STUDIES ON THE BIOLOGY OF STONEFLIES

by

S. G. KHOO

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CONTENTS

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I. GENERAL INTRODUCTION

The periodic occurrence of adult stoneflies in temperate and northern latitudes is a well-known phenomenon. Studies on the ecology and general life-histories of this group of insects have been made by various authors (e.g. Hynes 1941, 1958, 1961, 1962; Brinck 1949; Illies 1952; Rauser 1962, 1963). The effect of environmental conditions such as temperature on the growth of many species has also been discussed by these authors. Brinck (1949) mentioned that most stoneflies in southern Scandinavia pass the summer in stages (egg and nymphulae) which are resistant to high temperatures and a small amount of oxygen. Hynes (1961, 1962) showed that in many species, the period during which very small specimens can be found is much longer than the flight period. He suggested the possibility of a delayed hatching of some eggs as has been observed in the Ephemeroptera by Macan (1957) and later confirmed by Illies (1959) from laboratory studies on the hatching of the eggs of Baetis spp. A corollary to the above phenomenon is the occurrence of a short flight period in species which show a prolonged hatching period as well as in some of those which do not. Various hypotheses as to the causes of short emergence periods have been put forward by authors dealing with different groups of aquatic insects (e.g. Ephemeroptera: Ide 1935, Beskot 1953, Macan 1958; Plecoptera: Hynes 1962; Odonata: Corbet 1954, 1956). Corbet has shown from

experimental studies that the changing photoperiod is involved in the seasonal regulation of the dragonfly, Anax imperator, but as regards the Plecoptera (and the Ephemeroptera) no studies have previously been made to investigate the factors influencing seasonal periodicity and the various phenomena associated with it.

The absence of such investigations is due largely to the insufficient information that can be derived from field data regarding the early stages of the life-cycles of these insects. The length of adult-life and the egg-laying habits of stoneflies are still largely unknown and the period during which egg-laying occurs in nature cannot be determined just from the presence of adults at certain times of the year. Again very little is known about the biology of the eggs or of the early stages of stoneflies and it is therefore difficult to say whether the occurrence of small nymphs for a greater part of the year is due to delayed hatching of some eggs or to delayed growth of the nymphs. Delayed hatching of some eggs or delayed growth of young nymphs could be due to the immediate effects of unfavourable environmental conditions resulting in a general retardation of growth and development or to the ability of the species conderned to undergo diapause as distinct from quiescence. A detailed analysis of field data is therefore dependent upon a direct study of these aspects of stonefly biology. It is also from such studies that some of the factors influencing the seasonal periodicity of stoneflies and the various phenomena correlated with it could be usefully ascertained. It was on this premise that the present laboratory and field investigations were carried out. During the course of this research it was observed, for the first time, that several

species of stoneflies showed the phenomenon of diapause and preliminary studies were carried out to investigate the factors influencing diapause in some of the species concerned.

II. LABORATORY INVESTIGATIONS

1. Introduction

Previous studies on the biology of stoneflies have been based mainly on field investigations and very little work has been done in the laboratory. There have been a few random records on the incubation periods of the eggs of several species but the attempts at rearing the newly-hatched nymphs in the laboratory have not been very successful. The difficulties which the early workers found in rearing of the eggs and the newly-hatched nymphs under laboratory conditions have resulted in a big gap in our knowledge regarding the early stages of stoneflies. Much work, however, has been done in rearing full-grown nymphs to maturity in order to correlate the immature stages with the adult insects. The important studies by Hynes (1941, 1958, 1963a, b) and Brinck (1949) have contributed immensely towards the taxonomy of the nymphs of stoneflies occurring in Britain. Hynes (1941, 1942) and Brinck (1949) studied in detail the feeding habits of stonefly nymphs and adults and the results of these investigations now provide sufficient information for the rearing of stoneflies in the laboratory. The present laboratory studies are divided into 3 sections, namely, the adults, the eggs and the nymphs.

2. Materials and Methods

For the laboratory investigations,'a cold room' (approximately 6 x 6 x 7 feet) in which the temperature and light periods could be regulated to simulate the conditions in the stream was used.

The temperature was adjusted about once a fortnight according to the average of the stream temperatures measured during each period at the two stations in River Terrig where many field studies were carried out. The source of light was an ordinary 100 W lamp connected to a time-switch and the photoperiod was adjusted once a week to correspond to that experienced in Manchester at that period..

The studies on the biology of adult stoneflies, particularly the length of adult-life and the egg-laying habits, were carried out by keeping them in the manner that has been described by Hynes (1942). The adults were obtained by collecting fairly mature nymphs from various localities in North Wales and then rearing these nymphs in enamel pie-dishes in the cold room. Whenever possible, the adults that were collected from the field were also kept for study. Pairs of adults were kept and allowed to mate in 1 x 2 in. tubes, the bottoms of which were covered by wire guaze. Into each of these tubes were inserted a blob of cotton wool soaked with water and a twig covered with green algae. Larger tubes were used for the larger species. The adults were kept in the cold room but they were brought out daily for a few hours into room temperature and then returned. The adults mated, fed (in the case of the Filipalpia) and drank very well under these conditions. The tubes were regularly examined and whenever necessary the adults were tranferred to new tubes with fresh twigs and cotton wool. Very often it was necessary to keep the females in separate tubes after mating in order to avoid the constant

harrassing by the males which may occur even when the females are in the process of egg-laying. Egg-laying usually occurred soon after bringing the adults out into room temperature. Adults that were observed to extrude eggs were allowed to oviposit by transferring them into new tubes containing a piece of twig and then inverting these tubes onto petri-dishes containing stream water. Although most of the species successfully extruded and deposited their eggs under these conditions it was found necessary to induce egg-extrusion by some species, such as Nemoura cinerea, Leuctra nigra, Nemurella picteti and Capnia bifrons by first inverting the tubes containing these adults over a petri-dish of water. Those females that did not lay any eggs after some time were returned to their previous upright position and the procedure was repeated daily until they laid their eggs. Besides the study on the length of adult-life, observations were also made on the mating behaviour. Altogether, a total of more than one thousand adults belonging to 25 species was studied.

The emergence of adults occurred quite satisfactorily from nymphs kept in still water in enamel pie-dishes in the cold room. These dishes were covered with a glass plate to prevent the adults from escaping. Stream water collected from River Terrig was used for the rearing of these nymphs and stones were left in the dishes to allow mature nymphs to crawl out of the water for emergence. The newly-emerged adults were removed daily from these dishes. The Filipalpian nymphs were fed with decaying leaves collected from the stream or with alder or sycamore leaves.

that had been soaked in stream water. The Setipalpian nymphs were fed mainly on mayfly nymphs.

The eggs that were laid in petri-dishes were kept in the cold room as well as at room temperature. It was necessary to change the water regularly to avoid a layer of scum that tends to form over the surface after some time. Eggs attacked by fungus were removed to prevent the hyphae from extending to other developing eggs. The development of the eggs was followed by regularly examining the petri-dishes under a monocular microscope. Camera Lucida drawings were made of the various stages of development in the eggs of several species.

The rearing of the nymphs through their complete life-cycles in the laboratory was done by keeping the newly-hatched nymphs in petri-dishes or enamel pie-dishes containing stream water, some fine detritus and pieces of decaying sycamore or alder leaves. The young Setipalpian nymphs were fed with tiny oligochaetes that were fairly abundant among the decaying leaves collected from the stream. In rearing Capnia bifrons, it was found useful to "inoculate" the dishes containing stream water with some fine detritus and to leave them standing for one or two days before introducing newly-hatched nymphs. This was to let the detritus settle firmly on to the bottom of the dish so as to provide a hold for the nymphs. Otherwise, there is a strong tendency for the newly-hatched nymphs of C. bifrons (unlike other species of stonefly) to float to the surface of the water.

In following through the number of instars of stoneflies,

about 10 to 15 newly-hatched nymphs of each species were kept individually in small vessels (e.g. 1 x ½ in. polythene caps of tubes) so that it was possible to find the tiny exuviae. When the nymphs were fairly large they were transferred to petri-dishes and ultimately to enamel pie-dishes when they were ready for emergence. The nymphs were examined once every few days for their exuviae and in this way the number of moults and the rate of moulting were determined. The exuviae collected were then mounted in polyvinyl lactophenol to which was added lignin pink to stain the chitin. The increase in size between instars was determined by the measurements on the length of the hind tibia and of the whole exuviae,

3. The Biology of the Adults

(a) Introduction

The biology of adult stoneflies has been described in varying detail by several authors. Schoenemund (1924) and Kuhtreiber (1934) have written a great deal about their behaviour. Hynes (1941, 1942) studied in detail the feeding habits and brachypterous condition of some species at high altitudes. Brinck (1949, 1955) has given very detailed accounts of their biology, especially their mating habits, and he has also discussed the phenomenon of wing-polymorphism. Very little, however, is known about the length of adult-life and the egg-laying habits. Although emphasis has been placed on these two topics mention will

also be made of various other aspects of the biology of adult-stoneflies on which observations have been made.

(b) Emergence

The emergence of stoneflies has been described by many authors who have dealt with the ecology of these insects. Hynes (1941) mentions that "small species seem to emerge at any time of the day or night, but it seems that the Perlodidae and species of the genus Perla emerge only at night". Brinck (1949) states that the Setipalpia will always emerge in the morning or by night while the hiemal Filipalpia with an early flight period usuallyemerge in the morning and those with a late flight period emerge at any time of day. In the latter, he mentions that emergence occurs in humid surroundings. Humidity is considered by Brinck to be an important factor in determining at what time of day emergence occurs since "the chitinous skin becomes hard and brittle when drying which will check emergence". From the present observations in the field it appears that the smaller species usually emerge in the day, particularly in the morning when the sun is shining, while the larger Setipalpia (e.g. Perlodes microcephala) which migrate some distance from the water's edge seem to emerge in the night or early morning. In the laboratory, under conditions in the cold room, emergence of the various species studied seemed to occur at any time of the day or night. Here conditions were always humid since the nymphs were kept in

dishes that were covered by a glass plate. The apparent lack of a concentrated emergence to any particular time of the day seems, however, to be due to the absence of an appropriate stimulus in the cold room. In nature, the warming of the water by the sun's radiation probably acts as a stimulus causing the fully mature nymphs of the smaller species to emerge during the day. In the laboratory, this stimulus could be produced by bringing the dishes out of the "cold room" and placing them for a short period, with the glass cover removed, underneath a lamp at room temperature. When mature nymphs of C. bifrons with fully or partially black wing pads were subjected to this stimulus it was observed that many of them crawled out of the water and emerged successfully after the chitinous skins had been dried. Several nymphs which were probably not ready for emergence returned to the water after some time. Thus, it appears that the stimulus for a concentrated emergence of mature nymphs is the warming up of the water during the day and that low humidity is not a limiting factor in the emergence of C. bifrons. This probably applies to the other smaller species as well. In any case, emergence in nature often occurs on the under-surfaces of stones that protrude above the water level and here the humidity must certainly be quite high. However, humidity and other factors such as air temperature may be important in determining the time of emergence in the larger Setipalpia which migrate a considerable distance from the

water's edge.

(c) Size of adults in relation to flight period and wing-polymorphism

It was observed that in any one locality the adults which emerged during the early part of the flight period tended to be larger in size than those which emerged later. During the course of this study, nymphs of C. bifrons were collected from River Terrig just before the beginning of the flight period and were kept in the cold room. The emergence of adults was then recorded. From laboratory as well as from field investigations it was found that the emergence of this species occurred from February to May. The peak emergence period lies, however, during March and April as can be seen from the figures in Table 1, which shows the number of adults which emerged in the cold room during the first and second half of each month of 1962 and 1963. The mean size of adults in relation to the flight period, based on a study of 145 adults which emerged in 1963, is also shown in the same table. From the results of this study it can be seen that there is a marked decrease in the size of the adults towards the end of the emergence period.

From laboratory studies on the nymphs (Chapter II, 5) it has been found that the number of instars in C. bifrons (and in other species) varies. Thus, it is possible that these small adults might have emerged from nymphs that had not reached the complement of instars necessary to produce

	February		March		April		May	
	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	
Male	6.0	6.0	6.5	6.1	5.5	5.3	4.7	Mean size in mm.
	4	19	60	48	30	19	4	Adults emerged : '62 and '63
Female	9.0	9.0	8.8	8.3	7.8	7.3	7.1	Mean size in mm.
	3	8	39	50	24	23	15	Adults emerged : '62 and '63

Table 1.

an average-sized adult such as that found during the early part of the emergence period. If this is so, then there must be some environmental factors acting on the half-grown nymphs towards the end of this period and which induce the differentiation of adult characters. The environmental factors likely to influence growth and development are temperature and photoperiod. During April and May there are (i) a rapid rise in stream temperature and (ii) an increasingly long photoperiod. Of these two factors, there is reason to believe that it is the increasingly long photoperiod which might be involved since, in 1963, the temperatures in the cold room did not rise above 10°C till the end of May. Also, during the study on diapause in this species (Chapter IV) it was found that although the newly hatched nymphs were sensitive to both temperature and photoperiod, the sensitivity to temperature decreased in the later instars. It was possible to induce emergence of normal-sized adults as early as September by subjecting some non-diapause 4th and 5th

instar nymphs during June to summer temperatures of 14.5°C but under a changing photoperiod corresponding to that from October to February.

The effect of long photoperiod could also be seen in an experiment in which some rather small nymphs, collected from River Terrig on January 1963, were kept under conditions of a constant photoperiod of 16½ hours of light per day and under temperatures varying daily from 4 to 12°C. It was found that these nymphs moulted only 2 or 3 times in a period of 1½ to 2 months after which adult characters began to differentiate. Although these nymphs did not emerge successfully the adult characters were clearly visible. It is also worth pointing out that in these nymphs the wing pads of the females were only partially developed and had they emerged successfully the adults would have been short-winged even though the nymphs were collected from a locality where the females were long-winged.

Thus, besides the influence of environmental factors on the differentiation of adult characters in half-grown nymphs it is possible that brachyptery in C. bifrons (and probably also in other wing-polymorphic species of stonefly) may also be a result of such factors. Therefore, if, as a result of low temperature, late breaking of diapause or competition for food, the nymphs of C. bifrons are unable to grow rapidly enough to reach the complement of instars necessary for an early emergence, changing environmental factors such as an

increasingly long photoperiod might be able to induce the differentiation of adult characters in half-grown nymphs. In extreme cases of earlier instars this might possibly result in the emergence of short-winged adults. However, a word of caution must be added to the above hypothesis as the size of the nymphs need not always be associated with the stage of development. There may be other factors involved as well and it has been observed that nymphs destined for diapause were smaller than non-diapause nymphs of the same instar.

(d) Mating habits

The females of most of the species studied appeared to be fairly receptive soon after emergence but the males of several species seemed to have a maturation period of a few days before attempting to mate. If, however, the female should be attacked by males too soon after crawling out of the nymphal skin, she avoids mating by curling the tip of her abdomen upwards and by attempting to shake off the males. Mating in the females does not appear to be dependent upon the maturation of the eggs.

The maturation period of the males is fairly short in some species such as Capnia bifrons, Leuctra inermis, Taeniopteryx nebulosa and Perlodes microcephala where mating occurred on the same day as emergence. In C. bifrons mating was observed to occur about 45 minutes after the emergence of the adults of both sexes.

In Leuctra hippopus, Amphinemura sulcicollis, Amphinemura standfussi, Nemurella pictetti, Protonemura praecox and Chloroperla torrentium mating was not observed till 1 to 3 days after the emergence of the males.

The maturation period seemed to be longer in species such as Nemoura avicularis, Nemoura cambrica, Nemoura cinerea, Nemoura erratica, Diura bicaudata and Isoperla grammatica where mating was observed only about 4 to 7 days after emergence of the males.

In Isogenus nubecula the males never attempted to mate till 10 to 13 days after emergence.

In many species, mating was observed to last from a few minutes to several hours, but in some species such as N. erratica and N. pictetti the adults were commonly seen to be in a copulatory position for as long as 7 to 8 days, and during this period the males were not observed to feed at all.

Various instances of abnormal mating behaviour have been described among stoneflies. Hynes (1941) mentions that he once saw a male C. bifrons attempting to mate with a female Leuctra nigra which had been in contact with a ready female of C. bifrons and he concludes that "the males seem to be attracted only to females ready to copulate, and this attraction seems to be due to some substance extruded by the female". Brinck (1949) states that a male C. bifrons always tries to mate when finding a female even after she has been dead for several days but he mentions that he never saw a male attacking females

of other species. During the course of the present study, observations have been made of the males of C. bifrons attempting to mate with each other. The same behaviour was also observed in D. bicaudata. Two males of this species which were kept together soon after their emergence were observed to attempt mating with each other four days later. Observations were also made of a male P. microcephala making several unsuccessful attempts to mate with a female I. nubecula. However, such abnormal behaviour does not occur if the males are allowed to mate with the females of their own species soon after their maturation period or if they have not been kept separate for too long a period after a previous mating.

The drumming of the male abdomen against the substratum as described by Brinck (1949) was also observed in several of the species studied (e.g. T. nebulosa, C. bifrons, Leuctra geniculata, P. microcephala). In the laboratory, drumming was not observed if the males were allowed to mate fairly soon after emergence, but drumming was often seen when females were introduced into tubes containing males that had been kept separate for several days after emergence. In spite of the drumming, the females never seemed to respond or to be attracted to the males. Brinck (1949) observed the same pattern of behaviour but he suggests that the females may be stimulated by it preceding mating. It seems more likely, however, that this drumming of male abdomen and the various abnormal mating habits described earlier are just an unusual

reaction of excited males that had been prevented from mating for some time.

(e) Feeding habits

There had been previously contradictory accounts regarding the feeding of adult stoneflies (Schoenemund 1924; Kühtreiber 1934; Newcomer 1918; Wu 1923; Frison 1929, 1935; Neeracher 1910) and it was not until 1941 that Hynes showed that the Setipalpia only drink water but do not feed as adults whereas the Filipalpia are able to drink water as well as to feed on green algae and lichens growing on twigs and tree-trunks. This study has been confirmed by Brinck (1949).

