

THE UNIVERSITY OF HULL

" Studies on host-parasite relationships of some species
of digenean larvae with the marine prosobranch Littorina
littorea L. "

being a Thesis submitted for the Degree of

Doctor of Philosophy

in the University of Hull

by

Edna May Robson, B.Sc.

June 1969

CONTENTS

Section	Page
I. INTRODUCTION	1
II. GENERAL TOPOGRAPHY AND ECOLOGY OF THE AREAS STUDIED	2
III. THE OCCURRENCE OF LARVAL DIGENEA IN <u>LITTORINA</u>	
<u>LITTOREA</u> L. ON THE NORTH YORKSHIRE COAST ..	14
Introduction	14
Historical Review	14
Material and Methods	16
Results	17
A. The species of Digenea infecting <u>Littorina</u>	
<u>littorea</u> L. on the North Yorkshire coast	17
B. Seasonal variations in the incidence of	
digenean infections of <u>Littorina littorea</u>	
at Scalby Rocks	29
C. Intensity of infection	44
D. A laboratory experiment to ascertain if	
naturally infected specimens of <u>Littorina</u>	
<u>littorea</u> L. lose their infection with time	44
E. An experiment to attempt infection of	
<u>Littorina littorea</u> with <u>Cryptocotyle lingua</u>	47
Discussion	50
IV. THE EFFECT OF SOME LARVAL TREMATODES ON THE BREEDING	
CYCLE AND SEX RATIO OF <u>LITTORINA LITTOREA</u> L. ..	60
Introduction	60
Material and Methods	60
Results	70
A. The annual reproductive cycle of <u>Littorina</u>	
<u>littorea</u>	70

CONTENTS

Section	Page
B. The sex ratio of <u>Littorina littorea</u> ..	85
Discussion	102
V. THE EFFECT OF TREMATODE LARVAE ON THE CARBOHYDRATE CONTENT OF THE FOOT AND HEPATOPANCREAS OF <u>LITTORINA</u> <u>LITTOREA</u>	115
Historical Review	115
The Seasonal Variation in the Glycogen Content of the Foot and Hepatopancreas of <u>Littorina</u> <u>littorea</u>	122
MATERIAL AND METHODS	
A. Collection and laboratory treatment of <u>Littorina littorea</u>	122
B. Isolation and hydrolysis of glycogen ..	125
C. Estimation of carbohydrate in the hydrolysate	130
D. Identification of sugars in the hydrolysate	138
RESULTS	139
Experiment I. The Effect of Storage on the Physiological Condition of <u>Littorina littorea</u> as Indicated by the Glycogen Content	163
Material and Methods	163
Results	164
Discussion	169
Experiment II, The Effect of Starvation on the Carbohydrate Content of the Foot and Digestive Gland of <u>Littorina littorea</u>	170
Material and methods	170
Results	171
Discussion	174

CONTENTS

Section	Page
Experiment III. <u>In vitro</u> experiment to ascertain if the Rediae of <u>Cryptocotyle lingua</u> and <u>Himasthla leptosoma</u> and the Sporocysts of <u>Cercaria A</u> were capable of digesting and/or Removing Glycogen from a Pure Glycogen Solution made up in sea water	177
Experiment IV. Quantitative Determinations of the Glycogen Content of Sporocysts of <u>Cercaria A</u> ..	178
DISCUSSION	180
VI. SUMMARY AND CONCLUSIONS	197
VII. ACKNOWLEDGEMENTS	201
VIII. REFERENCES	202
APPENDIX	i

I.

INTRODUCTION

The purpose of this work was to study the effects of larval digeneans on the seasonal carbohydrate content and the reproductive biology of Littorina littorea L. at Scalby Rocks, North Yorkshire. Observations on infection with the different species of larvae were also made throughout the survey.

There have been many studies on the effects of trematode larvae on the carbohydrate content of their molluscan hosts, but in the main these have been based on histochemical tests and at isolated periods of time (see review by Cheng & Snyder 1962a). It was thought therefore that this aspect of host-parasite relationships afforded itself for further study from a quantitative and seasonal aspect.

This work falls into three more or less distinct sections; with an additional one which deals with the topography and ecology of the collecting grounds. The geology of the area is included in this to give a more complete picture of the nature of the coast.

II. GENERAL TOPOGRAPHY AND ECOLOGY OF THE AREAS STUDIED

The common or edible periwinkle, Littorina littorea L. is one of the most abundant of intertidal gastropods and the largest of British periwinkles, occasionally attaining a height of 38 mm. (Fretter & Graham, 1962). During the present survey the largest specimen collected, a female infected with Cryptocotyle lingua, had a shell height of 42.9 mm. L. littorea is generally found between H.W.N.T. and L.W.E.S.T. (Fretter & Graham, 1962); occurrences below L.W.S.T. are confined to this species of Littorina but are rare. L. littorea is less tolerant of surf than Littorina saxatilis (Evans, 1947) and like Littorina littoralis its lower limit of vertical distribution is raised on surf swept shores. Its upper limit of distribution appears to be raised by splash (Moore, 1940) and it may occur at a lower level on stones and gullies than on a furoid covered shore.

L. littorea may extend up estuaries, apparently indifferent to the lowered salinity and frequent presence of pollution. It may occur in sheltered bays or shores exposed to the open sea, though it is infrequent on chalk and limestone rocks and on shores which are exposed to the full force of surf.

At low tide L. littorea tends to aggregate in damp sheltered places, clusters being frequently seen in shallow pools and rock depressions. In adverse conditions they tend to remain inert.

L. littorea feeds at high tide on calm days and at low tide if the substratum is not too dry and if the humidity is high (Thamdrup, 1935). Its food consists of microorganisms, young sporelings of weeds and other attached organisms. It will also eat decaying plant and animal matter.

The specimens of L. littorea used in this investigation were collected from four localities on the North Yorkshire coast, namely South Bay (Scarborough); Scalby Rocks; Scalby Pipe and Burniston Bay (Fig. 1). Scalby Pipe is immediately south of the small headland shown as Scalby Ness.

The North Yorkshire coastline immediately north and south of Scarborough is characterised by cliffs of soft boulder clay overlying horizontally bedded rocks of the Jurassic Period, the latter forming the actual sea shore. Scalby is typical of this formation, as are South Bay (Scarborough) and Burniston Bay, which lie south and north of Scalby respectively. The Jurassic rocks in these areas are nearly all estuarine deposits of sandstones and shales. Their appearance and physical constituents vary considerably within very short distances and the resultant differential weathering has led to the sea shore presenting a gnarled and ridged appearance at low tide.

Scalby

At Scalby, two areas, Scalby Rocks (Grid Ref. TA 038905) and

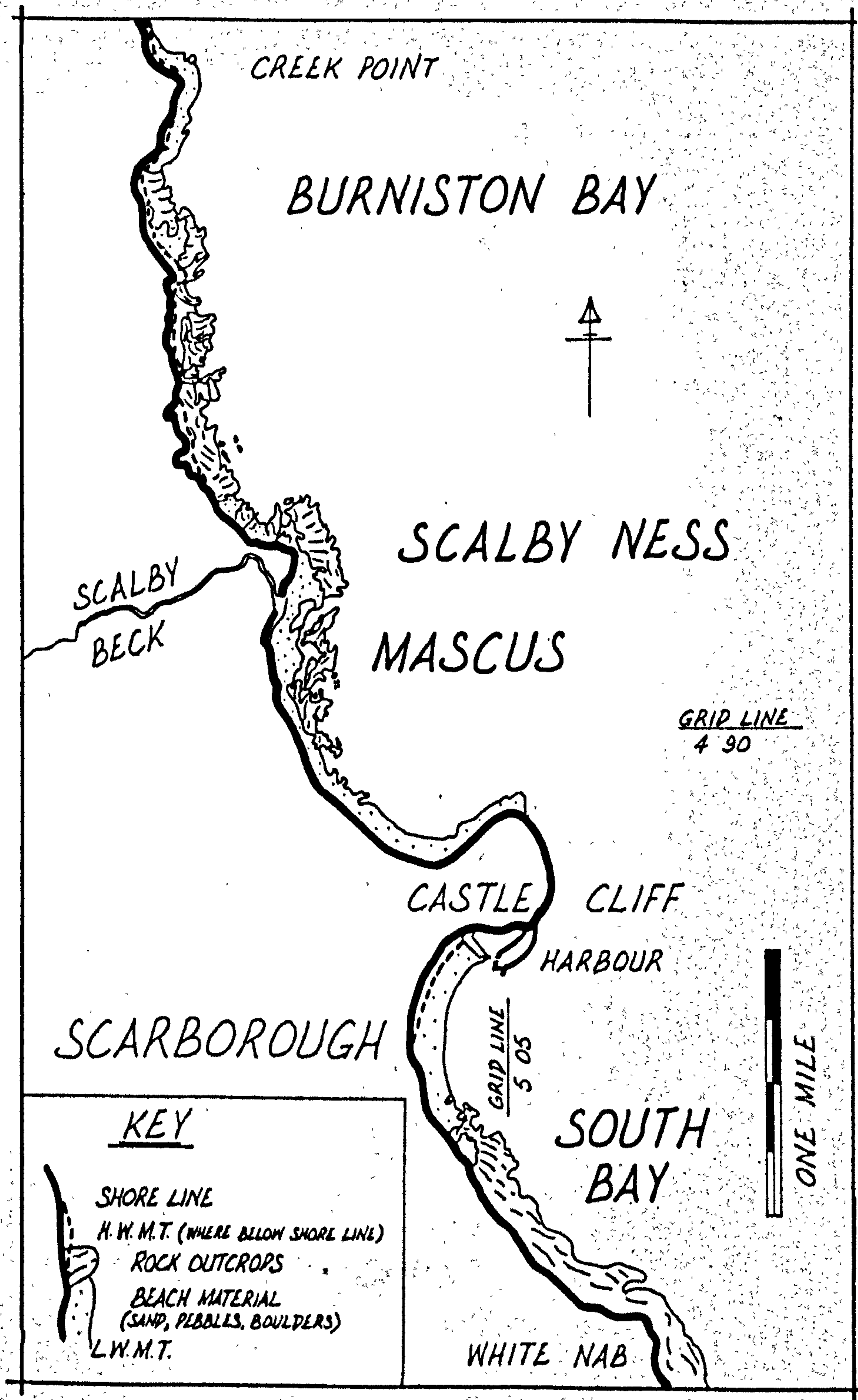


FIG 1 LOCATION OF COLLECTING GROUNDS

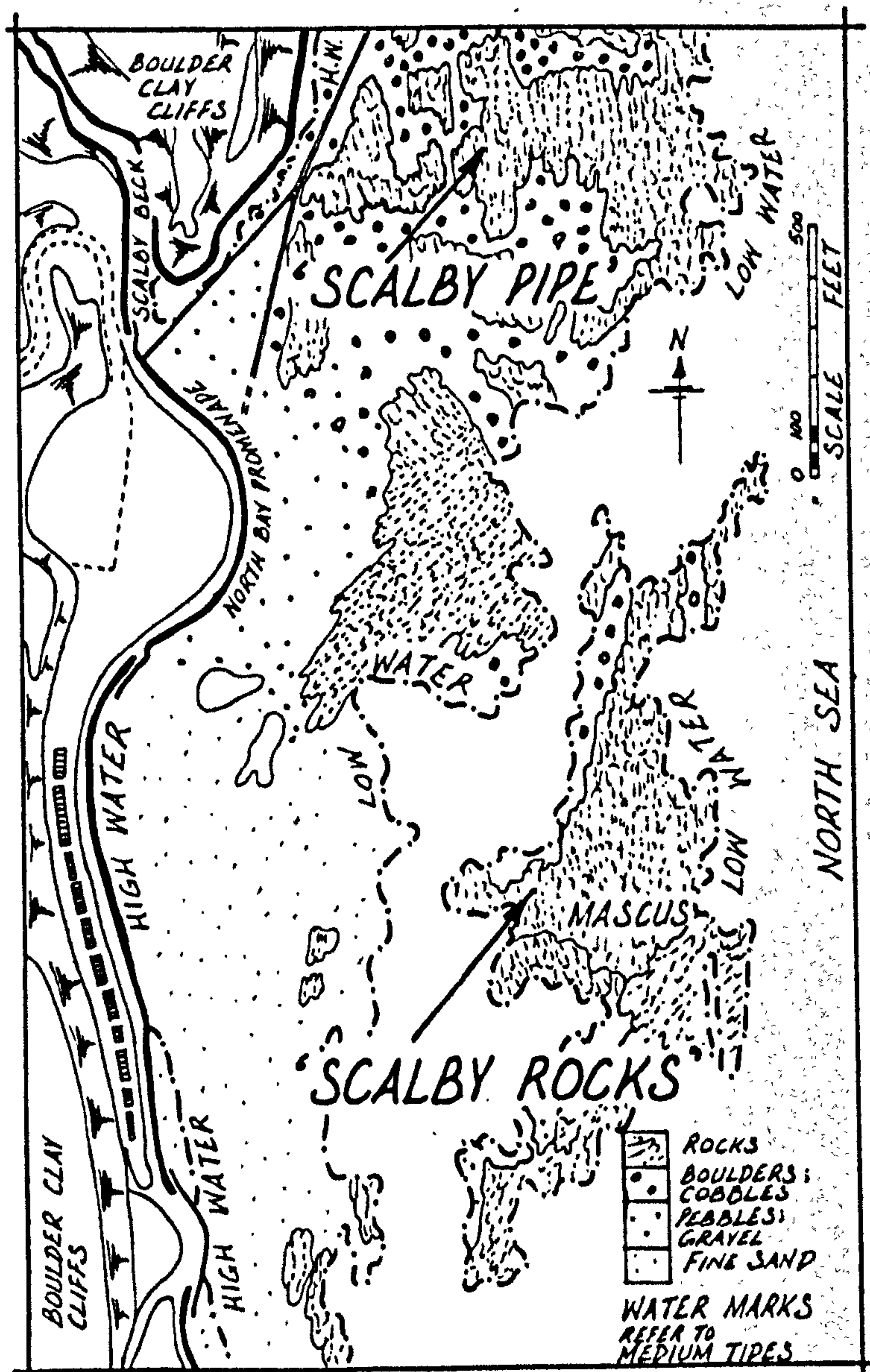


FIG 2 LOCATION OF
'SCALBY ROCKS' & 'SCALBY PIPE'

Scalby Pipe (Grid Ref. TA 038909) were selected for a preliminary investigation of the infection levels of L. littorea with digenean larvae. The location of the two collection areas is shown in Fig. 2. Scalby Rocks are named "Mascus" on Ordnance Survey maps. The unusual name "Mascus" possibly originated from the wrecking in that area of the coasting sailing vessel "Damascus" in the early part of the last century.

Mascus is approached from Scalby promenade by a beach which is alternately sandy and rocky. The rocks, or reefs, themselves rise steeply from the beach to a plateau which is effectively an island at most states of the tide, as it is accessible from land for about four hours only during spring tides, and at neap tides is cut off throughout the tidal cycle (Plates 1 and 2). All high tides completely submerge this plateau. Between the extremes of tide levels the view from the land is often that of a fairly flat island about 250 feet long parallel to the shore line and about 200 feet wide, but at low tide springs it can be seen that the rocks dip gently and continuously towards the sea in a north easterly direction. The rock surface on Mascus is heavily barnacled and sea lettuce (Ulva lactuca) occurs in sparse patches; to the seaward side of the plateau species of Fucus are prominent, as well as numerous young Mytilus edulis. The composition of the rock is almost entirely sandstone. Both calcareous and ferruginous

cementing materials are present and their alternations have probably caused the differential erosion which has led to the ridged appearance of the reefs (Plate 3).

The area on Mascus from which L.littorea were collected is within the plateau; however, being 2' 6" to 3' 6" lower, it forms a residual pool, when the tide exposes the rest of the plateau, of approximately 40 square yards of water surface, with an average depth of 6" to 8" and a maximum of 12" (Plate 4). This pool is littered with gravel, pebbles and shells, its sides and bed are stained with iron from the sandstone but closer examination shows the presence of a much softer, shaley, material than is seen elsewhere on the plateau, this no doubt being the reason the pool was formed.

Mascus Rock is not very resistant, as sandstones go, but it stands out from the beach by virtue of being much harder than any of its surrounding rocks, which are intercalations of grey calcareous ferruginous sandstone and grey mudstone. The sandstone, compared with Mascus Rock, contains a proportionately larger quantity of the soft calcareous matter and less of the more resistant iron minerals. They are nevertheless harder than the mudstone which is not compact enough to be called shale and is scarcely harder than the beach sand itself. Some pure calcareous matter is also evident in this area as small

patches of soft chalk. The beach sand contains a large amount of silt, especially further out to sea. This has built up since early 1968 due to the tipping of local excavations into the bay. In certain low areas, which form shallow channels for the outlet of Scalby Beck (Fig. 2), the sand is very soft and dirty, and impossible to walk on without sinking several inches below the surface. This again may be due to the tipping or it may be due to the saturation of the underlying mudstone.

The pattern of the shore to the landward side of Mascus Rock, then, is that of a sandy beach which slopes steeply away from the promenade and further out is punctuated by soft weathered grey sandstones. The beach sand lies quite thinly in places over an extremely soft mudstone which is also seen on the surface in a few areas.

Mascus, or Scalby Rocks, presents a slightly atypical area of study in that, as stated earlier, it is virtually an island. It is visited regularly by petrels, gulls and waders as a roost and feeding ground, especially when isolated from the shore. Disturbance by humans is negligible except at low tide and in any case is confined mainly to the short summer holiday season.

Scalby Pipe, 500 yards to the north of Scalby Rocks, is more accessible to humans than the latter. This area consists

of a rocky wave cut platform of similar nature to Mascus (or Scalby Rocks), but has a more broken boulder like appearance. The periwinkles in this area were somewhat smaller than those on Mascus, and their shells were often pitted and erroded.

The bay at Scalby is bounded on its south side by barren sandstone cliffs (Castle Cliff), about 200 feet high, on which stands the remains of Scarborough Castle. These cliffs are favourite nesting and roosting sites for a number of seabirds. The main colony consists of some one thousand plus pairs of kittiwake (Rissa tridactyla). These are present throughout the spring, summer and autumn but only occasional immature birds are seen during the winter months. The other two species which nest on the headland are the herring gull, Larus argentatus Pont. and the fulmar petrel, Fulmarus glacialis. There are about 50 pairs of herring gulls and 75 pairs of fulmars but the numbers fluctuate annually. In 1968 the breeding population of the headland reached saturation point and the herring gull was found nesting on several roof tops in Scarborough. The common gull, Larus canus, and the greater black-backed gull, Larus marinus, are also common around the headland but are non-resident. The common gull roosts on the coast and tends to feed inland and the greater black-backed gull is common around the harbour (to the south of Castle Cliff, Fig. 1) as a scavenger during the winter months.

The sea temperature referred to in this work was taken at the base of Mascus on its southerly seaward side.

South Bay, Scarborough (Grid Ref. TA 051871)

The boulder clay cliffs in this area are about 200 feet in height and are covered by thick foliage which has a stabilising influence against erosion as have the numerous winding footpaths leading to the sea shore, which form benches in the cliff side. In the more unsound areas small retaining walls have also been built, and a promenade runs along much of the shoreline. A sandy beach is present at the northern end of the bay but becomes sparse to non-existent a quarter of a mile south of the harbour where the Jurassic bedrock is exposed. Red brown ferruginous, heavily fissured, sandstones and grey siliceous sandstones are present, as well as very soft calcareous shalestones. All these outcrop at various points in thin horizontal beds. The harder siliceous sandstones are seen frequently to stand proud of their surround of shale or soft sandstone, but generally the sea shore is fairly flat with a gentle dip towards the sea. No reefs or sand banks are visible at any tidal state. The coast line at South Bay runs S.S.E. to N.N.W.

The area supports a large periwinkle population, similar in height to those at Mascus. This region is readily accessible to the public and is, therefore, favoured by very few sea birds.

Burniston Bay (Grid Ref. TA 028934)

Burniston Bay lies about three miles north of Scarborough. The cliffs, largely boulder clay, attain some 75 feet in height. The base of the cliffs are composed of thin alternating beds of fairly hard siliceous sandstone, decomposed sandstone and impure shalestone, the last type being severely shattered and forming a small scree at the foot of the cliff. These lower rocks are only a little more stable than the clay, but better coastal protection is provided beyond the cliff base by a small wave cut platform of quartzose sandstone. Beyond this ridge the sea shore outcrops are covered with varying thicknesses of non-indigenous boulders, cobbles and coarse sand and gravel. Again sandstone and shales prevail and their local variations seem still more acute than at Scalby or South Bay, Scarborough. Ferruginous and calcareous types of sandstone are found separately and together, and very hard deposits of quartzite stand out as severe ridges from the main rock. Ironstone is present, sometimes in large nodules, also specks of feldspar and thin bands of coal. The shalestones however are in less quantity here and are confined to low lying areas.

The shore line, which faces due east, bears out the erratic nature of the rocks in its rugged, ridged and nodular variations; these variations, however, are on a small scale, so that in relief the shore can be described as fairly flat.

The periwinkle population at Burniston was not very large, but the animals present were of a similar stature to those found on Mascus. Seabirds were not common, despite the relatively isolated nature of the habitat.

PLATE 1. Scalby Rocks (Mascus) at L W S T taken from
Scalby Ness (TA 036911) facing due south.
Mascus is seen as an island in the middle
distance.

PLATE 2. Scalby Rocks (Mascus) 2 hours after L W S T .

PLATE 3. Typical view of plateau of Mascus.

PLATE 4. Collecting pool at Mascus. White area in mid-
ground is one foot square.



PLATE 1



PLATE 2



PLATE 3

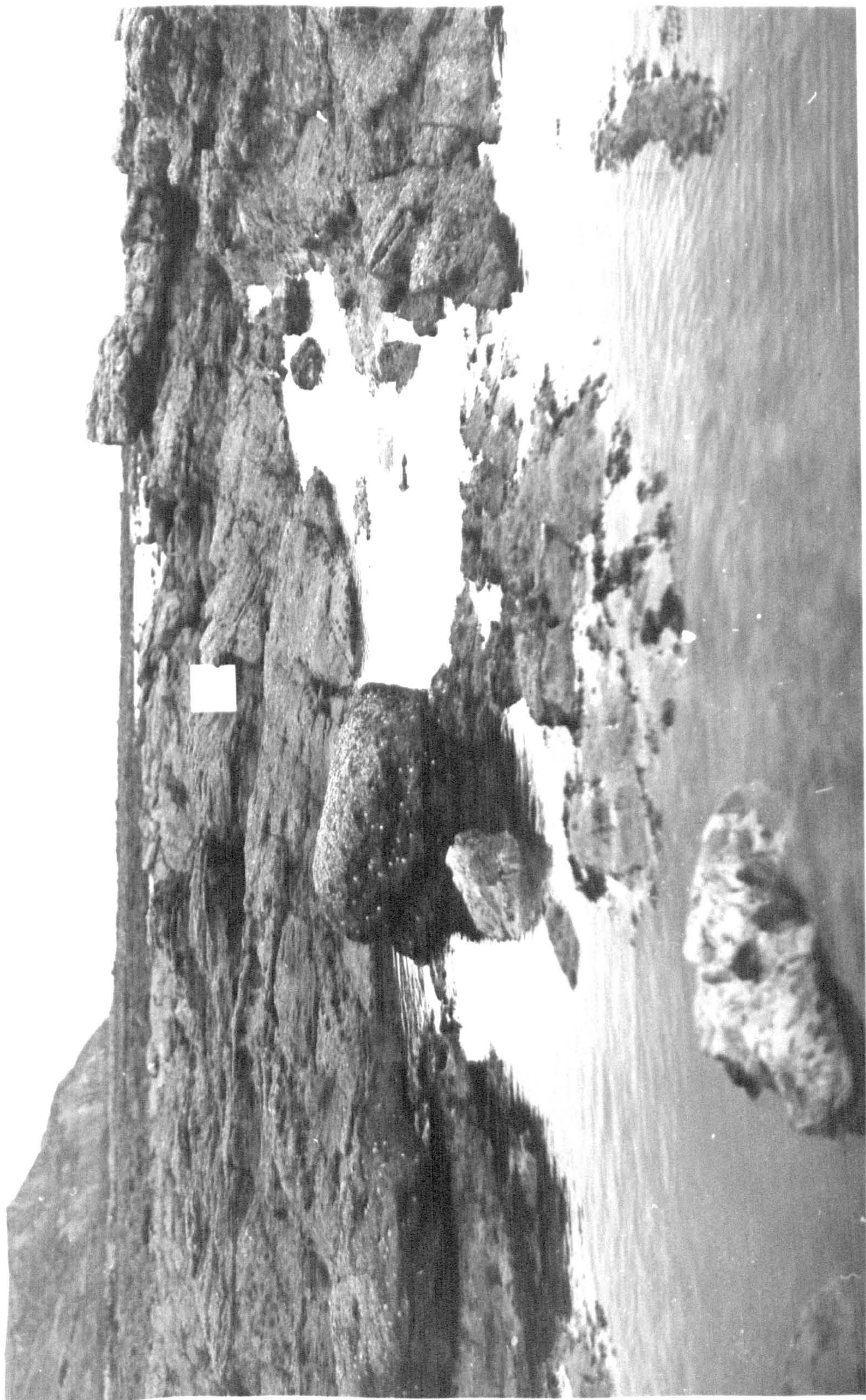


PLATE 4

III. THE OCCURRENCE OF LARVAL DIGENEA IN LITTORINA LITTOREA L.
ON THE NORTH YORKSHIRE COAST

INTRODUCTION

The present study was made in order to determine the different species of larval Digenea infecting one species of common intertidal mollusc, namely L.littorea, on the North Yorkshire coast, particular attention being paid to seasonal fluctuations in the incidence of infection. Only larvae undergoing asexual reproduction in the periwinkle, and thus using it as a primary intermediate host, were considered.

A preliminary survey of the incidence of infection of L.littorea with larval Digenea was made by taking samples of the periwinkle populations from four localities near Scarborough (Figs. 1 and 2). The survey showed the incidence of infection to be highest at Scalby Rocks (Tables 1 and 3) and this location was chosen for further study.

HISTORICAL REVIEW

There have been many reports on the occurrence of larval digeneans in littoral molluscs, but in the main these have been concerned with describing newly discovered or little known larvae. Major contributions on the morphology and taxonomy of marine cercariae have been made by Lebour (1907, 1908, 1911, 1914) for molluscs of the Northumberland and Scottish coasts; Rothschild (1935 a,b; 1936 a,b; 1937, 1938 a, b, c; 1941 a) at Plymouth

✓ TABLE 1. DIFFERENCES IN % INFECTION OF LITTORINA LITTOREA AT SOUTH BAY
(SCARBOROUGH), SCALBY PIPE AND BURNISTON BAY

	No. Exam.	% infected with:				Double Infs.	Total Parasitised
		<u>C.lingua</u>	<u>Cercaria A</u>	<u>H.leptosoma</u>	<u>C.lebouri</u>		
<u>SOUTH BAY</u>							
24.4.66	106	1.0	0.0	4.7	0.0	0.0	5.7
10.5.66	259	0.8	1.1	0.8	0.0	0.0	2.7
8.6.66	143	2.1	2.8	0.0	0.0	0.7	5.6
20.6.66	168	0.6	0.0	1.2	0.0	0.0	1.8
7.7.66	113	0.0	0.9	0.0	0.0	0.0	0.9
16.8.66	131	1.5	0.8	0.0	0.0	0.0	2.3
1.9.66	89	4.5	0.0	0.0	0.0	0.0	4.5
Total Obs.	1009	1.3	0.9	0.9	0.0	0.1	3.2
<u>SCALBY PIPE</u>							
5.2.66	81	0.0	1.2	4.9	0.0	0.0	6.1
20.4.66	253	1.2	7.5	5.9	0.0	0.4	15.0
1.6.66	164	4.9	0.6	2.4	0.0	0.0	7.9
7.7.66	126	6.3	3.2	1.6	0.8	0.0	11.9
16.8.66	121	12.4	14.9	3.3	0.0	1.6	32.2
30.8.66	126	5.6	7.9	4.0	0.0	0.0	17.5
2.11.66	323	10.2	19.8	3.7	0.0	1.9	35.6
Total Obs.	1194	6.2	9.8	3.8	0.1	0.8	20.7
<u>BURNISTON BAY</u>							
21.3.66	60	0.0	0.0	0.0	0.0	0.0	0.0
6.5.66	69	0.0	0.0	0.0	0.0	0.0	0.0
1.6.66	114	1.8	0.0	0.0	0.0	0.0	1.8
7.7.66	110	2.7	0.9	0.0	0.0	0.0	3.6
16.8.66	68	4.4	0.0	0.0	1.5	1.5	7.4
30.8.66	13	0.0	0.0	0.0	0.0	0.0	0.0
Total Obs.	434	1.9	0.2	0.0	0.2	0.2	2.3

and Naples; Sinitzin (1911) on the Black Sea; Palombi (1934, 1938, 1940) Gulf of Naples; Miller (1925 a,b) at Puget Sound, Washington and Dry Tortugas, Florida; Stunkard (1932, 1938, 1950, 1957, 1964, 1966 and 1968) at Woods Hole, Massachusetts and Roscoff, Finistère; Cable (1956, 1963) at Puerto Rico, Curacao and Jamaica; and Holliman (1961) at Apalachee Bay, Florida. Holliman's work also includes an extensive bibliography on the marine cercariae of the world.

However, there have been very few publications relating to the seasonal changes of infection with larval digeneans in marine molluscs. The major contributors are Miller & Northup (1926) for Nassa obsoleta at Woods Hole, Mass.; Sindermann & Farrin (1962) for Littoreia at Boothbay Harbour, Maine and James (1964, 1968 a) for Littorina saxatilis (Mont.) on the coast of Wales.

MATERIAL AND METHODS

Specimens were collected soon after low tide springs and after transporting to the laboratory they were kept in an aerated, circulating sea-water aquaria at about 10°C and supplied with Fucus sp., also gathered from Scalby Rocks. The animals were examined within 14 days.

The following records were made for each animal: the length of shell and breadth at the widest part of the body whorl, measured to 0.1 mm; sex and reproductive condition using the criteria of

Williams (1964) (see p.63); the species, intensity, state of maturity and location of digenean larvae.

L.littorea were divided into 6 size classes, (Classes A to F), with the following shell height ranges: up to 10.0 mm; 10.1 - 15.0 mm; 15.1 - 20.0 mm; 20.1 - 25.0 mm; 25.1 - 30.0 mm and animals greater than 30.0 mm. The monthly sample of each group varied and was dependant upon its abundance at the time of collection.

Shells were cracked with the aid of a vice and the specimens washed in filtered sea water and examined under a stereo-binocular dissecting microscope in order to find non-emerging cercariae and their parthenitae.

Representative specimens were fixed in Bouin's or Carnoy's (6:3:1) fluids, paraffin embedded and sectioned at 5 - 10 μ . They were then stained with Mallory's triple connective tissue stain or Erlich's haematoxylin and eosin for confirmation of location and intensity of infections and the amount of tissue damaged by the larvae.

RESULTS

A. The species of Digenea infecting Littorina littorea L. on the North Yorkshire coast

Four species of digenean larvae were recorded from Littorina littorea L. on the North Yorkshire coast, all of

which were readily distinguishable from each other. Their systematic classification follows that of Holliman (1961) in his check list of known marine cercariae. Holliman used La Rue's (1957) scheme of classification as a basis for a key to Superfamilies.

Systematic list of larval Digenea found in Littorina littorea L.

Anepitheliocystidia La Rue, 1957

Echinostomida La Rue, 1957

Echinostomata Szidat, 1939

Echinostomatoidea Faust, 1929

Echinostomatidae Looss, 1902

Himasthla leptosoma (Creplin
1829) Dietz 1909. (= H. secunda
= Cercaria Echinostomum secundum
(Nicoll, 1896) Dietz, 1909,
Lebour, 1911.

Paramphistomata Szidat, 1936

Notocotyloidea La Rue, 1957

Notocotylidae Lühe, 1909

Cercaria lebouri Stunkard, 1932

Epitheliocystidia La Rue, 1957

Opisthorchiida La Rue, 1957

Opisthorchiata La Rue, 1957

Opisthorchioidea Faust, 1929

Heterophyidae Odhner, 1914

Cryptocotyle lingua (Creplin,
1825) Fischoeder, 1903

Stunkard 1930; Syns: Cercaria
lophocerca, Lebour 1907
(nec. Filippi, 1857)

Plagiorchiida La Rue, 1957

Plagiorchiata La Rue, 1957

Plagiorchioidea Dollfus, 1930

Cercaria A

(a) Himasthla leptosoma is an echinostome cercaria which develops in redia in the haemocoel of the digestive gland and gonad of L. littorea. The life cycle includes a free living miracidium and cercaria, and many species of lamelli-branches serve as second intermediate hosts and shore birds as final hosts (Lebour, 1911). Hydrobia ulvae (Pennant) has also been recorded as a first intermediate host of H. leptosoma by Rothschild (1936 a) at Plymouth.

In heavy infections with H. leptosoma the visceral mass of L. littorea is a brilliant orange, and can be readily distinguished from uninfected specimens. The colour is imparted by the rediae which are of a bright pinkish-orange

when fully grown; very young rediae are colourless. In heavy infections very little host tissue is left and almost the whole visceral spire is occupied by rediae. When the rediae are mature they may be found invading the gills and haemal spaces of the mantle region.

The rediae of H.leptosoma are constantly changing shape and the cercariae may be distinguished from the other species found in this survey by the collar of 29 spines borne on the anterior end.

(b) Cercaria lebouri develops in rediae which occupy the interlobular lymph spaces (haemocoel) of the digestive gland of L.littorea. The cercariae, which are tri-occulate monostome notocotylids, emerge from the rediae before they are fully developed. The life cycle is unknown but the cercariae escape from the intermediate host and after a short free-living existence encyst on weeds, rock surfaces or on shells of molluscs. The adult probably occurs in a marine bird. James (1968 b) suggests that it may be the larval form of Catatropis verrucosa (Frölich) which occurs mainly in marine ducks and geese. Apart from L.littorea larval development also takes place in L.littoralis L., Littorina obtusata, Littorina neritoides L., and L.saxatilis.

C.lebouri is readily distinguishable from the other three

species by the presence of 3 deeply-pigmented eye spots and 2 ventrally situated locomotor pockets at the postero-lateral corners of the body.

(c) Cryptocotyle lingua is a heterophyid pleurolophocercous cercaria which develops in redia in the haemocoel of the digestive gland and gonad of L.littorea.

The life cycle includes a free-swimming cercaria. Many species of rock-pool and sub-littoral fishes act as second intermediate hosts and many marine birds, including gulls, as final hosts. Mammals, including dogs and cats, have also been recorded as final hosts (Stunkard 1930, Christensen & Roth 1948, Smyth 1962).

The appearance of a periwinkle infected with C.lingua is very unhealthy, the digestive gland being a sickly greyish-colour in mature infections and light brown in immature infections. As in infections with H.leptosoma, C.lingua feeds upon its host's gonad ultimately destroying it completely.

The rediae are colourless, and may be very active and constantly changing shape. In mature infections the rediae appear to be covered with minute black spots, corresponding to the eye spots of the enclosed cercariae. The latter are markedly phototactic. C.lingua is distinguishable from the

other 3 species by the paired eyes and the pleurolophocercous tail, which is about three times the length of the body.

(d) Cercaria A, an unidentified distomate xiphidocercaria, was the larvae most commonly found infecting L.littorea.

Specimens of L.littorea infected with Cercaria A are readily recognized when the shell is removed by the orange colour of the compact mass of sporocysts. Mature infections tend to be the more deeply pigmented, and, whereas light infections are confined to the region adjacent to the stomach, heavy infections are more widespread. When large the mass of sporocysts may cause protrusion of the body wall and the pressure set up may rupture the tunica propria. Histological examination showed that the region occupied by the parasite was virtually devoid of host tissue, only fragments of host tissue occurring between individual sporocysts. Host tissue adjacent to the mass showed signs of degeneration whilst unparasitized regions looked normal.

The location of the sporocysts suggest that infection is acquired by ingestion of the infective egg stage, which hatches into a miracidium in the stomach, thence migrating through the stomach wall on the outside of which it develops into a mother sporocyst.

Measurements were made on preserved material, living cercariae and sporocysts in 200 ml of sea water being killed by pouring into 200 ml of steaming 10% formalin (Holliman 1961). Specimens were measured under a coverslip with the minimum amount of fluid to prevent distortion from pressure.

When viewed in a mass the sporocysts are orange but are virtually colourless when examined separately. They are oval in shape, measuring 0.475 - 0.815 mm (average 0.689 mm) long and 0.195 - 0.439 mm (average 0.292 mm) wide. A birth pore, seen only when the cercariae were emerging, lies toward one end of the sporocysts. It is, however, possible that emergence of cercariae rupture the body wall of the sporocyst in this area. Eight to twenty cercariae (average 13) in different stages of development lie in the cavity of the sporocyst.

The cercariae are oval when viewed from above and in mature cercariae the body is virtually opaque being filled with dense cells, those at the periphery staining deeply with a 0.5% solution of nile blue sulphate in sea water. However, nile blue sulphate is toxic to the cercariae and death quickly ensues. The cercarial body measures 0.20 - 0.27 mm (average 0.24 mm) long and 0.08 - 0.11 mm (average 0.09 mm) wide. An acetabulum, only present in near mature cercariae, is seen as a prominent protruberance when viewed laterally. It lies just behind the mid-body and measures 0.04 mm in diameter, being

about the same size as, or slightly larger than, the oral sucker. The latter bears a simple pointed stylet (0.014 mm long) in its dorsal wall. The mouth is subterminal. Both oral and ventral suckers are surrounded by small pointed spines, and the body, but not the tail, is covered with minute, backwardly directed cuticular spines. The tail is recessed into the body and appears to be transversally striated, the transverse muscles being stronger than the longitudinal ones. The excretory vesicle is Y-shaped, with the arms reaching to the level of the anterior margin of the ventral sucker. The two main collecting ducts enter the stem of the vesicle. The connections of the flame-cells with the excretory collecting ducts have not been ascertained, but there are at least 12 flame-cells per side ($3 + 3 + 3 + 3$), and from the siting of what appear to be other flame-cells it seems probable that the formula will be $2(3 + 3 + 3 + 3 + 3 + 3) = 36$. Both the body and tail are very contractile and are constantly changing shape, but when fully extended the tail is almost as long as the body.

The body is contracted and bent ventrally when swimming and lashing of the tail drives the animal upward and forward in the water. When the tail is at rest the larva sinks to

the bottom. The cercariae do not seem to respond to light in any way. They are not strong swimmers and die without encysting on the bottom of containers.

Within the sporocysts the cercariae are very active but when the sporocysts are released in sea water their walls are relatively immobile. When mature, the cercariae are shed into the water, they then penetrate and encyst in their second intermediate host, which may be another mollusc or a crustacean.

The main feature differentiating this species from the other three found in L.littorea during this survey is the presence of the pointed stylet at its anterior end.

To date, experiments to determine the adult form of the xiphidocercariae have not been successful. L.littorea found, by isolation, to be shedding the xiphidocercariae were kept in a circulating sea water aquarium, and supplied with Fucus sp. from the 23rd May 1967 to 14th August 1968; during this time they regularly shed cercariae. In an attempt to find the second intermediate host several species of marine gastropods, including uninfected L.littorea, bivalves and crustaceans (mainly Cancer and Carcinus maenus) were kept in the same aquarium as the infected specimens of L.littorea. It was found that both uninfected L.littorea and those previously



infected with the xiphidocercariae acquired heavy metacercarial infections in the mantle and throughout the visceral mass.

Tissue of L.littorea containing these metacercariae were force fed to 3 herring gulls, Larus argentatus Pont., aged about 3 months, some 100 to 200 metacercariae being given to each gull. The birds were killed and examined 17, 23 and 31 days respectively after infection. All organs together with the alimentary canal were examined but no trematode infections were found. Prior to this experiment, metacercarial cysts were fed to laboratory hamsters, rats and mice of various ages, and ducklings and chicks. Two specimens from each group of experimental animals were autopsied 1, 2, 4 and 6 days after infection and others after 2, 4 and 6 weeks. Negative results were given by all animals. Controls were examined from each group with the exception of the experiment using gulls where only 3 gulls were available.

Thus no further light was shed on the life cycle. The cercariae were found to leave the first intermediate host, L.littorea, and penetrate L.littorea, L.saxatilis, L.littoralis and Carcinus maenus. The metacercariae were found in the haemocoel of the digestive gland and gonad of the molluscs, and in the haemocoel of the hepatopaneas of the crab.

The family relationship of this distomate plagiorchoid

xiphidocercaria is unknown. It is similar to Cercaria roscovita Stunkard, 1932, Cercaria parvicaudata Stunkard & Shaw, 1931 and Cercaria opaca Holliman, 1961, the adult stages of which have not been determined. It is also similar to the cercaria of Renicola thaidus Stunkard, 1964. The cercaria of these encyst in the gills, walls of the supra-branchial chambers, mantle and foot of Mytilus edulis and Pecten irradians. Stunkard fed these metacercariae to experimental hosts, but adults were only found in the kidneys of two baby gulls, Larus argentatus. He did not however think that gulls were the natural definitive hosts. From the adult characteristics, Stunkard places this cercaria in the family Renicolidae in the Plagiorchioidea. Table 2 compares the characteristics of these four cercariae with those of the present xiphidocercaria. Differences in size between them may result from conditions under which the observations were made, such as pressure of the coverslip.

The present cercaria seems to resemble C. parvicaudata the most closely. If this is the case it will be the first record to my knowledge of this species in Britain and indeed outside of Woods Hole, Massachusetts.

The literature abounds with spuriously named cercariae; because of this the xiphidocercariae will be referred to as Cercaria A until it has been positively identified with a

TABLE 2

Host	<u>Cercaria oprea</u>	<u>Remicola thaidus</u>	<u>Cercaria roseoviridis</u>	<u>Cercaria parvicaudata</u>	<u>Cercaria A</u>
	<u>Littorina littorea</u>	<u>Thais lanillus - T. lanellona</u>	<u>Littorina saxatilis microlinaea</u>	<u>Littorina littorea. L. saxatilis. L. oblongata</u>	<u>Littorina littorea</u>
Sporocyst	Orange coloured, .352-.556 (.462) long, .163-.291 (.222) wide	.5-1.26 long. .2-.4 wide, birth-pore at anterior end	Lemon coloured, .7-1.5 long, .25-.45 wide	Orange coloured, .3-.9 long, .15-.35 wide	Orange coloured, .475-.815 (.689) long, .195-.439 (.292) wide
Cercaria	.209-.240 (.228) long .097-.107 (.103) wide	.25-.38 long .06-.13 wide	.15-.3 long .06-.12 wide	.14-.36 long .06-.12 wide	.20-.27 (.24) long .08-.11 (.09) wide
Tail	.209-.275 (.222) long	.08-.13 long .029-.076 wide	.08-.3 long, subterminal	.06-.3 long, subterminal	.13-.17 (.15) long, .016-.021 (.02) wide, subterminal
Acetabulum	Post-central, .037-.058 (.037) in diameter	Post-central, .038-.043 in diameter	Post-central, .034-.05 in diameter	Post-central, .034-.05 in diameter	Post-central, .04 in diameter
Stylet	Rod-like with conical point, .009 long	Hastate, .008-.010 long	Simple, .016-.018 long, .002-.003 wide	Simple, .015 long, .0032 wide	Simple, .014 long
Cuticula	Covered with minute spines	Sharp pointed spines	Sharp pointed spines	Sharp pointed spines	Sharp pointed spines
Refractive granules	Pairly numerous scattered throughout body	Many in interstices between cystogenous glands	Very numerous in intercellular spaces	Very numerous in intercellular spaces	Very numerous throughout body
Excretory vesicle	Y-shaped	Y-shaped	Y-shaped, collecting ducts enter stem of Y	Y-shaped, collecting ducts enter stem of Y	Y-shaped, collecting ducts enter stem of Y
Flame-cell formula	Probably $2[(3+3) + (3+3)] = 24$	$2[(3+3+3) + (3+3+3)] = 36$	$2[(3+3+3) + (3+3+3)] = 36$	$2[(3+3+3) + (3+3+3)] = 36$	Probably $2[(3+3+3) + (3+3+3)] = 36$
Family		Remicolidae	Probably Plagiorchidae	Probably Plagiorchidae	
Locality	Salt marsh, St. Marks Light, Wakulla County, Florida	Woods Hole & Cape Cod, Mass.; Boothbay Harbor, Maine; Friday Harbor, Washington	Roscoff, Finistère	Woods Hole, Mass.	North Yorkshire Coast

NOTE: all measurements in millimeters

previously described cercaria or until its adult stage has been established by life history studies.

B. Seasonal variations in the incidence of digenean infections of Littorina littorea at Scalby Rocks

(i) All size classes

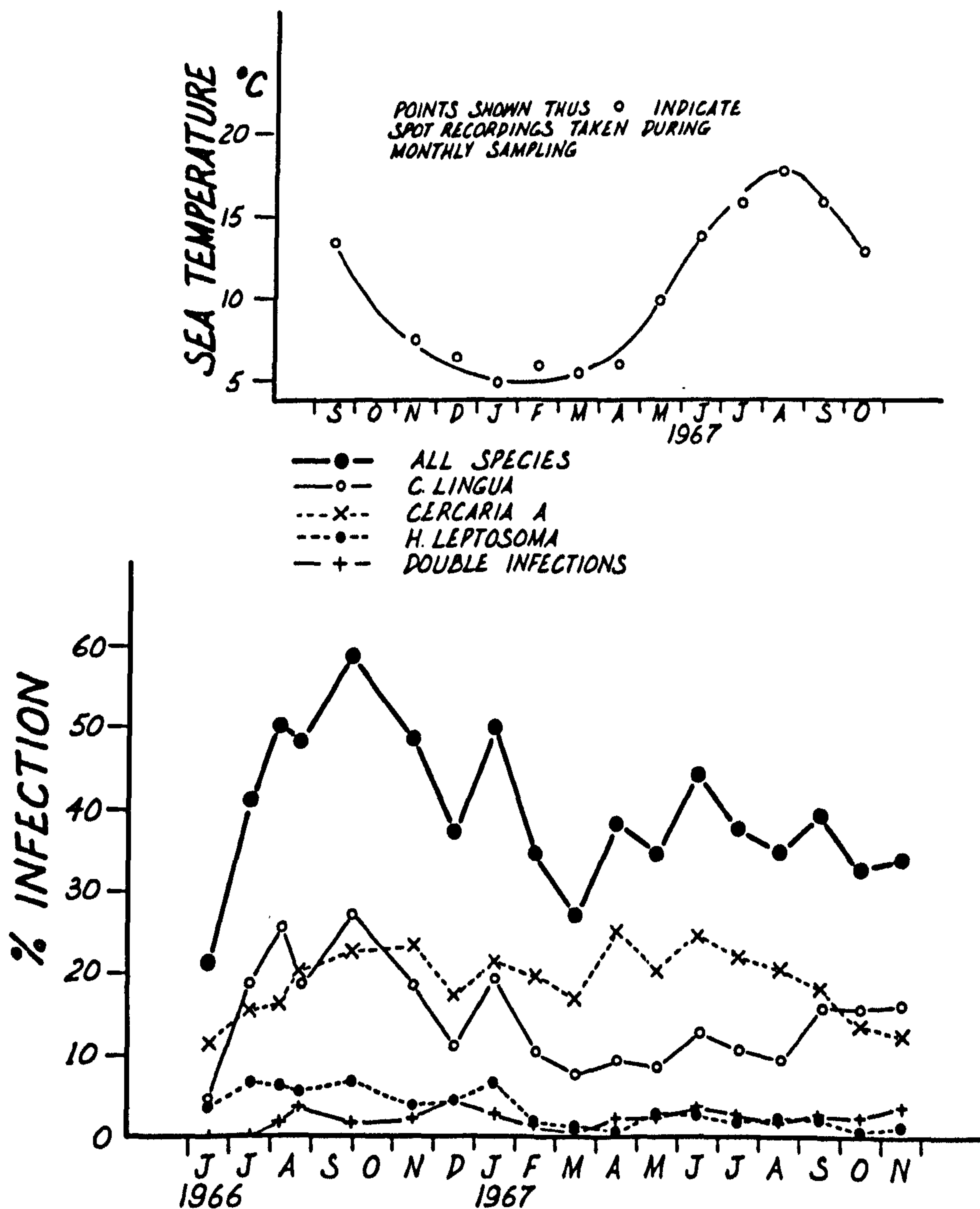
Of 5,975 specimens examined at intervals from February 1966 to November 1967, 2,363 or 39.5% were found to be parasitised with larval Digenea, comprising 19.5% infected with Cercaria A, 14.2% with C. lingua, 3.2% with H. leptosoma, 2.4% harbouring double infections, and 0.1% infected with C. lebouri, the identity of the remaining 0.1% was not recorded. The monthly fluctuations of infection are shown in Fig. 3 and Table 3.

Although the incidence of infection changed erratically, there was a peak in the incidence of total parasitised L. littorea during the period from August to November 1966 which reached 58.9% in September 1966, followed by a trough in March 1967 (27.2%) and a less pronounced peak in June 1967 (44.0%). The greater incidence of infection in 1966 reflected the influence of C. lingua and to a lesser extent that of H. leptosoma rather than Cercaria A (Fig. 3).

When the different species of larvae are considered separately the erratic nature of the incidences of infection are not quite so apparent.

TABLE 3. MONTHLY FLUCTUATIONS IN % INFECTION OF LITTORINA LITTOREA AT SCALBY ROCKS; MALES
AND FEMALES OF ALL SIZE CLASSES

Date	No. Exam.	% infected with:				<u>H. leptosoma</u>	<u>C. lebouri</u>	Double Infs.	Species Unrec.	Total Parasitised
		<u>C. lingua</u>	<u>Cercaria A</u>	<u>H. leptosoma</u>	<u>C. lebouri</u>					
5.2.66	97	10.3	0.0	1.0	0.0	0.0	0.0	0.0	3.1	14.4
1.6.66	104	4.8	11.5	3.9	0.0	0.0	0.0	0.0	0.9	21.1
7.7.66	133	18.8	15.8	6.8	0.0	0.0	0.0	0.0	0.0	41.4
16.8.66	216	25.9	16.2	6.5	0.0	0.0	1.9	0.0	0.0	50.5
31.8.66	175	18.9	20.0	5.1	0.6	0.0	3.4	0.0	0.0	48.0
20.9.66	394	27.4	22.6	6.9	0.0	0.0	1.8	0.2	0.0	58.9
2.11.66	313	18.5	23.0	3.8	0.0	0.0	3.5	0.0	0.0	48.8
1.12.66	289	11.1	17.3	4.5	0.0	0.0	4.5	0.0	0.0	37.4
12.1.67	423	19.2	21.0	6.6	0.0	0.0	2.8	0.0	0.0	49.6
11.2.67	304	10.5	19.7	2.0	0.3	0.0	2.0	0.3	0.0	34.8
14.3.67	408	7.6	17.2	1.5	0.2	0.0	0.7	0.0	0.0	27.2
10.4.67	341	9.7	25.2	0.9	0.0	0.0	2.3	0.0	0.0	38.1
9.5.67	475	8.6	20.4	2.7	0.2	0.0	2.5	0.2	0.0	34.7
6.6.67	436	12.8	24.5	3.0	0.0	0.0	3.7	0.0	0.0	44.0
10.7.67	568	10.9	22.2	1.9	0.0	0.0	2.4	0.0	0.0	37.5
7.8.67	361	9.7	20.8	2.2	0.3	0.0	1.9	0.0	0.0	34.9
4.9.67	353	15.9	18.1	2.8	0.3	0.0	2.6	0.0	0.0	39.7
2.10.67	353	15.6	13.6	0.8	0.3	0.0	2.3	0.0	0.0	32.6
2.11.67	232	15.9	12.5	1.3	0.0	0.0	3.9	0.0	0.0	33.6
Total Obs.	5975	14.2	19.5	3.2	0.1	0.0	2.4	0.1	0.0	39.5



✓ FIG 3 MONTHLY FLUCTUATIONS IN:
PERCENTAGE INFECTION OF L. Littorea
AND SEA TEMPERATURE

The xiphidocercaria, Cercaria A, was the most abundant of all species found infecting L.littorea at Scalby Rocks, accounting for over 50% of the parasitised animals, this total being made up of 1,165 single and 145 double infections.

The level of infection with Cercaria A remained fairly constant between September 1966 and August 1967, but there was a steady decline from September to November 1967 which did not repeat the trend observed during the corresponding period in the previous year when a slight increase occurred (Fig. 3).

