that non-infested female and infested Thais lapillus foot tissues have a similar lipid content (mg. g^{-1}) and that non-infested male foot tissue has a significantly ($p<0.05$) higher value. Similar treatment of the visceral component data (Tables 3.105 to 3.107) showed that non-infested male and infested viscera lipid contents (mg.g^{-1}) were similar, but that non-infested female visceral tissue contained significantly more lipid per unit fresh mass.

The lipid mass (mg) percentages of dry tissue mass (mg) data, calculated from the tissue fresh to dry mass ratios (Fig. 3.6, 3.7,and Tables 3.108 to 3.112) reflects the differences in visceral water contents (Table 3.112), but are still significantly different as for lipid content when treated similarly (Tables 3.113 to 3.120).

Fig. 3.1

Foot, mantle, digestive gland and gonad or parasite percentages of total dry body mass in infested and non-infsted male and female Thais lapillus in February, March and April, 1981, 1982.

FEBRUARY MARCH AND APRIL.

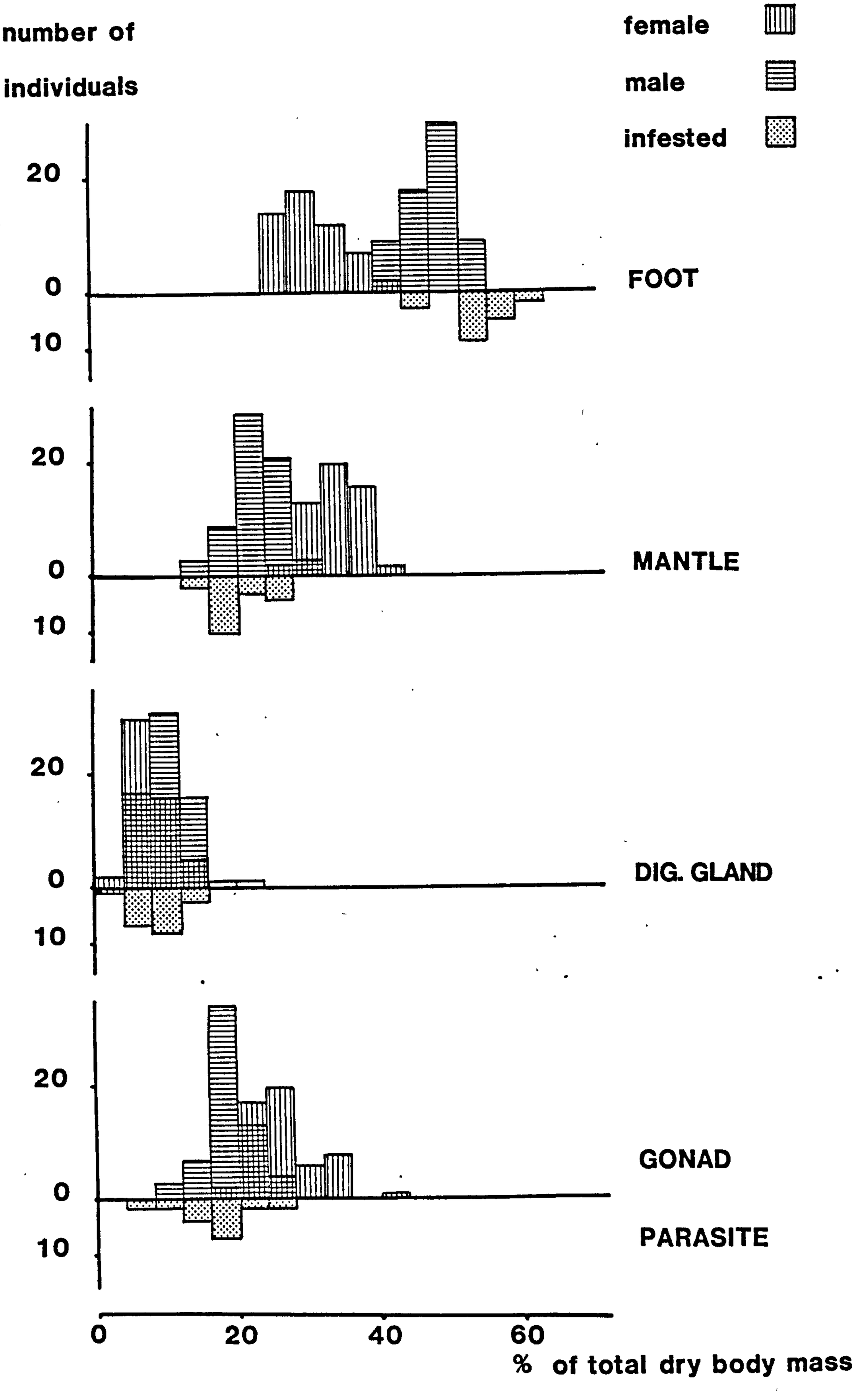


Fig. 3.2

Foot, mantle, digestive gland and gonad percentages of total body dry mass in non-infested male and female Thais lapillus in June and July, 1981, 1982.

JUNE AND JULY

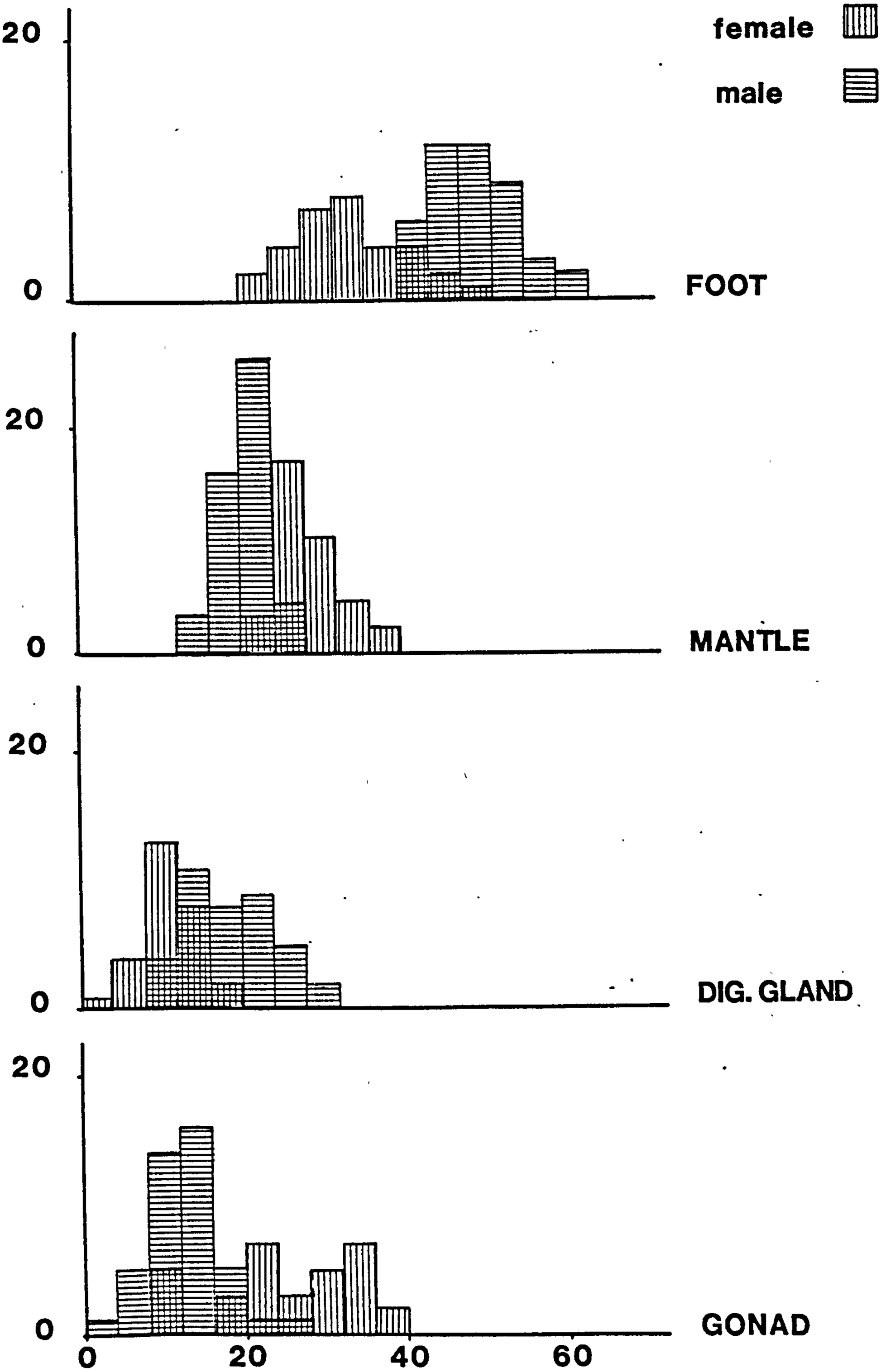


Fig. 3.3

Foot, mantle, digestive gland and gonad or parasite percentages of total dry body mass in infested and non-infested male and female Thais lapillus in August 1982.

AUGUST

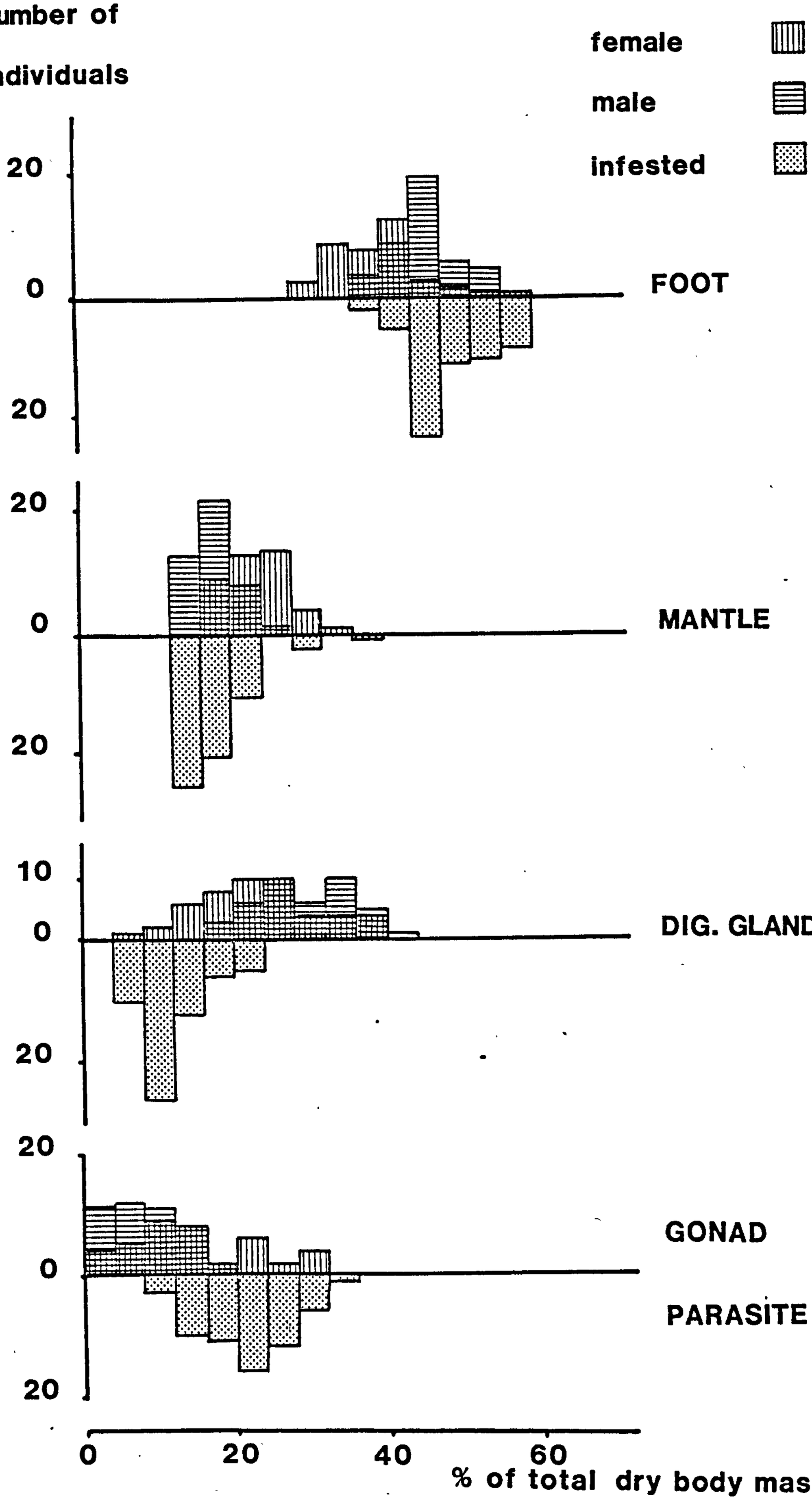

number of individuals
female
male
infested
20
0
20
FOOT
20
0
20
MANTLE
10
0
20
DIG. GLAND
20
0
20
GONAD
PARASITE
0
20
40
60
% of total dry body mass

Fig. 3.4

Foot, mantle, digestive gland and gonad or parasite percentages of total dry body mass for infested and non-infested male and female *Thais lapillus* in October, 1982.

OCTOBER

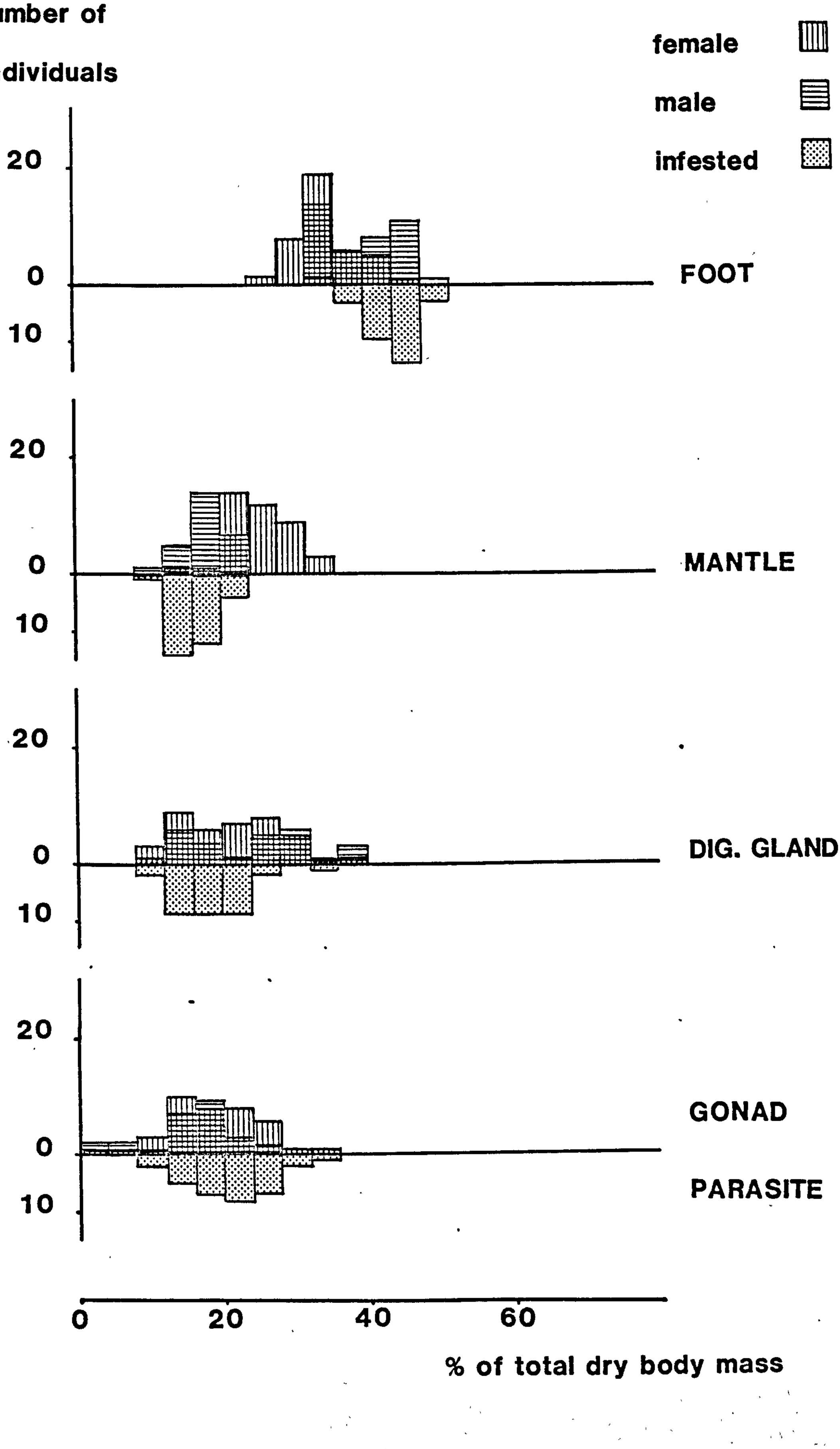

number of individuals
female
male
infested
20
0
10
FOOT
20
0
10
MANTLE
20
0
10
DIG. GLAND
20
0
10
GONAD
PARASITE
0
20
40
60
% of total dry body mass

Fig. 3.5

October 1982 fresh tissue lipid contents of infested and non-infested male and female Thais lapillus foot and viscera components.

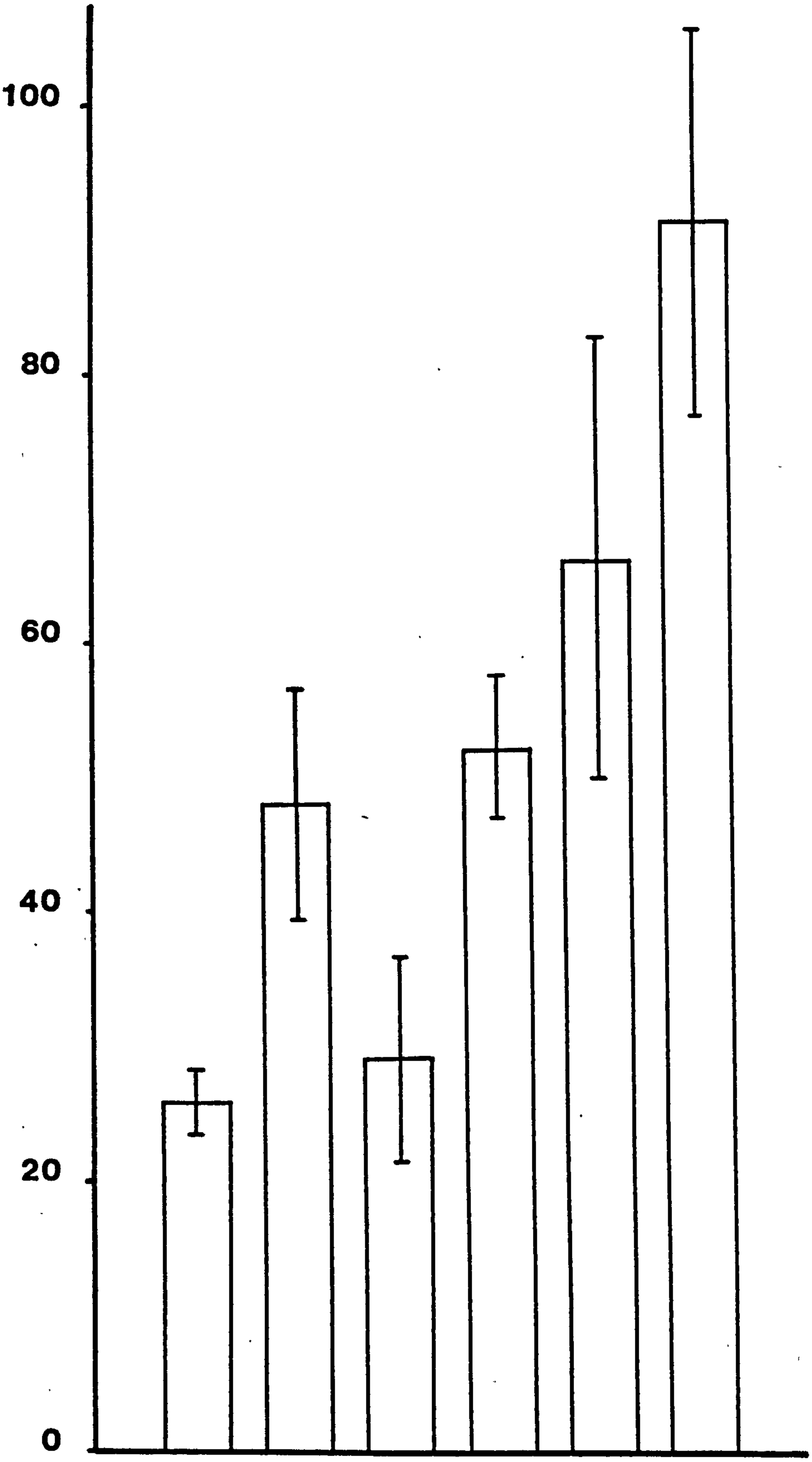

total lipid per mass
fresh tissue (mg.g-1)
100
80
60
40
20
0
I
M
F
I
M
F
FOOT
VISCERA

Fig. 3.6

Dry mass: fresh mass ratio for infested and non-infested male and female Thais lapillus in October 1982 - Foot component (including head, foot and mantle).

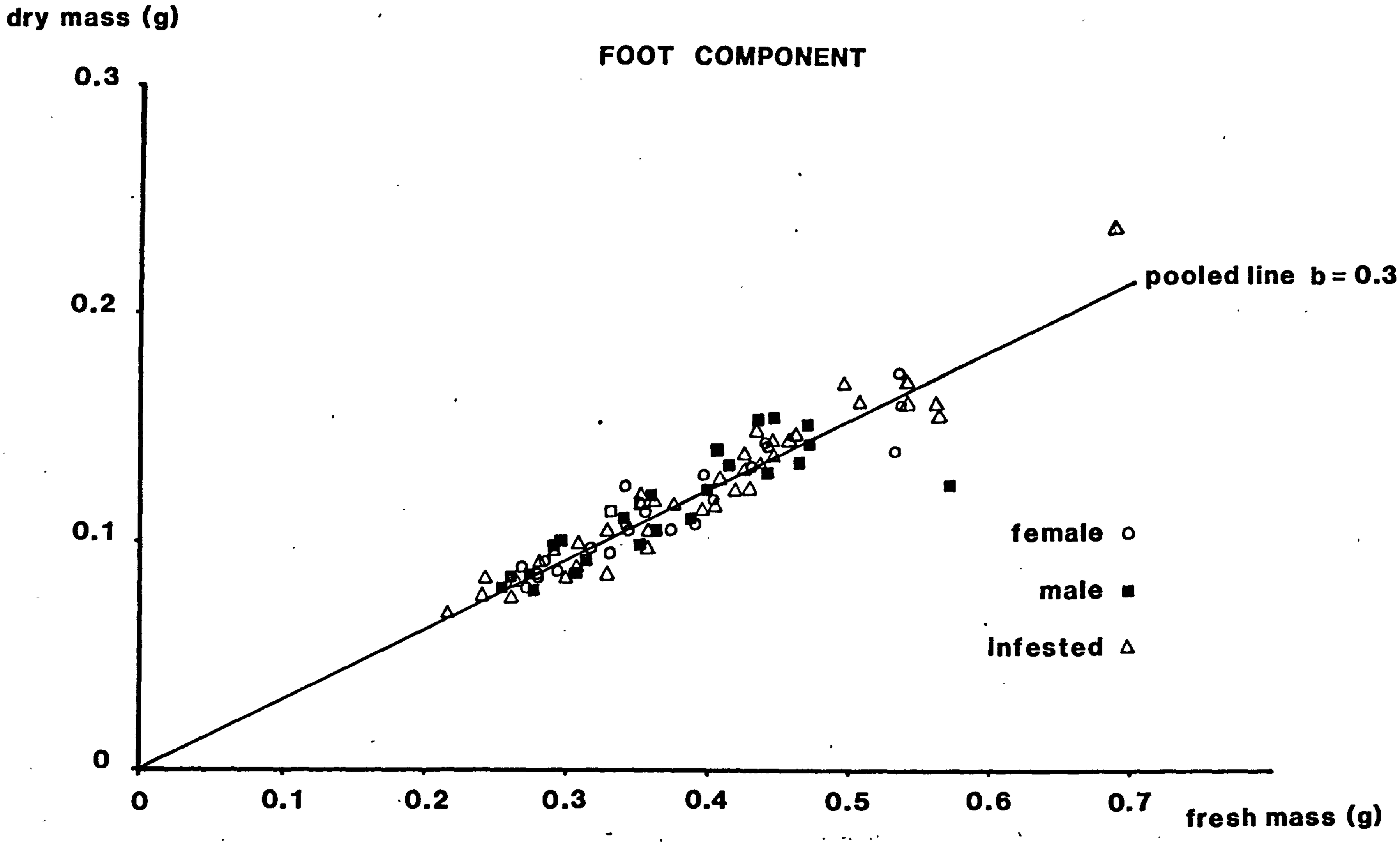

FOOT COMPONENT
dry mass (g)
0.3
0.2
0.1
0
pooled line b = 0.31
female
male
infested
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
fresh mass (g)

Fig. 3.7

Dry mass: fresh mass ratio for infested and non-infested male and female Thais lapillus in October 1982 - Viscera component (including digestive gland and gonad).

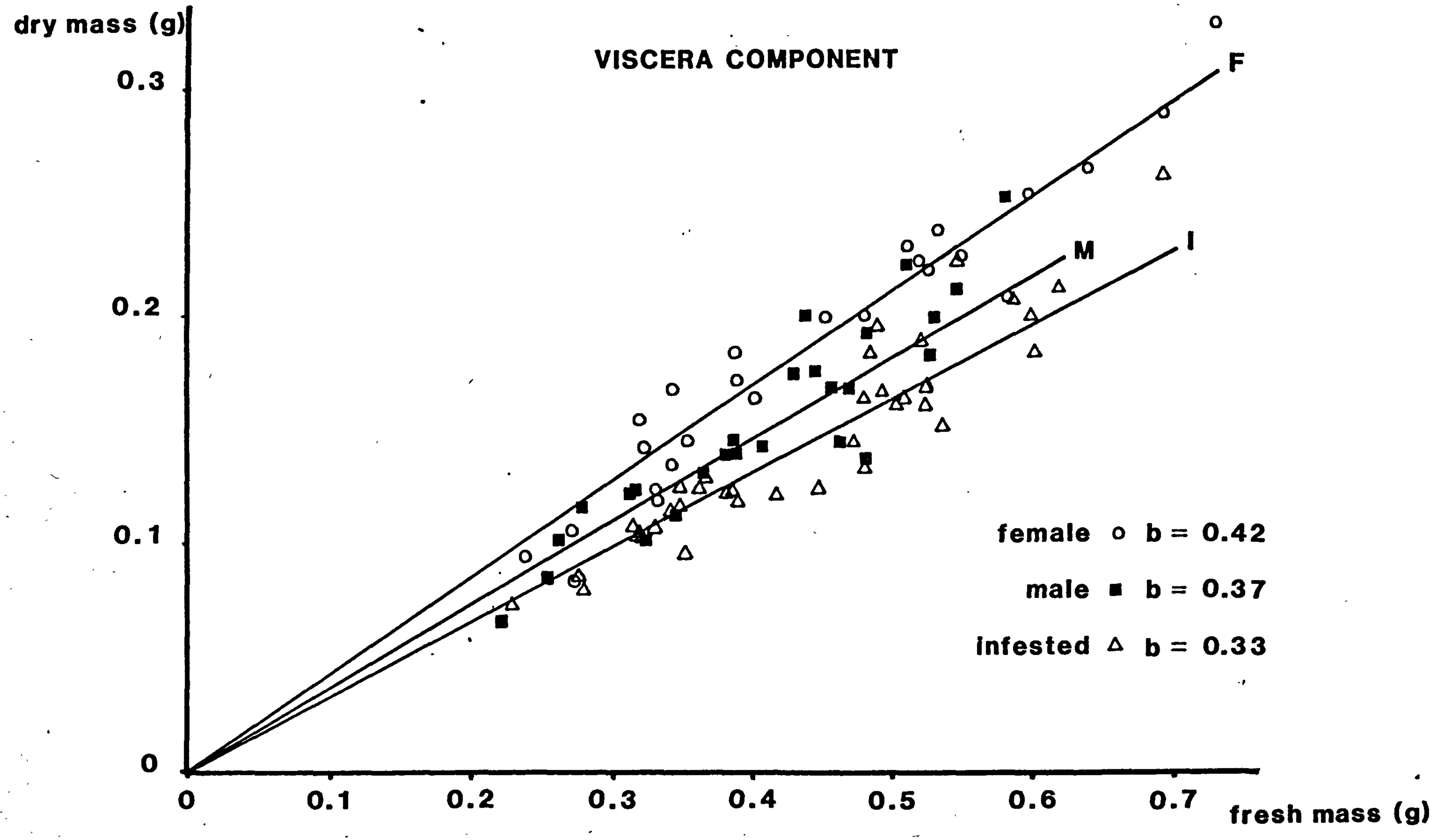

VISCERA COMPONENT
dry mass (g)
0.3
0.2
0.1
0
F
M
I
female ○ b = 0.42
male ■ b = 0.37
infested △ b = 0.33
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
fresh mass (g)

TABLE 3.1. FOOT PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH AND APRIL.

	MALE	FEMALE	INFESTED
n	66	53	19
Σx	3189.21	1665.45	1040.45
$\Sigma(x^2)$	154882.72	53325.11	57341.43
$\bar{x}$	48.32	31.42	54.76

TABLE 3.2. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	11588.56	2	5794.28	366.96
ERROR	2132.28	135	15.79	
TOTAL	13720.84	137		

TABLE 3.3. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	MALE	FEMALE	MSR
54.76	48.32	31.42	
		23.34	2.57
	6.44		2.50
		16.90	1.77

TABLE 3.4. MANTLE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH AND APRIL.

	MALE	FEMALE	INFESTED
n	66	53	19
Σx	1497.22	1800.89	376.32
$\Sigma(x^2)$	34742.63	61773.51	7651.56
$\bar{x}$	22.69	33.98	19.81

TABLE 3.5. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	4774.23	2	2387.11	206.98
ERROR	1556.98	135	11.53	
TOTAL	6331.21	137		

TABLE 3.6. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	MSR
33.98	22.69	19.81	
		14.17	2.20
	11.29		1.52
		2.88	2.14

TABLE 3.7. DIGESTIVE GLAND PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH AND APRIL.