Hynes (1942) also showed that feeding in the adults is an essential part of the life history of N. cinerea and he suggests that this would appear to apply to the whole of the Filipalpia. Brinck (1949) in his studies on Swedish stone-flies was thus faced with the question as to whether the winter stoneflies such as T. nebulosa and Capnia atra, which belong to the Filipalpia, do feed as adults since these "emerge in immense numbers from large rivers and lakes when it is still winter and no food is found in the immediate vicinity". He dissected the mature nymphs and young adults of these species but found that the eggs were small and undeveloped. He concluded that the females cannot produce eggs immediately and that a maturation period is necessary. However, he mentions that the adults females of T. nebulosa must mature rapidly since almost all the adults that had fed were found

to contain well-developed eggs. During the course of this study it was found that the adults of T. nebulosa laid viable eggs within 24 hours of emergence (much earlier than any other stoneflies, including the Setipalpia). Two mature female nymphs of this species were brought back from the field to the laboratory on the evening of 15th March, 1963 and by the next morning the adults were found to have emerged. These adults were allowed to feed and mate and by the same afternoon (1.10 p.m.) both were observed to lay viable eggs. Although the adults had been feeding a few hours earlier, it seems unlikely that this short feeding period could have affected the maturation of the eggs, but it is possible that the maturation of some eggs could have occurred in the last nymphal instar. If this is so, then the laying of the first batch of eggs might not necessarily be dependent upon the feeding of the adults and as such would be of adaptive significance in stoneflies which emerge early in the year.

(f) Length of adult-life

It is a common statement that adult stoneflies live only for a few days but there has been little study of this aspect of adult-life. The only worthwhile information on the length of adult-life has been that by Hynes (1942) in which he recorded a maximun length of adult-life of 43 days for a male and 50 days for a female N. cinerea under laboratory conditions.

The data on the maximum length of adult-life of 25 species of stoneflies reared under conditions in the cold room are shown in Table 2. For N. cinerea and P. microcephala the results were based, unfortunately, on very few specimens and it was not surprising that the maximum length of adult-life recorded for the former species is shorter than that given by Hynes (1942). The data for Leuctra moselyi and Dinocras cephalotes were based only on adults collected from the field while the rest of the species were based on adults collected from the field as well as on those which emerged in the laboratory.

From Table 2, it can be seen that adult stoneflies can, in fact, live for a fairly long period of time. Even among the Setipalpia which do not feed as adults, the maximum length of adult-life recorded for several species (D. bicaudata, I. grammatica, P. microcephala and I. nubecula) was about a month. One female I. nubecula lived for as long as 40 days. Most of the Flilpalpian species showed a maximum length of adult-life of about a month or more. Adult females of L. hippopus, A. sulcicollis, N. cambrica, P. praecox and B. risi showed a maximum length of adult-life of about 2 months while one female of C. bifrons lived for 83 days. However, the usual length of adult-life for C. bifrons was about 2 months.

The length of adult-life may be influenced by environmental factors such as temperature. Some adults of C. bifrons

	Source of Material	No. of Adults	Max. length of Adult-life in days Male	Female	Interval (days) between Emergence and Egg-laying	Egg-batches	Max. no. of egg-batches	Max. total No. of eggs
CAPNIIDDAE Klapalek								
Capnia bifrons (Newman)	a	349	65	83	29 - 80	-	1	713
LEUCTRIDAE Klapalek								
Leuctra fusca (Linne)	a	52	38	38	12 - 14	1 - 5	4	673
L. geniculata (Stephens)	g	26	33	32	9 - 16	3 - 5	4	2089
L. hippopus (Kempney)	a	64	49	63	11 - 18	4 - 12	4	928
L. inermis Kempney	b, d	29	19	32	12 - 19	3 - 8	3	620
L. moselyi Morton	b	16	20	20	?	1 - 7	6	1294
* L. nigra (Olivier)	á	15	42	28	18	-	1	125
NEMOURIDAE								
Amphinemura sulcicollis Ris	a	30	62	60	14	2 - 9	4	1208
A. standfussi (Stephens)	a, b	27	45	41	9 - 10	5 - 7	4	830
* Nemoura avicularis (Morton)	a	28	53	51	33	-	1	160
N. cambrica (Stephens)	a	39	44	55	17	3 - 11	4	1357
N. cinerea (Retzius)	b, h	5	32	37	21 - 34	5 - 20	3	1443
N. erratica Claasen	a	19	37	47	-	-	-	-
Nemurella pictetii Klapalek	b	41	22	25	5 - 14	3 - 14	2	1079
Protonemura praecox (Morton)	a	40	53	57	12 - 13	1 - 11	6	> 2000

P. P. meyeri (Pictet)	a	15	26	33	5 - 9	1 - 6	9	>2000
TAENIOPTERYGIDAE Klapalek								
Brachyptera risi (Morton)	a, d	54	31	67	?	8 - 11	4	1466
Taeniopteryx nebulosa (Linne)	f	39	24	30	1	1 - 7	4	1811
CHLOROPERLIDAE Okamoto								
Chloroperla torrentium (Pictet)	a	58	18	21	7 - 15	1 - 9	4	56
* C. tripunctata (Scopoli)	b, d	25	12	14	?	-	1	56
PERLODIDAE Klapalek								
Diura bicaudata (Linne)	b, c	53	26	28	4 - 14	2 - 7	5	938
Isogenus nubecula Newman	e	15	23	40	17 - 39	-	1	250
Isoperla grammatica (Poda)	a	21	27	37	5 - 17	2 - 8	5	339
Perlodes microcephala (Pictet)	e	4	28	20	4 - 6	6	2	375
PERLIDAE McLachlan								
Dinocras cephalotes (Curtis)	d	17	17	9	?	1 - 3	5	2069

* - Egg-laying occurred in only one female.

Source of material: (a) - River Terrig (b) - Afon Hirnant

(c) - Lake Bala (d) - Horseshoe Pass

(e) - Bangor-is-y-coed (SJ/388454) (f) - Llangollen

(g) - Logger-heads (SJ/199626) (h) - Nant y Ffrith (SJ/255534)

Table 2

which emerged during February and early March were kept throughout at room temperature and the maximum length of adult-life recorded was 30 days for a female which mated and laid fully-developed eggs within that period. Adults emerging during the same period but which were kept in the cold room had a life span about twice as long. Under conditions in the cold room it was found that the adults emerging later in the flight period tend to have a shorter period of adult-life than those which emerge earlier. This was also observed in L. hipropus which, like C. bifrons, has a fairly long emergence period, from February to May. This decreasing length of adult-life towards the later part of the emergence period is partly due to the increasing temperature and partly to the type of adults since many of the adults which emerged late in the flight period seemed to die before egg-laying.

(g) Egg-laying habits

Various authors have described the manner in which the eggs are carried and later deposited in the water. McLachlan (1864) states that L. geniculata carries its egg-mass "from the upcurved last segment to the base of the posterior wings, all along the dorsal surface of the abdomen". Percival and Whitehead (1928) and Brinck (1949) mention that the tip of abdomen carrying the eggs curls upwards so that the egg-mass appears apical or dorsal in position but it never

extends along the dorsal surface of abdomen. During the present study it was found that the first egg-batch laid by L. geniculata is often unusually large (well over 800 eggs) as compared with other Leuctra spp. and the egg-mass tends to be elongated rather than spherical in shape. As such, McLachlan's statement is not without foundation.

Although in most species the manner in which the eggs are carried is fairly similar to the general pattern that has been previously described, in Brachyptera risi there is an interesting variation. In this species, the tip of abdomen is never curled upwards during egg-laying but instead the abdomen is arched so that when the eggs are being laid they accumulate on the concave ventral surface.

Hynes (1941) and Brinck (1949) have described the manner in which the different species of stoneflies deposit their eggs into the water. Although in most species the eggs first accumulate as a mass before being deposited yet in N. picteti the females were often observed to crawl into the water to extrude their eggs. The same behaviour was also seen occasionally in C. bifrons but the adults of this species mostly extrude their eggs while above the water and later deposit them in a single mass.

Of 25 species of adult stoneflies that were reared in the laboratory only one (N. erratica) did not succeed in laying eggs. It was found that the adults of most species that were collected from the field extruded eggs successfully

when they were kept in tubes containing a blob of wet cotton wool and a piece of twig covered with green algae. However, none of the adults of C. tripunctata, B. risi and N. erratica which emerged in the laboratory succeeded in laying eggs under these conditions. In B. risi and N. erratica it was found that these adults had mated and fed very well and their abdomen were fully distended with eggs when they died. The adults of L. nigra and N. avicularis which emerged in in the laboratory also had difficulty in laying eggs under the same conditions. Of the 14 females of N. avicularis that were reared, only one extruded a batch of 160 eggs after the thirty-third day of her life. Dissection of several females which died before egg-laying showed 400-500 well-developed eggs within the oviducts. Hynes (1942) had a similar difficulty in getting the adults of N. cinerea to lay eggs under laboratory conditions. It was late in this study(after all the N. erratica and N. avicularis had died) when it occurred to me that it might be possible to induce the gravid females to extrude eggs by inverting the tubes containing the adults over a dish of water as had been done for C. bifrons. Then it was found that all 4 females of N. cinerea which were treated in this manner extruded eggs without any difficulty. One female L. nigra was also subjected to this treatment and she managed to extrude a batch of eggs before dying. It appears, therefore, that the females of several species of stoneflies which had

been reared throughout the whole of their adult-life in captivity, need some kind of stimulus for egg-extrusion whereas those that were collected from the field did not seem to require such stimulus. In the case of N. cinerea, L. nigra and probably also N. erratica and N. avicularis this stimulus might be the presence of a body of water in the vicinity. In B. risi there was no difficulty in getting adults collected from the field to lay eggs but all attempts to get adults that emerged in the laboratory to extrude eggs met with no success.

In the laboratory, it was observed that egg-laying occurred mainly during the day particularly when the adults were brought out of the cold room into room temperature. The females of C. bifrons are ovoviviparous and the eggs are normally laid only when the embryos are fully-developed. If, however, the females were placed in direct sunshine or near a lamp at room temperature, they occassionally laid eggs in which the embryos were still undeveloped or only partially so. These eggs never survived, but eggs containing fully developed embryos have been dissected from females that had died before oviposition and have hatched successfully on coming into contact with water. Virgin females of A. standfussi and N. picteti have also been observed to lay eggs nine and fourteen days respectively after emergence. This is in contrast to the statement by Miller (1939) that virgin females of Pteronarcys proteus

do not lay eggs.

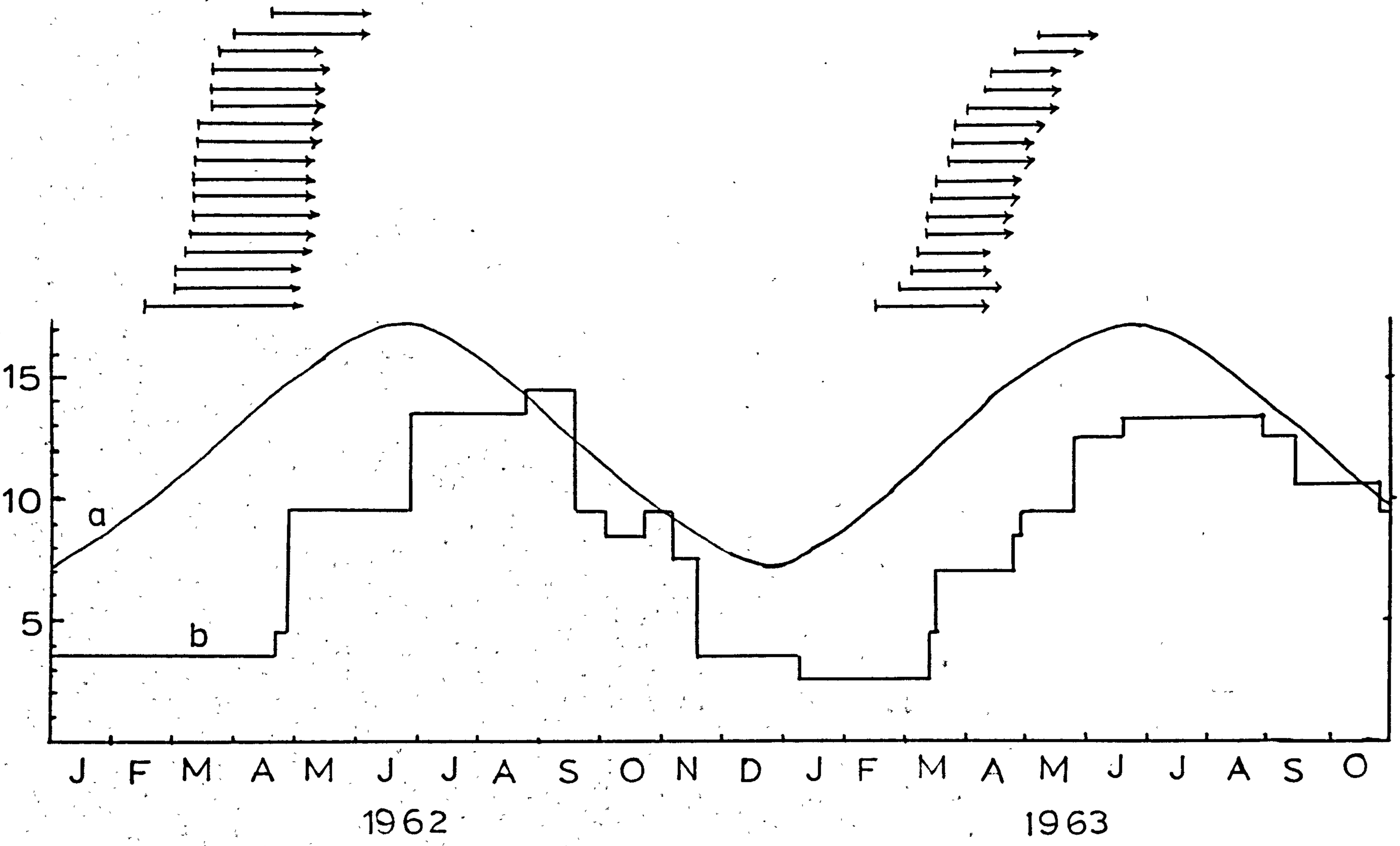
Fertilisation in most species seems to occur when the eggs are being extruded as mature eggs dissected from the oviducts of mated females of B. risi, N. erratica and N. avicularis never developed when kept in water. That these adults had successfully mated was shown by the presence of sperms in the spermathecae. In C. bifrons, however, fertilisation must occur while the eggs are still in the oviducts since this species is ovoviviparous. There is no spermatheca present in the C. bifrons and this has also been found to be true of C. atra by Brinck (1955).

The data on various aspects of the egg-laying habits, such as the interval between emergence and the laying of the first batch of eggs, the interval between egg-batches, the maximum number of egg-batches and the maximum total number of eggs recorded for each species are shown in Table 2. The data for L. moselyi, D. cephalotes, B. risi and C. tripunctata were based only on adults collected from the field. In the 2 last species it was found that none of the females that emerged in the laboratory laid any eggs.

From the results obtained, it can be seen that most of the species laid several batches of eggs. In L. nigra, N. avicularis and C. tripunctata only one female of each species succeeded in laying eggs and therefore the single egg-batch recorded may be unusual. In C. bifrons, however,

the females certainly lay only a single batch of eggs and they die soon afterwards. That the female of this species should oviposit only once in her short life-time is not surprising since the fertilised eggs require some time to develop into complete embryos within the adult. In I. nubecula only a single batch of eggs was obtained from each of the 3 females that oviposited.

The maximum number of eggs recorded for C. bifrons was 713 but the usual size of an egg-batch was around 300-500 eggs. There is a tendency for the smaller-sized adults to lay fewer eggs. The interval between emergence and egg-laying (i.e. laying of fully-developed eggs) varied from 29 to 80 days - this being influenced by temperature. One female which emerged in 12th March 1962 and which was kept at room temperature was found to lay fully-developed eggs after 23 days. Those which emerged during the same period but were kept in the cold room showed an interval of about 2 months before egg-laying. The influence of temperature on the interval between emergence and egg-laying is shown in figure 1 . Those which emerged early tended to show a longer interval between emergence and egg-laying than those which emerged late in the year. In 1963, this interval was generally shorter than in 1962. This was because the temperatures in the cold room were higher during March and April of 1963 than for the same period in 1962. Also, in 1963 the adults were intentionally left for longer



a = Photoperiod in hours/day b = Temp. in °C

⟼ Interval between emergence & egg-laying

Fig. 1

periods at room temperature.

L. geniculata laid the largest number of eggs among the various Leuctra spp. The first egg-batch was quite large and there was a decline in the size of later batches, e.g. 881, 493, 300 and 415 eggs. The females began to lay eggs about 9 to 16 days after emergence and the interval between egg-batches varied from 3 to 5 days. The maximum length of the egg-laying period was about 10 days.

In L. fusca, the maximum total number of eggs recorded was 673 and the size of consecutive egg-batches was quite irregular, e.g. 230, 141, 79 and 223 eggs. Egg-laying occurred about 12 to 14 days after emergence.

In L. hippopus, egg-laying occurred about 11 to 15 days after emergence and continued for 2 to 2½ weeks. Although the maximum number of egg-batches was four, yet the maximum total number of eggs recorded was from a female which laid only 3 batches, viz. 450, 290 and 188 eggs on 2 25.4.62, 3.5.62 and 15.5.62 respectively.

Egg-laying in L. inermis occurred about 12 to 19 days after emergence and most of the females laid only 1 or 2 batches of eggs. One female, however, laid 3 egg-batches, viz. 437, 117 and 65 eggs on 18.6.62, 21.6.62 and 25.6.62 respectively.

Most of the females of L. mosdyi collected from the field laid several batches of eggs and the size of 6 consecutive batches laid by a female from 13.5.62 to

4.9.62 was 130, 337, 50, 387, 180 and 310 eggs. The size of the successive egg-batches was thus quite variable. The interval between batches ranged from 1 to 7 days but the usual period was 4 to 5 days.

Only a single adult of L. nigra successfully laid a batch of 125 eggs after 18 days.

The usual size of an egg-batch in A. sulcicollis was around 200 - 350 eggs and the average interval between egg-batches was 3 to 4 days. An example of the size of the consecutive egg-batches from one female was 345, 345, 300 and 218 eggs. There was, however, no definite correlation between the number and the size of consecutive egg-batches and in 2 individuals the first egg-batch was found to be smaller than the 4th batch of eggs.