The larval stages of C.lingua were found in 893 L.littorea, of these 47 occurred in combination with the larvae of another species. Infections with C.lingua show a much more pronounced cyclical pattern than those of Cercaria A, with the highest levels being found during the periods July - November 1966 and September - November 1967 with a seasonal minimum between December 1966 and August 1967 disturbed only by the surprisingly high incidence in January 1967 (19.2%). As with Cercaria A the 1967 peak was lower than that recorded in 1966 (Fig. 3).

The echinostome, H.leptosoma, was found in small numbers throughout the year with no pronounced peaks of infection. As with the former species the incidences were higher in 1966 than in 1967 (Fig. 3). Threlfall (1965, 1967) records the adult stage as occurring irregularly throughout the year in herring gulls from North Wales without any seasonal trend.

A fourth species found in L.littorea at Scalby Rocks, namely C.lebouri, was found only infrequently (Table 3); this may be because it normally parasites a different molluscan species, but when the opportunity arises it is capable of developing in L.littorea.

Of the specimens of L.littorea examined between August 1966 and November 1967, 145 or 2.4% were found to be harbouring the larval stages of two species of digenean trematodes (Fig.3 Table 3). When concurrent infections with two trematode species were encountered it was generally the case that one was more dominant than the other. In most of the double infections seen the more active redial stages of C.lingua or H.leptosoma tended to be the dominant member, while the sporocysts of Cercaria A were subordinate. The rediae of H.leptosoma were frequently seen browsing among the sporocyst masses of Cercaria A, and they were also found attached to the sporocysts by means of their powerful sucking pharynx. A similar situation has been reported in the fresh water gastropod Indoplanorbis exustus, where the sporocyst of Schistosoma spindale were devoured by rediae of Echinostoma malayanum (Heyneman & Umathevy 1968).

It was very rare for both members of a double infection to shed cercariae at the same time, although it has been seen

occasionally with H.leptosoma and Cercaria A. In most double infections it was usual for the sporocysts of Cercaria A to show signs of degeneration, even when Cercaria A was the dominant member. In infections where either H.leptosoma or C.lingua was dominant, the sporocysts of Cercaria A were often only visible as a fibrous, granular mass representing the remains of the degenerated sporocysts. The more sedentary nature of the sporocyst of Cercaria A, coupled with the lack of a pharynx probably accounts for its regression and the ensuing destruction by rediae of C.lingua or H.leptosoma. The rediae of the last two both have a pharynx and considerable locomotory power. In no case was a double infection recorded in which both members had a redial stage in their development.

Lie et al. (1968) attribute dominance of the redial stage to either (i) direct redial antagonism, by predation, or (ii) indirect antagonism and retardation. They considered that in many cases of double infections, predation was apparently more responsible than indirect antagonism for regression of the sporocysts of the subordinate species. The mechanism of indirect antagonism is not known, but Lie et al. (1968) suggest it may be caused by (a) the liberation of inhibitory substances by rediae or sporocysts, (b) by the production of inhibitory substances or cellular reaction by the snail host, (c) by

competition for nutrients, or (d) by other causes, yet unknown.

Of the 145 cases of double infections in L.littorea found at Scalby Rocks, 97 were infected with H.leptosoma and Cercaria A. Of these 97, the echinostome was dominant in 81 and subordinate in 3 cases, while in the remaining 13 infections it was judged that both parasites were of equal standing. Forty-seven double infections of C.lingua and Cercaria A were seen, in 44 of which C.lingua was dominant, 1 where Cercaria A was dominant and 2 where they were of equal standing. Only 1 infection of C.lebouri and Cercaria A occurring together was recorded.

As shown earlier the incidence of infection with H.leptosoma was 3.2% of 5,975 L.littorea and that of Cercaria A was 19.5% (Table 3). Hence, the expected percentage incidence of double infections with these two species occurring by chance at Scalby Rocks is $\frac{3.2}{100} \times \frac{19.5}{100} = 0.6\%$, whereas the observed incidence was 1.6%.

Similarly 14.2% of L.littorea were infected with C.lingua, and the expected percentage incidence of double infections with Cercaria A is $\frac{14.2}{100} \times \frac{19.5}{100} = 2.8\%$, and the observed incidence was 0.8%.

Only 0.1% of specimens were infected with C.lebouri and the expected incidence of double infections with Cercaria A is $\frac{0.1}{100} \times \frac{19.5}{100} = 0.02\%$, which was the proportion observed.

Thus, the observed incidence of double infections bore little resemblance to that expected by chance alone, the last case being inconclusive as only one such infection occurred. Of the other two combinations, C. lingua with Cercaria A occurred less frequently, and H. leptosoma with Cercaria A occurred more frequently than was predicted by chance alone, reflecting respectively instances of mutual antagonism and a "super-sensitivity" between different parasite combinations. The latter mechanism has been suggested by several workers among them Ewers (1960), Bourns (1963) and Bowers (1965) to account for instances in which the number of double infections were greater than predicted by chance. Cort et al. (1937) reported mutual antagonism between Diplostomum flexicaudum and Plagiorchis muris in Lymnaea^a emarginata.

(ii) Variations in percentage infection with host size

The incidences of infection of L. littorea with digenean larvae at Scalby Rocks with relation to host size are summarized in Fig. 4 and Table 4. Very few animals under 15.0 mm in length were infected but it should be noted that this size range comprises animals which have only recently settled on the shore together with slightly older animals which are attaining sexual maturity for the first time (i.e. "neuters", stage I and IIa animals). However, in animals exceeding 15 mm in length the trend was for the incidence of infection to increase steadily with

TABLE 4. NUMBER OF INFECTED LITTORINA LITTOREA OF EACH SIZE CLASS (MONTHLY DATA POOLED)

Shell height	No. Exam.	No. infected with:			<u>H.leptosoma</u>	<u>C.lebouri</u>	Double Infs.	Species Unrec.	Total Parasitised
		<u>C.lingua</u>	<u>Cercaria A</u>						
up to 10.0mm Class A	275	1	1		0	0	0	0	2
10.1-15.0mm Class B	334	2	2		0	0	0	0	4
15.1-20.0mm Class C	751	22	96		4	0	4	1	127
20.1-25.0mm Class D	3200	289	835		74	3	85	5	1291
25.1-30.0mm Class E	1203	397	221		88	4	46	0	756
above 30.0mm Class F	212	135	10		27	0	10	1	183
All size classes	5975	846	1165		193	7	145	7	2363

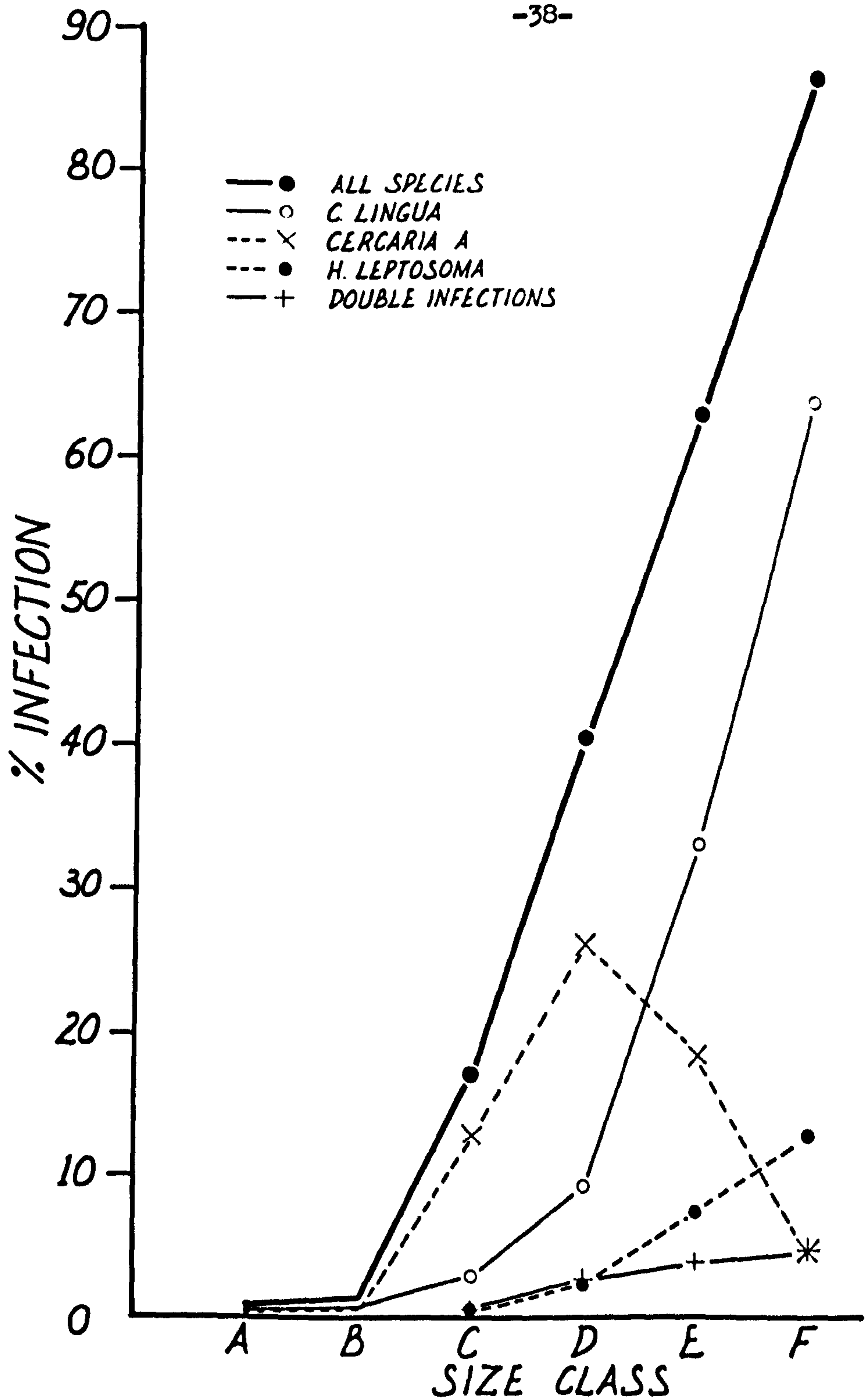


FIG 4 PERCENTAGE INFECTION
OF L. Littorea OF EACH SIZE CLASS
(MONTHLY DATA POOLED)

size reaching a level of 86.3% in Class F, the largest L.littorea examined.

It is clear from Fig. 4 that the individual species of parasites do not contribute equally to the incidence of infection in each host size-class. Thus, in animals greater than 15.1 mm but less than 25.1 mm Cercaria A was present in more L.littorea than either C.lingua or H.leptosoma, whereas in animals larger than 25.1 mm the incidence of infection with Cercaria A fell sharply with increase in the size of the host. On the other hand, the incidence of infection with C.lingua was fairly low in animals less than 25.1 mm (9.0%) and it was only in animals larger than this that C.lingua played a significant part in the proportion of infected periwinkles. In contrast to infection with Cercaria A, the proportion of animals infected with C.lingua increased steadily with size (Fig. 4).

A similar condition was also shown by H.leptosoma, a preference being shown for the larger animals with a peak incidence (12.7%) in animals of Class F. The situation was much the same regarding the prevalence of double infections, namely a greater proportion of double infections occurring in the larger animals.

(iii) Variations in percentage infection with sex

The seasonal levels of infection in male and female

**TABLE 5. MONTHLY FLUCTUATIONS IN % INFECTION OF LITTORINA LITTOREA AT SCALBY ROCKS; MALES
OF ALL SIZE CLASSES**

	No. Exam.	% infected with:		<u>C. lebouri</u>	<u>H. leptosoma</u>	<u>C. lebouri</u>	Double Infs.	Species Unrec.	Total Parasitised
		<u>C. lingua</u>	<u>Cercaria A</u>						
5.2.66	51	9.7	0.0	0.0	0.0	0.0	0.0	4.0	13.7
1.6.66	46	4.4	13.0	0.0	4.4	0.0	0.0	0.0	21.8
7.7.66	65	18.5	18.5	0.0	4.6	0.0	0.0	0.0	41.6
16.8.66	96	25.0	16.7	0.0	7.3	0.0	2.1	0.0	51.1
31.8.66	82	14.7	25.6	0.0	3.6	0.0	3.6	0.0	47.5
29.9.66	205	29.8	22.4	0.0	8.3	0.0	2.0	0.0	62.5
2.11.66	146	20.6	21.9	0.0	4.1	0.0	3.4	0.0	50.0
1.12.66	141	10.6	18.4	0.0	3.6	0.0	3.6	0.0	36.2
12.1.67	183	19.1	25.7	0.0	4.9	0.0	3.3	0.0	53.0
11.2.67	153	9.8	16.3	0.0	2.6	0.0	2.0	0.0	30.7
14.3.67	177	7.3	18.1	0.0	2.3	0.0	1.1	0.0	28.8
10.4.67	174	6.9	27.6	0.0	1.7	0.0	1.7	0.0	37.9
9.5.67	219	10.0	18.7	0.5	3.7	0.5	2.3	0.0	35.2
6.6.67	202	13.9	25.2	0.0	3.0	0.0	1.5	0.0	43.6
10.7.67	261	11.1	24.1	0.0	2.3	0.0	2.3	0.0	39.8
7.8.67	136	10.3	21.3	0.7	1.5	0.7	3.7	0.0	37.5
4.9.67	159	13.2	22.0	0.0	4.4	0.0	1.9	0.0	41.5
2.10.67	154	18.2	16.2	0.0	0.0	0.0	1.3	0.0	35.7
2.11.67	118	15.3	12.7	0.0	0.8	0.0	6.8	0.0	35.6
Total Obs.	2768	14.3	20.6	0.1	3.4	0.1	2.3	0.1	40.8

TABLE 6. MONTHLY FLUCTUATIONS IN % INFECTION OF LITTORINA LITTOREA AT SCALBY ROCKS;
FEMALES OF ALL SIZE CLASSES

No. / % infected with:		C. lingua		Cercaria A	H. leptosoma	C. lebouri	Double	Species	Total
Exam.							Inf.	Unrec.	Parasitised
5.2.66	42	9.5	0.0	2.4	0.0	0.0	0.0	2.4	14.3
1.6.66	55	5.5	10.9	3.6	0.0	0.0	0.0	1.8	21.8
7.7.66	64	20.3	12.5	9.4	0.0	0.0	0.0	0.0	42.2
16.8.66	117	27.4	16.2	6.0	0.0	0.0	1.7	0.0	51.3
31.8.66	89	23.6	15.7	6.8	1.1	3.4	0.0	0.0	50.6
28.9.66	170	27.6	24.7	5.9	0.0	1.8	0.0	0.0	60.0
2.11.66	143	18.2	25.9	4.2	0.0	4.2	0.0	0.0	52.5
1.12.66	122	12.3	20.4	6.6	0.0	6.6	0.0	0.0	45.9
12.1.67	211	21.8	16.6	8.5	0.0	2.8	0.0	0.0	49.7
11.2.67	138	12.3	24.5	2.2	0.0	2.2	0.0	0.0	41.2
14.3.67	207	8.2	18.8	1.0	0.5	0.5	0.0	0.0	29.0
10.4.67	141	14.9	25.5	0.0	0.0	3.5	0.0	0.0	43.9
9.5.67	208	8.2	26.4	2.4	0.0	3.4	0.5	0.0	40.9
6.6.67	210	13.8	25.7	3.8	0.0	5.2	0.0	0.0	48.5
10.7.67	278	11.9	22.7	1.8	0.0	2.9	0.0	0.0	39.2
7.8.67	194	10.3	23.7	3.1	0.0	1.0	0.0	0.0	38.1
4.9.67	161	18.0	17.4	1.9	0.0	3.1	0.0	0.0	40.4
2.10.67	189	14.3	12.2	1.6	0.5	3.2	0.0	0.0	31.8
2.11.67	101	18.8	13.9	2.0	0.0	1.0	0.0	0.0	35.7
Total Obs.	2828	15.4	20.4	3.6	0.2	2.7	0.2	0.2	42.3

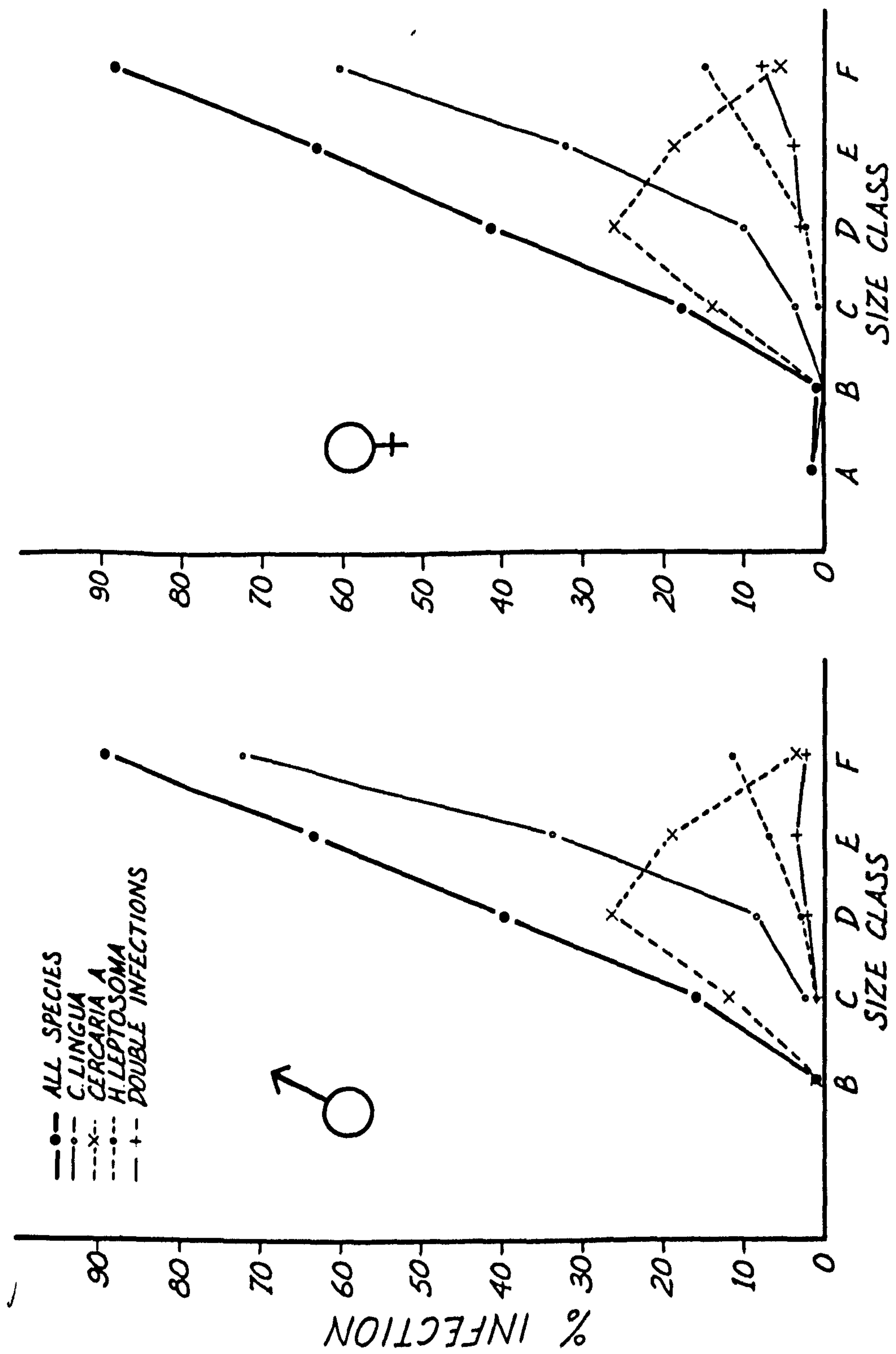


FIG 5 PERCENTAGE INFECTION OF *L. Littorea*
OF EACH SIZE CLASS, MALES AND FEMALES SEPARATELY
(MONTHLY DATA POOLED)

TABLE 7. NUMBER OF INFECTED LITTORINA LITTOREA OF EACH SIZE CLASS, MALES AND FEMALES SEPARATELY (MONTHLY DATA POOLED)

Shell height	No. Exam.	No. infected with:				Double Infs.	Species Unrec.	Total Parasitised
		<u>G. lingua</u>	<u>Cercaria A</u>	<u>H. leptosoma</u>	<u>C. lebouri</u>			
<u>MALES</u>								
up to 10.0mm Class A	20	0	0	0	0	0	0	0
10.1-15.0mm Class B	131	0	1	0	0	0	0	1
15.1-20.0mm Class C	396	9	47	3	0	4	0	63
20.1-25.0mm Class D	1563	127	413	41	2	38	2	623
25.1-30.0mm Class E	561	190	106	38	0	21	0	355
above 30.0mm Class F	97	70	3	11	0	2	0	86
All size classes	2768	396	570	93	2	65	2	1128
<u>FEMALES</u>								
up to 10.0mm Class A	55	1	0	0	0	0	0	1
10.1-15.0mm Class B	142	0	1	0	0	0	0	1
15.1-20.0mm Class C	341	12	47	1	0	0	1	61
20.1-25.0mm Class D	1558	156	408	33	0	45	2	644
25.1-30.0mm Class E	625	202	116	51	3	24	0	396
above 30.0mm Class F	107	65	6	16	0	8	0	95
All size classes	2828	436	578	101	3	77	3	1198

L.littorea of all size classes are summarized in Tables 5 and 6; on the whole both sexes tend to have similar cycles of infection. The overall pattern of infection with the different species for the different size classes was very similar for both males and females (Fig. 5 Table 7). To summarise, Cercaria A was much more prevalent in animals up to 25.1 mm, while C.lingua and H.leptosoma predominated in L.littorea larger than 25.1 mm.

C. Intensity of infection

The intensity of infection in parasitised animals was graded according to the proportion of host tissue affected, five levels being recognised, namely: very light, light, moderate to heavy, heavy and very heavy. Surprisingly, very few light infections were recorded. The intensity of infection of the majority of infected animals was generally moderate to heavy to very heavy. Levels of infection classified as light were generally immature. Thus, it is possible that some of the animals classified as uninfected were carrying light infections since it is difficult to find single sporocysts in the tissue of L.littorea.

D. A laboratory experiment to ascertain if naturally infected specimens of Littorina littorea lose their infection with time.

About 150 L.littorea were collected from Scalby Pipe on 23rd May 1967. On arrival in the laboratory they were placed

in individual 50 ml crystallising dishes and covered with bacteriologically filtered sea water, thus preventing the introduction of cercariae which may filter through with the sea water when paper is used. They were maintained at about 14°C, the water being changed daily. They were examined daily during the following week for cercarial emission.

By the end of the week 86 animals had shed cercariae - (49 C.lingua, 35 Cercaria A and 2 H.leptosoma). The infected snails were marked with marine paint on the tip of the shell, a different colour being used for each of the three species of Digenea and then transferred to a sea water aquarium (at about 10°C) and supplied regularly with Fucus sp. fronds until 6th December 1967. The Fucus had been collected from South Bay, Scarborough, to minimize the risk of introducing fresh infections - L.littorea at Scarborough being only sparsely infected (Table 1).

On 6th December 1967, after a period of six and a half months from collection, the animals were measured, removed from the shells and their tissues examined for the presence of larval digeneans.

The results are summarized in Table 8. Of the 64 animals alive at the end of the experiment 40 carried infections of C.lingua, 17 Cercaria A, and 3 had mixed infections of H.leptosoma and Cercaria A. Only 4 snails, which had previously shed

cercariae, were apparently uninfected.

TABLE 8. LABORATORY EXPERIMENT TO ASCERTAIN IF NATURALLY INFECTED SPECIMENS OF LITTORINA LITTOREA LOSE THEIR INFECTION WITH TIME

Shell height	No. Exam.	Uninfected	No. infected with			Double Infections
			<u>C. lingua</u>	<u>Cercaria A</u>	<u>H. leptosoma</u>	
15.1-20.0mm						
Class C	20	2	12	6	0	0
20.1-25.0mm						
Class D	34	2	20	9	0	[*] 2(E+X) 1(X+E)
25.1-30.0mm						
Class E	9	0	7	2	0	0
Above 30.0mm						
Class F	1	0	1	0	0	0
All size classes	64	4	40	17	0	2(E+X) 1(X+E)

* E = H. leptosoma; X = Cercaria A.

The leading letter is the dominant species.

On 2nd September 1967, 14 dead animals were removed from the aquarium - 11 of these had previously been recorded as shedding the xiphidocercaria, Cercaria A, and the other 3 those of C. lingua. Three of the dead animals infected with Cercaria A belonged to Class C (15.1 - 20.0 mm shell height) with all the remainder belonging to Class D (20.1 - 25.0 mm).

Thus, of the 78 animals examined, 8 of the original 86

having disappeared from the aquarium, only 4 were uninfected. Therefore, during the course of the experiment, lasting six and a half months, only 4 of the remaining 78 specimens had lost their infections.

It is worth emphasising that a larger number of animals infected with Cercaria A died off during the experiment than did L.littorea infected with C.lingua. This throws some light on the drop in the percentage of animals larger than 25.0 mm infected with Cercaria A (Fig. 4).

E. An experiment to attempt infection of Littorina littorea with Cryptocotyle lingua

L.littorea of various sizes were collected from South Bay, Scarborough (an area of low infection Table 1) on 23rd May 1967. On return to the laboratory they were isolated in crystallising dishes, about ten snails per group, and examined daily for shed cercariae over the next 10 days. L.littorea which had not shed cercariae during this period were divided into two groups and kept in circulating, aerated, sea water aquaria at about 10°C. They were supplied with Fucus also collected from South Bay. One of the groups was used as a control since it was possible that some specimens of L.littorea isolated as uninfected were carrying immature infections.

On 21st June 1967 about 50 adult specimens of C.lingua were obtained from a lesser black-backed gull and about another 30 from an immature herring gull chick, both birds coming from

Newborough Warren, Nature Reserve, Anglesey. The adult C. lingua were removed from their hosts' gut and washed well in bacteriologically filtered sea water to remove gut debris. The specimens were transported to the laboratory in bacteriologically filtered sea water, kept at about 18°C in an insulated container.

On the 22nd June the adult specimens of C. lingua were transferred to watch glasses containing bacteriologically filtered sea water, any contained eggs being transferred to fresh bacteriologically filtered sea water. The water was changed twice daily. The containers were kept in the laboratory where the temperature fluctuated daily between 18.5° and 22.0°C. The eggs were examined daily for development and on 4th July miracidia were very active within the eggs, embryonation thus having taken 12 days.

On 6th July L. littorea from the experimental group were placed in watch glasses containing the embryonated eggs, a minimum of 40 eggs per snail, and kept there without food for 7 days (the water being changed twice daily), after which they were returned to the sea water aquarium where food was available. By this time all the eggs had disappeared. Two specimens from this group were fixed in Bouin's fluid 2, 7, 21, 28 and 44 days respectively after exposure to the eggs.

The original purpose of the experiment was to establish an experimental infection of C.lingua in L.littorea and using these experimentally infected animals to study what effect infection with C.lingua had on the carbohydrate content and reproductive state of the host over a known period from initial infection. Unfortunately this aim could not be achieved because of the paucity of C.lingua eggs obtained. Instead the snails exposed to embryonated eggs of C.lingua were fixed and examined histologically in the hope of learning more about the sites of entry of the infective stages and their rate of development in L.littorea. After fixation the specimens were embedded in paraffin and serially sectioned at 10 μ . Representative slides were stained with Mallory's triple connective tissue stain in order to locate the sites of any infection which had been successfully established. However all snails were negative.

Earlier attempts to obtain a supply of eggs by establishing adult infections of C.lingua in laboratory hosts had been unsuccessful. Inshore fish (mainly butterflyfish and scorpion fish) were exposed to the cercariae of C.lingua and the resultant metacercariae were then fed to rats, mice, hamsters and chicks. However, results were negative.

DISCUSSION

The variations in the incidences of infection with larval Digenea in Littorina littorea from the four localities studied on the North Yorkshire coast were surprisingly large in view of their close proximity to each other. These local differences in incidence of infection may be related to: (i) the behaviour of the parasites' intermediate and/or final hosts, (ii) the effects of the environmental conditions on the susceptibility of the intermediate host to infection, or to their effects on the free-living stages of the parasite, or (iii) the physiological or genetical resistance of the intermediate host.

Of these possible causes the behaviour of the final host seems to be the most feasible explanation. At both the Scalby collecting areas (Figs. 1 and 2) the shore was usually covered with bird faeces and it was normal for large numbers of seabirds to be visiting these two areas. The birds (largely kittiwakes, herring gulls, fulmar petrels, common gulls and greater black-backed gulls) come from Castle Cliff, a short headland between Scalby (or North Bay, Scarborough) and South Bay, Scarborough. The public are warned off the cliff face because of its instability, hence the attraction as a roosting and nesting ground. Scalby Rocks was especially popular as a feeding ground when the tide was on the turn, isolating the area from the shore. In marked contrast, seabirds were seen only rarely at Burniston Bay and South Bay, Scarborough. Their absence is probably explained by the easy access for the public to these areas and the unsuitability of the cliffs for roosting.

Assuming the presence of seabirds accounts for the much higher incidence of infection of L.littorea at Scalby compared with that found at South Bay (Scarborough) and Burniston Bay; also that the accessibility of Scalby Pipe collecting ground to humans, by disturbing the final hosts (seabirds) of the digenean parasites, accounts for the lowered incidence of infection at Scalby Pipe (compared with Scalby Rocks), what factors contribute to the seasonal changes in infection found in the Scalby Rocks population of L.littorea?

The incidence will be influenced directly by the number of trematode infective stages in proportion to the number of suitable molluscan hosts. The susceptibility of the latter may vary with age, season and physiological state. The natural death of snails, or the accelerated death of infected specimens, could result in fluctuations in percentage infection. One would also expect a lowering of the incidence of infection at that time of the year when there is a larger number of juvenile L.littorea, that is towards the end of the breeding season (Fig. 8). Juveniles will not have been exposed to infection for as great a length of time as the rest of the population.

If the adult trematodes are present in the final host during all times of the year, then one would expect the larval stages to be present in the intermediate host throughout the year. However, the numbers of the infective stage may vary at different times of the year as a result of the seasonal activity of the gulls. The latter may

migrate or they may not use the shore as a feeding ground all the year round (as instanced by the herring gull, Larus argentatus, which commonly feeds inland during the winter months). The incidence and intensity of the adult parasite may also vary with season. This would result in fluctuations in the number of infective stages available in a given molluscan habitat.

Seasonal climatic changes may also contribute to variation in infection by acting on the parasite's free-living pre-molluscan stage, for example, extremes of temperature may kill the egg or miracidial stage or may alter the physiological or behavioural response of the larval stages.

A cyclical trend of infection of L.littorea with C.lingua was observed (Fig. 3) with peaks occurring between July and November 1966 and between September and November 1967, separated by a trough during spring and summer coinciding with the period when cercariae are emerging from the intermediate host. Sindermann & Farrin (1962) found that cercarial emergence ceased below 10°C. I found emergence ceased below 8°C. Sindermann (1964) found that during the winter months, infections of C.lingua in L.littorea regressed, so that mature infections were absent. A phenomenon he showed experimentally to be related to decreasing environmental temperature and in a limited way to light.

Sindermann & Farrin (1962) have also studied the larval ecology

of C.lingua in L.littorea at Boothbay Harbour, Maine, where they found peak incidences of infection between December and April and troughs for the warmer part of the year, corresponding to the period of greatest cercarial emergence. This is similar to the condition at Scalby Rocks except that I found the greatest incidence to occur during late autumn and early winter - this difference is possibly related to local climatic conditions.

Threlfall (1965, 1967) in his survey of the helminth fauna of the herring gull, Larus argentatus, in northern Caernarvonshire and Anglesey, found that the adult stages of C.lingua exhibited a heterogeneous distribution with an annual trough for the period June-July and a peak incidence in winter (October-November). We may reasonably suppose that a larger number of mollusc infecting stages occurs when a greater number of gulls are infected. If this is so we would expect a peak incidence of infection to occur in L.littorea a few weeks after the maximum level is attained for the adult stage, assuming the infective egg takes about two to three weeks to embryonate. Stunkard (1930) found that in the laboratory eggs embryonate in about 10 days when kept in sea water at 20°C, and I found that eggs which had been teased from the uteri of adult C.lingua took 12 days to embryonate when kept in sea water, the temperature of which fluctuated daily between 18.5°C and 22°C (p.48).

Discrepancies between the theoretical and observed peaks in the

incidences of infection of L.littorea with C.lingua might be explained by the ability of the different stages of the parasite to alter their length of development depending on temperature and other climatic conditions.

One theory put forward to account for seasonal variations in incidence of infection of molluscs with larval digeneans is that infected snails can lose their infection by shedding all their cercariae. A steady decrease in the percentage infection of snails (mainly Indoplanorbis exsustus) was found by Sewell (1922). He considered that this might be attributed to a certain percentage of infected snails ultimately recovering from the disease. Similarly Standen (1963) suggested that snails (Australorbis glabratus) infected with Schistosomes continue to shed cercariae for varying periods of time but that some did tend to lose their infection.

If this were the case regarding L.littorea and C.lingua, one would expect a lower incidence of infection to occur after the period of cercarial emission, but at Scalby Rocks a period of peak incidence follows. Moreover the results of my experiment (p.44) suggest that L.littorea do not lose their infection of C.lingua with time. Indeed Rothschild (1942) reported that a specimen of L.littorea infected with C.lingua was still shedding cercariae seven years after collection, with no opportunity for reinfection to have taken place. This implies that infections once acquired, persist for a considerable time, possibly until the death of the host. Cort (1941) found no evidence to

suggest recovery from an old infection with digenean larvae in any of the fresh-water snails examined by him. Kendall (1963) reports that loss of infection of Fasciola hepatica was observed in the abnormal host Lymnaea stagnalis, but not in the preferred host, Lymnaea truncatula.

The case of Lymnaea may be slightly different in that they tend to live for less than a year. Stirewalt (1954) found that at maintenance temperatures below the optimum A. glabratus tended to lose their infections of Schistosoma mansoni; at temperatures apparently optimal for development they retained their infections until death.

Sindermann & Farrin (1962) observed that L. littorea migrated from the high tide zone to the mid and low tide zones in late autumn and returned to the high tide zone in early spring; they suggested that infections with C. lingua immobilized the host and it was then unable to take part in seasonal migrations. They attributed the higher winter incidences of infection in the high tide zone to failure to migrate. This is amplified by the work of Lambert & Farley (1968) who showed experimentally that L. littorea infected with C. lingua still migrated but that they responded more slowly to the cold stimulus and migrated to a lesser extent.

Thus the early winter increase in incidence of infection observed at Scalby Rocks might have resulted from uninfected snails moving further seawards, and the decrease in incidence during winter and spring by uninfected animals returning to the area. However, the trend in peak

incidence does not coincide exactly with the lowest sea temperature (Fig. 3). This may be because in England (Smith & Newell 1955, Williams 1964) L.littorea do not seem to undergo such extensive migrations as have been reported for populations of L.littorea in North America, where the annual winter migration seawards is attributed to the more severe winter conditions (Gowanloch & Hayes 1926, Hayes 1929, Sindermann & Farrin 1962).

It was found that L.littorea infected with C.lingua were seldom capable of reproducing, and either exhibited characteristics of spent animals or else their reproductive state was not discernable - stage X - (Fig. 8). Thus, the peak levels of infection obtained in early winter corresponded to the time of the year when the uninfected animals had finished breeding and were in the spent condition (Fig. 8). It seems highly probable that infection with C.lingua is acquired when L.littorea is in this reproductive state. This is further supported by my observations (Fig. 4) that the proportion of animals infected with C.lingua increases with size. It is also during the winter that immature rediae are present in greatest numbers.

James (1968a) also observed that seasonal variations in percentage infection of Microphallus pygmaeus in adult hosts of Littorina saxatilis tenebrosa was inversely correlated with the breeding cycle. He also considered that this inverse correlation was related to the fact that only spent hosts were infected.

The incidence of infection of L.littorea with Cercaria A at Scalby Rocks remained fairly constant between September 1966 and August 1967. The presence of a fairly constant level of infection indicates that the mollusc infecting stage is continually present. The final host of the xiphidocercariae is unknown, but the adult may occur either in an avian host which visits the shore or it may occur in inshore fish. Until the adult stage is known it is difficult to surmise what effects the behaviour of the final host has on the pattern of infection incidence in the molluscan host.

From the data (Fig 8; Table 10) on the reproductive condition of L.littorea infected with Cercaria A, it is seen that the hosts, unlike those infected with C.lingua, still exhibited characteristics of mature spawning animals. This observation coupled with the relationship between percentage infection and the size of hosts infected with Cercaria A (Figs. 4 and 5) suggests that the hosts acquire their infections at a much earlier age, and, in contrast to infections of C.lingua, do not have to be in the spent state in order for an infection to be established. In fact, infection is probably acquired prior to or during the first breeding season.

A decrease in the proportion of L.littorea infected with Cercaria A at Scalby Rocks was found for animals larger than 25 mm (Figs. 4 and 5). This could be due to (i) an increased immunity with age, which has been suggested by some earlier workers or (ii) an increase in mortality of infected specimens as the parasite mass

grows in volume and causes more damage. Ameel (1934) observed a case of immunity increasing with age, small (about 1.0 mm) but not larger specimens of Pomatiopsis lapidaria (Sny) being susceptible to infection with Paragonimus. More recently Sogandares-Bernal (1965) reported that susceptibility of P.lapidaria in the 2.1 - 3.0 mm size range to infection with the miracidia of Paragonimus kellicotti Ward, 1908 was approximately double that of the 3.1 - 4.0 mm specimens. In my experiment conducted to see if L.littorea lost their infections of larval digeneans with time (p.44), a greater proportion of animals infected with Cercaria A died than did those infected with C.lingua. The majority of the hosts infected with Cercaria A which died belonged to size class D, 20.1 - 25.0 mm shell height. These facts suggest that the decrease in percentage infection with Cercaria A in animals greater than 25 mm resulted from death of infected snails rather than to age immunity, though the latter may still play some part in the observed relationship between host size and percentage infection. This aspect may be clarified further when the adult stage of Cercaria A has been found, and eggs are available for infection experiments.

From September 1967 until the end of the observations in November 1967, a steady decline in the percentage infection of L.littorea with Cercaria A was observed (Fig. 3) - this decline did not occur over the corresponding period of the previous year. For all species of digenean larvae the incidence of infection at the end

of 1967 was lower than the corresponding period in 1966. This may reflect changes in the final host, either in terms of its parasite burden or in its behaviour - it may no longer visit the area as frequently as before.

Thus, we see that the incidence of infection may be influenced by factors affecting the intermediate host, the final host and the free-living stages of the parasite, all these factors acting separately or in combination.

IV. THE EFFECT OF SOME LARVAL TREMATODES ON THE BREEDING
CYCLE AND SEX RATIO OF LITTORINA LITTOREA L.

INTRODUCTION

The reproductive cycle of a population of L.littorea on the Welsh coast has been studied by Williams (1964), and observations on the sex ratio reported by Pelsoneer (1926) and Moore (1937). The effects of parasitic digenetic trematode larvae on the gonad and digestive gland of L.littorea, as revealed by histological examination, have been described by Rees (1936). However, studies have not previously been directed towards the influence of larval digeneans on the reproductive biology of L.littorea. Observations were made, therefore, on the sex ratio and reproductive condition of a population of L.littorea so that the influence, if any, of natural infections with trematode larvae could be determined.

MATERIAL AND METHODS

(a) Population sampled

A survey of the reproductive cycle in a population of L.littorea was made by taking monthly samples from Scalby Rocks between September 1966 and November 1967. During the survey 14 collections were made, totalling 4,957 individuals, including animals from all the size classes classified earlier on p. 17, and consisting of 1,947 parasitised and 3,010

uninfected L.littorea. The animals were examined alive as soon as possible after collection, and in any case within one week. In addition to an assessment of the reproductive state, the height and width of the shell, and the presence and intensity of any digenean infection were recorded.

(b) Determination of reproductive condition

Several workers investigating the breeding cycle of various species of gastropod molluscs have recognised developmental stages. Moore (1937), Lysaght (1941) and Orton, Southward & Dodd (1956) analyzed the various reproductive stages that could be recognized from a macroscopic and, in some cases, histological examination of the animal. Although studying amphineurans and not gastropods, Tucker & Giese (1962) and Giese & Araki (1962) assessed the reproductive condition of their specimens by determining what they called the "gonad index" (that is the ratio of gonad volume to wet weight of animal $\times 100$) and by microscopic examination of sections and smears of the reproductive tissues. The use of the gonad index¹ and histological sections to determine the reproductive

1. Some authors (see review by Giese 1959) prefer to define the gonad index as i) ratio of gonad volume to wet weight of the animal $\times 100$; or ii) ratio of gonad weight to wet weight of the animal $\times 100$; or iii) ratio of gonad volume to volume of the animal.

state presents practical difficulties when fairly large numbers of animals are involved as both techniques are time consuming. In the case of L.littorea assessment of the reproductive condition using the gonad index is further complicated by the fact that the gonad is intermingled with the digestive gland and is not a discrete isolated organ. The use of quantitative factors such as length of penis and size of ova is also handicapped by the time required to make such an assessment especially when large numbers of specimens are studied.

The reproductive condition of animals used during the present study was assessed following the classification described by Williams (1964) for L.littorea in the Aberystwyth area. This method is based on an examination of the gross anatomy of the reproductive structures, the various reproductive states being readily recognizable. The anatomy of the reproductive structures of L.littorea has been described by Linke (1933, 1934) and more recently by Fretter & Graham (1962), thus only the main features will be described.

The sex of L.littorea is difficult to ascertain without removing the animal from its shell. The male is then distinguishable from the female by the presence of a coiled

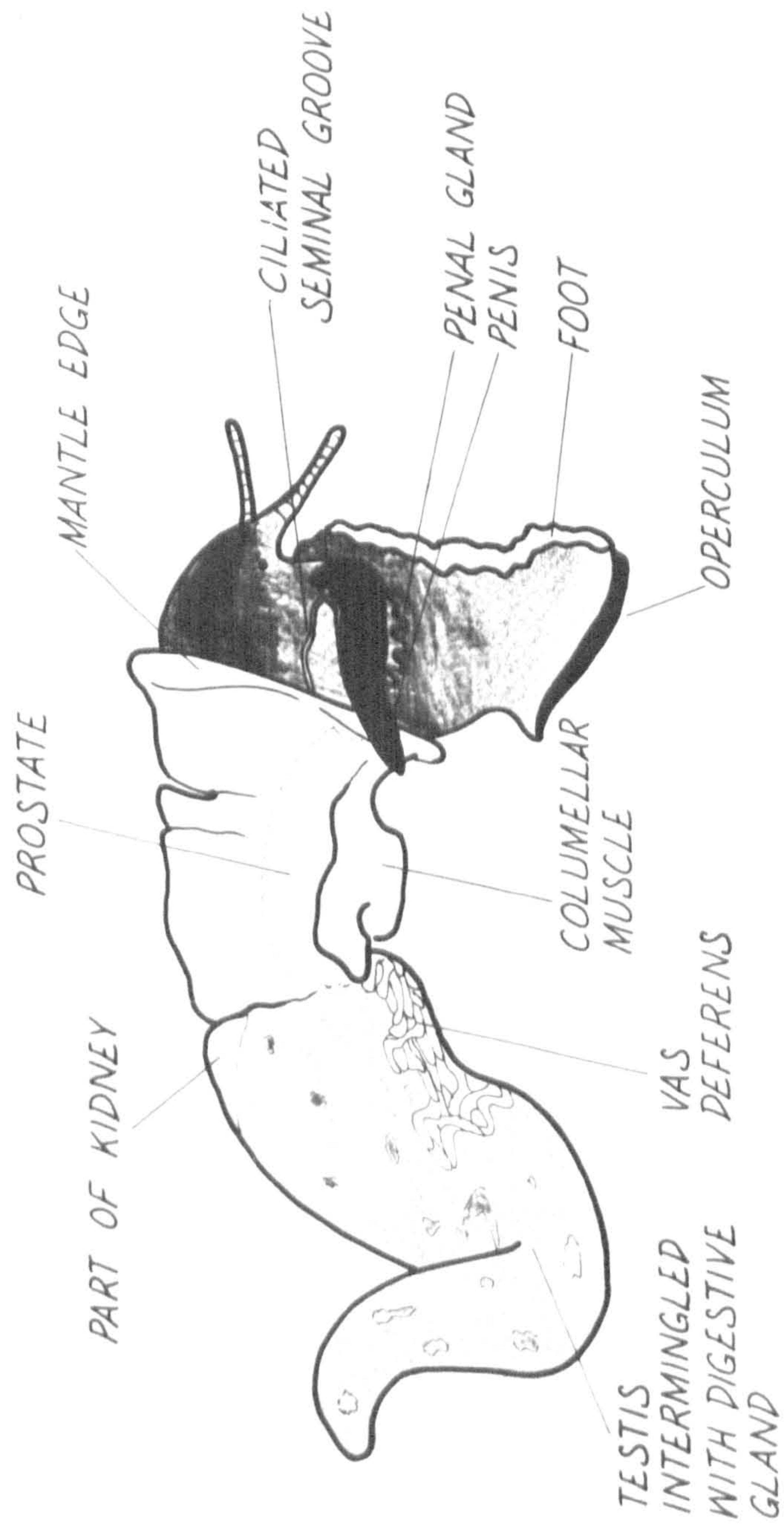
tube, the vas deferens, lying along the inner side of the visceral mass close to the columellar muscle; also by the presence of a penis, normally hidden within the mantle cavity, posteroventral to the right tentacle (Fig. 6). In the female the distal part of the duct which carries the ova from the ovary to the exterior is very glandular, comprising the capsule gland with its associated covering and albumen glands (Fig. 7). In both sexes the gonad is intermingled with the digestive gland and, depending on whether the animal is breeding or not, greater or lesser areas of gonad lie over the digestive gland, especially on the columellar side of the visceral mass whorls, and obscure it from view. The remainder of the reproductive apparatus diminishes outside the breeding season, to be reformed when the next one approaches.

Stages in the reproductive cycle

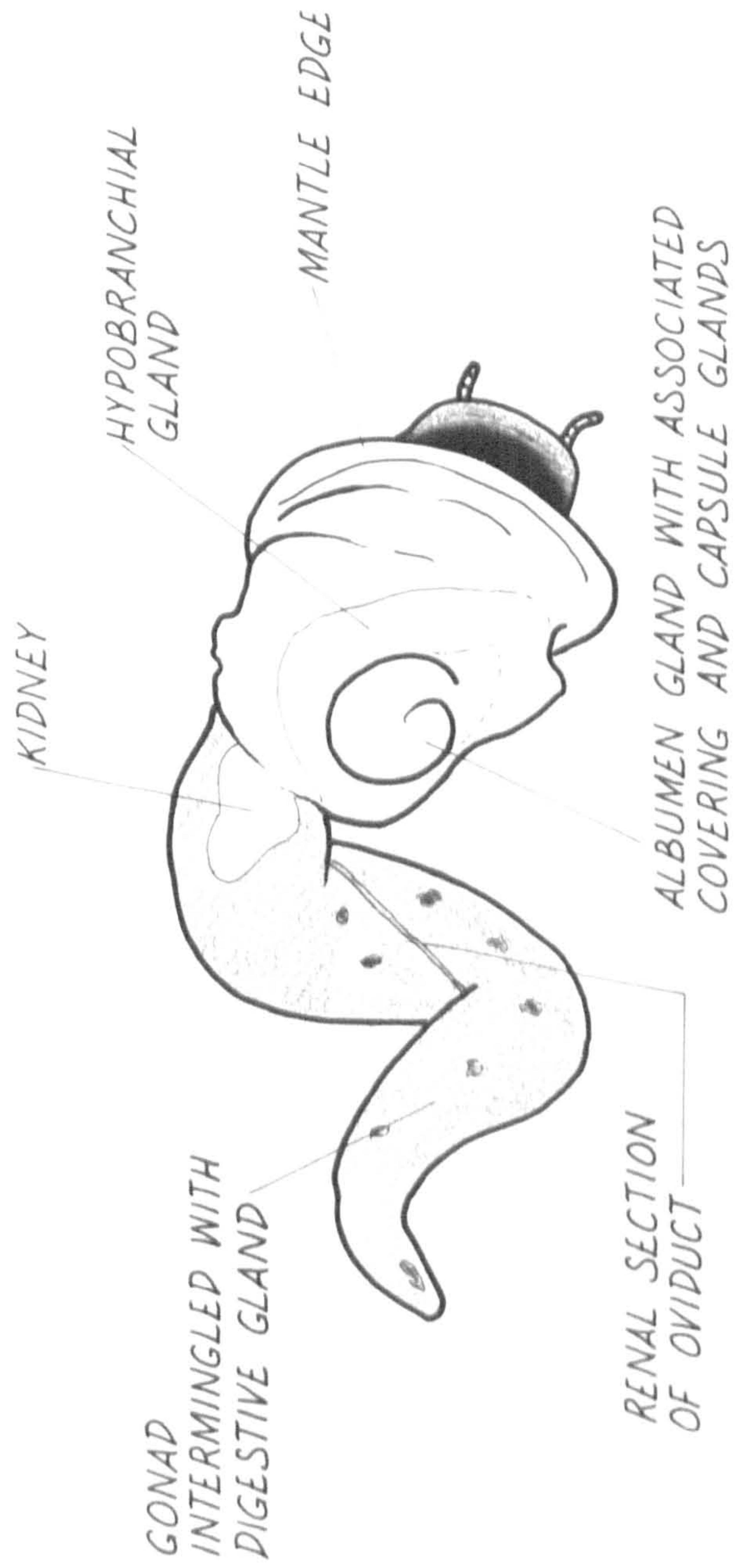
The state of the gonad and other reproductive structures during the reproductive cycle of L. littorea may be divided into a number of arbitrary stages (Williams, 1964). The essential features of each stage are:

"Neuter" animals

These are individuals in which neither male nor female structures are discernible; they were for the most part confined to class A animals (i.e. up to 10.0 mm shell height),



MATURE MALE *Littorina Littorea*, REMOVED FROM SHELL
FIG 6 (VIEWED FROM RIGHT)



MATURE FEMALE Littorina Littorea, **REMOVED FROM SHELL**
FIG 7 (VIEWED DORSALLY AND FROM RIGHT)

although a few were found in size class B (10.1 - 15.0 mm) and 4 were found in class C (15.1 - 20.0 mm). The maximum, minimum, and average shell height and number of "neuter" animals collected each month for classes A and B are presented in table 9.

Stage I (virgin animals)

Female: the capsule, albumen and covering glands are vestigial; the ovary is not yet detectable macroscopically. The ovipositor is usually heavily pigmented.

Male: the penis is rudimentary and the prostate is just visible, but of the other structures there is no trace.

Stage IIa (animals maturing for the first time)

Female: the ovary is now seen as diffuse, light pink areas; and the capsule, albumen and covering glands are now small but visible.

Male: the penis is enlarged slightly and the beginnings of the yellow-cream testis are now recognizable, though still scarce. The vas deferens is gently coiled, perhaps containing a little sperm.

Stage III (fully mature and spawning animals)

Female: the albumen and capsule glands are now very swollen and stand out a brilliant white; the oviduct is swollen and contains large well-rounded pink or grey ova.

TABLE 9. SIZE RANGE OF "NEUTER" UNINFECTED LITTORINA LITTOREA AT SCALBY ROCKS

	<u>CLASS A up to 10.0mm</u>			<u>CLASS B 10.1-15.0mm</u>		
	Shell height mm Range	Average	No. of "neuters"	Shell height mm Range	Average	No. of "neuters"
28.9.66	5.0- 9.9	7.9	13	10.8-12.1	11.6	3
2.11.66	4.0- 9.4	7.3	4	11.2-12.6	11.7	3
1.12.66	5.5-10.0	8.1	16	11.0-11.9	11.5	2
12.1.67	9.8	9.8	1	11.9	11.9	1
11.2.67	3.5- 9.9	7.8	4	10.9-13.5	11.6	6
14.3.67	6.0- 9.0	7.3	7	11.8-14.9	12.6	6
10.4.67	6.4- 9.9	7.9	5 ^a	10.2-14.6	12.0	8
9.5.67	3.6- 9.5	6.2	32	10.1-13.8	11.9	12
6.6.67	5.5- 9.1	7.5	12	10.1-12.5	10.4	4
10.7.67	2.9- 9.2	5.4	27	10.5-11.1	10.8	2
7.8.67	3.8- 9.1	6.8	28	10.5	10.5	1
4.9.67	4.0- 9.9	6.6	30	11.8	11.8	1
2.10.67	6.2- 9.9	8.0	4	10.8	10.8	1
2.11.67	6.2- 9.9	8.1	11	10.1-11.6	10.7 ^b	2
Total Obs.	3.5-10.0	6.9	194	10.1-14.9	11.7	52

(a) Plus one L.littorea (8.0mm) infected with Cercaria A

(b) Plus one L.littorea (13.8mm) infected with C.lingua

Note: not shown in table four uninfected L.littorea of class C

The diffuse light pink ovary is very extensive.