	MALE	FEMALE	INFESTED
n	66	53	19
Σx	1226.66	862.99	330.72
$\Sigma(x^2)$	23293.90	14407.41	5869.80
$\bar{x}$	18.56	16.28	17.41

TABLE 3.8. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	156.29	2	78.15	10.95
ERROR	964.16	135	7.14	
TOTAL	1120.45	137		

TABLE 3.9. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	INFESTED	FEMALE	MSR
18.56	17.41	16.28	
		2.28	1.05
	1.15		1.50
		1.13	1.53

TABLE 3.10. GONAD OR PARASITE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN FEBRUARY, MARCH AND APRIL.

		MALE	FEMALE	INFESTED
	n	66	53	19
	Σx	1680.53	1642.54	444.87
	$\Sigma(x^2)$	43219.94	51533.36	10829.64
	$\bar{x}$	25.46	30.99	23.41
Back Transformed	$\bar{x}$	18.48	26.51	15.79

TABLE 3.11. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1231.88	2	615.94	56.51
ERROR	1471.55	135	10.90	
TOTAL	2703.43	137		

TABLE 3.12. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	MSR
30.99	25.46	23.41	
		7.58	2.14
	5.53		1.49
		2.05	2.08

TABLE 3.13. FOOT PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN JUNE AND JULY.

	MALE	FEMALE	INFESTED
n	44	33	16
Σx	2197.64	1142.18	857.63
$\Sigma(x^2)$	110887.78	41054.69	46320.72
$\bar{x}$	49.95	34.61	53.60

TABLE 3.14. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	5820.12	2	2910.06	87.49
ERROR	2995.90	90	33.29	
TOTAL	8816.02	92		

TABLE 3.15. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	MALE	FEMALE	MSR
53.60	49.95	34.61	
		18.99	4.31
	3.65		4.13
		15.34	3.26

TABLE 3.16. MANTLE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN JUNE AND JULY.

	MALE	FEMALE	INFESTED
n	44	33	16
Σx	895.61	945.22	306.24
$\Sigma(x^2)$	18547.82	27570.84	6031.01
$\bar{x}$	20.36	28.64	19.14

TABLE 3.17. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1596.42	2	798.21	72.98
ERROR	984.33	90	10.94	
TOTAL	2580.75	92		

TABLE 3.18. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	MSR
28.64	20.36	19.14	
		9.50	2.47
	8.28		1.87
		1.22	2.37

TABLE 3.19. DIGESTIVE GLAND PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN JUNE AND JULY.

	MALE	FEMALE	INFESTED
n	44	33	16
Σx	1087.79	623.87	297.79
$\Sigma(x^2)$	27578.60	12075.24	5598.07
$\bar{x}$	24.72	18.91	18.61
Back Transformed $\bar{x}$	17.49	10.50	10.19

TABLE 3.20. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE

Source of variance	SS	DF	MS	F
TREATMENTS	811.51	2	405.76	35.72
ERROR	1022.23	90	11.36	
TOTAL	1833.75	92		

TABLE 3.21. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS

MALE	FEMALE	INFESTED	MSR
24.72	18.91	18.61	
		6.11	2.41
	5.81		1.90
		0.30	2.59

TABLE 3.22. GONAD OR PARASITE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN JUNE AND JULY.

		MALE	FEMALE	INFESTED
	n	44	33	16
	ξx	878.31	978.58	372.49
	$\xi(x^2)$	18289.81	30213.83	9494.24
	$\bar{x}$	19.96	29.65	23.28
Back Transformed	$\bar{x}$	11.66	24.48	15.62

TABLE 3.23. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1780.70	2	890.35	28.88
ERROR	2774.87	90	30.83	
TOTAL	4555.57	92		

TABLE 3.24. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	INFESTED	MALE	MSR
29.65	23.28	19.96	
		9.69	3.14
	6.37		4.15
		3.32	3.98

TABLE 3.25. FOOT PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN AUGUST.

	MALE	FEMALE	INFESTED
n	43	38	59
ξx	1960.81	1513.13	2910.48
$\xi(x^2)$	89989.66	61615.66	145016.58
$\bar{x}$	45.60	39.82	49.33

TABLE 3.26. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	2090.80	2	1045.40	42.34
ERROR	3382.39	137	24.69	
TOTAL	5473.19	139		

TABLE 3.27. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	MALE	FEMALE	MSR
49.33	45.60	39.82	
		9.51	2.50
	3.73		2.41
		5.78	2.68

TABLE 3.28. MANTLE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN AUGUST.

	MALE	FEMALE	INFESTED
n	43	38	59
Σx	752.95	909.05	1034.52
$\Sigma(x^2)$	13492.33	22324.73	19301.91
$\bar{x}$	17.51	23.92	17.53

TABLE 3.29. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1133.37	2	566.68	37.91
ERROR	2048.31	137	14.95	
TOTAL	3181.68	139		

TABLE 3.30. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	INFESTED	MALE	MSR
23.92	17.53	17.51	
		6.41	2.08
	6.39		1.95
		0.02	1.88

TABLE 3.31. DIGESTIVE GLAND PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN AUGUST.

		MALE	FEMALE	INFESTED
n		43	38	59
Σx		1405.52	1051.49	1178.85
$\Sigma(x^2)$		46578.23	30266.54	24410.43
$\bar{x}$		32.69	27.67	19.98
Back Transformed	$\bar{x}$	29.16	21.57	11.68

TABLE 3.32. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	4166.28	2	2083.14	107.10
ERROR	2664.08	137	19.45	
TOTAL	6830.36	139		

TABLE 3.33. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	FEMALE	INFESTED	MSR
32.69	27.67	19.98	
		12.71	2.14
	5.02		2.38
		7.69	2.22

TABLE 3.34. GONAD OR PARASITE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN AUGUST.

	MALE	FEMALE	INFESTED
n	43	38	59
Σx	654.87	816.64	1606.62
$\Sigma(x^2)$	11317.06	19743.43	44770.49
$\bar{x}$	15.23	21.49	27.23
Back Transformed $\bar{x}$	6.90	13.42	20.94

TABLE 3.35. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	3595.27	2	1797.64	54.03
ERROR	4557.97	137	33.27	
TOTAL	8153.24	139		

TABLE 3.36. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	FEMALE	MALE	MSR
27.23	21.49	15.23	
		12.00	2.80
	5.74		2.90
		6.26	3.11

TABLE 3.37. FOOT PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	27	40	31
Σx	1156.4	1404.89	1361.50
$\Sigma(x^2)$	49825.05	50021.84	60130.40
$\bar{x}$	42.83	35.12	43.92

TABLE 3.38. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1644.00	2	822	59.61
ERROR	1310.01	95	13.79	
TOTAL	2954.01	97		

TABLE 3.39. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	MALE	FEMALE	MSR
43.92	42.83	35.12	
		8.80	2.18
	1.09		2.40
		7.71	2.27

TABLE 3.40. MANTLE PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	27	40	31
ξx	487.80	1024.83	510.47
$\xi(x^2)$	9026.87	27013.90	8645.32
$\bar{x}$	18.07	25.62	16.47

TABLE 3.41. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1711.00	2	855.50	67.15
ERROR	1210.46	95	12.74	
TOTAL	2921.46	97		

TABLE 3.42. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	MSR
25.62	18.07	16.47	
		9.15	2.10
	7.55		2.18
		1.60	2.31

TABLE 3.43. DIGESTIVE GLAND PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	27	40	31
Σx	643.11	838.27	577.22
$\Sigma(x^2)$	17189.46	19636.25	11411.88
$\bar{x}$	23.82	20.96	18.62

TABLE 3.44. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	390.21	2	195.11	4.03
ERROR	4604.18	95	48.47	
TOTAL	4994.39	97		

TABLE 3.45. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	FEMALE	INFESTED	MSR
23.82	20.96	18.62	
		5.20	4.50
	2.86		4.26
		2.34	4.09

TABLE 3.46. PARASITE OR GONAD PERCENTAGE OF WHOLE BODY DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	27	40	31
ξx	607.70	1000.04	839.67
$\xi(x^2)$	14433.06	26028.60	23262.45
$\bar{x}$	22.51	25.00	27.09
Back Transformed $\bar{x}$	14.65	17.86	20.73

TABLE 3.47. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	302.59	2	151.30	6.25
ERROR	2300.95	95	24.22	
TOTAL	2603.54	97		

TABLE 3.48. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	FEMALE	MALE	MSR
20.73	17.86	14.65	
		6.08	3.18
	2.87		2.89
		3.21	3.01

TABLE 3.49. SEASONAL DIFFERENCES IN PARASITE PERCENTAGE OF HOST TOTAL BODY DRY MASS.

		MARCH	JULY	AUGUST	OCTOBER
	n	19	16	59	31
	Σx	444.87	372.49	1606.62	839.67
	$\Sigma(x^2)$	10829.64	9494.24	44770.49	23262.45
	$\bar{x}$	23.41	23.28	27.23	27.09
Back transformed	$\bar{x}$	15.79	15.62	20.94	20.73

TABLE 3.50. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	369.82	3	123.27	5.37
ERROR	2775.71	121	22.94	
TOTAL	3145.53	124		

TABLE 3.51. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

AUGUST	OCTOBER	MARCH	JULY	MSR
27.23	27.09	23.41	23.28	

3.95 3.61

3.82 3.38

0.14 2.84

3.81 3.94

3.68 3.73

0.13 4.34

TABLE 3.52. ASH PERCENTAGE OF TISSUE DRY MASS FOR THAIS LAPILLUS COLLECTED IN APRIL.

		Treatments		
	Blocks	MALE	FEMALE	Σx
FOOT	n	6	6	
	$\bar{x}$	7.06	6.18	
	Arcsine	15.29	14.32	177.66
VISCERA	n	6	6	
	$\bar{x}$	10.57	6.16	
	Arcsine	18.87	14.25	198.72
	Σx	204.96	171.42	376.38

TABLE 3.53. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	46.90	1	46.90	10.55
Blocks	18.44	1	18.44	4.22
Interaction	19.96	1	1.96	4.57
Error	87.37	20	4.37	
Total	172.67	23		

TABLE 3.54. MEAN ASH PERCENTAGES OF TISSUE DRY MASS (+-95% confidence limits).

MALE	FEMALE
8.81(+-1.83)	6.17(+-1.01)

TABLE 3.55. TISSUE CALORIC VALUES (J.mg^{-1}) FOR MALE AND FEMALE THAIS LAPILLUS COLLECTED IN APRIL (1981).

		Treatments		
	Blocks	MALE	FEMALE	Σx
FOOT	n	8	8	
	$\bar{x}$	22.85	22.71	364.48
VISCERA	n	8	8	
	$\bar{x}$	22.85	25.59	386.88
	Σx	365.60	385.76	751.36

TABLE 3.56. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	12.76	1	12.76	
Blocks	15.72	1	15.72	
Interaction	15.62	1	15.62	6.08
Error	72.08	28	2.57	
Total	116.18	31		

TABLE 3.57. TISSUE CALORIC VALUES ($J.mg^{-1}$) FOR MALE AND FEMALE THAIS LAPILLUS COLLECTED IN APRIL 1981.

	MALE		FEMALE	
	Foot	Viscera	Foot	Viscera
n	8	8	8	8
Transformed $\bar{x}$	22.85	22.85	22.71	25.59
Variance	1.72	3.19	2.63	3.14

TABLE 3.58. ONE WAY ANOVA TABLE CALCULATED FROM THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENT	44.10	3	14.70	5.72
ERROR	72.08	28	2.57	
TOTAL		31		

TABLE 3.59. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE Viscera	MALE Foot	MALE Viscera	FEMALE Foot	SSR
25.59	22.85	22.85	22.71	
			2.88	2.21
		2.74		2.00
	2.74			1.66

TABLE 3.60. TISSUE CALORIC VALUES FOR NON-INFESTED MALE AND FEMALE FOOT AND VISCERAL COMPONENTS (+-95% confidence limits).

MALE Foot	MALE Viscera	FEMALE Foot	FEMALE Viscera
	22.80(+-0.64)		25.59(+-1.25)

TABLE 3.61. ASH PERCENTAGE OF TISSUE DRY MASS FOR THAIS LAPILLUS COLLECTED IN JUNE.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	4	4	4	
FOOT	$\bar{x}$	10.62	16.83	11.74	156.76
VISCERA	n	4	4	4	
VISCERA	$\bar{x}$	12.54	15.31	12.58	161.72
VISCERA	Σx	92.64	128.56	97.26	318.47

TABLE 3.62. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	94.96	2	45.65	23.65
Blocks	1.03	1	1.03	0.17
Interaction	12.23	2	6.12	3.17
Error	34.73	18	1.93	
Total	142.95	23		

TABLE 3.63. JUNE ASH PERCENTAGE OF TISSUE DRY MASS FOR THAIS LAPILLUS.

	MALE	FEMALE	INFESTED	PARASITE
n	8	8	8	4
Σx	92.65	128.58	97.28	59.73
$\Sigma(x^2)$	1090.43	2078.39	1201.68	903.58
$\bar{x}$	11.58	16.07	12.16	14.93

TABLE 3.64. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	104.97	3	34.99	14.11
ERROR	56.93	24	2.48	
TOTAL	164.60	27		

TABLE 3.65. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

15.69+-1.00 (FEMALE, PARASITE) 11.87+-0.84 (INFESTED, MALE)

FEMALE	PARASITE	INFESTED	MALE	MSR
16.07	14.93	12.16	11.58	
			4.49	2.01
		3.91		2.01
	1.14			2.47
		2.77		2.47
			0.58	2.01

TABLE 3.66. TISSUE CALORIC VALUES ($J.mg^{-1}$) THAIS LAPILLUS COLLECTED IN JUNE.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	5	5	5	
	$\bar{x}$	24.30	23.36	23.14	354.00
VISCERA	n	5	5	5	
	$\bar{x}$	26.23	26.06	25.57	389.30
	Σx	252.65	247.10	243.55	743.30

TABLE 3.67. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	4.21	2	2.11	1.08
Blocks	41.75	1	41.75	21.41
Interaction	0.77	2	2.52	1.29
Error	46.73	24	1.95	
Total	151.25	29		

TABLE 3.68. t-TEST FOR COMPARISON BETWEEN MEAN THAIS LAPILLUS VISCERAL CALORIC VALUE AND THAT OF PARORCHIS ACANTHUS REDIAE IN JUNE.

	N	$\bar{x}$	$\Sigma(x)^2$	$\Sigma(x^2)$	S	t
Parasite	5	23.44	13740.53	2781.69	8.40	1.92
Viscera	15	25.96	151577.85	10187.33	5.87	$<p=0.05$

TABLE 3.69. ASH PERCENTAGE OF TISSUE DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	5	5	5	
	$\bar{X}$	7.56	6.95	5.17	
	Arcsine $\bar{X}$	15.92	15.28	13.12	221.60
VISCERA	n	5	5	5	
	$\bar{X}$	7.09	4.25	5.00	
	Arcsine $\bar{X}$	15.42	11.87	12.87	200.80
	Σx	156.70	135.75	129.95	422.40

TABLE 3.70. TWO WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	39.55	2	19.78	
Blocks	14.36	1	14.36	
Interaction	15.94	2	7.75	8.23
Error	22.62	24	0.94	
Total	92.02	29		

TABLE 3.71. ASH PERCENTAGE OF FOOT TISSUE DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	5	5	5
Transformed $\bar{x}$	15.92	15.28	13.12
Variance	1.80	0.09	0.73
Back transformed $\bar{x}$	7.56	6.95	5.17

TABLE 3.72. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	Sums of squares	Degrees of freedom	Mean squares	F
TREATMENTS	21.49	2	10.75	12.31
ERROR	10.47	12	0.87	
TOTAL	31.96	14		

TABLE 3.73. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	FEMALE	INFESTED	SSR
15.92	15.28	13.12	
		2.80	1.58
	0.64		1.29

TABLE 3.74. FOOT TISSUE MEAN ASH PERCENTAGES OF DRY MASS. (+-95% confidence)

MALE	FEMALE	INFESTED
7.25 (+-0.65)		5.17(+-0.81)

TABLE 3.75. ASH PERCENTAGE OF VISCERA TISSUE DRY MASS FOR THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	5	5	5
Transformed $\bar{x}$	15.42	11.87	12.87
Variance	0.60	0.88	1.56
Back transformed $\bar{x}$	7.09	4.25	5.00

TABLE 3.76. ONE WAY ANOVA TALE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	33.58	2	16.78	16.56
ERROR	12.15	12	1.01	
TOTAL	45.70	14		

TABLE 3.77. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	INFESTED	FEMALE	SSR
15.42	12.87	11.87	
		3.55	1.67
	2.55		1.39

TABLE 3.78. VISCERAL TISSUE MEAN ASH PERCENTAGES OF DRY MASS (+-95% confidence limits).

MALE	FEMALE	INFESTED
7.09 (+-0.84)		4.62(+-0.61)

TABLE 3.79. TISSUE CALORIC VALUES ($J.mg^{-1}$) FOR *THAIS LAPILLUS* COLLECTED IN OCTOBER.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	6	6	6	
	$\bar{x}$	25.81	26.38	26.08	469.59
VISCERA	n	6	6	6	
	$\bar{x}$	29.21	30.61	30.62	542.66
	Σx	330.11	341.95	340.19	1012.25

TABLE 3.80. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	6.80	2	3.40	2.64
Blocks	148.31	1	148.31	114.97
Interaction	2.12	2	1.06	0.82
Error	38.62	30	1.29	
Total	195.85	35		

TABLE 3.81. TISSUE CALORIC VALUES ($J.mg^{-1}$) FOR *THAIS LAPILLUS* VISCERA COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED	PARASITE
n	6	6	6	6
Transformed $\bar{x}$	29.21	30.61	30.62	28.69
Variance	5.21	0.20	0.59	0.44

TABLE 3.82. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	17.57	3	5.88	3.64
ERROR	32.17	20	1.61	
TOTAL	49.74	23		

TABLE 3.83. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

INFESTED	FEMALE	MALE	PARASITE	SSR
30.62	30.61	29.21	28.69	
			1.93	2.05
				1.85
				1.53

TABLE 3.84. VISCERAL TISSUE MEAN CALORIC VALUES ($J.mg^{-1}$) (+- 95% confidence limits).

MALE	FEMALE	INFESTED AND PARASITE
29.78 (+- 0.055)		

TABLE 3.85. SEASONAL DIFFERENCES IN INFESTED FOOT TISSUE CALORIC VALUE ($J.mg^{-1}$).

	JUNE	OCTOBER
N	5	6
$\bar{x}$	23.14	26.08
ξx	115.68	156.46
$\xi(x^2)$	2681.55	4082.75
S	0.89	
t	5.17 (>0.001)	

TABLE 3.86. SEASONAL DIFFERENCES IN INFESTED VISCERA CALORIC VALUE ($J.mg^{-1}$).

	JUNE	OCTOBER
N	5	6
$\bar{x}$	25.57	30.62
ξx	127.85	183.73
$\xi(x^2)$	3307.18	5629.04
S	2.13	
t	3.88 (>0.01)	

TABLE 3.87. SEASONAL DIFFERENCES IN NON-INFESTED THAIS LAPILLUS MALE FOOT CALORIC VALUES ($J.mg^{-1}$).

	APRIL	JUNE	OCTOBER
n	8	5	6
Σx	182.80	121.50	154.86
$\Sigma(x^2)$	4188.12	2955.80	4001.18
$\bar{x}$	22.85	24.30	25.81

TABLE 3.88. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	30.16	2	15.08	12.89
ERROR	18.73	16	1.17	
TOTAL	48.89	18		

TABLE 3.89. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

OCTOBER	JUNE	APRIL	MSR
25.81	24.30	22.85	
		2.96	1.55
	1.51		1.74
		1.45	1.63

TABLE 3.90. SEASONAL DIFFERENCES IN NON-INFESTED THAIS LAPILLUS MALE VISCERA CALORIC VALUES ($J.mg^{-1}$).

	APRIL	JUNE	OCTOBER
n	8	5	6
ξx	182.80	131.15	175.26
$\xi(x^2)$	4200.24	3445.07	5144.82
$\bar{x}$	22.85	26.23	29.21

TABLE 3.91. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	126.56	2	63.28	15.03
ERROR	67.44	16	4.21	
TOTAL	194.00	18		

TABLE 3.92. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

OCTOBER	JUNE	APRIL	MSR
29.21	26.23	22.85	
		6.36	2.94
	2.98		3.29
		3.38	3.10

TABLE 3.93. SEASONAL DIFFERENCES IN NON-INFESTED FEMALE THAIS LAPILLUS FOOT CALORIC VALUE ($J.mg^{-1}$).

	APRIL	JUNE	OCTOBER
n	8	5	6
ξx	181.71	116.78	158.27
$\xi(x^2)$	4145.75	2741.58	4176.5
$\bar{x}$	22.71	23.36	26.38

TABLE 3.94. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	49.22	2	24.61	11.55
ERROR	34.10	16	2.13	
TOTAL	83.32	18		

TABLE 3.95. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

OCTOBER	JUNE	APRIL	MSR
26.38	23.36	22.71	
		3.67	2.09
	3.02		2.34
		0.65	2.20

TABLE 3.96. SEASONAL DIFFERENCES IN NON-INFESTED FEMALE THAIS LAPILLUS VISCERA CALORIC VALUE ($J.mg^{-1}$).

	APRIL	JUNE	OCTOBER
n	8	5	6
Σx	204.70	130.32	183.68
$\Sigma(x^2)$	5253.47	3435.08	5624.05
$\bar{x}$	25.59	26.06	30.61

TABLE 3.97. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	107.40	2	53.70	19.25
ERROR	44.69	16	2.79	
TOTAL	152.09	18		

TABLE 3.98. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

OCTOBER	JUNE	APRIL	MSR
30.61	26.06	25.59	
		5.02	2.39
	4.55		2.68
		0.47	2.52

TABLE 3.99. COMPARISON BETWEEN MEAN CALORIC VALUES FOR PARORCHIS ACANTHUS REDIAE FOR JUNE AND OCTOBER SAMPLES.

	N	$\bar{x}$	$\xi(x)^2$	$\xi(x^2)$	S*
JUNE	5	23.44	13740.53	2781.69	8.40
OCTOBER	6	28.69	29632.18	4939.16	0.09

* Variances not assumed equal

d = 4.03 for f = 4 degrees of freedom

therefore $p \leq 0.02$.

TABLE 3.100. OCTOBER TISSUE LIPID CONTENT (mg LIPID PER g FRESH TISSUE) FOR THAIS LAPILLUS.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	4	4	4	
	$\bar{x}$	48.07	29.05	25.94	412.24
VISCERA	n	4	4	4	
	$\bar{x}$	66.03	91.21	51.96	836.80
	Σx	456.40	481.04	311.60	1249.04

TABLE 3.101. TWO WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	2095.31	2	1047.65	
Blocks	7510.11	1	7510.11	
Interaction	2217.29	2	1108.64	25.72
Error	775.80	18	43.10	
Total	12598.51	23		

TABLE 3.102. OCTOBER FOOT TISSUE LIPID CONTENT (mg LIPID PER g FRESH TISSUE) FOR THAIS LAPILLUS.

	MALE	FEMALE	INFESTED
n	4	4	4
$\bar{x}$	48.07	29.05	25.94
Variance	29.63	24.17	2.43

TABLE 3.103. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	1148.54	2	574.27	30.63
ERROR	168.71	9	18.75	
TOTAL	1317.25	11		

TABLE 3.104. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	FEMALE	INFESTED	SSR
48.07	29.05	25.94	
		22.13	8.55
	19.02		6.93

TABLE 3.105. OCTOBER VISCERAL TISSUE LIPID CONTENT (mg LIPID PER g FRESH TISSUE) FOR THAIS LAPILLUS.

	MALE	FEMALE	INFESTED
n	4	4	4
$\bar{x}$	66.03	91.21	51.96
Variance	108.37	82.91	11.08

TABLE 3.106. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	3164.05	2	1582.03	23.45
ERROR	607.09	9	67.46	
TOTAL	3771.14	11		

TABLE 3.107. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	SSR
91.21	66.03	51.96	
		39.25	16.22
	25.18		13.14

TABLE 3.108. DRY MASS/WET MASS RELATIONSHIP FOR FOOT TISSUE FROM THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	26	23	39
ξx	8.039	7.1097	12.0070
$\xi(x^2)$	2.505	2.2082	3.7122
$\bar{x}$	0.309	0.3091	0.3079

TABLE 3.109. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	3.6x10-5	2	0.00002	0.0350
ERROR	0.04586	85	0.00057	
TOTAL	0.0459	87		

Mean dry mass/wet mass slope = 0.3086(+-0.0049)
(95% confidence)

TABLE 3.110. DRY MASS/FRESH MASS RELATIONSHIP FOR VISCERAL TISSUE FROM THAIS LAPILLUS COLLECTED IN OCTOBER.

	MALE	FEMALE	INFESTED
n	26	24	40
ξx	9.5696	10.1859	13.1187
$\xi(x^2)$	3.5702	4.3536	4.3429
$\bar{x}$	0.3681	0.4244	0.3280

TABLE 3.111. ONE WAY ANOVA TABLE CALCULATED FOR THE DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	0.1398	2	0.0699	49.9286
ERROR	0.1190	87	0.0014	
TOTAL	0.2588	89		

TABLE 3.112. GT2 TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	MSR
0.4244	0.3681	0.3280	
		0.0964	0.0237
	0.0563		0.0260
		0.0401	0.0231

TABLE 3.113. OCTOBER LIPID % OF TISSUE DRY MASS FOR MALE, FEMALE AND INFESTED THAIS LAPILLUS.

		Treatments			
	Blocks	MALE	FEMALE	INFESTED	Σx
FOOT	n	4	4	4	
	$\bar{x}$	15.47	9.37	8.37	
	Arcsine	23.14	17.78	16.81	57.73
VISCERA	n	4	4	4	
	$\bar{x}$	17.85	21.72	15.74	
	Arcsine	24.95	27.76	23.37	76.08
	Σx	48.09	45.54	40.18	133.81

TABLE 3.114. TWO WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variation	SS	DF	MS	F
Treatments	65.07	2	32.53	
Blocks	224.40	1	224.40	
Interaction	67.35	2	33.68	17.13
Error	35.39	18	1.97	
Total		23		

TABLE 3.115. OCTOBER LIPID % OF FOOT TISSUE DRY MASS FOR MALE FEMALE AND INFESTED THAIS LAPILLUS.

	MALE	FEMALE	INFESTED
	4	4	4
Replicates	14.74	10.65	7.82
	13.41	9.26	8.53
	16.69	7.17	8.14
	17.04	10.39	9.99
Arcsine transformed $\bar{x}$	23.14	17.78	16.81
Back transformed $\bar{x}$	15.47	9.37	8.37

TABLE 3.116. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	92.93	2	46.47	29.68
ERROR	14.01	9	1.57	
TOTAL	107.02	11		

TABLE 3.117. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

MALE	FEMALE	INFESTED	SSR
23.14	17.78	16.81	
		6.33	2.47
	5.36		2.03

TABLE 3.118. OCTOBER LIPID % OF VISCERA TISSUE DRY MASS FOR MALE, FEMALE AND INFESTED THAIS LAPILLUS.

	MALE	FEMALE	INFESTED
	4	4	4
Replicates	16.1	19.82	15.12
	16.0	24.38	14.77
	17.2	20.08	16.07
	22.0	22.58	17.01
Arcsine $\bar{x}$ transformed	24.9	27.76	23.37
Back $\bar{x}$ transformed	17.8	21.72	15.74

TABLE 3.119. ONE WAY ANOVA TABLE CALCULATED FOR THE TRANSFORMED DATA ABOVE.

Source of variance	SS	DF	MS	F
TREATMENTS	39.4	2	19.75	8.34
ERROR	21.3	9	2.37	
TOTAL	60.8	11		

TABLE 3.120. SNK TEST FOR SIGNIFICANT DIFFERENCES BETWEEN MEANS.

FEMALE	MALE	INFESTED	SSR
27.76	24.95	23.37	
		4.39	3.03
	2.81		2.46

DISCUSSION

The results for April and June non-infested male and female Thais lapillus tissue caloric values do not demonstrate a decrease in tissue energy ($J.mg^{-1}$) as a result of reproduction. (Loss of tissue mass associated with reproduction is discussed in Chapter 4). The increased male visceral caloric value in June is probably the result of resumed feeding as the males leave the aggregations, while the females remain to lay their egg capsules (Feare 1969). The considerable lipid contribution by female T.lapillus to their egg capsules (Bayne 1968) does not seem to have any effect on the caloric value of the tissues. The result is to some extent supported by the conclusion of Stickle (1975) who noted that it was the component proportion of T. lamellosa that changed through the seasonal and reproductive cycles, rather than their biochemical constituents.

The presence of the larval stages of the digenean trematode Parorchis acanthus has no effect on the tissue caloric values ($J.mg^{-1}$) of the host in either June or October (Tables 3.66 and 3.67, 3.79 and 3.80). In infested animals the tissue caloric value increases from early summer to autumn, prior to winter aggregation, in a similar manner to non-infested males and females. The results of this study are in partial agreement with those of Burky and Hornbach (1979), who calculated the calorific values of uninfested, and infested individuals of the pulmonate snail Succinea ovalis with Leucochloridium variae from the total tissue carbon contents, as described by Russell-Hunter et. al. (1978). The authors of the former study found no difference

between the calculated calorific values of the tissues of non-infested and infested hosts or of parasite, all of which lay between approximately 20.34 and 21.93 $J.mg^{-1}$ (converted here from Kcal for the sake of comparison). The low calorific value of Succinea ovalis was commented upon by Slobodkin and Richman (1961) who attributed it to the inactive life-style of the species. The parasite, Leucochloridium variae has a similar caloric value to that obtained for parasitic helminths by Calow and Jennings (1974) of 21.904 $KJ.g^{-1}$. The latter authors suggested that the low caloric values of parasitic helminths are associated with the host environment being one of constant food availability; hence no storage compounds need be assimilated in the tissues, and the maximum amount of the available energy can be expended on reproduction.

The caloric value of Thais lapillus tissue can be as high as 30.60 J.mg (Table 3.57), while that of the trematode Parorchis acanthus rises from 23.44 $J.mg^{-1}$ in June to an energy level similar to that for infested and non-infested viscera - 28.69 $J.mg^{-1}$ - in October. The caloric values for P. acanthus rediae in June and October are significantly different from each other, (Table 3.99), but are not significantly lower than the caloric values of their host viscera. This suggests that, for this species of larval trematode at least, the tissue caloric values are closely allied to those of the free-living host, and do not reflect the reduced need for energy storage postulated by Calow and Jennings (1974).