In A. standfussi the size of egg-batches was around 150 - 250 eggs and the example of the successive egg-batches recorded from one female was 211, 243, 117 and 204 eggs. Egg-laying occurred about 9 to 10 days after emergence and the interval between egg-batches was 5 to 7 days.

Only a single female of N. avicularis laid eggs, on the 33rd day of her life. The size of the egg-batch was 160 eggs. It is likely, however, that more than one batch is normally laid. One female which died without ovipositing on the 33rd day was dissected and 400 to 500 mature eggs were found.

The interval between emergence and egg-laying in N.

cambrica was 17 days. The interval between egg-batches varied from 3 to 11 days but the usual period was 3 to 4 days. The size of the first egg-batch was often more than 500 eggs and there was a fall in the size of the later batches, e.g. 706, 222, 106 and 66 eggs laid on 15.5.62, 22.5.62, 25.5.62 and 29.5.62 respectively.

Egg-laying in N. cinerea occurred about 21 to 34 days after emergence. The size of the first egg-batch was around 900 to 1000 eggs but the size of successive batches fell very sharply, e.g. 908, 352 and 183 eggs laid on 13.7.62, 3.8.62 and 7.8.62 respectively.

In N. picteti the usual interval between emergence and egg-laying was around 7 to 9 days and the usual period between egg-batches was 7 to 12 days. In several females the size of the first and second egg-batches was about 200 to 300 eggs but in some other individuals the size of the first egg-batch was 600 to 700 eggs and the second was 400 to 500 eggs.

P. praecox laid a very large number of eggs and the maximum length of egg-laying period was one month. The usual size of an egg-batch was between 400 - 600 eggs and there was no significant fall in the size of consecutive batches. Egg-laying occurred about 12 to 13 days after emergence and the usual period between egg-batches was around 5 to 8 days.

P. meyeri laid an equally large number of eggs as did

P. praecox. The maximum length of the egg-laying period was about 3½ weeks. The size of an egg-batch was usually between 300 - 500 eggs. The females begain to lay eggs about 5 to 9 days after emergence and the usual period between egg-batches was 2 to 4 days.

None of the adults of B. Fisi which emerged in the laboratory laid any eggs. However, the maximum number of egg-batches recorded from adults collected from the field was 4. The size of the consecutive egg-batches recorded from one female collected on 1.5.62 was 369, 274, 283 and 540 eggs laid on 18.5.62, 29.5.62, 6.6.62 and 17.6.62 respectively. The egg-laying period was fairly long and there was no definite decrease in the size of successive batches.

The females of T. nebulosa began to lay eggs within 24 hours of emergence and, as has been discussed earlier, it is possible that the maturation of the first batch of eggs may occur in the last nymphal instar. The size of the first egg-batch was very large (between 960 to 1000 eggs) but the size of the consecutive batches was around 200 - 350 eggs, e.g. 945, 212, 355 and 299 eggs laid on 16.3.63, 20.3.63, 25.3.63 and 2.4.63 respectively.

Egg-laying in C. torrentium occurred about 7 to 15 days after emergence. The adults usually laid 2 batches of eggs. The size of the first egg-batch was commonly between 30 to 40 while the second batch was below 20.

The maximum total number of eggs recorded was from a female which laid 2 egg-batches, viz. 41 and 15 eggs on 12.6.62 and 18.6.62 respectively. In one female which oviposited 4 times, the size of consecutive batches was as follows: 28, 15, 4 and 5 eggs laid on 28.6.6.2, 29.6.62, 1.7.62 and 3.7.62 respectively.

Only a single batch of 56 eggs was obtained from a female C. tripunctata collected from the field.

Egg-laying in D. bicaudata occurred around 4 to 14 days after emergence and the length of the egg-laying period was about 1½ to 2 weeks. There was a definite tendency towards a decrease in the size of successive egg-batches, e.g. (a) 292, 259, 167, 133, 87 eggs, (b) 302, 123, 145, 56 eggs, and (c) 247, 152, 106, 45 eggs.

Only a single batch of eggs was recorded from each of the three females of I. nubecula that oviposited. Egg-laying occurred so long after emergence that the females were observed to be very weak after oviposition and they died soon afterwards.

The length of the egg-laying period in I. grammatica was about 2 to 2½ weeks and the interval between emergence and egg-laying varied from 5 to 17 days. There was a tendency towards a fall in the size of successive egg-batches, e.g. 140, 134, 31, 38 eggs laid on 30.5.62, 4.6.62, 7.6.62 and 14.6.62 respectively.

Egg-laying in P. microcephala occurred 4 to 6 days

after emergence and only a single female laid 2 batches of eggs, viz. 320, 155 on 1.5.63 and 7.5.63 respectively.

D. cephalotes laid a large number of eggs and the size of the consecutive egg-batches of one female collected from the field was as follows: 1485, 163, 174, 119 and 128 eggs laid between 17.6.62 and 23.6.62.

From the results of this study, it can be seen that in the Setipalpia as a whole there is a definite tendency towards a decrease in the size of consecutive egg-batches. In this suborder, the eggs are usually fully-developed when the females emerge and no feeding occurs during the adult-stage. In the Filipalpia, however, the eggs are usually still immature at the time of emergence of the females and maturation of the eggs occurs essentially during adult-life. Thus, the size of the egg-batches is very much dependent upon the length of the feeding period during the adult-stage. In T. nebulosa it was found that the first egg-batch was considerably larger than successive batches even though the interval between emergence and oviposition was much shorter than the interval between egg-batches. In this species, however, it is possible that the maturation of some of theeggs occurs in the last nymphal instar.

4. The Biology of the Eggs

(a) Introduction

The eggs of many species of stoneflies have been figured or described by various authors (e.g. Samal 1923, Smith 1923, Wu 1923, Percival and Whitehead 1928, Kuhtreiber 1934, Helson 1935, Miller 1939, Hynes 1941 and Brinck 1949). Miller (1939) studied the detailed embryology of Pteronarcys proteus (Newman) and reported the incubation periods under varying conditions of temperature. There have been only scanty record of the incubation periods of British species by Percival and Whitehead (1928), Hynes (1941) and Brinck (1949). The possibility of a delayed hatching of some eggs as was suggested by Hynes (1961, 1962) is not shown in any of the previous records on the incubation of stonefly eggs. However, the occurrence of a long incubation period has been observed in P. proteus by Miller. He found that the development of the embryo was complete in about 5½ months, but that under natural conditions the naiad remained dormant over winter, and hatched about 10 months after oviposition. The fatal effect of several hours' desiccation of the eggs of this species has also been mentioned by Miller. Hynes (1958), however, when studying the effect of 2 months' drought on the fauna of a small mountain stream, found that those species of stoneflies which were present in the egg-stage were able to survive the dry period.

During the present investigations on the hatching of

the eggs, observations were also made on the main stages of embryonic development, but no attempt was made to study the detailed embryology of the various species. The chief purpose of these observations was to enable correlation of the main stages of embryonic development with the data on incubation and hatching periods. The effects of partial and complete desiccation on the eggs were also studied.

(b) Morphology of eggs and ecological significance of morphological structures.

Figure 2 shows the eggs of the various species that were studied. The drawings were made with the aid of a camera lucida soon after the eggs were laid. Only one species of Leuctra, L. moselyi is shown in the figure since the eggs of the other species of this genus were quite similar in shape and size. In general, the present observations agree with the descriptions given by Percival and Whitehead (1928), Hynes (1941) and Brinck (1949). There are, however, a few differences from the previous descriptions of some species and also some interesting features which have not been previously mentioned.

The eggs of stoneflies are usually surrounded by a gelatinous covering which absorbs water and expands fairly rapidly. In C. bifrons, however, this covering is very characteristically absent. Hynes (1941) mentioned the presence of a sticky membrane surrounding the egg of Capnia nigra which, he stated in 1958, was a misidentification of

Figure 2 - Eggs of:-

A - Leuctra moselyi

B - Amphinemura standfussi

C - Amphinemura sulcicollis

D - Protonemura praecox

E - Protonemura meyeri

F - Capnia bifrons

G - Nemoura avicularis

H - Nemoura cambrica

I - Nemurella picteti

J - Nemoura cinerea

K - Taeniopteryx nebulosa

L - Chloroperla torrentium

M_1 & M_2 - Chloroperla tripunctata

N - Diura bicaudata

O - Isogen's nubecula

P - Dinocras cephalotes

Q - Perlodes microcephala

R - Isoperla grammatica

S - Brachyptera risi

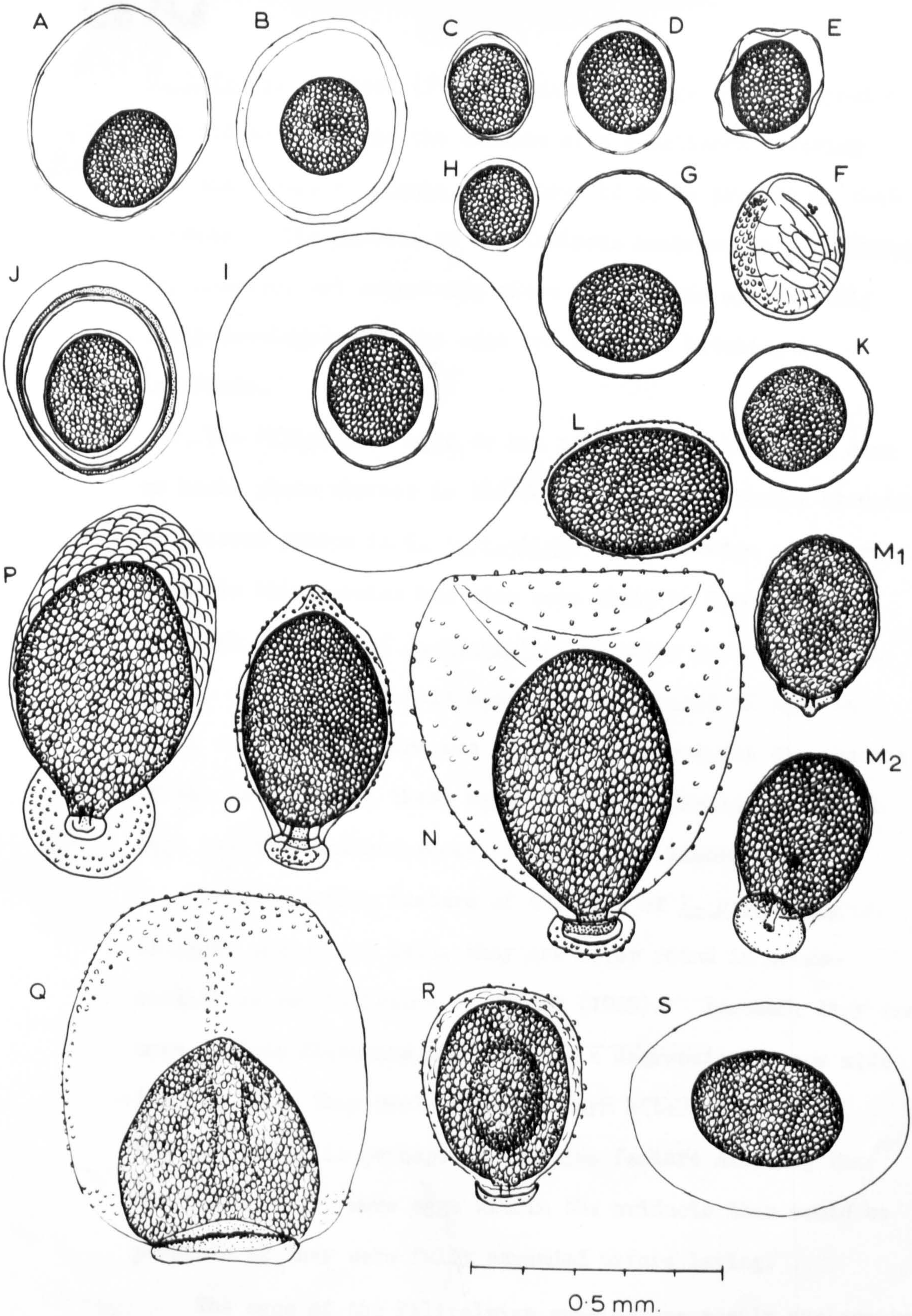


Fig. 2

Figure 2 - Eggs of:-

A - *Leuctra moselyi*

B - *Amphinemura standfussi*

C - *Amphinemura sulcicollis*

D - *Protonemura praecox*

E - *Protonemura meyeri*

F - *Capnia bifrons*

G - *Nemoura avicularis*

H - *Nemoura cambrica*

I - *Nemurella picteti*

J - *Nemoura cinerea*

K - *Taeniopteryx nebulosa*

L - *Chloroperla torrentium*

M_1 & M_2 - *Chloroperla tripunctata*

N - *Diura bicaudata*

O - *Isogenus nubecula*

P - *Dinocras cephalotes*

Q - *Perlodes microcephala*

R - *Isoperla grammatica*

S - *Brachyptera risi*

C. bifrons. Brinck (1949) studied the eggs of this species but did not indicate the absence of a gelatinous covering and his figure of Capnia atra shows it to be present in that species. The absence of a gelatinous membrane in C. bifrons is, however, not surprising since the embryos are normally fully-developed when the eggs are laid and hatching is immediate.

The Filipalpian eggs do not possess an attachment disc or basal plate whereas in the Setipalpian eggs such a structure is present except in C. torrentium. The absence of a basal plate in this species has also been noted by Hynes (1941). Although the eggs of I. grammatica normally possess a basal plate, one female of this species laid a batch of eggs in which no such structure was present. Except for the absence of the basal plate, these eggs were quite similar to normal eggs and they hatched successfully in the laboratory.

An interesting feature of the eggs of I. grammatica is that, when first laid, they are never round in cross-section as was indicated by Brinck (1949). Instead, they are more or less flattened and there is a depression on one side (Figure 2). They swell up a few days after oviposition, the flattening is perhaps an adaptive feature allowing the females to carry more eggs within the oviducts than would be possible if they were fully expanded before laying.

The eggs of the Filipalpian species are mostly unpigmented, but those of B. risi are orange or light brown in colour.

Among the Setipalpia, the eggs of C. torrentium, C. tripunctata, I. grammatica and I. nubecula are usually light brown while those of D. cephalotes, D. bicaudata and P. microcephala are often dark brown in colour. It is, however, not uncommon to find some eggs in an egg-batch which are much lighter coloured than the best. There does not appear to be any difference in the rate of development between eggs of different pigmentation.

The gelatinous covering of the eggs of Setipalpian species disappears fairly rapidly after it has expanded but in most of the Filipalpian species there is a gradual shrinkage of this covering and it is often still visible just before hatching. In N.picteti and B. risi, however, there is no obvious shrinkage of this membrane during the course of development. Another interesting feature regarding the gelatinous covering of Filipalpian eggs is the presence of several layers surrounding the egg body, e.g. in N. cinerea and N. picteti. In N. cinerea the outermost layer of this covering dissolves away within about an hour whereas the remaining layers show a gradual shrinkage during development. There is also a difference in the degree of expansion of different layers, e.g. A. standfussi as compared with L. moselyi.

The ecological significance of the gelatinous covering and of the basal plate as a means of anchorage has been discussed by Brink (1949). However, the differences in

the nature and behaviour of the gelatinous covering suggest a variety of other possible functions besides attachment. One of these functions is probably the protection of the eggs against injury. Filipalpian eggs usually have a thin chorion whereas in Setipalpia the chorion is usually hard and thick. In the latter group there is, except in C. torrentium, an efficient means of attachment by the basal plate and the eggs are not easily detached once they have become anchored to the substratum. The protective effect of the gelatinous membrane is thus of less significance in the Setipalpia and it disappears quite rapidly after expansion. In the Filipalpia, with the notable exception of B. risi, the means of attachment is much less effective and the eggs are quite easily dislodged. In this group, the gelatinous covering does not shrink very rapidly and it is therefore probably of importance as a buffer if the eggs should be dislodged during a flood. Another possible function of the gelatinous covering is to enable the eggs, once the covering has expanded, to survive short periods of exposure to the atmosphere during drought.

(c) Effect of desiccation

It was found that eggs which were not deposited into water were unable to withstand exposure to the atmosphere for any length of time; a few hours' desiccation was

sufficient to kill them. However, eggs in which the gelatinous covering had fully expanded as a result of water absorption were able to survive fairly long periods of exposure.

Eggs of N. picteti that were laid onto a piece of twig in water were able to develop after the twig had been removed from the water and kept in a dry tube for 7 days. At the end of this period the twig was found to be quite dry but the gelatinous coverings of the eggs were still moist and the eggs hatched successfully when they were replaced in water.

Eggs of B. risi were able to survive after being removed from water and left exposed to a damp atmosphere for 12 days. Some eggs that were completely desiccated did not survive when they were returned to water.

The eggs of stoneflies appear to be able to develop successfully as long as they remain moist and the gelatinous covering seems to be able to retain moisture for some length of time. Thus, the ability of the eggs to survive periods of drought as was observed by Hynes (1958) is probably because they were not completely desiccated during the drying up of the stream. It is likely that the humidity down among the stones was quite high and this would enable the gelatinous covering to remain moist during the period of drought.

(d) Visible changes in the embryonic development of the eggs.

The various stages in the embryonic development of the eggs of B. risi, T. nebulosa and I. grammatica are shown in figures 3, 4 and 5 respectively. Figure 6 shows the later stages of embryonic development and the hatching of the eggs of D. bicaudata. It has been possible to follow in fair detail the development in B. risi because of the slight pigmentation of the oval-shaped eggs and because of the manner of attachment.

The gelatinous covering of the eggs of B. risi began to expand about 10 to 30 minutes after oviposition. This covering was at first transparent but it soon became opalescent. When fully expanded the gelatinous covering attaches the egg very firmly to the substratum and it spreads over the egg in the same manner as the albumen spreads over the yolk of a fried hen's egg.