Male: the penis is very well developed; the swollen vas deferens is much coiled, white and full of active sperm. The testis is very extensive, usually light yellow-cream.

Stage IV (partially spent animals)

The gonads have started to regress.

Female: the ovary, though still extensive, now assumes a red to reddish-brown follicular appearance. The oviduct is no longer swollen and contains only a few well-developed ova. The capsule and albumen glands are not as prominent, being less swollen and of a yellow-cream colour in contrast to the brilliant white of the fully mature and spawning animals.

Male: the penis is still well developed, though not as swollen as in the spawning males. The vas deferens remains highly coiled but is now usually yellow-brown and not swollen. The testis is still extensive but has taken on a browner appearance.

Stage V (spent animals)

The reproductive apparatus is now much reduced but it is still possible to distinguish between the sexes.

Female: the follicular ovary becomes scarce and very compact, and of the same colour as in stage IV. The oviduct

lacks ova and is a very fine dark red or red-brown tube. The capsule and albumen glands become a dark yellow-brown and are very poorly developed.

Male: the penis is much reduced in size, weak and very inconspicuous against the similarly pigmented (charcoal-black) head region. The dark brown vas deferens lacks sperm and has become more thread like. The regressed testis is confined to localized dark brown areas.

Stage IIb (recovering spent animals) .

These are animals which have previously spawned, and their reproductive tissue is reforming for the next breeding season.

Female: the capsule and albumen glands are light yellow-brown, they have increased in size and are often swollen in contrast to their much reduced condition in stage V. The follicular, light-pink ovary becomes extensive, and the oviduct is full of a light pink fluid containing small ova.

Male: the yellow-cream testis is quite extensive. The vas deferens is gently coiled and yellowish, possibly containing small numbers of sperm. The penis is almost as well developed as in the fully mature and spawning animals.

After stage IIb the animals pass into the mature and spawning condition (stage III) of the next breeding season.

As outlined above, the reproductive stages are fairly

clear cut, but some animals were examined which showed characteristics of two adjacent stages. When these intermediate forms occurred their numbers were divided equally into the two stages, above and below, to simplify tabulation and graphical representation.

In parasitised animals especially, and for a few uninfected animals, individuals were examined which could not be placed in any of the categories described above; these animals have been allocated to stage X. They could, however, be sexed; the male by the presence of a minute penis, the female by the usually much reduced and darkish brown albumen gland and associated capsule and covering glands. The other reproductive structures in either sex were unrecognizable.

RESULTS

A. THE ANNUAL REPRODUCTIVE CYCLE OF LITTORINA LITTOREA

The results of the survey are summarized in Tables 9 to 13 and Figs. 8 to 12. The relative proportion of specimens in each developmental stage at each collection is expressed as a percentage.

(i) Uninfected Animals

In the overall picture of the breeding cycle all animals from all size classes are considered together (Fig. 8, Table 10).

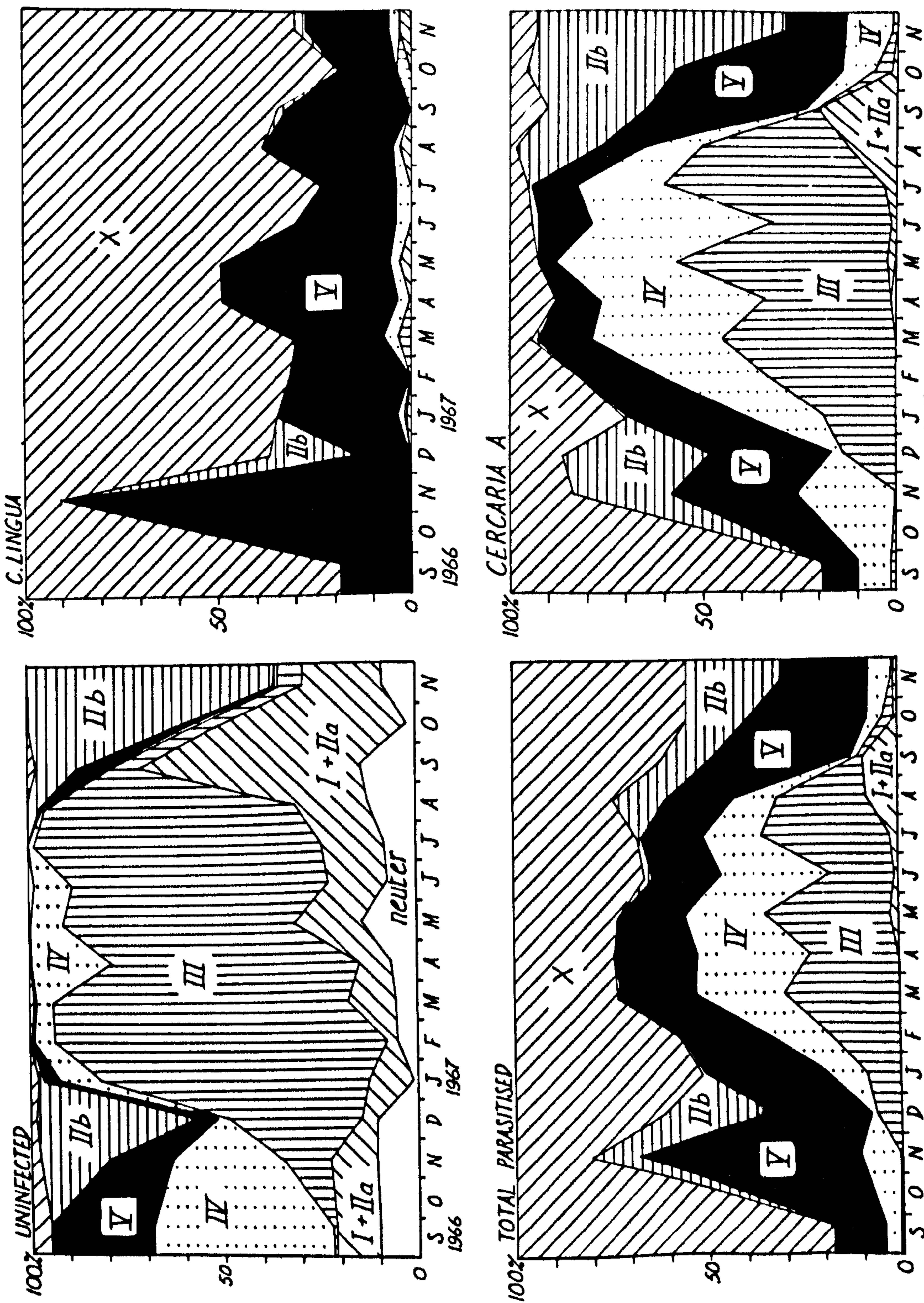


FIG 8 BREEDING CYCLE OF *Littoreia* MALES & FEMALES OF ALL SIZE CLASSES: UNINFECTED AND THOSE INFECTED WITH SPECIES INDICATED

PROPORTION OF L.LITTOREA IN EACH REPRODUCTIVE STAGE :
UNINFECTED AND THOSE INFECTED WITH THE SPECIES INDICATED
(males and females of all size classes)

UNINFECTED	"neuters"	REPRODUCTIVE STAGES (%)							No. Exam		REPRODUCTIVE STAGES (%)							No. Exam
		I-IIa	III	IV	V	IIb	X	"neuters"			I-IIa	III	IV	V	IIb	X		
20.9.66	10.0	11.3	0.6	46.6	26.6	0.0	4.4	160		0.0	0.0	0.4	4.1	13.3	0.0	82.2	230	
2.11.66	11.3	11.3	12.3	28.2	17.0	17.9	1.9	53		0.0	0.0	0.0	10.7	38.1	11.6	19.7	56	
1.12.66	10.4	4.2	34.2	2.8	5.3	41.7	1.2	174		0.0	0.0	6.5	1.4	24.8	29.0	38.3	107	
12.1.67	1.0	11.6	69.1	10.8	4.4	1.0	2.1	194		0.0	0.5	8.4	10.2	31.7	0.3	49.0	202	
11.2.67	3.1	2.5	85.8	5.8	0.3	0.0	0.3	197		0.0	0.0	18.9	16.0	22.3	0.0	42.7	103	
14.3.67	5.2	12.5	76.4	4.9	0.4	0.0	0.7	288		0.0	0.0	30.0	22.7	19.6	0.5	27.3	110	
10.4.67	7.0	7.7	63.9	20.9	0.0	0.0	0.3	201		0.7	0.0	22.9	29.1	21.7	0.0	25.6	129	
9.5.67	14.3	17.2	59.6	8.8	0.2	0.0	0.0	308		0.0	2.2	32.3	20.9	17.3	0.0	26.9	160	
6.6.67	7.1	15.7	66.1	9.6	1.1	0.4	0.0	239		0.0	0.8	16.8	28.4	18.2	1.1	34.7	190	
10.7.67	8.2	16.6	73.8	1.4	0.0	0.0	0.0	355		0.0	2.1	33.3	15.3	16.4	0.5	32.4	213	
7.8.67	12.4	18.8	65.0	0.2	0.4	3.2	0.0	234		0.0	8.3	22.6	11.3	18.6	13.3	25.8	124	
4.9.67	14.1	57.3	7.7	0.5	7.7	10.3	2.3	220		0.0	9.9	0.8	1.5	36.3	13.4	38.2	131	
2.10.67	2.2	49.8	6.4	1.7	2.8	36.7	0.0	233		0.0	0.4	4.0	3.3	31.1	16.2	44.7	114	
2.11.67	8.4	20.1	6.5	0.7	0.3	64.0	0.0	154		0.0	1.3	0.6	5.8	22.4	25.0	44.9	78	
Total Obs.	8.3	19.2	50.3	8.1	3.3	10.0	0.8	3010		0.1	1.8	13.2	13.4	23.0	5.8	40.7	1947	
CERCARIA A																		
20.9.66	0.0	0.0	1.1	9.1	9.1	0.0	80.7	88		0.0	0.0	0.0	0.9	17.6	0.0	81.5	108	
2.11.66	0.0	0.0	0.0	16.0	42.0	26.0	16.0	25		0.0	0.0	0.0	4.5	86.4	0.0	9.1	22	
1.12.66	0.0	0.0	13.7	2.9	31.4	38.2	13.7	51		0.0	0.0	0.0	0.0	15.0	21.7	63.3	30	
12.1.67	0.0	0.0	18.9	20.1	31.1	0.6	29.3	62		0.0	1.2	0.6	1.9	30.9	0.0	65.4	81	
11.2.67	0.0	0.0	33.1	27.1	21.1	0.0	18.6	39		0.0	0.0	0.0	0.0	31.3	0.0	68.8	32	
14.3.67	0.0	0.0	45.1	33.8	14.8	0.7	5.6	71		0.0	0.0	3.3	3.3	23.3	0.0	70.0	30	
10.4.67	1.2	0.0	33.0	42.4	11.8	0.0	11.8	85		0.0	0.0	0.0	3.1	45.3	0.0	51.3	33	
9.5.67	0.0	2.6	54.2	31.3	4.7	0.0	7.3	96		0.0	2.6	0.0	2.6	43.6	0.0	51.3	39	
6.6.67	0.0	1.4	30.5	46.7	13.8	1.9	5.7	103		0.0	0.0	0.0	3.5	26.3	0.0	70.2	57	
10.7.67	0.0	3.6	56.4	23.0	11.5	0.8	4.8	126		0.0	0.0	0.0	3.2	19.4	0.0	77.4	62	
7.8.67	0.0	12.7	37.3	15.3	11.3	22.0	1.3	73		0.0	2.9	0.0	1.5	33.8	0.0	61.8	34	
4.9.67	0.0	20.6	0.0	3.2	40.3	26.2	9.5	63		0.0	0.0	0.0	0.0	32.0	2.0	66.0	50	
2.10.67	0.0	1.0	3.2	8.3	42.7	38.5	4.2	48		0.0	0.0	3.6	0.0	14.6	0.0	81.8	35	
2.11.67	0.0	0.0	1.7	12.1	15.3	63.8	6.9	29		0.0	2.7	0.0	2.7	21.6	2.7	70.3	37	
Total Obs.	0.1	3.1	28.8	23.4	18.3	10.0	16.1	1003		0.0	0.6	0.3	1.8	27.9	1.3	67.9	670	
MINASTHIA LEPTOSOMA																		
20.9.66	0.0	0.0	0.0	1.9	13.0	0.0	85.2	27		0.0	0.0	0.0	0.0	0.0	0.0	100.0	7	
2.11.66	0.0	0.0	0.0	23.0	30.0	0.0	23.0	4		0.0	0.0	0.0	0.0	20.0	0.0	80.0	3	
1.12.66	0.0	0.0	0.0	0.0	30.8	38.5	30.8	13		0.0	0.0	0.0	0.0	15.4	0.0	84.6	13	
12.1.67	0.0	0.0	3.7	9.3	24.1	0.0	63.0	27		0.0	0.0	0.0	0.0	58.3	0.0	41.7	12	
11.2.67	0.0	0.0	0.0	4.3	8.3	0.0	83.3	6		0.0	0.0	0.0	0.0	0.0	0.0	100.0	6	
14.3.67	0.0	0.0	0.0	0.0	66.7	0.0	33.3	6		0.0	0.0	0.0	0.0	0.0	0.0	100.0	3	
10.4.67	0.0	0.0	0.0	0.0	0.0	0.0	100.0	3		0.0	0.0	18.8	6.3	37.3	0.0	37.3	8	
9.5.67	0.0	0.0	0.0	19.2	26.3	0.0	53.7	13		0.0	0.0	0.0	0.0	25.0	0.0	75.0	12	
6.6.67	0.0	0.0	0.0	7.1	14.3	0.0	78.6	14		0.0	0.0	0.0	14.3	21.4	0.0	64.3	14	
10.7.67	0.0	0.0	0.0	13.6	31.8	0.0	54.6	11		0.0	0.0	0.0	0.0	33.7	0.0	64.3	14	
7.8.67	0.0	0.0	0.0	12.5	12.5	0.0	75.0	8		0.0	0.0	0.0	14.3	38.6	0.0	37.1	7	
4.9.67	0.0	0.0	0.0	0.0	50.0	0.0	50.0	10		0.0	0.0	12.3	0.0	12.3	0.0	75.0	8	
2.10.67	0.0	0.0	0.0	0.0	66.7	0.0	33.3	3		0.0	0.0	0.0	0.0	62.3	0.0	37.3	8	
2.11.67	0.0	0.0	0.0	0.0	100.0	0.0	0.0	3		0.0	0.0	0.0	0.0	22.2	0.0	77.8	9	
Total Obs.	0.0	0.0	0.7	7.1	27.3	3.4	61.5	148		0.0	0.0	2.0	2.8	27.0	0.0	68.2	126	

Mature (stage III) animals were found as early as December 1966, when they comprised 34.2% of the total uninfected sample, most of the remainder being "neuters" (10.4%) and animals maturing for at least the second time (stage IIb - 41.7%). Mature animals were most abundant between January and August 1967, with the main peak in February and March (85.8% & 76.4%), and a smaller one in July (73.8%). Between August and September 1967 the proportion of stage III animals had fallen sharply from 65.0% to 7.7%, with stage I and IIa animals (57.3%) forming the greater bulk of the September sample. In 1967 the animals passed quickly through the partially spent (stage IV) and spent (stage V) conditions to the recovering state (stage IIb), and in November 1967, 64.0% of the sample were in stage IIb. The remainder of the November 1967 sample was largely comprised of virgin (stage I) animals and those attaining sexual maturity for the first time (stage IIa).

The reproductive cycle of males and females had a similar pattern (Figs. 9 and 10, Tables 11 and 12). Comparable proportions of sexually mature males (38.9%) and females (37.1%) appeared in December 1966, and their respective breeding periods were identical. The only noticeable difference was that the proportion of mature

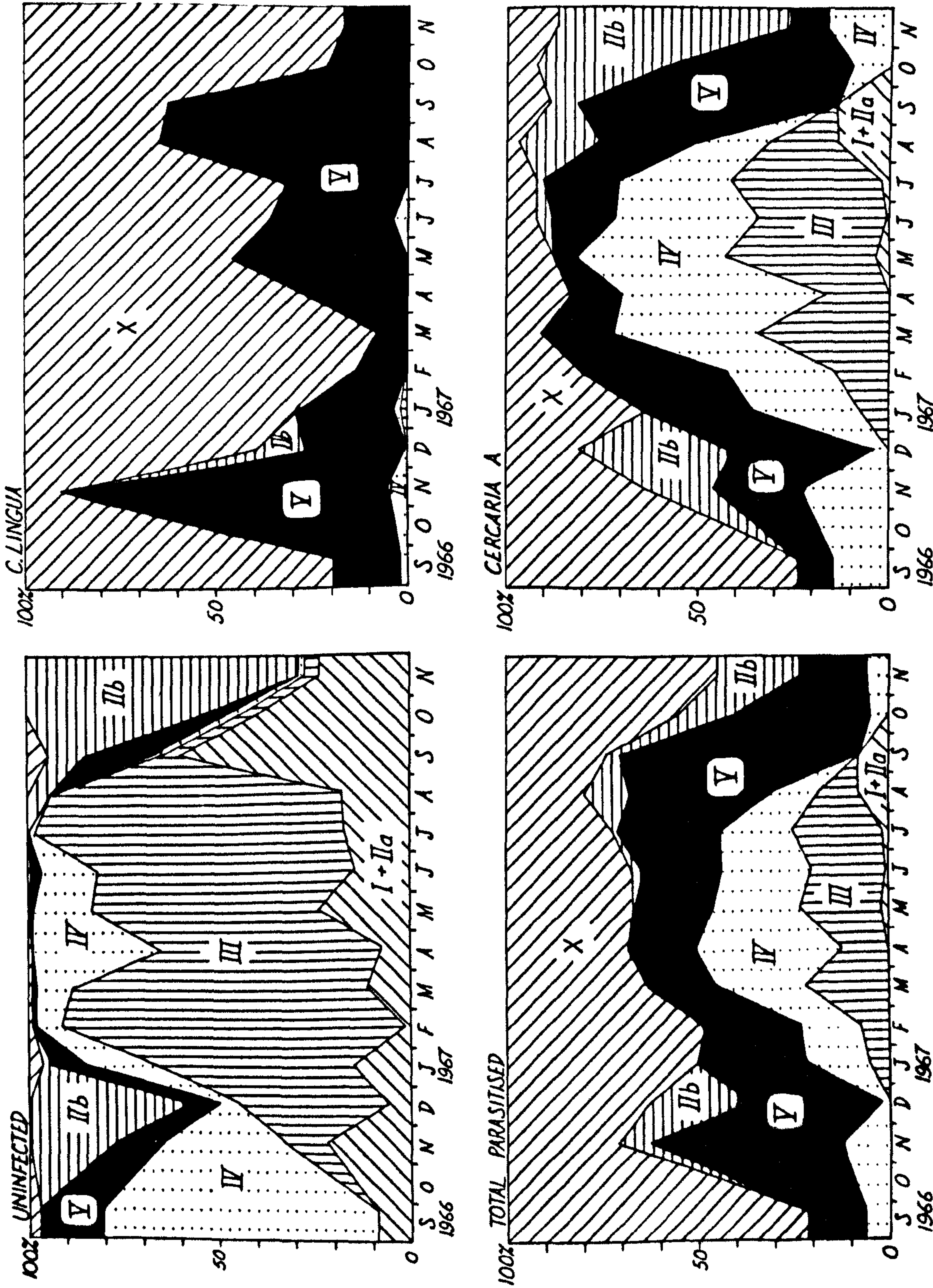


FIG 9 BREEDING CYCLE OF *LITTOREA* MALES OF ALL SIZE CLASSES: UNINFECTED AND THOSE INFECTED WITH SPECIES INDICATED

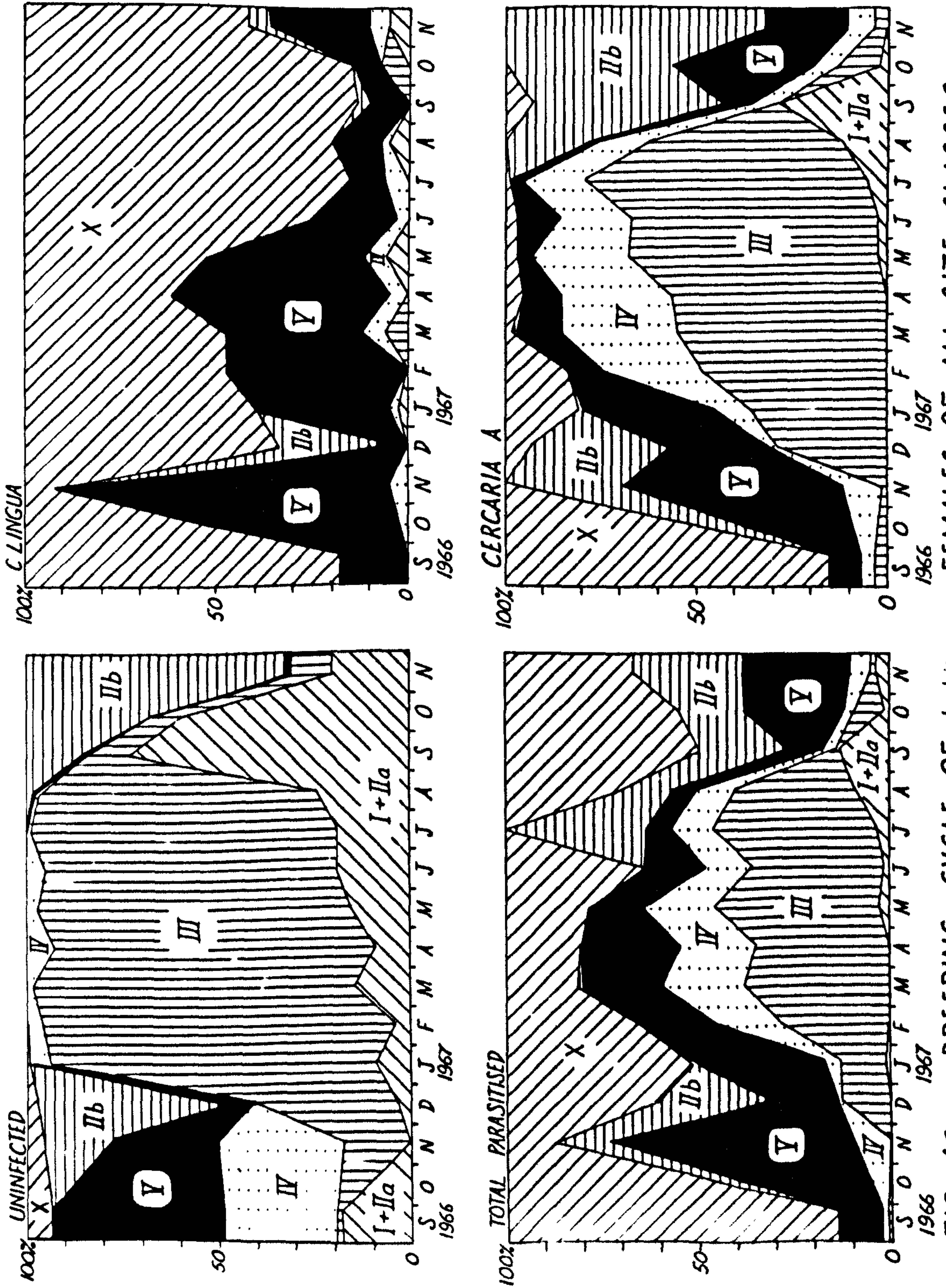


FIG 10 BREEDING CYCLE OF *L. Littorea* FEMALES OF ALL SIZE CLASSES: UNINFECTED AND THOSE INFECTED WITH THE SPECIES INDICATED

TABLE 11

DATE	REPRODUCTIVE STAGES (X)								REPRODUCTIVE STAGES (X)								No. Exam
	"neuters"	I+IIa	III	IV	V	IIb	I	"neuters"	I+IIa	III	IV	V	IIb	I			
TOTAL PARASITIZED																	
20.9.66	7.9	1.3	0.0	71.7	16.5	0.0	2.6	76	0.0	0.0	0.0	6.3	14.8	0.0	76.9		
2.11.66	7.4	14.8	11.1	31.5	15.0	22.2	0.0	27	0.0	0.0	0.0	12.5	50.0	4.5	29.2		
1.12.66	2.2	5.9	38.9	5.0	8.9	41.1	0.0	90	0.0	0.0	0.0	2.0	33.3	27.5	37.3		
12.1.67	3.5	11.6	51.7	18.6	8.7	2.5	3.5	86	0.0	0.0	5.2	16.5	28.9	0.0	49.5		
11.2.67	0.9	0.9	89.6	7.1	0.5	0.0	0.9	106	0.0	0.0	7.6	15.2	25.0	0.0	52.2		
14.3.67	2.8	9.1	77.4	9.1	0.8	0.0	0.8	126	0.0	0.0	21.6	25.5	17.7	0.0	37.3		
10.4.67	3.2	4.6	57.9	33.3	0.0	0.0	0.9	108	0.0	0.0	12.1	38.6	17.4	0.0	31.8		
9.5.67	9.5	14.8	59.5	15.9	0.3	0.0	0.0	142	0.0	2.0	21.1	25.0	21.1	0.0	32.9		
6.6.67	1.8	13.2	67.1	14.9	2.2	0.9	0.0	114	0.0	0.6	19.5	25.9	21.0	2.5	33.0		
10.7.67	10.5	7.0	61.2	1.3	0.0	0.0	0.0	157	0.0	1.4	25.6	19.2	26.9	1.0	27.2		
7.8.67	9.4	8.2	76.5	0.0	0.0	5.9	0.0	85	2.0	6.0	10.0	15.0	37.0	12.0	20.0		
4.9.67	12.4	47.9	7.0	1.1	17.2	9.1	5.4	93	0.0	7.6	0.0	0.0	62.9	3.8	25.8		
2.10.67	18.7	21.7	4.0	4.0	6.6	45.0	0.0	99	0.0	0.0	0.0	4.6	36.4	15.5	43.6		
2.11.67	13.2	10.5	4.0	1.3	0.0	71.1	0.0	76	0.0	0.0	0.0	6.0	17.9	21.4	54.8		
CRYPTOCOTYLE LINCU																	
20.9.66	0.0	0.0	0.0	14.1	9.8	0.0	76.1	46	0.0	0.0	0.0	1.6	18.0	0.0	80.3		
2.11.66	0.0	0.0	0.0	22.7	22.7	18.2	36.4	11	0.0	0.0	0.0	5.0	85.0	0.0	10.0		
1.12.66	0.0	0.0	0.0	3.9	36.5	40.4	19.2	26	0.0	0.0	0.0	0.0	26.7	13.5	60.0		
12.1.67	0.0	0.0	7.5	27.7	28.7	0.0	36.2	42	0.0	0.0	1.4	1.4	25.7	0.0	71.4		
11.2.67	0.0	0.0	14.0	28.0	38.0	0.0	20.0	25	0.0	0.0	0.0	0.0	13.5	0.0	86.7		
14.3.67	0.0	0.0	34.4	37.5	18.8	0.0	9.4	32	0.0	0.0	0.0	0.0	7.7	0.0	92.3		
10.4.67	0.0	0.0	16.7	53.1	13.5	0.0	16.7	48	0.0	0.0	0.0	0.0	25.0	0.0	75.0		
9.5.67	0.0	3.7	39.0	39.0	6.1	0.0	12.2	41	0.0	0.0	0.0	0.0	45.5	0.0	54.6		
6.6.67	0.0	1.0	33.3	37.5	16.7	3.9	7.8	51	0.0	0.0	0.0	5.6	32.1	0.0	64.3		
10.7.67	0.0	2.4	38.9	29.4	19.8	1.6	7.9	63	0.0	0.0	0.0	0.0	31.0	0.0	69.0		
7.8.67	3.5	10.4	17.2	13.0	25.9	20.7	3.5	29	0.0	0.0	0.0	0.0	64.3	0.0	35.7		
4.9.67	0.0	14.3	0.0	0.0	67.1	7.1	11.4	35	0.0	0.0	0.0	0.0	61.9	0.0	38.1		
2.10.67	0.0	0.0	0.0	10.0	48.0	34.0	8.0	25	0.0	0.0	0.0	0.0	21.4	0.0	78.6		
2.11.67	0.0	0.0	0.0	16.7	10.0	60.0	13.3	15	0.0	0.0	0.0	0.0	16.7	0.0	83.3		
DOUBL. INFECTIONS																	
20.9.66	0.0	0.0	0.0	2.9	20.6	0.0	76.5	17	0.0	0.0	0.0	0.0	0.0	0.0	100.0		
2.11.66	0.0	0.0	0.0	0.0	50.0	0.0	50.0	2	0.0	0.0	0.0	0.0	0.0	0.0	100.0		
1.12.66	0.0	0.0	0.0	0.0	50.0	50.0	20.0	5	0.0	0.0	0.0	0.0	20.0	0.0	80.0		
12.1.67	0.0	0.0	11.1	27.8	16.7	0.0	44.4	9	0.0	0.0	0.0	0.0	66.7	0.0	33.3		
11.2.67	0.0	0.0	0.0	0.0	0.0	0.0	100.0	3	0.0	0.0	0.0	0.0	0.0	0.0	100.0		
14.3.67	0.0	0.0	0.0	0.0	50.0	0.0	50.0	4	0.0	0.0	0.0	0.0	0.0	0.0	100.0		
10.4.67	0.0	0.0	0.0	0.0	0.0	0.0	100.0	3	0.0	0.0	0.0	0.0	66.7	0.0	33.3		
9.5.67	0.0	0.0	0.0	18.8	31.3	0.0	50.0	8	0.0	0.0	0.0	0.0	20.0	0.0	80.0		
6.6.67	0.0	0.0	0.0	0.0	16.7	0.0	83.3	6	0.0	0.0	0.0	33.3	0.0	0.0	66.7		
10.7.67	0.0	0.0	0.0	25.0	14.7	0.0	35.3	6	0.0	0.0	0.0	0.0	66.7	0.0	33.3		
7.8.67	0.0	0.0	0.0	0.0	50.0	0.0	50.0	2	0.0	0.0	0.0	20.0	20.0	0.0	60.0		
4.9.67	0.0	0.0	0.0	0.0	57.1	0.0	42.9	7	0.0	0.0	0.0	0.0	33.3	0.0	66.6		
2.10.67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	100.0	0.0	0.0		
2.11.67	0.0	0.0	0.0	0.0	100.0	0.0	0.0	1	0.0	0.0	0.0	0.0	25.0	0.0	75.0		

TABLE 12 PROPORTION OF L.LITTOREA IN EACH REPRODUCTIVE STAGE :
UNINFECTED AND THOSE INFECTED WITH THE SPECIES INDICATED
(females of all size classes)

	REPRODUCTIVE STAGES (%)								REPRODUCTIVE STAGES (%)								
	"neuters"	I+IIa	III	IV	V	IIb	I	No. Exam	"neuters"	I+IIa	III	IV	V	IIb	I	No. Exam	
UNINFECTED												TOTAL PARASITISED					
20.9.66	17.7	0.0	1.5	29.4	44.2	0.0	7.4	66	0.0	0.0	1.0	1.5	11.5	0.0	86.5	102	
2.11.66	0.0	0.0	17.5	32.5	27.5	17.5	5.0	20	0.0	0.0	0.0	9.4	64.1	14.1	12.5	32	
1.12.66	0.0	2.3	37.1	0.5	2.5	53.8	3.0	66	0.0	0.0	12.5	0.9	17.0	30.4	39.5	56	
12.1.67	2.8	6.1	84.4	4.7	0.9	0.0	0.9	106	0.0	1.0	11.4	4.5	34.5	0.5	48.6	105	
11.2.67	3.1	0.6	91.4	4.9	0.0	0.0	0.0	81	0.0	0.0	28.6	16.7	20.2	0.0	35.1	57	
14.3.67	3.1	11.2	83.3	1.7	0.0	0.0	0.7	147	0.0	0.0	37.5	22.0	21.2	0.9	18.6	59	
10.4.67	2.5	6.3	83.5	7.6	0.0	0.0	0.0	79	0.0	0.0	34.7	19.4	26.6	0.0	19.4	62	
9.5.67	12.3	2.9	81.2	3.7	0.0	0.0	0.0	122	0.0	2.4	42.9	19.1	14.3	0.0	21.5	84	
6.6.67	11.1	7.9	75.5	5.6	0.0	0.0	0.0	108	0.0	1.0	34.5	12.8	15.7	0.0	36.5	102	
10.7.67	10.7	8.0	79.6	1.8	0.0	0.0	0.0	169	0.0	2.8	42.7	11.5	6.4	36.7	0.0	109	
7.8.67	7.5	16.7	72.5	0.4	0.8	2.1	0.0	120	0.0	8.8	31.1	10.1	6.1	14.2	29.7	74	
4.9.67	15.1	57.8	10.9	0.0	1.0	15.1	0.0	96	0.0	12.3	1.5	3.1	9.2	25.1	50.8	65	
2.10.67	33.3	25.6	8.5	0.0	0.0	31.8	0.9	129	0.0	0.9	7.6	2.5	26.3	17.0	45.8	59	
2.11.67	12.3	7.7	10.8	0.0	0.8	68.5	0.0	65	0.0	2.8	1.4	5.6	27.8	23.2	33.5	36	
CRYPTOCOTYLE LINGUA																	
20.9.66	0.0	0.0	2.4	3.6	8.3	0.0	85.7	42	0.0	0.0	0.0	0.0	17.0	0.0	83.0	47	
2.11.66	0.0	0.0	0.0	10.7	57.1	32.8	0.0	14	0.0	0.0	0.0	4.2	87.5	0.0	8.5	12	
1.12.66	0.0	0.0	28.0	2.0	26.0	36.0	8.0	25	0.0	0.0	0.0	0.0	3.5	30.0	66.7	15	
12.1.67	0.0	0.0	34.5	10.0	34.5	1.4	20.0	35	0.0	2.2	0.0	2.2	34.8	0.0	60.9	46	
11.2.67	0.0	0.0	47.1	26.5	8.8	0.0	17.7	34	0.0	0.0	0.0	0.0	47.1	0.0	52.9	17	
14.3.67	0.0	0.0	53.9	30.8	11.5	1.5	2.6	39	0.0	0.0	5.9	5.9	35.3	0.0	52.9	17	
10.4.67	0.0	0.0	55.6	29.2	9.7	0.0	5.6	36	0.0	0.0	0.0	4.7	57.1	0.0	38.1	21	
9.5.67	0.0	1.8	65.5	25.5	3.6	0.0	3.6	55	0.0	5.9	0.0	5.9	41.2	0.0	47.1	17	
6.6.67	0.0	1.3	64.8	10.5	11.1	0.0	3.7	54	0.0	0.0	0.0	3.5	20.7	0.0	75.9	29	
10.7.67	0.0	4.8	73.8	16.7	3.2	0.0	1.6	63	0.0	0.0	0.0	6.1	9.1	0.0	84.9	33	
7.8.67	0.0	12.0	50.0	13.0	2.2	22.8	0.0	46	0.0	5.0	0.0	2.5	12.5	0.0	80.0	20	
4.9.67	0.0	28.6	0.0	7.1	7.1	50.0	7.1	28	0.0	0.0	0.0	0.0	10.4	3.5	86.2	29	
2.10.67	0.0	2.2	10.9	6.3	37.0	43.5	0.0	23	0.0	0.0	7.4	0.0	7.4	0.0	85.2	27	
2.11.67	0.0	0.0	3.6	7.1	21.4	67.9	0.0	14	0.0	5.5	0.0	5.5	26.5	5.5	57.9	19	
DOUBLE INFECTIONS																	
20.9.66	0.0	0.0	0.0	0.0	0.0	0.0	100.0	10	0.0	0.0	0.0	0.0	0.0	0.0	100.0	3	
2.11.66	0.0	0.0	0.0	50.0	50.0	0.0	0.0	2	0.0	0.0	0.0	0.0	25.0	0.0	75.0	4	
1.12.66	0.0	0.0	0.0	0.0	18.8	43.8	37.5	8	0.0	0.0	0.0	0.0	12.5	0.0	87.5	8	
12.1.67	0.0	0.0	0.0	0.0	27.8	0.0	72.2	18	0.0	0.0	0.0	0.0	50.0	0.0	50.0	6	
11.2.67	0.0	0.0	0.0	16.7	16.7	0.0	66.7	3	0.0	0.0	0.0	0.0	0.0	0.0	100.0	3	
14.3.67	0.0	0.0	0.0	0.0	100.0	0.0	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1	
10.4.67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	30.0	10.0	20.0	0.0	40.0	5	
9.5.67	0.0	0.0	0.0	20.0	20.0	0.0	60.0	5	0.0	0.0	0.0	0.0	28.6	0.0	71.5	7	
6.6.67	0.0	0.0	0.0	12.5	12.5	0.0	75.0	8	0.0	0.0	0.0	9.1	27.5	0.0	63.6	11	
10.7.67	0.0	0.0	0.0	0.0	20.0	0.0	80.0	5	0.0	0.0	0.0	0.0	12.5	0.0	87.5	8	
7.8.67	0.0	0.0	0.0	16.7	0.0	0.0	83.3	6	0.0	0.0	0.0	0.0	50.0	0.0	50.0	2	
4.9.67	0.0	0.0	0.0	0.0	33.3	0.0	66.7	3	0.0	0.0	20.0	0.0	0.0	0.0	80.0	5	
2.10.67	0.0	0.0	0.0	0.0	66.7	0.0	66.7	3	0.0	0.0	0.0	0.0	50.0	0.0	50.0	6	
2.11.67	0.0	0.0	0.0	0.0	100.0	0.0	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1	

females remained fairly constant from January 1967 to July 1967 ($83.5 \pm 8\%$) whereas the proportion of mature males during this period exhibited two peaks, the first in February (89.6%), and the second in July (81.2%), with an intermediate fall to about 60% in April and May (Fig.9).

The proportion of uninfected L.littorea, shown as a percentage in each developmental stage, of the three main size classes (classes C- 15.1 - 20.0 mm; D- 20.1 - 25.0 mm; and E- 25.1 - 30.0 mm) is summarized in Table 13.

A similar overall reproductive pattern was exhibited by each class. The main difference among the classes is the preponderance of stage I and IIa animals in Class C and the absence of these stages in animals of class E.

A few stage IIa animals were recorded in class D, but these were usually at the lower end of the size range.

Again, males and females were broadly alike in all classes.

Smaller animals of both sexes appear to reach maturity earlier in the year than larger animals, a considerably greater proportion of animals of class C, compared with those of classes D or E, being at stage III by December 1966 - (males plus females of class C, 72.7%; D, 40.1%; and class E, 12.5%; Fig. 11).

Sea and air temperatures were lowest between December

TABLE 13 PROPORTION OF UNINFECTED L. LITTOREA AT EACH REPRODUCTIVE STAGE, FOR MALES AND FEMALES OF SIZE CLASSES C, D AND E

REPRODUCTIVE STAGES (%)																		
	"neuters"	I-IIa	CLASS C (15.1-20.0mm)					I-IIb	I	No. Exam	"neuters"	CLASS D (20.1-25.0mm)						
			III	IV	V	III	IV					V	III	IV	V	I-IIa		
29.9.66	0.0	0.0	0.0	28.1	34.4	0.0	0.0	0.0	37.5	16	0.0	0.0	0.0	69.7	30.3	0.0	0.0	94
2.11.66	0.0	0.0	62.5	0.0	25.0	12.5	0.0	0.0	0.0	4	0.0	0.0	0.0	32.6	15.2	34.8	0.0	23
1.12.66	0.0	6.8	72.7	0.0	0.0	20.5	0.0	0.0	0.0	22	0.0	0.0	2.1	0.5	5.7	49.5	2.1	96
12.1.67	0.0	61.7	25.6	2.9	0.0	0.0	0.0	0.0	11.8	17	0.0	0.0	1.7	10.6	4.7	1.7	0.0	118
11.2.67	0.0	0.0	97.7	2.3	0.0	0.0	0.0	0.0	0.0	22	0.0	0.0	0.0	4.2	0.0	0.0	0.7	130
14.3.67	0.0	4.4	95.6	0.0	0.0	0.0	0.0	0.0	0.0	69	0.0	0.0	1.4	4.6	0.7	0.0	0.7	141
10.4.67	0.0	13.4	71.4	13.4	0.0	0.0	0.0	0.0	1.8	56	0.0	0.0	0.0	29.4	0.0	0.0	0.0	114
9.5.67	0.0	26.0	74.0	0.0	0.0	0.0	0.0	0.0	0.0	73	0.0	0.0	3.7	11.8	0.4	0.0	0.0	135
6.6.67	0.0	19.2	73.0	0.0	1.4	0.0	1.4	0.0	0.0	73	0.0	0.0	0.5	15.5	1.4	0.0	0.0	112
10.7.67	0.0	30.6	69.4	0.0	0.0	0.0	0.0	0.0	0.0	85	0.0	0.0	2.6	1.0	0.0	0.0	0.0	193
7.8.67	0.0	50.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	40	0.0	0.0	5.8	0.0	0.0	4.0	0.0	138
4.9.67	0.0	89.8	3.1	0.0	0.0	0.0	7.1	0.0	0.0	49	0.0	0.0	46.7	1.1	14.1	16.9	5.4	92
2.10.67	0.0	60.8	6.8	0.0	1.4	0.0	31.0	0.0	0.0	37	0.0	0.0	14.0	3.2	2.7	68.8	1.1	93
2.11.67	0.0	20.0	10.0	0.0	0.0	0.0	70.0	0.0	0.0	20	0.0	0.0	5.0	0.0	0.0	86.3	0.0	80
CLASS E (25.0-30.0mm)																		
29.9.66	0.0	0.0	7.7	26.9	57.7	0.0	0.0	0.0	7.7	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.11.66	0.0	0.0	0.0	50.0	34.6	7.7	0.0	0.0	7.7	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.12.66	0.0	0.0	12.5	10.9	12.5	64.1	0.0	0.0	0.0	32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12.1.67	0.0	0.0	72.7	18.2	6.8	0.0	0.0	0.0	2.3	44	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11.2.67	0.0	0.0	81.5	8.5	0.0	0.0	0.0	0.0	0.0	27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.3.67	0.0	0.0	65.9	34.1	0.0	0.0	0.0	0.0	0.0	22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.4.67	0.0	0.0	85.7	14.3	0.0	0.0	0.0	0.0	0.0	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9.5.67	0.0	0.0	57.7	42.3	0.0	0.0	0.0	0.0	0.0	26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.6.67	0.0	0.0	61.5	38.5	0.0	0.0	0.0	0.0	0.0	13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.7.67	0.0	0.0	84.2	15.8	0.0	0.0	0.0	0.0	0.0	19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.8.67	0.0	0.0	68.1	4.6	9.1	18.2	0.0	0.0	0.0	11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.9.67	0.0	0.0	12.5	0.0	37.5	50.0	0.0	0.0	0.0	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.10.67	0.0	0.0	14.7	5.9	20.6	58.8	0.0	0.0	0.0	17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.11.67	0.0	0.0	5.6	5.6	2.8	86.0	0.0	0.0	0.0	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

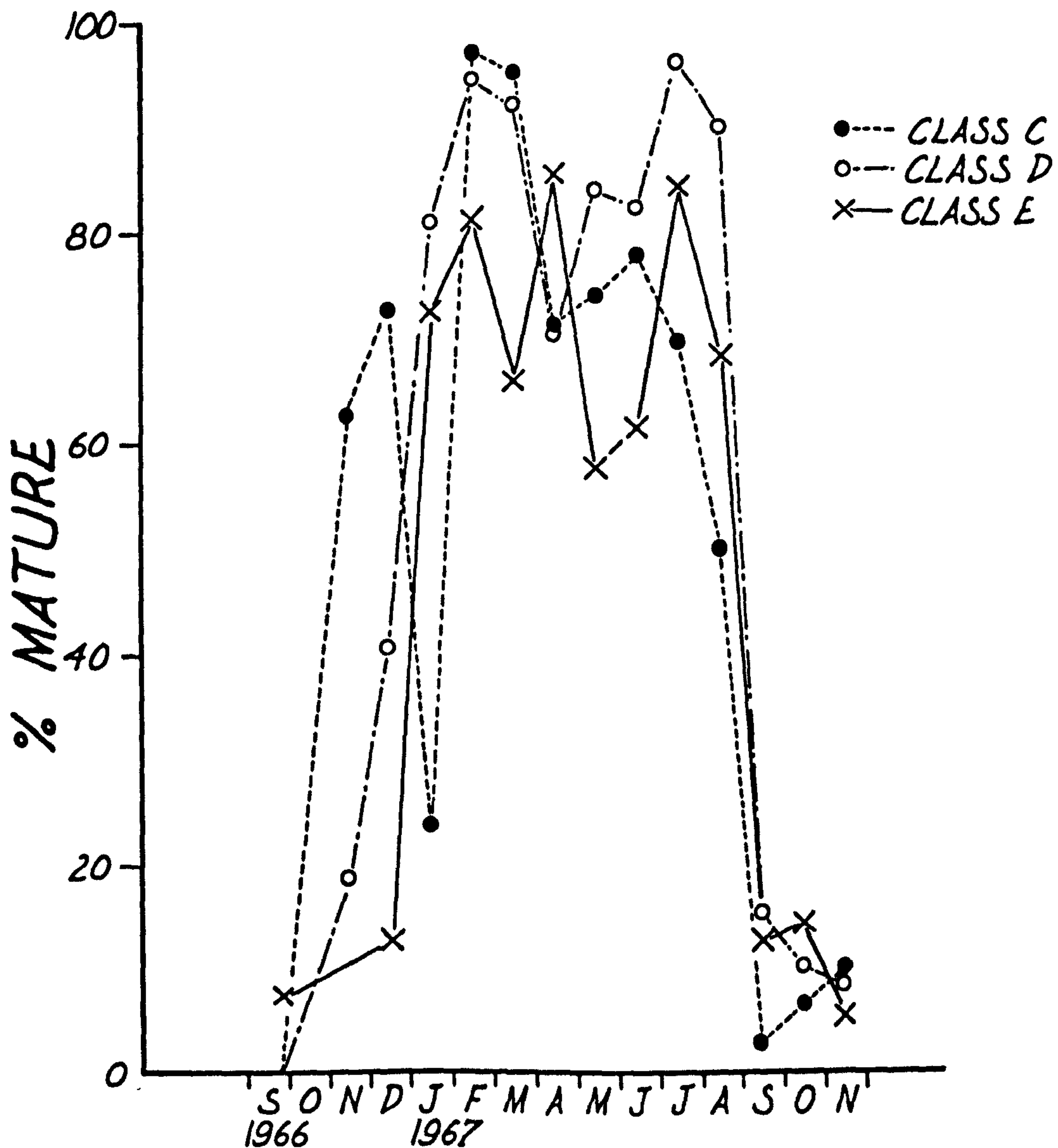


FIG 11 PROPORTION OF MATURE
L. Littorea MALES AND FEMALES OF
SIZE CLASSES C, D, AND E

1966 and April 1967, and highest in July, August and September 1967 (Fig. 17, Appendix Table A). In the uninfected population at Scalby Rocks mature and maturing animals were recorded during the period when the sea temperature was at its minimum, the breeding season extending through this period to the seasonal maximum temperature in August 1967. The sea temperature fell in September 1967 and very few mature animals were found in this month's sample.

(ii) Infected Animals

(a) Total parasitised animals

Infected males and females of all sizes had a similar reproductive cycle to the uninfected animals (Fig. 8; Table 10), but the proportion of mature animals each month was very much lower, and the variations more erratic, reaching individual peaks in March (30.0%), May (32.5%) and July (33.3%). Between these peaks the proportion fell to 22.9% in April and 16.8% in June. In contrast to the uninfected animals there was a large number of animals each month whose reproductive stage could not be ascertained (stage X). As stated earlier, animals assigned to this class could still be sexually identified; the male by the presence of a minute penis, the female by the usually much reduced and darkish brown albumen gland region. The other reproductive structures could not be

discerned. In some specimens the penis was almost completely absent.

When considered separately the infected males and females (Figs. 9 and 10; Tables 11 and 12) showed the same general monthly distribution of reproductive stages as the entire sample of infected animals. The most noticeable difference between the sexes was the larger proportion of mature and spawning females found throughout the period of study, the highest percentage of mature parasitised males for any month being 23.6% (July 1967) compared with 42.9% (May 1967) for the parasitised females. These values were, however, much lower than the maximum percentage of mature males (89.6% in February 1967) and females (91.4% also in February) recorded for the uninfected animals (Figs. 9 and 10; Tables 11 and 12).

When the reproductive activity of animals infected with C. lingua and those infected with Cercaria A are considered separately striking differences between these two infections are immediately noticed.

(b) Littorina littorea infected with Cryptocotyle lingua

Very few L. littorea infected with the sporocysts and rediae of C. lingua exhibited any characteristics of mature, spawning, animals (Fig. 8; Table 10). Recognizably mature animals were found only in January (0.6%), March (3.3%) and October (3.6%)

1967, and throughout the period of study over 50% of the animals infected with C.lingua, excepting those in the November 1966 sample, were allocated to stage X, while most of the remaining animals fell within stage V (spent animals). Only 4 of the 670 specimens examined exhibited characteristics of animals maturing for the first time (stage IIa).

Both males (Fig. 9; Table 11) and females (Fig. 10; Table 12) infected with C.lingua showed the same general pattern of reproductive condition. It is worth emphasising that the total number of both mature and maturing females infected with C.lingua did not exceed 7.4% in any one month in contrast to the maximum of 91.4% recorded in February 1967 for mature uninfected females.

(c) Littorina littorea infected with Cercaria A

There was a moderate number of L.littorea infected with Cercaria A that could be classified as spawning mature animals (Fig. 8; Table 10) but whether or not these animals lay eggs which developed into viable larvae is not known, and was not tested because of the length of time it would have taken for the veliger larvae to metamorphose and grow to a size capable of reproducing (but both the shed eggs and sperm looked normal). Unlike the animals infected with C.lingua it was possible to assign most individuals to a particular reproductive stage, and only a few animals were classed as indeterminates (stage X).

There were proportionately more animals infected with the xiphidocercaria in the partially spent and spent conditions compared with the proportion of these stages in uninfected L.littorea.

The most noticeable difference between male and female L.littorea infected with Cercaria A (Figs. 9 and 10; Tables 11 and 12) was the much larger proportion of females each month which showed the characteristics of stage III (mature and spawning) animals. The proportion of females infected with Cercaria A that were classified as mature and spawning increased steadily from the start of the breeding season in December 1966 (28.0%) reaching a peak in July 1967 (73.8%). This July percentage was closely similar to the same month's 79.6% for uninfected females at stage III; however, whereas this high proportion of uninfected stage III females had been maintained since January 1967 with little variation (Fig. 10, Table 11) the proportion of stage III females infected with Cercaria A had shown a gradual increase throughout the breeding season (Fig. 10). The highest percentage of stage III males infected with Cercaria A was recorded in May 1967 (39.0%). For most months a larger proportion of partially spent and spent forms were recorded for infected males compared with the proportion of this stage in females infected with Cercaria A.

The overall picture of the reproductive state of animals infected with Cercaria A is approximately midway between the condition seen in uninfected L.littorea and in those infected with C.lingua.

- (d) Littorina littorea infected with Himasthla leptosoma or harbouring double infections (i.e. infected with two species of digenean larvae).

L.littorea infected with H.leptosoma or harbouring double infections were not very common and, when encountered, the reproductive structures were usually poorly developed, seldom exhibiting characteristics of mature spawning animals (stage III) (Table 10). The cyclical pattern of the reproductive condition obtained during the survey (Fig. 12) was similar to that exhibited by the animals infected with C.lingua. However the number of animals harbouring these infections was too small to enable any conclusions to be drawn relating to the effects of these parasites on the reproductive potential of their hosts.

B. THE SEX RATIO OF LITTORINA LITTOREA

Animals used in the sex ratio studies included those collected from Scalby Rocks between February 1966 and August 1966, as well as those collected specifically for the survey of the reproductive cycle.