The decreased fresh weight to dry weight ratio of infested Thais lapillus viscera (including Parorchis acanthus rediae) (Fig. 3.7) is in agreement with the increased water content reported for Schistosoma mansoni infested Biomphalaria glabrata by Becker (1980). There is a posibility that in the latter instance the increased water content could be the result of a disproportionately large haemolymph volume in infested snails caused by the effects of the parasite on the endocrine organs, the Dorsal Bodies, of the host snail as described by Geraerts (1976). In the case of T. lapillus infested with P. acanthus the similarity of the foot tissue fresh:dry mass ratio to those for non-infested male and female snails (Fig. 3.6), suggests that the disproportionately large fresh mass:dry mass ratios of infested visceral tissue are probably due to the replacement of gonad and digestive gland tissues with rediae.

The non-infested male and female viscera lipid percentages of tissue dry mass (17.85% and 21.72% respectively) are lower than the level of approximately 30% given for October specimens by Lambert and Dehnel (1974), and the figure of more than 23% for November specimens obtained by Stickle (1975). The techniques used by Stickle (1975) for lipid extraction did not include structural lipids which were extracted both in the present study and in that of Lambert and Dehnel (1974), and consequently the lipid level values obtained by Stickle (1975) are slightly reduced (by approximately 5% (Giese, 1969)). The difference in lipid level between T. lapillus and T. lamellosa may be due to either interspecific or geographical variations.

The overall reduced lipid content of the infested dogwhelks is not reflected in their tissue caloric values for October which are similar to those of non-infested animals. This indicates a change in the metabolism of the infested snails, and perhaps confirms the suggestions of Stickle (1971, 1973, 1975) and Stickle and Bayne (1982) that lipid is not stored as a respiratory reserve, to be used during starvation in Thaisid snails, but has a reproductive function, as also suggested for Patella vulgata (Blackmore 1969), and Littorina littorea (Williams 1970). The fact that lipid content and tissue dry mass percentages of infested animals are reduced overall for the whole animal, when compared with non-infested Thais lapillus, is in opposition to the reports of increased lipid levels in infested Physa occidentalis by Hurst (1927), and Helisoma trivolvis by Cheng and Snyder (1962). James (1965) suggested that increased lipid deposition in the digestive gland cells of infested L. saxatilis could be linked with increased anaerobic respiration by infested tissues, but there is no evidence of this on infested T. lapillus (Chapter 5). The elevated lipid level of the female visceral mass as with that of non-infested male and infested individuals suggests that it is the female gonad which is causing the elevation of the visceral lipid content.

The elevation of the lipid level of non-infested male foot tissue as compared with non-infested female and infested foot tissues is surprising. It was noted by Giese (1969) that gastropod foot tissue served no storage function whatsoever. Studies on T. lamellosa by Stickle (1975) and

Lambert and Dehnel (1974) found no evidence of enhanced lipid storage in male foot tissues. The elevated lipid level is probably due to the inclusion of organs other than purely foot muscle tissue in the 'Foot' component of this study. Whether the lipid is stored for sexual or survival purposes might be determined if the centre (if one exists) of lipid deposition in this component of male Thais lapillus was to be located.

It is dificult to find a biological explanation for the similar caloric values of infested and non-infested T. lapillus visceral tissues which have significantly different lipid percentages of dry mass.

The relative abilities of infested and non-infested T. lapillus individuals to endure periods of cold has not been investigated. The studies of Vernberg and Vernberg (1963, 1968), Vernberg (1969) and Lunetta and Vernberg (1971) on Nassarius obsoletus carrying trematode infestations suggest that infested snails would be less able to survive prolonged cold periods. Tallmark and Norgren (1976) also reported reduced thermal tolerance in infested N.reticulatus, giving further weight to the hypothesis that larval trematodes do effect this aspect of their host's metabolism. The fact that some infested dogwhelks have many rows of aperture teeth (Feare 1970a reports having found an individual with 8 rows of teeth), on the other hand, suggests that some, if not all, infested animals can survive the winter temperatures of the North Yorkshire Coast.

The organ component indices of Thais lapillus calculated in this study are not directly comparable with those of Stickle (1973) and Belisle and Stickle (1978) for T. lamellosa and T. haemostoma respectively, because these authors include the tissue water content and the shell, which were both excluded in the present investigation. Tissue dry masses were used to construct the component proportions, as Lambert and Dehnel (1974) did for T. lamellosa, rather than fresh masses as first described by Giese (1969). Different components were used in this investigation to all the others mentioned for Thaisids because the treatment with boiling water of the tissues allowed the visceral mass to be divided into digestive gland and gonad or parasite components, which was considered to be more important than interspecific comparisons.

In the February, March and April samples there was a clear division between male and female mantle component percentages of whole body dry mass, resulting from the swollen capsule gland in the mantle complexes of females. This relatively heavy organ, and the fact that ovary tends to be more massive than testis in T lapillus individuals, causes the smaller foot percentage of dry mass observed for female snails. The infested animals have the highest foot proportion of the total dry mass (Fig.3.1, Table 3.3), because they have damaged or regressed accessory reproductive organs in the mantle, so that they form a smaller proportion of the whole body dry weight than either male or female mantle components (Fig. 3.1, Table 3.6). The mean digestive gland component percentage of infested snails

lies between those of male and female animals, of which the male percentage is significantly greater than that for females (Fig 3.1, Table 3.9). The redial populations in infested snails probably have higher water contents than the volume of ovary that they replace in previously female hosts (Fig. 3.7); this will contribute towards their significantly smaller percentage of total body dry mass than that for ovary in non-infested females (Fig. 3.1,Table 3.12). Male gonad contributes a similar proportion to the total dry mass of non-infested males to the redial standing crop in infested snails.

The organ components of non-infested male and female Thais lapillus collected in June 1981 and July 1982 show little evidence of changing relative proportions following reproduction (Fig. 3.2) although there are some females with much lower gonad percentages of dry mass than found in the early spring collection. The prolonged period of low temperatures in the early months of the summer of 1982 may well have delayed egg capsule deposition by the female T. lapillus at Robin Hoods Bay.

Individuals from the small sample of infested T. lapillus have foot and mantle component dry mass percentages similar to those of non-infested male T. lapillus individuals, and the parasite dry mass percentage is similar to that of non-infested testis in normal males which is less than that of the ovary in non-infested females (Tables 3.13-3.24). This means that dogwhelks which were female before they became infested are still supporting a smaller proportional mass of rediae than the gonad mass which has been replaced.

(Table 3.24). Although infested dogwhelks have less digestive gland tissue than non-infested male individuals it is apparently not reduced by potentially harmful amounts by the feeding Parorchis acanthus rediae as it is a similar percentage of the total dry mass as the digestive gland of non-infested female Thais lapillus (Table 3.21).

By August the male and female gonad dry mass percentage of the whole body dry mass tends to be exceeded by the dry mass of the digestive gland (Fig. 3.3, Table 3.3). The infested T. lapillus at this time of year have significantly less digestive gland relative to their total dry mass than non-infested females, who in turn also have a significantly lower digestive gland percentage of total body dry mass than non-infested males (Table 3.33). As the gonads of the non-infested breeding animals become reduced in size after the breeding season (Figs. 3.1 and 3.3), and Feare 1970b), and the parasite percentage of dry mass slightly increases (Table 3.51) as infestations develop, the infested dogwhelks have, by August, to support a standing crop of rediae which is proportionately greater than the gonad of both non-infested male and female T.lapillus individuals (Fig. 3.3, Table 3.36). Until August the infested dogwhelks appeared to be at no major disadvantage (except the loss of their reproductive ability) as a result of supporting an infestation of P.acanthus rediae in place of their own gonads.

In October the gonads of non-infested male and female T. lapillus individuals are maturing and developing before the animals aggregate for winter (Fig. 3.4). The proportion of

gonad dry mass to total body dry mass in non-infested female snails is similar to that of the Parorchis acanthus redial mass in infested snails but the male gonad proportion is significantly smaller than both of these (Fig. 3.4, Table 3.48); this indicates that the period of 1982 when infested animals carried a proportion of parasite larger than the proportion of gonad in female non-infested snails was August and September only, when feeding rates are greatest (Bayne and Scullard 1978b), and hence the parasite load can be most easily supported and compensated for. The digestive gland percentage of the total body dry mass of infested snails is again similar to that of non-infested females, but significantly less than for males (Table 3.45), further demonstrating the relatively moderate effects of the infestation on this host organ.

The effects of the presence of P. acanthus rediae on the host tend to cause a reduction in the digestive gland and mantle percentage of total dry mass and the total replacement of gonad with parasite. The digestive gland component of infested Thais lapillus was proportionately lighter than that found in non-infested female snails only during August. The mantle component of infested snails is always reduced because of the regression and physical destruction of the associated glands.

The average proportion of parasite in infested T.lapillus in August and October slightly exceeds 20% and is slightly less than 16% earlier in the year. (Tables 3.49). There are very few published accounts of the relative masses of parasite and host tissue as larval trematode infestations of

gastropod molluscs, probably because of the difficulty in separating small sporocysts or rediae from snail digestive gland. The size of Parorchis acanthus rediae (greater than 3.5mm in length according to Rees (1966)) and the consolidating effect of the 30 second immersion in boiling water on both parasite and snail tissues, made the complete separation of rediae from digestive gland possible by the procedure described above (p 51).

Burky and Hornbach (1979) found that the percentage dry weight of Leucochloridium variae brood sacs and sporocysts in Succinea ovalis constituted 23.8% (SD+-6.6%) of the combined dry mass of host and parasite. The fresh mass percentages of Acanthatrium anaplocami sporocysts in Nitocris dilatatus, and Echinostoma revolutum rediae in Physa sayii have been calculated by the writer from information provided by Cheng (1971) as 16% and 27% respectively.

These examples suggest that larval trematodes can be responsible for between 15% and 25% of the soft body mass of infested snail hosts. This study had demonstrated seasonal differences in infestation percentages of total dry mass of the hosts soft body for P.acanthus rediae in Thais lapillus. In conclusion, the similar tissue caloric values of P. acanthus rediae infested T. lapillus host tissue and tissues of the same tissue component in non-infested snails at the same time of year indicate that the presence of the parasite does not cause a major overall nutrient depletion of the tissues. The difference in lipid in male, female and

infested tissues indicates sexual differences in lipid accumulation between male and female Thais lapillus, and reduced lipid percentages of tissue dry mass in castrated infested animals. The suggestion by other authors that lipid is used by marine gastropods as a reproductive energy source rather than for body maintenance is one possible interpretation of the result obtained here.

The fact that October tissue caloric values do not reflect the differences in lipid distribution described above may indicate that lipid is synthesised in non-infested dogwhelks from protein since this is present in large quantity (Stickle 1975, Belisle and Stickle 1978), and lipid and protein levels were shown by Lambert and Dehnel (1974) to mirror one another in T. lamellosa.

The changes in organ components between February and October for non-infested T. lapillus males and females are due to the different reproductive states and levels of feeding activity shown by the snails. Infested animals have lower parasite percentages of tissue dry mass at the beginning of the year (on average about 16%), than in the later summer and autumn when mean infestation percentages rise to over 20%. Only during August was the parasite standing crop percentage of the soft body dry weight found to exceed that of ovary in non-infested females. The male gonad was never a significantly greater proportion of the total body dry mass of non-infested animals than the parasite standing crop of infested dogwhelks, and was smaller than the parasite percentage of dry mass for most of the year.

The implications of the differences in the energy partitioning of hosts of different sexes which lose their sexual characteristics on infestation with Parorchis acanthus rediae are hard to predict. It was reported by Moore (1938a) that large Thais lapillus are more frequently female. Some factor of the physiology of female T. lapillus individuals may allow them to grow larger than males during immaturity (as Moore (1938a) found no evidence of adult growth), to invest more energy in reproduction (Hughes 1972, and Chapter 4) to remain in aggregation longer, thus feeding for a shorter period during the year (Feare 1969), and to become more common in older year classes, suggesting increased longevity (Feare 1970b). Host sex selection by larval trematodes has been postulated in earlier studies on marine prosobranchs by Rees (1936) for Littorina littorea, Lysaght (1941) for L. neritoides, Berry (1962) for L. saxatilis, and Crewe (1951) for Patella vulgata. In all these examples larger snails tended to be female, and infestations were more common in larger animals. Whether this parasite distribution pattern is as a result of active host sex selection by the invading miracidia is not clear. The increasing incidence of females in the older year classes of T lapillus (noted by Feare 1970b), and sex reversal from male to female by ageing limpets (Orton 1946) may provide explanations for the parasite distributions, rather than host selection by the miracidia.

The present study demonstrates the considerable change in organ component indices undergone by female T. lapillus as a result of infestation with Parorchis acanthus. The

replacement of gonad with parasite, which only during August exceeds the proportional mass of a normal ovary, and the reduction in size of the capsule gland are the two most marked effects. The proportional mass of the digestive gland is not significantly reduced from the normal condition except in August. Whether female 'vigour' is required for an individual Thais lapillus to support an infestation of Parorchis acanthus has not been investigated, but the evidence of Feare (1970b) suggests that the infestation will be of greater duration in the longer-lived female host if that characteristic is not lost as a result of the infestation, in a similar manner to sexually related structures.

CHAPTER FOUR

COMPARISONS BETWEEN THE ENERGY EXPENDED ON REPRODUCTION BY NON-INFESTED *THAIS LAPILLUS* ADULTS AND THE PRODUCTION OF *PARORCHIS ACANTHUS* LARVAL STAGES IN INFESTED HOSTS.

INTRODUCTION

In a general discussion on the strategy of host castration Baudoin (1974) suggested that, by reducing their host's reproductive effort, castrating parasites gain advantage as a result of increased host survivorship, growth, and energy availability. All these effects are considered beneficial to the parasite, but not the host; thus the parasite that utilizes the hosts reproductive energy for its own purposes increases its Darwinian fitness. The fact that castrating parasites tend to be proportionately larger in comparison to their hosts than non-castrating parasites is suggested by Baudoin to be evidence of this trend.

In his review Wright (1966) concluded that destruction of gastropod gonads by larval trematodes is rarely the direct result of such infestation. Gonads may be physically damaged or consumed by large rediae in the digestive gland - as happens in the case of *Cryptocotyle lingua* rediae in *Littorina littorea* (Rees, 1936) - or by nutritional deprivation as a result of infestation. This can be achieved either by a "blocking layer" of inactive sporocysts as described by Rees (1936) for *L. littorea* infested with *Cercariae emasculans*, or by depletion of host nutritional resources by the parasite. The latter is, as discussed by

Baudoin (1974), not reconcilable with the associated growth effects in castrated gastropods. Baudoin suggested that any nutritional depletion associated with the infestion would have to be selective, and not a total reduction of nutrient levels within the host. Wright (1966) observed that, despite the absence of direct damage to the gonad and accessory sexual organs of infested gastropods, there is commonly (particularly in Basommatophoran pulmonates) a general reduction in fecundity, and he cited the work of Coehlo (1954), Najarian (1961) and Etges and Gresso (1965), in support of this statement.

By way of summarizing a review of current literature, and his own findings for Nassarius obsoletus infested with, and castrated by, sporocysts of Zoogonus rubellus, Cheng et al. (1973), proposed a simple classification for the "types" of parasitic castration in molluscs, which is as follows:-

Mechanical Castration:-	Parasite in gonads
Chemical Castration:-	Direct type: parasite in gonads
	Indirect type:parasite not in gonads

Cheng et al. did not limit the cause of chemical castration to the production of a castrating hormone but included also the production of enzymes which destroy the gonadal tissue. In the case of Z. rubellus infestations of N. obsoletus he suggested that it is the presence of some highly specific chemical factor produced by the Z. rubellus sporocysts which causes the castration of the snail host, as the sporocysts could not possibly ingest the gonadal tissue.

Individuals of the Basommatophoran pulmonate species Lymnaea stagnalis when infested with Trichobilharzia ocellata were reported by McClelland and Bourns (1969), to have significantly reduced rates of egg production, increased growth rates and extended life spans, in comparison with non-infested controls. These authors suggested that this may be the result of a hormonal secretion from the sporocysts, although they had no evidence of such a secretion. Baudoin (1974) speculated that the continual production of a castrating hormone by the parasite would be a significant drain upon its resources, which would need to be compensated for by any advantages for the parasite resulting from the reduced reproductive effort of the host, unless the hormone was an end-product or by-product of the parasite's normal metabolism.

The effects of Schistosoma mansoni larval stages on their Basommatophoran hosts Biomphalaria glabrata and B. pfeifferi have been extensively studied. It was reported by Olivier and Mao (1949) that the target organ for the infestive stages of Schistosoma mansoni in Biomphalaria glabrata was the digestive gland and that secondary involvement of the ovotestis only occurred in heavy infestations, or late in the development of an infestation. This finding was later confirmed by Pan (1963, 1965). Becker (1970) suggested that it might be the high concentrations of nutrients in the haemolymph surrounding the digestive gland which made it the focal colonisation site for the S. mansoni sporocysts. B. glabrata individuals harbouring S. mansoni sporocysts have been found to have considerably reduced haemolymph protein

and free amino-acid concentrations (Brand and Files, 1947, Gilbertson et al., 1967, Lee and Cheng, 1972, Gress and Cheng, 1973, Anteson and Williams, 1975, Becker and Hirtbach, 1975, Stanislawsky and Becker, 1979 and Stanislawsky et al., 1979). Similarly, glucose and carbohydrate concentrations are reduced (Brand and Files, 1947, Christie and Foster, 1970, Cheng and Lee, 1971 and Christie et al., 1974). Glucose is the most important monosaccharide present in the haemolymph of Biomphalaria glabrata (Becker, 1980), although others are present (an unpublished observation by Flinzer cited by Becker, 1980). The haemolymph glucose levels of infested B. glabrata were significantly lowered within 21 days after infestation (Cheng and Lee, 1971), and a similar effect was noted for haemolymph proteins (Gilbertson et al., 1967 and Lee and Cheng, 1972). The similarity between the haemolymph nutrient levels of infested B. glabrata and non-infested individuals which have been starved for 5 days was noted by Carter and Bogtish, 1975 and Becker, 1980). It was suggested by Becker (1980) that in their natural environment B. glabrata individuals may quite often experience similar short periods of starvation.

It was reported by Looker and Etges (1979) that 23 days after infestation with Schistosoma mansoni, B. glabrata individuals had significantly reduced rates of egg production when compared with non-infested controls. The 23 day time interval was found in the same study to coincide with the proliferation of S. mansoni daughter sporocysts through the digestive gland, and so nutrient depletion is

thought by these authors to be the factor involved in reducing the reproductive rate of the infested B. glabrata.

The digestive glands of Biomphalaria glabrata and B. pfeifferi were investigated by Pan (1965) and Meuleman (1972) respectively, for evidence of pathological damage as a result of S. mansoni infestation. Both authors concluded that any damage caused by the parasite to the organ was minimal. Thus, as Becker (1980) suggests, the depletion in nutrient levels within the host snail would seem to be the result of competion between the snail and parasite for the available substrates, rather than a reduction in the ability of the host snail to assimilate food.

Meuleman (1972) cited a number of authors who had found that the egg-laying potential of snails of Biomphalaria species was reduced by schistosome infestations still in their pre-patent period. Having obtained similar results to the previous authors, Meier and Meier-Brook (1981) suggested that the reduction in egg laying by B. glabrata harbouring pre-patent infestations of Schistosoma mansoni of only 2 weeks duration is caused by the mother sporocysts releasing some factor (possibly a hormone) which suppresses the host's reproduction.

The findings of the above authors contrast with those of Looker and Etges (1979) as discussed previously. An alternative mechanism to that of Meier and Meier-Brook (1981) for the reduction of the reproductive potential of infested B. glabrata can be drawn from the information contained in the review of Becker (1980), which could apply

to both studies, and is as follows.

The observations of Krupa and Bogtish (1972), Krupa et al. (1975) and Carter and Bogtish (1975) quoted by Becker (1980), that Schistosoma mansoni sporocysts have very high metabolic rates could well result in a significant haemolymph glucose level reduction in hosts harbouring pre-patent infestations. (Pan (1965) reported that S. mansoni infestations can increase their mass by 150 to 200 times in just 6 days in Biomphalaria glabrata hosts maintained at 28°C). This hypothesis is in agreement with the suggestion of Looker and Etges (1979) that, as there is no evidence of galactogen mobilisation from the albumen gland in infested B. glabrata, it is the haemolymph glucose concentration which controls the reproductive ability of the snails.

Increased urea cycle activity was observed by Schmale and Becker (1977) in infested and starved B. glabrata; this explained the increased concentrations of urea found in the haemolymph of infested and starved snails (Becker and Schmale, 1975; Schmale and Becker, 1975).

Becker (1980) suggested that as adult S. mansoni, S. haematobium and Fasciola hepatica have been reported by Senft, (1967) and Kurelec, (1975) respectively, to require arginine for reproduction, larval S. mansoni may also require arginine for the production of cercariae or daughter sporocysts. The S. mansoni sporocysts therefore may have been selected for their ability to simulate starvation in their snail host, thus increasing the catabolism of

proteins, resulting in arginine being produced as a by-product of the urea production required to dispose of the increased nitrogenous waste.

Lymnaea stagnalis individuals infested with Trichobilharzia ocellata were found, as stated above, to produce fewer eggs and grow faster than non-infested snails by McClelland and Bourns (1969). There was no evidence of mechanical damage to gonad or digestive gland in infested snails, but the reproductive tract failed to develop and the ovotestis regressed as a result of parasitisation. The above authors postulated that the increased growth rate may have been possible because of the production of cercariae by infested snails being less energetically demanding than the snails normal reproductive effort. In a later publication Bourns (1974) reported that the carbohydrate contribution of infested Lymnaea stagnalis into cercarial production was only aproximately 33% of the amount contributed into reproduction by non-infested snails. Similarly, the protein contribution towards cercarial production was approximately 60% of the contribution made for reproduction. This evidence supported the earlier theory of McClelland and Bourns (1969), that the production of cercariae and associated cessation, or limitation, of reproduction in infested L. stagnalis left them with a net energy gain in comparison with non-infested snails, which manifested itself in the enhanced growth rates of infested individuals.

If parasite-induced nutrient level reduction, as opposed to a secretion by the parasite, is the cause of the reduction in reproductive effort of infested L. stagnalis, it must be

as a result of the limited availability of a specific substrate, or range of substrates, used by the host to monitor its physiological state with regard to reproduction (Baudoin 1974).

As a result of extirpating the lateral lobes of the cerebral ganglia (a centre of endocrine activity in pulmonates) from Lymnaea stagnalis individuals, Geraerts (1976) reported effects similar to those characteristic of snails carrying larval trematode infestations - enhanced growth and suppression of female reproductive activity. The growth resulting from the removal of the lateral lobes was however shown to be abnormal by Scheerboom and Dogterom (1978), because it resulted, not from increased tissue masses, but a disproportionate increase in haemolymph volume.

A "tentative" scheme of factors controlling galactogen and glycogen synthesis was proposed by Veldhuijzen and Cuperus (1976) which involved the release of a neurohormone from the lateral lobes, which acts upon the Dorsal Bodies to produce Dorsal Body Hormone which then either stimulates the production of galactogen in the albumen gland directly, or does so via the gonad. (Galactogen production in the albumen gland is part of female reproductive activity). If haemolymph glucose concentrations are low, galactogen may be catabolised, and the albumen gland decrease in size. If Lymnaea stagnalis snails are fed on a carbohydrate-rich diet, which will raise their haemolymph glucose concentration, they have been shown by Veldhuijzen and van Beek (1976), to as much as double their female reproductive activity.

This relationship was taken further by Scheerboom (1978), who demonstrated that the relationships between growth of the soft body tissues and reproductive activity were linear with respect to the amount of lettuce assimilated by similar sized Lymnaea stagnalis individuals. It may be significant that the minimum ration for growth was 5mg lettuce assimilated per day, whereas the minimum ration for reproduction was 7mg of lettuce assimilated per day. It can be hypothesised that instead of producing a blocking agent for the neurohormone produced by the lateral lobes, or for Dorsal Body Hormone, Trichobilharzia ocellata sporocysts could lower the haemolymph glucose level to an intermediate level which allows growth, but not reproduction.

T. ocellata infestations in L.stagnalis were found by Sluiters et al. (1980) to be either 'high-productive' (HP), or 'low-productive' (LP), depending on the numbers of cercariae released. Snails with HP infestations produced more cercariae, reproduced less and grew at faster rates than snails with LP infestations, and non-infested controls. The number of miracidia the snail was initially exposed to had some effect on whether the final infestation was going to be HP or LP. Some L. stagnalis individuals exposed to only one miracidium had HP infestations, but animals exposed to the maximum dose of 4 miracidia were more often in the HP category. The authors suggest that this result is caused by the incomplete commandeering of the snails' reproductive energy in LP infestations. The mechanism employed by the parasite is suggested to be either a reduction in gonadotrophic hormones or a de-sensitising of their targets

as a result of the presence of the sporocysts (Sluiters 1981).

A possible mechanism for de-sensitisation of the gonadotrophic hormone receptor targets could be the reduction in haemolymph glucose levels as a result of the presence of Trichobilharzia ocellata sporocysts, in line with the scheme proposed by Veldhuijzen and Cuperus (1976). It may be significant that the gonad in infested Lymnaea stagnalis individuals does not show signs of retarded growth until 19 days after infestation, by which time daughter sporocysts are beginning to appear in the digestive gland and ovotestis region of the snail (Sluiters 1981). Although the above author believes that the parasites are secreting a hormone, if T. ocellata sporocysts resemble those of Schistosoma mansoni with respect to having a high metabolic rate (Krupa and Bogtish, 1972, Krupa et al., 1975, and Carter and Bogtish 1975) and growth rate (Pan 1965), a resultant haemolymph glucose depletion (Becker, 1980) could lead to a decline in female reproductive activity, as described by Lookers and Etges (1979) for starved L. stagnalis individuals.

With regard to HP and LP infestations of Sluiters et al. (1980); if the sporocysts can commandeer enough glucose in the early stages of the infestation to prevent the normal development of the gonad and accessory organs they will have less competition for nutrients from the snail than if they allow the reproductive system to develop during the prepatent period of the infestation.

As the above consideration of published information will have indicated the mechanisms involved in the suppression of reproduction in trematode-infested Basommatophoran pulmonates are as yet unknown. It is possible to suggest that either the action of hormones (Geraerts 1975, Sluiters 1981, Meier and Meier-Brook 1981), or haemolymph glucose concentrations (Looker and Etges, 1979) is the controlling factor. The presence of larval trematodes has been reported to deplete the haemolymph of glucose, as infestations of Biomphalaria glabrata with Schistosoma mansoni (Cheng and Lee 1971), and haemolymph glucose level has been shown to be related to reproductive effort in Lymnaea stagnalis (for example, Veldhuijzen and Van Beek 1976). The existence of a hormone or hormonal blocking agent of sporocyst origin has yet to be confirmed.

In contrast to the above examples of the castration of freshwater snails the mechanisms involved in castration of Littorina littorea (Rees, 1936), and Nassarius obsoletus (Cheng, et. al., 1973), are more easily observed, and quite clearly of a different nature, although Robson and Williams (1971) reported reduced glucose levels in Littorina littorea infested with Cryptocotyle lingua.

Parorchis acanthus rediae were reported by Cooley (1962) to directly ingest the gonad tissue of their host Thais lamellosa, using the pharynx in a similar manner to that described by Rees (1936) for C. lingua rediae, which are responsible for the castration of their host L. littorea. In the light of the work of McClelland and Bourns (1969) and Bourns (1974) the present writer decided to compare the

reproductive effort of non-infested male and female T. lapillus with the costs of parasite production to infested snails.

Parasite production can be separated into two categories - the production of cercariae and the growth and/or multiplication of rediae.

The production of cercariae by an infestation of Cryptocotyle lingua in an individual Littorina littorea was reported by Rothschild (1939) to have averaged 300 per day for three years. In a further communication about the same individual Meyerhof and Rothschild (1940), reported that the infestation had persisted for seven years, and had produced approximately 5,500,000 cercariae during the first five years of observation.

Rees (1948) found that 4 individual Thais lapillus infested with Parorchis acanthus produced a daily average of approximately 1400 cercariae at temperatues between 15°C and 18°C. The snails were maintained in a 12 hours light and 12 hours dark regime and the vast majority of cercariae were released in the light. As the above author had selected her 4 infested Thais lapillus subjects on the grounds of apparently similar levels of infestation, which suggests that only individuals with well established infestations were used, a more accurate estimate of mean field production of P. acanthus cercariae might be obtained from a random sample of infested individuals maintained in conditions similar to those outlined above.

Both Rothschild (1939) and Rees (1948) found that the production of cercariae by larval digeneans was significantly reduced at low temperatures and Rees (1948) reported that infested T. lapillus did not release P. acanthus cercariae at temperatures below 12°C. The fact that low temperatures induced Fasciola hepatica rediae to produce daughter rediae rather than cercariae was reported by Kendall (1964). In a review including the results of a number of investigations into cercarial production, Dinnick and Dinnick (1964) were cited by Whitfield and Evans (1983) as having found the same effect in F. gigantica infestations in Lymnaea natalensis maintained at water temperatures below 16 °C.

Rees (1980) described three stages in the life of Parorchis acanthus rediae. Stage I rediae are small, active rediae, considered by Rees to be young. Stage II rediae are larger, less active and may contain a few developing rediae or cercariae. Finally, Stage III rediae are fully grown rediae which have virtually lost their ability to move, and may contain as many as 50 cercariae. It was also noted by Rees that stages I and II rediae are present only in spring and early summer, whereas larger stage III rediae are present all the year round. It was decided by the present author to investigate the variation in the mean individual redial mass and mean infestation mass of P. acanthus infestations of Thais lapillus at Robin Hoods Bay in 1982 so that an estimate of the redial population production in an average infested T. lapillus could be estimated.

Estimates of Reproductive Energy Expenditure by Non-Infested Male and Female Thais Lapillus at Robin Hoods Bay (1981).

METHOD

Non-infested T. lapillus from Robin Hoods Bay or Scarborough were sampled monthly from February to June, with the majority of animals being initially in aggregation but by June dispersing onto the shore to feed. The snails taken were killed by immersion in boiling water for 30 seconds. Shell measurements were determined as described in Chapter 2, and the component dry weights determined as described in Chapter 3. After being removed from the shell, T. lapillus individuals could be sexed by the presence or absence of a penis to the right of the head (the former condition being male, the latter female). The vas deferens was also often apparent in males, close to the inner surface of the visceral whorls running between the digestive gland and viscera. The capsule gland of the females caused them to have a distinctive area of enlarged glandular tissue above the mantle, which could be used as a criterion for sexing.

The colour and condition of the gonad, and appearance of the vas deferens in the male and the capsule gland in the mantle of the female were the parameters used to separate animals into 'ripe' and 'spent' categories, as described by Feare (1970b). The more detailed histological techniques used by Feare could not be applied to the gonads in the present study as the latter were required for a gravimetric investigation.