Quite soon after oviposition there appeared in the centre of the egg a dark spot which was probably the fusion nucleus (Stage I A). After the first day, this nucleus could be seen to have undergone one or two divisions as indicated by the presence of 2 or 4 dark spots in the egg. Further divisions of these cleavage nuclei occurred till the 6th to 9th day when numerous nuclei were observed (Stage I B). Very soon afterwards these nuclei could no longer be seen (Stage I C). This stage probably corresponds to the migration of the cleavage cells to the surface of

Figure 3 - Embryonic development in B. risi.

I A - I C - Division of fusion nucleus and ultimate formation of germ disc.

II A - II B - Invagination of germ disc to form early embryo and the cleavage of yolk.

III A - III C - Lengthening of embryo and its arching into the yolk.

IV A - IV B - Broadening and shortening of embryo till the stage prior to blastokinesis.

V A - V C - Post-blastokinesis development.

For details refer text.

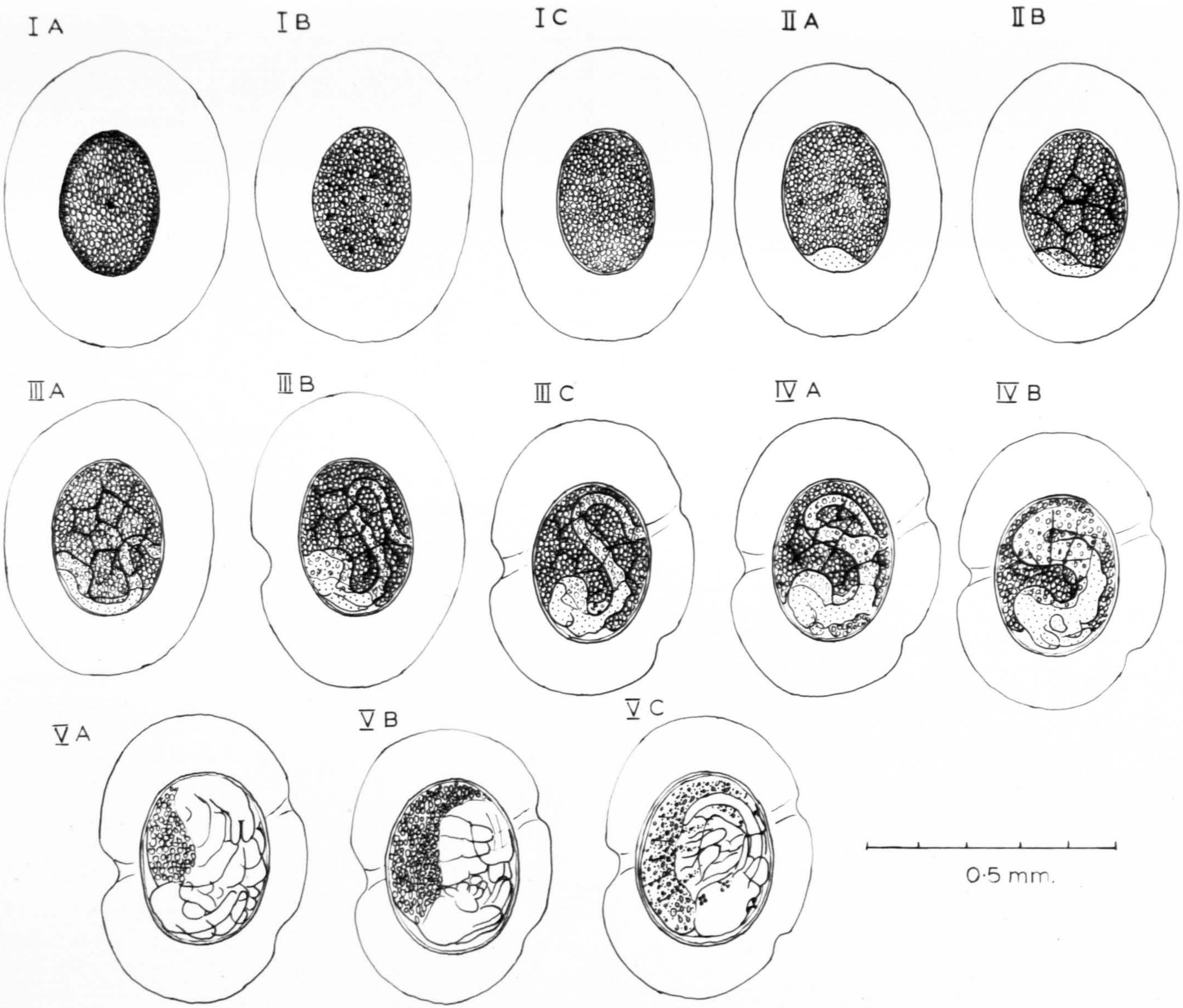


Fig. 3

the yolk to form the primary epithelium and later the germ disc as has been observed by Miller (1939) in P. proteus. In B. risi all the eggs seemed to undergo diapause at this stage and no further change was observed till 2 to 3½ months later (depending on the egg-batch) when some eggs began to show a depression in the yolk (Stage II A -"Yolk depression"). This stage probably corresponds to the invagination of the germ disc to form an early embryo. In P. proteus and in many other species (e.g. P. praecox, L. hippopus, T. nebulosa) which do not undergo diapause, the invagination of the germ disc occurs very soon after its formation.

The time taken for post-diapause development in B. risi (i.e. the interval between "yolk depression" and the first hatching of the eggs) was about 1½ to 2 months. One day after the invagination of the germ disc, cleavage of the yolk occurred (Stage II B - "Yolk cleavage and depression"). At this stage it could be seen that the embryo became slightly elongated but its position remained unchanged. The next three stages (Stage III A to III C), lasting about a week, showed the lengthening and the dorsal arching of the embryo into the yolk. Stage III A was reached after about 5 to 6 days of post-diapause development and the embryo at this stage appeared to be still unsegmented. However, the protocephalic lobes were already sharply differentiated from the narrow protocorm and the embryo was beginning to arch into the yolk near its posterior end. Stage III B showed a more pro-

Figure 4 - Embryonic development in T. nebulosa.

Stages corresponding to those in B. risi. (fig. 3).

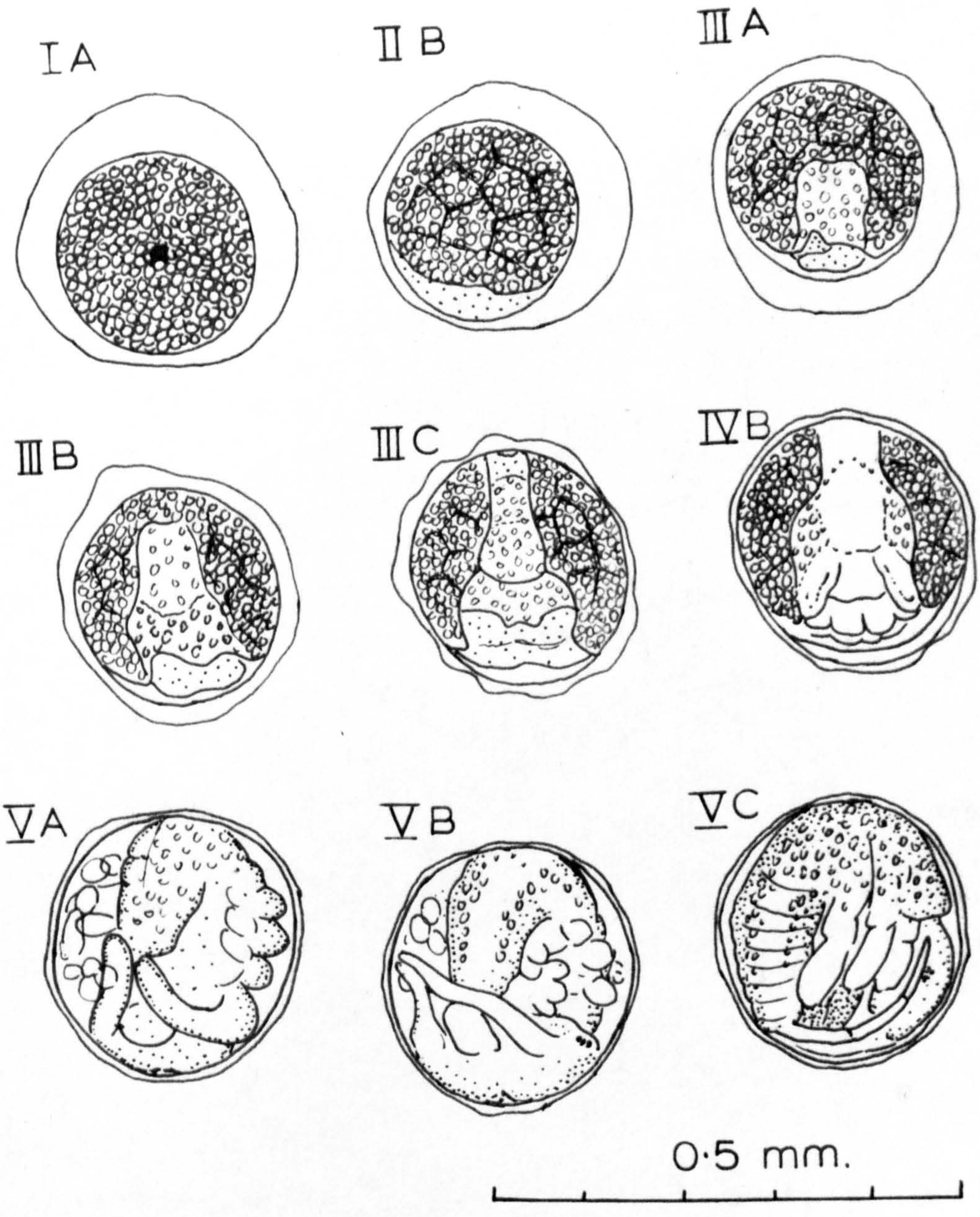


Fig. 4

nounced arching of the embryo and a great increase in length. Slight evidence of segmentation of the embryo was nowvisible. Evidence of further segmentation occurred in Stage III C when the embryo appeared to have reached its maximum length. The next stage, which was about 3 days later, was the shortening and broadening of the embryo as well as the appearance of limb-buds (Stage IV A). By about the 22nd day of post-diapause development, the embryo was found to have reached the stage (Stage IV B) which was quite close to blastokinesis (i.e. the rupture of the amnion and serosa, and the associated movements of the embryo which result in its becoming more or less C-shaped).[1] Blastokinesis was not observed but it probably occurred between the 23rd and 25th day after the breaking of diapause. Stage V A showed the embryo on the 25th day of post-diapause development and this was probably the stage just after blastokinesis. Instead of lying sideways, the embryo seemed to lie with its frontal surface more or less upwards. At this stage the antennal and cercal buds had not been fully-elongated and the eyes were still absent. However, the size of the embryo had increased considerably. By the next day, the embryo was found to have reverted to its sideways position (Stage V B). On the 33rd day after the breaking of diapause the embryo was almost fully-developed (Stage V C). The egg-tooth and the eyes were already present and the antennal and cercal buds appeared to have reached their maximum lengths; but

the antennae and cerci were still unsegmented. Hatching occurred about 2 weeks after this stage was reached. The above descriptions were based on eggs reared under conditions in the cold room.

The various stages of the embryonic development of T. nebulosa and I. grammatica are shown in figures 4 and 5 respectively. The stages that are indicated correspond roughly to those described for B. risi but the length of each stage varies between the species. In T. nebulosa as in most other Filipalpian species, the orientation of the embryo, as observed under a microscope, was quite variable because of the spherical nature of the egg. Thus, in contrast to B. risi where the flattened gelatinous covering holds the egg in one position, the developing embryos can often be seen in different views while the eggs

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Footnote 1.

Blastokinesis, as designated by Wheeler (1893), refers to the oscillatory movements or flexions of the germ band during development. Johannsen and Butt (1941) use the term to include all displacements, rotations, or revolution of the embryo within the egg since the flexions of the embryo in some cases cannot be clearly distinguished from shifts due to growth in length and to later contraction. Miller (1939) refers to it as a process during which occurred the rupture of the amnion and serosa and the slipping of the embryo out of the yolk. The term is used here in the same sense as that used by Miller.

Figure 5 - Embryonic development in I. grammatica

Stages I A to V C as in B. risi (fig. 3).

I A to IV B - side view of egg.

V C - dorsal view of egg.

VI - empty egg-shell (dorsal view).

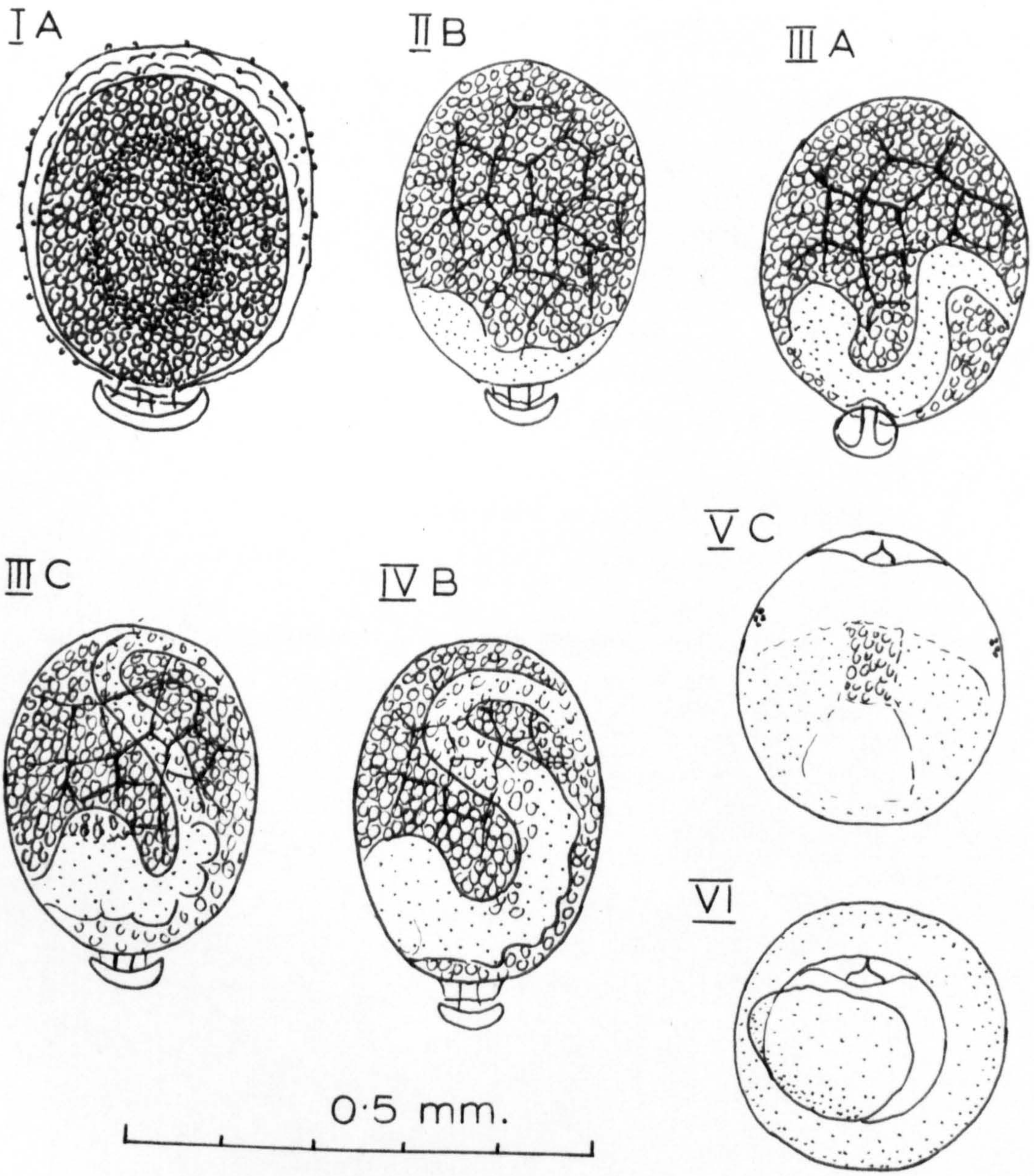


Fig. 5

are still attached to the bottom of the petri-dish. Also, because of the lack of pigmentation in the eggs, the details of the various stages are not as clearly observed as in the eggs of B. risi.

There is no occurrence of a diapause at any particular embryonic stage in T. nebulosa. The drawings shown in figure 4 were made from the same egg reared at room temperature. The development of the egg was complete on the 12th day but hatching occurred on the 15th day. Stage II B was reached 3 days after oviposition and by the 7th day the embryo was found to be in Stage III C. A day later Stage IV B was reached and blastokinesis seemed to occur between the 8th and 10th day. At Stage V B, which was on the 11th day of development, the eyes were vaguely visible but the antennae were still unsegmented. On the 12th day the embryo was fully developed and movements were observed. The cerci were seen to be fully segmented (Stage V C). Hatching occurred 3 days later.

In L.grammatica the incubation period of the eggs at room temperature was about a month. The eggs were seen to be in Stage II B about 7 days after oviposition. The dorsal arching of the embryo (Stage III A) occurred a day later and by the 12th day of development the embryo had reached Stage III C, and segmentation was clearly visible. Soon after this stage, the broadening of the embryo occurred and around the 16th day the embryo was observed to be in Stage IV B.

Figure 6 - Revolution of embryo and hatching in D. bicaudata.

A - Stage of diapause prior to revolution of embryo.

B - Embryo in the process of revolution.

C - Completion of revolution of embryo.

D - Dorsal view of C.

E - Hatching.