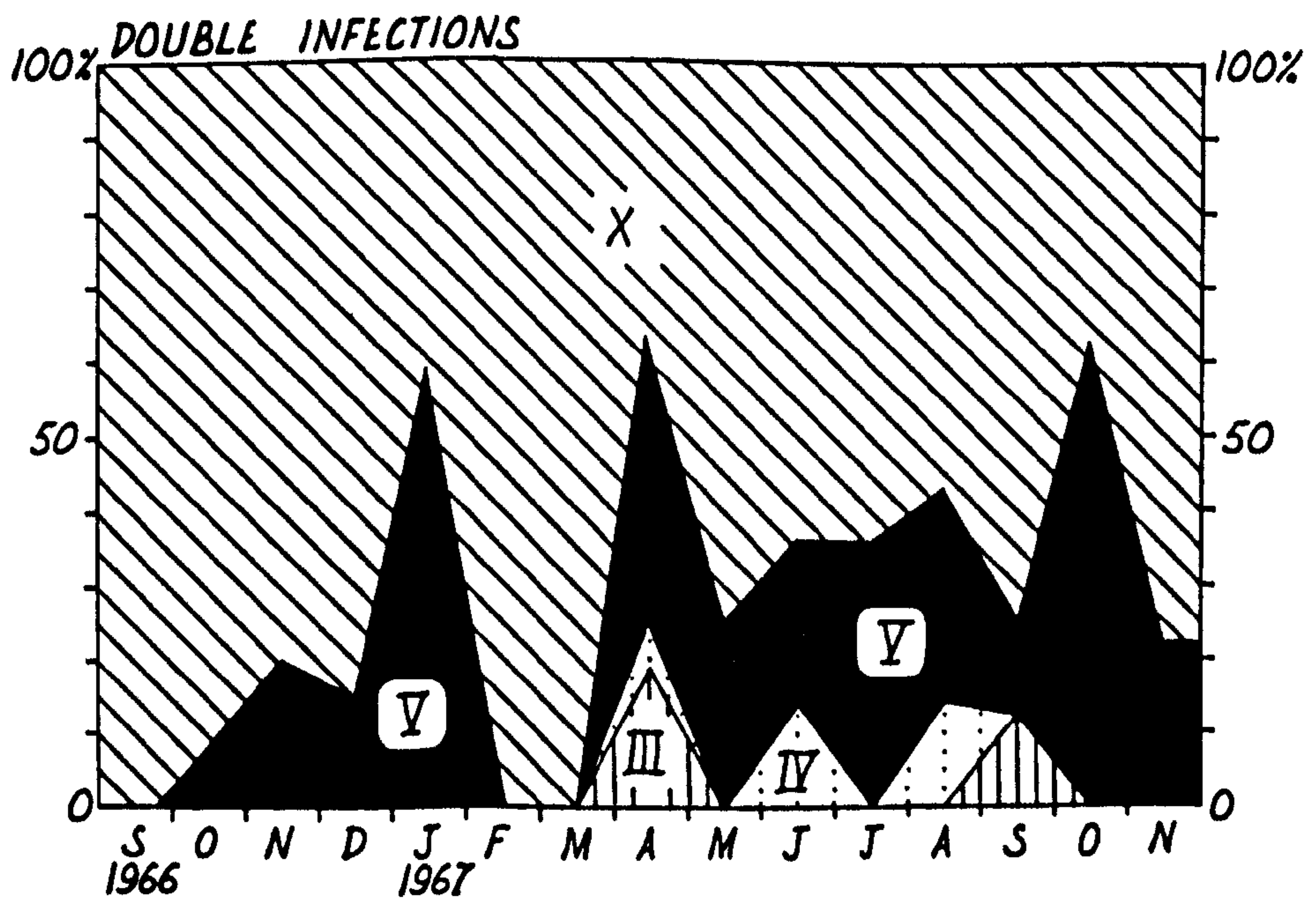
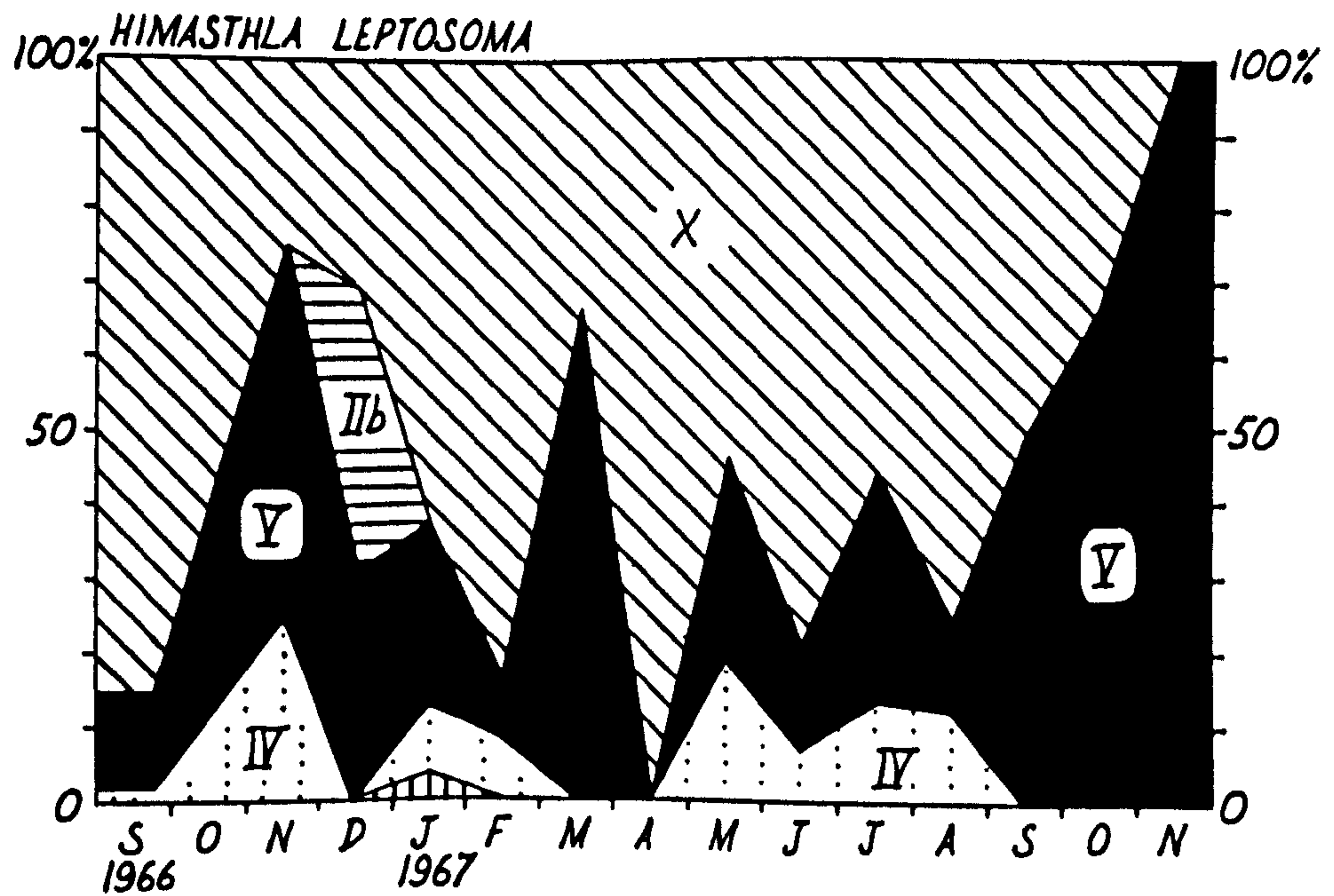


FIG 12 BREEDING CYCLE OF L. Littorea MALES AND FEMALES INFECTED WITH THE SPECIES INDICATED

(i) Sex ratio of uninfected Littorina littorea

Of the 3,525 uninfected animals examined 1,640 were males, 1,630 females, and 255 were too young for their sex to be determined. The results are shown graphically in Fig. 13 as the percentage of males, females and "neuters" collected each month for the various size classes, and also for the entire size range. The actual numbers are given in Table 14.

The confidence interval statistical test (Simpson, Roe & Lewontin, 1960) was used to ascertain if the sex ratio differed significantly from the 1:1 ratio. This is the ratio one would expect to occur if L.littorea conformed to the genetical theory which predicts that in species with regular sexual reproduction equal numbers of male and female zygotes are formed at fertilization.

Confidence interval test : worked example

Sex ratio among uninfected L.littorea at Scalby Rocks for total number of animals that could be sexed.

Number of animals	N	=	3270
Number of males	n_m	=	1640
Number of females	n_f	=	1630
Proportion of males	n_m / N	=	.502

continued

95% confidence interval for proportion of males :

$$= n_m / N \pm 1.96 \sqrt{\frac{(.502)(.498)}{3270}}$$

$$= .502 \pm (1.96)(.0087) = .502 \pm .017$$

or from .485 - .519

Since this interval includes the hypothetical value of .50 there is no reason to reject the hypothesis that this sample had the normal Mendelian sex ratio.

Although considerable monthly variation occurred in the proportion of males and females of each size class collected (Fig. 13) the confidence interval was only calculated for total numbers of each size class (in a similar manner to the above worked example). The intervals appear in Table 15. In classes containing "neuter" animals the sex proportion was based on the number of male and female animals only. In the animals studied the 95% confidence interval includes the hypothetical value of .50 for all but those in the first and third size classes (i.e. up to 10.0 mm and 15.1 - 20.0 mm shell height).

When the proportion of males in the 15.1 - 20.0 mm size class is considered, the 95% confidence interval only just fails to incorporate the hypothetical value of .50. This value, however, is just included if the class is tested at the 98% level

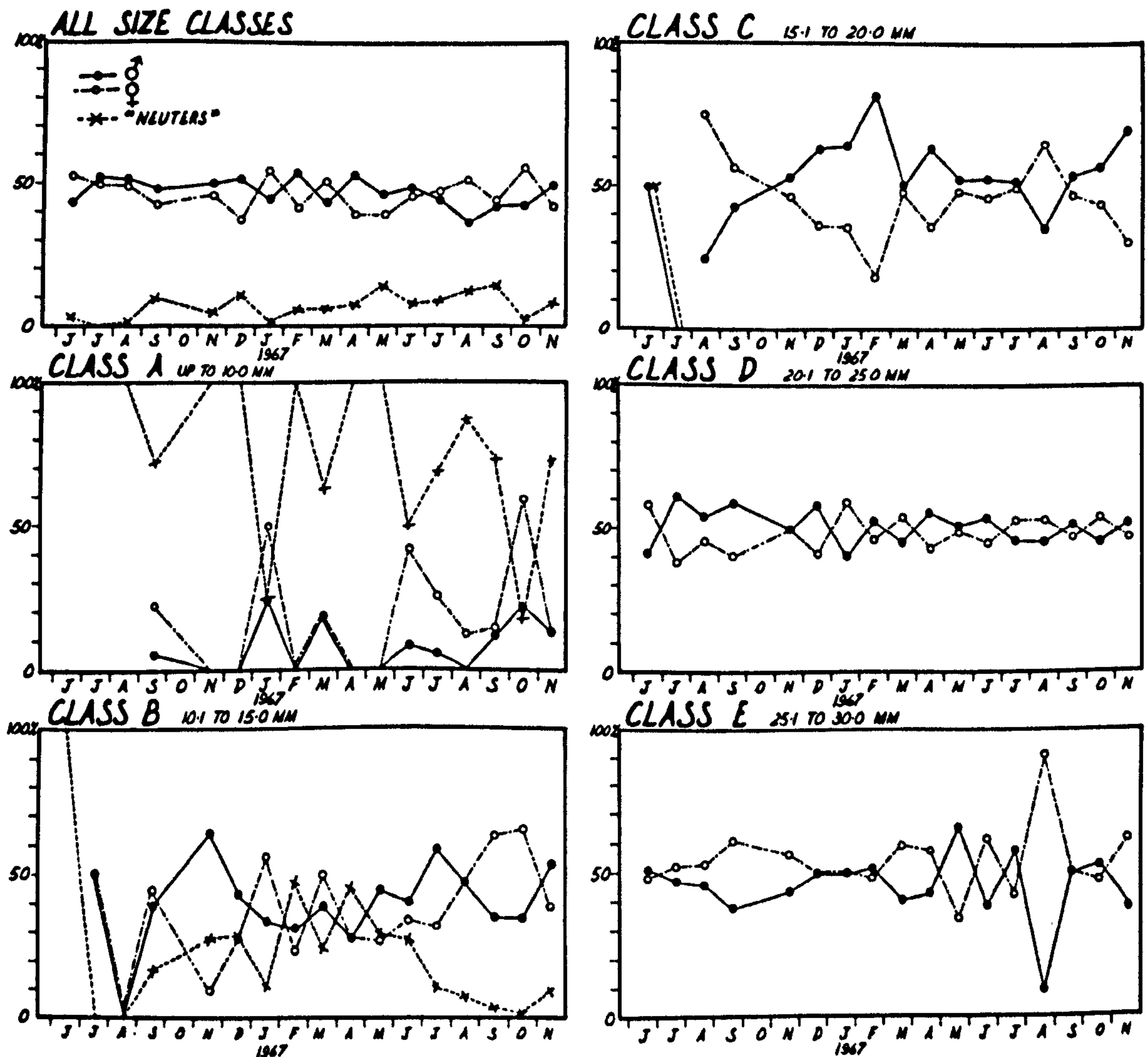


FIG 13 SEX RATIOS IN DIFFERENT SIZE CLASSES AND FOR ENTIRE SIZE RANGE OF UNINFECTED *L. Littorea*

TABLE 14

DIVISION OF UNINFECTED L.LITTOREA AND TOTAL PARASITISED L.LITTOREA AT SCALBY ROCKS
INTO SEX AND SIZE CLASS

UNINFECTED

	CLASS A (up to 10.0mm)		CLASS B (10.1-15.0mm)		CLASS C (15.1-20.0mm)		CLASS D (20.1-25.0mm)		CLASS E (25.1-30.0mm)		CLASS F (above 30.0mm)		ALL SIZE CLASSES	
	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂
5.2.66	0	0	0	0	0	7	0	38	0	0	0	0	0	43
1.6.66	0	0	2	0	1	1	0	18	0	0	0	0	3	36
7.7.66	0	0	0	0	0	0	0	16	0	0	0	0	0	36
16.8.66	1	0	0	0	0	0	0	22	0	0	0	0	1	47
31.8.66	1	0	0	0	0	1	0	27	0	0	0	0	1	43
29.9.66	13	1	3	7	0	7	9	56	0	0	0	0	16	76
2.11.66	4	0	3	7	0	8	7	47	0	0	0	0	7	73
1.12.66	16	0	2	3	0	14	8	56	0	0	0	0	13	90
12.1.67	1	1	1	3	0	11	6	48	0	0	0	0	2	86
11.2.67	4	0	6	4	0	18	4	69	0	0	0	0	10	106
14.3.67	7	2	6	16	0	35	34	64	0	0	0	0	15	126
10.4.67	5	0	8	5	1	36	20	64	0	0	0	0	14	108
9.5.67	32	0	12	18	0	38	35	68	0	0	0	0	44	142
6.6.67	12	2	4	6	1	39	34	61	0	0	0	0	17	114
10.7.67	27	2	2	11	0	43	42	90	0	0	0	0	29	157
7.8.67	28	4	1	6	0	14	26	64	0	0	0	0	29	85
4.9.67	30	5	1	10	0	26	23	48	0	0	0	0	31	93
2.10.67	4	5	1	22	0	21	16	42	0	0	0	0	5	99
2.11.67	11	2	2	11	0	14	6	42	0	0	0	0	13	76
Total Obs.	196	20	54	130	5	353	280	940	0	266	279	0	255	1640

TOTAL PARASITISED

	CLASS A (up to 10.0mm)		CLASS B (10.1-15.0mm)		CLASS C (15.1-20.0mm)		CLASS D (20.1-25.0mm)		CLASS E (25.1-30.0mm)		CLASS F (above 30.0mm)		ALL SIZE CLASSES	
	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂
5.2.66	0	0	0	0	0	1	0	5	0	1	0	0	0	7
1.6.66	0	0	0	0	0	0	0	4	0	3	7	0	0	10
7.7.66	0	0	0	0	0	0	0	5	0	15	15	0	0	27
16.8.66	0	0	0	0	0	0	0	11	0	26	32	0	0	43
31.8.66	0	0	0	0	0	0	0	16	0	16	24	0	0	39
29.9.66	0	0	1	1	0	6	2	58	0	50	38	0	0	128
2.11.66	0	0	1	1	0	6	4	35	0	24	29	0	1	73
1.12.66	0	0	0	0	0	4	1	29	0	17	16	0	0	51
12.1.67	0	0	0	0	0	4	3	49	0	37	40	0	0	97
11.2.67	0	0	0	0	0	3	7	24	0	15	24	0	0	47
14.3.67	0	0	0	0	0	8	6	31	0	7	16	0	0	51
10.4.67	1	0	0	0	0	8	7	43	0	13	16	0	1	60
9.5.67	0	0	0	0	0	5	10	47	0	21	23	0	0	77
6.6.67	0	0	0	0	0	6	3	57	0	21	16	0	0	88
10.7.67	0	0	0	0	0	5	7	75	0	20	28	0	0	104
7.8.67	0	0	0	0	0	2	6	37	0	11	21	0	0	51
4.9.67	0	0	0	0	0	3	2	42	0	19	21	0	0	66
2.10.67	0	0	0	0	0	1	0	33	0	19	14	0	0	35
2.11.67	0	0	0	0	0	1	0	22	0	16	14	0	0	42
Total Obs.	1	0	1	1	0	63	61	623	0	353	396	0	2	1128

of confidence, resulting in an interval range of .496 - .590. It is possible that this size class in nature exhibits the same 50 : 50 distribution of male and female L.littorea : however larger numbers of animals need to be examined before the true relationship between the proportion of the sexes in this size class is revealed. In animals up to 10.0 mm there appear to be significantly fewer males than females, but it would be wrong to draw any conclusions from this as the number of animals whose sex could be determined was only 74 out of a total in this size range of 270.

TABLE 15. 95% CONFIDENCE INTERVAL FOR MALES AND FEMALES PRESENT IN A 1:1 HYPOTHESIS FOR EACH SIZE CLASS AND TOTAL SIZE RANGE OF UNINFECTED LITTORINA LITTOREA AT SCALBY ROCKS

<u>Shell height</u>	<u>Interval Range of Males</u>	<u>Proportion of Males</u>	<u>N</u>
Up to 10.0 mm Class A	.167 to .373	.270 *	74
10.1 - 15.0 mm Class B	.421 to .539	.480	271
15.1 - 20.0 mm Class C	.504 to .582	.543 *	613
20.1 - 25.0 mm Class D	.484 to .530	.507	1854
25.1 - 30.0 mm Class E	.427 to .521	.474	435
Above 30.0 mm Class F	.262 to .694	.478	23
All size classes	.485 to .519	.502	3270

cont.

x = proportion of males significantly different at the 95% confidence interval.

N = Number of males and females in each group

Thus, apart from the inconclusive evidence mentioned above uninfected L.littorea considered as a whole exhibit a 1:1 sex ratio with little significant deviation.

Pelseneer (1926) observed that the sexes were present in approximately equal numbers in the smaller L.littorea (13 - 15 mm), but in a sample of 192 individuals greater than 22 mm in height he found only 32.8% to be males. Similarly at Plymouth, Moore (1937) found approximately equal numbers up to 25 mm. Above this size the proportion of males fell sharply to about one quarter of the animals examined. The results obtained during the present survey do not confirm fully the findings of these two authors. At Scalby Rocks an examination of the different size classes indicated that the sex ratio remains approximately the same at all sizes in this locality. However, as Rothschild (1938 b) stated:- "In nature the relative number of males and females of Peringia ulvae seem to vary considerably, according to both size (age) and distribution, and other unknown factors, and therefore each sample must be examined separately".

(ii) Sex ratio of parasitised Littorina littorea

Tables 14 and 16 show the actual number each month of male

TABLE 16

DIVISION OF L.LITTOREA INFECTED WITH C.LINGUA AND L.LITTOREA INFECTED WITH CERCARIA A
AT SCALBY ROCKS INTO SEX AND SIZE CLASSCRYPTOCOPILE LINGUA

	CLASS A (UP TO 10.0mm)		CLASS B (10.1-15.0mm)		CLASS C (15.1-20.0mm)		CLASS D (20.1-25.0mm)		CLASS E (25.1-30.0mm)		CLASS F (above 30.0mm)		ALL SIZE CLASSES	
	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂
5-2.66	0	0	0	0	0	1	0	3	4	0	1	0	0	5
1.6.66	0	0	0	0	0	0	0	0	0	0	1	2	0	2
7.7.66	0	0	0	0	0	0	2	2	3	0	0	6	0	12
16.8.66	0	0	0	0	0	0	2	2	2	0	14	19	0	24
31.8.66	0	0	0	0	0	0	3	3	3	0	3	11	0	12
29.9.66	0	0	0	0	0	1	16	16	16	0	30	23	0	61
2.11.66	0	0	0	0	0	2	6	6	6	0	15	13	0	30
1.11.66	0	0	0	0	0	0	7	7	7	0	0	7	4	15
12.1.67	0	0	0	0	0	0	10	71	17	0	18	17	0	35
11.1.67	0	0	0	0	0	0	6	1	6	0	6	11	0	15
14.3.67	0	0	0	0	0	1	4	6	5	0	5	8	0	13
10.4.67	0	0	0	0	0	1	4	5	5	0	5	12	0	12
9.5.67	0	0	0	0	0	1	9	7	10	0	10	6	0	22
6.6.67	0	0	0	0	0	1	13	16	10	0	10	10	0	28
10.7.67	0	0	0	0	0	1	14	14	11	0	10	11	0	29
7.8.67	0	0	0	0	0	0	5	6	8	0	8	10	0	14
4.9.67	0	0	0	0	0	0	4	14	15	0	15	15	0	21
2.11.67	0	0	0	0	0	0	12	14	14	0	14	10	0	28
2.11.67	0	0	0	0	0	0	7	8	9	0	9	9	0	18
Total Obs.	0	1	0	0	0	9	127	156	190	202	0	70	0	396

-93-

CERCARIA A

	CLASS A (UP TO 10.0mm)		CLASS B (10.1-15.0mm)		CLASS C (15.1-20.0mm)		CLASS D (20.1-25.0mm)		CLASS E (25.1-30.0mm)		CLASS F (above 30.0mm)		ALL SIZE CLASSES	
	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂	"neuters"	♂
5-2.66	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.6.66	0	0	0	0	0	0	4	2	0	0	0	0	0	0
7.7.66	0	0	0	0	0	0	3	2	0	0	0	0	0	0
16.8.66	0	0	0	0	0	0	5	10	10	9	0	0	0	12
31.8.66	0	0	0	0	0	0	11	5	10	7	0	0	0	16
29.9.66	0	0	0	0	0	4	29	32	13	8	0	0	0	21
2.11.66	0	0	0	0	0	4	21	23	6	10	0	0	0	46
1.11.66	0	0	0	0	0	3	17	17	6	5	0	0	0	32
12.1.67	0	0	0	0	0	3	33	21	11	10	0	0	0	26
11.2.67	0	0	0	0	0	3	15	18	7	9	0	0	0	47
14.3.67	0	0	0	0	0	6	25	26	1	6	0	0	0	35
10.4.67	1	0	0	0	0	6	36	27	6	3	0	0	0	35
9.5.67	0	0	0	0	0	4	33	36	4	11	0	0	1	48
6.6.67	0	0	0	0	0	5	38	49	8	2	0	0	0	41
10.7.67	0	0	0	0	0	2	57	53	4	8	0	0	0	51
7.8.67	0	0	0	0	0	2	27	53	0	9	0	0	0	63
4.9.67	0	0	0	0	0	3	30	23	2	3	0	0	0	29
2.11.67	0	0	0	0	0	1	19	20	3	3	0	0	0	35
2.11.67	0	0	0	0	0	1	10	11	4	3	0	0	0	25
Total Obs.	1	0	0	1	0	47	413	408	106	116	0	3	1	570

and female L.littorea parasitised with 1) C.lingua 2) Cercaria A and 3) all infections (i.e. C.lingua, Cercaria A, H.leptosoma, mixed infections and the infrequent C.lebouri infections summed).

The sex distribution as a percentage in each monthly collection for the various size classes is shown in Figs. 14 to 16. A total of 2,328 parasitised animals - 1128 males, 1198 females and 2 "neuters" - were examined.

As was found in the uninfected animals considerable monthly variation occurred in the proportion of males and females of each size class collected, but again the 95% confidence interval was only calculated for the total number of animals in each size class (in which sufficient parasitised individuals were found), and for the entire size range for L.littorea infected with a) C.lingua, b) Cercaria A and c) all species of digenean larvae. The intervals appear in Table 17 which shows that the number of males and females do not differ significantly from each other at the 5% level of significance, the hypothetical value of .50 being included in the interval range.

From the results obtained in this survey parasitism has not altered the expected 1:1 sex ratio, which was found in uninfected animals of L.littorea infected either with C.lingua or with Cercaria A or with all species of digenean larvae considered together.

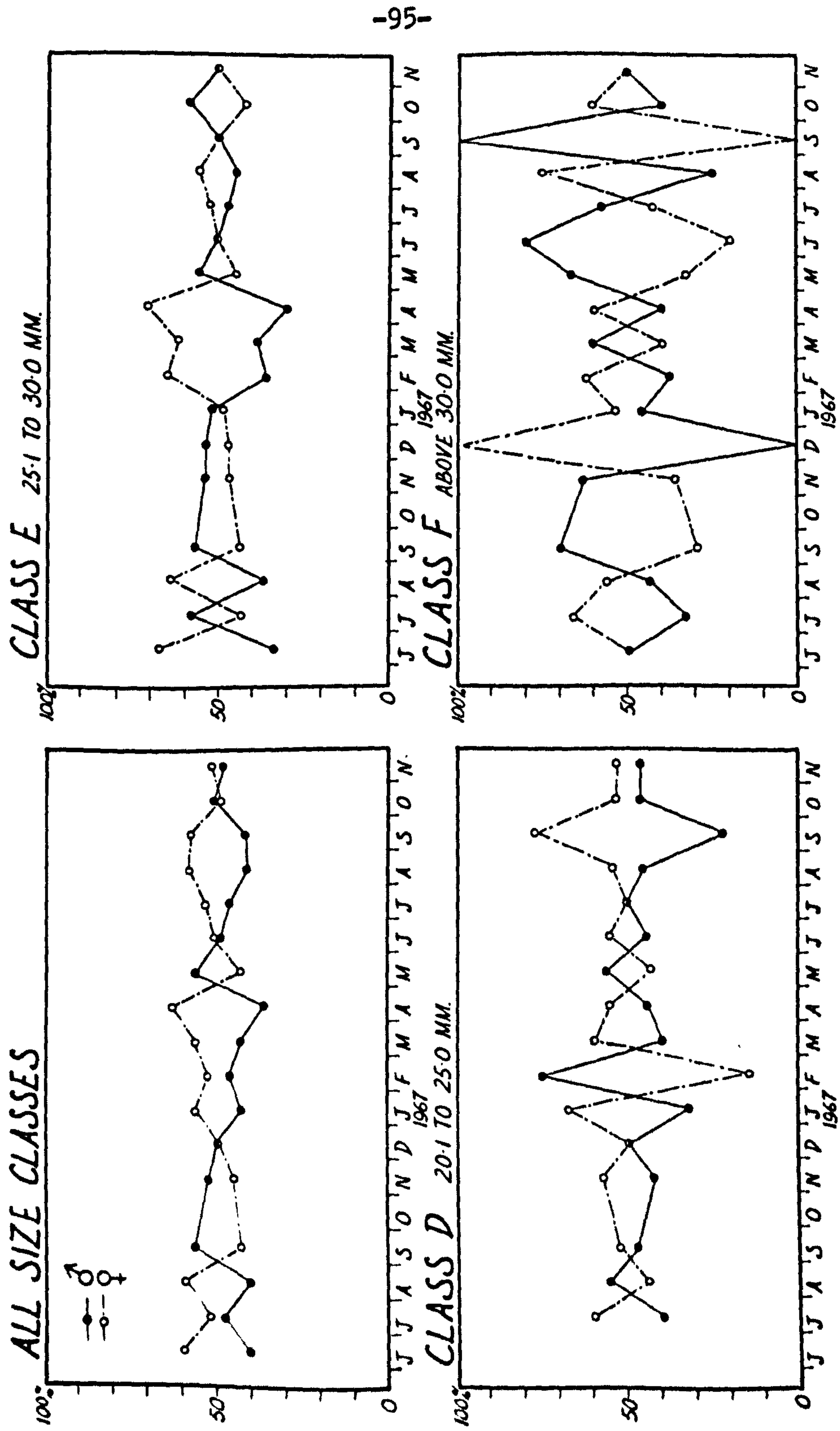


FIG 14 SEX RATIOS IN DIFFERENT SIZE CLASSES AND FOR THE ENTIRE SIZE RANGE OF *L. Littorea* INFECTED WITH *C. Lingua*

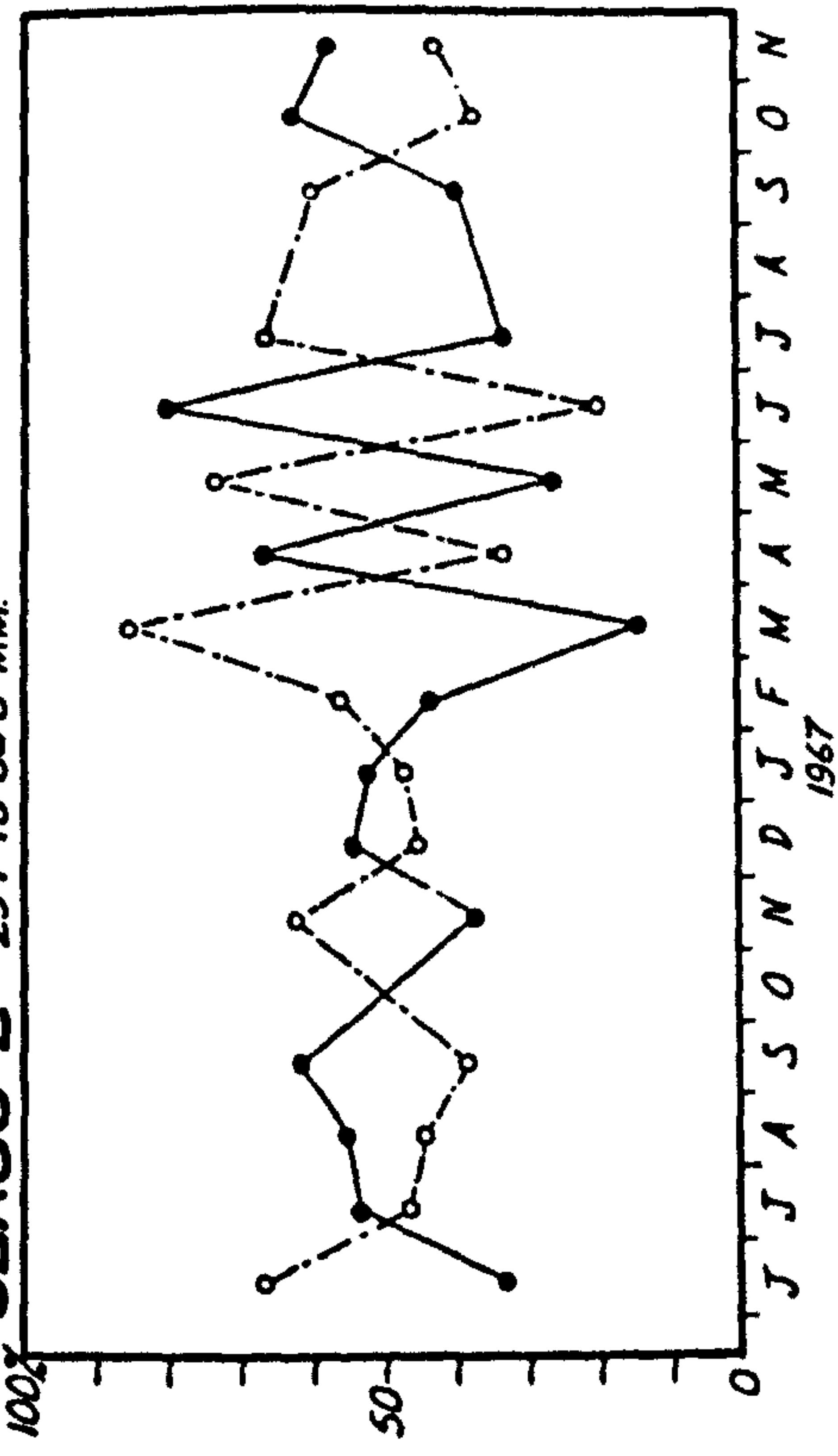
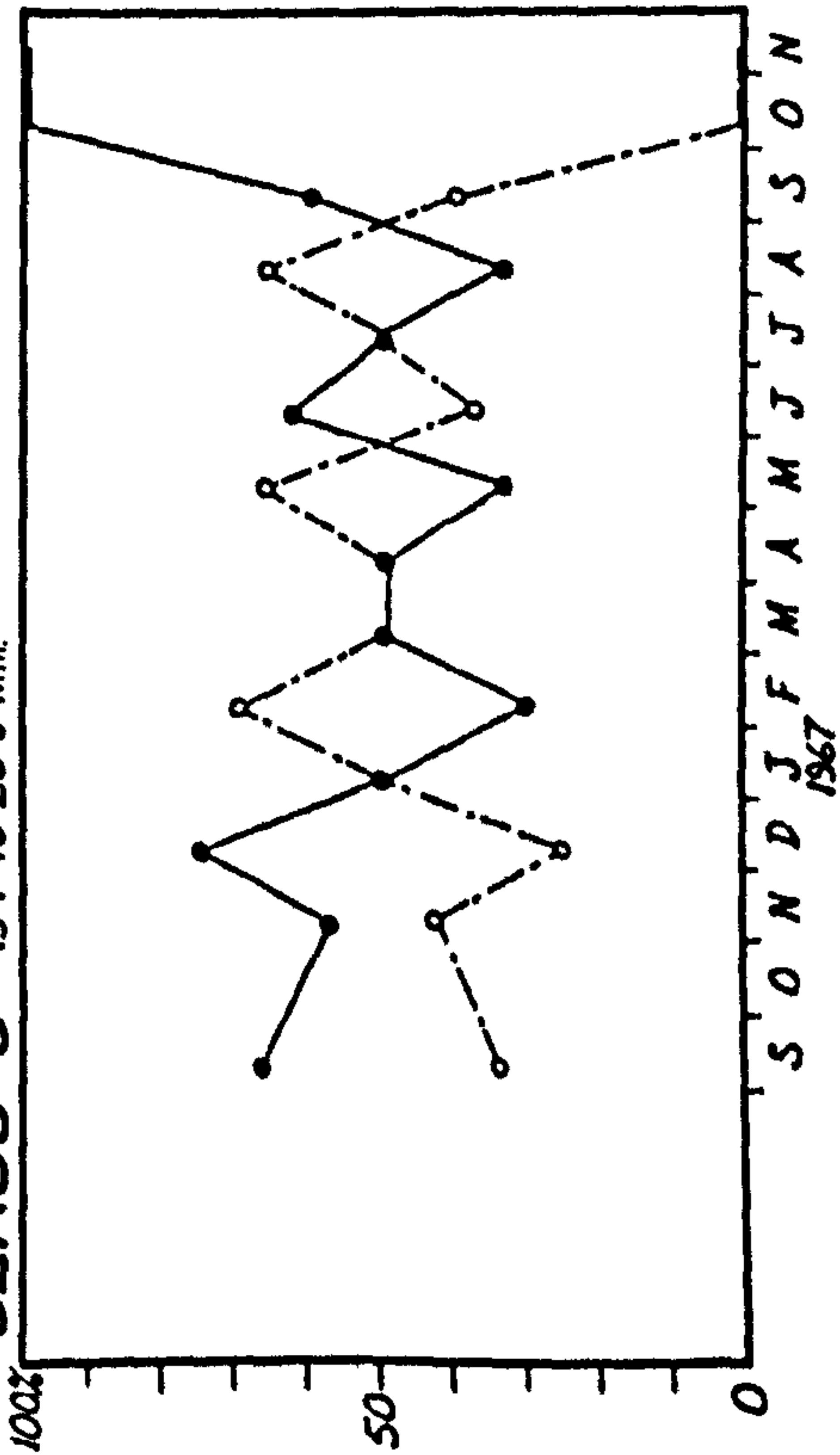
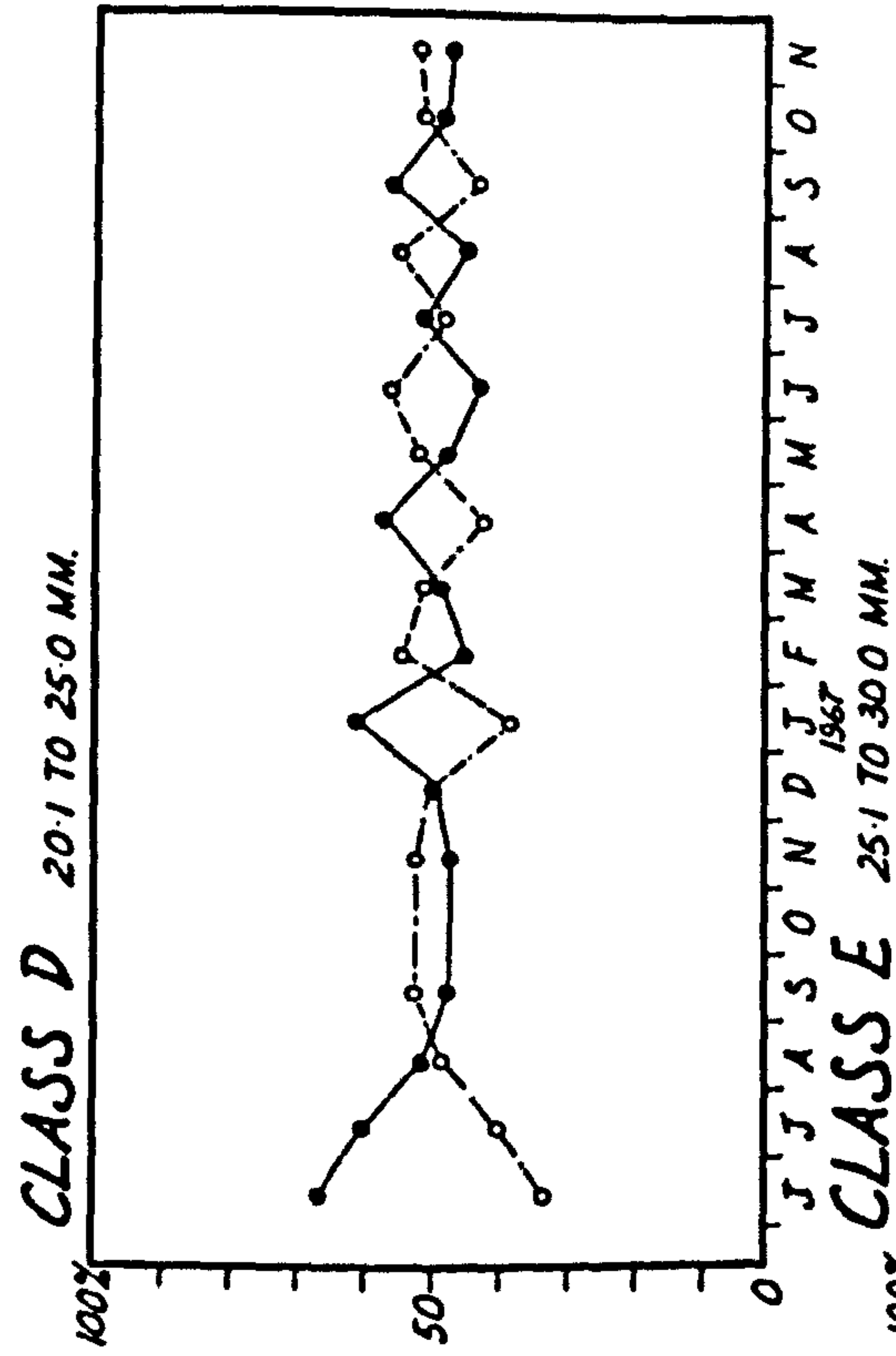
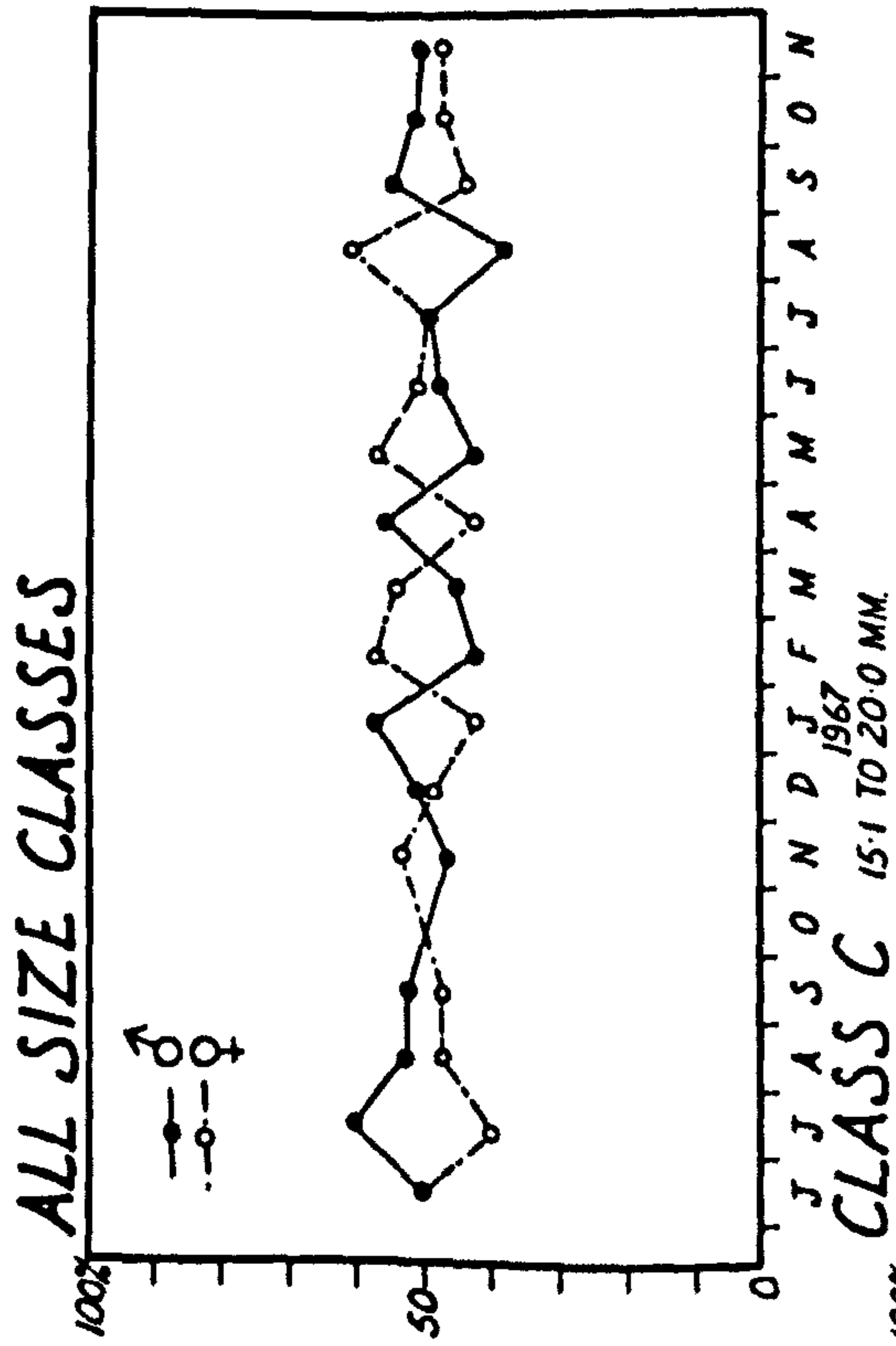