Ripe males could be distinguished by their enlarged, white vas deferens. The gonad colour was not always brick-red as described by Feare, but the presence of distinctive white patches of sperm in the seminiferous tubules within the testis was used as an indicator of ripeness. The ripe ovary was, as described by Feare, enlarged and of an obvious granular nature. This granular appearance is enhanced in spent ovaries but they are clearly in a shrunken condition. The capsule gland was as useful an indicator of female reproductive condition as the gonad, being extremely swollen and white in ripe, pre-reproductive snails.

The reproductive effort for male and female non-infested Thais lapillus at Robin Hoods Bay and Scarborough was calculated for 1981 from the change in the Log dry body mass to Log shell length regressions for individuals changing from pre-reproductive to post-reproductive conditions. As T. lapillus remain in special breeding aggregations to reproduce (Moore 1938b) the total weight loss includes not only weight loss from egg and capsule production,by females, but also sperm transfer in males, and respiration whilst aggregating. Infested animals from April and June samples were measured and dissected as above to establish Log dry body mass to Log shell length regressions for infested animals at these two times, one prior to the reproduction by non-infested animals, and one preceeding it.

The calculated mass changes for hypothetical non-infested male and female and infested T. lapillus adults were converted into ash free dry masses and subsequently caloric values using the methods and results described above in

Chapter 3.

RESULTS

The Log shell length (mm) to Log dry body mass (mg) regressions for pre- reproductive and post-reproductive male dogwhelks collected in 1981 are shown in Fig. 4.1. The ANOVA tables for the regressions (Tables 4.1 and 4.2) show that both regressions have a significant F. ratio ($p \leq 0.001$). The results of ANCOVA on the comparison between the regression coefficients are contained in Table 4.3, with pre- and post-reproductive slopes not different ($p > 0.05$), adjusted means different ($p \leq 0.001$).

The Log shell length (mm) to Log dry body mass (mg) regressions for pre-reproductive (egg deposition) and post-reproductive female Thais lapillus collected in 1981 are shown in Fig. 4.2. The ANOVA tables for the regressions (Tables 4.4. and 4.5) show that both regressions have a significant F ratio ($p \leq 0.01$). The results of ANCOVA on the comparison between the regression coefficients are contained in table 4.6, with pre- and post-reproductive slopes not different ($p > 0.05$), adjusted means different ($p < 0.001$).

The Log shell length (mm) to Log dry body mass (mg) regressions for April and June (1981) samples of infested T.lapillus are shown in Fig. 4.3. The ANOVA tables for the regressions are Tables 4.7 and 4.8. The F ratio for the April sample is significant to a probability level ≤ 0.05, so although the body mass to shell size relationship is weaker for the April sample than for June, it is still significant. The F ratio for the June sample (Table 4.8) is

significant to $p \leq 0.01$.

The results of a t-test on the comparison of the two regression coefficients are contained in Table 4.9, with the April and June slopes not different ($p > 0.05$), adjusted means different ($p \leq 0.001$).

Fig. 4.4. shows the relative percentages of ripe and spent male and female Thais lapillus in monthly samples collected from March to June (1981). It can be seen that by May all males collected had spent gonads, as a result of sperm transfer, and by June all females had spent gonads, having deposited their egg cases.

Estimate of Annual Energy Expenditure on Parorchis acanthus Cercarial Production by a Hypothetical Infested Thais lapillus Host.

METHOD

Daily temperatures for both air and water, obtained from a thermocouple embedded in the rock surface in the middle shore zone at Robin Hoods Bay, were used for the year November 1981 to October 1982 inclusive. The highest daily air or water temperatures always occurred in the daytime, so whenever these highest temperatures were above 12 ^{o}C conditions were taken as suitable for cercarial release of Parorchis acanthus from T. lapillus (Rees 1948).

Productivity of P. acanthus cercariae from individual T. lapillus taken from Robin Hoods Bay in June and July, was investigated in the laboratory at 15°C. This temperature was chosen so as to not inhibit, or unduly encourage the release

of cercariae (Rees 1948), and because it represented a reasonable average shore temperature. During the investigation a 12 hours light, 12 hours dark lighting regime was maintained. Cercarial production was monitored over 48 hours. An average daily cercarial production was calculated by pooling the data, and dividing by 2. A short investigation was designed to try and negate the likelihood of obtaining unnatural results caused by laboratory confinement. Infested Thais lapillus were identified as such by the presence of Parorchis acanthus metacercarial cysts on their opercula. The number of cysts was recorded, so that any subsequent increase in the number could be added to the cercarial production count.

The snails were kept in small polythene bags containing 200 ml of seawater. The top of each bag was left open so that air could enter and the bags packed into an empty aquarium tank. The snails were transferred to new bags with clean water after 24 hours. The old seawater was poured from each bag into a crystallising dish and the number of cercariae present counted under a stereomicroscope at X10 magnification. Each polythene bag was slit open, and the number of metacercarial cysts adhering to it added to the cercarial count. The number of cysts on the operculum and shell of the snail was re-checked, and any increase included in the total cercarial production for that period. Counted samples of cysts were then dried under vacuum for 24 hours at 60 $^{\circ}$C, and weighed to the nearest 0.00001g on a Metler HL52 balance. The mass per cercaria was determined simply by dividing the total dry mass by the number of cysts counted.

The caloric value and ash content of the cercariae was taken to be the same as that for Parorchis acanthus rediae in which they originate. The methods used to determine ash content and caloric value are those described in Chapter 3.

RESULTS

Cercarial production by P. acanthus rediae infesting T.lapillus hosts maintained under laboratory conditions at 15°C and 12 hours dark/light is shown in Table 4.10. The average production value shown at the bottom of the table is used in subsequent estimates of the cost of cercarial production.

The correlations between cercarial productivity and infestation mass (mg), mass of 30 entire rediae (mg) and mass of snail host (mg) respectively are shown in Table 4.11. All of these factors can be seen to be unrelated to cercarial productivity, at least over such a short period as 48 hours. (The period of the investigation was intentionally short because of the deterioration in the health of Thais lapillus maintained in the described laboratory conditions for a long period of time).

A summary of information from a rock-embedded shore temperature probe at Robin Hood's Bay is given in Table 4.12. The number of days when the water temperature exceeds 12 °C can be taken as the number of days on which cercarial release occurred (Rees, 1948).

Since the metacercaria represents the cercaria minus tail and surrounded by a cyst wall produced by glands within the

cercarial body, the mass of the metacercaria should closely resemble that of the original cercaria. The loss of the cercarial tail and the use of energy stores during activity by the cercaria will tend to err the cercarial mass estimate towards the low side, which will make the subsequent estimates of the cost of cercarial production a little conservative, and so may be safely overlooked. The results of 5 estimates of metacercarial mass are shown in Table 4.13.

The June and October redial ash percentages of dry mass and redial caloric values ($J.mg^{-1}$ dry mass) summarised in Table 4.14. The mean values from this table are used in the following estimates of cercarial mass and caloric value, as cercaria are produced throughout the period June to October inclusive.

Estimate of Annual *Parorchis acanthus* Redial Population Growth Increment in a Hypothetical Infested *Thais lapillus* Host.

METHOD

Infested *T. lapillus* hosts were collected from Robin Hoods Bay in March, July, August and October (1982) and measured, boiled and cracked open as described above (Chapter 2). The removal of *P. acanthus* rediae and subsequent determinations of dry weight, ash content and caloric value were performed as described above (Chapter 3).

RESULTS

The summarised results for total redial infestation dry masses (g) for March, July, August and October, 1982, are shown in Table 4.15. The ANOVA table for a one way analysis of variance on the data (Table 4.16) shows that there is a significant variation between the average infestation masses for different months ($p \leq 0.01$). The result of the GT2 test for significant difference between means (summarised in Table 4.17) is that the October and August infestations are significantly heavier than March and July infestations.

The sizes of the infested individuals used for the above comparison of infestation masses were compared as follows. The shell lengths (mm) of the infested Thais lapillus subjects collected in March, July, August and October 1982 are summarised in Table 4.18. The result of a one way analysis of variance of the data is shown in Table 4.19, where the F value obtained indicates that there is a significant difference between the class means ($p < 0.01$). The October sample mean shell length is shown to be slightly smaller than the July, March and August sample means by the GT2 test for significant differences between means contained in Table 4.20. Thus the increase in infestation masses in August and October samples (Table 4.18) is not associated with increasing host size.

The positive relationship between the dry mass of host tissue and the dry mass of parasite tissue within infested dogwhelk individuals collected in July (1981) and August (1981 and 1982) is shown in Fig.4.5. The results for these

two months only were used so as to minimise effects of seasonal changes in host body mass. The sample correlation coefficient (r) for the relationship is significant, being significant to $p<0.001$.

The summarised data for the seasonal differences in the dry weights (mg) of samples of 30 entire P. acanthus rediae taken from an infesting population are shown in Table 4.21, with the ANOVA table (Table 4.22) showing that the calculated F value is significant, with a value greater than P=0.01. The result of the GT2 test for differences between paired means in Table 4.23 is that the mean masses of samples of 30 entire rediae for October and August samples are similar, and significantly greater than the July and March redial masses, which are not significantly different to each other.

The summarised results of seasonal estimates of the numbers of Parorchis acanthus rediae in infested Thais lapillus subjects in Table 4.24, and the result of a one way analysis of variance in Table 4.25, is that there is no significant difference between samples.

The significantly positive relationship between the mass of a sample of 30 entire rediae (mg), and the total mass the infestation (mg), shown in Fig.4.6, has a sample correlation coefficient (r) significant to $p< 0.001$ for 107 degrees of freedom.

The significantly positive relationship between the numbers of rediae within an infesting redial population and the total mass of the infestation is shown in Fig 4.7, and has a

sample correlation coefficient (r) that is significant to ≤0.001 for 107 degrees of freedom.

The following estimates of reproductive effort by non-infested males and females, or the weight increase of infested dogwhelks over the same period, and the weight increase of the redial population through the summer, were all calculated for hypothetical male, female or infested individuals of the approximate average shell length for infested dogwhelks found in this study - 32mm.

ESTIMATE OF ENERGY COST OF <u>PARORCHIS</u> <u>ACANTHUS</u> CERCARIAL PRODUCTION

Average numbers of cercarial produced at 15°C = 243+-69
Number of days when seawater temperature > 12°C = 88
Caloric value of each cercaria = 0.093 J
(Using June + October/2 redial CV.)
TOTAL ENERGY EXPENDITURE = 1988.71 J
+-564.70
(95% conf.)

ESTIMATES OF ENERGY INVESTMENT IN REDIAL STANDING CROP

Average infestation mass (mg)				(March)		30.57
					32.98	
"	"	"	"	(July)	(+- 5.82)	36.25
					(95% conf)	
"	"	"	"	(August)		49.45
					50.48	
"	"	"	"	(October)	(+- 2.97)	51.78

<u>P. acanthus</u> redial tissue caloric values (J.mg)

March and July 25.33 (Table 3.99)
August and October 29.78 (Table 3.99)

Average infestation (March) Caloric value (J) (July) 835.38 (+- 147.42)

Average infestation (August) Caloric value (J) (October) 1503.29 (+- 88.45)

ANNUAL INCREASE IN ENERGY CONTENT OF REDIAL POPULATION (J) = 667.91 (+-235.87)

TOTAL CERCARIAL + REDIAL PRODUCTION (J) = 2656.62 (+-800.57)

NET ENERGY EXPENDITURE BY THAIS LAPILLUS CHANGING FROM PRE-REPRODUCTIVE TO POST REPRODUCTIVE CONDITIONS COMPARED WITH NET ENERGY GAINS FOR FEEDING INFESTED SNAILS.

MALES

Soft body dry mass before reproduction	95% conf.	267.68 254.68mg 242.32
Soft body dry mass after reproduction		192.81 180.87mg 169.68
Soft body dry mass lost during reproduction		98.00 73.81mg 49.51
April visceral Caloric value (Table 3.90)		22.80 J.mg-1
Total energy lost during reproduction		2234.40 -1682.87 J. 1128.83

FEMALES

Soft body dry mass before reproduction	95% conf.	354.03 340.17mg 326.84
Soft body dry mass after reproduction		258.57 237.23mg 217.65
Soft body dry mass lost during reproduction		136.38 102.94mg 68.27
April visceral CV (Table 3.96)		25.59 J.mg-1
Total energy lost during reproduction		3489.96 -2634.23J. 1747.03

INFESTED

April soft body dry mass	95% conf.	355.39 310.60mg 271.45
June soft body dry mass		406.71 375.77mg 347.20
Soft body dry mass gained		135.26 65.17mg 8.19
June visceral CV (Table 3.86)		25.33 J mg-1
Total energy gain		3426.14 +1650.76 J 207.45

Fig. 4.1

Log total soft body dry mass versus Log shell length regressions for pre-reproductive and post-reproductive non-infested male Thais lapillus for 1981.

(Regression equations and F values are shown in Tables 4.1 and 4.2).

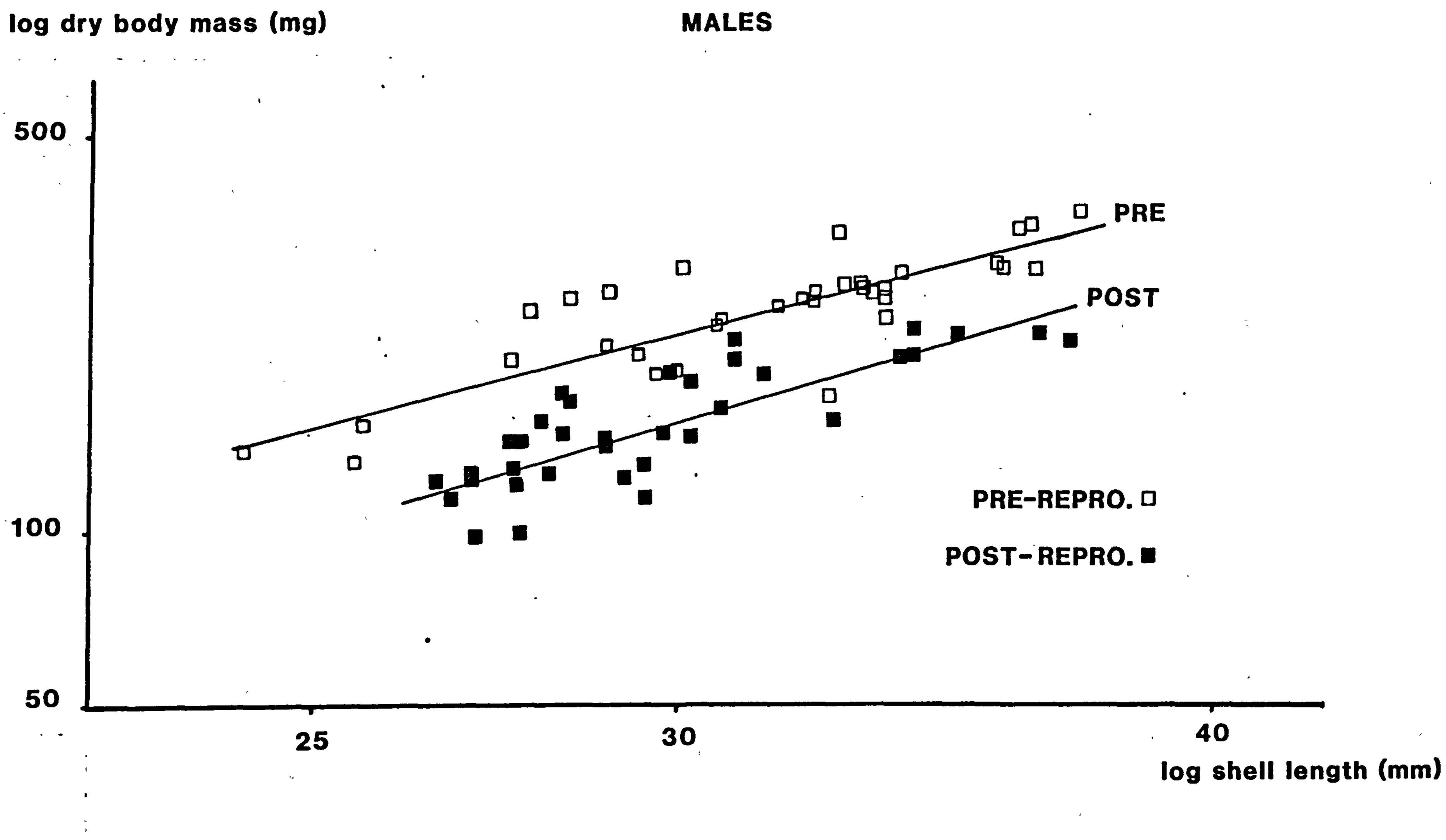
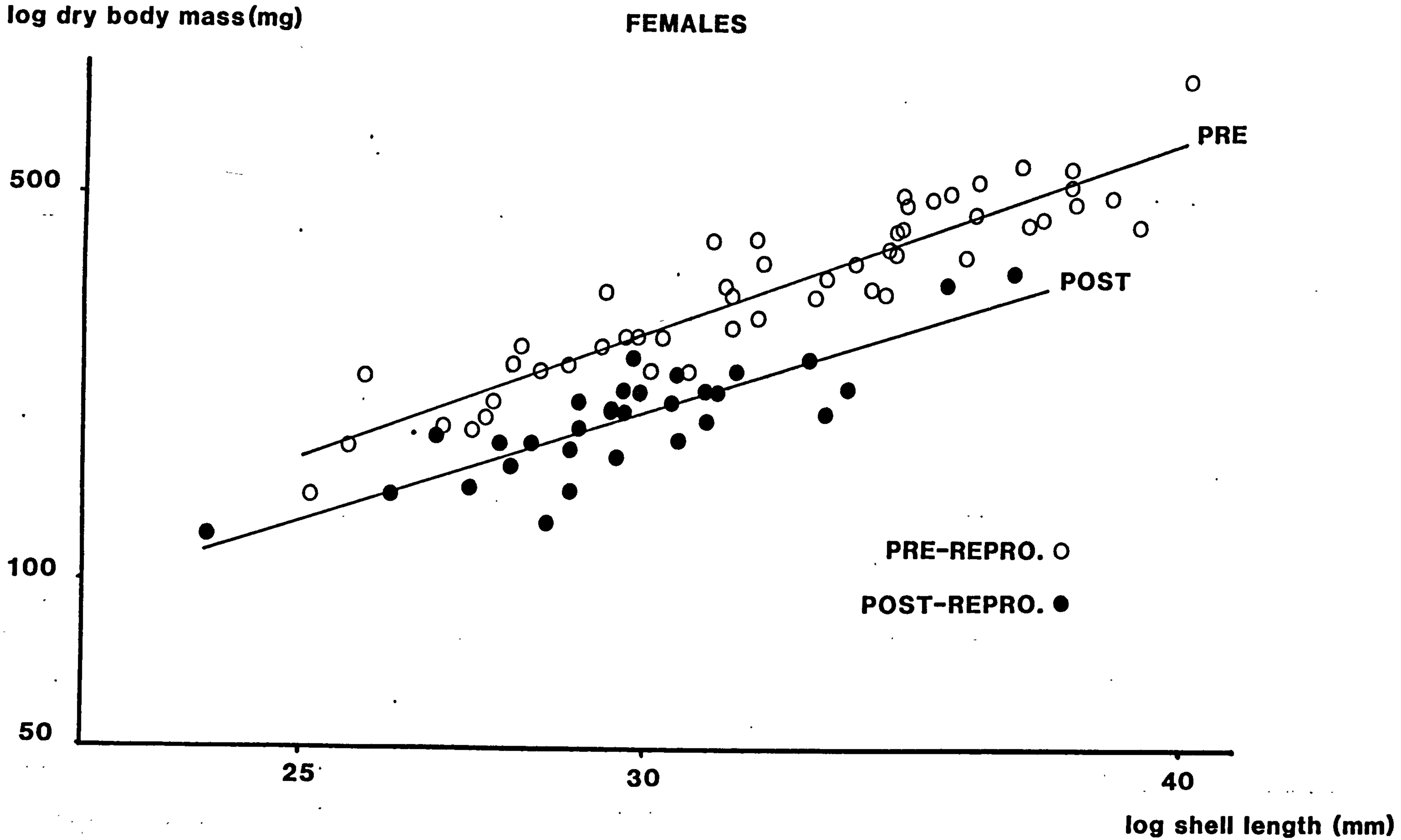

MALES
log dry body mass (mg)
500
100
50
25
30
40
log shell length (mm)
PRE
POST
PRE-REPRO.
POST-REPRO.

Fig. 4.2

Log total soft body dry mass versus Log shell length regressions for pre-reproductive and post-reproductive non-infested female Thais lapillus for 1981.

(Regression equations and F values are shown in Tables 4.4 and 4.5).


log dry body mass (mg)
FEMALES
500
100
50
PRE
POST
PRE-REPRO. ○
POST-REPRO. ●
25
30
40
log shell length (mm)

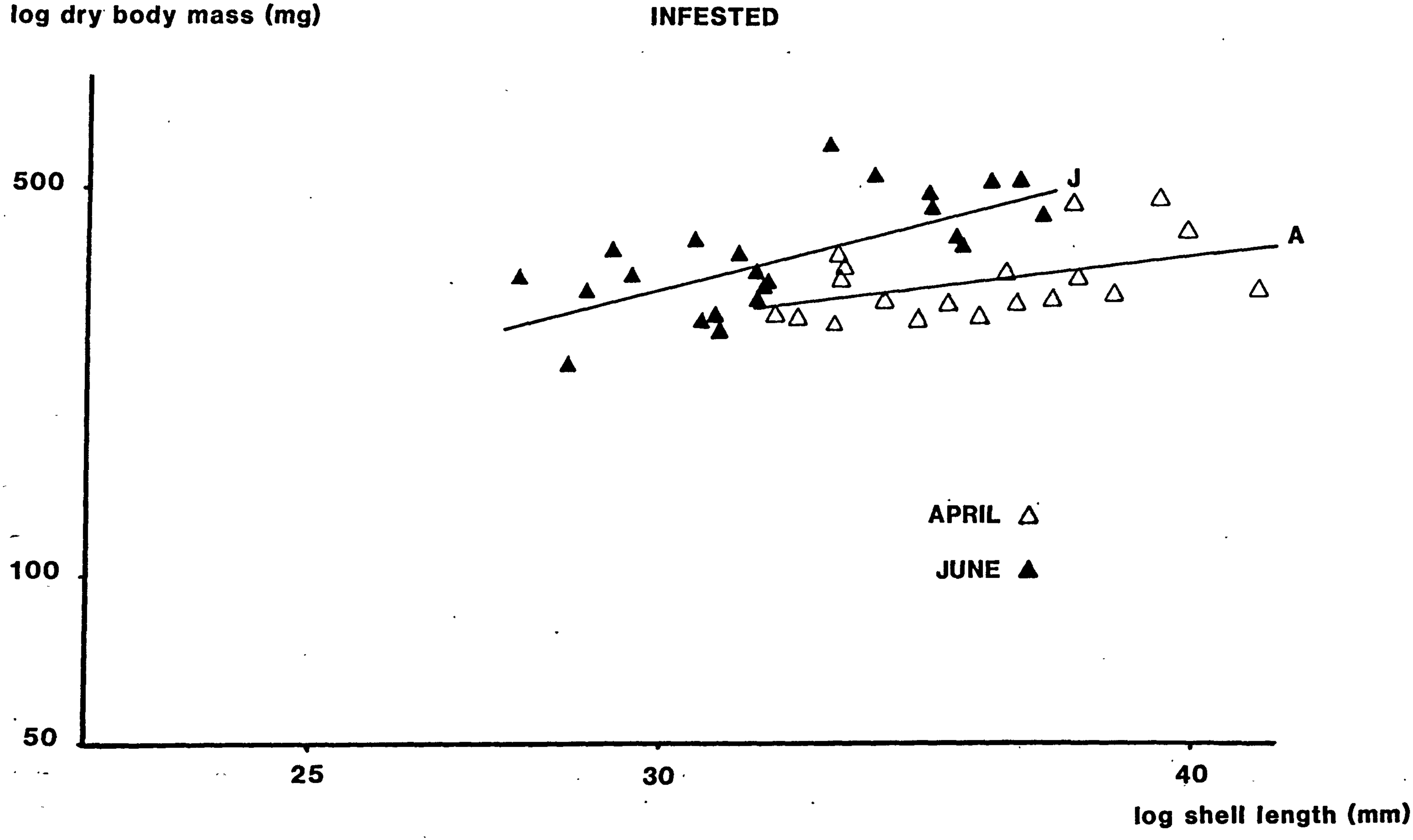
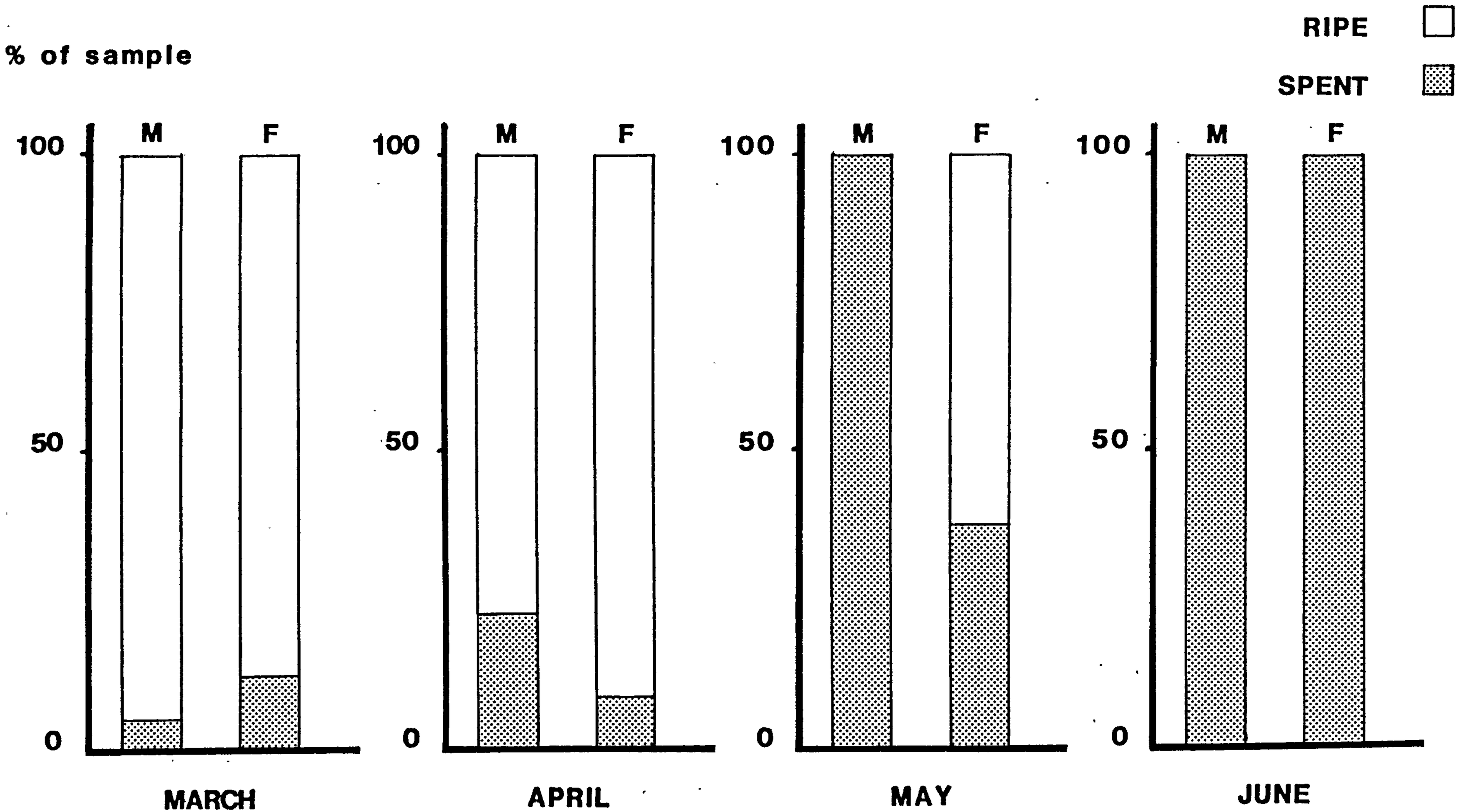

log dry body mass (mg)
INFESTED
500
100
50
J
A
APRIL △
JUNE ▲
25
30
40
log shell length (mm)

Fig. 4.4

Proportions of ripe and spent Male and Female Thais lapillus collected during March to June inclusive, 1981.


% of sample
RIPE
SPENT
100
50
0
M
F
MARCH
APRIL
MAY
JUNE

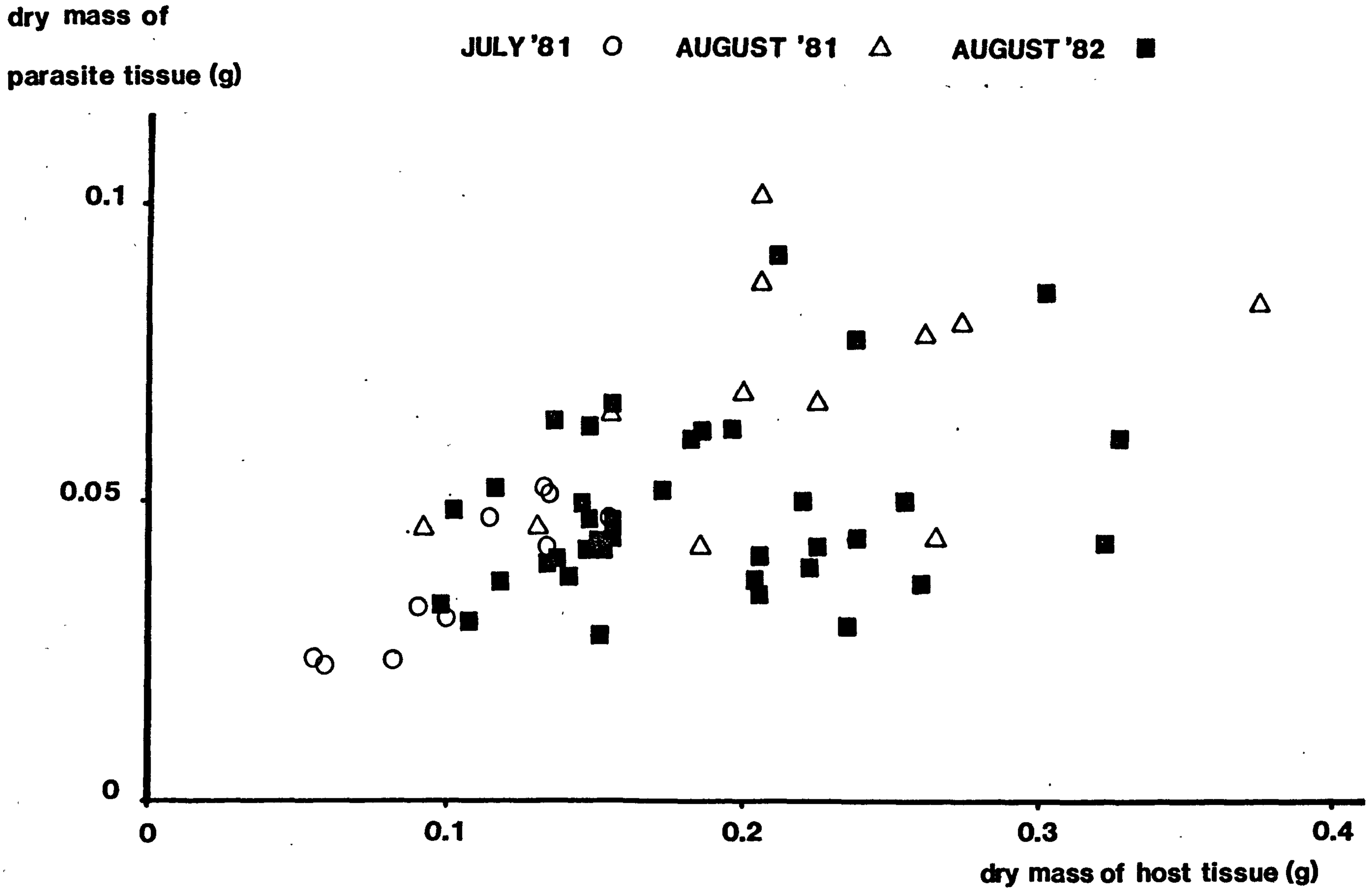

dry mass of
parasite tissue (g)
JULY '81
AUGUST '81
AUGUST '82
0.1
0.05
0
0
0.1
0.2
0.3
0.4
dry mass of host tissue (g)

Fig. 4.6

Correlation between the dry mass of 30 rediae and the total dry mass of *Parorchis acanthus* infestations in individual *Thais lapillus* collected in March, July, August and October, 1981.