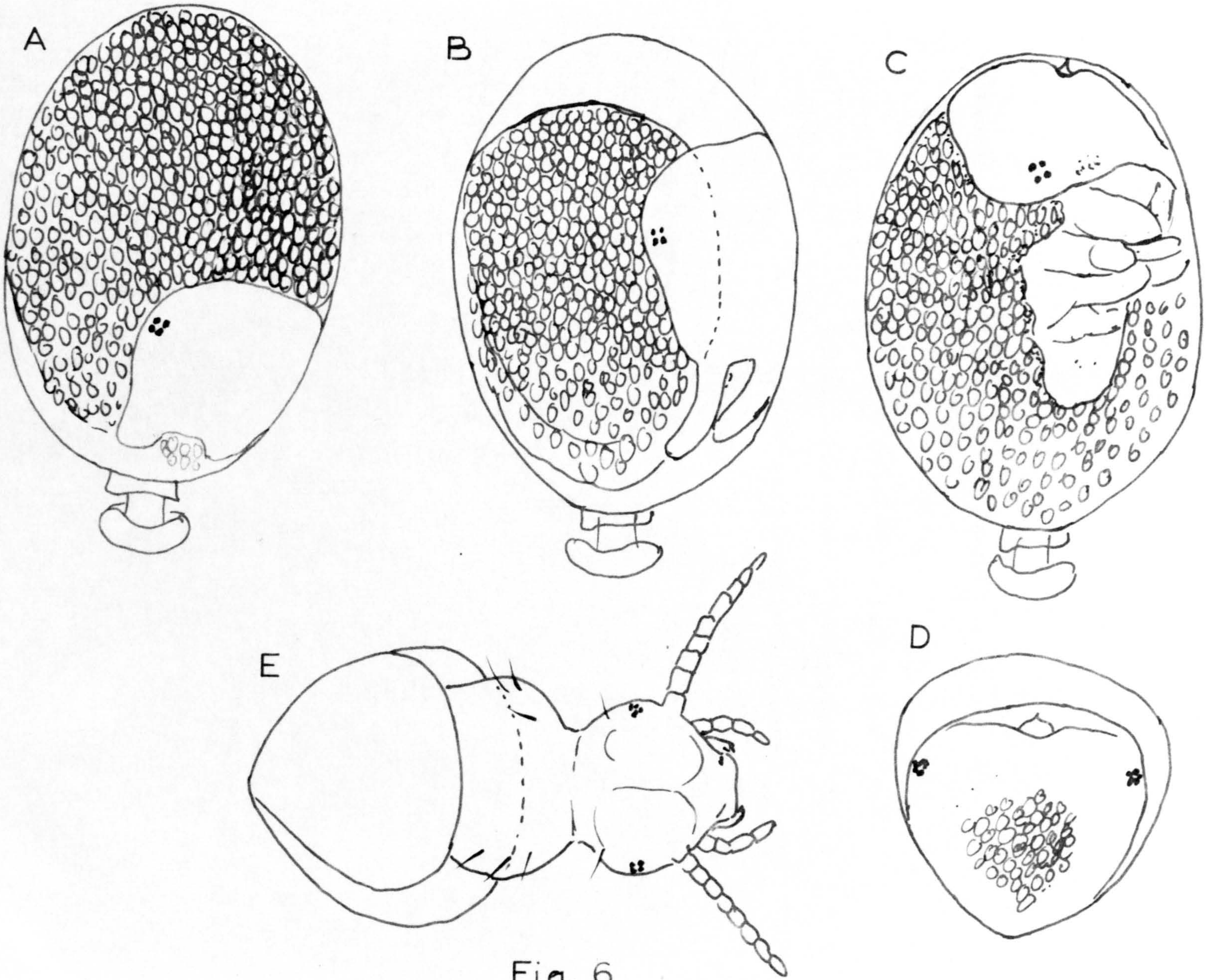


Fig. 6

Blastokinesis was not seen and by the 26th day the embryo was almost fully developed (Stage V C). Hatching occurred about 4 days later.

One interesting feature in the embryonic development of I. grammatica and of the various other Setipalpian species that were studied is that blastokinesis involves the revolution of the embryo in the longitudinal axis of the egg whereas in B. risi this was not so. In I. grammatica it can be seen that prior to blastokinesis, at Stage IV B, the head of the embryo was ventral in position but after blastokinesis (Stage V C) the head came to lie dorsally and eclosion occurred at this end.

The stages in the revolution of the embryo and of the hatching of the eggs of D. bicaudata are shown in Figure 6. Blastokinesis seemed to occur fairly rapidly once the process had been initiated and for this reason the intermediate stage (Figure 6 - B) was not often observed. What is indeed interesting in this species is that the eggs can remain in diapause for several months at (A) which corresponds to stage IV B that has been described for B. risi, T. nebulosa and I. grammatica. However, in the pre-blastokinesis stage of D. bicaudata the eyes were already visible even though the antennal buds had not yet elongated. The revolution of the embryo seems to involve the "pulling" of the head backwards till it comes to lie on the dorsal end of the egg where

eclosion occurs. Further details of the pattern of behaviour of the eggs of this species are given in Chapter IV on diapause and in the following section on the hatching of eggs.

(é) Hatching of eggs

The hatching of the eggs of 24 species was studied. Figures 7 to 17 show the incubation periods, hatching periods and the percentage hatching of some representative egg-batches of the various species under the conditions indicated. The temperature conditions and the photoperiods in the cold room are shown with each figure whereas the room temperatures are shown in figure I in the Appendix. The horizontal connections below the base line in figures 7 to 17 indicate the length of the incubation periods, i.e. the interval between oviposition and the first hatching of the eggs. Those marked with an asterisk indicate that the data were based on several egg-batches that were laid during the period.

Cannia bifrons

The data regarding the hatching of the eggs of C. bifrons are not shown in the figures. In this species, the eggs are normally deposited only when the embryos are fully-developed and hatching is immediate. Eggs that were laid prematurely, i.e. when the embryos were incompletely developed, never survived in water.

The hatching of most of the eggs occurred within one

Figures 7 to 17

The length of incubation periods and hatching of eggs.

Temperature conditions and photoperiods in the cold room are shown:

P - photoperiods in hours of light per day

T - temperature in °C.

Room temperatures are shown in Figure I in Appendix.

Temperature in refrigerator: 3 - 5°C.

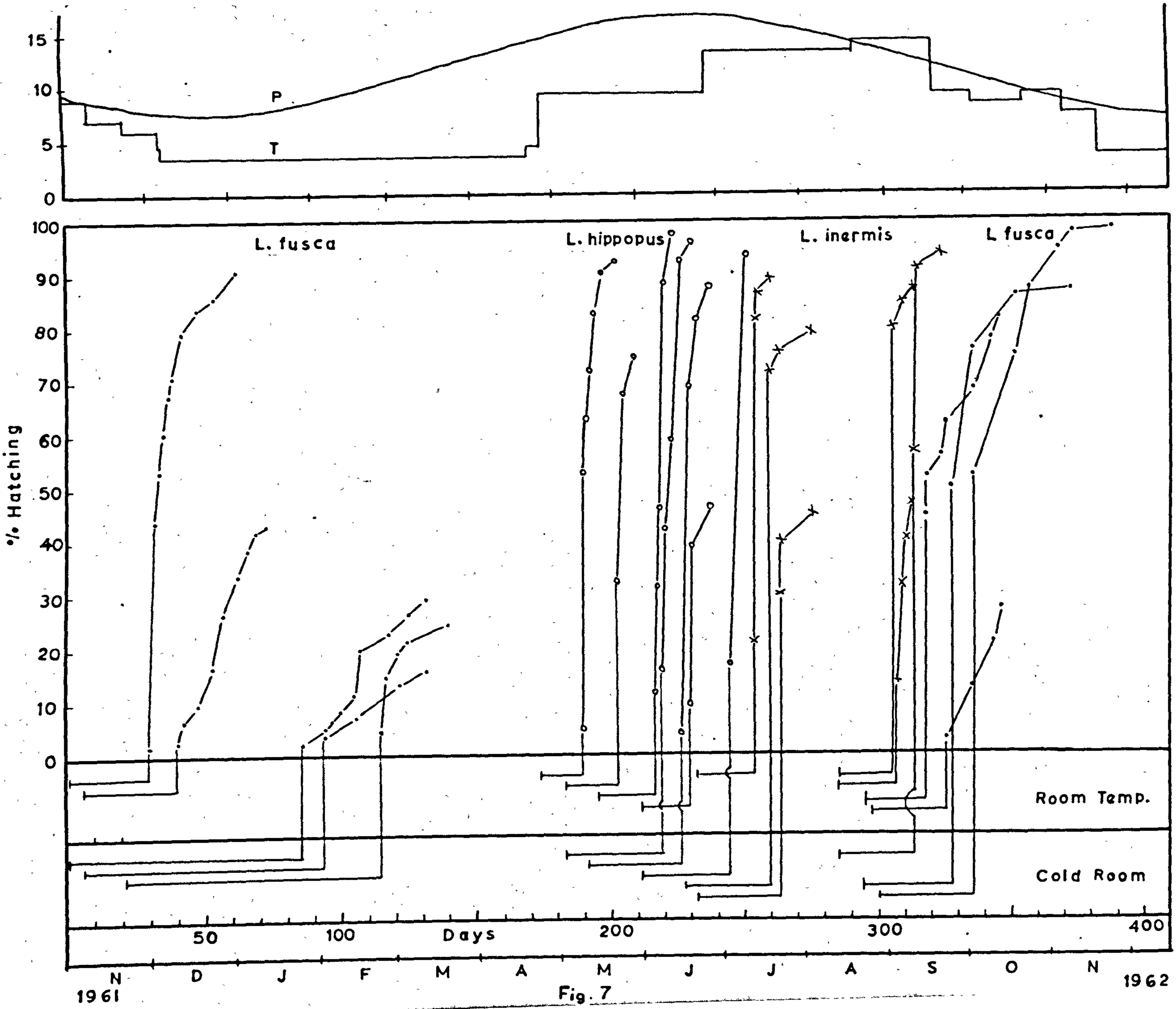

15
10
5
0
P
T
100
90
80
70
60
50
40
30
20
10
0
% Hatching
L. fusca
L. hippopus
L. inermis
L fusca
Room Temp.
Cold Room
50
100
Days
200
300
400
N
D
J
F
M
A
M
J
J
A
S
O
N
1961
1962

Fig. 7

minute after oviposition and the few remaining eggs usually hatched after a few minutes or sometimes after several hours. Thus, the hatching and incubation periods of the eggs are extremely short and this has also been found previously by Hynes (1941) and Brinck (1949).

The percentage of hatching was very high and there were sometimes only 2 to 10 undeveloped eggs in any single egg-batch.

Leuctra fusca (Figure 7)

Seventeen egg-batches were obtained in 1961, 1962 and 1963 and the results for several of these are shown in the figure.

The eggs which were laid in late August began to hatch in the cold room in late September after an average incubation period of 33 days while those that were laid in November began to hatch only in late January or in February after an average period of 89 days. At room temperature, the incubation period of eggs laid during both these periods varied from 22 to 33 days. The long incubation period, in the cold room, of eggs that were laid in November was due to the slow rate of embryonic development resulting from the falling temperatures during this time.

The hatching period of a single egg-batch extended for about 1 to 1½ months. This fairly long hatching period seems to be due to the irregular rate of development since the eggs in any one batch were often observed to be in

different stages of development. It was found that the fully-developed embryos were unable to survive within the eggs for more than a few days if they failed to hatch.

Although a large number of eggs developed successfully both in the cold room and at room temperature, the percentage of hatching was quite variable. It was found that the low percentage of hatching was due largely to the degeneration of the fully-developed embryos within the eggs. In 1961, the eggs which began to hatch from December to January at room temperature showed a lower percentage of hatching than the eggs which began to hatch from late November to December. In 1962, the eggs which began to hatch from late September to October at room temperature also showed a lower percentage of hatching than those which hatched from the second week of September to early October. In both cases, the low percentage of hatching was apparently correlated with the high room temperature (Maximum of 23.5°C) during January and October of 1962, and it seems likely that the high temperature prevented the successful hatching of a large number of fully-developed embryos.

In 1962, in the cold room, the eggs which began to hatch in January or February also showed a lower percentage of hatching than those which hatched in September or October. In this case the unsuccessful hatching of a large percentage of the fully-developed embryos was probably associated with the extremely low temperature (3.5°C) in the cold room.

In 1964, it was found that a batch of eggs (not shown in the figure) which was laid on 19.11.63 and kept in the cold room began to hatch in late February when the temperature was 5° to 6°C. The percentage of hatching recorded was 94%. It is thus possible that the low temperature of 3.5°C could have resulted in the decreased activity of the fully-developed embryos and as such prevented many of them from successfully breaking out of the egg-shells.

The incubation period of the eggs of this species has been recorded by Brinck (1949) as 24 days at 18°C.

Leuctra hippopus (Figure 7)

More than 30 egg-batches were studied. The incubation periods of the eggs kept at room temperature varied from 15 to 21 days. In the cold room, eggs laid in early May began to hatch in June, after a period of 36 days at 9.5°C. Eggs laid in early June began to hatch after 33 days. This slightly shorter incubation period was due to the rising temperature of the cold room to 13.5°C in late June.

Unlike in L. fusca, the hatching period in this species was quite short, lasting only about 7 to 13 days.

The percentage of hatching was usually high and it was observed that fully-developed embryos degenerated when they did not hatch within a few days.

Brinck(1949) recorded an incubation period of 21 days at 18°C.

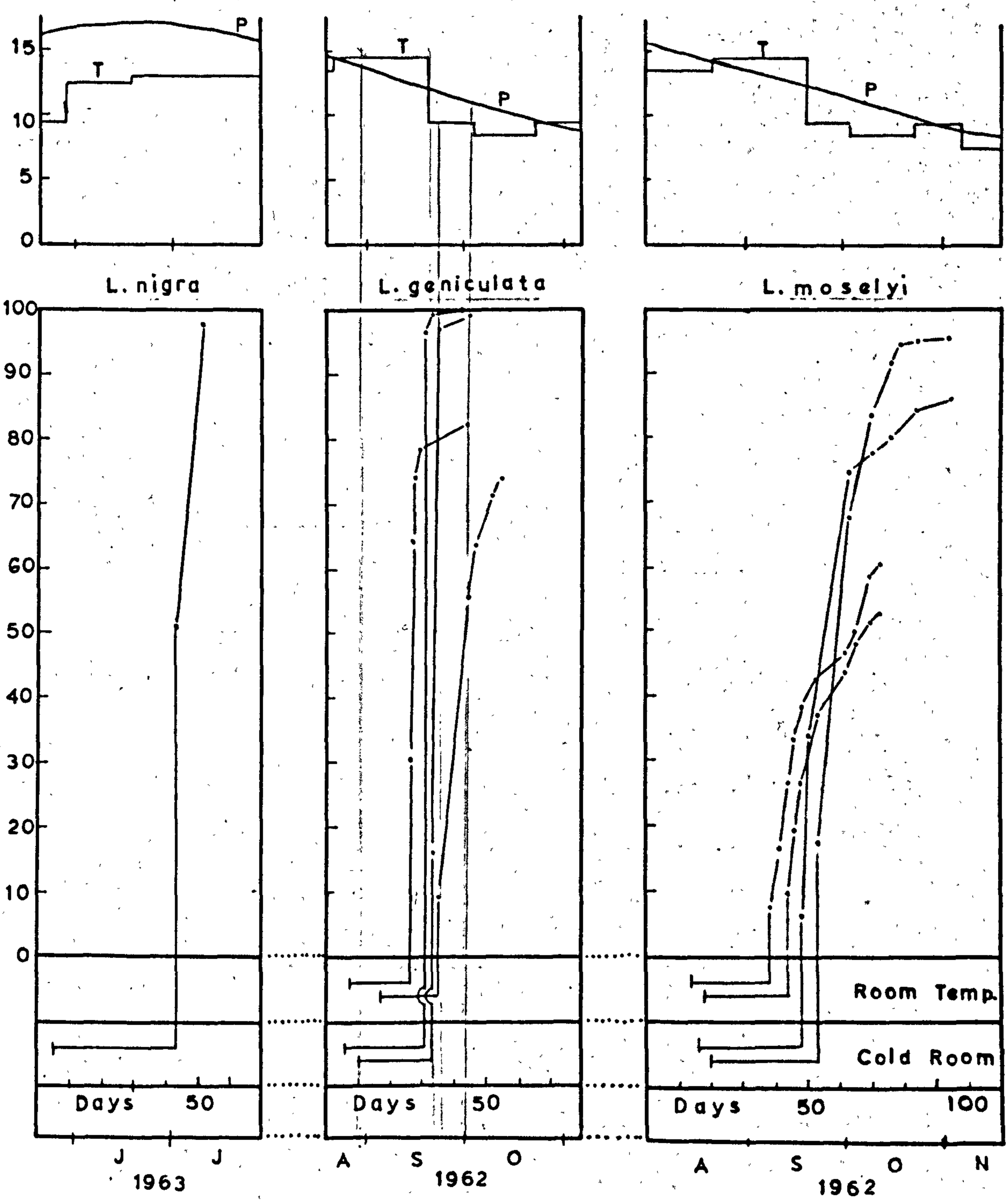

L. nigra
L. geniculata
L. moselyi
P
T
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
% Hatching
Room Temp.
Cold Room
Days
50
100
J
J
A
S
O
N
1963
1962
1962

Fig. 8

Leuctra inermis (Figure 7)

Ten egg-batches were obtained from June to August of 1962 and the results of 6 representative batches are shown in the figure.

The incubation period at room temperature was 21 days. In the cold room, the average incubation period of eggs laid in June was 31 days while that of eggs laid in August, when the temperature was higher, was 28 days.

The percentage of hatching was quite variable both at room temperature and in the cold room. Of the 10 batches that were studied, it was found that 4 showed more than 90% hatching, 2 between 80-90%, 2 between 70-80% and 2 below 60%. One interesting feature of the percentage of hatching in this species is that, unlike in L. fusca or L. hippopus, the remaining unhatched eggs consisted mainly of seemingly undeveloped eggs rather than of eggs containing degenerate embryos. Whether these eggs were truly undeveloped or were in diapause at an early germ disc stage was unfortunately not determined as these eggs were discarded after some time. However, on the basis of the present data, the hatching period is around 1 to 2½ weeks.

Hynes (1941) recorded an incubation period of 28 days at 15°C and a hatching period of 20 days.

Leuctra geniculata (Figure 8)

Thirteen egg-batches were studied but only 4 representative batches are shown in the figure.

The average incubation period at room temperature was 18 days and in the cold room it was 24 days at 14.5°C.

The development of the eggs, unlike that of L. fusca, was quite regular and this was reflected in the short hatching period. In the cold room, hatching was almost over within a few days but at room temperature it continued for about 2½ weeks although most of it occurred during the first week.

A lower percentage of hatching was obtained at room temperature than in the cold room. This was due largely to the degeneration of well-developed embryos within the eggs.