FIG 15 SEX RATIOS IN DIFFERENT SIZE CLASSES AND FOR THE ENTIRE SIZE RANGE OF *L. Littorea* INFECTED WITH *Cercaria A*

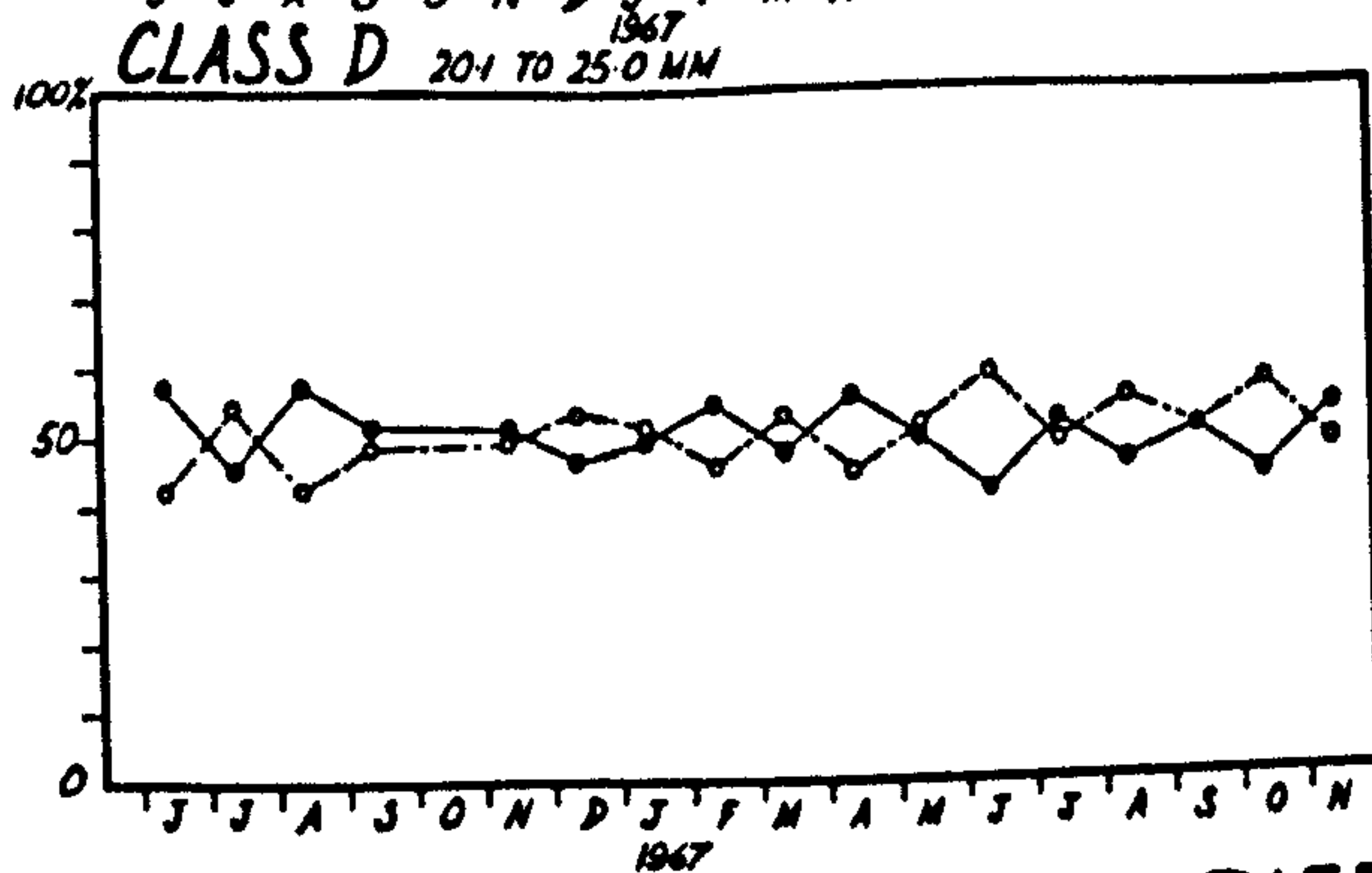
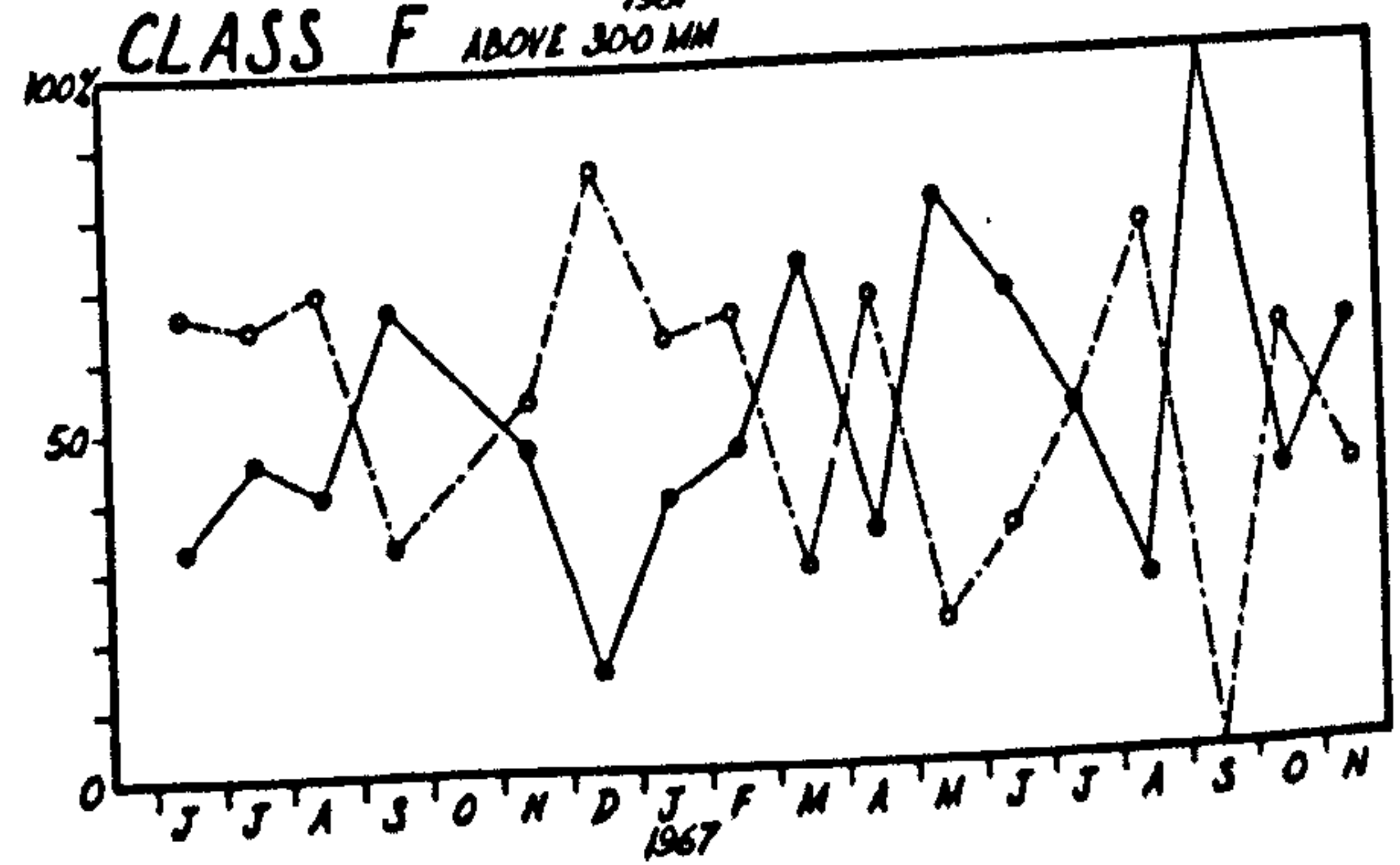
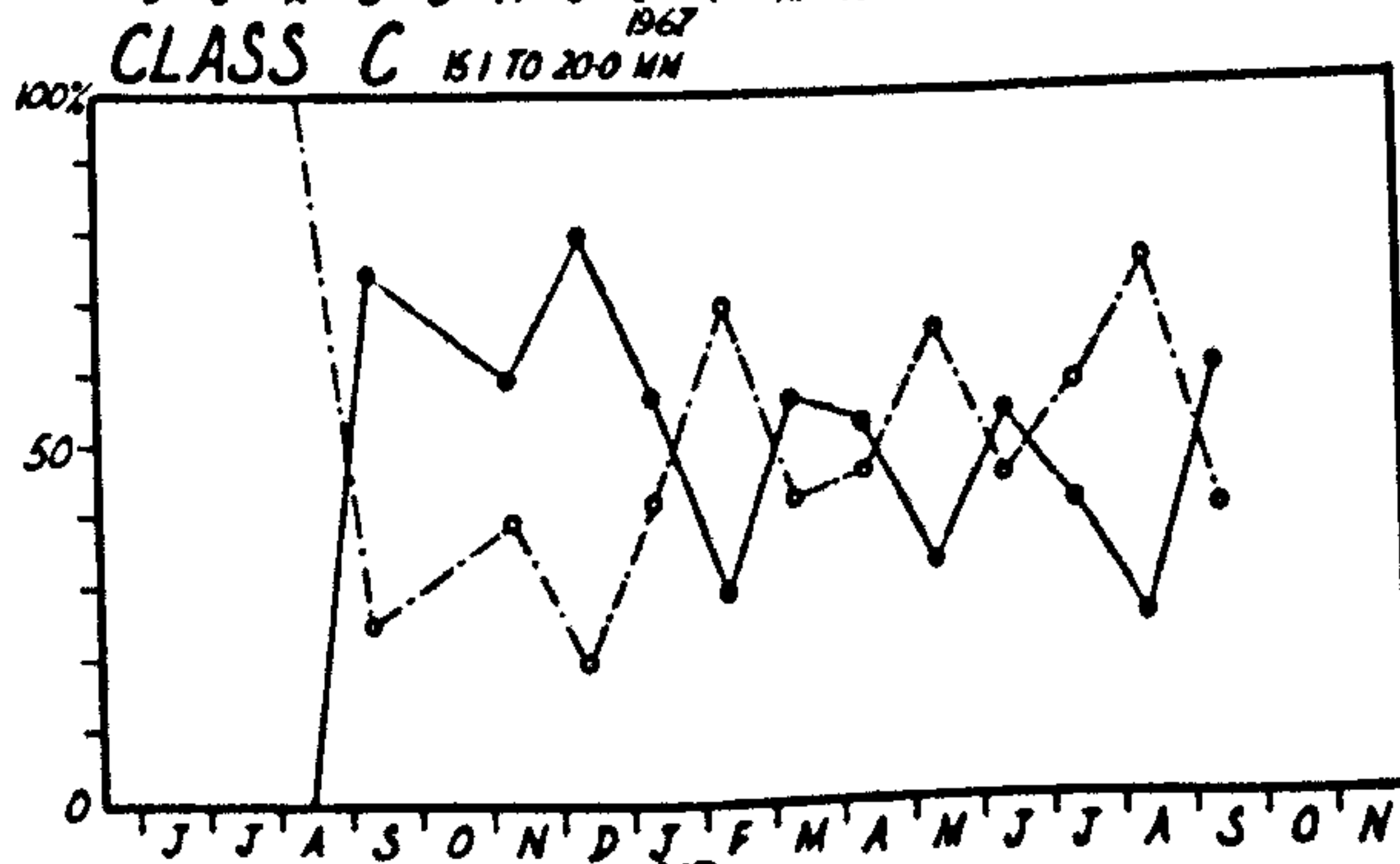
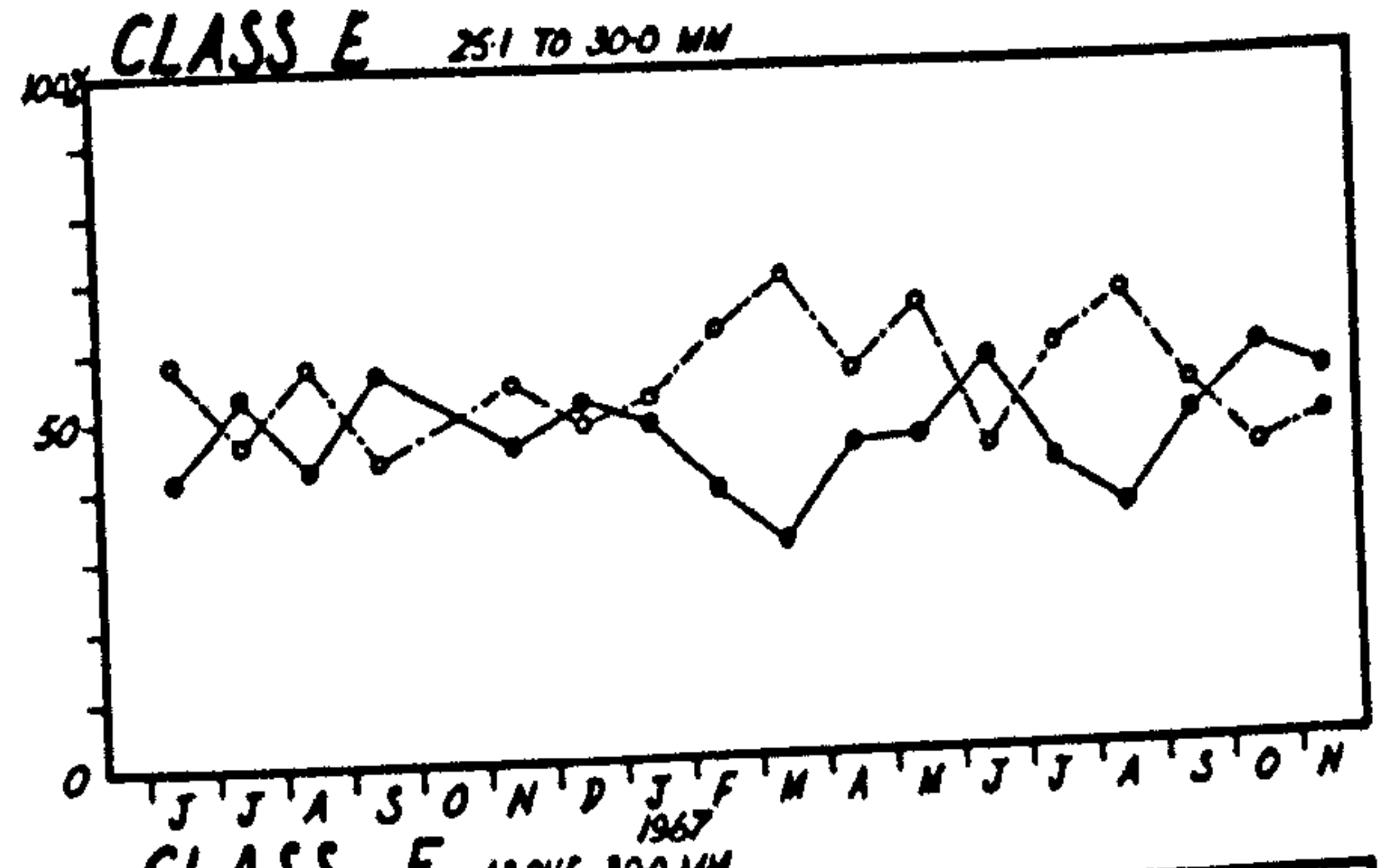
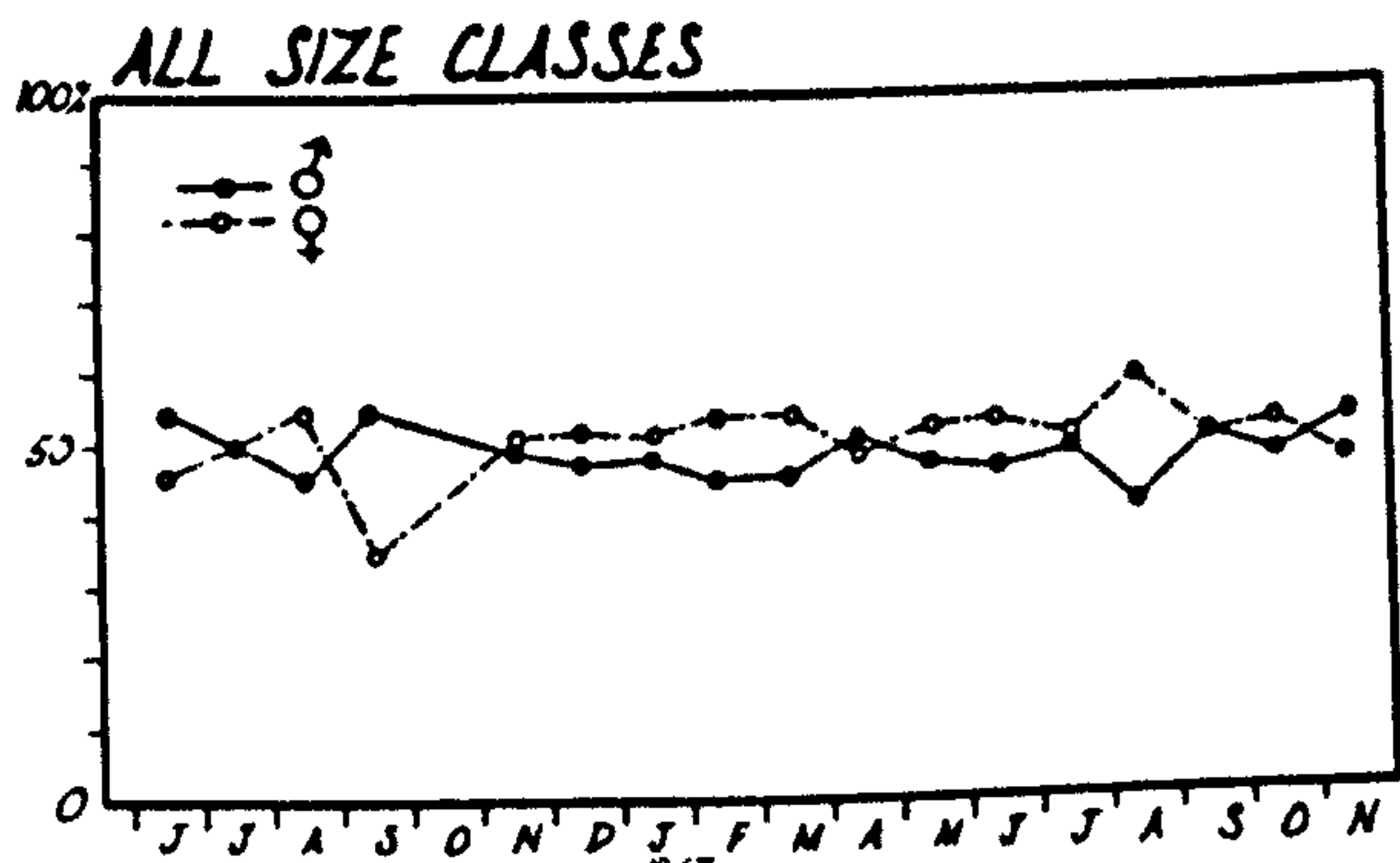


FIG 16 SEX RATIOS IN DIFFERENT SIZE CLASSES AND FOR ENTIRE SIZE RANGE OF TOTAL PARASITISED L. Littorea

TABLE 17. 95% CONFIDENCE INTERVAL FOR MALES AND FEMALES PRESENT IN A 1:1 HYPOTHESIS FOR EACH SIZE CLASS AND TOTAL SIZE RANGE OF L.LITTOREA INFECTED WITH: C.LINGUA ; CERCARIA A ; AND ALL SPECIES OF DIGENEAN LARVAE

Shell height	Interval Range of Males	Proportion of Males	No. Exam ♂+♀
<u>CRYPTOCOTYLE LINGUA</u>			
Up to 10.0mm (Class A)	----	.000	1
10.1-15.0mm (Class B)	----	----	0
15.1-20.0mm (Class C)	.132 to .726	.429	21
20.1-25.0mm (Class D)	.391 to .507	.449	283
25.1-30.0mm (Class E)	.436 to .535	.485	392
Above 30.0mm (Class F)	.435 to .603	.519	135
All Size Classes	.442 to .510	.476	832
<u>CERCARIA A</u>			
Up to 10.0mm (Class A)	----	----	0
10.1-15.0mm (Class B)	----	.500	2
15.1-20.0mm (Class C)	.397 to .603	.500	94
20.1-25.0mm (Class D)	.469 to .537	.503	821
25.1-30.0mm (Class E)	.411 to .543	.477	222
Above 30.0mm (Class F)	minus .022 to .688 ^a	.333	9
All Size Classes	.468 to .526	.497	1148
<u>TOTAL PARASITISED</u>			
Up to 10.0mm (Class A)	----	.000	1
10.1-15.0mm (Class B)	----	.500	2
15.1-20.0mm (Class C)	.420 to .596	.508	124
20.1-25.0mm (Class D)	.464 to .520	.492	1267
25.1-30.0mm (Class E)	.437 to .509	.473	751
Above 30.0mm (Class F)	.402 to .548	.475	181
All Size Classes	.465 to .505	.485	2326

(a) Test meaningless

Lysaght (1941) found that in a population of Littorina peritoides on the Plymouth Breakwater infected with two digenean cercariae and one metacercaria, all unidentified, the proportions of uninfected and infected males were similar. She did, however, find that the proportion of both infected and uninfected males compared with females was significantly higher in the smaller size classes than in the larger ones. This condition was not observed in the population of L.littorea at Scalby Rocks.

The phenomenon of sex-reversal is of normal occurrence in some gastropods, but it has not been recorded in L.littorea (Fretter & Graham, 1962). Wesenberg-Lund (1931) was the first to suggest that trematode parasites may be the cause of sex-reversal in species in which it does not normally occur. He found that in the hermaphrodite snail Succinea putris (L.) parasitised by Leucochloridium macrostoma (Rud. (= L.paradoxum Carus)) the female organs are first destroyed by the parasite, while the production of sperm - though greatly reduced - continues; finally however the mollusc may be completely sterilised. Krull (1935) suggested that infected specimens of Hydrobia ulvae (= Peringia ulva (Pennant) = Paludestrina ulvae) with a much reduced penis were in reality "sex-reversed" females, though they never functioned as males. The discovery by Rothschild (1938 b) of a gravid female of H.ulvae with a small penis lends support to

this idea; she did not however consider that all snails with a reduced penis were "sex-reversed" females. Rees (1936) and Lysaght (1941, 1943) found no occurrence of sex-reversal in infected specimens of L.littorea or Littorina neritoides.

During the course of the survey of L.littorea at Scalby Rocks there was no evidence to suggest that sex-reversal occurred in this species, either in the uninfected individuals or as a result of infection with larval digeneans. No transitional stages from male to female or vice versa were seen.

(iii) Sex ratios in Littorina littorea collected from other areas

Animals were also collected from South Bay, Scarborough, Scalby Pipe and Burniston Bay during the preliminary investigation on the incidence of trematode infections in L.littorea on the North Yorkshire Coast. The data was analysed statistically to ascertain if the sex ratios differed significantly from the 1:1 ratio. The 95 % confidence intervals are given in Table 18.

It will be noticed that the Scalby Pipe specimens (total size classes) did not bear out the 50 : 50 sex ratio which was found at Scalby Rocks and other areas of collection.

The Scalby Pipe figures when analysed for each size class (where numbers permitted) show that with the exception of uninfected animals in the size range 20.1 - 25.0 mm and also for total animals in this size range that the 50 : 50 basis of sex is borne out (Table 19).

TABLE 18. 95% CONFIDENCE INTERVAL FOR MALES AND FEMALES PRESENT IN A 1:1 HYPOTHESIS FOR TOTAL SIZE RANGE OF UNINFECTED L.LITTOREA AND TOTAL PARASITISED L.LITTOREA AT BURNISTON BAY, SOUTH BAY (SCARBOROUGH) AND SCALBY PIPE

	Interval Range of Males	Proportion of Males	No. Exam
<u>BURNISTON</u>			
Uninfected	.470 to .566	.518	415
Total Parasitised	minus .023 to .623 ^a	.300	10
Total Animals	.465 to .561	.513	425
<u>SOUTH BAY</u>			
Uninfected	.464 to .528	.496	967
Total Parasitised	.466 to .504	.485	31
Total Animals	.465 to .527	.496	998
<u>SCALBY PIPE</u>			
Uninfected	.415 to .479	.447 ^a	933
Total Parasitised	.435 to .561	.498	239
Total Animals	.428 to .486	.457 ^a	1172

(a) Test meaningless

^aProportion of males significantly different at the 95% confidence interval

No. Exam = males + females

TABLE 19. 95% CONFIDENCE INTERVAL FOR MALES AND FEMALES PRESENT IN A 1:1 HYPOTHESIS FOR EACH SIZE CLASS (where numbers were sufficient) OF UNINFECTED L.LITTOREA AND TOTAL PARASITISED L.LITTOREA AT SCALBY PIPE

	Interval Range of Males	Proportion of Males	No. Exam
<u>UNINFECTED</u>			
10.1-15.0mm (Class B)	.152 to .556	.364	22
15.1-20.0mm (Class C)	.421 to .503	.462	571
20.1-25.0mm (Class D)	.370 to .478	.424 ^a	314
25.1-30.0mm (Class E)	.264 to .694	.479	23
<u>TOTAL PARASITISED</u>			
15.1-20.0mm (Class C)	.477 to .691	.584	84
20.1-25.0mm (Class D)	.343 to .517	.430	128
<u>TOTAL ANIMALS</u>			
10.1-15.0mm (Class B)	.162 to .558	.360	25
15.1-20.0mm (Class C)	.440 to .516	.478	655
20.1-25.0mm (Class D)	.379 to .471	.425 ^a	442
25.1-30.0mm (Class E)	.363 to .657	.510	47

^a Proportion of males significantly different at the 95% confidence interval

DISCUSSION

Studies on the breeding season of L.littorea have been made by several workers. Among the earliest, Tattersall (1920) reported that the breeding season of L.littorea in Western Ireland extended from January to June, with a peak in April and early May, and pelagic larvae were commonly found in inshore waters from May to July. According to Elmhirst (1923), the season at Millport extended from January to July, on rare occasions being prolonged into August; the young were abundant on the shore in April. Further records of the main spawning period are summarized in Table 20.

The breeding period of L.littorea at Scalby Rocks extended over a greater period than has been reported hitherto by other workers in different localities. Mature and spawning animals were recorded as early as December 1966 and they were collected in large numbers up to and including the beginning of August 1967, the main peak occurring in February and March (Fig. 8, Table 10).

From the earlier literature and the results obtained during the present survey, it appears that L.littorea is probably capable of breeding at any time of the year; the duration and timing of the breeding season varying with locality and local environmental conditions.

Both Tattersall (1920) and Moore (1937) recorded mature male L.littorea in January, but mature females were never found before

TABLE 20 RECORDS ON THE BREEDING OF L. LITTOREA AT DIFFERENT LOCALITIES

-103-

Locality	Breeding Season (Peak Periods Shown in Parenthesis)	Observations on Larvae	Observations on Juveniles	Other Remarks	Author
Heligoland	January - June (March - May)	_____	_____	_____	Linke, 1933, 1934
Plymouth	December - Mid-March or April	_____	_____	The evidence suggests that although the winter months are favoured <u>L. littorea</u> is capable, at Plymouth anyway, of breeding throughout the year	Moore, 1937
Plymouth	_____	Found in plankton hauls throughout the year, but were most abundant in February and March	_____		Lebour, 1937, 1947
Kresund, Denmark	May - September	Early stages most abundant during June, July and August. None found in February and March	_____	_____	Thorson, 1946
Writstable, Kent	_____	Taken in plankton hauls from July to October. None in winter	5mm young abundant on shore from April to June	Abundance of young on shore between April and June, suggests eggs had been present in sea during winter months	Smith & Nevell, 1955
Aberystwyth	November - June (March)	_____	_____	_____	Williams, 1964

February. Similarly Williams (1964) stated that males attain sexual maturity before females. In the Scalby Rocks population, however, uninfected males and females attained sexual maturity at the same time, as judged by the criterion proposed by Williams (1964), (Tables 11 and 12, Figs. 9 and 10). However, at Scalby Rocks the proportion of mature females remained fairly constant throughout the breeding period (Fig. 10), while mature males were present in greatest proportions in February and June 1967 (Fig. 9). Assuming the results given in Table 11 are representative of the entire uninfected population, the first peak of mature spawning males might be largely attributable to older animals attaining maturity for at least the second time. Thus in November and December 1966, 22.2% and 41.1% respectively of males examined were maturing for at least the second time, after which very few stage IIb periwinkles were seen until August 1967. This suggests strongly that the numbers of fully mature (stage III) uninfected males were being sustained by those maturing for the first time. The seasonal picture for females is broadly similar, except that a greater proportion of the female population between January and August were mature, and they did not show any obvious fluctuations during this period (Fig. 10). It seems likely that during the winter the mature female population consisted of animals which had already reproduced in previous seasons; the spring and summer figures probably were maintained by the maturation of animals from the previous seasons broods.

During the 1966-1967 reproductive cycle of L.littorea at Scalby Rocks smaller animals of both sexes appeared, at first sight, to reach spawning condition before larger ones (Fig. 11). Thus, a considerably greater proportion of animals (72.7%) of class C (15.1 - 20.0 mm shell height) were at stage III in December 1966, compared with 40.1% of class D and 12.5% of class E (Table 13). However, this difference is probably a sampling illusion, the number of animals in the different size classes for December 1966 varying considerably (Table 13). For the other months the proportion of mature animals of different size classes bore a closer resemblance to each other, and the breeding season extended over the same period. It seems likely that at Scalby Rocks L.littorea of different sizes attain sexual maturity at about the same time of the year. This observation is not in agreement with the view of Williams (1964) that older specimens mature first. Further investigation into the subject might be fruitful.

Moore (1937) found that L.littorea at Plymouth attained average lengths of 14 mm, 17.4 mm, 22.4 mm and 25.4 mm at the end of their first, second, third and fourth winters respectively. He also found that they did not attain sexual maturity until they were about 15-18 months old, corresponding to a length of 17 mm. Williams (1964) at Aberystwyth studied a population of L.littorea which, compared with the results of Moore (1937) and the present study, was smaller in size and in growth rate; at the end of their first, second, third and fourth

years they had attained heights of 8-9 mm, 13-14 mm, 15.5-16.5 mm and 17.5 + mm respectively. Williams rarely found fully-developed gonads in animals less than 11-12 mm. Smith and Newell (1955) found that L.littorea at Whitstable, Kent measured 2-12 mm in height in their first year; 13-16 mm in height in their second year; and third year and older specimens were 17 mm and over. They also said that sexual maturity was attained during their second year. In the population studied at Scalby Rocks, animals maturing for the first time were usually confined to size class C (15.1 - 20.0 mm in length) with a few occurring among the smaller members of class D (20.1 - 25.0 mm). Thus at Scalby Rocks maturity was seldom encountered in animals less than 15 mm. These differences between the four populations may be genetic but may also be related to prevailing environmental conditions, especially climate, relative exposure to wave action and the availability of food.

The length of the pelagic larval life of L.littorea is generally considered to be 4 - 5 weeks, depending on water temperature and food supply, (Linke 1933, Smith & Newell 1955) but under unfavourable conditions it may be prolonged considerably (Thorson 1946). This may explain the continual presence of "neuter", stage I and IIa animals throughout the year. Although a marked peak in their numbers (71.4% of total uninfected) occurred in September 1967 (Fig. 8), there was a gradual build-up to and fall-off from this figure indicating, perhaps,

a considerable variation in the duration of larval life as well as a protracted spawning season. "Neuters" are the present season's brood, stage I animals being made up of the present and late developers from the previous season's brood, and stage IIa animals are upwards of a year old.

My findings on the time and extent of maturation of L.littorea at Scalby Rocks in relation to seasonal temperatures are in general agreement with the conclusions reached by Williams (1964) for L.littorea at Aberystwyth. He considered that animals were maturing when the sea temperature was at its seasonal lowest, and spawning in greatest numbers as the temperature rose. At Scalby Rocks the greatest proportion of maturing individuals (stage IIa, IIb) occurred in November-December 1966 and October-November 1967 (Fig. 8, Table 10) as the sea temperature approached its annual minimum (Fig. 17). Katkansky and Sparks (1966) reported a comparable condition for the Pacific Oyster, Crassostrea gigas; they considered that gonadal development appeared to be related to rising temperatures, while resorption of the gonad was related to falling temperatures.

Giese (1959) considers that work on the reproductive cycles of a number of marine invertebrates points to an endogenous rhythm as determining the pattern of the annual reproductive cycle but it is timed by environmental variables which are as yet unspecified.

The factors responsible for the initiation of gonad development

and their relation to the events preceding spawning are still speculative. However, it is unlikely that temperature alone is the environmental factor which induces the seasonal reproductive activity of marine invertebrates. Sastry (1968) considered that gonad development of the bay scallop, Aequipecten sp., was initiated when food was abundant and temperatures had reached a certain minimum level; gonad growth and gametogenesis being activated by these environmental conditions. Gonad activity appeared to be limited by the decrease in both temperature and food production following the spawning period.

The reproductive activity of L.littorea is considered in relation to temperature, abundance of food and carbohydrate content of the foot and digestive gland in a later section.

The pattern of the reproductive activity of L.littorea parasitised by digenean larvae at Scalby Rocks when the effects of all the species of parasites are considered together, to a certain extent resembles the cyclical nature shown by the uninfected periwinkles, but it is evident (Fig. 8) that the reproductive activity of the host was deleteriously affected by the presence of the parasitic larval stages. In particular the proportion of mature animals was drastically reduced, the maximum percentage (33.3%) recorded for any of the months throughout the survey being approximately two-fifths of the maximum for uninfected animals (85.8% - Table 10). Throughout the duration of

the survey, only 15.2% of parasitised animals were recorded as mature and spawning, compared with 50.3% of uninfected animals (Table 10). Also when the parasitised animals are considered separately for each species of parasite marked differences between them are apparent.

Very few L.littorea (0.5%) infected with C.lingua were found which exhibited characteristics of mature, spawning animals, whereas in periwinkles infected with Cercaria A a very much larger proportion (28.8%) were classified as such, and were presumably capable of breeding. Another noticeable difference between animals harbouring these two parasites was that very few periwinkles infected with C.lingua were considered to be maturing for the first time (stage IIa), whereas stage I and IIa specimens were found among the animals infected with Cercaria A (Fig. 8; Table 10). The peak for these two stages in the Cercaria A infected hosts was reached in September 1967. However, the proportion (20.6%) of these maturing animals was much lower than in the uninfected animals (57.3%). The results of these observations on the reproductive activity of animals infected with Cercaria A compared with that of C.lingua infected host, suggests that Cercaria A may be acquired at a much earlier stage in the life of the host, most likely before it has begun its first breeding season. This inference is further supported by my observations on the relationship between percentage infection and host size (Figs. 4 and 5). It was found that the percentage infection with C.lingua

increased proportionately with host size, whereas Cercaria A was much more prevalent in animals with a shell height less than 25.1 mm, and above this height the proportion of animals infected with Cercaria A decreased sharply (Fig. 4). However, it is possible that the larger size of animals infected with C. lingua may be due to the effects of the parasite rather than entirely to a difference in the initial size of the host at infection.

Williams (1964) recorded a reduction in growth rate of L. littorea during the breeding season, this being most noticeable in animals maturing for the first time. He considered that growth was depressed at this time of the year because of intense reproductive activity. Yonge (1949) also recorded that growth of L. littorea was reduced during spawning, all the food reserves presumably being utilized for gamete production. The effect of C. lingua on the reproductive system of its host usually manifests itself as an inability on the part of the host to produce gametes. If the observations of Williams (1964) and Yonge (1949) may be taken as typical for all populations of L. littorea, one may conclude that infection with C. lingua sporocysts and rediae could result in an increase in height of its host, since breeding is inhibited. The suggestion was originally put forward by Moore (1937) that parasitic castration might account for the increase in size of infected L. littorea. However, Rothschild (1941) artificially castrated L. littorea by treatment with X-rays, and found that they

did not grow faster or larger than the controls.

Gigantism, reduction in egg output or complete sterility have been recorded for several molluscs from differing habitats infected with larval digeneans. Rothschild (1941b) stated that primary infections probably caused accelerated growth in Littorina neritoides. Hydrobia ulvae (= Peringia ulvae) collected from their natural habitat also showed a similar effect (Rothschild 1938b) and in a controlled laboratory experiment Rothschild & Rothschild (1939) concluded that infected H. ulvae did grow larger than uninfected animals. Sturrock (1966), however, did not consider that gigantism occurred as an effect of infection with larval digeneans. She found that Biomphalaria pfeifferi infected with Schistosoma mansoni showed an initial burst of growth over a period of four weeks which began two weeks before the onset of cercarial shedding regardless of length of prepatent period. The growth curve then followed that of control snails until death, infected snails having a shorter life-span. Incidentally she also found that complete sterilization occurred only when infection was acquired after the onset of egg laying, and even when eggs were laid by infected snails these had a high sterility rate.

An increase in growth rate (Chernin 1960, Pan 1963) and a depression of egg laying (Pan 1963) has also been reported in Australorbis glabratus infected with the sporocyst of Schistosoma mansoni. Wesenberg-Lund (1934) was the first to point out that

infected snails are sometimes abnormally large, and suggested this was due to their overcompensating for their parasitic burden by eating more. However, Chernin (1960) found that A.glabratus infected with S.mansoni tended to grow more rapidly than uninfected snails when both were fed identical rations under controlled bacteriologically sterile conditions. If the parasites were withdrawing food as suggested by Wesenberg-Lund (1934), then the parasitised snails might have been expected to be smaller than control snails, which was not the case. Parasitic castration did not seem to have played any significant part in the accelerated growth of infected snails since most of the snails were small and sexually immature. Moreover, the difference in growth became apparent within one week of infection.

The cause of gigantism in snails infected with larval trematodes, if it is a real phenomenon, is as yet unknown. However Deuel (1957) showed that some carotenoid pigments are the precursors of the growth promoting Vitamin A. Thus the release of the pigment into the haemocoel from the histolysed molluscan digestive gland as a result of parasitism may be an explanation for gigantism (James 1965). This is partially inferred from my observations at Scalby Rocks on L.littorea parasitised with C.lingua or H.leptosoma, the foot of which was usually yellow-brown. Willey and Gross (1957) considered that the pigments were derived from the release of carotenoids from the hepatopancreas. The foot of hosts infected with Cercaria A was usually the normal greyish-white colour.

These observations coupled with the finding that animals infected with C.lingua or H.leptosoma were as a rule larger than those infected with Cercaria A, lends some support to the Vitamin hypothesis.

The observations recorded during the present survey seem to suggest that C.lingua and Cercaria A affect their host in different ways and/or with varying severity which might be correlated with initial time of infection in the life cycle of L.littorea. The observations of Sturrock (1966) that sterilization occurred only if the infection was acquired after the onset of egg laying may be one of the reasons why C.lingua affects the reproductive activity of its host to a greater extent than Cercaria A. It is likely that Cercaria A infects the host at an early age, probably before L.littorea has passed through its first breeding season. On the other hand, in view of the paucity of stage IIa animals infected with C.lingua, this infection is most likely acquired at a later stage in the life of the host, probably after it has gone through at least one breeding cycle. This view is supported by the infection level data correlated with host size (Fig. 4). In some way the C.lingua infected host is then unable to prepare itself for the next breeding season and support the parasite at the same time. When the parasite is acquired earlier in life, as with Cercaria A, the animals seem to be more fitted to cope with this additional burden, in so far as the host is able to breed. However, on the evidence available it seems likely that animals infected with Cercaria A die off sooner than do those infected with C.lingua.

L.littorea infected with H.leptosoma or harbouring mixed infections were seldom found in the mature and spawning condition, 0.7% and 2.0% respectively (Table 10), this being similar to the condition found for hosts infected with C.lingua. Unlike Cercaria A both C.lingua and H.leptosoma have a very active redial stage in their development.

In conclusion, a number of factors can be recognised at work in the parasitisation of L.littorea, the principal of which are:

- (i) the initial time of infection, together with the reproductive state and the size of host,
- (ii) the effect of the parasite on the metabolism of the host, and
- (iii) the differential effects of parasites, especially their location and the nature and duration of their juvenile stages.

V. THE EFFECT OF TREMATODE LARVAE ON THE CARBOHYDRATE CONTENT
OF THE FOOT AND HEPATOPANCREAS OF LITTORINA LITTOREA

HISTORICAL REVIEW

The literature reveals surprisingly few reports of the effect of digenean trematode larvae on the glycogen content of their molluscan intermediate hosts; indeed there are few comprehensive papers on the glycogen content of molluscs in general. Claude Bernard was the first to report the occurrence of glycogen in the hepatopancreas of some gastropod and bivalve molluscs. Since his account in 1850 there have been many isolated measurements of the glycogen content of molluscs, most of which have been reviewed by Martin (1961) and Goddard & Martin (1966).

In spite of the early discovery of the occurrence of glycogen in molluscs very little interest has been focused on its seasonal change apart from that produced by hibernation. Von Brand (1931) followed the changes in total polysaccharides in Helix pomatia during the entire year and found a marked deposition of polysaccharide, amounting to about 4% of wet weight, during the three months prior to hibernation. The level then declined steadily during hibernation and thereafter variably during the first month of activity and the period of egg laying. May (1934 a-d) reported a similar yearly cycle in H. pomatia for both glycogen and galactogen.

Most reports on the seasonal cycle of polysaccharides of marine molluscs have been centred around the commercially important oyster. As early as 1907 Milroy established that the glycogen content of the oyster changed with season and stated that:- "As regards seasonal variation there is a gradual rise in the percentage from the beginning of August until the middle or end of October. This is succeeded by a fall which reaches its minimum about the middle of December. From that period onwards the percentage rises until it reaches its maximum some time between the beginning of April and early in May. The percentage then falls until it reaches its second minimum early in August". Mitchell (1915), Russel (1923) and Okazaki & Kobayashi (1929) also reported seasonal changes in glycogen content of oysters. They all recorded the lowest levels as occurring during the breeding season and suggested that this was related to spawning. It has since been established that there is a complicated interaction of seasonal, nutritional and breeding factors. Couteaux-Bargeton (1946) followed the glycogen content of the gonads, digestive complex and labial palps of Crassostrea angulata (= Gryphaea angulata) through a complete year. The gonads showed the greatest loss during the breeding season, but made a rapid recovery, most likely at the expense of the other organs, during the winter months. Galtsoff (1964) reported a similar condition for Crassostrea virginica Gmelin.

Considerable storage of carbohydrate has been reported for other bivalves such as Mytilus edulis (Fraga, 1958), Tivela stultorum (Giese, Hart, Smith & Cheung, 1967) and Teredo pedicellata (Greenfield, 1953). This is in contrast to the condition found in the chitons Katherina tunicata, Mopalia hindsii (Giese & Araki, 1962) and Cryptochiton stelleri (Tucker & Giese, 1962) in which lipids are more important as a store.

There have been very few reports in the literature on changes in the seasonal glycogen content of marine gastropods. Barry & Munday (1959) studied seasonal changes in the glycogen level of the foot and hepatopancreas of Patella sp. (probably P. vulgata), finding that both the foot and hepatopancreatic glycogen showed a well defined seasonal variation, and the shedding of gametes coincided with a sharp decrease in the concentration of tissue glycogen between November and January. Unlike oysters, however, the glycogen levels remained low throughout the winter.

Glycogen is the most widely distributed polysaccharide, and in many animals is the only polysaccharide present. In some gastropods, however, the picture is complicated by the occurrence of a polymer of galactose units, galactogen, which has been isolated from a number of freshwater and terrestrial gastropods. It has been reported as occurring in the egg and albumen gland of

Helix pomatia (May, 1934 a-d, Baldwin & Bell, 1938, and others), Lysanaea sp. (Holm, 1946; Horstmann, 1956; and McMahon, von Brand & Nolan, 1957) and also of Viviparus sp. and Ariophanta sp. (Meenakshi, 1954), as well as in the uterus and eggs of the prosobranch Pila sp. (Meenakshi, 1954). McMahon et al (1957) have also recorded galactogen as occurring in Australorbis glabratus, Otala lactea and Helix aspersa.

Apart from glycogen and galactogen, other polysaccharides have been isolated from several molluscan species. For example a galactose-fucose polysaccharide was identified in the thick walled uterus of females of the operculate snails Lanistes boltenianus and Pomacea zeileki, only glycogen being present in the males (McMahon et al, 1957). Horstmann (1959) found that the eggs of the marine opisthobranch Aplysia depilans and Aplysia limacina, and the prosobranch Dolium galea contained a galactose-fucose sulphated polysaccharide, but was unable to detect either glycogen or galactogen. Lloyd & Lloyd (1963) isolated a sulphated polysaccharide from the viscera of Patella vulgata, which on hydrolysis yielded galactose, fucose and xylose. They considered that the polysaccharide was derived, at least partially, from undigested algal sources.

Emerson (1965, 1966) examined the polysaccharide content of several species of intertidal prosobranch including two species of

Littorina, namely L.scutulata (Gould, 1849) and L.planaxia (Philippi, 1847). Of the species he examined only Calliostoma ligatum (Gould, 1849), Tegula eisenii, Fissurella volcana and Acmaea limatula contained galactose which was present in both males and females; it was thought, therefore, to have been derived from algal sources. This conclusion is further borne out by the absence of galactose in the carnivorous species Emerson studied.

At the present time galactogen has not been isolated in any appreciable concentration from marine gastropods and from the literature reviewed above, it seems unlikely that galactogen is of any great importance in marine gastropods.

The only other report on the biochemical composition of Littorina sp. is that of Emerson & Duerr (1967) who studied the effect of starvation on the chemical composition of Littorina planaxis (Philippi, 1847). They found only a slight decrease in the polysaccharide content after 70 days starvation, and protein nitrogen failed to decrease with starvation; however they did find a significant decrease in the total lipid content. They suggested that lipids are the main source of stored energy in L.planaxis during starvation.

The carbohydrate metabolism of some adult digenetic trematodes has been fairly well studied (see reviews by von Brand 1952, 1960

and 1966; Read 1961 and Smyth 1966), but until the early part of the 1960's with the publication of the work of Cheng and co-workers, little attention had been paid to the metabolism of intra-molluscan digenean larvae.

Most of the work pertaining to the effects of trematode larvae on their molluscan hosts has been studied from a histological and histochemical aspect. The literature on this subject, including the effect of the parasite on the carbohydrate metabolism of their molluscan host, has been reviewed and summarized by Cheng & Snyder (1926 a, b), Cheng (1963a), James (1965), Smyth (1966) and Wright (1966). Wright (1966) concluded that "there is ample evidence that infections of larval trematodes severely deplete the glycogen reserves of their molluscan hosts".

Since these reviews there have been only a few reports on the effects of trematode larvae on the glycogen content of their molluscan hosts. Thus, Zischke & Zischke (1965), by histochemical methods, found a decrease in the amount of liver glycogen in the snail Stagnicola palustris infected with the redial stage of Echinostoma revolutum. Cheng & Burton (1966) also reported a reduction in the quantity of glycogen in tissues of the American oyster, Crassostrea virginica (Gmelin), which were in close proximity to the sporocysts of a species of Bucephalus Von Baer; this substantiates the earlier findings of Cheng and his co-workers.

James & Bowers (1967) reported similar observations for Cardium edule L. infected with the sporocysts of Cercaria bucephalopsis haimaena Lacaze-Duthiers, 1854. Finally, Negus (1968) employing histochemical methods, was unable to find evidence of glycogen removal by the sporocysts of Cercaria doricha Rothschild, 1935 from the surrounding connective tissue of its molluscan host, the marine prosobranch Turritella communis Risso; this is contrary to the findings of other authors.

As early as 1920 Faust, using histochemical methods, recorded a reduction in glycogen content in the liver of a number of fresh-water gastropods infected with several species of larval trematodes. Hurst (1927) confirmed the findings of Faust for Physa occidentalis Tryon infected with the rediae of Echinostoma revolutum Froelich by using histochemical and also biochemical methods. Von Brand & Files (1947) employing biochemical methods showed a considerable reduction in total body glycogen content of Australorbis glabratus when infected with Schistosoma mansoni larvae. Blumenthal (in Cheng 1967) measured the quantitative changes in the glycogen content in the marine gastropod Nassarius obsoletus infected with the rediae of Stephanostomum tenue and found there was significantly less glycogen in the infected hosts.

Until the present work was undertaken, no studies had been reported on the seasonal changes in glycogen content of healthy

molluscs compared with those infected with trematode larvae. The nearest approach to this has been the work of Solère, Prom & Huguet (1965) who compared the seasonal changes in the glycogen content of Mytilus galloprovincialis Lmk. infected with the copepod Mytilicola intestinalis Steuer with that in the uninfected animal; the glycogen content was always higher in the latter.

THE SEASONAL VARIATION IN THE GLYCOGEN CONTENT OF THE FOOT AND
HEPATOPANCREAS OF LITTORINA LITTOREA

MATERIAL AND METHODS

A. Collection and laboratory treatment of Littorina littorea

Determinations of the glycogen content of the marine periwinkle L.littorea were made at approximately monthly intervals between August 1966 and November 1967, using both biochemical and histochemical methods. Separate determinations were made on the hepatopancreas and foot muscle of each animal.

The animals were collected each month from Scalby Rocks (Fig. 2) soon after low tide springs. On arrival at the laboratory they were placed in a circulating sea water aquarium at about 10°C, no attempt being made to simulate tidal fluctuations.

The animals to be used for the carbohydrate determinations were kept without food overnight to ensure that the results were not influenced by any undigested algae in the alimentary system. North (1954) noted that food passed completely through the digestive tract

of Littorina planaxis and Littorina scutulata in $2\frac{1}{2}$ to 6 hours. Overnight starvation also ensured that as much grit as possible was expelled from the digestive system, the presence of which, one need hardly point out, would result in an under-estimation of the proportionate glycogen content of the tissue.

To minimize any variation in the proportionate glycogen content of the animals due to size animals with a shell height of 20 - 30 mm (classes D and E) were preferred for the carbohydrate estimations. This size range also formed the greatest proportion of the population. Whenever possible the monthly determinations were carried out on 20 uninfected and 20 parasitised animals, with an approximately equal proportion of males and females in each group. In all, the carbohydrate content of 685 L.littorea, consisting of 288 uninfected and 397 parasitised specimens, was estimated during the 15 months covered by the survey.

The following records were made for each periwinkle: the length of its shell and its breadth at the widest part of the body whorl (using sliding Vernier calipers calibrated in 0.1 mm); the sex and the reproductive stage using the criteria of Williams (1964) and detailed on page 63 ; also the species, intensity, state of maturity and location of any digenean larvae.

The shells were cracked with the aid of a vice and the animals

removed with the minimal amount of damage to the tissues. The hepatopancreas together with intermingled gonad, and the foot of each animal, were weighed separately on a torsion balance (capacity 200 mg) and treated as described below for the extraction of glycogen (p.129). A small piece of the visceral mass of each animal analyzed for its carbohydrate content was fixed in Carnoy's fluid (6:3:1), paraffin embedded and sectioned at 5 μ on a Cambridge Rocking Microtome (Cambridge Instrument Company Ltd.), for both a histological examination and a histochemical study of glycogen. For histochemistry sequential sections were stained for polysaccharide by either periodic acid - Schiff (PAS) technique (Pearse, 1960) or Best's ammoniacal carmine (Casselman, 1962), the latter being specific for both glycogen and galactose. Control sections were processed in the same way after treatment with either: 1) a 1% diastase solution in phosphate buffer pH7 at 37° C for 2 hours - this digests glycogen, or 2) a freshly prepared 5% suspension of pectinase in distilled water, filtered before use (Grainger & Shillitoe, 1952) - this removes galactogen by enzymic digestion. Galactogen also gives a positive result with PAS and Best's Carmine. The controls verified that the substance giving a positive reaction with PAS and Best's Carmine, not staining after diastase but staining after pectinase treatment, was glycogen.

Tissue for quantitative determination of glycogen was taken from the region between the posterior edge of the stomach and the apex and that for fixation from the region anterior to the stomach (Figs. 6 and 7).

B. Isolation and hydrolysis of glycogen

A number of different methods have been introduced, not always successfully, for the quantitative isolation and estimation of glycogen. Pflüger in 1905 described a method for the extraction and determination of glycogen. This requires digestion of the tissue in hot concentrated potassium hydroxide (KOH) solution, precipitation of the liberated glycogen with ethanol, hydrolysis of the precipitate with mineral acid and determination of the glucose in the hydrolysate as reducing sugar. Good, Kramer & Somogyi (1933) modified Pflüger's method, thus enabling the determination to be carried out in several hours instead of two days as in the original procedure. This was made possible by shortening the KOH-digestion procedure - (20 minutes as against 3 hours) - and reducing the time required for alcoholic precipitation of glycogen - (15 minutes as against overnight). They considered that their method, combined with copper reduction of the neutralized hydrolysate, was the only satisfactory method for the determination of glycogen.

The method described by Good et al. (1933) was tested

using glycogen solutions of known concentrations prepared from commercially available oyster glycogen powder (obtained from B.D.H. England); the only modification being that after hydrolysis of the ethanol precipitated glycogen the glucose present was estimated colorimetrically using the anthrone reaction (p.35) instead of the copper reduction method used by Good et al., the latter requiring more manipulations and hence a longer estimation time. Using glycogen solutions with concentrations of the same order as those reported in the literature for other molluscs the percentage recovery of glycogen was found to be unsatisfactory. In fact, with concentrations of either 0.5 or 1.0 mg glycogen dissolved in 1.0 ml distilled water the amount recovered was usually less than 90% of the original (Table 21). Van Handel (1965) showed that when the method of Good et al. (1933) is used for the determination of low concentrations of glycogen, results are low and unreproducible. However, with the aid of a coprecipitant as little as 1 µg of glycogen could be isolated. Van Handel introduced the coprecipitant, usually Na_2SO_4 , at the ethanol stage. In the method used for the present survey of the carbohydrate content of tissues of Littoreia the Na_2SO_4 , which acts as an adhesion substance for the glycogen, was introduced at the KOH stage as this produced slightly better results. With this modification to the basic method of Good et al.

reproducible and high percentage recovery of glycogen was achieved (Table 21).

Several workers, among them Seifter et al. (1950), have attempted to purify the glycogen precipitated by ethanol from the KOH-digest by dissolving and redispersing the precipitate in distilled water, followed by a second precipitation with ethanol. With this procedure, however, the amount of glycogen recovered from solutions of known concentrations was lower than when only one precipitation was employed (Table 21).

The KOH extraction procedure was preferred to the trichloroacetic acid (TCA) method of extraction advocated by some workers, among them Kemp & Kits Van Heijningen (1954) and Carroll, Longley & Roe (1956). The latter method requires thorough homogenization and often repeated extractions to ensure complete removal of glycogen from the tissues; this is so time consuming as to make it impracticable when dealing with a large number of specimens at any one time.

Procedure for the isolation and hydrolysis of glycogen

REAGENTS

30% aqueous KOH

10% aqueous Na_2SO_4

95% Ethanol

2.5N H_2SO_4

TABLE 21. RECOVERIES OF GLYCOGEN AND GLUCOSE FROM PURE SOLUTIONS AS DETERMINED BY THE ANTHRONE REACTION

mgs glycogen per ml of test solution	Treatment	Recovery (%)
1.0	A. As p. 129 but lacking Coppt. i.e. basic Good, Kramer & Somogyi method	73
1.0		80
1.0		87
0.5		84
0.5		78
0.5		84
1.0	B. As A but with redispersal of glycogen in dist H ₂ O followed by a 2nd EtOH ppt. of glycogen	76
1.0		66
0.5		68
0.5		68
0.5	C. As A but with omission of KOH stage	62
0.5		62
1.0	D. 40 mins. hydrolysis of the known glycogen solns. with 2.5N H ₂ SO ₄ . KOH and EtOH stages omitted.	97
1.0		101
1.0		100
1.0		91
1.0		95
0.5		100
0.5		100
0.5		100
0.5		96
1.0	E. Anthrone reaction on untreated glycogen solutions	101
0.5		104
0.15	F. Anthrone reaction on pure glucose solutions without any prior treatment	98.8
0.1		99
0.1		100
0.05		96
0.025		93
1.0	G. Method of extraction used during present survey p. 129 i.e. as A but with Na ₂ SO ₄ as Coppt.	94
1.0		94
1.0		98
1.0		95
1.0		93
0.5		96
0.5		100
0.5		96
0.5		100
0.5		96
1.0	H. As G but with a 2nd EtOH ppt. stage	80
1.0		91
0.5		84
0.5		92

The reagents, prepared from A.R. grade chemicals, were dispensed by automatic zippettes, thus facilitating mixing of the reagents.

The tubes were capped with glass marbles for both the glycogen extraction procedure and the anthrone reaction.

METHOD

1. The foot and hepatopaneceas were quickly dissected out, washed in cold filtered sea water (4°C) - (to remove any adhering extraneous material) - and the excess moisture removed by blotting with filter paper. The tissues were then weighed immediately on a torsion balance (capacity 200 mg.) and placed with the minimum of delay into separate 15 ml graduated Pyrex centrifuge tubes each containing 1 ml 30% KOH and 2 drops of 10% Na_2SO_4 . Potassium hydroxide quickly penetrates tissues of this size to destroy any enzymatic action without resorting to the necessity of using pre-heated KOH (Barford 1948). After all the samples had been prepared in this way they were digested with occasional shaking in a gently boiling water bath for about 30 minutes or until the tissues had passed into solution. The tubes were then cooled in iced water.
2. The glycogen was then precipitated with 5 ml of 95% ethanol, and the tubes heated to boiling; heating causes coarse flocculation of the prscipitate. The tubes were cooled in iced water and

allowed to stand for one hour. At this stage the samples could be capped and left overnight at 4°C without the results being affected.

3. The tubes were then centrifuged in a MSE 'Mistral 4L' refrigerator centrifuge at 3,000 rpm at 0°C for 20 minutes. The supernatant was decanted and the precipitate washed with 1 ml of 95% ethanol. This helped to remove any alcohol soluble pigments that might be left adhering to the precipitate. After standing for 20 minutes the tubes were centrifuged for a further 5 minutes, also at 0°C and 3,000 rpm.

4. The supernatant was decanted and the residue - (which may be capped and stored at 4°C overnight) - hydrolysed for 40 minutes at 100°C with 2 mls of 2.5N H₂SO₄ with occasional agitation (longer periods of hydrolysis were employed where the residue was still incompletely dissolved). The tubes were then cooled and the contents diluted to known volume with distilled water, such that 1 ml of the hydrolysate contained between 0.005 and 0.15 mg per ml of glucose. The tubes were capped with "Parafilm" and inverted several times to ensure an homogeneous solution. 1 ml samples were withdrawn and the glucose content estimated colorimetrically by the anthrone reaction (p135).

C. Estimation of carbohydrate in the hydrolysate

Anthrone (9,10-dihydro-9-oxoanthracene) was first recognised by Dreywood (1946) to react specifically with many carbohydrates

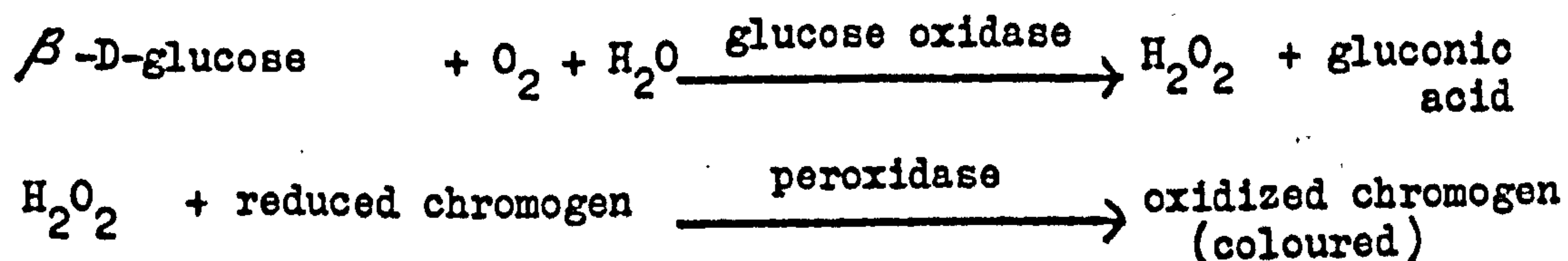
in concentrated sulphuric acid solutions to produce a characteristic blue-green colour. Two years later, Morris (1948) adapted the use of this organic reagent for the quantitative estimation of carbohydrates. The chemistry of the reaction has been studied by Sattler & Zirban (1948), who postulated that water is first split from the carbohydrate with the formation of a hydroxyaldehyde, this being subsequently converted to furfural or a furfural derivative, which was then presumed to condense with the active form of anthrone to give the characteristic blue-green product. Anthrone is the keto form of a tautomeric pair of which the enol form is anthranol (Zipf, Waldo & Dayton 1952). Whereas the keto form is indifferent to most reagents, the anthranol form is highly reactive and presumably it is this form which participates in the "anthrone-carbohydrate" reaction. This assumption is supported by the fact that the reaction is prevented by oxidizing agents and is continued or supported by reducing agents (Roe 1955).

In the original method described by Morris (1948) preparation of the samples and development of the reaction colour were both carried out at room temperature. Seifter et al. (1950) modified the method of Morris by preparing all the reagent tubes in an iced water bath prior to mixing and then heating for 10 minutes at 100°C. Since the colour produced is dependant upon the heat of reaction, this modification standardized the heating of the

solutions, thus giving rise to reproducible and accurate results.

The anthrone reaction is specific for all carbohydrates, not just glycogen and glucose (Koehler 1952, Mokrasch 1954); because of this the Glucostat method was considered in view of its alleged specificity for beta D-glucose (Saifer & Gerstenfeld 1958). For the Glucostat¹ reaction glycogen solutions of known concentration were treated with KOH solution, precipitated with ethanol, and hydrolysed with 2.5N H₂SO₄ as described in the glycogen extraction method (p.127). The hydrolysate was then neutralised with 2.5N sodium hydroxide, diluted to known volume, and the prepared reagent 'Glucostat' employed to measure the amount of glucose present.