($r = 0.584$ $n = 109$ $p \leq 0.001$).

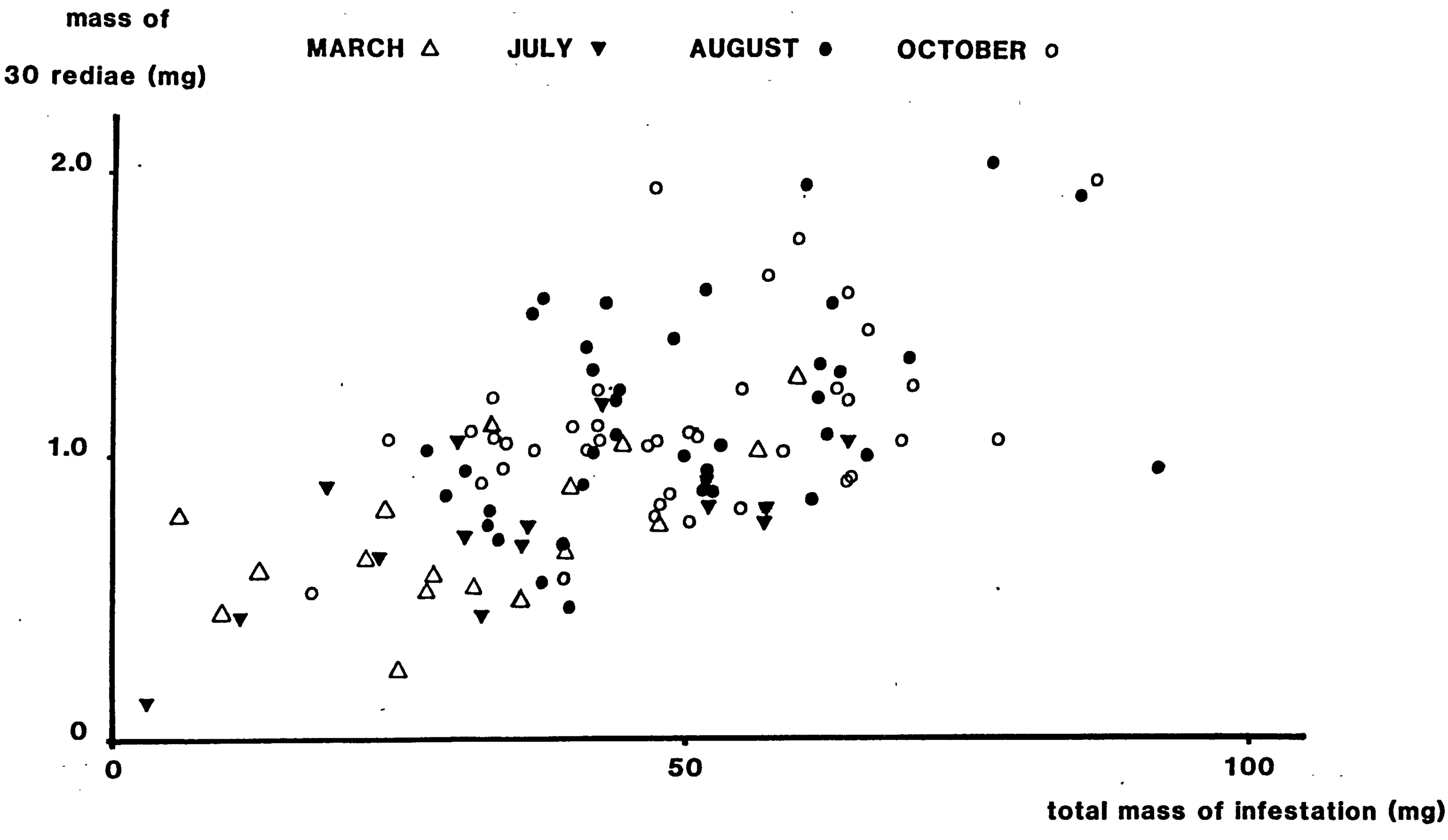
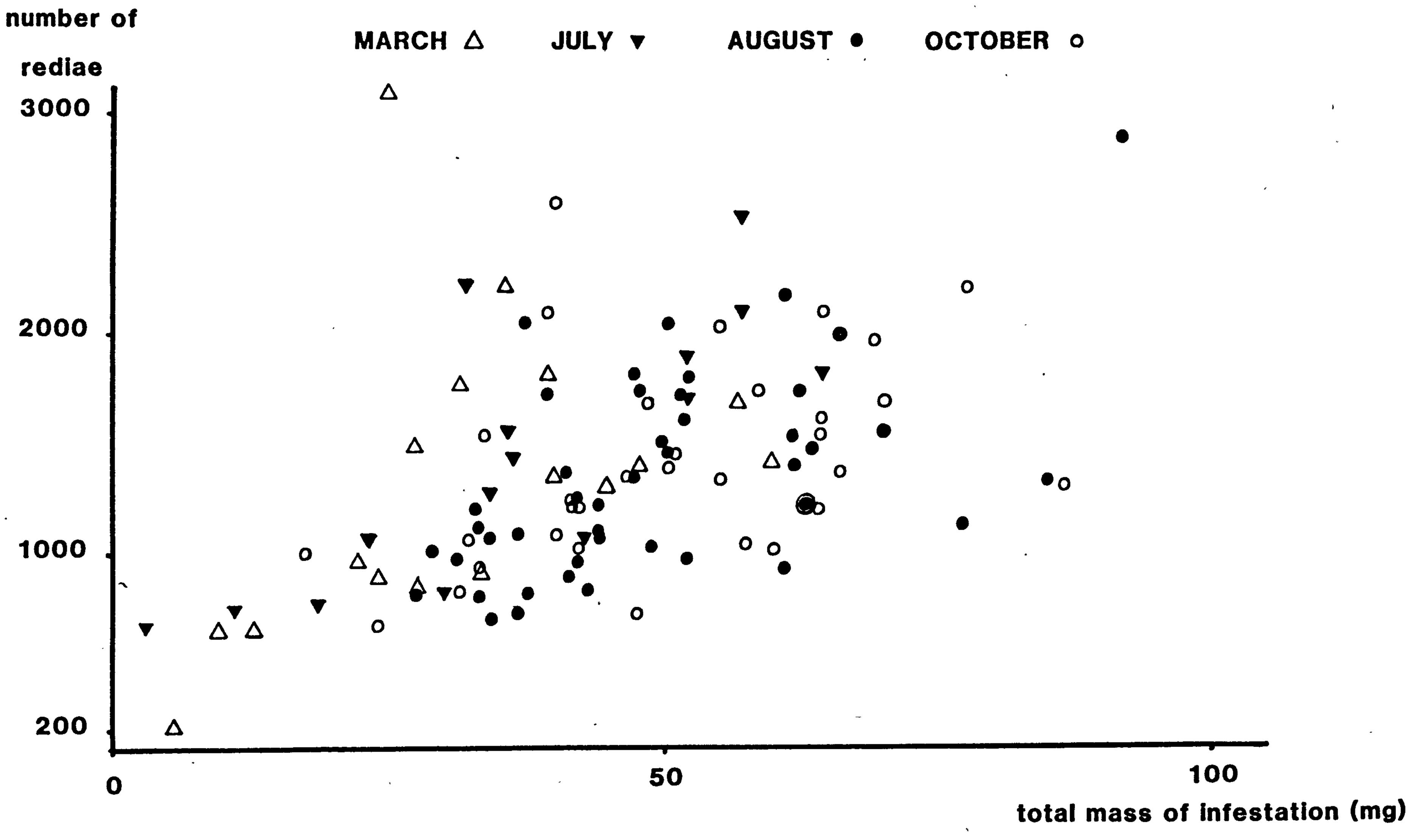

mass of
30 rediae (mg)
MARCH △
JULY ▼
AUGUST ●
OCTOBER o
2.0
1.0
0
0
50
100
total mass of infestation (mg)

Fig. 4.7

Correlation between the total numbers and total masses of rediae in infestation of Thais lapillus with Parorchis acanthus during March, July, August and October, 1981.

($r = 0.48$ $n = 109$ $p \leq 0.001$).


number of
rediae
3000
2000
1000
200
MARCH △
JULY ▼
AUGUST ●
OCTOBER ○
0
50
100
total mass of infestation (mg)

MALE THAIS LAPILLUS LOG SHELL LENGTH (mm) TO LOG DRY BODY MASS (mg) REGRESSIONS FOR PRE AND POST-REPRODUCTIVE CONDITIONS.

TABLE 4.1. PRE-REPRODUCTIVE

Source of variation	SS	DF	MS	F
REGRESSION	0.3070	1	0.3070	79.1849
ERROR	0.1318	34	0.0039	
TOTAL	0.4388	35		

TABLE 4.2. POST-REPRODUCTIVE

Source of variation	SS	DF	MS	F
REGRESSION	0.2784	1	0.2784	68.7167
ERROR	0.1297	32	0.0041	
TOTAL	0.4081	33		

TABLE 4.3. SUMMARY TABLE, ANCOVA ON ABOVE REGRESSIONS

Source of variation	DF	SS	MS	F
Between groups	1	0.680	0.680	0.803
Within groups	68	0.847	0.0125	
Common slope within groups	1	0.582	0.582	147.33
Differences between slopes	1	0.003	0.003	0.824
Differences between adjusted means	1	0.402	0.402	101.63

FEMALE THAIS LAPILLUS LOG SHELL LENGTH (mm) TO LOG DRY BODY MASS (mg) REGRESSIONS FOR PRE AND POST-REPRODUCTIVE CONDITIONS.

TABLE 4.4. PRE-REPRODUCTIVE

Source of variation	SS	DF	MS	F
REGRESSION	1.0920	1	1.0920	309.3220
ERROR	0.1659	47	0.0035	
TOTAL	1.2579	48		

TABLE 4.5. POST-REPRODUCTIVE

Source of variation	SS	DF	MS	F
REGRESSION	0.3037	1	0.3037	52.5405
ERROR	0.1445	25	0.0058	
TOTAL	0.4482	26		

TABLE 4.6. SUMMARY TABLE, ANCOVA ON ABOVE REGRESSIONS

SOURCE OF VARIATION	DF	SS	MS	F
Between groups	1	1.006	1.006	0.590
Within groups	74	1.706	0.023	
Common slope within groups	1	1.396	1.396	327.89
Differences between slopes	1	0.0002	0.0002	0.057
Differences between adjusted means	1	0.403	0.403	94.751

INFESTED THAIS LAPILLUS LOG SHELL LENGTH (mm) TO LOG DRY BODY MASS (mg) REGRESSIONS FOR APRIL AND JUNE.

TABLE 4.7.

APRIL

Source of variation	SS	DF	MS	F
REGRESSION	0.0173	1	0.0173	4.5360
ERROR	0.0611	16	0.0038	
TOTAL	0.0784	17		

TABLE 4.8.

JUNE

Source of variation	SS	DF	MS	F
REGRESSION	0.1079	1	0.1079	16.4962
ERROR	0.1438	22	0.0063	
TOTAL	0.2517	23		

TABLE 4.9. SUMMARY TABLE, ANCOVA ON ABOVE REGRESSIONS

Source of variation	DF	SS	MS	F
Between groups	1	0.017	0.017	0.051
Within groups	40	0.330	0.008	
Common slope within groups	1	0.114	0.114	20.64
Differences between slopes	1	0.094	0.094	2.029
Differences among adjusted means	1	8.954	8.954	16.179

TABLE 4.10. PRODUCTION OF PARORCHIS ACANTHUS CERCARIAE BY INFESTED THAIS LAPILLUS COLLECTED IN AUGUST 1982.

Snail no.	Cercariae produced in 48 hrs.	Daily average	Mass of infestations (mg)	Mass of 30 rediae (mg)	Mass of snail host (mg)
1	16	8	91.84	0.95	303.58
2	1001	500.5	52.43	0.87	167.65
3	620	310	36.75	-	155.35
4	835	417.5	-	-	-
5	58	29	39.28	0.68	261.00
6	1158	579	49.85	0.99	194.65
7	8	4	40.78	0.89	176.22
8	165	82.5	50.20	0.76	260.33
9	109	54.5	33.04	1.20	130.22
10	213	106.5	48.92	1.41	150.11
11	119	59.5	60.75	1.95	387.03
12	189	94.5	36.70	-	240.29
13	133	66.5	77.23	2.03	314.74
14	227	113.5	28.77	0.85	263.60
15	8	4	39.94	0.46	173.32
16	491	245.5	30.27	0.75	137.94
17	12	6	85.04	1.91	386.20
18	391	195.5	41.82	1.00	188.87
19	174	87	37.31	1.55	178.45
20	296	148	47.28	1.05	203.15
21	715	357.5	42.94	1.54	365.81
22	1050	525	43.81	1.19	281.87
23	202	101	43.77	1.07	194.75
24	802	401	34.17	1.44	239.54
25	147	73.5	41.19	1.36	245.94
26	1524	762	50.30	1.03	304.00
27	764	382	47.67	0.82	195.06
28	887	443.5	63.55	1.29	199.03
29	530	265	61.70	1.32	248.23
30	656	328	69.92	1.34	311.06
31	656	328	47.28	0.78	271.52
32	12	6	27.30	1.01	178.32
33	325	162.5	41.83	1.30	194.02
34	311	155.5	61.00	0.84	242.95
35	292	146	36.54	1.50	296.32
36	754	377	63.04	1.53	210.41
37	339	169.5	62.41	1.07	158.32
38	373	186.5	65.89	0.99	220.85
39	1434	717	44.12	1.23	200.69
40	1479	739.5	51.87	1.58	224.57
$\bar{x}$ (+-95% conf.)			243.44 (+-69.22)		

TABLE 4.11.CORRELATIONS BETWEEN CERCARIAL PRODUCTION AND INFESTATION DRY MASS, DRY MASS OF 30 PARORCHIS ACANTHUS REDIAE, AND DRY MASS OF THAIS LAPILLUS HOST TISSUES.

Correlation	Correlation coefficient r	DF	Significance
Daily cercarial production infestation mass (mg)	0.0143	37	Not sig.
Daily cercarial production mass of 30 rediae (mg)	0.0424	34	Not sig.
Daily cercarial production mass of host snail (mg)	0.0256	37	Not sig.

TABLE 4.12.SEA AND ROCK TEMPERATURE DATA FROM A ROCK EMBEDDED TEMPERATURE PROBE AT ROBIN HOODS BAY.

	Month	Number of days temp. > 12 C TIDE IN	TIDE OUT
'81	November*	0	0
	December	0	0
	January	0	0
	February	0	0
	March	0	4
	April	0	26
'82	May	0	26
	June *	1	20+4
	July	23	23
	August	31	31
	September	30	30
	October	3	17
	TOTAL	88	181

* Results corrected proportionally due to equipment failure

TABLE 4.13.ESTIMATES OF THE MASS OF A METACERCARIAL CYST OR CERCARIA OF PARORCHIS ACANTHUS.

No. of cysts	Dry mass of cysts (mg)	Individual dry mass (mg)	Mean mass (+-95% conf)(mg)
698	2.77	3.97	
795	2.82	3.76	
701	2.44	3.48	3.76 (+-0.53)
543	1.76	3.24	
962	4.18	4.35	

TABLE 4.14.AVERAGE ESTIMATES OF REDIAL ASH PERCENTAGES OF DRY MASS (DM) AND CALORIC VALUES (CV) ($J.mg^{-1}$).

	Ash % DM	CV (J)
JUNE	15.69	25.33
OCTOBER	4.62	29.78
MEAN	10.16	27.56

(See Chapter 3)

(Metacercarial) cercarial ash free dry mass = 3.38 g
(Redial) cercarial caloric value = 0.093 J

TABLE 4.15. INFESTATION DRY MASS (g) OF PARORCHIS ACANTHUS REDIAE IN INFESTED THAIS LAPILLUS COLLECTED IN 1982.

	March	July	August	October
n	19	14	39	31
Σx	0.58074	0.50746	1.9825	1.60509
$\Sigma(x^2)$	0.02188	0.02293	0.10389	0.09065
$\bar{x}$	0.03057	0.03625	0.04945	0.05178

TABLE 4.16. RESULTS OF ONE WAY ANOVA.

Source of variation	SS	DF	MS	F
TREATMENTS	0.00777	3	0.00259	12.95
ERRORS	0.01932	99	0.00020	
TOTAL	0.02709	102		

TABLE 4.17. GT2 TEST FOR DIFFERENCES BETWEEN MEANS.

October	August	July	March	MSR
0.05178	0.04945	0.03625	0.03057	
			0.02121	0.01119
		0.01553		0.01237
	0.00233			0.00924
			0.01888	0.01075
		0.01320		0.01197
			0.00568	0.01353

TABLE 4.18. COMPARISON OF MEAN SHELL LENGTHS FOR SAMPLES OF INFESTED THAIS LAPILLUS.

	March	July	August	October
n	19	14	39	31
Σx	605.90	456.70	1242.00	923.40
$\Sigma(x^2)$	19582.33	14972.31	39846.7	27682.22
$\bar{x}$	31.89	32.62	31.85	29.79

TABLE 4.19. RESULTS OF ONE WAY ANOVA.

Source of variation	SS	DF	MS	F
TREATMENTS	113.47	3	37.82	4.65
ERRORS	805.20	99	8.13	
TOTAL	918.67	102		

TABLE 4.20. GT2 TEST FOR DIFFERENCES BETWEEN MEANS.

July	March	August	October	MSR
32.62	31.89	31.85	29.79	
			2.83	2.494
		0.77		2.413
	0.73			2.728
			2.10	2.256
		0.04		2.167
			2.06	1.863

TABLE 4.21. SEASONAL DIFFERENCES IN DRY MASS FOR SAMPLES OF 30 REDIAE (g).

	March	July	August	October
n	19	15	46	31
Σx	0.01413	0.01123	0.05019	0.03559
$\Sigma(x^2)$	0.000012	0.000009	0.000060	0.000044
$\bar{x}$	0.00074	0.00075	0.00109	0.00115

TABLE 4.22. RESULTS OF ONE WAY ANOVA.

Source of variation	SS	DF	MS	F
TREATMENTS	3x10	3	1x10	10
ERRORS	11x10	107	1x10	
TOTAL	14x10	110		

TABLE 4.23. GT2 TEST FOR DIFFERENCES BETWEEN MEANS.

October	August	July	March	MSR
0.00115	0.00109	0.00075	0.00074	
			0.00041	0.000250
		0.00040		0.000270
	0.00006			0.000200
			0.00035	0.000234
		0.00034		0.000255
			0.00001	0.000297

TABLE 4.24. SEASONAL COMPARISON OF PARORCHIS ACANTHUS REDIAE NUMBERS.

	March	July	August	October
n	18	15	46	31
Σx	24138	19559	63662	43455
$\Sigma(x^2)$	40103812	30315137	98843206	66570463
$\bar{x}$	1341	1304	1384	1402

TABLE 4.25. RESULTS OF ONE WAY ANOVA.

Source of variation	SS	DF	MS	F
TREATMENTS	120749.6	3	40249.87	0.15
ERRORS	28940390.2	106	273022.55	
TOTAL	29061139.8	109		

DISCUSSION

The result of the reproductive effort estimate for Thais lapillus at Robin Hoods Bay in 1981 is about half that calculated by Hughes (1972) for a population of dogwhelks in Nova Scotia. His technique was to put dogwhelks of similar size in cages on the shore and divide the number and mass of egg capsules produced in a year by the number of females present. The male reproductive effort was assumed to be half of that for similar sized female T. lapillus, which seems to be a reasonable estimate when compared with the results obtained in the present investigation.

The method employed by myself for the estimate of reproductive effort is more comprehensive than that of Hughes because it includes factors such as weight loss through continued presence in aggregation and abstinance from feeding. (It should be noted that infested dogwhelks actually increased their body mass relative to their shell length from April to June, Fig.4.3). The technique used in this study would, for this reason, yield a larger estimate of reproductive effort than the method employed by Hughes. One possible source of error could have been the incorrect classification of T. lapillus individuals into 'Ripe' and 'Spent' classes. However, the distribution of spent animals (shown in Fig. 4.4) is very similar to that of Feare (1970b), which supports the accuracy of classification in this study. The smaller reproductive effort of T. lapillus individuals at Robin Hoods Bay in 1981 when compared with Nova Scotian dogwhelks (Hughes 1972) is probably the result

of annual and geographical variation rather than differences in technique.

The mean cercarial production value, shown in Table 4.10, for infested Thais lapillus collected in August 1982 is considerably lower than the mean value of approximately 1400 cercariae per day obtained by Rees (1948) for infested T. lapillus in similar laboratory conditions. This difference in result is probably attributable to the fact that Rees (1948) selected individual T. lapillus that appeared to be "uniformly parasitised". The criteria upon which this judgement was based were not elucidated by the author. The infested dogwhelks in the present investigation were selected for merely by the presence of Parorchis acanthus metacercarial cysts on their opercula (Feare 1969). The variance around the mean value for daily cercarcarial production is much greater in the present study than in that of Rees (1948). This may be as a result of the different selection of infested snail subjects, but as the effects of factors such as the nutritional state of the host snails (Kendall, 1949), or the ability of the parasite to utilize the snails nutrient resources (Sluiters et al., 1980) can effect the numbers of cercariae produced, the lower mean value for daily cercarial production obtained in this study may be more accurate than Rees' (1948) estimate. The daily production of cercariae by an individual infested T. lapillus was found to be unrelated to the mass of infestation, the mass of 30 sample rediae, or to the mass of the snail (Table 4.11).

The crude estimate of the total energy lost per year by an infested Thais lapillus individual as a result of producing an average number of cercariae on every day when the seawater temperature rises above 12°C, is on its own greater than the estimate of energy spent on reproduction by an average sized (32mm) male T. lapillus in 1981. The difference between cercarial output and male reproductive effort for 1981 could be more than compensated for by the energy input, resulting in a weight increase in infested dogwhelks, as a result of the early seasonal feeding activity of these animals whilst non-infested males remained in aggregation (Feare 1969). In 1982, when there was a cold spring, the infested dogwhelks collected in July had not increased their shell length related body weights above those of infested snails collected in March, so the compensatory weight gain observed for infested T. lapillus in 1981 may be an unreliable reward of infestation.

The roughly estimated average cercarial production requires considerably less energy than the estimated reproductive effort of an average female T. lapillus at Robin Hoods Bay in 1981. This is in agreement with the suggestion of McClelland and Bourns (1969) confirmed by Bourns (1974) for Lymnaea stagnalis infested with Trichobilharzia ocellata that there is an energy saving associated with the switch by an infested snail from its own reproduction to parasite production.

The weights of Parorchis acanthus rediae removed from infested dogwhelks collected from Robin Hoods Bay in 1982 revealed an increase in the infestation mass in snails of

similar size from spring to autumn samples. The average redial population calculated energy increase, when added to the estimate of average cercarial production, exceeds the energy spent on reproduction of both non-infested male and female Thais lapillus of average size. In 1981, this difference is again more than compensated for by the April to June weight gain as a result of early feeding by non-infested individuals, but as stated previously, there was no evidence of such a compensatory weight increase in 1982.

The increase of Parorchis acanthus infestation mass with increased size of host observed here is in general agreement with earlier studies on the increased levels of parasitism in larger snail hosts. For example Zischke (1967) found that Echinostoma revolutum rediae in Stagnicola palustris were present in increased numbers and size in larger snails which were exposed to the same miracidial dose as the smaller snails. It is possible that the availability of nutrients and space are combining to limit infestation sizes in smaller hosts. In the case of P. acanthus infestations in T. lapillus it is a possibility that because the larger hosts may have been infested for longer periods of time than smaller individuals, the annual boost of young rediae each spring (Rees, 1980) may have an accumulative effect causing an increase in the infestation mass proportional to the increased size of the host.

Although there is a significant positive relationship between the total mass of the infestation and both the mass of a sample of 30 entire rediae from an infestation, and

redial numbers (Figs. 4.6 and 4.7), it is interesting to note that no significant difference was found between average estimated redial numbers in infestations through the summer (Table 4.25), whereas there is a significant increase in redial mass during the same period (Table 4.23). It appears that increased summer infestation masses are the result of increased redial sizes rather than increased redial numbers.

The difficulty of accurately assessing the total numbers of rediae in spring infestations associated with the small size of very young stage I rediae (Rees 1980) may have resulted in under estimates of spring redial numbers. As a result of the technique used to estimate redial numbers a trend towards decreased numbers of rediae in infestations during the late summer, as compared with spring redial numbers, may have been overlooked. This would not, however, alter the conclusions drawn concerning the nature of the increase in infestation mass.

The average annual soft tissue mass and energy increments calculated for infested snails on page 105, suggest that the amount of energy invested into growth by infested snails is quite small in relation to the reproductive effort and cercarial production energy losses in non-infested and infested Thais lapillus individuals respectively, shown on page 106. The calculated value for the average growth of redial populations from spring to autumn 1982 (p.104) is very similar to the estimate of soft body growth increment. This may be more than a coincidental similarity. Rees (1980) described the increasing prevalance of small stage I and

stage II Parorchis acanthus rediae in infested dogwhelks during the spring and early summer, as opposed to those of late summer and autumn when the rediae were of the large stage III type. The results obtained in this investigation, as shown in Table 4.23, are that the average mass of 30 rediae from an infestation was found to increase significantly from spring and early summer to late summer and autumn, in agreement with Rees.

There are at least three possible explanations for the occurrence of greater numbers of stage I and II rediae in spring and autumn:

1) As stage I and stage II rediae are assumed to be younger than stage III rediae by Rees (1967, 1980), it may be that infested animals have been re-infested early in the year. Perhaps the tendency of infested snails to leave aggregation before non-infested individuals (Feare, 1969) renders them more susceptible to re-infestation than non-infested snails are to initial infestation. (The possible success of re-infestations of Parorchis acanthus in Thais lapillus should be considered with respect to the report of Lie et al. (1975), that Echinostoma lindoense rediae cannibalise the early stages of a new infestation of the same species).

2) Low temperatures may cause the rediae within the existing infestation to produce more rediae, as in Fasciola hepatica infestations of Lymnaea truncatula (Kendall 1964). It was reported by Rees (1980) that stage III rediae produced only cercariae and that stage I and stage II rediae were present only in spring and early summer. If the divisions between

season and redial development are as absolute as Rees suggests then the most probable mechanism by which stage I and II re-occur in spring infestations is re-infestation.

3) As a result of the low temperatures and starved condition of the over-wintering host (Feare 1970a), stage III rediae may regress or rejuvenate to stage II or even stage I rediae,as described for triclad turbellarians by Calow (1978). The effects of temperature and the nutritional state of the host on redial development are discussed by Gordon et al. (1934) and Kendall (1949) respectively.

Regardless of the mechanism responsible for their presence in Thais lapillus, the re-occurrence of stage I and stage II rediae which develop into stage III rediae as the year progresses, represents not only an energy demand on the host, but also a possible mechanism for the increased shell growth observed in infested snails, both in this study and by Feare(1970a). In support of the above mechanism the calculated soft body tissue mass increase that would be expected to cause the average shell growth increment is very similar to the increase in the redial mass within the snail over the summer.

The report of Donges (1971) that Isthimiophora melis rediae are capable of producing up to 104 generations of daughter rediae, and the observation of Meyerhof and Rothschild (1940), that an individual Littorina littorea remained infested with Cryptocotyle lingua rediae for a period of 8 years indicates that the above mechanism is a plausible hypothesis for the appearance of regular annual growth in

infested T. lapillus.

In conclusion, the reproductive effort of an average sized non-infested male Thais lapillus was found to be about two thirds that of a similar non-infested female dogwhelk. The rough estimate of the energy lost by an infested dogwhelk as a result of producing an average number of cercariae on all the days in the year when the sea temperatures were 12°C or greater, is similar to, but slightly more, than the reproductive effort of an average sized (32mm) male dogwhelk, but less than the amount of energy a 32mm female dogwhelk would expend on reproduction. This result is in agreement with the suggestion of McClelland and Bourns (1969), and findings of Bourns (1974), that reproduction is nutritionally more costly for a snail than parasite production. In 1981 it was found that infested T. lapillus were able to feed in early spring, whilst the non-infested animals remained in aggregation (Feare, 1971a), thereby achieving a significant increase in body mass. This was not observed for infested T. lapillus during the cold spring of 1982. Thus, in some years, the infested T.lapillus individuals will benefit from their early dispersion from spring reproductive aggregations (Feare 1971).

The increased mass of samples of 30 entire rediae from similar-sized infested T. lapillus individuals collected from Robin Hoods Bay in autumn as opposed to spring (1982) also comprises an energy drain to the host resulting from the presence of the larval trematodes - a situation that McClelland and Bourns (1969) and Bourns (1974) did not consider. When redial growth is taken into account,

previously non-infested male T. lapillus may expend more energy producing parasites than they would have done reproducing themselves and previously non-infested females may also expend slightly more energy on parasite production than on reproduction.