Leuctra moselyi (Figure 8)

Eighteen egg-batches were studied.

The incubation period at room temperature was about 25 days. In the cold room, the average incubation period of eggs laid in August was 33 days.

As in L. fusca, there was a fairly long hatching period of about 1 to 1½ months due to the irregular development.

The percentage of hatching was higher in the cold room than at room temperature, and in both, most of the remaining eggs contained fully-developed embryos which had degenerated.

Leuctra nigra (Figure 8)

Only one batch of eggs was obtained. They began to hatch after 38 days in the cold room and continued hatching for about 9 days.

Nemoura cambrica (Figure 9)

Nine egg-batches were studied.

The average incubation period at room temperature was 17 days. In the cold room, hatching occurred after about 30 days at 9.5°C.

There was only a very short hatching period varying from 4 to 14 days.

The percentage of hatching was generally quite high and remaining eggs were usually immature or contained fully-developed embryos which had degenerated.

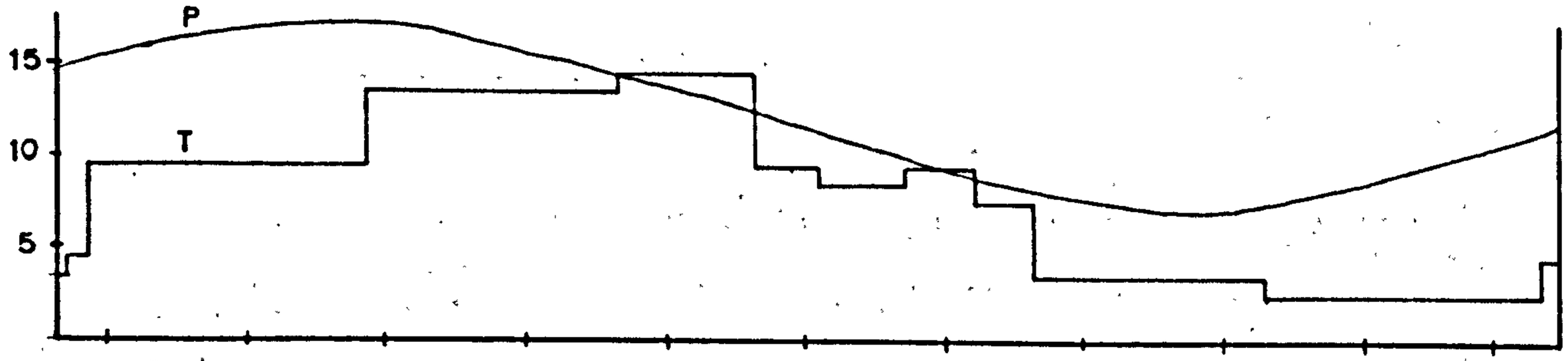
Nemoura cinerea (Figures 9 and 10)

Only five egg-batches laid by 2 adults of this species were studied. The egg-batches laid by the same adults are indicated by the same symbol in figure 9. The eggs of this species showed the most interesting pattern of development.

In figure 9 it can be seen that the incubation periods were 88 days and 122 days for the 2 egg-batches reared at room temperature while in the cold room the periods recorded were 54, 72 and 82 days respectively. Thus, unlike the other species that have been described here, the incubation period of N. cinerea was longer at room temperature than at the lower temperatures in the cold room.

There was a very prolonged hatching period of about 5 months in the cold room but this period was very much shorter at room temperature.

The percentage of hatching in the cold room was between


P
T
15
10
5

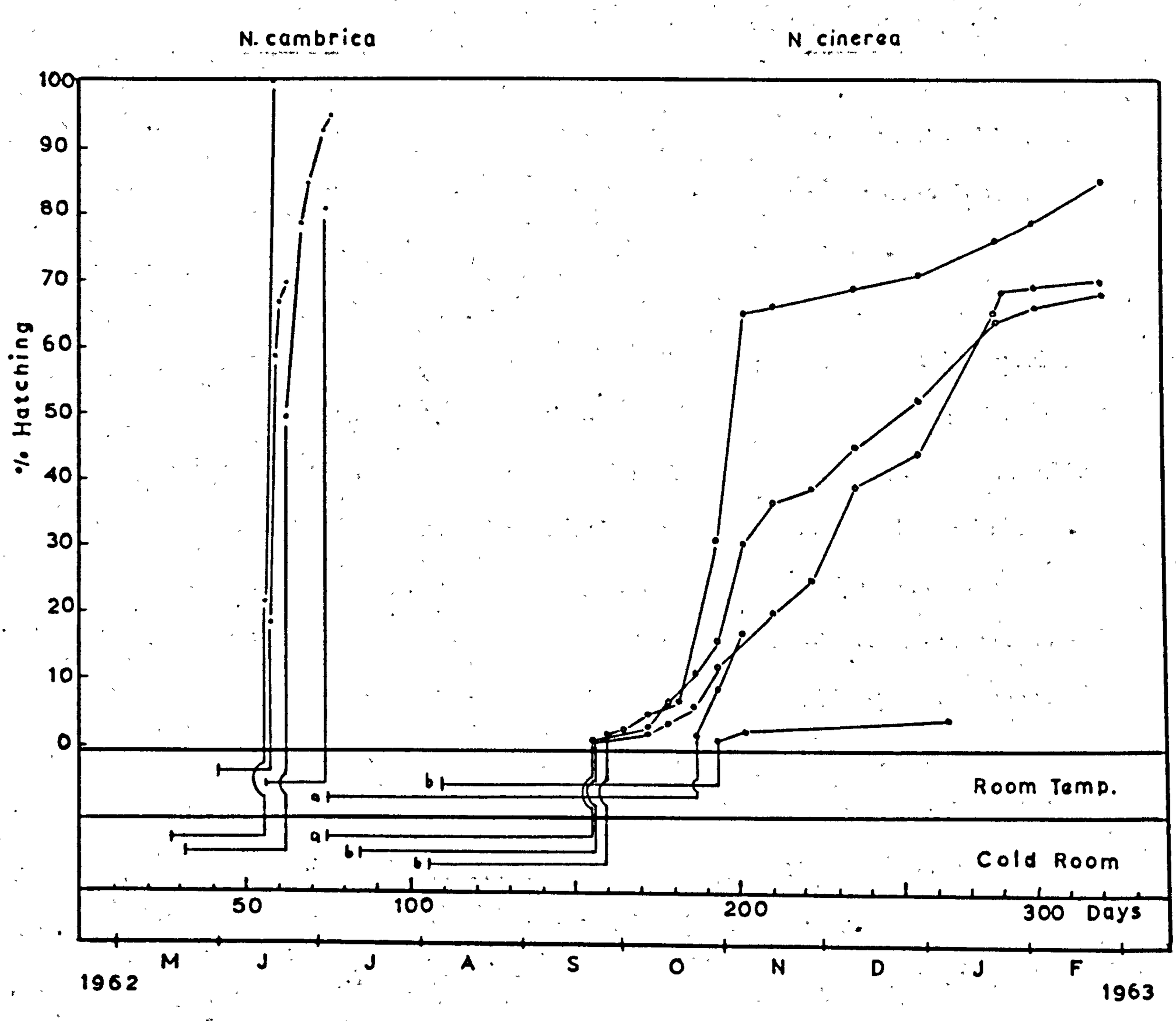

N. cambrica
N cinerea
% Hatching
100
90
80
70
60
50
40
30
20
10
0
Room Temp.
Cold Room
50
100
200
300 Days
M
J
J
A
S
O
N
D
J
F
1962
1963

Fig. 9

67% and 85% while at room temperature it was below 20%. It was observed that a large proportion of those which failed to hatch consisted in both cases of eggs in which fully-developed embryos had degenerated.

For an understanding of the above results it is necessary to describe the development under the differing conditions and to correlate the various stages of embryonic development with the environmental conditions.

At room temperature, the eggs laid on 3.7.62 and 7.8.62 showed an initial rapid development. About 4 days after oviposition they were found to be in the stage of "yolk cleavage and depression" (cf. Stage II B in figure 3). They remained at this stage for some time and no further change was observed till 20.9.62 when several eggs were found to be in the stage in which the embryos had elongated and were arching into the yolk (cf. Stages III A to III C in figure 3). By 4.10.62 many eggs with fully-developed embryos were observed but no hatching occurred till late October, by which time several of these embryos were beginning to degenerate. It is worth noting that, although the dates of oviposition of the 2 egg-batches were more than a month apart, the resumption of development and the beginning of hatching occurred at about the same date in each instance.

In the cold room the development of the eggs was quite erratic. Just as at room temperature, the eggs were at the stage of "yolk cleavage and depression" four days after

oviposition. Some, however, continued to develop without interruption until complete embryos were formed, about 3 to 5 weeks after being laid (e.g. some eggs laid on 2.8.62 were fully-developed on 21.8.62); others within the same batch did not reach stage III A until mid or late September. Although fully-developed embryos were found in August, hatching began only in late September. It is interesting to note that egg-batches laid about a month apart began hatching at the same period and that this occurred about a month earlier than at room temperature.

From the above observations, it appears that at room temperature, all the eggs undergo diapause at the stage of "yolk cleavage and depression". The breaking of diapause in the second half of September seems to be associated with the temporary fall in the room temperature to a minimum of 13°C during this period (Figure I in Appendix). Although fully-developed embryos were found in early October no hatching occurred during this period when the room temperature was above 15°C. Hatching began, however, in late October when the room temperature again fell for a short period to a minimum of 10°C. The low percentage of hatching resulting from the degeneration of fully-developed embryos, was probably because they were unable to survive within the eggs for too long a period at room temperature.

In the cold room, the temperature of 13.5°C to 14.5°C did not cause an interruption of development in some eggs


P
T
15
10
5
0

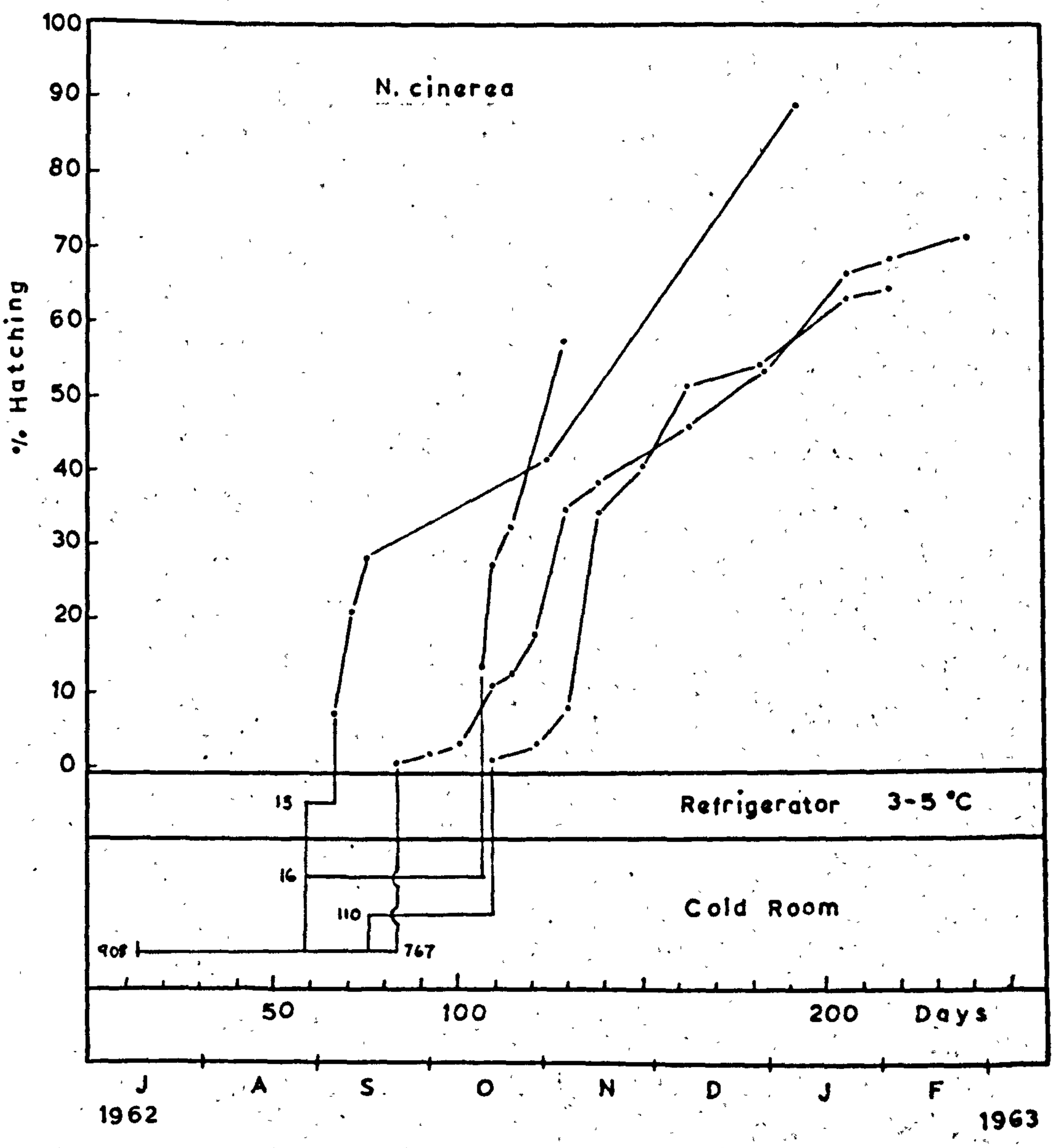

N. cinerea
% Hatching
100
90
80
70
60
50
40
30
20
10
0
15
16
110
908
767
Refrigerator 3-5 °C
Cold Room
50
100
200
Days
J
A
S
O
N
D
J
F
1962
1963

Fig. 10

which were fully-developed in August. The hatching of eggs in late September seems to be associated to the fall in temperature on 21.9.63 to 9.5°C.

The effect of falling temperatures on the hatching of the eggs can be seen in the following experiment, the results of which are shown in figure 10. A batch of 908 eggs laid on 13.7.62 was reared in the cold room. At different intervals the eggs, containing well-developed embryos, were removed and kept in separate dishes under the conditions shown. On 28.8.62, 31 eggs were removed and kept in two dishes. One dish containing 15 eggs was kept in a refrigerator at about 3 - 5°C while the other containing 16 eggs was left in the cold room. On 14.9.62 a batch of 110 well-developed eggs was again removed and left in a separate dish in the cold room. The hatching of these and of the remaining eggs was then recorded.

From the results shown in figure 10 it can be seen that hatching occurred much earlier among eggs that were transferred to the refrigerator than among eggs that were left in the cold room in which the temperature began to fall only on 21.9.62. Also, in the cold room, the eggs in which the embryos were fully-developed after 14.9.62 showed an earlier hatching than the eggs in which the embryos were fully-developed before this date. Those eggs in which the fully-developed embryos occurred later would have undergone diapause in the "yolk cleavage and depression" stage and they probably did not undergo diapause

again in the fully-developed stage. On the other hand, those eggs which did not show an interruption in the early embryonic development underwent diapause in the fully-developed stage. The long hatching period also suggests that the fully-developed embryos are able to survive within the eggs for long periods at low temperatures.

Nemoura avicularis (Figure 11)

Only a single egg-batch of 160 eggs was obtained, on 20,6.62.

The incubation period in the cold room was 19 days and hatching occurred over about 9 days. Fully-developed embryos that did not hatch within a short period were found to degenerate.

Nemurella pictoti (Figure 11)

Fifteen egg-batches were studied.

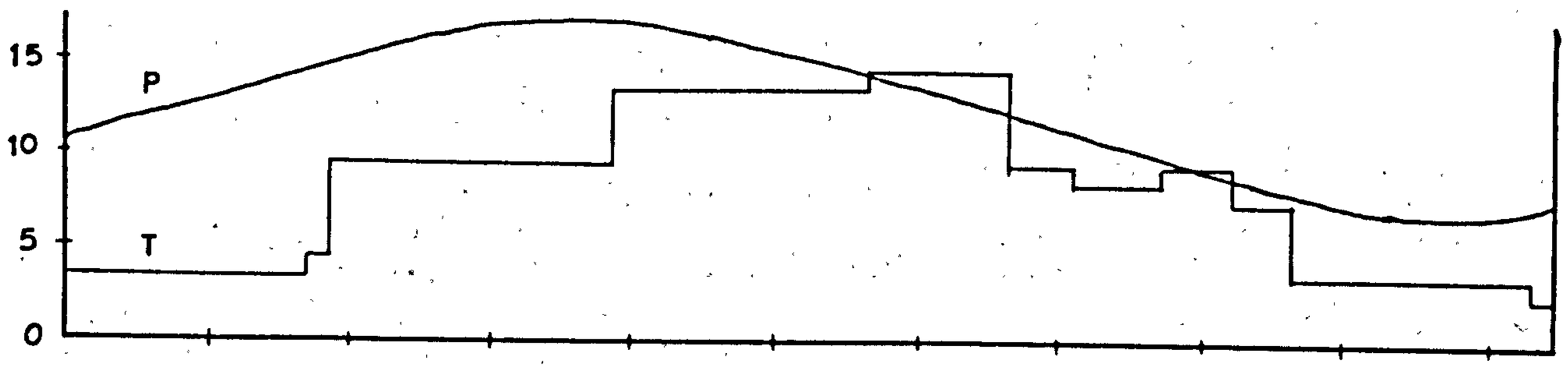
The incubation period at room temperature was 14 days, and in the cold room the eggs began to hatch after 16 days at 14.5°C. Eggs that were kept at about 3 - 5°C showed an incubation period of 71 days.

The hatching period extended over 3 weeks in the cold room but a large percentage of the eggs hatched within the first week.

The percentage of hatching was generally high under all the conditions studied.

Protonemura praecox (Figure 11)

More than 20 egg-batches were studied.