Glucostat is a coupled enzyme system; the reaction (simplified) taking place may be written as:-



However the Glucostat method was found to be unsatisfactory as the percentage recovery of hydrolysed glucose from glycogen solutions of known concentration was low, often less than 50% of the original (Table 22). However, Glucostat reacting with pure

1. Glucostat reagent obtained from the Worthington Biochemical Corp. Freehold, New Jersey - Method 1A in their handbook (July 1963 edition).

TABLE 22. RECOVERIES OF GLYCOGEN AND GLUCOSE FROM PURE SOLUTIONS AS DETERMINED BY THE GLUCOSTAT REACTION

mgs glycogen per ml of test solution		Treatment	Recovery (%)
1.0	40 mins .	A. As p.129 but acid hydrolysing times varied as shown. Glucostat reaction at room temperature.	35
1.0	40 mins .		28
0.5	40 mins .		30
0.5	40 mins .		26
1.0	90 mins .		39
1.0	90 mins .		32
0.5	90 mins.		36
0.5	90 mins .		48
1.0	150 mins .		41
0.5	150 mins .		36
0.5	150 mins .		48
1.0	40 mins,	B. Hydrolysis of the known glycogen solutions with 2.5N H ₂ SO ₄ , KOH and EtOH stages omitted Glucostat reaction at room temperature	36
1.0	90 mins.		24
1.0	150 mins.		39
1.0	} glucose	C. Treatment of known glucose solutions with 2.5N H ₂ SO ₄ for 40 mins. Glucostat reaction at room temperature	93
1.0			94
1.0		D. As A but with 40 mins. hydrolysis with 2.5N H ₂ SO ₄ . Glucostat reaction at 37°C	70
1.0			73
1.0			69
1.0			70
0.5			68
0.5			68
0.5			70
0.5			70
0.5			66
0.2	} glucose	E. Glucostat reaction at room temperature on pure glucose solutions without any prior treatment	94.5
0.15			99.4
0.1			99
0.075			98.4
0.075			100
0.05			100
0.05			102
0.01			100
0.005			100

glucose solutions gave much higher recovery rates (>94% - Table 22).

It is possible that the low recovery of glucose from known glycogen solutions may be attributed either to an inhibition of the development of the final colour reaction brought about by substances competing with the chromogen as hydrogen donors (Fales et al. 1961) (these inhibitors may be the reagents used during the extraction procedure or they may be present in the tissues being analyzed), or else to incomplete hydrolysis of the isolated glycogen to glucose by the 2.5N H_2SO_4 . Table 22 summarizes the effects of various acid hydrolysing times on the percentage recovery of glycogen followed by the Glucostat reaction performed at room temperature and 37°C. Table 22 also includes information on the percentage recovery of glucose from glucose solutions of known concentration as determined by the Glucostat reaction carried out at room temperature. The results obtained show high recovery when glucose solutions made up in distilled water are treated with the Glucostat reagent (Table 22), more than 94.5% of the initial glucose present being accounted for. The incomplete hydrolysis of glycogen to glucose seems the more feasible explanation since hydrolysis of known glycogen solutions with acid without any prior treatment still fails to result in high percentage recovery (Table 22).

Failure to ensure complete hydrolysis is of no importance in the anthrone reaction as this reagent gives the same colour reaction with glycogen as with an equal amount of glucose (Seifer et al. 1950; Van Handel 1965). In the method used for the present studies a short period of hydrolysis (approximately 30 minutes) was employed to bring the precipitated glycogen into solution much quicker than if it had been dissolved in distilled water.

The anthrone reaction also has an advantage over both the Glucostat and the reduction methods (e.g. Nelson-Somogyi copper reduction method - Somogyi, 1952) in that only one reagent and the different samples require handling, thus shortening the time required for the technical manipulations.

Procedure for the anthrone reaction

The anthrone reagent (B.D.H.), 0.2% solution in 95% H_2SO_4 (500 ml conc. H_2SO_4 sp.g. 1.84 + 25 ml distilled water) was made up fresh and placed in a refrigerator for 30 minutes before use. It was discarded if not used within three hours.

The anthrone reagent made up in a concentrated H_2SO_4 was preferred to that of Trevelyan & Harrison (1952) in which the reagent (0.2% solution) is made up in a more dilute acid (500 ml conc. H_2SO_4 + 200 ml distilled water). When this less concentrated reagent was tried, the anthrone was precipitated out

of solution on reaction with the carbohydrate solution under test.

As in the extraction procedure, the anthrone reagent was dispensed with automatic zippettes, and all tubes were capped with glass marbles.

Glucose standards made up in distilled water (0.005 - 0.2 mg glucose A.R. per ml) were prepared from a 1 mg per ml stock glucose solution.

METHOD

1. 5 ml of the anthrone reagent was pipetted into 16 x 150 mm test tubes and kept in an iced water bath. 1 ml of the diluted hydrolysate was carefully layered on top of the reagent.
2. A distilled water reagent blank and glucose standards (0.005 - 0.2 mg per ml) were prepared in the same way.
3. When all the tubes had been so prepared, the contents were thoroughly mixed by shaking and returned to the iced water bath.
4. When all the samples had been mixed, the tubes were transferred en masse in a wire rack to a vigorously boiling water bath for 10 minutes - (the water bath was of an opaque nature since the colour reaction is affected by light - Roe 1955).
5. The tubes were then cooled for at least 2 minutes in running tap water.
6. The optical density of the resultant green colour was measured

in a Hilger Spekker absorptiometer at 620 m μ using an orange filter, the instrument having been set previously to give 100% transmission with the reagent blank. The absorptiometer cells had a light path of 5 mm.

A graph was constructed with a line passing through the standard points and the glucose concentration of the samples determined.

Total amount of glucose in sample = volume of diluted hydrolysate $\times$ mg glucose in 1 ml of diluted hydrolysate as determined by the anthrone reaction. Owing to doubts which exist as to the correct conversion factor the glucose figures were not converted into equivalent amounts of glycogen.

Following convention, glycogen is defined as all anthrone sensitive material with an absorption peak at 620 m μ precipitable with ethanol after KOH digestion. KOH digestion destroys any free tissue glucose that could react with anthrone.

The colour developed obeys Beer's Law over the range 0.005 to 0.2 mg per ml of glucose and is stable for several hours at room temperature. Because the anthrone reagent is not completely stable and because the colour produced is dependant upon the heat of reaction, a standard curve was calibrated for each set of determinations.

It is essential that every care should be taken to avoid

contamination of samples by extraneous materials that can react with the anthrone reagent, for example, filter paper, dust, dirty glass ware, thus resulting in a false positive result.

D. Identification of sugars in the hydrolysate

The nature of the carbohydrates reacting with the anthrone reagent were identified by one dimensional descending paper chromatography. Two solvent systems were used : n-butanol : acetic acid : water (6:1:1 v/v) or n-propanol : ethyl acetate : water (7:2:1 v/v).

The tissues were digested in 30% KOH as described earlier (p.127); the "glycogen" was then precipitated with 95% ethanol, re-dissolved in water (to purify the "glycogen"), followed by re-precipitation with 95% ethanol; the precipitate was then hydrolysed for 3 hours with 1 ml of 1N H_2SO_4 . The hydrolysate was neutralized with solid barium carbonate and the resulting precipitate of barium sulphate removed by centrifugation. Aliquots (10 μ l) of the clear supernatant were spotted on Whatman no. 1 chromatographic paper; reference sugars were included with each run. The chromatograms were developed for 48 hours, this resulting in an adequate separation of the sugars in the standard solutions.

The chromatograms were dried thoroughly in a fume-cupboard and the sugars were located by spraying with either para-anisidine or diphenylamine (Block, Durrum & Zweig 1958) followed by heating of the chromatograms to 100°C or 90°C respectively.

Samples from hydrolysates of the following tissues were chromatographed: a) foot muscle; b) hepatopancreas together with intermingled gonad from both male and female specimens; c) albumen gland; d) hepatopancreas of L.littorea infected with C.lingua; and e) hepatopancreas of L.littorea infected with Cercaria A.

In all the tissue samples glucose was the only sugar located. It thus seems fairly certain that the carbohydrate extracted from the tissues and estimated by the anthrone reaction was glycogen.

RESULTS

The results of the survey are summarized in Tables 23 to 29 and Figs. 17 to 24. The glycogen content of the tissues is expressed as milligrams of glucose per 100 mg wet weight of tissue (mg %).

Although each species of parasite is included in the results for total parasitised animals, only two species were present in sufficiently large numbers to be worthy of separate consideration. These were C.lingua and Cercaria A.

Students 't' test for small numbers (Maxwell Little, 1962), unless otherwise stated, was used to investigate the statistical significance, if any, of differences between sample means.

(1) Seasonal variation in the glycogen content of the foot and hepatopancreas of uninfected Littorina littorea

The glycogen content of the hepatopancreas for both sexes

combined exhibited a well defined seasonal variation (Fig.17). In 1966 the glycogen content rose sharply from the August level (2.97 mg %) to the September peak (5.35 mg %). It then fell off gradually and reached its annual minimum in April and May 1967 (average 0.85 mg %). Subsequently it rose with increasing steepness to the annual maximum in October 1967 (7.70 mg %, Table 23). The succeeding monthly mean suggested the onset of the winter decline observed in the previous year. In other words uninfected L.littorea show a pronounced seasonal cycle in which the glycogen level is lowest in April and May and highest during the period September and October, with the mid points of highest and lowest content occurring approximately six months apart.

The glycogen concentration of the foot (Fig. 17) showed a similar, though less well defined, pattern but generally at a much lower level than that of the hepatopancreas.

It should be emphasized that the decline in glycogen content corresponds with the falling sea temperature, and the increase approximately with the rising sea temperature, the peak in carbohydrate content occurring in 1967 just after the warmest time of the year (Fig. 17). However, the seasonal glycogen cycle also seems to be associated in some way with the annual reproductive cycle (Fig. 17). The glycogen content was at its lowest at a time when the majority of the uninfected animals were mature and

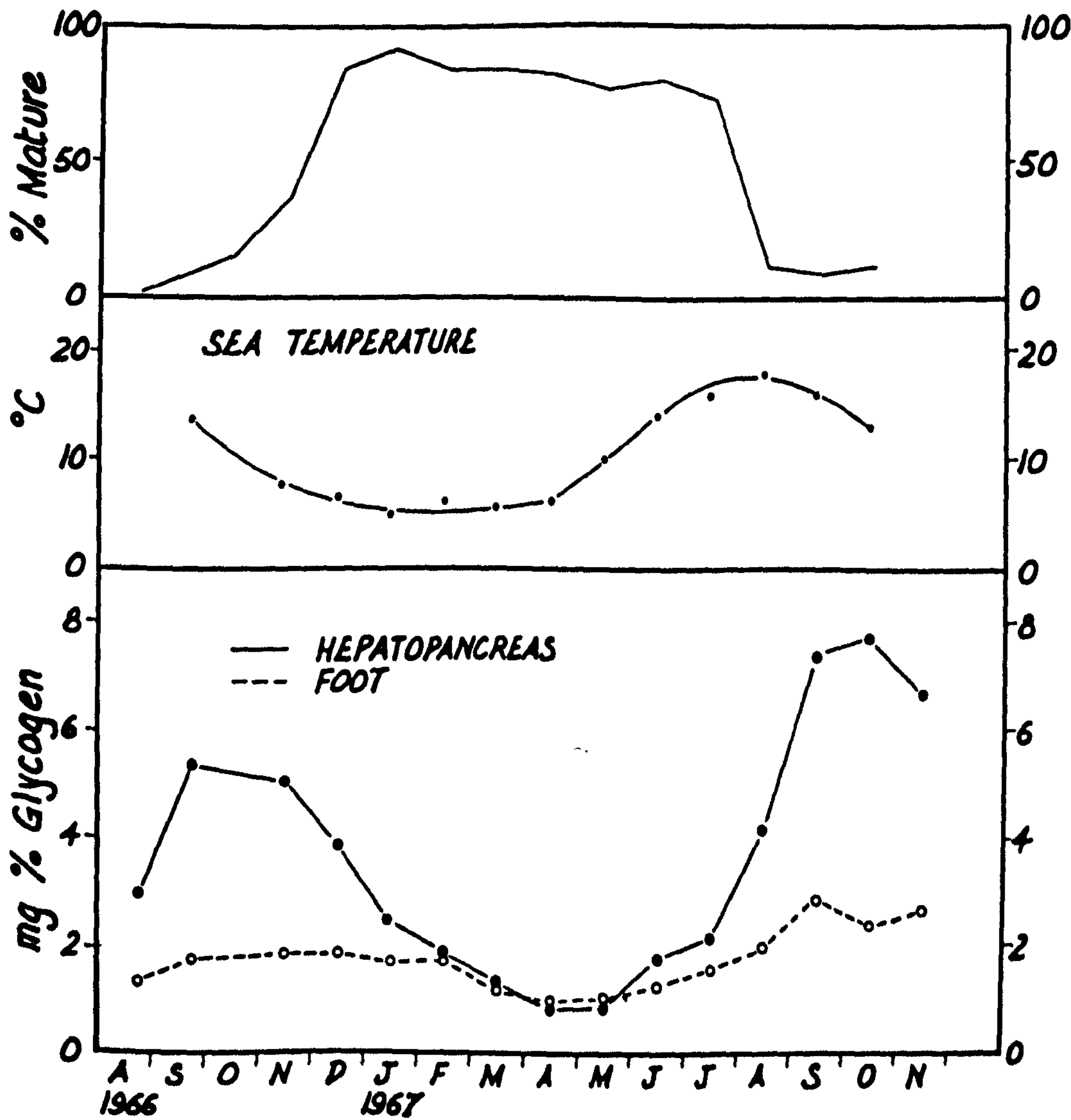


FIG 17 MATURITY CURVE FOR UNINFECTED L. Littorea ; SEA TEMPERATURE; GLYCOGEN CONCENTRATION OF UNINFECTED L. Littorea

TABLE 23

**AVERAGE GLYCOGEN CONTENT IN EACH MONTHLY SAMPLE OF L. LITTOREA (males and females)
EXPRESSED AS MG GLUCOSE PER 100 MG WET WEIGHT OF TISSUE**

Numbers preceded by + give standard error of mean . Numbers in parenthesis indicate numbers of animals in samples

	<u>UNINFECTED</u>		<u>TOTAL PARASITISED</u>		<u>CRYPTOCOTYLE LINGUA</u>		<u>CERCARIA A</u>	
	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot
16.8.66	2.97 $\pm$.39 (17)	1.36 $\pm$.12 (18)	1.08 $\pm$.15 (13)	1.13 $\pm$.08 (17)	1.14 $\pm$.19 (10)	1.24 $\pm$.09 (11)	0.80 $\pm$.13 (2)	0.85 $\pm$.25 (3)
28.9.66	5.35 $\pm$.43 (10)	1.76 $\pm$.17 (11)	2.09 $\pm$.20 (68)	1.20 $\pm$.04 (66)	1.48 $\pm$.09 (31)	1.24 $\pm$.06 (28)	3.29 $\pm$.62 (13)	1.11 $\pm$.11 (13)
2.11.66	5.04 $\pm$.28 (16)	1.84 $\pm$.09 (16)	2.07 $\pm$.25 (24)	1.45 $\pm$.12 (24)	1.62 $\pm$.08 (15)	1.51 $\pm$.14 (15)	2.84 $\pm$.59 (9)	1.35 $\pm$.22 (9)
1.12.66	3.87 $\pm$.22 (18)	1.87 $\pm$.11 (19)	2.62 $\pm$.28 (18)	1.50 $\pm$.10 (20)	1.87 $\pm$.14 (6)	1.74 $\pm$.19 (8)	3.30 $\pm$.54 (6)	1.39 $\pm$.18 (6)
12.1.67	2.22 $\pm$.27 (15)	1.70 $\pm$.11 (15)	2.43 $\pm$.18 (7)	1.42 $\pm$.10 (7)	1.33 $\pm$.09 (6)	1.39 $\pm$.12 (6)	2.69 $\pm$.21 (14)	1.52 $\pm$.16 (14)
11.2.67	1.90 $\pm$.22 (15)	1.72 $\pm$.20 (12)	1.86 $\pm$.25 (23)	1.35 $\pm$.10 (23)	1.36 $\pm$.25 (9)	1.51 $\pm$.16 (9)	1.84 $\pm$.40 (11)	1.22 $\pm$.15 (11)
14.3.67	1.28 $\pm$.10 (39)	1.28 $\pm$.05 (40)	1.59 $\pm$.14 (37)	1.19 $\pm$.11 (36)	1.36 $\pm$.15 (16)	1.58 $\pm$.21 (15)	1.61 $\pm$.21 (17)	0.87 $\pm$.09 (17)
10.4.67	0.82 $\pm$.11 (18)	0.95 $\pm$.08 (18)	1.11 $\pm$.06 (17)	0.93 $\pm$.09 (20)	1.19 $\pm$.06 (9)	1.28 $\pm$.06 (9)	0.97 $\pm$.10 (7)	0.57 $\pm$.06 (10)
9.5.67	0.87 $\pm$.07 (19)	1.02 $\pm$.06 (19)	1.16 $\pm$.09 (24)	0.88 $\pm$.06 (24)	1.33 $\pm$.12 (10)	1.09 $\pm$.11 (9)	0.92 $\pm$.10 (11)	0.72 $\pm$.05 (12)
6.6.67	1.77 $\pm$.14 (16)	1.24 $\pm$.05 (16)	1.32 $\pm$.13 (21)	1.27 $\pm$.08 (23)	1.05 $\pm$.07 (8)	1.48 $\pm$.07 (10)	1.33 $\pm$.16 (9)	1.13 $\pm$.11 (9)
10.7.67	2.11 $\pm$.18 (18)	1.52 $\pm$.08 (18)	2.19 $\pm$.25 (24)	1.58 $\pm$.12 (23)	1.92 $\pm$.23 (11)	1.82 $\pm$.12 (11)	2.42 $\pm$.46 (11)	1.32 $\pm$.21 (10)
7.8.67	4.14 $\pm$.21 (19)	1.93 $\pm$.08 (20)	3.28 $\pm$.30 (21)	1.86 $\pm$.13 (20)	2.58 $\pm$.58 (10)	2.06 $\pm$.22 (10)	3.83 $\pm$.40 (9)	1.59 $\pm$.12 (8)
4.9.67	7.34 $\pm$.42 (18)	2.82 $\pm$.13 (20)	4.53 $\pm$.42 (22)	2.48 $\pm$.08 (23)	3.20 $\pm$.30 (11)	2.58 $\pm$.08 (11)	5.50 $\pm$.69 (8)	2.26 $\pm$.14 (9)
2.11.67	7.70 $\pm$.36 (20)	2.37 $\pm$.07 (19)	3.80 $\pm$.49 (21)	1.89 $\pm$.13 (20)	2.51 $\pm$.11 (11)	1.97 $\pm$.13 (10)	5.22 $\pm$.83 (10)	1.80 $\pm$.22 (10)
2.11.67	6.68 $\pm$.24 (16)	2.61 $\pm$.10 (17)	4.53 $\pm$.55 (21)	2.06 $\pm$.07 (21)	1.74 $\pm$.11 (10)	2.12 $\pm$.09 (10)	7.10 $\pm$ 1.00 (9)	2.00 $\pm$.12 (9)

breeding. The September to October glycogen peaks, on the other hand, occurred at a time when the proportion of the population that were in the recovering spent stage (Stage IIb) or maturing for the first time (stage IIa) was at its highest (Fig. 8).

Uninfected male and female L.littorea both show similar seasonal cycles of glycogen content (Fig. 18). However, in the male the hepatopancreatic glycogen fell below that of the foot during the period March-May, (Table 24) and the extremes of concentration shown by the hepatopancreatic glycogen were greater in the males than in the females (Fig. 18). The graph gives the immediate impression that the two curves are "out of phase", the males leading the females by a gap of between 1 and 4 weeks. The differences between the glycogen concentration of the hepatopancreas of uninfected males and females for the same month were shown by Students 't' test to be significant for 8 of the 15 months studied (Table 26). However, if the observations on the glycogen content of the digestive gland for uninfected animals are analyzed by an analysis of variance for disproportionate subclass numbers (Snedecor 1962) the difference between the sexes is not significant, but the difference between the months which has resulted in the very pronounced seasonal cycle is highly significant as is also the interaction between months and sex (Table 27).

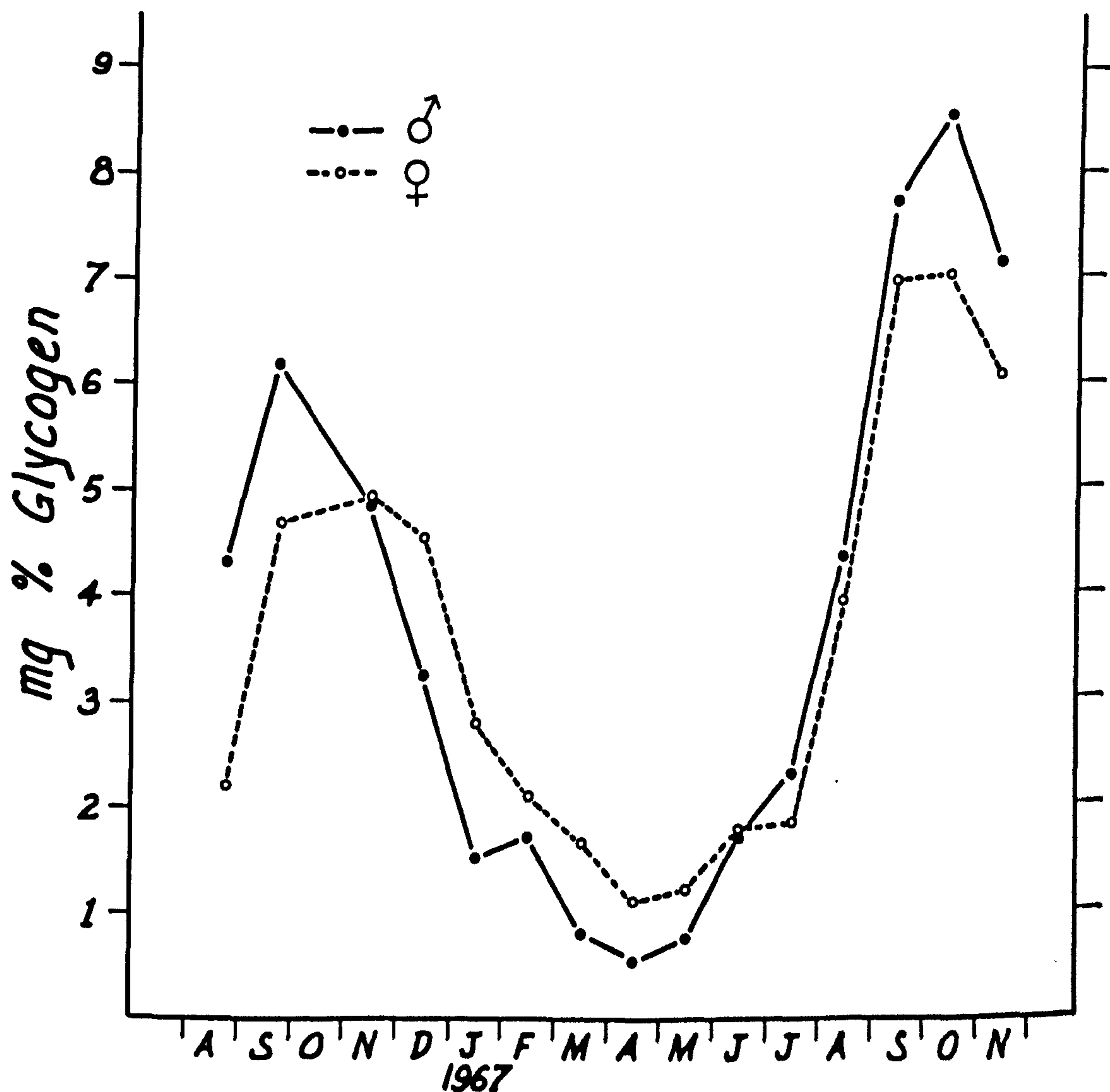


FIG 18 GLYCOGEN CONCENTRATION (WET WEIGHT) OF HEPATOPANCREAS OF UNINFECTED L. Littorea (MALES COMPARED WITH FEMALES)

TABLE 24

AVERAGE GLYCOGEN CONTENT IN EACH MONTHLY SAMPLE OF L. LITTOREA (males) EXPRESSED
AS MG GLUCOSE PER 100 MG WET WEIGHT OF TISSUE

Numbers preceded by $\pm$ give standard error of mean . Numbers in parenthesis indicate
numbers of animals in samples.

	<u>UNINFECTED</u>		<u>TOTAL PARASITISED</u>		<u>CRYPTOCOTYLE LINGUA</u>		<u>CERCARIA</u>	
	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot
16.8.66	4.34 $\pm$.65 (6)	1.52 $\pm$.22 (8)	0.90 $\pm$.12 (4)	1.13 $\pm$.17 (4)	0.93 $\pm$.49 (2)	1.34 $\pm$.04 (2)	0.67 (1)	0.62 (1)
28.9.66	6.21 $\pm$.73 (4)	1.68 $\pm$.18 (4)	1.98 $\pm$.27 (41)	1.20 $\pm$.06 (37)	1.34 $\pm$.10 (13)	1.32 $\pm$.11 (9)	2.75 $\pm$ 1.14 (6)	1.05 $\pm$.19 (6)
2.11.66	4.88 $\pm$.38 (7)	1.69 $\pm$.11 (7)	2.36 $\pm$.58 (9)	1.60 $\pm$.20 (9)	1.56 $\pm$.14 (6)	1.70 $\pm$.21 (6)	3.96 $\pm$ 1.41 (3)	1.41 $\pm$.45 (3)
1.12.66	3.29 $\pm$.12 (10)	1.65 $\pm$.11 (11)	2.52 $\pm$.34 (8)	1.69 $\pm$.18 (8)	1.97 $\pm$.12 (4)	2.00 $\pm$.28 (4)	2.82 $\pm$.50 (2)	1.29 $\pm$.03 (2)
12.1.67	1.53 $\pm$.29 (7)	1.48 $\pm$.13 (7)	2.62 $\pm$.33 (9)	1.35 $\pm$.16 (9)	1.53 (1)	1.74 (1)	2.91 $\pm$.36 (7)	1.33 $\pm$.19 (7)
11.2.67	1.71 $\pm$.08 (8)	1.49 $\pm$.11 (6)	1.29 $\pm$.14 (9)	1.23 $\pm$.17 (9)	1.27 $\pm$.16 (4)	1.42 $\pm$.27 (4)	1.31 $\pm$.23 (5)	1.08 $\pm$.21 (5)
14.3.67	0.81 $\pm$.10 (18)	1.21 $\pm$.07 (18)	1.57 $\pm$.24 (15)	1.24 $\pm$.23 (15)	1.15 $\pm$.10 (8)	1.61 $\pm$.43 (7)	1.39 $\pm$.34 (4)	0.71 $\pm$.13 (5)
10.4.67	0.54 $\pm$.09 (3)	0.94 $\pm$.07 (9)	1.20 $\pm$.11 (6)	0.84 $\pm$.15 (9)	1.30 $\pm$.11 (3)	1.39 $\pm$.08 (3)	1.10 $\pm$.19 (3)	0.57 $\pm$.08 (6)
9.5.67	0.78 $\pm$.07 (15)	1.05 $\pm$.07 (15)	1.23 $\pm$.13 (13)	0.99 $\pm$.08 (13)	1.38 $\pm$.17 (5)	1.24 $\pm$.11 (5)	0.89 $\pm$.14 (6)	0.74 $\pm$.06 (6)
6.6.67	1.74 $\pm$.24 (7)	1.36 $\pm$.08 (7)	1.52 $\pm$.25 (10)	1.49 $\pm$.06 (12)	0.96 $\pm$.08 (5)	1.47 $\pm$.10 (7)	1.68 $\pm$.20 (3)	1.50 $\pm$.10 (3)
10.7.67	2.34 $\pm$.30 (9)	1.61 $\pm$.11 (9)	2.09 $\pm$.32 (14)	1.54 $\pm$.14 (14)	1.99 $\pm$.34 (7)	1.80 $\pm$.13 (7)	2.12 $\pm$.72 (5)	1.17 $\pm$.30 (5)
7.8.67	4.35 $\pm$.38 (9)	1.92 $\pm$.08 (10)	3.73 $\pm$.48 (9)	1.96 $\pm$.16 (8)	3.30 $\pm$.97 (3)	2.37 $\pm$.23 (3)	3.76 $\pm$.76 (4)	1.56 $\pm$.05 (3)
4.9.67	7.71 $\pm$.67 (9)	2.85 $\pm$.23 (10)	5.00 $\pm$.84 (8)	2.49 $\pm$.12 (10)	3.33 $\pm$.30 (4)	2.74 $\pm$.13 (4)	6.36 $\pm$ 1.32 (3)	2.27 $\pm$.18 (5)
2.10.67	8.55 $\pm$.63 (9)	2.32 $\pm$.08 (8)	3.85 $\pm$.82 (10)	1.90 $\pm$.20 (10)	2.47 $\pm$.19 (6)	1.85 $\pm$.16 (6)	5.91 $\pm$ 1.49 (4)	1.99 $\pm$.48 (4)
2.11.67	7.15 $\pm$.31 (9)	2.53 $\pm$.07 (9)	4.27 $\pm$.52 (11)	1.93 $\pm$.87 (11)	1.56 $\pm$.17 (5)	1.93 $\pm$.08 (5)	6.83 $\pm$ 1.71 (5)	1.98 $\pm$.18 (5)

TABLE 25 AVERAGE GLYCOGEN CONTENT IN EACH MONTHLY SAMPLE OF L.LITTOREA (females) EXPRESSED

AS MG GLUCOSE PER 100 MG WET WEIGHT OF TISSUE

Numbers preceded by + give standard error of mean . Numbers in parenthesis indicate number of animals in samples

	<u>UNINFECTED</u>		<u>TOTAL PARASITISED</u>		<u>CRYPTOCOTYLE LINGUA</u>		<u>CERCARIA A</u>	
	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot
16.8.66	2.23 ± .41 (11)	1.23 ± .14 (10)	1.16 ± .19 (9)	1.14 ± .10 (13)	1.19 ± .22 (8)	1.22 ± .11 (9)	0.93 (1)	0.97 ± .38 (2)
20.9.66	4.72 ± .40 (6)	1.80 ± .26 (7)	2.25 ± .27 (27)	1.21 ± .06 (29)	1.56 ± .14 (18)	1.20 ± .08 (19)	3.75 ± .67 (7)	1.17 ± .13 (7)
2.11.66	4.97 ± .53 (7)	1.97 ± .17 (7)	1.90 ± .22 (15)	1.36 ± .15 (15)	1.65 ± .11 (9)	1.39 ± .18 (9)	2.28 ± .50 (6)	1.32 ± .28 (6)
2.12.66	4.59 ± .31 (8)	2.18 ± .16 (8)	2.69 ± .43 (10)	1.38 ± .12 (12)	1.69 ± .37 (2)	1.47 ± .22 (4)	3.54 ± .79 (4)	1.44 ± .28 (4)
12.1.67	2.83 ± .36 (8)	1.89 ± .14 (8)	2.33 ± .32 (16)	1.46 ± .13 (16)	1.30 ± .10 (5)	1.32 ± .12 (5)	2.47 ± .19 (7)	1.71 ± .24 (7)
11.2.67	2.11 ± .39 (7)	1.94 ± .37 (6)	2.23 ± .38 (14)	1.43 ± .12 (14)	1.43 ± .20 (5)	1.59 ± .21 (5)	2.29 ± .69 (6)	1.34 ± .21 (6)
14.3.67	1.69 ± .09 (21)	1.33 ± .06 (22)	1.61 ± .18 (22)	1.16 ± .11 (21)	1.56 ± .26 (8)	1.55 ± .15 (8)	1.68 ± .26 (13)	0.93 ± .11 (12)
10.4.67	1.10 ± .14 (9)	0.96 ± .15 (9)	1.05 ± .07 (11)	0.99 ± .11 (11)	1.13 ± .07 (6)	1.23 ± .08 (6)	0.88 ± .10 (4)	0.58 ± .11 (4)
9.5.67	1.22 ± .14 (4)	0.90 ± .07 (4)	1.09 ± .12 (11)	0.75 ± .08 (11)	1.29 ± .19 (5)	0.89 ± .16 (4)	0.96 ± .16 (5)	0.69 ± .09 (6)
6.6.67	1.80 ± .16 (9)	1.15 ± .05 (9)	1.17 ± .12 (9)	1.13 ± .04 (9)	1.21 ± .08 (3)	1.51 ± .04 (3)	1.16 ± .18 (6)	0.94 ± .09 (6)
10.7.67	1.58 ± .21 (3)	1.43 ± .11 (9)	2.32 ± .41 (10)	1.64 ± .21 (9)	1.79 ± .22 (4)	1.86 ± .28 (4)	2.67 ± .64 (6)	1.46 ± .31 (5)
7.8.67	3.94 ± .20 (10)	1.94 ± .15 (10)	2.95 ± .38 (12)	1.79 ± .19 (12)	2.28 ± .73 (7)	1.92 ± .30 (7)	3.88 ± .47 (5)	1.61 ± .20 (5)
4.9.67	6.97 ± .50 (9)	2.79 ± .13 (10)	4.26 ± .50 (14)	2.48 ± .10 (13)	3.12 ± .46 (7)	2.49 ± .08 (7)	4.98 ± .79 (5)	2.26 ± .25 (4)
2.10.67	7.00 ± .23 (11)	2.40 ± .12 (11)	3.76 ± .61 (11)	1.87 ± .17 (10)	2.57 ± .12 (5)	2.15 ± .21 (4)	4.76 ± .95 (6)	1.68 ± .22 (6)
2.11.67	6.08 ± .23 (7)	2.71 ± .19 (8)	4.81 ± 1.01 (10)	2.20 ± .09 (10)	1.93 ± .10 (5)	2.31 ± .10 (5)	7.44 ± 1.05 (4)	2.03 ± .15 (4)

TABLE 26. SIGNIFICANCE PROBABILITIES OF DIFFERENCE BETWEEN MEANS FOR HEPATOPANCREAS GLYCOGEN CONCENTRATION CALCULATED BY STUDENT'S t TEST APPLIED TO SEVEN COMBINATIONS AS INDICATED

1. Uninfected males versus uninfected females
2. Uninfected versus total parasitised males and females
3. Uninfected versus total parasitised males
4. Uninfected versus total parasitised females
5. Uninfected males and females versus those infected with C. lingua
6. Uninfected males and females versus those infected with Cercaria
7. Males and females infected with Cercaria versus those infected with C. lingua

COMBINATIONS

	1	2	3	4	5	6	7
16.8.66	t 3.327 P < .01	5.143 < .001	4.140 < .01	2.755 < .02	3.472 < .01	1.870 > .05	0.756 > .4
29.9.66	t 1.957 P > .05	6.092 < .001	4.657 < .001	4.134 < .001	13.250 < .001	2.555 < .02	4.278 < .001
1.11.66	t 0.135 P > .8	7.681 < .001	3.422 < .01	6.476 < .001	11.242 < .001	3.797 < .001	2.638 < .02
1.12.66	t 4.235 P < .001	3.518 < .01	2.321 < .05	3.394 < .01	5.166 < .001	1.180 > .2	2.565 < .05
12.1.67	t 3.002 P < .02	0.581 > .5	2.393 < .05	0.999 > .3	2.024 > .85	1.359 > .1	4.192 < .001
11.2.67	t 0.889 P > .3	0.111 > .9	1.559 > .1	0.187 > .8	1.769 > .05	0.130 > .8	1.051 > .3
14.3.67	t 6.627 P < .001	1.837 > .05	3.138 < .01	0.398 > .6	0.418 > .6	1.356 > .1	0.984 > .3
10.4.67	t 3.345 P < .01	2.311 < .05	4.539 < .001	0.330 > .7	2.319 < .05	0.819 > .3	1.907 > .05
9.5.67	t 3.017 P < .01	2.443 < .02	3.194 < .01	0.605 > .5	3.505 < .01	0.436 > .6	2.633 < .02
6.6.67	t 0.224 P > .8	2.337 < .05	0.715 > .4	2.895 < .02	3.490 < .01	1.994 > .05	1.566 > .1
10.7.67	t 1.289 P > .2	0.232 > .8	0.539 > .5	0.935 > .3	0.647 > .5	0.722 > .4	0.972 > .3
7.8.67	t 0.987 P > .3	2.289 < .02	3.986 < .01	2.217 < .05	3.072 < .01	0.758 > .4	1.727 > .1
4.9.67	t 0.882 P > .3	4.694 < .001	2.631 < .02	3.690 < .01	7.085 < .001	2.380 < .05	3.373 < .01
2.10.67	t 2.396 P < .05	6.338 < .001	4.468 < .001	4.008 < .001	10.492 < .001	3.211 < .01	3.387 < .01
2.11.67	t 2.625 P < .02	3.210 < .01	4.520 < .001	0.992 > .3	15.612 < .001	0.525 > .6	5.608 < .001

The difference between means was taken to be significant if P was less than .05
The degrees of freedom are obtainable from the data in Tables 23 to 25
t = Student's t -statistic

TABLE 27. VARIANCE TABLE: GLYCOGEN CONTENT OF HEPATOPANCREAS
OF UNINFECTED MALES VERSUS UNINFECTED FEMALES

Source	Sum of squares	d.f.	Mean Square	F	P
Sex	0.54	1	0.54	0.617	>.250
Months	1450.458 - 0.6604 = 1449.798	14	103.557	118.352	<.005
Interaction	66.624	14	4.758	5.437	<.005
Within subclasses	211.96	242	0.875		

d.f. = Degrees of freedom

The seasonal variation in the amount of hepatopancreatic glycogen was further substantiated by histochemical studies. The intensity and volume of glycogen staining areas were in the main greater in animals which were found, from quantitative measurements, to have a high glycogen content, than in those with lower values. In a few cases animals with low quantitative glycogen values gave large positive histochemical reactions and vice versa. These instances may be fortuitous in that the volume of tissue taken for the histochemical examination was very small compared with that used for the quantitative estimation. The histochemical studies showed that a lot of the glycogen was stored in the connective tissues of the visceral mass. The

connective tissue is situated between the branches of the digestive gland and gonadial tubules. Glycogen was also located in the digestive gland itself, but very little was found in the gonads. The volume of connective tissue was seen to increase at the end of the breeding season to occupy the areas originally filled by gonadial tissue; and to regress prior to the next breeding season as the gonad once again matured. The deposition of glycogen was found to be very heavy in the vicinity of the stomach.

(ii) Seasonal variation in the glycogen content of the foot and hepatopancreas of *Littorina littorea* infected with digenean larvae.

(a) Total parasitised animals

The parasitised animals, like those uninfected, showed a cyclical variation in glycogen content for both the hepatopancreas and foot (Figs. 19 and 20; Table 23). The patterns were similar, with the infected animals having a lower level except during the seasonal low of the uninfected specimens occurring between January-May 1967 (Fig. 19).

The monthly means for the hepatopancreatic glycogen of infected males and females (Fig. 21, Tables 24 and 25) were very similar to each other, in notable contrast to the differences recorded earlier for uninfected animals, and no "out of phase" feature was discernable.

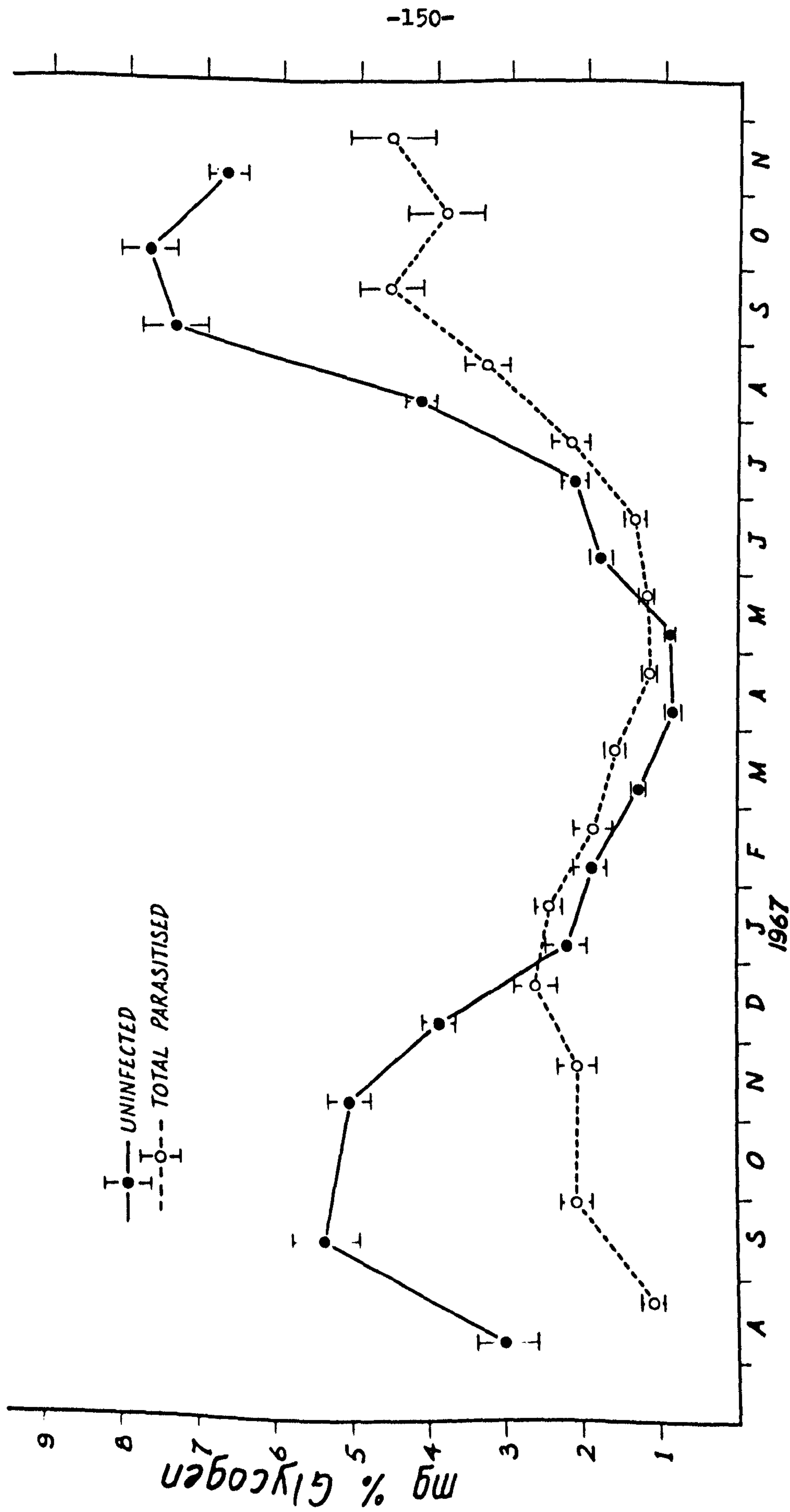


FIG 19 GLYCOGEN CONCENTRATION (WET WEIGHT) of HEPATOPANCREAS of *L. Littorea* MALES & FEMALES : UNINFECTED AND TOTAL PARASITISED (MEAN $\pm$ S.E.)

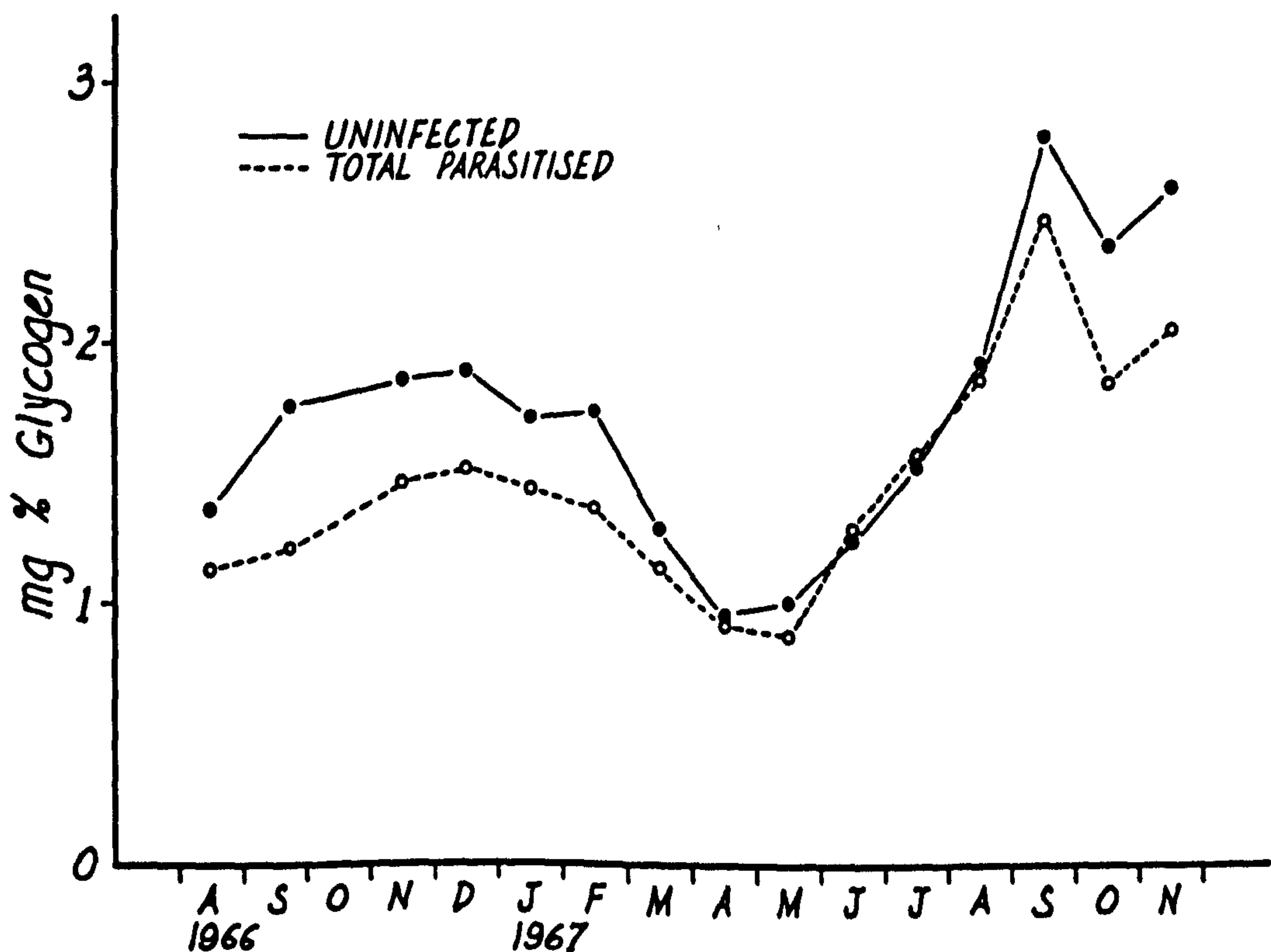


FIG 20 GLYCOGEN CONCENTRATION (WET WEIGHT)
of FOOT of L. Littorea MALES & FEMALES :
UNINFECTED AND TOTAL PARASITISED

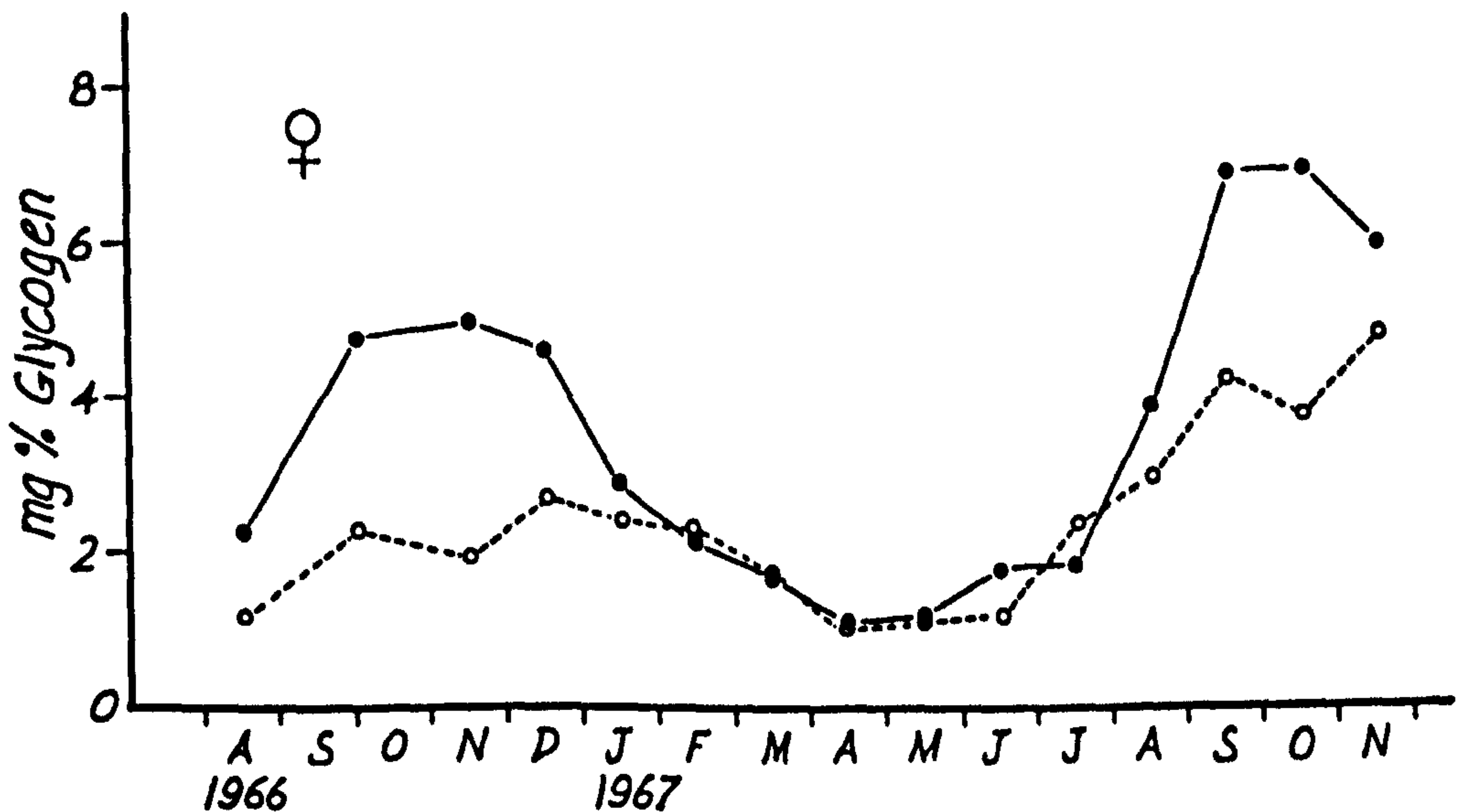
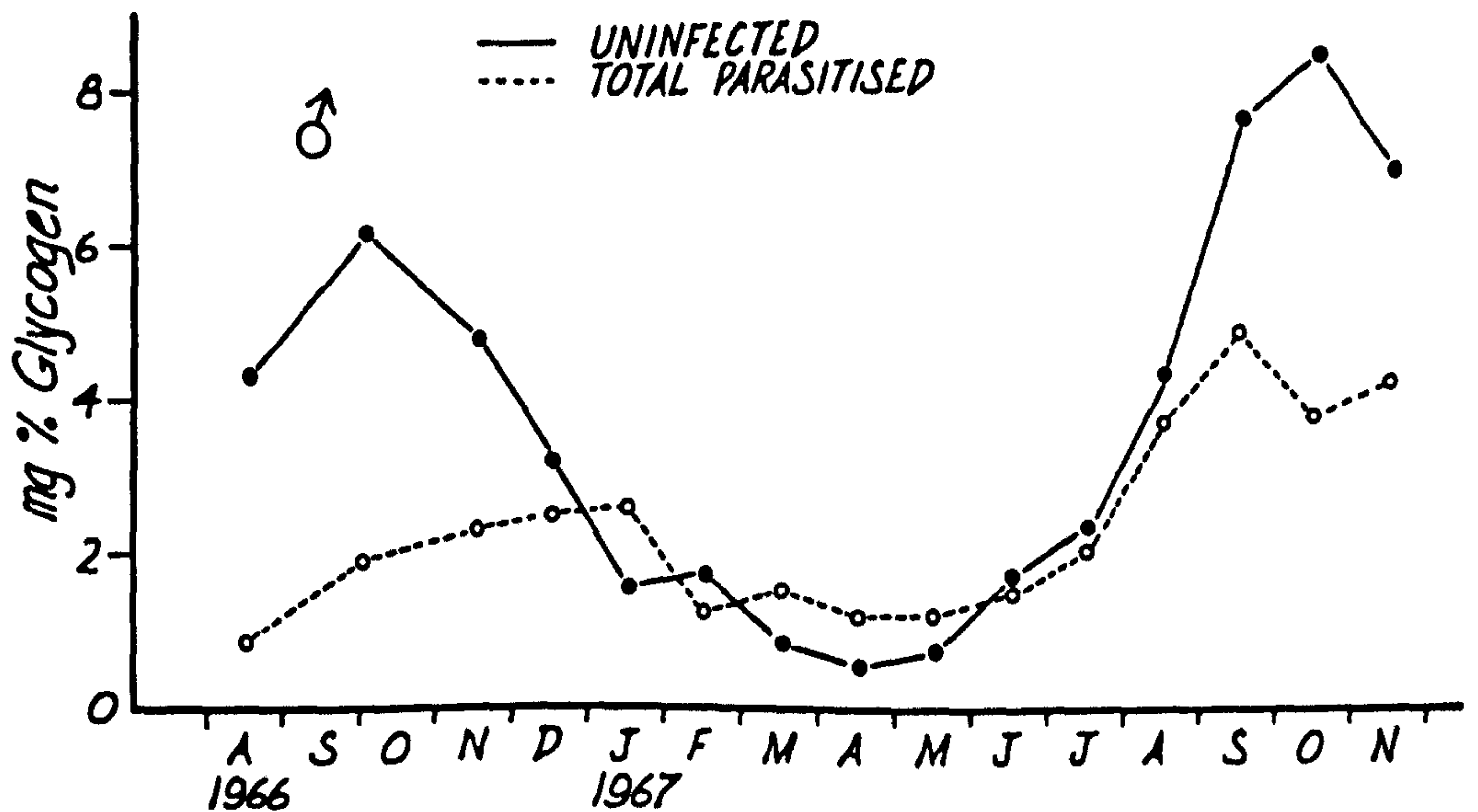


FIG 21 GLYCOGEN CONCENTRATION
(WET WEIGHT) OF HEPATOPANCREAS OF L. Littorea:
UNINFECTED AND TOTAL PARASITISED
(MALES & FEMALES SEPARATELY)

The glycogen concentration of the digestive gland of uninfected animals compared with those of the total parasitised animals are shown in Fig. 19. The differences between their monthly means were shown to be significant at the 5% level or less for 11 of the 15 months studied (Table 26). It was during the period January-May (i.e. in the breeding season) that the glycogen content of infected L.littorea was higher than that recorded for uninfected animals. However, this increase was only significant in April and May. The difference is especially significant (P generally $< .001$) outside the breeding period, when the uninfected animals are in the recovering spent state (stage IIb) or the maturing state (stage IIa) (Fig. 8). An analysis of variance for disproportionate subclass numbers shows that variation due to infection is highly significant ($P < .005$ - Table 28) as is also variation due to months, and the variation resulting from an interaction between months and infection. This indicates that parasitisation by larval Digenea had a real effect on the glycogen content of the host.