The increase in size of rediae within the infesting redial population during the year (also observed by Rees 1980), provides a possible mechanism for the continued growth of infested dogwhelks, which is supported by the similarity in redial population increments and the annual increase in body mass of infested Thais lapillus, calculated from the average annual increase in shell length, and the Log body mass to Log shell length regression.

CHAPTER FIVE

EFFECTS OF PARORCHIS ACANTHUS INFESTATIONS ON THE OXYGEN UPTAKE OF THAIS LAPILLUS HOSTS.

INTRODUCTION

The effects of larval trematode infestations on oxygen consumption by their gastropod hosts has not been the subject of many studies. The first indication of respiratory metabolism changes was the discovery by Hurst and Walker (1935) that Lymnaea stagnalis infested with larval trematodes produced more heat than non-infested individuals.

In a later study Becker (1964) found that trematode infested L. palustris had a lower rate of oxygen uptake than normal individuals. The two studies thus indicate that there could be an increase in anaerobic respiration in infested lymnaeids.

Vernberg and Vernberg (1967) reported that Nassarius obsoleta infested with the sporocysts of Zoogonus lasius and Lepocreadium setiferoides had thermal metabolic responses differing from those of normal snails. Infested individuals which had been acclimated to low temperatures respired at a slightly increased rate to non-infested animals similarly acclimated, when subjected to a temperature of 35°C. Warm acclimated infested individuals had increased oxygen consumption at 10°C when compared with normal snails.

Vernberg and Vernberg justified the experimental use of high and low temperatures by describing the intertidal zone as an area of temperature extremes. At all other experimental

temperatures, for both warm and cold acclimated animals, there was no difference in oxygen uptake between infested and non-infested Nassarius obsoleta. Very similar results were obtained for N. reticulatus from Sweden, by Tallmark and Norgren (1976) where the snails were infested with an unidentified Microphallid digenean. The relatively minor effects of trematode infestation on the thermal metabolic responses of N. obsoleta can, at least in part, be explained by the decreased activity of cytochrome c oxidase in infested individuals (Vernberg, 1969).

In a study on infested Lymnaea stagnalis, the rate of increase of oxygen requirement with increasing body mass for normal snails was found by Duerr (1967) to be greater than those for snails infested by sporocysts or metacercariae of Cotylurus flabelliformis.

The relationship between body mass and oxygen uptake differed depending on whether the snail was harbouring sporocysts or metacercariae. Generally, the oxygen uptake of an organism increases in proportion with a fractional power of its body weight thus

$$Y = a.X^{b}$$

which transforms to a straight line thus

$$\log Y = \text{Log } a + b.\text{Log } X$$

where:-

Y = Oxygen uptake per unit of time

X = Body Weight

a = a constant - logarithmic intercept on the Y axis

b = a constant - slope of the logarithmic regression line

The slope (b) obtained by Duerr for the Log n oxygen uptake ($ml.h^{-1}$) as against Log n fresh body mass (g) for non-infested Lymnaea stagnalis was 1.0+-0.05 (at 95% confidence level) which was reduced to 0.77+- 0.22 in animals shedding unidentified xiphidiocercariae, to 0.49 +- 0.45 by the presence of Cotylurus flabelliformis sporocysts, and to 0.31+- 0.21 by the presence of C. flabelliformis metacercarial cysts. Duerr explained the reduction in oxygen uptake in larger infested animals by suggesting that the sporocyst or metacercarial mass required less oxygen than the snail tissue it replaced and that there was an increased amount of dead and dying host tissue as a result of the infestation.

Lee and Cheng (1971) found variations in oxygen uptake between non-infested Biomphalaria glabrata and individuals carrying infestations of Schistosoma mansoni in different stages of development. This work was to some degree repeated, and considerably improved upon by Meakins (1980) using the same species of host and parasite. In his study

Meakins demonstrated a reduction in the oxygen uptake for body mass slope of infested animals when compared with that for non-infested snails, but the average rate of oxygen uptake was increased in infested snails. Lightly and heavily infested snails were used at three different temperatures, and all showed a reduction in slope during the period of the infestation. In heavily infested snails oxygen uptake returned to normal (i.e. control) level after 10 weeks of infestation, whereas it remained significantly elevated in lightly infested animals. Infested Biomphalaria glabrata became lighter than non-infested animals of similar shell diameter during and after cercarial emission. Earlier, Lee and Cheng (1971) had found that B. glabrata have only 25 percent of the haemolymph glucose present in normal animals. Taking these observations into account Meakins (1980) suggested that the change in the oxygen uptake for body mass regression slope in infested B. glabrata is due to a change in the substrates respired.

B. pfeifferi infested with larval schistosomes were noted by Meuleman (1972) to have a reduced number of smaller, crystolysed mitochondria than non-infested snails. The results of these studies on Biomphalaria species could indicate a reduction in the efficiency of the hosts aerobic metabolism, as a result of larval trematode infection.

In the early seventies a number of illuminating studies were made into the enzyme activity of infested and non-infested digestive glands of Littorina saxatilis and the sporocysts of the digenean Microphallus similis by James et al.. Glycolytic enzymes in the sporocysts and infested digestive

glands were found to be generally more active than those in non-infested tissues by Marshall, McManus and James (1974). Both host digestive glands and sporocysts were shown to have a full complement of tricarboxcylic acid cycle enzymes (McManus and James, 1975a), but it was suggested that the balance of activity of succinate dehydrogenase between the oxidation of succinate and the reduction of fumarate indicates that both species are facultative anaerobes. This supports some earlier evidence of Marshall, McManus and James (1974), that Microphallus similis could be a facultative anaerobe, because of the presence of both pyruvate kinase and a relatively active phosphoenolpyruvate carboxykinase combined with the low level of lactic dehydrogenase, for which Bryant (1972) is cited as having suggested a facultative anaerobic respiratory pathway. As a result of allowing the sporocysts of M. similis and digestive gland tissue from infested and non-infested Littorina saxatilis to respire anaerobically in vitro, McManus and James (1975b) suggested a new pathway for anaerobic glucose metabolism for both host and parasite, which they felt was indicative of convergent evolution.

Experiments by McManus and James (1975c) on the aerobic respiration of whole L. saxatilis (infested and non-infested) digestive glands indicate that the deeper snail tissues, as well as the M. similis sporocyst mass (which of course lacks any oxygen transport system), have to respire anaerobically, whereas surface tissues respire aerobically. The oxidative metabolism of the parasite is shown to be less efficient than that of the host by this

study. The summary of these studies is that the increased activity of the anaerobic respiration pathways in M. similis sporocysts and infested digestive gland tissue from L. saxatilis hosts, as compared with non-infested animals, is that infested L. saxatilis should require less oxygen per unit weight than non-infested snails.

Narayanan and Venkateswararao (1980) found that infested Lymnaea luteola tissue had a much greater affinity for succinate than normal, coupled in the former with a lowered endogenous oxygen consumption and inhibition of succinate oxidation by increased malonate availability. The reduced ATP levels found in infested L. luteola tissue, as compared with the non-infested condition, by Narayanan et al. (1982) coupled with the earlier work by the first two authors should result in reduced oxygen uptake by infested (as opposed to non-infested) L. luteola. However, the work of Narayanan (1978) is cited in the later publication for having found no evidence of increased anaerobic respiration as a result of the infestation.

On finding no evidence of enhanced glycolytic enzyme activity in Nassarius obsoleta infested with Zoogonus rubellus sporocysts Schilansky, Levin and Fried (1977) suggested that the findings of Marshall et al. (1974) with respect to enzyme activity in Littorina saxatilis infested with Microphallus similis, applied only to that particular example, and was not representative of all larval trematode infestations in snails.

The infested snail can thus be envisaged as an individual with a reduction in, or alteration of, respiratory substrates, possibly altered enzyme characteristics, mitochondrial disturbance and with a significant proportion of the viscera being potentially anaerobic. How all these factors will combine to effect the oxygen consumption of the infested snail cannot be easily predicted.

The factors that affect the oxygen uptake of marine invertebrates were arranged by Newell (1973) into one of three groups - tidal, seasonal and endogenous.

Tidal dependent factors include the proportion of time the animal is exposed to air, the temperatue fluctuations to which it is exposed and the time that is available for feeding.

Seasonal factors include temperature and photoperiod. (In the case of aggregating Thaisid prosobranchs, the ability to obtain food, especially on exposed shores, should also be included here).

Endogenous factors include activity, stage of development and body size. Stickle and Duerr (1970) and Stickle (1971, 1973), found that the sex of the individual should also be included for Thais lamellosa.

Sandison (1966) and McMahon and Russell-Hunter (1977) investigated a range of marine gastropods which live at different littoral and sub-littoral levels and found that thermal responses and recovery from desiccation differed between lower shore and upper shore species. The three sub-

littoral gastropod species studied by McMahon and Russell-Hunter did not make any metabolic adjustments to cope with increasing temperature, and did not have increased oxygen uptake in air, whereas the mid-shore Mesogastropod Littorina littorea had a band of metabolic compensation between 20°C and 30°C. L. littorea also had a higher rate of oxygen consumption in air, as did all four species (including T. lapillus) investigated by Sandison (1966). Littorina saxatilis , the highest shore species studied by McMahon and Russell-Hunter (1977) was shown to go into a reversible torpor as a response to temperatures above 25°C up to a maximum of 44°C.

A clear tidal periodicity of oxygen uptake was found by Sandeen, Stephens and Brown (1954) in Urosalpinx cinereus, and a less obvious one in Littorina littorea although both species were found to display diurnal periodicity of oxygen uptake. Sandison (1966) found that L. littorea, had a daily periodicity of oxygen uptake from air, and a tidal periodicity of oxygen uptake from water. The higher shore littorinid L. saxatilis had only a diurnal rhythm whilst exposed to air, and Thais lapillus displayed neither diurnal or tidal periodicity of oxygen uptake.

The periodicity of oxygen uptake in L.littorea may be further explained with reference to the work of Newell and Northcroft (1966) in which L. littorea (along with three other invertebrate species) was shown to have a fairly constant basal metabolism over a range of temperatures, but a variable maximal active oxygen uptake which is temperature dependant. Diurnal and tidal periodicity may well be due to

L. littorea switching from one metabolism to another as a result of environmental cues.

The active oxygen uptake rate (as demonstrated by Newell and Northcroft 1967) of L. Littorea and Mytilus edulis was shown to remain temperature depend nt throughout the year, but the quiescent rate was found to be temperature dependent, in the range occurring on the shore, by Newell and Pye (1970a). The upper thermal tolerance temperatures of both species also reflected the environmental temperatures experienced at the different seasons. In the same study this seasonal oxygen uptake was shown to be present in cell free tissue homogenates of Littorina littorea and Mytilus edulis. In a later study Newell and Pye (1970b) demonstrated a similar effect in individuals of the same two species as used previously which had been acclimated to different temperatures in the laboratory. In a further investigation the same authors (1971a) demonstrated that L. littorea mitochondrial oxygen uptake was also temperature dependent, so long as there was abundant Krebs cycle substrates to metabolise.

Newell and Pye (1971a) reported that low concentrations of pyruvate (0.01 - 0.1mM) limited the oxygen uptake by crude mitochondrial preparations from L. littorea, and that pyruvate concentrations of around 2.0mM lifted the oxygen uptake rate into a temperature dependent level similar to the active level reported for L. littorea by Newell and Northcroft (1966).

In 1978 Bayne and Scullard reported that the summer oxygen uptake rates of Thais lapillus individuals were elevated above, and less temperature sensitive than, those found for individuals collected in winter. Snails of the related species T. lamellosa had been found by Stickle (1973) to have seasonal variations in their oxygen uptake, but these two studies also include the effects of the endogenous states of the animals. T. lapillus was found to have elevated oxygen uptake during drilling and ingestion of prey by Bayne and Scullard (1978). Nassarius reticulatus was similarly shown to increase its oxygen uptake rate when stimulated by the presence of food, or chemical extracts from food, and for two or three days after feeding, by Crisp, et al., also in 1978. A period of sudden increase in the oxygen uptake of male Thais lamellosa was reported by Stickle (1973) to occur between February and March which is the period when male T. lamellosa disperse out of winter aggregations and start feeding.

Starvation has been reported to both increase (Stickle and Duerr, 1970 and Stickle, 1971) and decrease (Bayne and Scullard, 1978) oxygen uptake by Thaisids. Subsequently Stickle and Bayne (1982) found no starvation-induced reduction in oxygen uptake rates of T. lapillus individuals. The seasonal study of Stickle (1973) shows that, at least for males, the lowest oxygen uptake values were for animals in their last month of aggregation (February) after the winter period of irregular, or no, feeding, although their respiration rates will have acclimated to low temperatures. Having performed a study together on T. lapillus Stickle and

Bayne (1982), on finding that there was no decrease in oxygen uptake by starved animals (as was found in the two studies by Stickle on T. lamellosa quoted previously) sought to explain the different result obtained by Bayne and Scullard (1978). On the one hand Bayne and Scullard used T. lapillus subjects of which it was known that they were feeding actively prior to their period of starvation. These authors noted an early sharp decline in oxygen uptake in the first few days of starvation, after which the oxygen uptake rate remained constant. On the other hand Stickle and Duerr (1970), Stickle (1971), Stickle (1973) and Stickle and Bayne (1982) used animals collected from the shore, with unknown feeding histories. Their results were all similar, having fairly constant, tending to increasing, oxygen uptake by starving subjects. A significant weight related oxygen uptake rate was calculated for starving Thais lapillus by Stickle and Bayne. Having explained the differences in their earlier results in the latter paper the authors do not comment on the relative accuracy of the two different methods.

The root of the discrepancy is the definition of normal, or basal, oxygen uptake for T. lapillus taking into account the frequency of feeding. In the summer active feeding periods the oxygen uptake rate was reported by Bayne and Scullard (1978) to be so elevated as a result of feeding, that increasing temperatures had no effect on it. This suggests that a study of the effects of starvation on oxygen uptake for T. lapillus collected in the summer months ought to include the initial few days post - feeding when there is a

rapid drop in oxygen requirement, whereas in the winter months when the snails are in, or around, aggregations and feeding is at best irregular, the method employed by Stickle and Duerr (1970), Stickle (1971), and Stickle and Bayne (1982) may be more suitable.

The similarity of the effects of starvation, or trematode infestation of snail hosts has been commented on, for example, by James (1965) in Littorina saxatilis infested with Microphallus similis, and Becker (1980) in Biomphalaria glabrata infested with Schistosoma mansoni.

Active Littorina littorea were shown by Newell and Pye (1971b,c) to increase their oxygen uptake by 300% to 500% of the inactive level, and Nassarius reticulatus was shown by Crisp (1978) to elevate its oxygen uptake level by 20% of the inactive level during strenuous activity.

Stickle (1973) found differences in the oxygen uptake of male and female Thais lamellosa, with seasonal variations caused by copulatory activity and lipogenesis. The lipid a female T. lamellosa, or T. lapillus contributes towards her egg capsules must therefore incur some partitioning of, or addition to, her oxygen uptake.

Early workers on the relationship between oxygen uptake and animal size found the slope (b) of the logarithmic relationship to be around 0.67 (Rubner 1902, and Brody 1945, cited by Duerr, 1967). This meant that oxygen uptake by larger organisms increased with their surface area, and thus the 'surface laws' were formulated. Hemmingsen (1950, cited by Duerr, 1967) obtained a slope of 0.73 for his range of

organisms, and concluded that it was their internal "vital" surfaces which were the important ones. In a later study metazoan organisms of the size range that encompass gastropod molluscs were shown by Zeuthen (1953) to have a Log oxygen uptake to Log body mass slope (b) of 0.76 - 0.80. In 1955 Davison theorised that organisms which increased their oxygen uptake in relation to the total cell surface area would have a slope (b) equal to 1.0, as if the oxygen consumption increased with body mass.

Bertalanffy (1957) reviewing previous literature applied all the slopes previously calculated or deduced by classifying organisms into three different types, depending on their respiratory apparatus and mechanisms. He predicted that prosobranch snails would be in the first group, with organisms that respire in proportion with their surface area; in these organisms (b) is 0.67. Land pulmonate snails included in the third group with animals which respire in proportion with their body mass (b = 1.0). Pond snails, such as Lymnaea stagnalis were placed in an intermediate group, with slopes of 0.75 to 0.80.

Duerr (1967) found that non-infested Lymnaea stagnalis had a slope of 1.0, with very little variation, and implied that other studies must have used a mixed batch of infested and non-infested snails to get their results, as his study found that individuals infested with Cotylurus flabelliformis had shallower regression slopes than normal snails. In the light of his findings, Duerr (1967), disputes the existence of the intermediatory respiratory group hypothesised by Bertalanffy (1957).

It is worth noting that Duerr's experimental technique entailed monitoring the oxygen uptake of a pulmonate pond snail in only 2 ml of water (reported by Duerr as insufficient to cover the pneumostome in all cases). This makes the result extremely interesting, because a pulmonate pond snail out of water has a weight specific oxygen uptake in a manner similar to that predicted for land pulmonates by Bertalanffy (1957).

The size-metabolic rate relationship for male Thais lamellosa was found by Stickle (1973) to change from a minimum slope of 0.19 to a maximum slope of 1.02 in consecutive months. The female slope changed from 0.42 to 0.98 during the year. Stickle obtained respiration data for T. lamellosa for 11 consecutive months, but for 6 of these months either, or both male and female snails showed no relationship between oxygen uptake and size (dry body mass). Bayne and Scullard (1978) and Stickle and Bayne (1982) applied constant slopes throughout their experiments on T. lapillus, using b = 0.511 and b = 0.60 respectively, for combined samples of male and female snails.

Newell (1971b) demonstrated that tissue slices from whole Littorina littorea of different sizes had a similar size-metabolic rate relationship to the whole animal, but when the cells were disrupted the resultant homogenates did not show significant variation. Newell concluded that the cell surfaces control the size-related oxygen uptake of L. littorea.

The work of Duerr (1967) indicates that a size - oxygen uptake slope reduction may be caused by the presence of Parorchis acanthus in Thais lapillus. Bayne and Scullard (1978) and Stickle and Bayne (1982) found, and used, similar values of b for T. lapillus, but agreement between authors seems to be rare in this field. The effects of factors other than size cannot be totally excluded from a study into the effects of larval trematodes on the size - respiratory rate of the snail hosts and so must be included in the design of the investigation.

Oxygen Electrode Respirometry

METHOD

Thais Lapillus from Robin Hoods Bay were collected in March, and June 1982, starved and acclimatised to 10 C in the laboratory for 1 to 2 weeks before measurement of their oxygen uptake using a Radiometer E5046 oxygen electrode in a small sealed chamber as illustrated in Fig. 5.1. The apparatus was constructed by Dr. J.W. Grahame, then at the Wellcome Marine Laboratory at Robin Hoods Bay, and differed from that of Crisp, Davenport and Shumway (1978) in that the electrode outer sleeve was fixed to the lid of the chamber which itself was sealed to the rest of the chamber with vaseline during use. The snail to be studied was cleaned and dropped, foot upwards, into the chamber, which was partially filled with seawater and subsequently topped up so that the meniscus was standing proud above the vaseline seal.

The seawater used in the respirometer chamber was always taken from a single carboy which had been stored in the dark

for 5 to 6 years in the basement of the laboratories at Robin Hoods Bay. The investigations of Brand et al. (1937, 1939) showed that plankton populations died out within a matter of weeks in similar, but warmer (20 to 25°C), conditions, and populations of decomposing bacteria were found to disappear after 9 weeks by Waksman and Renn (1936). Brand et al. (1937) showed that there was no residue of toxic nitrogenous breakdown products in aged seawater, with the nitrogen being converted to nitrates. The seawater for the respirometer was kept in the dark until within no more than 24 hours before use, during which time it was covered, and maintained at 10°C in the water bath so that the temperature could equilibrate before use. The aged seawater was thus considered to contain a negligible amount of micro-organisms or organic matter.

When the chamber lid and electrode were lowered on to the body of the vessel excess seawater was displaced through a small pressure release hole to the side of the electrode. This method prevented air bubbles remaining in the chamber because of under-filling, but also protected the membrane from pressure increases which might cause minute alterations of the critical distance from the membrane to the electrode. The pressure release hole was quickly plugged with vaseline after the chamber lid and electrode were in position. The whole chamber was then placed in the 10°C water bath which was subjected to a constant level of illumination whilst the electrode was in use. The seawater in the chamber was mixed with a pneumatically driven magnetic stirrer. The snail subject was suspended above the stirrer 'flea' on a plastic

mesh platform in the chamber.

The decline in oxygen tension within the vessel was monitored on a Radiometer PHM71 Mk. 2 Acid/Base Analyser, with a PHA934 PO2 module unit, and recorded on a Servoscribe chart recorder. The snails behaviour was observed, and categorised as being either 'Active' or 'Inactive' accordingly. The Thais lapillus were dropped into the chamber foot side up to try and prevent them from being so active as to dislodge the chamber lid and electrode, breaking the seal, as happened occasionally.

The oxygen uptake rate was calculated using the gradient of the declining straight line relationship between oxygen tension within the chamber, and time. Once a straight line relationship was achieved, the gradient remained constant until low oxygen tensions, when a compensatory lower oxygen uptake response from the subject would result. The snail was removed from the chamber once there was sufficient trace on the chart recorder to be sure that a straight line decline had been attained.

To remove the dogwhelk from the chamber after it's oxygen uptake had been determined, it was first necessary to remove the vaseline plug from the pressure equalising hole so that before the lid and electrode were lifted away from the body of the vessel, the pressure was equalised. The excess vaseline was wiped off the lid with tissues and none was allowed to come into contact with the electrode membrane. The small cavity between the electrode membrane and the plastic mesh protecting it was filled with oxygenated

seawater at 10 °C by Pasteur pippette, making sure that no air bubbles were trapped in the protective mesh, or adhering to the membrane. The electrode was then suspended in a beaker of aerated aged seawater in the 10°C water bath to re-equilibrate. This seawater also contained 1mg. l^{-1} of Streptomycin, so that bacterial colonisation of the membrane would not occur. The chamber was kept filled with distilled water between 'runs' to prevent air bubbles being trapped in the plastic mesh protecting the snail from the stirrer, and to prevent colonisation of the chamber by marine organisms.

After being removed from the chamber the dogwhelk was measured, as described in Chapter 2, and then dropped into a measuring cylinder containing a known volume of seawater. The resultant change in volume was recorded as the volume of the snail. Then the snail was killed and, after its shell cracked open, it was sexed, dissected and its component dry weights determined, all as described previously in Chapter 2.

Once the electrode was re-stabilised, the constant read out was adusted to 158mmHg, the oxygen tension at 760mmHg of air pressure, before the next dogwhelk was treated as above. The initial calibration of the apparatus was achieved by setting up the chamber as normal with a new electrode, except for the addition of a small amount of sodium dithionite to the seawater. This reacts with all the dissolved oxygen in the seawater and, as described by Davenport (1976), thus provides a method for determining the zero point of the scale. The oxygen electrode meter and the chart recorder were set to zero as soon as no further decline in the oxygen

tension occurred. The seawater containing sodium dithionite was carefully rinsed off the electrode before it was allowed to re-equilibrate, as above, prior to use.

The rate of oxygen depletion by the electrode was ascertained by recording the decline in oxygen tension in a chamber set up normally but without an occupant. This value was then deducted from all subsequent values for individual snails. Oxygen volume changes within the respirometer chamber were calculated from the oxygen partial pressure changes recorded by the electrode and the chart recorder. The chart recorder range was expanded so that four widths of chart paper (400 divisions) was equal to the 0 - 160 mmHg full scale deflection of the PO_2 meter. Thus each chart division was equal to 160/400 = 0.4 mmHg. and the record on the chart recorder could be converted to pressure change in mmHg. By the law of partial pressure, at 760mmHg air pressure the partial pressure of oxygen is 158 mmHg. At 760mmHg air pressure, and 10°C, 1 ml of seawater (34.8‰) contains 6.44 μl (the Bunsen Coefficient). The total volume of the respirometer was ascertained by weighing the chamber first empty, and then full of distilled water, the difference in weight being taken as the volume of the chamber in ml. The volume of the Thais lapillus in the chamber was ascertained, as described above, and deducted from the chamber volume to give the volume of seawater and thus oxygen. By knowing the initial volume and pressure of oxygen, the volume decrease could be calculated from the following equation:

$$\Delta VO_2\ (\mu l O_2.h^{-1}) = \left[\frac{(Vc - Vs) \times 6{\cdot}44}{158}\right] \times \Delta PO_2$$

ΔVO_2 is the change in oxygen volume (μl) per hour as a result of oxygen uptake by the individual T. lapillus or the O_2 electrode.

Vc is the volume of the respirometer chamber (ml)

Vs is the volume of the snail (ml)

158 is the partial pressure of oxygen (mmHg) at 760 mmHg air pressure

ΔPO_2 is the change in the partial pressure of oxygen (mmHg) per hour.

6.44 is the volume of oxygen in $\mu l.ml^{-1}$ of seawater at 10°C and 760 mmHg air pressure.

Dr. J.W. Grahame had previously determined the salinity of the aged seawater by silver nitrate titration as 34.01‰. This salinity very closely approximated to 19‰ chlorinity, allowing the oxygen saturation coefficient to be taken directly from the table of values compiled by Fox, as shown in Sverdrup, Johnson and Flemming, 1942). The aerated seawater was thus considered to contain 6.44 ml. l^{-1} of oxygen.

Log oxygen uptake ($\mu l O_2.\ h^{-1}$) versus Log body dry mass (mg) regressions were calculated, and Analysis of Covariance was applied as described by Sokal and Rohlf (1981), using computer programmes written by Mr. R. Willows.

RESULTS

Figs. 5.2 and 5.3 show the Log oxygen uptake ($\mu l O_2.h^{-1}$) against Log dry body mass (mg) regression lines for

non-infested male and female, and infested Thais lapillus in both Active (Fig.5.2) and Inactive (Fig.5.3) conditions. The regression coefficients and Y intercepts for the above lines are shown in Tables 5.1 and 5.2.

Table 5.3 shows the results of analysis of Covariance (ANCOVA) of the regressions for Log oxygen uptake ($\mu lO_2.h^{-1}$) against Log dry body mass (mg) for Active non-infested male and female, and infested T. lapillus. All three regression lines have variance ratios significant to $p \leq 0.01$. There are no significant differences between mean values of oxygen uptake in non-infested male and female, and infested snails, but the slopes are significantly different from a pooled slope fitted through each group of points ($p \leq 0.01$). The use of Hochberg's GT2 test, and a Tukey-Kramers test (as described in Sokal and Rohlf 1981) indicated that no individual slope is significantly different from the other two, so it is concluded that all the lines are different from each other.

Table 5.4 shows the results for ANCOVA of the regressions for Log oxygen uptake ($\mu lO_2.h^{-1}$) against Log dry body mass (mg) for Inactive non-infested male and female, and infested Thais lapillus. All three regression lines have variance ratios significant to $p \leq 0.01$. There are no significant differences between mean values of oxygen uptake in non-infested male and female, and infested snails, but the slopes are significantly different from a pooled slope fitted through each group of points ($p \leq 0.01$). The use of a Hochberg's GT2 test, and a Tukey-Kramers test (as described in Sokal and Rohlf; 1981) indicated that no individual slope is significantly different from the other two, so it is

concluded that all the lines are different from each other.

Table 5.5 shows the results of ANCOVA of the regressions of the Log oxygen uptake ($\mu l O_2 . h^{-1}$) against Log dry body mass (mg) for Active and Inactive non-infested male T. lapillus. As described before the regressions are significant. The mean values for the oxygen uptake of the two groups are very significantly different ($p \leq 0.001$). The group slopes are not significantly different from a pooled slope fitted through the separate means ($p \geq 0.1$). The adjusted means (or intercepts) are very significantly different from each other ($p \leq 0.001$ for the variance ratio of errors among intercepts over errors from the common slope). Thus the two lines are considered to be different from each other, with the Active line being significantly elevated above the Inactive condition.

Table 5.6 shows the results of ANCOVA of the regressions of the Log oxygen uptake ($\mu l O_2 . h^{-1}$) against Log dry body mass (mg) for Active and Inactive non-infested female Thais lapillus. As described before, the regressions are significant. The mean values for the oxygen uptake of the two groups are very significantly different ($p \leq 0.001$). The group slopes are not significantly different from a pooled slope fitted through the separate means ($p \geq 0.1$). The adjusted means (or intercepts) are very significantly different ($p \leq 0.001$ for the variance ratio of errors among intercepts over errors from the common slope). Thus the two lines are considered to be different from each other, with the Active line being significantly elevated above the Inactive condition.

Table 5.7 shows the results of ANCOVA of the regressions of Log oxygen uptake ($\mu lO_2.h^{-1}$) against Log dry body mass (mg) for Active and Inactive Parorchis acanthus infested T. lapillus. As described before the regressions are significant. The mean values for the oxygen uptake of the two groups are very significantly different ($p \leq 0.001$). The group slopes are not significantly different from a pooled slope fitted through the separate means ($p > 0.1$). The adjusted means (or intercepts) are very significantly different from each other ($p \leq 0.001$ for the variance ratio of errors among intercepts over the errors from the common slope). Thus the two lines are considered to be different from each other, with the Active line being significantly elevated above the Inactive condition.

Fig. 5.1

Oxygen electrode and chamber (see text).