15
10
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P
T

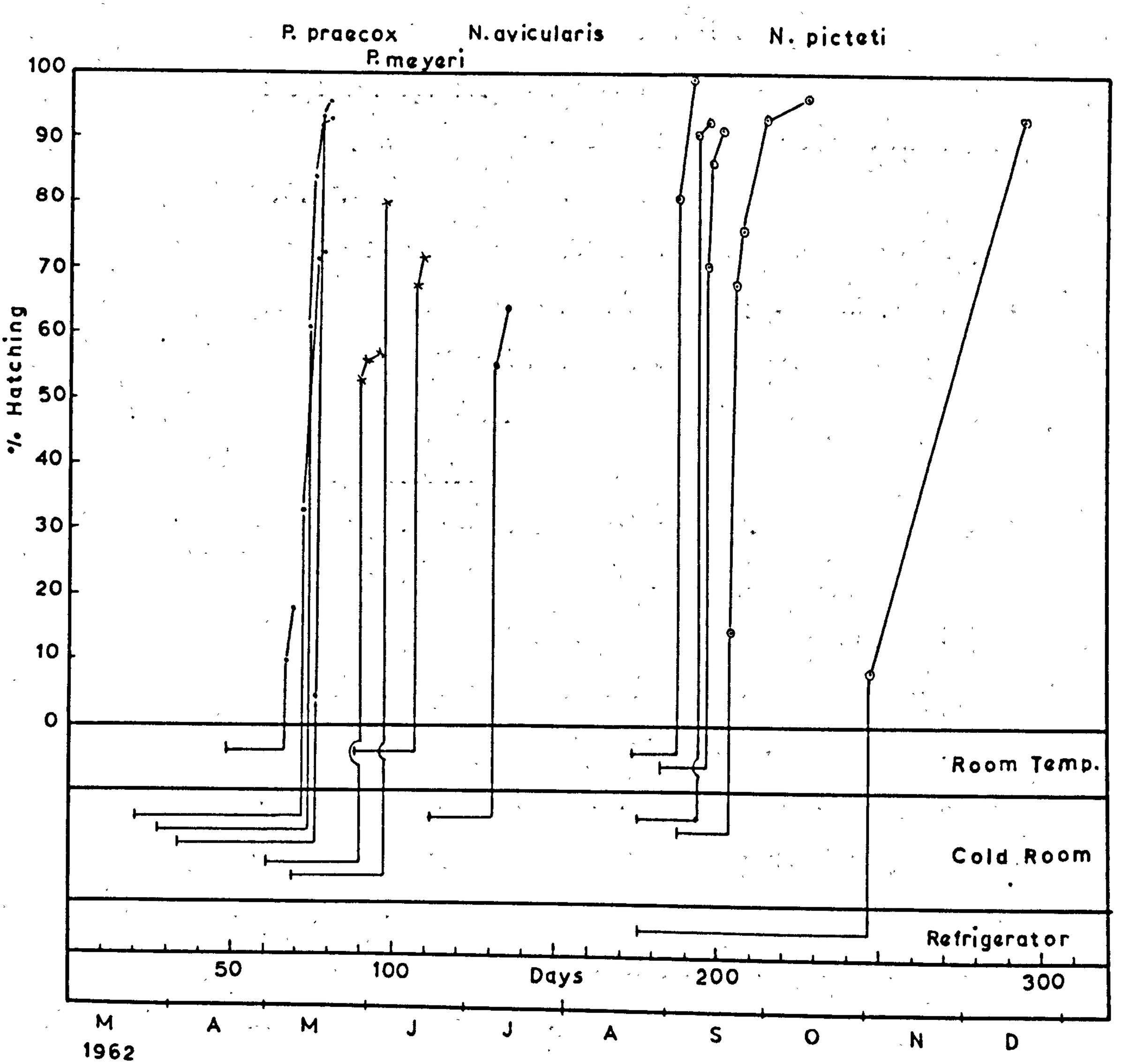

P. praecox
N. avicularis
P. meyeri
N. picteti
100
90
80
70
60
50
40
30
20
10
0
% Hatching
Room Temp.
Cold Room
Refrigerator
50
100
Days
200
300
M
A
M
J
J
A
S
O
N
D
1962

Fig. 11

At room temperature, the eggs began to hatch 18 days after oviposition. In the cold room, the eggs laid on 12.3.62 began to hatch after 59 days while those laid on 10.4.62 began to hatch after 33 days. The eggs laid between these two dates showed an incubation period varying from 42 to 51 days. The rising temperature of the cold room during April resulted in a shortening of the incubation periods of eggs laid during this period.

The hatching of most of the eggs occurred within a few days to a week. It was observed that fully-developed embryos degenerated when they did not hatch during this period.

A lower percentage of hatching was obtained at room temperature than in the cold room due to the degeneration of the fully-developed embryos.

Hynes (1941) recorded an incubation period of 18 days at 15°C.

Protonemura meveri (Figure 11)

More than 30 egg-batches were obtained from late April to May 1962.

The incubation period of the eggs at room temperature was 18 days; in the cold room it was 29 days at 9.5°C.

Hatching occurred for only a few days to a week and the fully-developed embryos degenerated if they did not hatch within this period.

The percentage of hatching was fairly high and the

remaining unhatched eggs were undeveloped or contained degenerate embryos.

Taeniopteryx nebulosa (Figure 12)

A total of 12 egg-batches was studied.

The average incubation period at room temperature was 14 days. In the cold room, the eggs laid 1.3.62 began to hatch after 61 days while those laid on 16.3.63 began to hatch after 35 days. The shorter incubation period recorded in 1963 was due to the higher temperatures in the cold room during March and April of this year than during the same period of 1962.

The hatching period was short and lasted for about 10 days in the cold room. The fully-developed eggs soon degenerated if they did not hatch within this period.

The percentage of hatching was very much higher in the cold room than at room temperature. The low percentage of hatching under the latter condition was due to the degeneration of fully-developed embryos.

The incubation period recorded by Percival and Whitehead (1923) was 20 days at 15°C. Hynes (1941) recorded an incubation period of 24 to 26 days at the same temperature.

Brachyptera risi (Figure 12)

Seven egg-batches laid by 3 adults were studied in 1962 and the results of 6 batches are shown in figure 12. Egg-batches laid by the same female are indicated by the same symbol (e.g. $a_1 - a_3$, $b_1 - b_2$ and c_3 were laid by 3 different

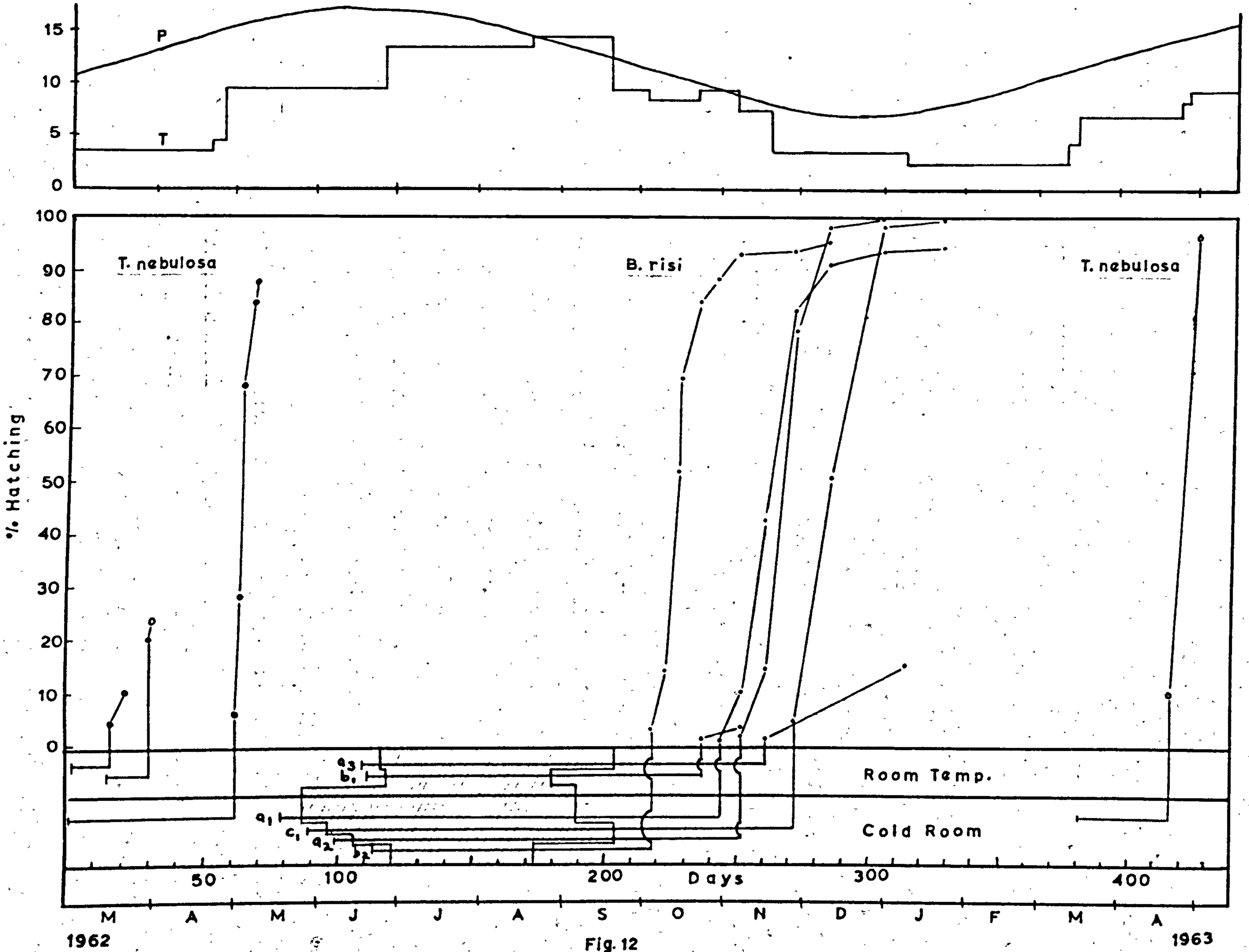

15
10
5
0
P
T
100
90
80
70
60
50
40
30
20
10
0
% Hatching
T. nebulosa
B. risi
T. nebulosa
Room Temp.
Cold Room
a_3
b_1
a_1
c_1
a_2
b_2
50
100
200
Days
300
400
M
A
M
J
J
A
S
O
N
D
J
F
M
A
1962
1963

Fig. 12

females). As has been described earlier in the chapter, all the eggs of B. risi undergo diapause at the "germ disc" stage and the breaking of diapause is indicated by the appearance of a depression in the yolk which corresponds to the invagination of the germ disc to form the early embryo. The shaded areas in figure 12 show the periods of diapause of the different egg-batches. The breaking of diapause among the eggs of any one batch was very variable. Even at the time of hatching the eggs in any one batch could be seen to be in various stages of development and some were only just breaking diapause during this period. Hence, the shaded areas indicate only the inception and the first breaking of diapause.

The eggs of B. risi showed a very long incubation period. At room temperature, egg-batches a_3 and b_1 began to hatch after 152 and 126 days respectively. In the cold room, the incubation periods of a_1, a_2, b_2 and c_1 were 165, 174, 105 and 183 days respectively.

A very high percentage of hatching (94 - 100%) was obtained from eggs reared in the cold room and the hatching period extended for a period of $1\frac{3}{4}$ to $2\frac{3}{4}$ months. This long hatching period was observed to be largely due to the irregular breaking of diapause. At room temperature, the percentage of hatching recorded was rather low (below 15%) in spite of the fact that most if not all of the eggs developed successfully to the stage of a complete embryo.

It appears that high temperatures are unfavourable for the hatching of the eggs even though they do not inhibit the development of the embryos. The fully-developed embryos degenerate within the eggs if they do not hatch within a short period.

In figure 12 it can be seen that the length of diapause was quite variable between the egg-batches. Batches b_1 and b_2 showed a much shorter period of diapause than the batches a_1 to a_3 and c_1 and they showed a correspondingly shorter incubation period. The initiation and breaking of diapause do not seem to be influenced by the environmental conditions experienced by the eggs as can be seen by the varying periods during which the eggs began to undergo and to break diapause. At room temperature, egg-batch b_1 began to break diapause in late August when there was no fall in the temperature (Fig.I - Appendix). In the cold room, the breaking of diapause in egg-batches b_2 and a_2 occurred in August and early September respectively, before any fall in the temperature had taken place. The length of diapause, however, seems to be associated with the adults. It is perhaps interesting to point out that egg-batches a_1 to a_3 and c_1 were laid by adults collected from River Terrig and kept in the cold room while b_1 and b_2 were laid by an adult collected from a different locality (Horseshoe Pass in N. Wales) and kept at room temperature.

Although the breaking of diapause was not dependent upon a fall in temperature, the diapause period could be

prolonged by low temperatures.

A batch of 274 eggs laid by the same adult as a_1 to a_3 was obtained on 29.5.62 and was kept in the cold room. The eggs were still in diapause when they were transferred on 24.8.62 to a refrigerator at about 3 - 5°C where they were left for nearly 6½ months. With the exception of 4 eggs that hatched between late January and early February of 1963, all the remaining eggs showed no sign of development during the period in the refrigerator. The eggs were removed from the refrigerator on 4.3.63 and were divided into separate batches and kept under 4 different conditions as indicated in Table 3. The box in the cold room experienced temperatures

29.5.62 - 23.8.62	24.8.62 - 3.3.63	4.3.63 onwards
Cold room Normal photo-periods	Refrigerator 3 - 5°C Complete dark-ness for most of the time.	1. Cold room Normal photoperiods
		2. Box in bold room Decreasing photo-periods
		3. Box at room temperature Normal photoperiods
		4. Box at room temperature Decreasing photo-periods

Table 3.

which varied daily from the temperature of the cold room to about 2°C higher. The decreasing photoperiods indicated were about 4½ months ahead of the normal photoperiodic regime. The eggs were observed to break diapause in May 1963 in all the 4 sets of conditions. Hatching occurred

from July to September in the cold room. At room temperatures, fully-developed embryos were observed in both boxes during July but only 2 eggs hatched in box 4. Thus, the eggs showed an incubation period of about 14 months and they remained in diapause for 11 to 12 months. It is interesting to note that the breaking of diapause did not occur immediately after the eggs were transferred from the refrigerator to the various conditions. The long diapause period seems to be due to the chilling of the eggs and further studies (Chapter IV) did not show any influence of photoperiods on the eggs of this species.

Amphinemura sulcicollis (Figure 13)

Nineteen egg-batches laid during June 1962 were studied.

The average incubation period at room temperature was 16 days and hatching continued for 24 days. In the cold room, the eggs began to hatch after 26 days and continued to do so for about a week.

The percentage of hatching was quite high at both temperatures. The fully-developed embryos degenerated if they did not hatch within a few days.

Amphinemura standfussi (Figure 13)

Seventeen egg-batches were studied.

At room temperature, none of the eggs developed beyond the "yolk depression" stage (cf. Stage II A in figure 3) and most remained at the "germ disc" stage (cf. Stage I C in

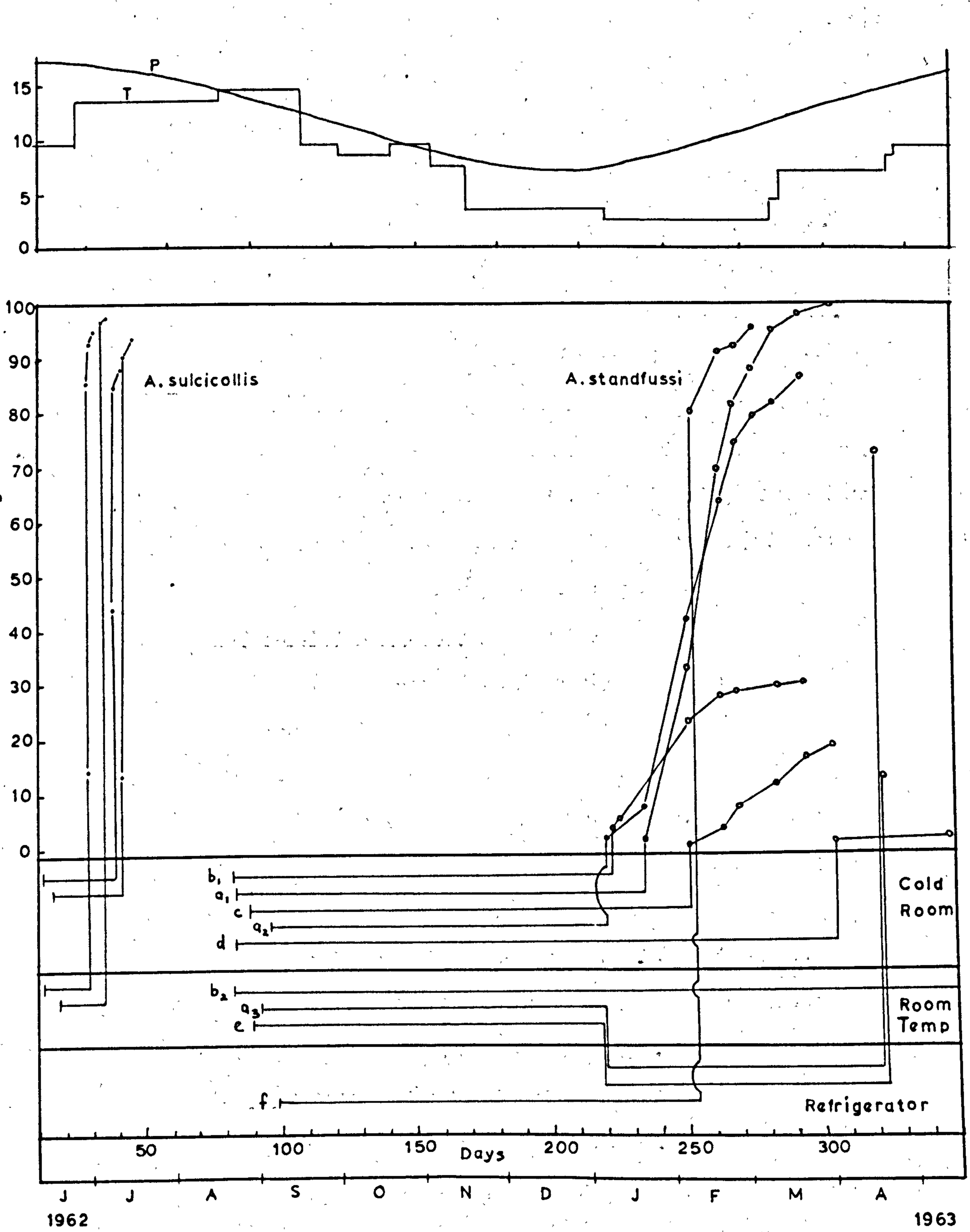

P
T
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
A. sulcicollis
A. standfussi
b1
a1
c
a2
d
b2
a3
e
f
Cold Room
Room Temp
Refrigerator
50
100
150
Days
200
250
300
J
J
A
S
O
N
D
J
F
M
A
1962
1963

Fig. 13

figure 3) till they finally died. They were able, however, to survive for a fairly long period in this dormant stage and eggs that were transferred to a refrigerator (about 3 to 5°C) after slightly more than 4 months at room temperature were observed to resume development, e.g. egg-batches (a_3) and (e), laid on 6.9.62 and 1.9.62 respectively and transferred on 8.1.63, were found to have hatched on 19.4.63 after a total incubation period of about 7½ months.