These differences between means were significant when the results were analysed separately by sex, but to a slightly lesser degree for the females (only 8 of the 15 months) than for the males (12 of the 15 months) (Table 26).

**TABLE 28. VARIANCE TABLE: GLYCOGEN CONTENT OF HEPATOPANCREAS
OF UNINFECTED VERSUS TOTAL PARASITISED L.LITTORINA
(males plus females)**

Source	Sum of squares	d.f.	Mean Square	F	P
Infection	180.366	1	180.366	111.405	<.005
Months	1533.948 - 6.087 = 1540.035	14	110.002	67.95	<.005
Interaction	311.306	14	22.236	13.74	<.005
Within sub- classes	1002.385	619	1.619		

The glycogen concentrations of the foot in the parasitised animals showed a seasonal cycle parallelling that of the hepatopancreas, but at a lower level throughout.

Figure 20 compares the differences between the glycogen levels of the foot of uninfected animals (males plus females) with those of the parasitised animals. Significant differences between them were found to occur only in September, November and December 1966, and September, October and November 1967. Throughout the breeding period, which corresponded to the months when the glycogen levels were at their lowest, significant differences did not occur. (Table 29).

When the parasitised animals are separated into those infected with the Cercaria A and those infected with the parthenitae of C.lingua striking differences are observed in the glycogen content of the tissues.

TABLE 29. SIGNIFICANCE PROBABILITIES OF DIFFERENCE BETWEEN MEANS FOR FOOT
GLYCOGEN CONCENTRATION CALCULATED BY STUDENT'S t TEST FOR
UNINFECTED VERSUS TOTAL PARASITISED L.LITTOREA

16.8.66	t P > .1	9.5.67	t P > .1
29.9.66	t P < .001	6.6.67	t P > .8
2.11.66	t P < .05	10.7.67	t P > .7
1.12.66	t P < .02	7.8.67	t P > .6
12.1.67	t P > .1	4.9.67	t P < .05
11.2.67	t P < .05	2.10.67	t P < .01
14.3.67	t P > .8	2.11.67	t P < .001
10.4.67	t P > .8		

The difference between means was taken to be significant if P was less than .05
The degrees of freedom are obtainable from the data in Table 23
t = Student's t-statistic

(b) Littorina littorea infected with Gyptocotyle lingua

Animals infected with C.lingua exhibit a seasonal glycogen cycle for the hepatopaneas but at a lower level than that of uninfected animals with the exception of March-May 1967 when the position is reversed (Fig. 22). The maximum value reached by C.lingua infected animals was less than half that of the uninfected animals (1.87 mg% as against 5.35 mg% in 1966; and 3.20 mg% compared with 7.70 mg% in 1967; Table 23, Fig. 22).

The differences between the monthly means for hepatopaneatic glycogen in the uninfected L.littorea and those infected with C.lingua were significant for all but 4 of the monthly observations, namely, January, February, March and July 1967 (Table 26).

Significant differences also occurred between the monthly glycogen values for the digestive gland of animals infected with C.lingua and those infected with Cercaria A for all but 7 of the monthly means (namely August 1966; February, March, April, June, July and August 1967 - Table 26). With the exception of the April and May figures the glycogen level of the hepatopaneas is lower for L.littorea infected with C.lingua than for those infected with Cercaria A. (Fig. 22).

Throughout the period studied the glycogen content of the foot closely approached the level found in the digestive gland, and during the Scalby population's breeding season (January - June) rose slightly above it, with the single exception of May

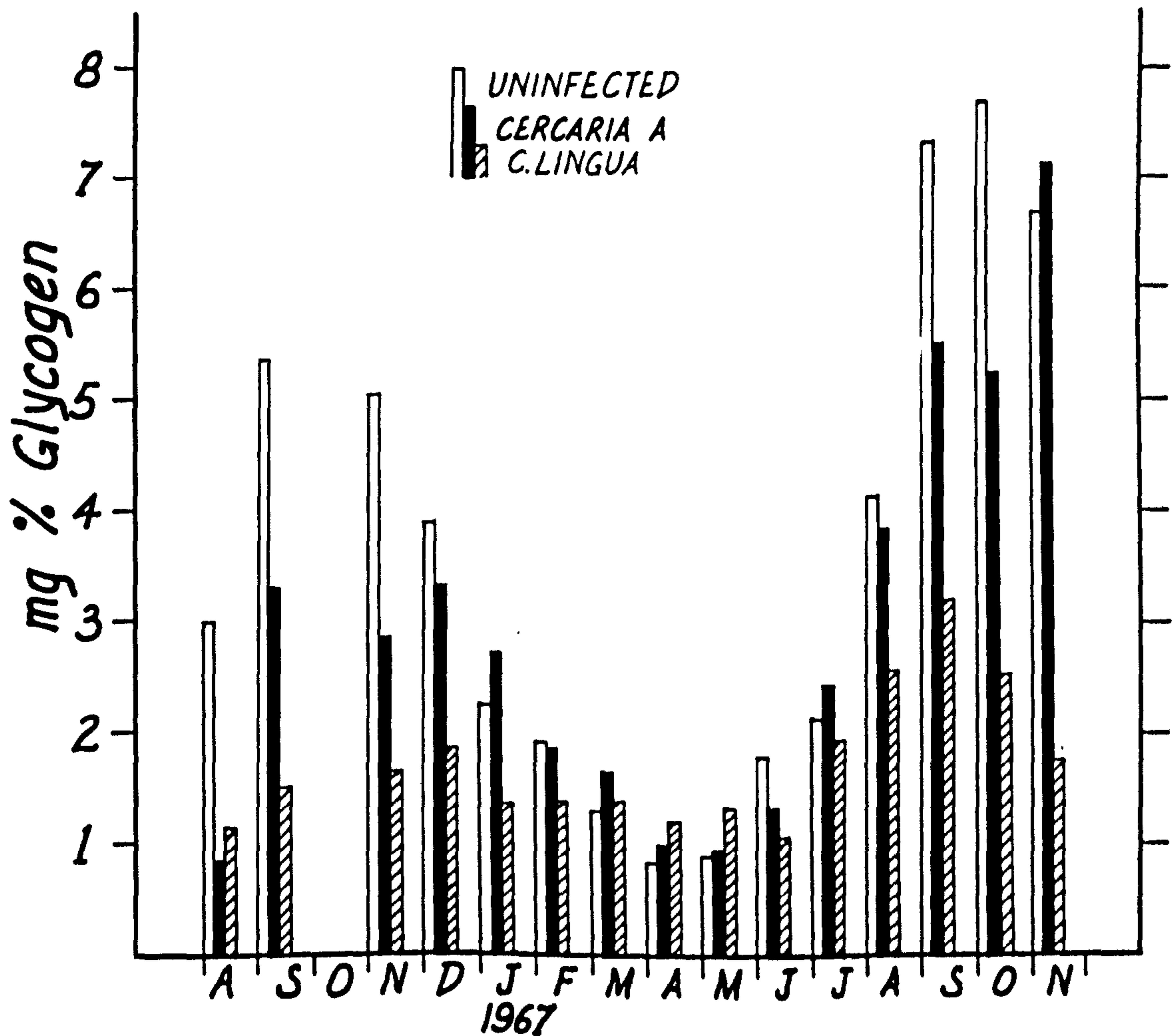


FIG 22 GLYCOGEN CONCENTRATION (WET WEIGHT) OF HEPATOPANCREAS OF L. Littorea MALES & FEMALES: UNINFECTED, INFECTED WITH Cercaria A, AND INFECTED WITH C. Lingua

(Table 23).

Both males and females (Figs. 23, 24; Tables 24 and 25) infected with C.lingua exhibit similar conditions.

(c) Littorina littorea infected with Cercaria A.

Animals infected with Cercaria A also showed a seasonal cycle in respect of their glycogen content but this bore a greater resemblance to the cycle in the uninfected animals (Fig. 22). The difference between the glycogen monthly means for the hepatopancreas of L.littorea infected with Cercaria A and those of uninfected animals only differed significantly in September and November 1966 and September and October 1967 (Table 26), these periods corresponding to the seasonal glycogen maximum for uninfected animals. This is in striking contrast to the corresponding relationship between uninfected animals and those infected with C.lingua. These were significantly different from each other in 11 of the 15 months studied (Table 26).

It is worth at this stage drawing attention to the relationship of these two parasites to the reproductive condition of their hosts. The pattern for the proportion of mature, breeding animals is similar to the glycogen picture, in that for L.littorea infected with Cercaria A the proportion capable of breeding, or at least exhibiting characteristics of this condition, was generally half way between the uninfected animals and C.lingua, the latter seldom showing mature characteristics (Figs. 8 and 22).

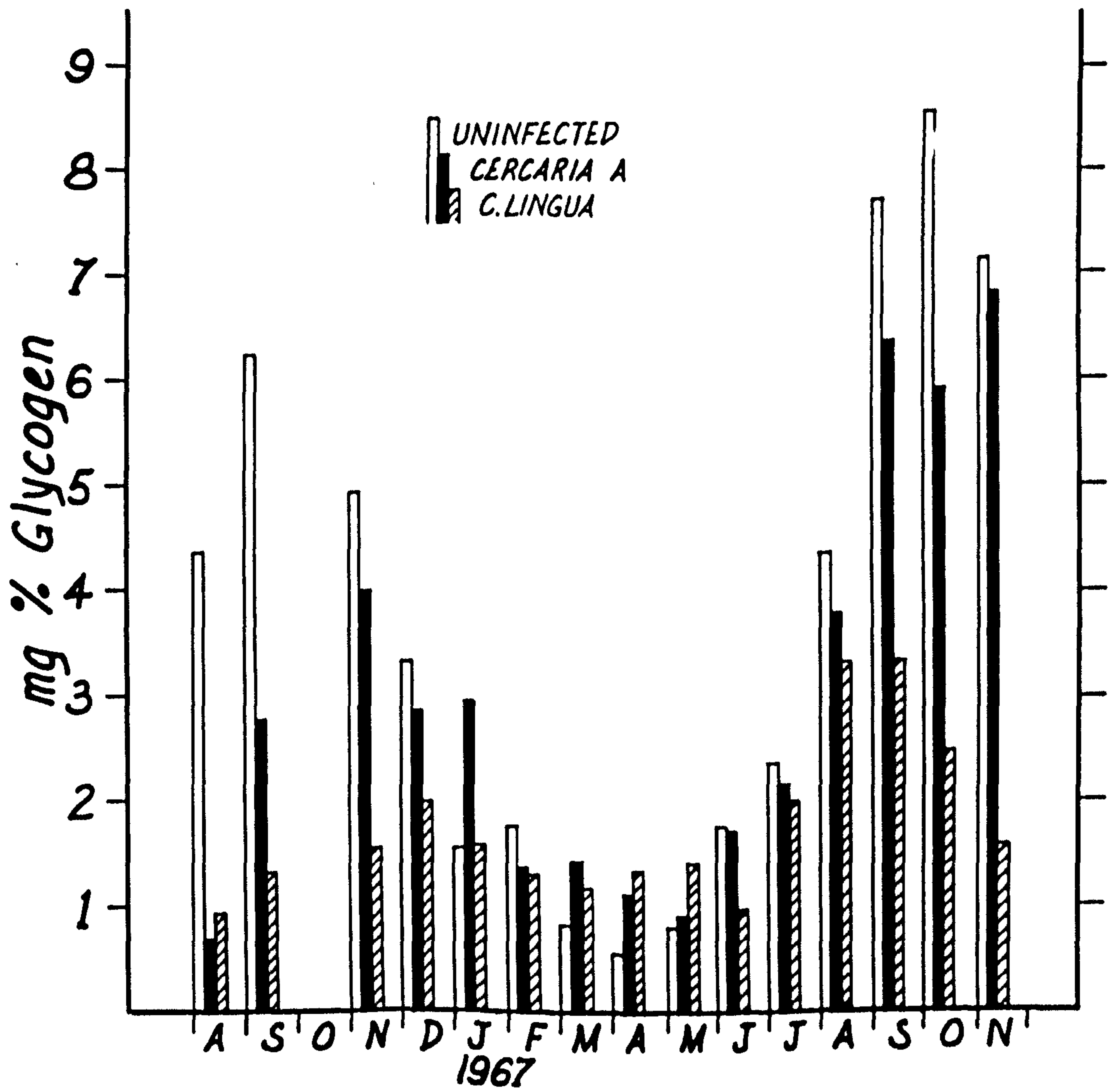


FIG 23 GLYCOGEN CONCENTRATION (WET WEIGHT) OF HEPATOPANCREAS OF L. Littorea MALES: UNINFECTED, INFECTED WITH Cercaria A, AND INFECTED WITH C. Lingua

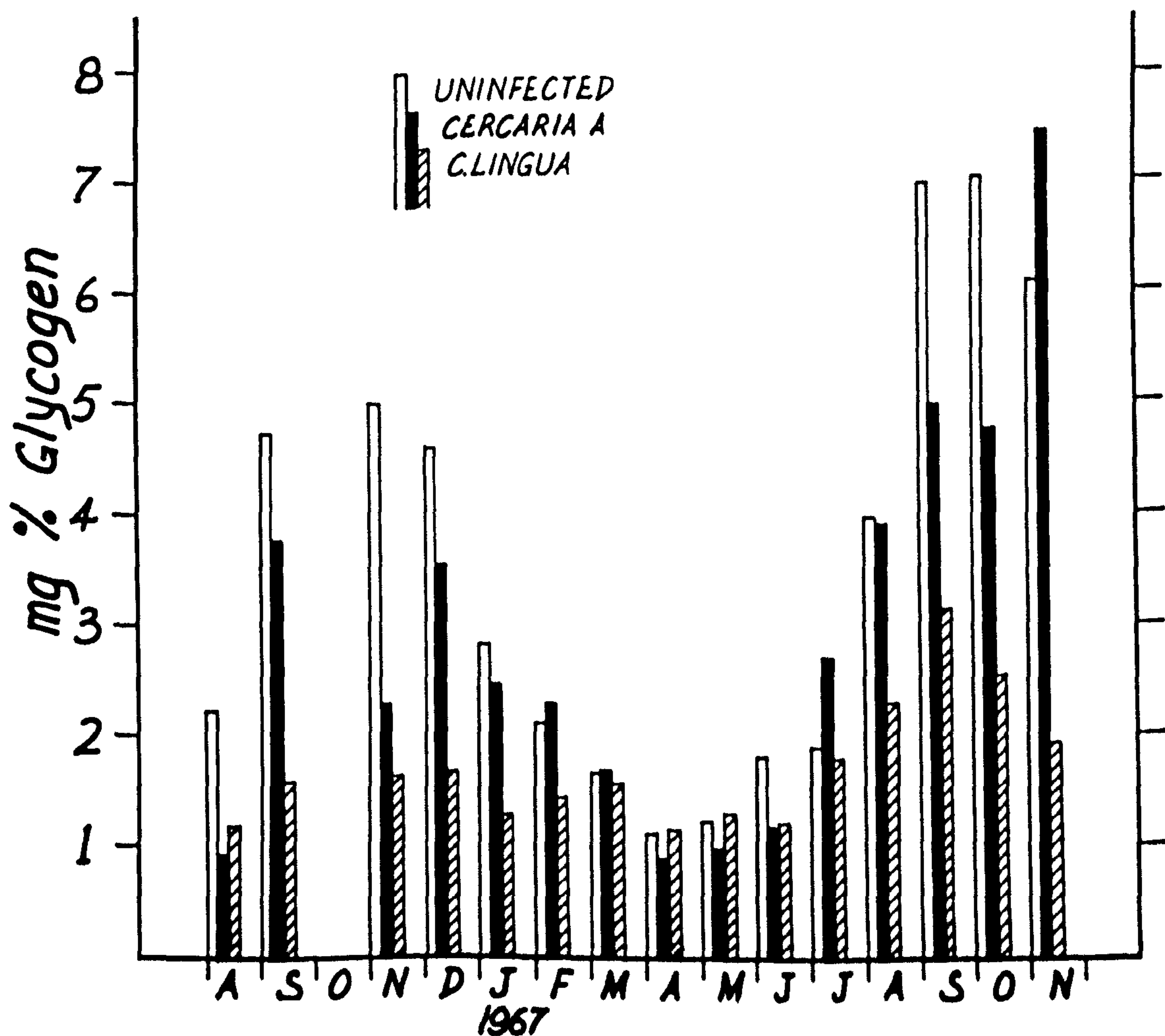


FIG 24 GLYCOGEN CONCENTRATION (WET WEIGHT) OF HEPATOPANCREAS OF L. Littorea FEMALES: UNINFECTED, INFECTED WITH Cercaria A, AND INFECTED WITH C. Lingua

The glycogen level in the foot of L.littorea infected with Cercaria A follows a similar seasonal pattern to that of the hepatopaneas; but unlike the condition in C.lingua infected animals the glycogen level in the foot never rose above that of the hepatopaneas (Table 23).

Similar seasonal cycles are shown by both male and female L.littorea infected with Cercaria A (Figs. 23 and 24, Tables 24 and 25).

- (d) Littorina littorea infected with Himasthla leptosoma or harbouring double infections (i.e. infected with two species of digenean larvae).

L.littorea infected with the echinostome or harbouring double infections were not very common and as explained previously too few were analysed for carbohydrate content to enable conclusions to be drawn relating to the effect of the presence of these parasites on the glycogen content of their hosts.

The recorded hepatopaneatic glycogen value for L.littorea infected with larval trematodes are probably higher than the true value, as the method used estimated the glycogen present in both the host tissue and in the larvae themselves. This was especially the case for hosts harbouring larval stages of C.lingua, where the latter were usually present in very large numbers, the host tissue in many cases being practically non-existent. The larvae

of Cercaria A were present in a more discrete localized manner and it was possible in many cases to estimate the glycogen content of the host's tissue only.

It was seen that the glycogen content of both parasitised and uninfected animals varies widely not only with season but also between individuals examined at the same time.

In parasitised L.littorea the histochemical tests also substantiated the variations in the amount of glycogen found by the quantitative anthrone method. In the case of Cercaria A the distribution of glycogen in the tissue distant to the sporocysts was similar to that of the uninfected animals, but in the tissue adjacent to the parasite mass the number and size of the glycogen granules was slightly smaller. The sporocysts themselves contained appreciable glycogen deposits, which were often heavy in the walls of the sporocysts.

In L.littorea infected with C.lingua the rediae occupied a large volume of the visceral mass and were randomly dispersed throughout the gland. Gonadial tissue was rarely seen. The connective tissue usually showed signs of degeneration as did the digestive gland with numerous vacuoles occupying the visceral hump. It was often the case that the remaining tissue contained amounts of glycogen comparable to the uninfected animals,

but the volume of host tissue was considerably less. Glycogen deposits were also present in the parasites but in lower concentrations than in uninfected host tissue.

EXPERIMENT I

The effect of storage on the physiological condition of *Littorina littorea* as indicated by the glycogen content.

At the beginning of the observations on the seasonal carbohydrate cycle of *L.littorea* it was considered advisable to measure the effect, if any, of laboratory conditions on the carbohydrate content of specimens. Thus, the carbohydrate content of a group of animals was estimated at intervals over a period of four weeks.

MATERIAL AND METHODS

L.littorea were collected on 28th September 1966 from Scalby Rocks. They were transported to the laboratory and within 1½ hours of collection they were put into an aquarium, with circulating aerated sea water maintained at about 10°C. They were supplied with abundant *Fucus* sp. collected from the same area, the *Fucus* fronds being replenished as soon as they showed signs of decay.

Samples for analysis of their carbohydrate content, consisting of about 40 animals, were removed from the aquarium and estimated fresh on the 6th October (Day 8) and on the 29th October (Day 31). A batch of animals was also estimated the day after their arrival at the laboratory (Day 1). As for the monthly glycogen deter-

minations food was removed from the aquarium the night prior to glycogen estimation.

During this four week period they were kept in the laboratory the majority of periwinkles clustered above the water mark, but were returned daily to the water to minimise any change in carbohydrate content due to dessication, also to encourage feeding.

The carbohydrate content of the foot and digestive gland was determined using the KOH extraction procedure and the Anthrone method as described on page 127. Students 't' test was also used. The amount of glycogen present was expressed as mg glucose per 100 mg wet weight of tissue.

It was unfortunate that it was not practicable to have equal numbers of parasitised and uninfected animals. The number of uninfected L.littorea was low, being about one third that of the infected specimens. This discrepancy in numbers was due to the high incidence of infection (58.9% total parasitised) at this time of the year. However, it is still possible to draw some general conclusions from the results.

RESULTS

The results are summarized in Fig. 25 and Table 30; the data from the September and November 1966 monthly determinations are

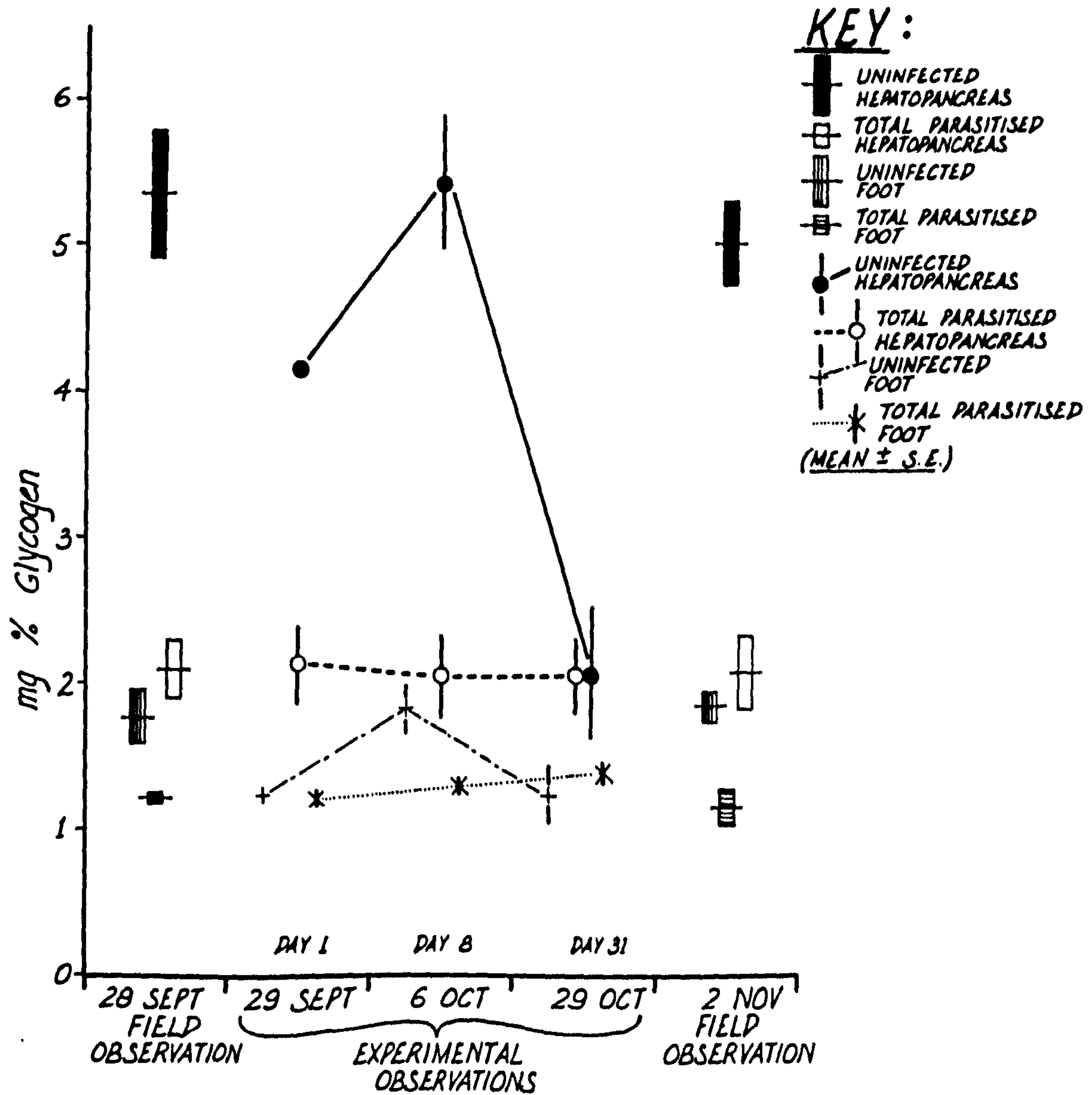


FIG 25 EFFECT OF STORAGE ON GLYCOGEN CONCENTRATION (WET WEIGHT) OF FOOT & HEPATOPANCREAS OF UNINFECTED & TOTAL PARASITISED *L. Littorea* MALES & FEMALES COMPARED WITH SEASONAL FIELD DATA

TABLE 30. EFFECT OF STORAGE ON THE GLYCOGEN CONCENTRATION, expressed as mg glucose per 100 mg wet weight of tissue, OF THE FOOT AND HEPATOPANCREAS OF UNINFECTED AND TOTAL PARASITISED L.LITTOREA

<u>Males + Females</u>	<u>UNINFECTED</u>		<u>TOTAL PARASITISED</u>	
	Dig. gland	Foot	Dig. gl.	Foot
29.9.66 Seasonal	5.35 $\pm$.43 (10)	1.76 $\pm$.17 (11)	2.09 $\pm$.20 (68)	1.20 $\pm$.04 (66)
29.9.66 Day 1	4.15 (1)	1.20 (1)	2.13 $\pm$.26 (38)	1.18 $\pm$.05 (39)
6.10.66 Day 8	5.43 $\pm$.46 (9)	1.82 $\pm$.18 (10)	2.04 $\pm$.29 (30)	1.28 $\pm$.07 (27)
29.10.66 Day 31	2.07 $\pm$.44 (8)	1.22 $\pm$.20 (7)	2.04 $\pm$.26 (32)	1.38 $\pm$.07 (33)
2.11.66 Seasonal	5.04 $\pm$.28 (16)	1.84 $\pm$.09 (16)	2.07 $\pm$.25 (24)	1.45 $\pm$.12 (24)
<u>Males</u>				
29.9.66 Seasonal	6.21 $\pm$.73 (4)	1.68 $\pm$.18 (4)	1.98 $\pm$.27 (41)	1.20 $\pm$.06 (37)
29.9.66 Day 1	----- (0)	----- (0)	2.15 $\pm$.39 (22)	1.19 $\pm$.07 (22)
6.10.66 Day 8	6.21 $\pm$.73 (4)	1.68 $\pm$.18 (4)	1.78 $\pm$.36 (19)	1.21 $\pm$.10 (15)
29.10.66 Day 31	2.31 $\pm$.76 (4)	1.20 $\pm$.40 (3)	2.07 $\pm$.30 (20)	1.38 $\pm$.10 (21)
2.11.66 Seasonal	4.88 $\pm$.38 (7)	1.69 $\pm$.11 (7)	2.36 $\pm$.58 (9)	1.60 $\pm$.20 (9)
<u>Females</u>				
29.9.66 Seasonal	4.72 $\pm$.40 (6)	1.80 $\pm$.26 (7)	2.25 $\pm$.27 (27)	1.21 $\pm$.06 (29)
29.9.66 Day 1	4.15 (1)	1.20 (1)	2.09 $\pm$.32 (16)	1.16 $\pm$.08 (17)
6.10.66 Day 8	4.83 $\pm$.47 (5)	1.91 $\pm$.28 (6)	2.47 $\pm$.47 (11)	1.28 $\pm$.09 (12)
29.10.66 Day 31	1.83 $\pm$.53 (4)	1.24 $\pm$.25 (4)	2.03 $\pm$.53 (11)	1.38 $\pm$.13 (11)
2.11.66 Seasonal	4.97 $\pm$.53 (7)	1.97 $\pm$.17 (7)	1.90 $\pm$.22 (15)	1.36 $\pm$.15 (15)

Numbers preceded by $\pm$ give S.E. of mean

Numbers in parenthesis indicate numbers of animals in each sample

included for comparison.

In the parasitised animals (for males + females) the glycogen content of the digestive gland did not differ significantly throughout the duration of the experiment (Fig. 25), the variation being less than 0.1 mg%. The foot muscle showed only a slight rising change in glycogen content, but again this was not significant.

The picture presented by the uninfected animals was however strikingly different from this (Fig. 25), the carbohydrate level in the digestive gland showing a significant drop ($P < .001$ - Table 31) of about 62% between the estimations of the 6th and 29th October, from 5.43 mg% to 2.07 mg%. It was at about this time that the carbohydrate content of the natural population began to show the seasonal decline (Fig. 17). However, the decrease shown by the animals kept in the laboratory was much steeper and the level differs very significantly ($P < .001$) from the comparable (2nd November 1966) seasonal figures - 5.04 mg% for the latter as against 2.07 mg% for the experimental animals.

The foot of the uninfected animals, unlike that of the parasitised animals, also showed a pronounced decrease in the mg% glycogen content between 6th and 29th October. The decrease was not as significant as for the digestive gland but was still within the 5% level of significance ($P < .05$ - Table 31). The amount found

TABLE 31. SIGNIFICANCE PROBABILITIES OF DIFFERENCE BETWEEN MEANS OF TISSUE GLYCOGEN CONCENTRATION (FOR THE COMBINATIONS SHOWN) IN EXPERIMENT I

<u>Foot uninfected</u>	<u>t</u>	<u>d.f.</u>	<u>P</u>
6.10.66 versus 29.10.66 Day 8 Day 31	2.174	15	< .05
29.10.66 versus 2.11.66 Day 31 Seasonal	3.327	21	< .01
6.10.66 versus 2.11.66 Day 8 Seasonal	0.132	25	> .8
<u>Hepatopancreas uninfected</u>			
6.10.66 versus 2.11.66 Day 8 Seasonal	0.796	23	> .4
29.10.66 versus 2.11.66 Day 31 Seasonal	5.789	22	< .001
6.10.66 versus 29.10.66 Day 8 Day 31	5.283	15	< .001
<u>Parasitised versus uninfected</u>			
6.10.66 hepatopancreas Day 8	5.724	37	< .001
6.10.66 Foot Day 8	3.693	35	< .001
29.10.66 hepatopancreas Day 31	0.059	38	> .9
29.10.66 Foot Day 31	0.852	38	> .3
2.11.66 hepatopancreas Seasonal	7.681	38	< .001
2.11.66 Foot Seasonal	2.377	38	< .05

t = Student's t-statistic
d.f. = Degrees of freedom
P = Probability calculated by Student's t test

in the foot of animals examined on the 29th October was also significantly lower ($P < .01$) than for the corresponding seasonal animals, (2nd November 1966) whereas the difference between the mg% glycogen content of the animals of 6th October and the seasonal November figures was not significant ($P > .8$); this was also the case for the digestive gland ($P > .4$).

Both male and female L.littorea showed the same general pattern (Table 30).

It should be noted that the mg% glycogen content of parasitised versus uninfected animals, for both the digestive gland and foot, differ significantly from each other in the estimation of 6th October ($P < .001$ for both organs). This is also the case with the November field animals (P for digestive gland $< .001$; P for foot $< .05$). However, in the animals estimated on 29th October the difference is not appreciable (P for digestive gland $> .9$; P for foot $> .3$).

DISCUSSION

The experiment was conducted over too short a period to make a sensible comparison between laboratory and natural conditions. Its aim was to discover whether it was essential to estimate the glycogen levels as soon as possible after sampling and the conclusion was that it did appear to be essential.

EXPERIMENT II

The effect of starvation on the carbohydrate content of the foot and digestive gland of *Littorina littorea*

MATERIAL AND METHODS

About 300 *L.littorea* were collected from Scalby Rocks on the 12th January 1967, transported to the laboratory with a time lag of about $1\frac{1}{2}$ hours and put in an aquarium with circulating, aerated sea water maintained at about 10°C as in the previously reported experiment. They were kept without food overnight.

The next day about 40 animals were analysed for the seasonal carbohydrate data. The remainder were divided into 2 groups approximately equal in numbers and placed in separate aquaria maintained as above. Animals in group A were given a regular supply of Fucus for the duration of the experiment which continued until 27th June 1967. Group B animals were not supplied with food. About 20 *L.littorea* of approximately the same size, were analysed from each tank 11, 24, 113 and 166 days after collection. On the 18th May (day 127) the animals remaining in group B were divided into equal groups B₁ and B₂. Group B₂ animals were supplied with Fucus, whereas group B₁ continued to starve. This was an attempt to see if after their 4 months of starvation *L.littorea* would readily feed in the laboratory, and also to see if re-application of food had any noticeable effect on the glycogen level in the

digestive gland and foot.

The glycogen content was estimated as before, and expressed as mg glucose per 100 mg wet weight of tissue (mg%).

RESULTS

Figure 26 and Table 32 summarize the information obtained from the experimental animals; the relevant seasonal date (Table 23) is included in the graph for comparison.

Group A - L.littorea supplied continuously with food

During the period studied the digestive gland glycogen content of the uninfected natural population was at its annual minimum (Figs. 17 and 26). The experimental animals still exhibit this trough but at a slightly higher level than the corresponding natural date (Fig. 26). This elevation is significant in two cases only, the first being the corresponding May figures ($P < .001$) and the second, the experimental June animals (analysed on the 27th). When these are compared with the natural June animals (analysed on 6th) there is again a significant difference ($P < .02$) but this disappears when comparing them with the natural July animals (analysed on 10th) ($P > .2$).

The parasitised animals also showed a cyclical pattern but the differences between the means of the experimental and natural population were never significant .

Males and females showed similar trends (Table 32).



FIG 26 EFFECT OF STARVATION ON GLYCOGEN CONCENTRATION (WET WEIGHT) OF HEPATOPANCREAS OF UNINFECTED & TOTAL PARASITISED *L. Littorea* MALES & FEMALES, COMPARED WITH SEASONAL FIELD DATA

TABLE 32 EFFECT OF STARVATION ON THE GLYCOGEN CONTENT , expressed as mg glucose per 100 mg wet weight of tissue, OF THE FOOT AND HEPATOPANCREAS OF UNINFECTED AND TOTAL PARASITISED L.LITTOREA

Males + Females	GROUP A- FOOD SUPPLIED				GROUP B- STARVED			
	UNINFECTED		TOTAL PARASITISED		UNINFECTED		TOTAL PARASITISED	
	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot	Dig. gland	Foot
22.1.67 Day 11	2.67 $\pm$.58 (6)	1.59 $\pm$.19 (6)	2.17 $\pm$.34 (8)	1.27 $\pm$.11 (8)	2.50 $\pm$.31 (7)	1.83 $\pm$.22 (6)	2.17 $\pm$.54 (5)	1.39 $\pm$.18 (6)
4.2.67 Day 24	2.30 $\pm$.20 (8)	1.50 $\pm$.21 (7)	1.58 $\pm$.19 (12)	1.11 $\pm$.11 (12)	1.97 $\pm$.15 (7)	1.11 $\pm$.19 (9)	1.56 $\pm$.33 (11)	1.16 $\pm$.17 (10)
4.5.67 Day 113	1.73 $\pm$.21 (9)	1.24 $\pm$.11 (10)	1.52 $\pm$.20 (11)	1.23 $\pm$.17 (10)	1.20 $\pm$.15 (7)	1.04 $\pm$.12 (7)	0.87 $\pm$.15 (11)	0.76 $\pm$.12 (13)
27.6.67 Day 166	2.64 $\pm$.26 (4)	1.35 $\pm$.30 (4)	1.99 $\pm$.40 (10)	1.15 $\pm$.17 (9)	1.11 $\pm$.14 (8)	0.95 $\pm$.09 (8)	1.14 $\pm$.17 (6)	1.01 $\pm$.19 (4)
Males								
22.1.67 Day 11	2.47 $\pm$.67 (5)	1.50 $\pm$.19 (5)	2.02 $\pm$.18 (4)	1.43 $\pm$.10 (4)	1.96 $\pm$.19 (4)	1.67 $\pm$.31 (4)	2.10 $\pm$.62 (3)	1.43 $\pm$.23 (4)
4.2.67 Day 24	1.75 (1)	0.74 (1)	1.58 $\pm$.17 (7)	1.05 $\pm$.09 (7)	2.33 $\pm$.21 (2)	1.17 $\pm$.27 (4)	1.70 $\pm$.41 (8)	1.08 $\pm$.20 (7)
4.5.67 Day 113	1.31 $\pm$.29 (4)	1.02 $\pm$.07 (5)	1.70 $\pm$.38 (5)	1.37 $\pm$.30 (5)	0.93 $\pm$.04 (3)	0.80 $\pm$.06 (3)	0.68 $\pm$.11 (6)	0.55 $\pm$.15 (7)
27.6.67 Day 166	2.63 $\pm$.36 (3)	1.06 $\pm$.09 (3)	2.27 $\pm$.46 (6)	1.08 $\pm$.14 (5)	1.16 $\pm$.17 (3)	0.85 $\pm$.07 (5)	1.13 $\pm$.26 (3)	1.20 $\pm$.09 (3)
Females								
22.1.67 Day 11	3.67 (1)	2.075 (1)	2.33 $\pm$.71 (4)	1.11 $\pm$.17 (4)	3.22 $\pm$.39 (3)	2.15 $\pm$.05 (2)	2.28 $\pm$.85 (2)	1.30 $\pm$.38 (2)
4.2.67 Day 24	2.38 $\pm$.22 (7)	1.63 $\pm$.20 (6)	1.76 $\pm$.47 (4)	1.30 $\pm$.29 (4)	1.83 $\pm$.16 (5)	1.07 $\pm$.27 (5)	1.19 $\pm$.36 (3)	1.34 $\pm$.35 (3)
4.5.67 Day 113	2.06 $\pm$.24 (5)	1.46 $\pm$.15 (5)	1.37 $\pm$.18 (6)	1.09 $\pm$.16 (5)	1.39 $\pm$.21 (4)	1.23 $\pm$.15 (4)	1.11 $\pm$.29 (5)	1.01 $\pm$.15 (6)
27.6.67 Day 166	2.65 (1)	2.24 (1)	1.55 $\pm$.59 (4)	1.24 $\pm$.36 (4)	1.03 $\pm$.18 (3)	1.10 $\pm$.19 (3)	1.13 $\pm$.29 (3)	0.456 (1)

GROUP B2 - Starved for 127 days then food re-supplied, analysed on Day 166 (27.6.67)

	UNINFECTED		TOTAL PARASITISED	
	Dig. gland	Foot	Dig. gland	Foot
Males + Females	1.96 $\pm$.26 (6)	1.43 $\pm$.23 (6)	1.57 $\pm$.22 (8)	1.73 $\pm$.22 (7)
Males	1.82 $\pm$.36 (4)	1.18 $\pm$.19 (4)	1.29 $\pm$.18 (4)	1.62 $\pm$.44 (3)
Females	2.22 $\pm$.37 (2)	1.93 $\pm$.44 (2)	1.85 $\pm$.37 (4)	1.82 $\pm$.23 (4)

Numbers preceded by $\pm$ give S.E. of mean
Numbers in parenthesis indicate number of animals in each sample

Group B - L.littorea deprived of food

At the beginning (i.e. after 11 and 24 days) starvation had little noticeable effect on the uninfected animals (Fig. 26). However, in June, by which time the fed experimental animals were showing a fairly considerable rise in digestive gland glycogen level, that of the uninfected starved specimens was significantly lower ($P < .001$). The pattern for animals infected with trematode larvae was similar (Fig. 26), but the difference between fed and starved animals was not as striking ($P > .1$).

Group B₂ - Food re-supplied to L.littorea

A noticeable increase in glycogen content was seen in uninfected animals which had been kept without food for 127 days and then supplied with food for 39 days prior to estimation of their glycogen content (Group B₂). Values approximately halfway between that of starved and fed animals were recorded. In parasitised animals the effect of feeding after this period of starvation was not as noticeable.

For all three experimental groups (fed, starved and food re-supplied) the glycogen content of the foot exhibited a similar pattern to that of the digestive gland but with differences not as pronounced (Table 32).

DISCUSSION

The observation that the glycogen level of fed laboratory

animals tended to be higher than that of the animals used for the seasonal data is most likely related to the constancy of the experimental environment, with respect to water temperature, oxygen supply and availability of food. In other words they led a more sheltered life than animals on the shore where these factors are continually fluctuating.

The starved animals also showed faint signs of a seasonal cycle. It may therefore be concluded that the glycogen cycle is to some extent inherent and related to other factors apart from the availability of food.

Starvation had the most noticeable effect on the glycogen levels towards the end of the experiment, at a time when the natural tendency was for the percentage of glycogen to increase (Fig. 22). Availability of food even over the relatively short period of 39 days, as compared with the earlier period of starvation (127 days), had a pronounced effect on the level of glycogen recorded for the uninfected animals. However, in L.littorea infected with larval trematodes the effect was not quite as pronounced.

The glycogen content of the foot did not differ as markedly between different conditions as did that of the digestive gland (Table 19). This seems to imply that the digestive gland glycogen is the more labile store and in an emergency is drawn upon first. The foot glycogen is more stable and is called upon after long periods of stress.

These results are not in agreement with those of Emerson and Duerr (1967) who found, for another species of Littorina, an insignificant decrease in polysaccharide content, no decrease in protein nitrogen, but a significant decrease in total lipids for Littorina planaxis which had been starved for 70 days. They concluded that lipids were the main source of stored energy during starvation. However, these authors did not compare the results of starvation with that of the natural population over the same period. Their only source of comparison was with specimens collected at the same time as the experimental animals and frozen until the end of the experiment. It is thus possible that the lipid content of L.planaxis was decreasing even under natural conditions as was found by me for the carbohydrate content of L.littorea (Fig. 17).

Experiments I and II were performed starting at opposite points in the glycogen cycle i.e. peak and trough respectively. It was observed in experiment I that with the initial condition being a peak in the natural glycogen cycle, the specimens transferred to the laboratory appeared to lose glycogen more quickly than specimens in nature. It may be that the laboratory specimens conform to a glycogen cycle of a smaller amplitude than natural specimens but since the two experiments were not pursued through the period of one cycle it would be wrong to try to connect the observations.

EXPERIMENT III

In vitro experiment to ascertain if the rediae of Cryptocotyle lingua and Himasthla leptosoma and the sporocysts of Cercaria A were capable of digesting and/or removing glycogen from a pure glycogen solution made up in sea water

The rediae of C. lingua and H. leptosoma and the sporocysts of Cercaria A were teased from their surrounding host's hepato-pancreas, washed by sedimentation through four changes of bacteriologically filtered sea water, lightly centrifuged to compact them and then removed with a pipette. The species were incubated separately in 1% glycogen solutions made up in sea water, contained either in cavity slides sealed with a cover slip (Series A) or in small beakers open to the atmosphere (Series B). Groups of 10 to 20 rediae and a comparable volume of the sporocyst mass of Cercaria A were so treated. The containers were kept at room temperature (about 22°C) and gently agitated intermittently on a shaking table for 8 hours. The rediae and sporocysts remained mobile for the duration of the experiment.

The presence of glucose in the media was tested for using Benedict's reagent after the 1st, 3rd, 5th and 8th hour of incubation. Two samples each from Series A and B for each of the 3 species were tested on each occasion. All tests were negative. This suggests

that glycolytic enzymes were not secreted by the parasites. It is possible however that the glycogen had been broken down to glucose, but the latter had been absorbed instantaneously by the parasites. At the end of the experiment the amount of glycogen remaining in the incubation media was determined by the anthrone method. One millilitre of the incubation media was withdrawn and reacted directly with anthrone, without prior precipitation of glycogen and hydrolysis to glucose. The latter is not necessary since anthrone gives the same colour reaction with glycogen as with an equal amount of glucose (Seifer et al. 1950, Van Handel 1965). It was found that glycogen had not been removed from the media. This lends further support to the view that the rediae and sporocysts did not secrete glycolytic enzymes. It also suggests that they were incapable of absorbing glycogen molecules. However, Becker (1964) did find an increase in the respiratory quotient when the sporocysts of Cercaria helvetica Dubois XXX 1929, were incubated in a 1% glycogen solution. Becker (1964) suggested two ways in which the sporocysts absorbed glycogen either directly or after its breakdown to simple sugars which were then absorbed. It is clear that more work needs to be done.

EXPERIMENT IV

Quantitative determination of the glycogen content of sporocysts of Cercaria A

A few quantitative determinations on the amount of glycogen

TABLE 33. GLYCOGEN CONTENT (MG% WET WEIGHT) OF SPORO CYSTS OF CERCARIA A AND ADJACENT HOST HEPATOPANCREAS

Host hepatopaneas mg% glycogen	Sporocysts of <u>Cercaria A</u> mg% glycogen	Sex and Reproductive state of host	
3.68	1.81	♀	Unrec.
3.43	1.71	♀	Unrec.
0.37 ^a	0.74	♂	V
1.29	1.49	♂	V
2.06	0.44	♀	III
0.50 ^b	1.00	♂	IV
0.22 ^b	0.70	♂	IV
1.01	1.17	♀	IV
1.47	1.47	♂	Unrec.
3.78	1.27	♀	III
1.41	1.57	♂	III
0.61	0.67	♂	IV
1.12	1.31	♂	X
0.41	1.82	♂	III
0.70	1.42	♀	IV
0.81	1.26	♂	III
3.97	2.32	♂	IV
3.01	1.86	♀	IV
3.94	1.60	♂	Unrec.
0.40	0.94	♂	Unrec.
5.35	2.94	♀	III
1.27	0.98	♂	IV

(a) Host starved for 24 Days
(b) Host starved for 113 Days

in the sporocyst mass of Cercaria A were made. These are summarized in Table 33 together with the glycogen content of the host's hepatopancreas; the sex and reproductive state are also shown. Of the 19 determinations, there were 9 cases where the sporocysts contained more glycogen than the hepatopancreas of their host, 9 where the hosts' hepatopancreas had more glycogen than the sporocysts of Cercaria A, and in the remaining case the sporocysts contained the same amount of glycogen as the hosts' hepatopancreas.

Quantitative determinations of glycogen content were not made for the rediae of C.lingua and H.leptosoma.

DISCUSSION

The amount of glycogen present in the hepatopancreas and foot of uninfected L.littorea from Scalby Rocks had a definite seasonal cycle, being especially pronounced in the hepatopancreas (Fig. 17). A number of causes may have contributed to this.

The glycogen level was lowest in April and May and highest in September and October. The decline in glycogen content corresponded to the period of decreasing sea temperature and the increase approximately to the rising sea temperature, the lowest glycogen values being recorded soon after the attainment of the minimum sea temperature and the highest at about the time of maximum temperatures.

The quantity and quality of food available will affect the amount of glycogen in the tissues of L.littorea. Patil & Joshi (1967) have shown that the chemical composition of Ulva lactuca on the sea shores of Bombay changes from month to month. It is probably safe to assume that a similar situation is exhibited by the algal food sources of L.littorea at Scalby Rocks. Changes in the amount of carbohydrates in the algae would naturally affect the amount of nutrients available and it is likely that these changes will be reflected in the amount of carbohydrates stored in the tissues of L.littorea. Vegetation was comparatively scarce at the time when the carbohydrate level was low and was most abundant during the July to September period, when the glycogen concentration was on the increase. The carbohydrate level then fell during October and November (Fig. 17). This may have corresponded to the beginning of the over wintering period, which is the period when periwinkles are very inactive and are generally located under stones, at greater depths in rock pools, or hidden in rock crevices. L.littorea is known to be inactive at low temperatures (Gowanloch & Hayes 1926, Hayes 1929, Southward 1958, Newell 1958 and Williams 1964). Thus during this over wintering period they are presumably living off accumulated food reserves - among them carbohydrates (= glycogen).

A relationship was found between the reproductive condition and the seasonal glycogen cycle of L.littorea at Scalby Rocks (Fig. 17). The glycogen content was at its seasonal low during the period

when the majority of uninfected animals were mature and spawning. Peaks occurred at the time of the year when the majority were either maturing for the first time (stage IIa) or in the recovering spent state (stage IIb).

Similar relationships between the glycogen content of molluscan tissues and their reproductive state have been reported previously. Galtsoff (1964) states "Glycogen is the reserve material of the oyster [Crassostrea virginica]. It is stored primarily in the connective tissue of the mantle and labial palps. During the rapid proliferation of sex cells the reserve supply is used, and by the end of the reproductive cycle the amount of glycogen is at a minimum and the mantle is reduced from a thick heavy layer to a transparent membrane. Soon after spawning the oysters begin to form and store glycogen"

Ansell & Lander (1967) working with the hard-shell clam Venus mercenaria from Southampton Water found that in some years changes in the carbohydrate content resulted largely from the development of the gonad and from spawning and that storage of reserve carbohydrate was of little importance. However, in other years, the level remained high during spawning and then fell steadily through the winter months. Ansell & Lander (1967) considered that high carbohydrate content is associated with the establishment of reserves which may be utilised during the ensuing winter months and suggested that environmental factors had a strong influence and that the breeding cycle had a less direct bearing on the carbohydrate content.

It could be mooted that the pronounced seasonal glycogen cycle of the hepatopancreas of L.littorea at Scalby Rocks is more apparent than real in that the gonad and digestive gland are intermingled and inevitably both are used in a chemical estimation of the glycogen content. The amount of gonad increases prior to spawning and when the animals are ripe and mature it accounts for a large proportion of the visceral mass. Thus one could surmise the drop in the glycogen concentration observed at this time, to be a direct result of an increase in the volume of gonadial tissues, the digestive gland containing the same amount of glycogen as observed during the period of high glycogen values. However, this was not the case. Histochemical tests on tissues from the animals used for the quantitative estimations showed the seasonal variation in the amount of glycogen to be a true reflection of the amount of stored glycogen in the tissues. The differences between the values recorded for the extremes of glycogen concentration may not be quite as pronounced as the quantitative values suggest, since as the gonad develops it replaces some of the connective tissue of the visceral mass. The connective tissue was found to be rich in glycogen, whereas the gonad contained only sparse deposits. Examination of the quantitative results for foot glycogen of L.littorea also indicates a seasonal glycogen cycle (Table 23, Fig. 17) which, although not as pronounced as that of the hepatopancreas, is still evident, suggesting that the tissue glycogen has a definite seasonal

variation.