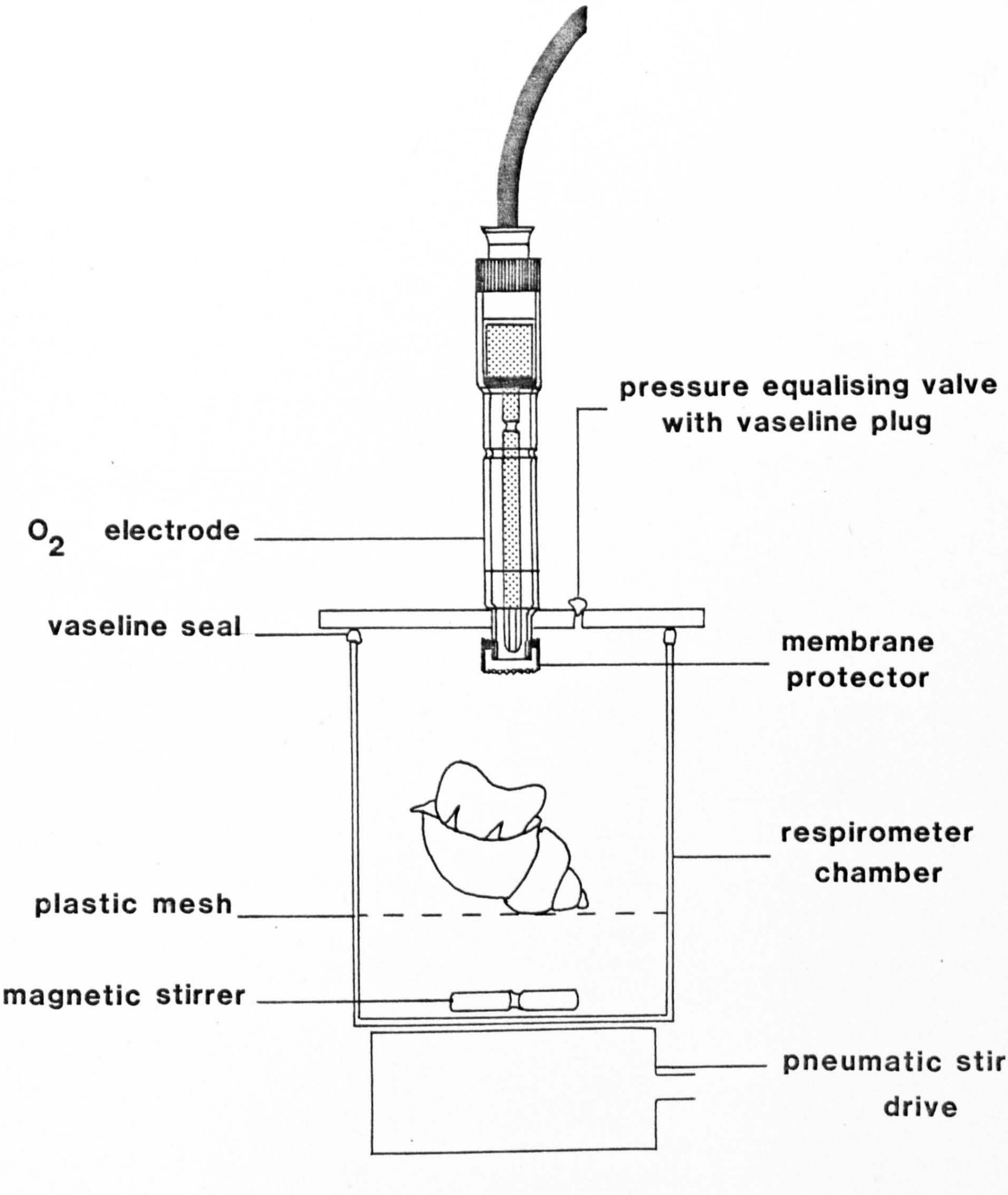

pressure equalising valve
with vaseline plug
O_2 electrode
vaseline seal
membrane
protector
respirometer
chamber
plastic mesh
magnetic stirrer
pneumatic stirrer
drive

Fig. 5.2

Log oxygen uptake versus Log dry body mass for infested and non-infested male and female Thais lapillus which were active in the respirometer chamber at 10°C.

(Regression equations and F values are shown in Tables 5.1 and 5.3).

ACTIVE

male ■

female ○

infested △

log oxygen uptake $\mu l O_2 h^{-1}$

100

50

10

M

F

100 200 400 800

log dry body mass (mg)

Fig 5.3

Log oxygen uptake versus Log dry body mass for infested and non-infested male and female Thais lapillus which remained inactive in the respirometer chamber at 10°C.

(Regression equations and F values are shown in Tables 5.2 and 5.4).

INACTIVE

male ■

female ○

infested △

log oxygen uptake $\mu l O_2 h^{-1}$

100

50

10

I

F

M

100 200 400 800

log dry body mass (mg)

REGRESSION COEFFICIENTS AND INTERCEPTS FOR LOG OXYGEN UPTAKE ($\mu l O_2 . h^{-1}$) AGAINST LOG DRY BODY MASS (mg) FOR INFESTED AND NON-INFESTED THAIS LAPILLUS.

TABLE 5.1.

ACTIVE

	b(+-standard error)	a
Non-infested MALE	0.589(+-0.0023) (with 27df)	2.328
Non-infested FEMALE	0.710(+-0.033) (with 18df)	0.933
INFESTED	1.360(+-0.033) (with 28df)	0.36

TABLE 5.2.

INACTIVE

	b(+-standard error)	a
Non-infested MALE	0.454(+-0.021) (with 22 df)	1.824
Non-infested FEMALE	1.282(+-0.049) (with 20 df)	0.014
INFESTED	1.003(+-0.013) (with 24 df)	0.106

TABLE 5.3.ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG OXYGEN UPTAKE ($\mu lO_2.h^{-1}$) AGAINST LOG DRY BODY MASS (mg) FOR ACTIVE NON-INFESTED MALE AND FEMALE AND INFESTED THAIS LAPILLUS.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.2383	0.2383	15.3770
Error (deviations from slope)	27	0.4184	0.0155	
MALE group total	28	0.6566		
Due to FEMALE regression	1	0.3292	0.3292	15.0533
Error (deviations from slope)	18	0.3937	0.0219	
FEMALE group total	19	0.7229		
Due to INFESTED regression	1	1.2139	1.2139	55.9390
Error (deviations from slope)	28	0.6076	0.0217	
INFESTED group total	29	1.8215		
Between groups	2	0.0964	0.0482	1.1440
Within groups	76	3.2011	0.0421	
Common slope within groups	1	1.5532	1.5532	70.6877
Error (deviations from common slope)	75	1.6479	0.2220	
Difference between slopes	2	0.2283	0.1141	5.8684
Error (deviations from slope within each group)	73	1.4197	0.0195	
Total (groups + within)	78	3.2974		
Common slope within study	1	1.4303	1.4303	
Error (deviations from common slope)	77	1.8671	0.0242	
Among intercepts	2	0.2192	0.1096	4.9883
Error (deviations from common slope)	75	1.6479	0.0220	

TABLE 5.4. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG OXYGEN UPTAKE ($\mu l O_2.h^{-1}$) AGAINST LOG DRY BODY MASS (mg) FOR INACTIVE NON-INFESTED MALE AND FEMALE, AND INFESTED THAIS LAPILLUS.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to MALE regression	1	0.1154	0.1154	9.7266
Error (deviations from slope)	22	0.2610	0.0119	
MALE group total	23	0.3763		
Due to FEMALE regression	1	0.7714	0.7714	33.5513
Error (deviations from slope)	20	0.4599	0.0230	
FEMALE group total	21	1.2313		
Due to INFESTED regression	1	1.1401	1.1401	78.9139
Error (deviations from slope)	24	0.3468	0.0145	
INFESTED group total	25	1.4869		
Between groups	2	0.0431	0.0215	0.4801
Within groups	69	3.0945	0.0449	
Common slope within groups	1	1.8361	1.8361	99.2176
Error (deviations from common slope)	68	1.2584	0.0185	
Difference between slopes	2	0.1908	0.0954	5.8990
Error (deviations from slope within each group)	66	1.0676	0.0162	
Total (groups + within)	71	3.1376		
Common slope within study	1	1.5093	1.5093	
Error (deviations from common slope)	70	1.6283	0.0233	
Among intercepts	2	0.3699	0.1849	9.9933
Error (deviations from common slope)	68	1.2584	0.0185	

TABLE 5.5. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSIONS OF LOG OXYGEN UPTAKE ($\mu l O_2 . h^{-1}$) AGAINST LOG DRY BODY MASS FOR ACTIVE AND INACTIVE NON-INFESTED MALE THAIS LAPILLUS.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to ACTIVE regression	1	0.2383	0.2383	15.3770
Error (deviations from slope)	27	0.4184	0.0155	
ACTIVE group total	28	0.6566		
Due to INACTIVE regression	1	0.1154	0.1154	9.7266
Error (deviations from slope)	22	0.2610	0.0119	
INACTIVE group total	23	0.3763		
Between groups	1	2.0943	2.0943	103.3999
Within groups	51	1.0330	0.0203	
Common slope within groups	1	0.3480	0.3480	25.4042
Error (deviations from common slope)	50	0.6850	0.0137	
Difference between slopes	1	0.0056	0.0056	0.4060
Error (deviations from slope within each group)	49	0.6793	0.0139	
Total (groups + within)	52	3.1273		
Common slope within study	1	0.1797	0.1797	
Error (deviations from common slope)	51	2.9476	0.0578	
Among intercepts	1	2.2626	2.2626	165.1602
Error (deviations from common slope)	50	0.6850	0.0137	

TABLE 5.6. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSION OF LOG OXYGEN UPTAKE ($\mu lO_2 . h^{-1}$) AGAINST LOG DRY BODY MASS (mg) FOR ACTIVE AND INACTIVE NON-INFESTED FEMALE THAIS LAPILLUS.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to ACTIVE regression	1	0.3292	0.3292	15.0533
Error (deviations from slope)	18	0.3937	0.0219	
ACTIVE group total	19	0.7229		
Due to INACTIVE regression	1	0.7714	0.7714	33.5513
Error (deviations from slope)	20	0.4599	0.0230	
INACTIVE group total	21	1.2313		
Between groups	1	1.2051	1.2051	24.6676
Within groups	40	1.9542	0.0489	
Common slope within groups	1	1.0112	1.0112	41.8235
Error (deviations from common slope)	39	0.9430	0.0242	
Difference between slopes	1	0.0894	0.0894	3.9817
Error (deviations from slope within each group)	38	0.8535	0.0225	
Total (groups + within)	41	3.1593		
Common slope within study	1	0.4180	0.4180	
Error (deviations from common slope)	40	2.7413	0.0685	
Among intercepts	1	1.7983	1.7983	74.3743
Error (deviations from common slope)	39	0.9430	0.2418	

TABLE 5.7. ANALYSIS OF COVARIANCE (ANCOVA) OF THE REGRESSION OF LOG OXYGEN UPTAKE ($\mu lO_2 . h^{-1}$) AGAINST LOG DRY BODY MASS (mg) FOR ACTIVE AND INACTIVE INFESTED THAIS LAPILLUS.

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F
Due to ACTIVE regression	1	1.2139	1.2139	55.9390
Error (deviations from slope)	28	0.6076	0.0217	
ACTIVE group total	29	1.8215		
Due to INACTIVE regression	1	1.1401	1.1401	78.9139
Error (deviations from slope)	24	0.3468	0.0145	
INACTIVE group total	25	1.4869		
Between groups	1	0.9555	0.9555	15.5959
Within groups	54	3.3084	0.0613	
Common slope within groups	1	2.3012	2.3012	121.0869
Error (deviations from common slope)	53	1.0072	0.0190	
Difference between slopes	1	0.0529	0.0529	2.8804
Error (deviation from slope within each group)	52	0.9544	0.0184	
Total (groups + within)	55	4.2639		
Common slope within study	1	1.6543	1.6543	
Error (deviation from common slope)	54	2.6096	0.0483	
Among intercepts	1	1.6024	1.6024	84.3184
Error (deviation from common slope)	53	1.0072	0.0190	

DISCUSSION

As stated in the introduction to this chapter, the oxygen uptake by marine gastropods is subject to many variables. The initial paragraphs of this discussion aim to justify the techniques used in this investigation to isolate the the effects of Parorchis acanthus rediae on the oxygen uptake of their Thais lapillus hosts.

Similar results were obtained in this investigation for animals collected in March and June. This similarity may, in part, have been associated with the long cold spring of 1982.

The temperature used for this study (10°C) was a little higher than the prevailing sea temperature at Robin Hoods Bay, but was not at an unnatural level. T. lapillus individuals infested with P. acanthus, and maintained at temperatures below 12°C were found not to release cercariae by Rees (1948). This observation was repeated in this investigation, so that oxygen uptake by cercariae released in the respirometer chamber did not require consideration.

The feeding/starvation studies of Bayne and Scullard (1978) and Stickle and Bayne (1982) with T. lapillus suggest that the one or two weeks laboratory holding time for subjects before use would not cause significant variations in oxygen uptake associated with starvation.

Salinity was not varied during the study with aged seawater from the same vessel being used in the respirometer chamber throughout the investigation.

Diurnal and tidal periodicities in oxygen uptake were found for some littoral gastropod species by Sandison (1966), but as stated in the introduction, this was not the case for Thais lapillus so that these variables were not recorded during this investigation.

The results obtained for active non-infested male and female, and infested T. lapillus (Fig. 5.2, Table 5.1) show that there is a much steeper increase in oxygen uptake with increasing dry body mass for infested animals (b = 1.360) than either non-infested males or females, who have quite similar slopes (b being around 0.6 and 0.7 respectively). These results for non-infested individuals are in agreement with the slopes obtained for this species by Bayne and Scullard (1978) and Stickle and Bayne (1982). The slopes for active non-infested male and female and infested T. lapillus are all significantly different from each other. In contrast previous investigations on T. lapillus by Bayne and Scullard (1978) and Stickle and Bayne (1982) pooled their results for male and female subjects. The slopes for non-infested male and female, and infested T. lapillus in active and inactive conditions are not significantly different (Tables 5.5, 5.6 and 5.7 respectively) which supports the method of Bayne and Scullard (1978) and Stickle and Bayne (1982) of fitting a line of similar slope for T. lapillus subjects in different experimental conditions. Although the above Log oxygen uptake versus Log dry body mass regression slopes are similar for active and inactive conditions, the elevation of oxygen uptake associated with activity causes non-infested male and female and infested snails to have significantly

elevated Y intercept values in the active as opposed to inactive condition (Tables 5.5, 5.6 and 5.7).

In both active and inactive states the larger infested individuals respired at a faster rate than non-infested Thais lapillus of either sex, while the sexual difference might be a seasonal effect with early spring being a period of reproductive activity for T. lapillus (Feare 1971). Stickle (1973) could not fit a significant Log oxygen uptake to Log dry body mass regression line for results obtained for female T.lamellosa at a similar time of year.

The increased oxygen uptake by T.lapillus infested with Parorchis acanthus is the opposite effect to that reported by Becker (1964) and Duerr (1967) for infested Lymnaea stagnalis. Lee and Cheng (1971) and Meakins (1980) found that mature infestations of Schistosoma mansoni in Biomphlaria glabrata had similar respiration rates to non-infested individuals. The increased activity of glycolytic enzymes from sporocysts of Microphallus similis, and infested digestive glands of Littorina saxat lis as compared with 'healthy' digestive glands, reported by Marshall, McManus and James (1974) suggests an increase in anaerobic respiration as a result of the infestation, with a consequent reduction in the size-related oxygen uptake.

The oxygen uptake effects noted in this investigation, for P. acanthus redial infestation of T. lapillus, may differ from the results obtained by the studies in the previous paragraph because they are sporocyst infestations.

It was reported by Rees (1966) that immature Parorchis acanthus rediae are capable of active movement, and thus although they are closely packed among the viscera, they may not form an inert mass with an anoxic interior as hypothesised by McManus and James (1975c) for Microphallus similis. The movement of the P. acanthus rediae, and the presence in each of a small but functional gut sac (Rees, 1966), may limit the damage to the host to localised tissue consumption, rather than areas of viscera degenerating as a result of interrupted nutrient and waste product circulation, in a similar manner as Rees (1936) reported for the redial infestations (Cryptocotyle lingua and Hismathla elongata) of Littorina littorea. In contrast, sporocyst infestations (particularly Renicola roscovita) of L. littorea were reported by Rees to form 'blocking layers' of immobile parthenitae, resulting in extensive host tissue death.

Thus, the areas of necrotic tissue envisaged by Duerr (1967) to cause the decrease in oxygen uptake by Cotylurus flabelliformis infested Lymnaea stagnalis are probably not present in infested Thais lapillus. In addition, the retention of relatively dormant metacercarial cysts of C. flabelliformis in the L. stagnalis host could be expected to cause a reduction in weight related oxygen consumption compared with 'healthy' individuals of the same species (Duerr 1967).

The rediae from infested dogwhelks collected in the early months of 1982 were significantly smaller than those taken later in the year from similar sized hosts (Table 4.23). The

March oxygen uptake elevation in larger infested dogwhelks may be as a result of the development of immature rediae (Rees, 1980), in a similar way to the increased oxygen uptake by immature infestations of Schistosoma mansoni in Biomphlaria glabrata as compared with 'healthy' individuals reported by Lee and Cheng (1971) and Meakins (1980).

Feare (1969) noted that Thais lapillus infested with Parorchis acanthus tended to disperse out of winter and reproductive aggregations before non-infested individuals. Stickle (1973) found a large increase in the oxygen uptake, and a steeper slope for oxygen uptake related to body mass (b = 1.02) for male T. lamellosa immediately after they had left aggregations and started to feed. The effects of very recent feeding (as described for T. lapillus by Bayne and Scullard (1978)) were reduced in this investigation by having a one to two weeks acclimatisation period for subjects prior to estimating their oxygen uptake. None the less, the results obtained for infested T. lapillus in this study could be attributable to a change in metabolic rate associated with a long term change in activity, as found for Littorina littorea and other invertebrate species by Newell and Northcroft (1967), which the non-infested individuals will make later in the year. In opposition to this possible explanation of the obtained results is the fact that in the present work the numbers of infested T. lapillus which were either active or inactive in the respirometer chamber were almost equal as was the case for non-infested males and females.

In conclusion, the significance of a particular value for the Log oxygen uptake versus Log body mass regression slope is highly dubious. The fact that larger infested T. lapillus respire at a greater rate than non-infested males and females in the early part of the year, appears at first sight to be contrary to the results of earlier studies until the different nature of the infestation is taken into account. The reason for the higher respiration rate is not clear and the significance to the infested Thais lapillus individuals of a small increase in oxygen uptake by larger infested snails as compared with non-infested individuals of similar sizes, possibly only in the early part of the year, is probably negligible in comparison with other effects of the infestation.

CHAPTER SIX

GENERAL DISCUSSION

Infestation of Thais lapillus hosts with the rediae of Parorchis acanthus causes a number of major changes in the snail. The observations of Feare (1970a) that large infested, castrated dogwhelks show signs of continued growth was based on the increased number of teeth rows in a relatively small sample of infested snails. The above observation has been confirmed in the present more extensive investigation. The increased number of aperture teeth rows in some infested individuals has been accompanied by other evidence of continued growth. The increased shell lip lengths of some infested snails found in this study, at the end of the summer active feeding period (described by Feare, 1971, Bayne and Scullard, 1977, amongst others), are indicative of summer growth and would probably result in new teeth rows being formed during the following winter aggregation. The infested dogwhelks were found to have thinner shell lips than normal adult snails, similar to those reported for growing immature dogwhelks (Moore, 1938a, Feare, 1970a, Coombs, 1973). Finally, the association of the ballooned second-to-last whorl with the presence of P. acanthus rediae was made by Feare (1970a), and in this study correlated with the number of aperture teeth rows. The ballooning of the higher whorls of the shell as a result of growth at the shell lip was not a deforming factor considered by Rothschild (1936) for infested Hydrobia ulvae which exhibited a similar shell deformity.

The shell increments between teeth rows calculated for a sample of infested dogwhelks from Scarborough South Bay, which were then converted into soft tissue energy increments, demonstrated that, when compared with the reproductive effort of non-infested hosts or the cercarial production of infested hosts, the energy spent on soft body growth was comparatively small. No attempt was made to estimate the energy invested in extra shell growth.

The rough estimate of average cercarial production energy demand for a year - assuming average cercarial release on every day with water temperatures above 12°C by an infested dogwhelk - was intermediate between male and female reproductive energy expenditures. When the redial population mass increase through the year is taken into account, the estimate of the average cost of the infestation is slightly elevated above the reproductive effort of even female Thais lapillus. The difference between male and female energy imputs into reproduction may influence the subsequent growth of infested dogwhelks. If a dogwhelk was male prior to infestation, it would have to expend more energy producing cercariae than it previously had on reproduction, whereas for a dogwhelk which was formerly female, there would be little change in energy loss as a result of infestation, but, as noted by Feare (1969), it would experience an extended annual feeding period.

There is a notable similarity between the mean annual increment calculated for the soft body tissue of infested T. lapillus, and the mean annual redial population mass increase, as stage I and stage II rediae (perhaps produced

during the cold winter months, as described for young rediae of Fasciola hepatica in Lymnaea truncatula by Kendall (1964)) develop into stage III rediae (Rees, 1980). This annual redial growth could possibly be a most important factor in the small annual growth increments observed in infested Thais lapillus.

The organ component analysis of infested and non-infested male and female T. lapillus demonstrated that proportionally the Parorchis acanthus redial population and the female ovary were similar; the main difference was that the parasite proportion of the total dry body mass of the host increased from spring to summer, whereas the ovary decreased in proportion from spring to summer, and began to rise again in the autumn. Only in the August collection were infested snails found to have a significantly higher mean proportion of parasite than females had ovary, and a significantly lower mean proportion of digestive gland than females. Male gonads made a proportionally smaller contribution to the total dry body mass of the whole snail whereas the digestive gland was proportionally larger than in the female and infested individuals. Despite the apparent advantages of having proportionally more digestive gland, and expending less energy on reproduction, male dogwhelks tend to be smaller than females, and Feare (1970a) noted that they had increased mortality rates, at least in their first year of adulthood (after which no age determination can be made reliably (Feare, 1970a, Coombs, 1973)). There may, as a result, also be an advantage to the parasite in infesting female rather than male dogwhelks because of the possible extended

life-span of female individuals.

The similarity of tissue caloric values for the same, or comparable, components of infested and non-infested male and female dogwhelks indicates that the presence of Parorchis acanthus rediae does not cause an overall depletion of tissue nutrients. This is a similar result to that of Burky and Hornbach (1979) for Leucochloridium variae in Succinea putris. The same seasonal differences in tissue energies are exhibited by both infested and non-infested snails, as well as the P. acanthus rediae, which do not possess significantly lower caloric values than the host visceral tissue. This result is contrary to the findings of Calow and Jennings (1974) that parasitic helminths have lower caloric values than free living helminths.

The metabolism of Thais lamellosa was originally thought to be mainly dependant on lipid (Stickle and Duerr 1970), but Stickle (1971, 1973) subsequently concluded that protein was in fact the most important respiratory substrate in both normal conditions, and during starvation, and that lipid was used as a reproductive energy source. Starving T. lapillus were also found to have a mainly protein based metabolism (Stickle and Bayne, 1982). The partioning of lipid into reproduction by non-infested female T. lapillus may explain the reduced lipid content of infested dogwhelks and decreased visceral caloric values.

The steeper incline of the size/oxygen uptake (metabolic rate) relationship for infested dogwhelks as compared with non-infested males and females, may be the result of the

relatively large rediae of Parorchis acanthus utilizing aerobic respiration (McDaniel and Dixon, 1967), the oxygen for which is available to them because, characteristically in infestation with large rediae (Rees, 1936) they do not severely hinder the flow of haemolymph through the snail tissues. Thus the size/oxygen uptake relationship of a sample of infested Thais lapillus represents a combination of T. lapillus and P. acanthus rediae size/metabolic rate relationships. The change in the above relationship for infested as opposed to non-infested dogwhelks is most probably less important to them than the other effects of the infestation.

The overall conclusion drawn from this investigation is that, although the distribution and expenditure of the resources of dogwhelks infested with P. acanthus differs from those of non-infested males and females, the infested animals do not appear to be subjected to excessive energy losses as a result of infestation. The fact that infested T. lapillus continue to grow, and that infestations with similar large redial stages can persist for many years in Littorina littorea (Rothschild, 1942) makes the conclusion of this study intuitively sensible. There can be no doubt that it is the castration of the gastropod host which allows both host and a comparatively heavy parasite population to exist together for any length of time.

REFERENCES

Anteson, R.K. and Williams, J.F., (1975). Selective depletion of hemolymph proteins of Biomphalaria glabrata infected with Schistosoma mansoni. J. Parasit., 61: 149.

Bailey, N.J.J., (1981). Statistical methods in biology, (Second edition). Hodder and Stoughton Ltd., Kent.

Baudoin, M., (1975). Host castration as a parasitic strategy. Evolution, Lancaster, Pa., 29: 335-352.

Bayne, C.J., (1968). Historical studies on the egg capsules of eight gastropod molluscs., Proc. malac. Soc. Lond., 38: 199-212.

Bayne, B.L. and Scullard, C., (1978a). Rates of oxygen consumption by Thais lapillus (L)., J. exp. mar. Biol. Ecol. 32: 97-111.

Bayne, B.L. and Scullard, C., (1978b). Rates of feeding by Thais (Nucella) lapillus (L).,Thais J. exp. mar. Biol. Ecol. 32: 113-129.

Becker, W., (1964). Der Einfluss von Trematoden auf den Gasstoffwechsel von Stagnicola palustris Mull., Z. Parasitkde., 25 (1): 77-102.

Becker, W., (1970). Untershungen uber die aus der Muttersporocyste von Schistosoma mansoni., 11. Die Wanderung dieser Stadien zur Mitteldarmdruse., Z. Parasitkde., 34: 226-241.

Becker, W., (1980). Metabolic interrelationship of parasitic trematodes and molluscs, especialy Schistosoma mansoni in Biomphalaria glabrata., Z. Parasitkde, 63 (2): 101-111.

Becker, W. and Hirtbach, E., (1975). Effect of starvation on total protein and hemoglobin concentration in the hemolymph of Biomphalaria glabrata. Comp. Biochem. Physiol. (A), 51: 15-16.

Becker, W. and Schmale, H., (1975). The nitrogenous products of degredation - ammonia, urea, uric acid - in the hemolymph of the snail Biomphalaria glabrata. Comp. Biochem. Physiol. (A), 51: 407-411.

Belisle, B.W. and Stickle, W.B., (1978). Seasonal patterns in the biochemical constituents and body component indexes of the muricid gastropod Thais haemastoma. Biol. Bull. mar. biol. Lab., Woods Hole, 155: 259-272.

Bertalanffy, L. von., (1957). Quantitative laws in metabolism and growth. Q. Rev. Biol., 32: 217-231.

Berry, A.J., (1962). The occurrence of a trematode larvae in a population of Littorina saxatilis (Olivi). Parasitology, 52: 237-240.

Blaber, S.J.M., (1970). The occurrence of a penis-like outgrowth behind the right tentacle in spent females of Nucella lapillus (L). Proc. malac. Soc. Lond., 39: 231-233.

Blackmore, D.T., (1969). Studies on Patella vulgata L. 11. Seasonal variation in biochemical composition. J. exp. mar. Biol. Ecol., 3: 213-245.

Bligh, E.G. and Dyer, W.J., (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: (8) 911-917.

Bourns, T.K.R., (1974). Carbohydrate and protein in Lymnaea stagnalis eggs and Trichobilharzia ocellata cercariae. J. Parasit., 60: No. 6.

Brand, T. von, and Files, V.S., (1947). Chemical and histochemical observations on the influence of Schistosoma mansoni infection on Australorbis glabratus. J. Parasit., 336: 476-482.

Brand, T. von, Rakestraw, N.W. and Renn, C.W., (1937). The experimental decomposition and regeneration of nitrogenous organic matter in sea water. Biol. Bull. mar. biol. Lab., Woods Hole, 72: 165-175.

Brand, T. von, Rakestraw, N.W. and Renn, C.W., (1939). Further experiments on the decomposition and regeneration of nitrogenous organic matter in sea and water. Biol. Bull. mar. biol. Lab., Woods Hole, 77: 285-296.

Brody, S., (1945). Bioenergetics and growth with special reference to the efficiency complex in domestic animals. Reinhold, New York.

Bryan, G.W., (1969). The effects of oil-spill removers ('detergents') on the gastropod Nucella lapillus on a rocky shore and in the laboratory. J. mar. biol. Ass. U.K., 49: 1067-91.

Bryant, C., (1972). The utilisation of carbon Z dioxide by Monizia expansa: aspects of metabolic regulation. In Van Der Bossche, H. (Ed.), Comparative Biochemistry of Parasites, Academic Press, New York: 417-432..

Burky, A.J. and Hornbach, D.J., (1979). Comparison of carbon and nitrogen content of infected and uninfected snails, Succinea ovalis and the trematode Leucochloridium variae. J. Parasit., 65 (3): 371-374,

Butler, P.A., (1953). The southern oyster drill. Proc. natn. Shellfish Ass., 44: 67-75.

Cable, R.M. and Martin, W.E., (1935). Parorchis avitus (Linton, 1914), a synonym of P. acanthus (Nicoll, 1906). J. Parasit. 21: 436-437.

Calow, P., (1978). Life cycles. An evolutionary approach to the physiology of reproduction, development and ageing. Chapman and Hall Ltd., London.

Calow, P. and Jennings, J.B., (1974). Calorific values in the phylum platyhelminthes: the relationship between potential energy, mode of life, and the evolution of entoparasitism. Biol. Bull. mar. biol. Lab., Woods Hole. 147: 81-94.

Carter, O.S. and Bogtish, B.J., (1975). Histologic and cytochemical observations of effects of Schistosoma mansoni on Biomphalaria glabrata. Ann. N.Y. Acad. Sci., 266: 380.

Cheng, T.C., (1971). Enhanced growth as a manifestation of parasitism and shell deposition in parasitized mollusks. In T.C. Cheng (Ed.), Aspects of the Biology of Symbiosis. Univ. Park Press, Baltimore, Maryland: 103-137.

Cheng, T.C. and Lee, F.O., (1971). Glucose levels in the mollusc Biomphalaria glabrata infected with Schistosoma mansoni. J. Invertebr. Pathol., 18: 395-399.

Cheng, T.C. and Snyder, R.W. Jr., (1962). Studies on host-parasite relationships between larval trematodes and their hosts. I. A review, II. The utilization of the hosts glycogen by the intra-molluscan larvae of Glypthelmins pennsylvaniensis Cheng, and associated phenomena. Trans. Am. microsc. Soc., 81: 209-228.

Cheng, T.C., Snyder, R.W. Jr., Rourke, A.W. and Blumenthal, A.B., (1966). Ionic calcium concentrations in non parasitized Nitocris dilatatus and those parasitized by the larvae of Prosthodendrium (Acanthatrium) anaplocami (Trematoda). Am. Zool., 6: 225.

Cheng, T.C., Sullivan, J.T. and Harris, K.R., (1973). Parasitic castration of the marine prosobranch gastropod Nassarius obsoletus by sporocysts of Zoogonus rubellus (Trematoda). Histopathology: J. Invertebr. Pathol. 21: 183-190.

Chernin, E., (1960). Infection of Australorbis glabratus with Schistosoma mansoni under bacteriologically sterile conditions. Proc. Soc. exp. Biol. Med., 105: 292-296.

Christie, J.D. and Foster, W.B., (1970). Effect of larval stages of Schistosoma mansoni on the albumen gland of Biomphalaria glabrata. J. Parasit., 56: 55-56.

Christie, J.D., Foster, W.B. and Stauber, L.A., (1974). ^{14}C-uptake by Schistosoma mansoni from Biomphalaria glabrata exposed to ^{14}C-glucose. J. Invertebr. Pathol., 23: 297-301.

Coelho, M.V., (1954). Acao das formas lavarias de Schistosoma mansoni sobre a reproducao de Australorbis glabratus. Publcoes avuls. Inst. Aggeu Magalhaes, 3: 39-50 (English summary 48-49).

Colman, J., (1933). The nature of the intertidal zonation of plants and animals. J. mar. biol. Ass. U.K., 18: 435-476.

Colton, H.S., (1922). Variation in the dog whelk Thais (Purpura) lapillus. Ecology, 3: 146-157.

Cooley, N.R., (1958). Incidence and life history of Parorchis acanthus, a digenetic trematode, in the southern oyster drill, Thais haemastoma. Proc. natn. Shellfish. Ass., 48: 174-188.

Cooley, N.R., (1962). Studies on Parochis acanthus (Trematoda:Digenea) as a biological control for the southern oyster drill, Thais haemastoma. Fishery Bull. Fish Wildl. Serv. U.S., 62: 77-91.

Coombs, V.A., (1973). Quantitative system for age analysis for dogwhelk Nucella lapillus. Proc. zool. Soc. Lond., 171: (Dec.) 437-448.

Cowell, E.B. and Crothers, J.H., (1970). On the occurrence of multiple rows of 'teeth' in the shell of the dog-whelk Nucella lapillus. J. mar. biol. Ass. U.K., 50: 1101-1111.

Crewe, W., (1951). The occurrence of Cercaria patellae Lebour (Trematoda) and its effect on the host; with notes on some other helminth parasites of British limpets. Parasitology, 41: 15-22.

Crisp, M., (1979). The effect of activity on the oxygen uptake of Nassarius reticulatus (Gastropoda, Prosobranchia). Malacologia, 18: 445-447.

Crisp, M., Davenport, J. and Shumway, S., (1978). Effects of feeding and of chemical stimulation on the oxygen uptake of Nassarius reticulatus (Gastropoda, prosobranchia). J. mar. biol. Ass. U.K., 58: 387-399.

Croll, N.A., (1966). Ecology of parasites. Harvard Univ. Press, Cambridge, Mass: 136.

Davenport, J., (1976). A technique for the measurement of oxygen consumption in small aquatic organisms. Lab. Pract., 25: 693-695.

Davison, J., (1955). Body weight, cell surface, and metabolic rate in anuran amphibia. Biol. Bull. mar. biol. Lab., Woods Hole, 109: 407-419.

Dinnick, J.A. and Dinnick, N.N., (1964). The influence of temperature on the succession of rediae and cercarial generations of Fasciola gigantica in a snail host. Parasitology, 54 (1): 59-65.

Donges, J., (1971). The potential number of redial generations in Echinostomatids (Trematoda). Int. J. Parasitol, 1: 51-59.

Duerr, G.F., (1967). Changes in the size-metabolic rate relationship of Lymnaea stagnalis appressa (Say) produced by digenetic trematode parasitism. Comp. Biochem. Physiol., 20: 391-398.

Etges, F.J., (1961a). Cercaria reynoldsi n.sp. (Trematoda: Echinostomatoidea) from Helisoma anceps (Menke) in Mountain Lake, Virginia. Trans. Am. microsc. Soc., 80 (2): 221-226.

Etges, F.J., (1961b). Contributions to the life history of the brain fluke of newts and fish, Diplostomulum scheuringi, Hughes, 1929 (Trematoda: Diplostomatidae). J. Parasit., 47: 453-458.

Etges, F.J. and Gresso, W., (1965). Effects of Schistosoma mansoni infection upon fecundity in Australorbis glabratus. J. Parasit., 51 (5): 757-760.

Farkas, T. and Herodek, S., (1964). The effect of environmental temperature on the fatty acid composition of crustacean plankton. J. Lipid Res., 5: 369-373.

Faust, E.C., (1924). The reactions of the miracidia of Schistosoma japonicum and S. haematobium in the presence of their intermediate hosts. J. Parasit., 10: 199-204, pl. 22.

Feare, C.J., (1969). The dynamics of a population of dogwhelks (Thais lapillus). Ph.D. Thesis, University of Leeds.

Feare, C.J., (1970a). Aspects of the ecology of an exposed shore population of dogwhelks (Nucella lapillus) (L). Oecologia, 5: 1-18.

Feare, C.J., (1970b). Reproductive cycle of the dogwhelk (Nucella lapillus) (L). Proc. malac. Soc. Lond., 39: 125-137.

Feare, C.J., (1971). The adaptive significance of aggregation behaviour in the dogwhelk Nucella lapillus (L). Oceologia, 7 (2): 117-126.

Geraerts, W.P.M., (1976). The control of growth by the neurosecretory light green cell hormone in the freshwater snail Lymnaea stagnalis. Gen. comp. Endocr., 29: 61-71.

Giese, A.C., (1969). A new aproach to the biochemical composition of the mollusc body. Oceanogr. mar. Biol. Ann. Rev., 7: 175-229.

Gilbertson, D.E., Etges, F.J. and Ogle, J.D., (1967). Free amino acids of Australorbis glabratus hemolymph: comparison of four geographic strains and effect of infection by Schistosoma mansoni. J. Parasit. 53: 565-568.

Gordon, R.M., Davey, T.H. and Peaston, H., (1934). The transmission of human bilharziasis in Sierra Leone, with an account of the life-cycle of the Schistosomes concerned, S. mansoni and S. haematobium. Ann. trop. Med. Parasit., 28: 323-418.

Gress, F.M. and Cheng, T.C., (1973). Alterations in total serum proteins and protein fractions in Biomphalaria glabrata parasitized by Schistosoma mansoni. J. Invert. Pathol., 22: 382-390.

Green, C.V. and Green, S.K., (1932). Shell growth in the periwinkle, Littorina littorea. Am. Nat., 66: 371-376.

Hayes, F.R., (1927). The effect of environmental factors on the development and growth of Littorina littorea. Trans. Nova Scotian Inst. Sci., 17: 6-13.

Hayes, F.R., (1929). Contribution to the study of marine gastropods. III Development, growth and behaviour of Littorina. Contr. Can. Biol. Fish., 4: 413-430.

Hemmingsen, A., (1950). The relation of standard (basal) energy metabolism to total fresh weight of living organisms. Rep. Steno. meml. Hosp., 4: 1-58.

Hughes, R.N., (1972). Annual production of two Nova Scotian populations of Nucella lapillus (L.). Oecologia, 8: 356-370.

Hurst, G.T. and Walker, C.R., (1935). Increased heat production in Poikilothermous animals in parasitism. Am. Nat., 69: 461-466.

James, B.L., (1965). The effects of parasitism by larval digenea on the digestive gland of the intertidal prosobranch Littorina saxatilis (Olivi) subsp. tenebrosa (Montagu). Parasitology, 55: 93-115.

Kemp, S. and Gravely, F.H., (1919). On the possible spread of Schistosomiasis in India. Ind. J. Med. Res. Calcutta, 7: 251-264.

Kendall, S.B., (1949). Nutritional factors affecting the rate of development of Fasciola hepatica in Lymnaea truncatula. J. Helminth., 23: 179.

Kendall, S.B., (1964). Some factors influencing the development and behaviour of trematodes in their molluscan hosts., in A.E. Taylor (Ed.) Host-Parasite Relationships in Invertebrate Hosts. Blackwell Scientific Publ., Oxford: 51-73.

Kendall, S.B. and Ollerenshaw, C.B., (1963). The effect of nutrition on the growth of Fasciola hepatica in its snail host. Proc. Nutr. Soc., 22: 41-46.

Kitching, J.A., Muntz, L. and Ebling, F.J., (1966). The ecology of Lough Ine. XV. The ecological significance of shell and body forms in Nucella. J. Anim. Ecol., 35: 113-126.

Køie, M., (1969). On the endoparasites of Buccinium undatum L. with special reference to the trematodes. Ophelia, 6: 251-279.

Krupa, P.L. and Bogtish, B.J., (1972). Ultrastructural phosphohydrolase activities in Schistosoma mansoni sporocysts and cercariae. J. Parasit., 58: 495-514.

Krupa, P.L., Lewis, L.M. and Delvecchio, P., (1975). Electron microscopy of anabain-inhibited potassium dependant transport adenosine triphosphatase activity in Schistosome sporocysts. Ann. N.Y. Acad. Sci., 226: 465-479.

Kurelec, B., (1975). Catabolic path of arginine and NAD regeneration in the parasite Fasciola hepatica. Comp. Biochem. Physiol. (B), 51: 151-156.

Lambert, P. and Dehnel, P.A., (1974). Seasonal variations in biochemical composition during the reproductive cycle of the intertidal gastropod Thais lamellosa (Gmelin) Gastropoda: Prosobranchia. Can. J. Zool., 52 (3): 305-318.

Largen, M.J., (1967). The diet of the dogwhelk Nucella lapillus (Gastropoda: Prosobranchia). Proc. zool. Soc., Lond., 151: 123-127.

Lauckner, G., (1980). Diseases of mollusca. In O. Kinne (Ed.) Diseases of marine animals. John Wiley and Sons, Interscience Publication: 311-424.

Lebour, M.V., (1907). On three mollusc-infesting trematodes. Ann. Mag. nat. Hist. (ser. 7) 19: 102-106.

Lebour, M.V., (1914). Some larval trematodes from Millport. Parasitology, 7: 1-11.

Lebour, M.V. and Elmhurst, R., (1922). A contribution towards the life history of Parorchis acanthus Nicoll, a trematode in the herring gull. J. mar. biol. Ass. U.K., 12: 829-832.

Lee, F.O. and Cheng, T.C., (1971). Schistosoma mansoni: respirometric and partial pressure studies in infected Biomphalaria glabrata. Expl. Parasit., 30: 393-399.

Lee, F.O. and Cheng, T.C., (1972). Schistosoma mansoni:- alterations in total protein and hemoglobin in the hemolymph of infected Biomphalaria glabrata. Expl. Parasit., 31: 203-216.

Lewis, J.R., (1964). The ecology of rocky shores. English Universities Press, London.

Lie, K.J., Heyneman, D. and Kostanian, N., (1975). Failure of Echinostoma lindoense to reinfect snails already harbouring that species. Int. J. Parasitol., 5: 483-486.

Linton, E., (1914). Notes on a viviparous distome. Proc. U.S. Natl. Mus., 7th Ser., 17 (102): 517-527.

Linton, E., (1928). Notes on trematode parasites of birds. Proc. U.S. Natl. Mus., 73 (1): 1-36 pls. 1-11.

Lookers, D.L. and Etges, F.J., (1979). Effect of Schistosoma mansoni infection on fecundity and perivitelline fluid composition in Biomphalaria glabrata. J. Parasitol., 65 (6): 880-885.

Lunetta, J.E. and Vernberg, W.B., (1971). Fatty acid composition of parasitized and non-parasitized tissue of the mud-flat snail, Nassarius obsoleta (Say). Expl. Parasit., 30: 244-248.

Lysaght, A.M., (1941). The biology and trematode parasites of the gastropod Littorina neritoides (L) on the Plymouth breakwater. J. mar. biol. Ass. U.K. 25: 41-67.

Manson-Bahr, P. and Fairley, N.H., (1920). Observations on Bilharziasis amongst the Egyptian Expeditionary Force. Parasitology, 12 (1): 33-71.

Marshall, I., McManus, D.P. and James, B.L., (1974). Glycolysis in the digestive gland of healthy and parasitized Littorina saxatilis rudis (Maton) and in the daughter sporocysts of Microphallus similis (Jäg) (Digenea: Microphallidae). Comp. Biochem. Physiol. (B), 49: 291-299.

McClelland, G. and Bournes, T.K.R., (1969). Effects of Trichobilharzia ocellata on growth, reproduction and survival of Lymnaea stagnalis. Expl. Parasit., 24 (2): 137-146.

McDaniel, J.S. and Dixon, K.E., (1967). Utilization of exogenous glucose by the rediae of Parorchis acanthus (Digenea: Philophthalmidae) and Cryptocotyle lingua (Digenea: Heterophyidae). Biol. Bull. mar. biol. Lab., Woods Hole, 133: 591-599.

McMahon, R.F. and Russell-Hunter, W.D., (1977). Temperature relations of aerial and aquatic respiration in 6 littoral snails in relation to their vertical zonation. Biol. Bull. mar. biol. Lab., Woods Hole, 132: 182-198.

McManus, D.P. and James, B.L., (1975a). Tricarboxcylic acid cycle enzymes in the digestive gland of Littorina saxatilis rudis (Maton) and in the daughter sporocysts of Microphallus similis (Jäg) (Digenea: Microphallidae). Comp. Biochem. Physiol. (B), 50: 491-495.

McManus, D.P. and James, B.L., (1975b). Anerobic glucose metabolism in the digestive gland of Littorina saxatilis rudis (Maton) and in the daughter sporocysts of Microphallus similis (Jäg) (Digenea: Microphallidae). Comp. Biochem. Physiol. (B) 51: 293-298.

McManus, D.P. and James, B.L., (1975c). Aerobic glucose metabolism in the digestive gland of Littorina saxatilis rudis (Maton) and in the daughter sporocysts of Microphallus similis (Jäg). Parasitkde., 46: 265-275.

Meakins, R.H., (1980). Studies on the physiology of the snail Biomphalaria glabrata (Say): effects of body size, temperature and parasitism by sporocysts of Schistosoma mansoni Sambon upon respiration. Comp. Biochem. Physiol. (A), 66: 137-140.

Meier, M. and Meier-Brook, C., (1981). Schistosoma mansoni: effect on growth, fertility and development of distal male organs in Biomphalaria glabrata exposed to miracidia at different ages. Z. Parasitkde, 66: 121-123.

Meuleman, E.A., (1972). Host-parasite interrelationships between the freshwater pulmonate Biomphalaria pfeifferi and the trematode, Schistosoma mansoni. Neth. J. Zool., 22 (4): 355-427.

Meyerhof, E. and Rothschild, M., (1940). A prolific trematode. Nature, Lond., 146: 367-368.

Moore, H.B., (1936). The biology of Purpura lapillus. Part I. Shell variation in relation to environment. J. mar. biol. Ass. U.K., 21: 61-89.

Moore, H.B., (1937). The biology of Littorina littorea. Part 1. Growth of the shell tisues, spawning, length of life and mortality. J. mar. biol. Ass. U.K., 21: 721-742.

Moore, H.B., (1938a). The biology of Purpura lapillus. Part II. Growth. J. mar. biol. Ass. U.K., 23: 57-66.

Moore, H.B., (1938b). The biology of Purpura lapillus. Part III. Life history and relation to environmental factors. J. mar. biol. Ass. U.K., 23: 67-74.

Moose, J.W., (1963). Growth inhibition of young Oncomelania nosphora exposed to Schistosoma japonicum. J. Parasit., 49: 151-152.

Morgan, P.R., (1972). The influence of prey availability on the distribution and predatory behaviour of Nucella lapillus (L). J. Anim. Ecol., 41 (2): 257.

Najarian, H.H., (1961). Egg-laying capacity of the snail Bulinus truncatus in relation to infection with Schistosoma haematobium. Tex. Rep. Biol. Med., 19 (2): 327-331.

Narayanan, R., (1978). Certain aspects of the impact of host-parasite relationship on the energy metabolism of a host. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India.

Narayanan, R. and Venkateswararao, P., (1980). Effect of xiphidiocercarial infection on oxidation of glycolitic and Krebs cycle intermediates in Lymnaea luteola. (Mollusca) J. Invertebr. Pathol., 36: 21-24.

Narayanan, R., Venkateswararao, P. and Damodaram Chowdary, V., (1982). Effect of xiphidiocercarial infection on the bioenergetic parameters of Lymnaea luteola. J. Invertebr. Pathol., 39: 277-280.

Newell, R.C., (1973). Factors affecting the respiration of intertidal invertebrates. Am. Zool., 13: 513-528.

Newell, R.C. and Northcroft, H.R., (1967). A re-interpretation of the effect of temperature on the metabolism of certain marine invertebrates. Proc. zool. Soc. Lond., 151: 277-298.

Newell, R.C. and Pye, V.J., (1970a). Seasonal changes in the effect of temperature on the oxygen consumption of the winkle Littorina littorea (L) and the mussel Mytilus edulis (L). Comp. Biochem. Physiol., 34: 385-397.

Newell, R.C. and Pye, V.J., (1970b). The influence of thermal acclimation on the relation between oxygen consumption and temperatue in Littorina littorea (L) and Mytilus edulis (L). Comp. Biochem. Physiol., 34: 367-383.

Newell, R.C. and Pye, V.J., (1971a). Temperature induced variations in the respiration of mitochondria from the winkle Littorina littorea (L). Comp. Biochem. Physiol., 40 (B): 249-261.

Newell, R.C. and Pye, V.J., (1971b). Variations in the relationship between oxygen consumption, body size and summated tissue metabolism in the winkle Littorina littorea. J. mar. biol. Ass. U.K., 51: 315-338.

Newell, R.C. and Pye, V.J., (1971c). Quantitative aspects of the relationship between metabolism and temperature in the winkle Littorina littorea (L). Comp. Biochem. Physiol., 38 (B): 635-650.

Nicoll, W., (1906). Some new and little-known Trematodes. Am. Mag. Nat. Hist., (ser. 7) 17: 513-527.

Nicoll, W., (1907). Parorchis acanthus, the type of a new genus of trematodes. Q. Jl. microsc. Sci., 51: 345-355.

Nolf, L.O. and Cort, W.W., (1933). On immunity reactions of snails to the penetration of the cercariae of the strigeid trematode Cotylurus flabelliformis (Faust). J. Parasit. 20 (1): 38-48.

Olivier, L. and Mao, C.P., (1949). The early larval stages of Schistosoma mansoni Sambon, 1907 in the snail host Australorbis glabratus (Say, 1818). J. Parasit., 35 (3): 267-275.

Orton, J.H., (1946). Biology of Patella in Great Britain. Nature, Lond., 158: 173-4.

Pan, C.T., (1963). Some biochemical and immunological aspects of host-parasite relationships. Generalized and focal tissue responses in the snail Australorbis glabratus, infected with Schistosoma mansoni. Ann. N.Y. Acad. Sci., 113 (1): 475-485.

Pan, C.T., (1965). Studies on the host-parasite relationship between Schistosoma mansoni and the snail Australorbis glabratus. Am. J. trop. Med. Hyg., 14: 931-976.

Parker, R.E., (1979). Introductory statistics for biology. (Second edition). Edward Arnold Ltd., London.

Pesigan, T.P., Hairston, N.G., Jauregui, J.J., Garcia, E.G., Santos, A.T., Santos, B.C. and Besa, A.A., (1958). Studies on Schistosoma japonicum infection in the Phillippines. 2. The molluscan host. Bull. Wld. Hlth. Org., 18 (4): 481-578. (French summary p 577).

Phillipson, J., (1964). A miniature bomb calorimeter for small biological samples. Oikos, 15 (1): 130-139.

Rees, G., (1937). The anatomy and encystment of Cercaria purpurae (Lebour 1911). Proc. zool. Soc. Lond., 107: 65-73.

Rees, G., (1939). Studies on the germ cell cycle of the digenetic trematode Parorchis acanthus Nicoll. Part I. Anatomy of the genitalia and gametogenesis in the adult. Parasitology, 31: 417-433.

Rees, G.,(1940). Studies on the germ cell cycle of the digenetic trematode Parorchis acanthus Nicoll. Parasitology, 32: 372-391.

Rees, G.,(1948). A study of the effect of light, temperature and salinity on the emergence of Cercaria purpurae Lebour, from Nucella lapillus (L). Parasitology, 38: 228-242.

Rees, G.,(1966). Light and electron microscope studies of the redia of Parorchis acanthus (Nicoll). Parasitology, 56: 589-602.

Rees, G.,(1967). The histochemistry of the cystogenous gland cells and cyst wall of Parorchis acanthus (Nicoll), and some details of the morphology and fine structure of the cercaria. Parasitology, 57: 87-110.

Rees, G., (1971). The ultrastructure of the epidermis of the redia and cercaria of Parochis acanthus (Nicoll). A study by scanning and emission electron microscopy. Parasitology, 62: 479-488.

Rees, G., (1980). Surface ultrastructure of the redia of Parorchis acanthus (Nicoll). (Digenea: Philophthalmidae).Z. Parasitkde., 63 (1): 33-46.

Rees, G., (1981). The ultrastructure of the epidermis of the redia of Parorchis acanthus (Nicoll) (Digenea: Philophthalmidae). Z. Parasitkde, 65: 19-30.

Rees, W.J., (1936). The effect of parasitism by larval trematodes on the tissue of Littorina littorea (Linne). Proc. zool. Soc. Lond., 2: 357-368.

Robson, E.M. and Williams, I.C., (1970). Relationships of some species of Digenea with the marine prosobranch Littorina littorea (L) 1. The occurrence of larval Digenea in L. littorea on the North Yorkshire coast. J. Helminth., 44: 153-168.

Robson, E.M. and Williams, I.C., (1971b). Relationships of some species of Digenea with the marine prosobranch Littorina littorea (L): III The effect of larval digeneans on the glycogen content of the digestive gland and foot of Littorina littorea. J. Helminth., 45: 381-401.

Rothschild, M., (1936). Gigantism and variation in Peringia ulvae (Pennant 1777), caused by infection with larval trematodes. J. mar. biol. Ass. U.K., 20: 537-546.

Rothschild, M.,(1939). A note on the life cycle of Cryptocotyle lingua (Creplin, 1825) (Trematoda). Novit. zool., 41: 178-180.

Rothschild, M.,(1941a). Observations on the growth and trematode infections of Peringua ulvae (Pennant 1777) in a pool in the Tamar Saltings, Plymouth. Parasitology, 33: 406-415.

Rothschild, M., (1941b). The effect of trematode parasites on the growth of Littorina neritoides (L). J. mar. biol. Ass. U.K., 25: 69-80

Rothschild, M., (1942). Seven years old infection of Cryptocotyle lingua in the winkle, Littorina littorea (L). J. Parasit., 28: 350.

Rothschild, A. and Rothschild, M., (1939). Some observations on the growth of Peringia ulvae (Pennant 1777) in the laboratory. Novit. zool., 41: 240-247.

Rubner, M., (1902). Die gesetze des energieverbrauchs bei der ernahrung. Deutche, Leipzig and Wien.

Russell-Hunter, W.E., Meadows, R.T., Apley, M.L. and Burky, A.J., (1968). On the use of a 'wet-oxidation' method for estimates of total organic carbon in mollusc growth studies. Proc. malac. Soc. Lond., 38: 1-11.

Sandeen, M.J., Stephens, G.C. and Brown, F.A., (1954). Persistent daily and tidal rhythms of oxygen consumption in two species of marine snails. Physiol. Zool., 27: 350-356.

Sandison, E.E., (1966). The oxygen consumption of some intertidal gastropods in relation to zonation. Proc. zool. Soc. Lond., 149: 163-173.

Scheerboom, J.E.M. and Dogterom, A.A., (1978). The effects of 'light green cells' and of the lateral lobes on growth and feeding of the pond snail Lymnaea stagnalis (L). Proc. K. ned. Akad. Wet. C., 81: 327-334.

Schilansky, M.M., Levin, L. and Fried, G.H., (1977). Metabolic implications of glucose phosphate dehydrogenae in two marine gastropods. Comp. Biochem. Physiol. (B), 56: 1-4.

Schmale, H. and Becker, W., (1975). Die ammonium-und harnstoff-konzentration in der hamolymphe von Biomphalaria glabrata unter dem einfluB einer infektion mit Schistosoma mansoni. Z. Parasitkde., 45: 237-241.

Schmale, H.B. and Becker, W., (1977). Studies on the urea cycle of Biomphalaria glabrata during normal feeding activity in starvation and with infection of Schistosoma mansoni. Comp. Biochem. Physiol. (B), 58: 321-330.

Senft, A.W., (1967). Studies in arginine metabolism by schistosomes II. Arginine depletion in mammals and snails infected with Schistosoma mansoni or Schistosoma haematobium. Comp. Biochem. Physiol., 21: 299-306.

Slobodkin, L.B. and Richman, S., (1961). Calories/gm in species of animals. Nature, Lond., 191: 299.

Sluiters, J.F., (1981). Development of Trichobilharzia ocellata in Lymnaea stagnalis and the effects of infection on the reproductive system of the host. Z. Parasitkde., 64 (3): 303-319.

Sluiters, J.F., Brussaard-Wust, C.M. and Meuleman, E.A., (1980). The relationship between miracidial dose, production of cercariae, and reproductive activity of the host in the combination Trichobilharzia ocellata and Lymnaea stagnalis. Z. Parasitkde., 63 (1): 13-26.

Sokal, R.R. and Rohlf, S.J., (1981). Biometry. (Second edition) Freeman, San Fransisco.

Staiger, H., (1954). Der chromosomendimorphismus bien prosobranchier Purpura lapillus in beziehung zur okologie der art. Chromosoma, 6: 419-478.

Staiger, H., (1957). Genetical and morphological variation in Purpura lapillus with respect to local and regional differentiation of population groups. Annee biol., 61: 251-8.

Stanislawsky, E. and Becker, W., (1979). Influences of semi-synthetic diets, starvation and infection with Schistosoma mansoni (Trematoda) on the metabolism of Biomphalaria glabrata (Gastropoda). Comp. Biochem. Physiol. (B), 63: 527-533.

Stanislawsky, E., Becker, W. and Muller, G., (1979). Alterations of free amino acid content in the hemolymph of Biomphalaria glabrata (Pulmonata) in starvation and after infection with Schistosoma mansoni (Trematoda). Comp. Biochem. Physiol. (B), 63: 477-482.

Stickle, W.B., (1971). Metabolic effects of starving Thais lamellosa immediately after spawning. Comp. Biochem. Physiol., 40 (3A): 627-634.

Stickle, W.B., (1973). The reproductive physiology of the intertidal prosobranch Thais lamellosa (Gmelin).I. Seasonal changes in the rate of oxygen consumption and body component indexes. Biol. Bull. mar. biol. Lab., Woods Hole., 144: 511-524.

Stickle, W.B., (1975). The reproductive physiology of the intertidal prosobranch Thais lamellosa (Gmelin). II. Seasonal changes in biochemical composition. Biol. Bull. mar. biol. Lab., Woods Hole, 148: 448-460.

Stickle, W.B. and Bayne, B.L., (1982). Effects of temperature and salinity on oxygen consumption and nitrogen excretion in Thais (Nucella) lapillus (L). J. exp. mar. Biol. Ecol., 58: 1-17.

Stickle, W.B. and Duerr, F.G., (1970). The effects of starvation on the respiration and major nutrient stores of Thais lamellosa. Comp. Biochem. Physiol., 33: 689-695.

Stunkard, H.W. and Cable, R.M., (1932). The life history of Parorchis avitus (Linton) a trematode from the cloaca of the gull. Biol. Bull. mar. biol. Lab., Woods Hole, 62: 328-338.

Stunkard, H.W. and Shaw, R.C., (1931). The effect of dilution of sea water on the activity and the longevity of certain marine cercariae, with descriptions of two new species. Biol. Bull. mar. biol. Lab., Woods Hole, 61: 242-271.

Sverdrop, H.V., Johnson, M.W. and Fleming, R.H., (1942). The oceans, their physics, chemistry, and general biology. Prentice-Hall, New York.

Tallmark, B. and Norrgren, G., (1976). The influence of parasitic trematodes on the ecology of Nassarius reticulatus (L) in Gullmar Fjord (Sweden). Zoon, 4: 149-156.

Veldhuijzen, J.P. and Van Beek, G., (1976). The influence of starvation and of increased carbohydrate intake on the polysaccharide content of various body parts of the pond snail Lymnaea stagnalis. Neth. J. Zool., 26: 106-118.

Veldhuijzen, J.P. and Cuperus, R., (1976). Effects of starvation, low temperature and the dorsal body hormone on the in vitro synthesis of galactogen in the albumen gland and the mantle of the pond snail Lymnaea stagnalis. Neth. J. Zool., 26: 119-135.

Vermeij, G.J., (1982). Phenotypic evolution in a poorly dispersing snail after the arrival of a predator. Nature, Lond., 299: 349-350.

Vernberg, W.B., (1969). Adaptations of host and symbionts in the intertidal zone. Am. Zool., 9: 357-365.

Vernberg, W.B. and Vernberg, F.J., (1963). Influence of parasitism on thermal resistance of the mud flat snail Nassarius obsoletus (Say). Expl.

Vernberg, W.B. and Vernberg, F.J., (1967). Interrelationships between parasites and their hosts. III. Effects of larval trematodes on the thermal metabolic response of their molluscan host. Expl. Parasit., 20: 225-231.

Wakman, S.A. and Renn, C.E., (1936). Decomposition of organic matter in sea water by bacteria. III. Factors influencing the rate of decomposition. Biol. Bull. mar. biol. Lab., Woods Hole, 70: 472-483.

Wesenberg-Lund, (1934). Contributions to the development of the Trematoda Digenea Part II. The biology of the freshwater cercariae in Danish freshwaters. D.K.D. Vidensk. Selsk. Skrifter, Naturv. og Math. Afd., 9 Raekke 3: 1-221.

Whitfield, P.J. and Evans, N.A., (1983). Parthenogenesis and asexual multiplication among parasitic helminths. Parasitology, 86: 121-160.

Williams E.E., (1964). Growth and distribution of Littorina littorea on a rocky shore in Wales. J. exp. mar. Biol. Ecol., 17: 47-58.

Williams, E.E., (1970). Seasonal variations in the biological composition of the edible winkle Littorina littorea (L). Comp. Biochem. Physiol., 33: 655-661.

Winfield, G.F., (1932). On the immunity of snails infested with the sporocysts of the strigeid, Cotylurus flabelliformis, to the penetration of is cercariae. J. Parasit., 19 (2): 130-133.

Wright, C.A., (1966). The pathogenesis of helminths in mollusca. Helminth. Abstr., 35 (3): 207-224.

Yamaguti, S., (1975). A synoptical review of life histories of digenetic trematodes of vertebrates. Published by Yagaku-Sha Ltd. Distributed by Keigaku Publishing Co. Ltd., Tokyo, Japan.

Zeuthen, E., (1953). Oxygen uptake as related to body size in organisms. Quart, Rev. Biol., 28: 1-12.

Zischke, J., (1967). Redial populations of Echinostoma revolutum developing in snails of different sizes. J. Parasit., 53: 1200-1204.

Zischke, J.A. and Zischke, D.P., (1965). The effects of Echinostoma revolutum larval infection on the growth and reproduction of the snail host Stagnicola palustris. Am. Zool., 5: 707-708.