Barry & Munday (1959) found that the glycogen content of both the foot and hepatopancreas of Patella exhibited seasonal variations, but, unlike the situation with L.littorea the amount of glycogen in the foot bore a much closer resemblance to that of the digestive gland. In L.littorea, the peak level for the foot was much lower than that for the hepatopancreas (Fig. 17). These differences may be related to the difference in habit of these two marine gastropods.

The factors referred to above may all contribute to the seasonal variations in glycogen content of L.littorea at Scalby Rocks. They may exert their influence either individually, or in combination, or in a succession of cause and effects.

Williams (1964) considered that male L.littorea attain sexual maturity before females, but from the reproductive evidence obtained for L.littorea at Scalby Rocks the sexes seemed to reach maturity at the same time (Figs. 9 and 10; Tables 11 and 12). There is however some evidence in support of Williams' observations. This is provided by the glycogen curves for male and female hepatopancreas (Fig. 18), which appear to be out of phase, that of the males leading the females by one to four weeks. If, as has been suggested earlier, the glycogen level is intimately associated with the reproductive cycle, this difference may be an indication of males maturing before females. Nevertheless, an analysis of variance applied to all the monthly data shows that there

is no significant difference between the glycogen content of the hepatopancreas of uninfected males and females (Table 27).

Parasitisation by larval Digenea was found to have a pronounced detrimental effect on the glycogen content of L.littorea (Figs. 19 and 20). A number of factors may have contributed to this, among them:

- (i) Lowered food consumption by the host.
- (ii) Impaired digestion and/or absorption of food.
- (iii) Production of toxic products by parasite interfering with carbohydrate metabolism of host.
- (iv) Excessive food consumption by parasite.
- (v) Disturbance of physiological balance of host.

Hurst (1927) found that Physa occidentalis Tryon infected with Echinostoma revolutum (Frölich) fed more voraciously than uninfected animals. However, this did not seem to occur with infected L.littorea, observations on animals maintained under laboratory conditions and known to be infected, on average, did not feed more or less than uninfected animals maintained under identical conditions.

The two main species infecting L.littorea at Scalby Rocks, namely C.lingua and Cercaria A, had markedly different effects on the glycogen content of their hosts. The proportionate hepatopancreatic glycogen content of L.littorea infected with the parthenitae of C.lingua was much lower than for L.littorea infected with the sporocysts of Cercaria A (Fig. 22). The glycogen content of hosts harbouring the latter was very

similar to that of uninfected animals. It was found that animals infected with Cercaria A were still capable of spawning, though in smaller numbers than uninfected animals. By comparison, L.littorea infected with C.lingua only rarely showed signs of developing gonads and were either in the spent state or their genitalia were greatly reduced and it was not then possible to assign the host to any reproductive stage.

In uninfected animals there seemed to be an inverse relationship between glycogen content and reproductive condition (Fig. 17). Animals infected with Cercaria A showed a similar pattern whereas L.littorea infected with C.lingua showed a much more flattened glycogen curve, which may be linked with their reproductive condition (Figs. 8 and 22).

In one sense it is surprising that animals infected with C.lingua have a low glycogen content. Healthy animals breed when the level is lowest and one would therefore expect that by not breeding, L.littorea infected with C.lingua would maintain a higher glycogen level. This was not the case however. Their glycogen level was continuously low, much lower than uninfected animals except during the latter's seasonal low. One is thus drawn to the possibility that it is the build up of the glycogen level prior to the breeding season that determines whether the animal will or will not reproduce. The accumulated glycogen reserves may be used up as the gametes develop (Figs. 8 and 22).

During the period of seasonal high in uninfected animals the average

hepatopancreatic glycogen content in L.littorea infected with C.lingua was about 50% lower (Fig. 22). This was the greatest difference in their levels. In contrast, animals infected with Cercaria A have only slightly less glycogen than uninfected L.littorea and can draw upon this during the maturation of the gametes. Linke (1934) also recognised the occurrence of parasitic castration in L.littorea parasitised with trematode larvae. He thought that this was possibly due to a combination of direct consumption, and inadequate feeding of the gonad on the part of the snail as a result of the rediae's demand for food for propagation.

If, then, one accepts that it is the glycogen cycle which influences the reproductive cycle, and that C.lingua by influencing the glycogen levels has an effect on reproduction, how is one to explain the difference observed between animals infected with C.lingua and Cercaria A?

Cercariae of C.lingua develop in rediae which are very mobile and active feeders. On the other hand, Cercaria A develop in sporocysts which are found in localised areas in the visceral mass and show little sign of movement. As mentioned earlier histological examination of L.littorea infected with Cercaria A showed that the area occupied by the parasite was virtually devoid of host tissue, adjacent host tissue showing signs of degeneration and tissue further away looking normal. Histochemical examination showed amounts of glycogen in this

part of the visceral mass comparable to uninfected animals. In contrast L.littorea infected with C.lingua, showed the sporocysts and rediae to be dispersed randomly throughout the visceral mass and the remaining host tissue to have a much more vacuolated appearance than normal animals. There were noticeably fewer glycogen staining areas, due in part at least to the much reduced host tissue.

Larvae acquire their food either by direct ingestion of host tissue by means of the redia's muscular pharynx or by assimilation in the form of smaller molecules, e.g. glucose, via the body wall. The latter method is the only one possible for sporocysts which do not have a gut. Recent electron microscope studies (Bils & Martin 1966; James, Bowers & Richards 1966; Krupa et al. 1966; Ginetsinskja et al. 1966; and Rees 1966 and 1967) have shown the integument of sporocysts, rediae and developing cercariae possess finger-like cytoplasmic projections that increase the surface area for exchange of substances (food, gases etc.). Ginetsinskja et al. (1966) liken the sporocysts' microvilli to the brush border of the intestinal epithelium of higher animals. Cheng (1963b) considered that mechanical damage caused by rediae to the hepatopancreatic cells is more severe than that resulting from lysis of cells by sporocysts. Intracellular debris was seen in the intestinal caecum of rediae of both C.lingua and H.leptosoma and it was noticed that cellular damage was greatest where the largest number of rediae were located. Direct ingestion of

the molluscan host's hepatopancreatic cells have been reported by several authors among them Cheng and James (1960), Cheng (1963)^b and Lie et al (1968). These different methods of acquiring food may partially contribute to the difference in glycogen values found for hosts harbouring parthenitae of C. lingua and Cercaria A.

Ginetzinskja et al (1966) considered that trematodes which have only a sporocyst stage and no redial stage in their life history are examples of a more perfect adaptation to their parasitic way of life. At first sight the different effects of Cercaria A and C. lingua on L. littorea seem to support this theory. Hosts infected with Cercaria A are able to carry on their normal bodily functions as well as support the parasite, whereas hosts infected with C. lingua are incapable of breeding and this indirectly affects the parasite in that it lessens the number of potential first intermediate hosts available for its own species. However, if the relationship shown earlier between percentage infection and size is straightforward, it may be inferred that Cercaria A kills off its host at an earlier "age" - percentage infection decreases in animals larger than 25 mm; whereas hosts infected with C. lingua live longer - percentage infection increases with size (Fig. 4). The latter relationship is obviously of greater advantage to the current redial population of C. lingua.

The rate of asexual multiplication and development of the parasite in the molluscan tissues is also likely to be a contributory factor to

the different effects of the various species on L.littorea. In animals which had been kept under laboratory conditions for several months, and also shielded from the possibility of reinfection, it was frequently the case that hosts infected with Cercaria A carried very light and often immature infections, whereas infections of C.lingua were usually much heavier. One may infer from this that C.lingua multiplies at a greater rate than does Cercaria A. The energy source for asexual reproduction are acquired initially from the nutrients of the host, and hence the faster rate of multiplication of C.lingua could explain the much lower glycogen values found in hosts infected with this species, than in hosts infected with Cercaria A. Coupled with this difference in the nutritional requirements for asexual multiplication is the difference in amount of glycogen stored in the cercarial's body. This is used during its free-living, non-feeding existence. Ginetzinskja (1960) studied the deposition and usage of glycogen in 10 species of freshwater cercariae. He concluded that the amount of stored glycogen was dependant on the longevity and activity of the cercariae, longer living and/or more active cercariae having more glycogen than short lived and/or relatively inactive cercaria (the rate of glycogen was also related to the environmental temperature). The shed cercariae of C.lingua were found to be more active than those of Cercariae A and also had a slightly longer free-living existence.

The requirement of a carbohydrate source of host origin among

intramolluscan trematode larvae appears to be one of absolute necessity. Simões & Coelho (1955) found that the larvae of S.mansoni stopped developing when their host Planorbis glabrata (= Australorbis glabratus) was removed from water, development being resumed on its return. The significance of this work becomes more apparent when considered together with the work of Magalhães & de Almeida (1956) who found the glycogen content in the hepatopancreas of P.glabrata to fall some 50% when kept out of water for 15 days, reaching 10 - 15% of the original just before death in about 75 days. It may be inferred from this that normal development of S.mansoni is correlated with the amount of glycogen in the host's digestive gland. This appears to be further corroborated by the work of Sindermann et al. (1957) who reported cercarial emission of Austrobilharzia variglandis (Miller & Northup) from the marine snail Nassarius obsoletus (Say) was reduced to a three - or four - day rhythm if the snails were starved and that feeding resulted in considerable emergence within 48 hours. Again, Brackett (1940) found that starved snails (Physa sp. and Lymnaea sp.) did not emit schistosome cercariae until they had been supplied with food. It may be assumed from these reports that the decrease of cercarial production in starved snails resulted from a decrease of liver glycogen, and that feeding resulted in a build up of glycogen which then enhanced cercarial production and emergence. In the present work it was

found that the period of greatest emission for C.lingua was during spring and summer, corresponding to the period when the glycogen content of uninfected and parasitised animals was at its lowest. At first sight this seems to contradict the earlier findings referred to above, since one would expect cercarial emergence to occur when L.littorea has a high glycogen content. However, it must be remembered that the low glycogen level in uninfected animals during spring and summer at least partially resulted from gamete production and since hosts infected with C.lingua were incapable of breeding, the glycogen stores of the host might therefore have been channelled for parasitic multiplication. The case of Cercaria A cannot be so explained since the cercariae are shed throughout the year and seemed to be associated less closely with the glycogen cycle. Neither was shedding as restricted by sea temperature as C.lingua, cercaria of which ceased to be shed below about 8°C.

Cheng & Snyder (1962a) using histochemical methods found a gradual reduction of the amount of glycogen in the hepatopancreas of Helisoma trivolvis infected with Glypthemins pennsylvaniensis Cheng, 1961 and simultaneously there was an increase in the amount of glycogen in the bodies of the developing cercariae within sporocysts. They also found that in heavy infections the host glycogen was reduced in cells not in intimate contact with the

sporocysts. Their conclusion was that the host's glycogen had been broken down to glucose and perhaps other simpler sugars which then passed through the hepatopancreatic cell wall, and the sporocyst walls into the brood chamber of the latter. Here it is resynthesised into glycogen and that which is not incorporated in glycogen synthesis is immediately catabolised for the production of energy. They suggested two possibilities to account for the reduction in the amount of host glycogen:

- 1) The parasites secreted a glycolytic enzyme which was absorbed into the host cells and catalyzed glycogen breakdown.
- 2) They secreted a substance which acted on the host's enzyme system stimulating it into increased catabolism of glycogen.

Of these the former did not seem to occur in either infections of C. lingua or Cercaria A. Rediae and sporocysts respectively of these parasites were incubated in a 1% glycogen solution made up in sea water (p.177) and at intervals the presence of glucose in the incubation medium was tested for using Benedict's Reagent. All tests were negative, thus inferring that the secretion of glycolytic enzymes by the parasite was unlikely. Cheng (1963a) performed a similar experiment with the daughter sporocysts of Glypthelmins pennsylvaniensis Cheng, 1961 and Glypthelmins quieta. In the light of his results he did not think the parasites were secreting a glycolytic enzyme. This conclusion appears reasonable since

evidences on membrane permeability have indicated fairly conclusively that large protein molecules such as carbohydrases, could not permeate the cell membrane of the host's hepatopancreatic cells (Cheng 1967). In view of Cheng's and my results it seems likely that the presence of sporocysts and rediae in some ways activates the host's native enzymes to increase the rate of glycogen catabolism.

McDaniel & Dixon (1967) found both the rediae of C. lingua and the hepatopancreas of L. littorea contained relatively large quantities of polysaccharide and glucose. They showed both the rediae and host hepatopancreas to be capable of absorbing exogenous glucose and incorporating considerable amounts of it into polysaccharide; they also found the apparent rates of glucose utilization by parasite and host tissue to be similar.

Utilization of carbohydrates by larval trematodes does not appear to hold true for all species of larval Digenea. Vernberg & Hunter (1963) by utilising the respiratory rate as an indicator of the metabolic rate reported that the metabolic rate of Himasthla quissitensis was not altered when glucose, mannose, ribose or fucose were added to the medium in which the rediae were maintained; however certain amino acids raised the respiratory rate. This may possibly be attributed to the experimental conditions, but if this occurred in vivo, it may help to account for the less drastic

effect of Cercaria A on the glycogen content of L.littorea compared with those of C.lingua, in that Cercaria A does not have a carbohydrate orientated metabolism. Its carbohydrate reserves are built up instead from some other source such as amino acids or lipids. Histochemical observations (using formol-calcium fixed frozen sections) on the lipids present in the sporocysts of Cercaria A and the rediae of C.lingua showed the latter to be virtually devoid of lipids, whereas the sporocysts of Cercaria A had very heavy deposits in their walls, and also in their contained cercariae. The lipids consisted largely of neutral fats - identified by Lillie's oil red O and Cain's Nile blue methods (Pearse 1960). Neutral fats were also present in the surrounding host tissue, being very heavy in the digestive gland and connective tissue bordering the gonadial tubules, but the gonadial tissues themselves did not contain lipids. From the relatively small number of animals examined it was not possible to draw any conclusions regarding the effects of the presence of digenean larvae on the lipid content of their hosts. In the animals examined parasitised L.littorea did not appear to have any more or less lipids than uninfected specimens.

Thus we see that the amount of glycogen stored in the tissues of uninfected or parasitised L.littorea at any one time is the balance resulting from the amount of glycogen formed and that broken down. This is well illustrated by Galtsoff's observations on the

oyster, Crassostrea virginica where the glycogen curve was increasing during the colder months of the year when feeding had ceased. Galtsoff (1964) postulated that the tissue glycogen continues to be resynthesized by the oyster from the carbohydrates accumulated with food during the period of active feeding or from indigenous sources of intermediary metabolism.

VI.

SUMMARY AND CONCLUSIONS

-This work was directed towards an investigation of the occurrence of larval digeneans using Littorina littorea L. as a first intermediate host and their effect on the reproductive biology of a population of L.littorea at Scalby Rocks, North Yorkshire. Their influence on the carbohydrate content of the host from a seasonal quantitative view point was also investigated. There follows a listed summary of the conclusions drawn during the course of the survey.

1. Four species of larval Digenea were recorded infecting L.littorea in the Scarborough area. These were Cryptocotyle lingua (a Heterophyid); Himasthla leptosoma (an Echinostome); Cercaria lebouri (a Notocotylid) and Cercaria A (a Xiphidocercaria) the family relationship of the last named was not determined.
2. The incidence of infection of L.littorea was examined at South Bay, Scarborough; Scalby Rocks; Scalby Pipe; and Burniston Bay. Considerable variation in the incidence of infection was found between the localities, it being virtually negligible at Burniston Bay and South Bay, Scarborough . The almost complete absence of infection was attributed to the unsuitability of these areas as a nest or roost for sea birds (a final host of digeneans). At Scalby Rocks 39.5% were found to be infected during the survey.

Scalby Rocks are almost completely inaccessible to the public, thus making it attractive to the final hosts as a roost and feeding ground.

3. The incidence of infection at Scalby Rocks had a fairly pronounced seasonal cycle which is felt to be related to the behaviour of the final host; age structure of the periwinkle population; behaviour and physiological state of L.littorea; and to the effect of environmental conditions on the pre-molluscan parasite stage.
4. The breeding period of L.littorea at Scalby Rocks extended from the beginning of December 1966 to the beginning of August 1967, with a peak in February-March. The greatest proportion of maturing individuals was found when the sea temperature was approximately at its lowest.
5. L.littorea infected with digenean larvae still showed a cyclical reproductive pattern resembling that of uninfected animals but the proportion of mature and spawning animals was greatly reduced. Hosts infected with C.lingua were generally in the spent condition and seldom showed characteristics of mature animals. This implied that infection had been acquired after they had been through one breeding season. Cercaria A did not have such a drastic effect on the reproductive biology of L.littorea, a considerable proportion of the hosts being capable of breeding. Their infection may have been acquired prior to or during the first breeding season.

6. Parasitisation by larval digeneans did not alter the 1:1 sex ratio found for the uninfected population.
7. The carbohydrate content of the hepatopancreas and foot of uninfected L.littorea had a definite seasonal cycle, being lowest in April and May and highest in September and October. The decline in glycogen content corresponded to the period of decreasing sea temperature and the increase approximately to the rising sea temperature. A relationship with the reproductive state was also found; glycogen levels were highest when the animals were maturing for the first time or in the recovering spent state; and lowest during the breeding season of L.littorea.
8. A seasonal glycogen cycle was also shown by parasitised hosts. The level was consistently lower than that of uninfected animals though the gap closed during the period of seasonal low. C.lingua had a much more deleterious effect on the glycogen content of their hosts than did Cercaria A. In the latter the levels were similar to that of the uninfected L.littorea. Hosts infected with C.lingua had much less glycogen during the period when the glycogen content of uninfected animals was at its highest. These differences are discussed in relation to the reproductive state of uninfected and parasitised animals.
9. The different effects of Cercaria A and C.lingua on their hosts are considered in terms of location and rate of development of the parasites; time of infection; and physiological condition of the host.

The interference of the parasites studied with the carbohydrate and reproductive biology of L.littorea is no doubt caused by a variety of mechanisms. Some of these can be recognised but their quantitative influence is not easily measured without an exhaustive series of laboratory experiments.

VII.

ACKNOWLEDGEMENTS

I would like to thank my Supervisor Dr. I.C.Williams for his guidance and advice.

I thank Professor P.G.'Espinasse and Professor J.G.Phillips for providing the facilities which enabled me to carry out this work.

I am indebted to Mrs. W.Baker for typing this thesis and to the Photographic Department of University College Hospital Medical School, London, for printing the Tables and Figures. Also to the Curator of Wood End Natural History Museum, Scarborough, for the information on the breeding population of sea birds on Castle Cliff.

Finally I am indebted to the University of Hull for providing the Research Studentship which enabled me to carry out this work.

VIII.

REFERENCES

- AMEEL, D.J. (1934). Paragonimus, its life history and distribution in North America and its taxonomy. Am. J. Hyg. 19, 279-317.
- ANSELL, A.D. & LANDER, K.F. (1967). Studies on the hard-shell clam, Venus mercenaria, in British Waters. III. Further observations on the seasonal biochemical cycle and on spawning. J. appl. Ecol. 4, 425-435.
- BALDWIN, E. & BELL, D.J. (1938). Preliminary investigation of galactogen from the albumen gland of Helix pomatia. J. Chem. Soc. 1461-1465.
- BARFORD, B. (1948). The distribution of glycogen in the rabbit liver. Acta physiol. scand. 16, 110-116.
- BARRY, R.J.C. & MUNDAY, K.A. (1959). Carbohydrate levels in Patella. J. mar. biol. Ass. U.K. 38, 81-95.
- BECKER, W. (1964). Der Einfluss Von Trematoden auf den Gasstoffwechsel von Stagnicola palustris Müll. Z. Parasitenk. 25, 77-102.
- BERNARD, C (1850). Sur un nouvelle fonction du foie chez l'homme et les animaux. Compt. rend. acad. sci. 31, 571-574.
- BILS, R.F. & MARTIN, W.E. (1966). Fine structure and development of the trematode tegument. Trans. Am. Microscop. Soc. 85, 78-88.
- BLOCK, R.J. DURRUM, E.L. & ZWEIG, G. (1958). "A manual of paper chromatography and paper electrophoresis." 2nd Ed. Acad. Press Inc. 484p. London . Ch. VI. Carbohydrates.

- BOURNS, T.K.R. (1963). Larval trematodes parasitizing Lymnaea stagnalis appressa Say in Ontario with emphasis on multiple infections. Can. J. Zool. 41, 937-941.
- BOWERS, E.A. (1965). Studies on some parasites of sea birds and molluscs. Ph.D. Thesis, University College, Swansea.
- BRACKETT, S. (1940). Studies on schistosome dermatitis. VI. Notes on the behaviour of schistosome cercariae. Am. J. Hyg. 31, 64-73.
- BRAND, T. von (1931). Der Jahreszyklus in Stoffbestand der Weinbergschnecke (Helix pomatia). Z. vergleich. Physiol. 14, 200-264.
- BRAND, T. von (1952). "Chemical physiology of endoparasitic animals" Acad. Press. N.Y.
- BRAND, T. von. (1960). Recent advances in carbohydrate biochemistry of helminths. Helminthological Abstr. 29, 97-111.
- BRAND, T. von (1966). "Biochemistry of parasites" Acad. Press, London.
- BRAND, T. von & FILES, V.S. (1947). Chemical and histological observations on the influence of Schistosoma mansoni infection on Australorbis glabratus . J. Parasit. 33, 476-482.
- CABLE, R.M. (1956). Scientific survey of Puerto Rico and the Virgin Islands. Marine cercariae of Puerto Rico. N.Y. Acad. Sci. 4 (16): 490-577.
- CABLE, R.M. (1963). Marine Cercariae from Curaçao and Jamaica. Z. Parasitenk. 23, 429-469.

CARROLL, N.V., LONGLEY, R.W. & Roe, J.H. (1956). The determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol.Chem. 220, 583-593.

CASSELMAN, W.G.B. (1962). "Histochemical technique". Methuen & Co. Ltd. London.

CHENG, T.C. (1963a). Biochemical requirements of larval trematodes. Annals N.Y. Acad. Sci. 113, 289-321.

CHENG, T.C. (1963b). The effects of Echinoparyphium larvae on the structure of and glycogen deposition in the hepatopancreas of Helisoma trivolvis and glycogenesis in the parasite larvae. Malacologia 1, 291-303.

CHENG, T.C. (1967). Advances in marine biology 5, Acad. Press. "Marine molluscs as hosts for symbioses"

CHENG, T.C. & BURTON, R.W. (1966). Relationships between Bucephalus sp. and Crassostrea virginica : A histochemical study of some carbohydrates and carbohydrate complexes occurring in the host and parasite. Parasitology 56, 111-112.

CHENG, T.C. & JAMES, H.A. (1960). The histopathology of Crepidostomum sp. infection in the second intermediate host, Sphaerium striatinum. Proc. helminth. Soc. Wash. 27, 67-68.

CHENG, T.C. & SNYDER, R.W. Jr. (1962a). Studies on host-parasite relationships between larval trematodes and their hosts. I. A review. II. The utilization of the host's glycogen by the intra-molluscan larvae of Glypthelmins pennsylvaniensis Cheng, and associated phenomena. Trans. Am. Microscop. Soc. 81, 209-228.

- CHENG, T.C. & SNYDER, R.W.Jr. (1962b). Studies on host-parasite relationships between larval trematodes and their hosts.
III. Certain aspects of lipid metabolism in Helisoma trivolvis (Say) infected with the larvae of Glypthelmins pennsylvaniensis Cheng, and related phenomena. Trans. Am. Microscop. Soc. 81, 327-331.
- CHERNIN, E. (1960). Infections of Australorbis glabratus with Schistosoma mansoni under bacteriologically sterile conditions. Proc. Soc. exp. Biol. Med., 105, 292-296.
- CHRISTENSEN, N.O. & ROTH, H. (1948). Investigations on internal parasites of dogs . Roy. Vet. Agric. Coll. Yearbook Reprint 1, 1-73.
- CORT, W.W., McMULLEN, D.B. & BRACKETT, S. (1937). Ecological studies on the cercariae in Stagnicola emarginata angulata (Sowerby) in the Douglas Lake region Michigan. J.Parasit. 23, 504-532.
- CORT, W.W. (1941). Ecological relations of the larval trematodes of fresh-water snails. In "A Symposium of Hydrobiology". University of Wisconsin Press, Madison, U.S.A. 9.115.
- COUTEAUX-BARGETON, M. (1946). Variations du taux de glycogène dans différents organes de l'huitre, Bull. Soc. Zool. France 71, 121-126.
- DEUEL, H.J.Jr. (1957). "The lipids" Vol. III. 1065 pp. New York: Interscience Publ. Inc.
- DREYWOOD, R. (1946). Qualitative test for carbohydrate material Ind. Eng. Chem . (Anal. Ed.) 18, 499.

ELMHIRST, R. (1923). Notes on the breeding and growth of marine animals in the Clyde Sea area. Rep. Scot. Mar. Biol. Ass. 1922, 19-43.

EMERSON, D.N. (1965). Summer polysaccharide content in seven species of West Coast intertidal Prosobranch snails. Veliger, 8, 62-66.

EMERSON, D.N. (1966). Further studies on the polysaccharides of West Coast Prosobranchs. Amer. Malacol. Union Report 1966, 74-75.

EMERSON, D.N. & DUERR, F.G. (1967). Some physiological effects of starvation in the intertidal Prosobranch Littorina planaxis. (Phillippi, 1847). Comp. Biochem. Physiol. 20, 45-53.

EVANS, R.G. (1947). The intertidal ecology of selected localities in the Plymouth neighbourhood. J. mar. biol. Ass. U.K. 27, 173-218.

EWERS, W.H. (1960). Multiple infections of trematodes in a snail. Nature, Lond. 186, 990.

FALES, F.W., RUSSELL, J.A. & FAIN, J.N. (1961). Some applications and limitations of the Enzymic, Reducing (Somogyi), and Anthrone Methods for estimating sugars. Clinical Chem. 7, 389-403.

FAUST, E.C. (1920). Pathological changes in the gastropod liver produced by fluke infections. Johns Hopkins Hosp. Bull. 31, 79-84.

FRAGA, F. (1958). Variación estacional de la composición química del mejillón (Mytilus edulis). II. Hidratos de carbono. Inv. Pesq. 11, 33-37.

FRETTER, V. & GRAHAM, A. (1962). "British Prosobranch Molluscs."
Ray Society, London.

GALSTOFF, P.S. (1964). The American Oyster, Crassostrea virginica
Gmelin. U.S. Fish Wildlife Serv., Fishery Bull. 64, 480pp.

GIESE, A.C. (1959). Comparative physiology : Annual reproductive
cycles of marine invertebrates. Ann. Rev. Physiol. 21, 547-576.

GIESE, A.C. & ARAKI, G. (1962). Chemical changes with reproductive
activity of the chitons, Katherina tunicata and Mopalia hindsii.
J. exp. Zool. 151, 259-267.

GIESE, A.C., HART, M.A., SMITH, A.M. & CHEUNG, M.A. (1967). Seasonal
changes in body component indices and chemical composition in
the Pismo clam Tivela stultorum . Comp. Biochem. Physiol. 22,
549-561.

GINETSINSKJA, T.A. (1960). Glycogen in the body of the cercariae
and dependence of its distribution on specific characters of
the parasite (In Russian). Dokl. Akad. Nauk. SSSR 135, 1012-
1015.

GINETSINSKJA, T.A., MASHAUSKII, V.F. & DOBROVOLSKII, A.A. (1966).
The ultrastructure of the outer membranes and the method of
feeding of rediae and sporocysts (In Russian). Dokl. Akad.
Nauk. SSSR 166, 1003-1004.

GODDARD, C.K. & MARTIN, A.W. (1966). Carbohydrate Metabolism, Ch.9,
in "Mollusca" Vol.II. Eds. Wilbur, K.M. & Yonge, C.M. Acad.
Press.

- GOOD, C.A., KRAMER, H. & SOMOGYI, M. (1933). The determination of glycogen. J. biol. Chem. 100, 485-491.
- GOWANLOCH, J.N. & HAYES, F.R. (1926). Contributions to the study of marine gastropods. I. The physical factors, behaviour and intertidal life of Littorina. Cont. Canad. Biol. Fish. N.S. 3, 133-165.
- GRAINGER, J.N.R. & SHILLITOE, A.J. (1952). Histochemical observations on galactogen. Stain Techn. 27, 81-86.
- GREENFIELD, L.J. (1953). Observations on the nitrogen and glycogen content of Teredo (Lyrodus) pedicellata De Quatrefages at Miami, Florida. Bull. Mar. Sci. Gulf. Carib. 2, 486-496.
- HAYES, F.R. (1929). Contribution to the study of marine gastropods. III. Development, growth and behaviour of Littorina. Contr. Can. Biol. Fish. N.S. 4, 413-430.
- HEYNEMAN, D. & UMATHEVY, T. (1968). Interaction of trematodes by predation within natural double infections in the host snail Indoplanorbis exustus. Nature, Lond. 217, 283-285.
- HOLLIMAN, R.B. (1961). Larval trematodes from Apalachee Bay Area, Florida, with a check list of known marine cercariae arranged in a key to their Superfamilies. Tulane studies in Zoology, 9, 2-74.
- HOLM, L.W. (1946). Histological and functional studies on the genital tract of Lymnaea stagnalis apressa Say. Trans. Am. Microscopio. Soc. 65, 45-68.

HORSTMANN, H.J. (1956). Der Galaktogengehalt der Eier von Lymnaea stagnalis Während der Embryonalentwicklung. Biochem. Zeit. 328, 342-347.

HORSTMANN, H.J. (1959). Untersuchungen über Polysaccharide aus den Eiern einiger mariner Mollusken. Pubbl. Staz. Napoli 31, 308-319.

HURST, C.T. (1927). Structural and functional changes produced in the gastropod mollusk, Physa occidentalis, in the case of parasitism by the larvae of Echinostoma revolutum. Univ. Calif. Publs. Zool. 29, 321-405.

JAMES, B.L. (1964). "Studies on larval trematodes from some littoral molluscs." Ph.D. Thesis, University College, Aberystwyth.

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.

JAMES, B.L. (1968a). Studies on the life-cycle of Microphallus pygmaeus (Levinsen, 1881) (Trematoda: Microphallidae). J. nat. Hist., 2, 155-172.

JAMES, B.L. (1968b). The occurrence of larval Digenea in ten species of intertidal prosobranch molluscs in Cardigan Bay. J. nat. Hist. 2, 329-343.

JAMES, B.L. & BOWERS, E.A. (1967). The effects of parasitism by the daughter sporocysts of Cercaria bucephalopsis haimaena Lacaze-Duthiers, 1854, on the digestive tubules of the cockle, Cardium edule L. Parasitology 57, 67-77.

- JAMES, B.L., BOWERS, E.A. & RICHARDS, J.G. (1966). The ultrastructure of the daughter sporocysts of Cercaria bucephalopsis haimaena Lacaze-Duthiers, 1854 (Digenea : Bucephalidae) from the edible cockle, Cardium edule L. Parasitology 56, 753-762.
- KATKANSKY, S.C. & SPARKS, A.K. (1966). Seasonal sexual pattern in the Pacific oyster Crassostrea gigas in Washington State, Fisheries Research Papers Wash. Dept. Fisheries 2, 80-89.
- KEMP, A. & KITS VAN HEIJNUNGEN, A.J.M. (1954). A colorimetric micro-method for the determination of glycogen in tissues. Biochem J. 56, 646-648.
- KENDALL, S.B. (1963). Some factors influencing the development and behaviour of trematodes in their molluscan hosts. p 51-73 in "Second Symposium of the British Society for Parasitology- Host-parasite relationships in invertebrate hosts." Ed. A.E.R.Taylor. Blackwell Scientific Publications, Oxford.
- KOEHLER, L.H. (1952). Differentiation of Carbohydrates by Anthrone Reaction Rate and Color Intensity. Anal. Chem. 24, 1576-1579.
- KRULL, H. (1935). Anatomische Untersuchungen an einheimischen Prosobranchiern und Beitrage zur Phylogenie der Gastropoden. Zool. Jb. (Anat. Ont.) 60, 399-464.
- KRUPA, P.L., BAL, A.K. & COUSINEAU, G.H. (1967). Ultrastructure of the rediae of Cryptocotyle lingua. J.Parasitol. 53, 725-734.
- LAMBERT, T.C. & FARLEY, J. (1968). The effect of parasitism by the trematode Cryptocotyle lingua (Creplin) on zonation and winter migration of the common periwinkle Littorina littorea L.

Can. J. Zool. 46, 1139-1147.

LA RUE, G.R. (1957). The classification of digenetic Trematoda: A review and a new system. Exp. Parasitol. 6, 306-349.

LEBOUR, M.V. (1907). Larval trematodes of the Northumberland Coast. Trans. Nat. Hist. Soc. Northumberland & Durham, n.s. 1, 437-454.

LEBOUR, M.V. (1908). Trematodes of the Northumberland Coast, No.II. Trans. Nat. Hist. Soc. Northumberland & Durham, n.s. 3, 28-45.

LEBOUR, M.V. (1911). A review of the British Marine Cercariae. Parasitology 4, 416-456.

LEBOUR, M.V. (1914). Some larval trematodes from Millport. Parasitology 7, 1-11.

LEBOUR, M.V. (1937). The eggs and larvae of the British Prosobranchs with special reference to those living in the plankton. J. mar. biol. Ass. U.K. 22, 105-166.

LEBOUR, M.V. (1947). Notes on the inshore plankton of Plymouth. J. mar. biol. Ass. U.K. 26, 527-547.

LIE, K.J., BASCH, P.F., HEYNEMAN, D., BECK, A.J. & AUDY, J.R. (1968) Implications for trematode control of interspecific larval antagonism within snail hosts. Trans. R. Soc. trop. Med. Hyg. 62, 299-319.

LINKE, O. (1933) Morphologie und Physiologie des Genitalapparatus der Nordseelittorinen. Wiss. Meeresuntersuch N.F. Abt. Helgoland 19, Nr. 5, 1-60.

- LINKE, O. (1934). Über die Beziehungen zwischen Keimdrüse und Soma bei Prosobranchiern. Zool. Anz. Suppl. 7, 164-175.
- LLOYD, P.F. & LLOYD, K.O. (1963). Sulphatases and sulphated polysaccharides in the viscera of marine molluscs. NATURE, Lond. 199, 287.
- 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.
- LYSAGHT, A.M. (1943). The incidence of larval trematodes in males and females of the gastropod Littorina neritoides (L.) on the Plymouth Breakwater. Parasitology 35, 17-22.
- MAGALHÃES B, & de ALMEIDA, A.M. (1956). Influência da dessecação sobre o teor de glicogênio dos tecidos em Australorbis glabratus. Publicações avuls. Inst. Aggen Magalhães 5, 1-5.
- MARTIN, A.W. (1961). The carbohydrate metabolism of the Mollusca. In "Comparative physiology of carbohydrate metabolism in heterothermic animals". (A.W.Martin, ed.) Univ. Washington Press, Seattle, Washington.
- MAXWELL LITTLE, J. (1962). "An introduction to the experimental method". Burgess Publishing Co.
- MAY, F. (1934a). Chemische und biologische Untersuchungen über Galaktogen (Chemischer Teil.) Z.Biol. 95, 277-297.
- MAY, F. (1934b). Chemische und biologische Untersuchungen über Galaktogen. (Biologischer Teil: Der Jahreszyklus im Galaktogen- und Glykogenbestand der Weinbergschnecke). Z. Biol. 95, 401-430.
- MAY, F. (1934c). Chemische und biologische Untersuchungen über

- Galaktogen. (Biologischer Teil: Galaktogen- und Glykogenehalt bei hungernden Weinberschnecken.) Z. Biol. 95, 606-613.
- MAY, F. (1934d). Chemische und biologische Untersuchungen uber Galaktogen. (Biologischer Teil: Galaktogennachweis bei verschiedenen Tierklassen und beim Menschen.) Z. Biol. 95, 614-634.
- McDANIEL, J.S. & 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, P. BRAND, T.von. & NOLAN, M.O. (1957). Observations on the polysaccharides of aquatic snails. J. cell comp. Physiol. 50, 219-240.
- MEENAKSHI, V.R. (1954). Galactogen in some common South Indian Gastropods with special reference to Pila. Current Science (India) 23, 301-302.
- MILLER, H.M. Jr. (1925a). A preliminary report on the larval trematodes infesting certain molluscs from Dry Tortugas . Yb. Carnegie Instn. Wash. 24, 232-238.
- MILLER, H.M. Jr. (1925b). Larval trematodes of certain marine gastropods from Puget Sound, Washington. Publs. Puget Sound mar. biol. Stn. 5, 75-89.
- MILLER, H.M. Jr. & NORTHUP, F.E. (1926). The seasonal infestation of Nassa obsoleta (Say) with larval trematodes. Biol. Bull.

mar. biol. Lab. Woods Hole 50, 490-508.

MILROY, J.A. (1907). Seasonal variations in the quantity of glycogen present in samples of oysters. Fisheries Ireland, Sci. Invest. 1907, 4, (1909) 3-12.

MITCHELL, P.H. (1915). Nutrition of oysters : glycogen formation and storage. Bull. U.S. Bureau Fisheries, 35, 153-161.

MOKRASCH, L.C. (1954). Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. J. Biol. Chem. 208, 55-59.

MOORE, H.B. (1937). The biology of Littorina littorea. Part I. Growth of the shell and tissues, spawning, length of life and mortality. J. mar. biol. Ass. U.K. 21, 721-742.

MOORE, H.B. (1940). The biology of Littorina littorea, Part II. Zonation in relation to other gastropods on stony and muddy shores. J. mar. biol. Ass. U.K. 24, 227-237.

MORRIS, D.L. (1948). Quantitative determination of carbohydrates by Dreywood's Anthrone Reagent. Science 107, 254-255.

NEGUS, M.R.S. (1968). The nutrition of sporocysts of the trematode Cercaria doricha Rothschild, 1935 in the molluscan host Turitella communis Risso . Parasitology 58, 355-366.

NEWELL, G.E. (1958). The behaviour of Littorina littorea (L.) under natural conditions and its relation to position on the shore. J. mar. biol. Ass. U.K. 37, 229-239.

NORTH, W.J. (1954). Size distribution , erosive activities, and gross

metabolic efficiency of the marine intertidal snails, Littorina planaxis and Littorina scutulata. Biol. Bull. mar. biol. Lab. Woods Hole 106, 185-197.

OKAZAKI, K. & KOBAYASHI, S. (1929). The seasonal variation in the glycogen content in the oyster, Ostrea circumpicta Fils. Sci. Repts. Tohoku Univ. Ser.D. 4, 183-191.

ORTON, J.H., SOUTHWARD, A.J. & DODD, J.M. (1956). Studies on the biology of limpets. II. The breeding of Patella vulgata L. in Britain. J. mar. biol. Ass. U.K. 35, 149-176.

PALOMBI, A. (1934). Gli stadi larvali dei Trematodi del Golfo di napoli. 1°. Contributo allo studio della morfologia, biologia e sistematica delle cercarie marine. Publicazioni Della Stazione Zoologica di Napoli 14, 51-93.

PALOMBI, A. (1938). Gli stadi larvali dei trematodi del Golfo di Napoli. 2°. Contributo allo studio della morfologia, biologia e sistematica delle cercariae marine: (il gruppo delle cercariae cotilocerche). Riv. Parassitol. 2, 189-206.

PALOMBI, A. (1940). Gli stadi larvali dei trematodi del Golfo di napoli. 3°. Contributo allo studio della morfologia, biologia e sistematica delle cercarie marine. Riv. Parassitol. 4, 7-30.

PAN, C. (1963). Some biochemical and immunological aspects of host-parasite relationships : generalized and focal tissue responses in the snail, Australorbis glabratus, infected with Schistosoma

- mansonii. Ann. N.Y. Acad. Sci. 113, 475-485.
- PATIL, B.A. & JOSHI, G.V. (1967). Seasonal variations in chemical composition of Ulva lactuca and sea water. Indian J. exp. Biol. 5, 236-238.
- PEARSE, A.G.E. (1960). "Histochemistry, theoretical and applied" 998 pp. 2nd. ed. (J.A.Churchill Ltd. London).
- PELSENEER, P. (1926). La proportion relative des sexes chez les Animaux et particulièrement chez les Mollusques. Mém. Acad. Roy. Sci. Belg. cl. Sci. 8, No. 11, 1-258.
- PFLÜGER, E. (1905). Das Glykogen und Seine Beziehungen Zur Zuckerkrankheit, Bonn, 2nd edition (1905) p. 53.
- READ, C.P. (1961). The carbohydrate metabolism of worms, In: "Comparative physiology of carbohydrate metabolism in heterothermic animals". p. 3-34. (A.W.Martin, ed.) Univ. Washington Press, Seattle, Washington.
- REES, G. (1966). Light and electron microscope studies of the rediae 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 cercariae. Parasitology 57, 87-110.
- REES, W.J. (1936). The effect of parasitism by larval trematodes on the tissues of Littorina littorea (Linné). Proc. Zool. Soc. Lond. 357-368.

- ROE, J.H. (1955). The determination of sugar in blood and spinal fluid with Anthrone Reagent. J. Biol. Chem. 212, 335-343.
- ROTHSCHILD, M. (1935a). Notes on the excretory system of Cercaria ephemera Lebour, 1907 (nec. Nitzsch). Parasitology 27, 171-174.
- ROTHSCHILD, M. (1935b). The trematode parasites of Turritella communis Lmk. from Plymouth and Naples . Parasitology, 27, 152-170.
- ROTHSCHILD, M. (1936a). A preliminary note on the trematode parasites of Peringia Ulvae (Pennant) 1777. Novit. zool. 39, 268-269.
- ROTHSCHILD, M. (1936b). 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. (1937). Note on the excretory system of the trematode genus Maritrema Nicoll, 1907, and the systematic position of the Microphallinae Ward, 1901. Ann. Mag. Nat. Hist. 10th Series, 19, 355-365.
- ROTHSCHILD, M. (1938a). Notes on the classification of cercariae of the Superfamily Notocotyloidea (Trematoda) with special reference to the excretory system. Novit. zool. 41, 75-83.
- ROTHSCHILD, M. (1938b). Further observations on the effect of trematode parasites on Peringia ulvae (Pennant) 1777. Novit. zool 41, 84-102.

- ROTHSCHILD, M. (1938c). The excretory system of Cercaria coronanda n.sp. together with notes on its life-history and the classification of cercariae of the Superfamily Opisthorchioidea Vogel 1934, (Trematoda). Novit.zool. 41, 148-163.
- ROTHSCHILD, M. 1941a). Observations on the growth and trematode infections of Peringia ulvae (Pennant) 1777 in a pool on 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). A seven year old infection of Cryptocotyle lingua Creplin in the wrinkle Littorina littorea L. J.Parasitol. 28, 350.
- ROTHSCHILD, A. & ROTHSCCHILD, M. (1939). Some observations on the growth of Peringia ulvae (Pennant) 1777 in the laboratory. Novit. zool. 41, 240-247.
- RUSSELL, E.S. (1923). Report on the seasonal variation in the chemical composition of oysters. Fishery Investigations London, Series II, 6. No.1, 2-24.
- SAIFER, A., GERSTENFELD, S. (1958). The photometric micro determination of blood glucose with glucose oxidase, J. Lab. clin. Med. 51, 448-460.
- SASTRY, A.N. (1968). The relationships among food, temperature and gonad development of the bay scallops Aequipecten irradians Lamark. Physiol. Zool. 41, 44-53.

- SATTLER , L. & ZERBAN, F.W. (1948). The Dreywood anthrone reagent as affected by carbohydrate structure. Science 108, 207.
- SEIFTER, S., DAYTON, S., NOVIC, B. & MUNTWYLER, E. (1950). The estimation of glycogen with the anthrone reagent. Archs. Biochem. 25, 191-200.
- SEWELL, R.B.S. (1922). Cercaria indicae. Indian J. Med. Research 10, (suppl) 1-370.
- SIMÕEL, B.F. & COELHO, M.V. (1955). Comportamento dos formas larvárias de Schistosoma mansoni em Australorbis glabratus (Mollusca : Planorbidae), sujeito a'estivação. Publicacoes avuls.Inst. Aggen Magalhães 4, 51-60.
- SIMPSON, G.G. ROE, A. & LEWONTIN, R.C. (1960). " Quantitative Zoology " 440pp. Harcourt, Brace & Company Inc, New York.
- SINDERMAN, C.J. (1964). Larval ecology of the trematode Cryptocotyle lingua. In: Proceedings of the first International Congress of Parasitology (1964) printed 1966 A1, 12-13.
- SINDERMAN, C.J. & FARRIN, A.E. (1962). Ecological studies of Cryptocotyle lingua (Trematoda: Heterophyidae) whose larvae cause " pigment spots" of marine fish. Ecology 43, 69-75.
- SINDERMAN, C.A. , A.ROSENFELD & STROM, L. (1957). The ecology of marine dermatitis-producing schistosomes. II. effects of certain environmental factors on emergence of cercariae of Austrobilharzia variglandis. J. Parasitol. 43, 382

- SINITZIN, D.F. (1911). Parthenogenetic generations of trematodes and its progeny in molluscs of the Black Sea (English translation of Russian text by A.M.Bagusin under the direction of H.B.Ward, presented to the British Museum, Natural History, by M.Rothschild). Mem. Acad. Imp. St. Petersb. (Cl. Phys;-Maths) 8. Ser. 30, 127 pp.
- SMITH, J.E. & NEWELL, G.E. (1955). The dynamics of the zonation of the common periwinkle (Littorina littorea (L.)) on a stony beach. J. Animal Ecology 24, 35-56.
- SMYTH, J.D. (1962). "Introduction to animal parasitology" The English Universities Press Ltd. London
- SMYTH, J.D. (1966). "The physiology of trematodes " University reviews in biology. Oliver & Boyd, Edinburgh and London.
- SNEDECOR, G.W. (1962). "Statistical methods ". Iowa State Univ. Press, 5th Ed. 1956, reprinted 1962.
- SOGANDARES-BERNAL, F. (1965). Studies on American Paragonimiasis I. Age immunity of the snail host. J.Parasitol. 51, 958-960.
- SOLÈRE, M., PROM, T. & HUGUET, R. (1965). Variations des glucides de l'hépatopancreas et du manteau de Mytilus galloprovincialis Lmk. non parasité et parasité par Mytilicola intestinalis Steuer au cours du cycle évolutif. Trav. Soc.Pharm. Montpellier 25, fasc.1. 24-27.
- SOMOGYI, M. (1952). Notes on sugar determination . J. Biol.Chem. 19-23.

- SOUTHWARD, A.J. (1958). Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution . J.mar. biol. Ass.U.K. 37, 49-66.
- STANDEN, O.D. (1963). In " Experimental chemotherapy" Vol.I. p.701-892. Ed. R.J.Schitzer & F.Hawkin. Acad. Press, New York and London.
- STIREWALT, M.A. (1954). Effect of snail maintenance temperatures on development of Schistosoma mansoni . Exp. Parasit. 3, 504.
- STUNKARD, H.W. (1930). The life history of Cryptocotyle lingua (Creplin) with notes on the physiology of the metacercariae. J. Morph. Physiol. 50, 143-191 .
- STUNKARD, H.W. (1932). Some larval trematodes from the coast in the region of Roscoff, Finestère. Parasitology 24, 321-343.
- STUNKARD, H.W. (1938). The morphology and life cycle of the trematode Himasthla quissetensis (Miller & Northup, 1926). Biol. Bull. mar. biol. Lab. Woods Hole. 75, 145-164.
- STUNKARD, H.W. (1950). Further observations on Cercaria parvicaudata Stunkard & Shaw, 1931. Biol. Bull. mar. biol. Lab. Woods Hole 99, 136-142.
- STUNKARD, H.W. (1957). The morphology and life history of the digenetic trematode Microphallus similis (Jagerskiöld, 1900) Baer, 1943. Biol. Bull. mar. biol. Lab. Woods Hole 112, 254-266.
- STUNKARD, H.W. (1964). Studies on the trematode genus Renicola: observations on the life-history, specificity and systematic

- position. Biol. Bull. mar. biol. Lab. Woods Hole, 126, 467-489.
- STUNKARD, H.W. (1966). The morphology and life history of the digenetic trematode, Himasthla littorinae sp. n. (Echinostomatidae). J. Parasitol. 52, 367-372.
- STUNKARD, H.W. (1968). The asexual generations, life-cycle and systematic relations of Microphallus limuli Stunkard, 1951 (Trematoda: Digenea). Biol. Bull. mar. biol. Lab. Woods Hole 134, 332-343.
- STUNKARD, H.W. & SHAW, C.R. (1931). The effect of the dilution of sea water on the activity and longevity of certain marine cercariae with descriptions of two new species. Biol. Bull. mar. biol. Lab. Woods Hole 41, 242-271.
- STURROCK, B.M. (1966). The influence of infection with Schistosoma mansoni on the growth rate and reproduction of Biomphalaria pfeifferi. Ann. trop. Med. Parasitol. 60, 187-197.
- TATTERSALL, W.M. (1920). Notes on the breeding habits and life history of the periwinkle. Fisheries, Ireland, Sci. Invest. 1920, I. 1-11.
- THAMDRUP, H.M. (1935). Beitrage zur Ökologie der Wattenfauna. Medd. Komm. Havundersøg., Kbh. Ser. Fiskeri 10, no.2 1-125.
- THORSON, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). Medd. Komm. Havundersøg. (Ser. Plankton), 4 no.1 523pp.

- THRELFALL, W. (1965). The helminth parasites of the herring gull (Larus argentatus Pontopp.) on the Newborough Warren Nature Reserve, Anglesey. Ph.D. thesis, University of Wales.
- THRELFALL, W. (1967). Studies on the helminth parasites of the herring gull, Larus argentatus Pontopp., in Northern Caernarvonshire and Anglesey. Parasitology 57, 431-453.
- TREVELYAN, W.E. & J.S.HARRISON (1952). Studies on the yeast metabolism
1. Fractionation and microdetermination of carbohydrates.
Biochem. J. 50, 298-303.
- TUCKER, J.S. & A.C.GIESE, (1962). Reproductive cycle of Cryptochiton stelleri (Middendorff). J.exp. Zool. 150, 33-43.
- VAN HANDEL, E. (1965). Estimation of glycogen in small amounts of tissue. Analyt. Biochem. 11 256-265.
- VERNBERG, W.A. & HUNTER, W.S. (1963). Utilization of certain substrates by larval and adult stages of Himasthla quissetensis. Exp. Parasit. 14, 311-315.
- WESENBERG - LUND, C.(1931). Contributions to the development of the Trematoda Digenea. Part I. The biology of Leucochloridium paradoxum. K. dansk. Vidensk. Selsk., 4, No.3. 1-142.
- WESENBERG - LUND, C. (1934). Contributions to the development of the Trematoda Digenea Part II. The biology of the fresh-water cercariae in Danish fresh water. D.Kgl. Dansk. Vidensk. Selsk. Skrifter. Naturw. Math. Afd. Raekke 9 (5), 1-223.

- WILLEY, C.H. & GROSS, P.R. (1957). Pigmentation in the foot of Littorina littorea as a means of recognition of infection with trematode larvae. J.Parasitol. 43. 324-327.
- WILLIAMS, E.E. (1964). The growth and distribution of Littorina littorea (L.) on a rocky shore in Wales. J.Anim. Ecology 33, 413-432.
- WRIGHT, C.A. (1966). The pathogenesis of helminths in the Mollusca Helminthological Abstracts 35, 207-224.
- YONGE, C.M. (1949). "The Sea Shore" Collins, London.
- ZIPF, R.E., WALDO, A.L. & DAYTON, M.S. (1952). Spectrophotometric analysis of carbohydrates and study of Anthrone Reagent. J.Lab. & Clin. Med. 39, 497-502.
- ZISCHKE, J.A. & ZISCHKE, D.P. (1965). The effect of Echinostoma revolutum larval infection on the growth and reproduction of the snail host Stagnicola palustris. Am. Zool. 5, 707(Abstract).

APPENDIX TABLE A

TEMPERATURE OF SEA , AIR , AND COLLECTING POOL RECORDED DURING
MONTHLY VISITS TO SCALBY ROCKS.

<u>DATE</u>	<u>TEMPERATURE °C</u>		
	<u>Sea</u>	<u>Air</u>	<u>Pool</u>
28.9.66	13.5	14.1	14.0
2.11.66	7.5	8.0	9.0
1.12.66	6.5	6.0	6.5
12.1.67	4.9	7.2	5.5
11.2.67	5.9	6.5	5.5
14.3.67	5.5	4.0	5.5
10.4.67	6.0	5.5	6.1
9.5.67	10.0	14.0	12.0
6.6.67	13.9	16.0	15.0
10.7.67	15.9	24.9	17.8
7.8.67	17.8	16.4	18.5
4.9.67	15.9	16.2	14.9
2.10.67	12.9	10.9	12.6