Eggs reared in the refrigerator showed an incubation period of about 5 months.

The incubation period in the cold room usually varied from 4 to 5½ months but one egg-batch (d) only began to hatch after 7½ months. The eggs seemed to be able to remain for a fairly long period at any of the 3 stages, viz. "germ disc" (Stage I C), "yolk depression (Stage II A) or "yolk cleavage and depression" (Stage II B). Although some eggs reached stage II B as early as 9.19.62, no further change was observed till late November when eggs in stages III A to III C were found. The resumption of development seems to have been associated with the fall in the temperature of the cold room to 3.5°C. Some eggs did not resume development until quite late and this was shown by the long incubation period of egg-batch (d).

The length of hatching period in the cold room was about 2½ months.

Some egg-batches (e.g. a_1 to a3 and f) showed a high

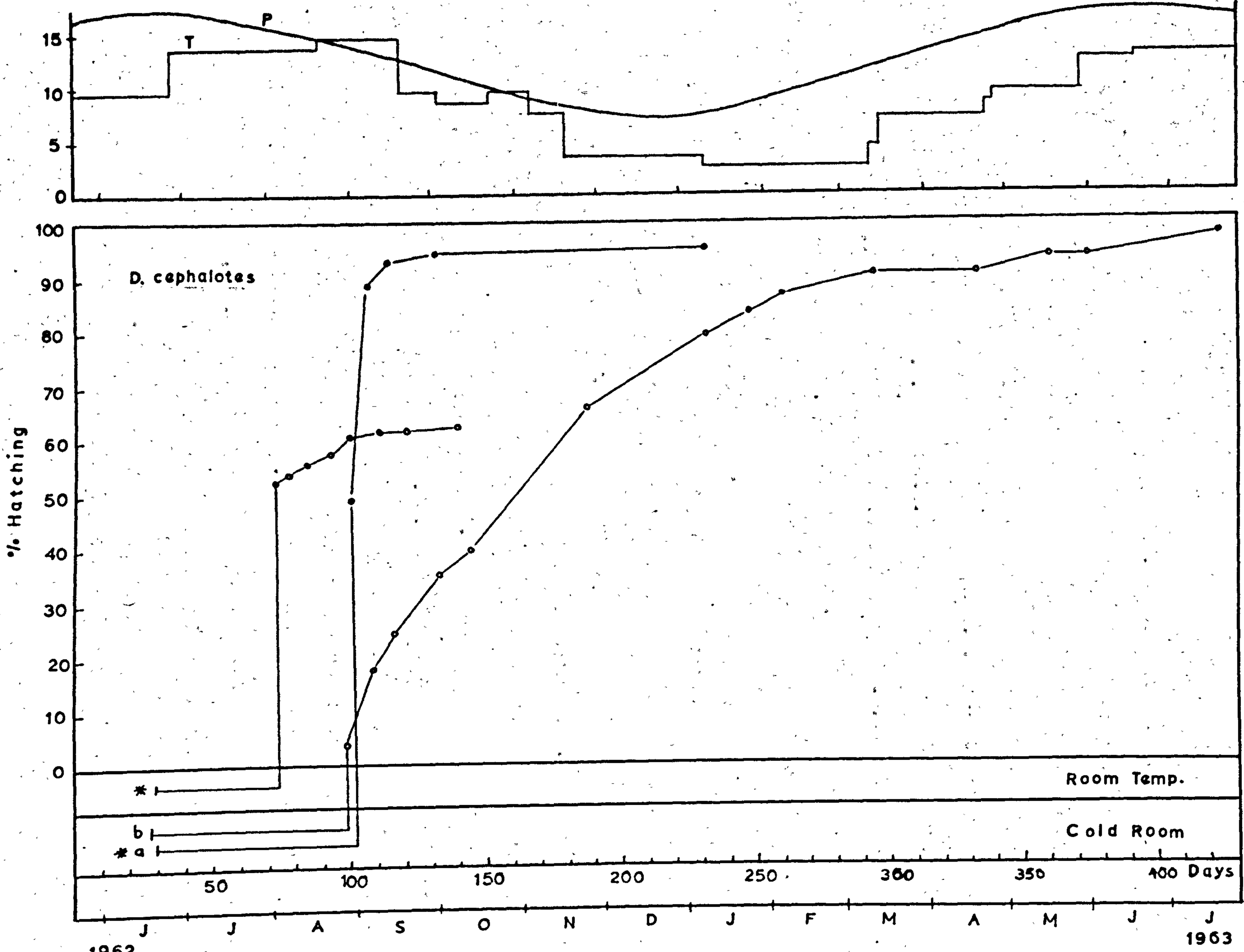

P
T
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
% Hatching
D. cephalotes
Room Temp.
Cold Room
b
a
50
100
150
200
250
300
350
400 Days
J
J
A
S
O
N
D
J
F
M
A
M
J
J
1962
1963

Fig. 14

percentage of hatching while others (e.g. b_1, c and d) laid by different adults showed only a very low percentage of hatching. The unhatched eggs were seemingly undeveloped but they could possibly have been in a state of diapause in stage I C.

Dinocras cephalotes (Figure 14)

Altogether 12 egg-batches were studied.

The average incubation period at room temperature was 44 days. In the cold room, eggs laid in June began to hatch after 71 days.

A lower percentage of hatching was obtained at room temperature than in the cold room and this was found to be due to the degeneration of fully-developed embryos.

Hatching usually continued for about 4 months in the cold room but a high percentage of the eggs hatched during the first 2 weeks, e.g. egg-batches (a). In egg-batch (b), however, there was a very prolonged hatching period of 11 months. This was a very large batch of 1485 eggs which were attached all along the inside of a 1 x 3 in.specimen tube (open at one end) that was kept in a large dish of water. The gradual and very prolonged hatching seems to be due to environmental conditions, possibly an oxygen gradient along the inside of the tube, since other egg-batches laid by the same adult onto petri-dishes showed a pattern of hatching similar to that of (a).

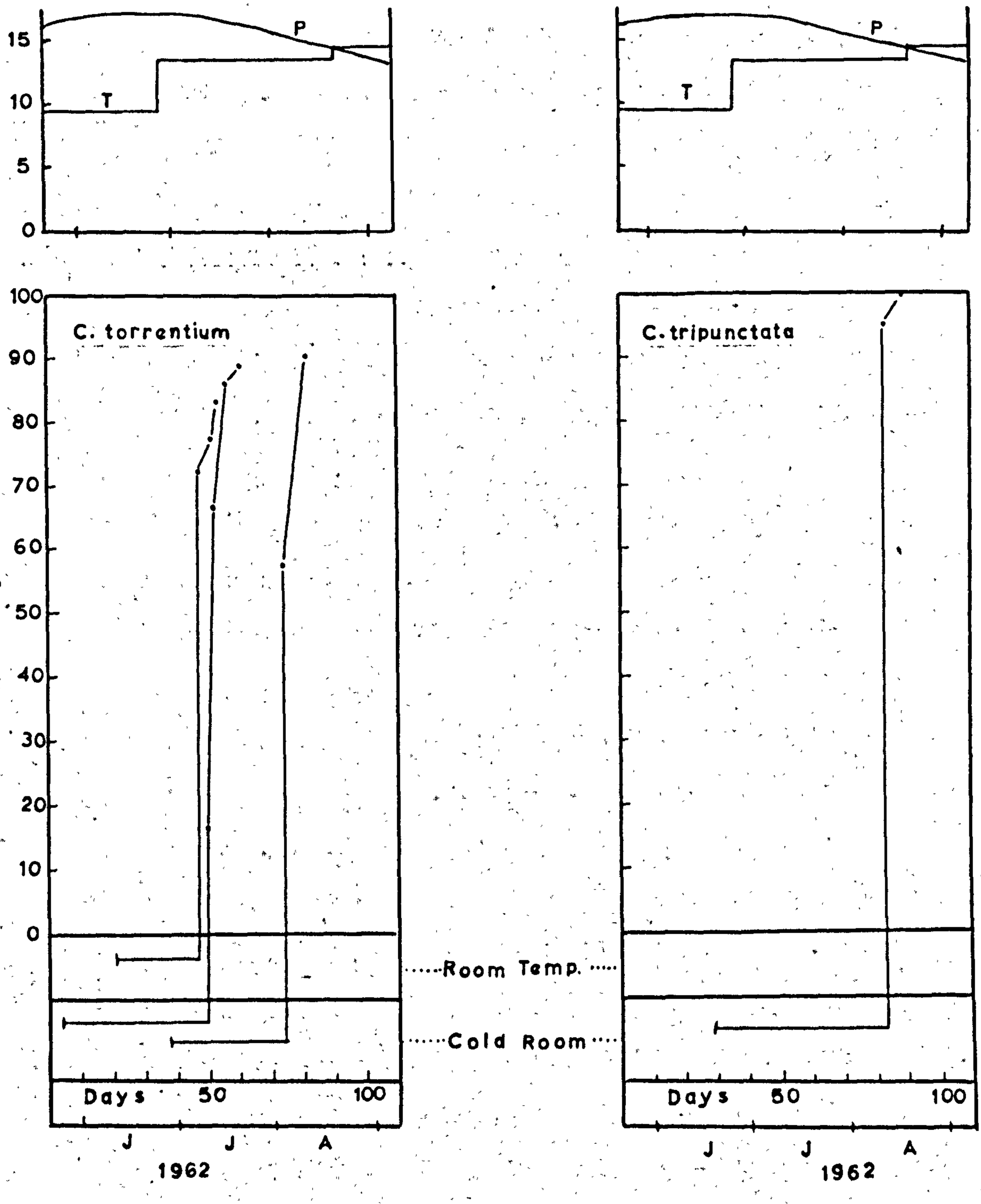

15
10
5
0
P
T
100
90
80
70
60
50
40
30
20
10
0
% Hatching
C. torrentium
C. tripunctata
.....Room Temp. .....
.....Cold Room.....
Days
50
100
J
J
A
1962

Fig. 15

The incubation period recorded by Hynes (1941) was 97 to 112 days at 15°C.

Chloroperla torrentium (Figure 15)

Eleven egg-batches were studied.

At room temperature, the eggs began to hatch after 25 days and continued hatching for about 7 days.

In the cold room, the eggs laid in late May or early June began to hatch after 46 days while those laid in late June began to hatch after 36 days due to the higher temperatures during the latter period. The hatching period was about 10 days. The fully-developed embryos soon degenerate if they do not hatch.

Hynes (1941) found that the eggs began to hatch after 28 to 61 days at 15°C and that the usual period was 30 days.

Chloroperla tripunctata (Figure 15)

Only one egg-batch was obtained during June 1962.

Hynes (1961) suggests that this species might have a very long incubation period, but the present study does not indicate this. The eggs reared in the cold room began to hatch after 56 days.

Diura bicaudata (Figure 16)

Twelve egg-batches were obtained during 1962 from 3 adults (a, b and c).

This species shows a most interesting pattern of development in that some adults lay only diapause eggs (e.g. b_1, 6_1 and c_2) while others lay non-diapause as well as diapause

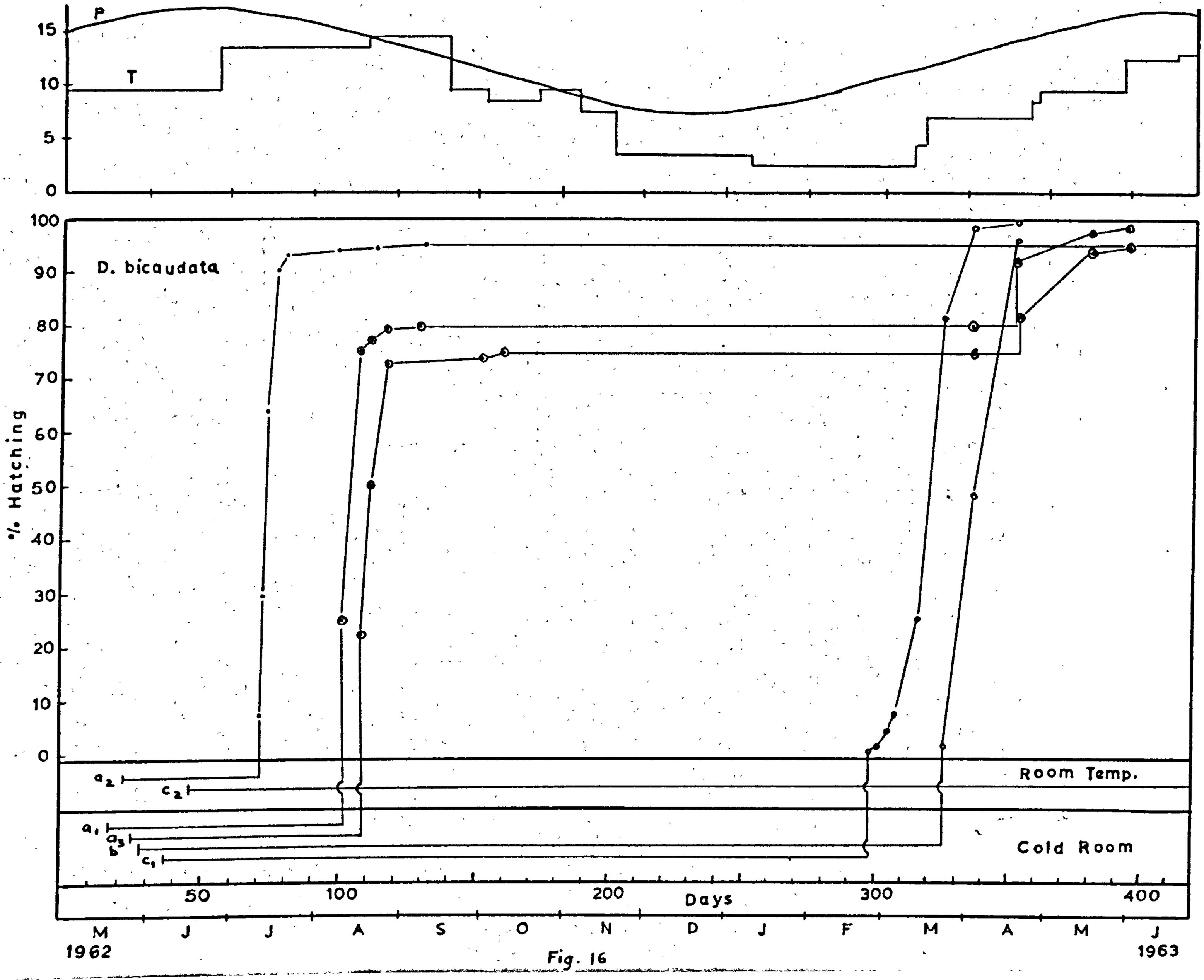

P
T
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
% Hatching
D. bicaudata
a2
c2
a1
a3
b
c1
Room Temp.
Cold Room
50
100
200
Days
300
400
M
J
J
A
S
O
N
D
J
F
M
A
M
J
1962
1963

Fig. 16

eggs within the same batch (e.g. a_1 to a_3). During the studies in 1962, the exact origin of the adults was not determined since the nymphs collected from two different localities (Afon Hirnant and Lake Bala) were reared together in the same enamel dish. However, further studies in 1963 (Chapter IV) have shown that adults from Lake Bala laid both diapause and non-diapause eggs while those from Afon Hirnant laid only diapause eggs.

The eggs undergo diapause at the stage just prior to the revolution of the embryo (Figure 6A). In diapause eggs, this stage was reached 3 to 4 months after oviposition but in non-diapause eggs the interval was shorter. At room temperature, the diapause eggs remained at this stage till they finally degenerated, e.g. a_2 and c_2. Egg batch c_2 consisted only of diapause eggs and therefore no hatching occurred. In a_2, a high percentage of the eggs were of the non-diapause type and hatching occurred 50 days after oviposition. The remaining diapause eggs never broke diapause at room temperature.

In the cold room, egg-batches a_1 and a_3 showed 2 distinct hatching periods. The non-diapause eggs began to hatch after about 85 days while the diapause eggs began to hatch after an average of 332 days. The eggs belonging to b and c_1 were all of the diapause type and the incubation periods were 298 and 262 days respectively. The breaking of diapause in b_1 and c_1 began to occur on 22.2.63 and 19.1.63 respectively

and in a_1 and a_3 on 12.3.63 and 22.2.63. The fairly long hatching period (about 1 to $1\frac{3}{4}$ months) of diapause eggs was due to the gradual breaking of diapause.

From the present study it can be seen that the breaking of diapause is dependent on the fall in the temperature during winter. Non-diapause eggs, however, were able to develop and to hatch successfully without any fall in temperature and the length of their incubation period was shorter at room temperature than in the cold room.

Hynes (1941) estimated that the eggs of D. bicaudata might take about 2 months to hatch in Lake Windermere while Brinck (1949) estimated an incubation period of about one month under natural conditions in Northern Sweden. It is very likely that Brinck was dealing with specimens of the 'Afon Hirnant' type in which the long incubation period could have resulted in the main hatching of the eggs around the beginning of the flight period and this would give rise to a false impression of a short incubation period. In any case, an incubation period of one month would be quite unlikely under the generally low temperatures in Northern Sweden. The estimation given by Hynes seems to be fairly close to the incubation period obtained for the non-diapause eggs and it is possible that the specimens occurring in Lake Windermere might be of the 'Lake Bala' type.

Isoperla grammatica (Figure 17)

Altogether 20 egg-batches were studied.

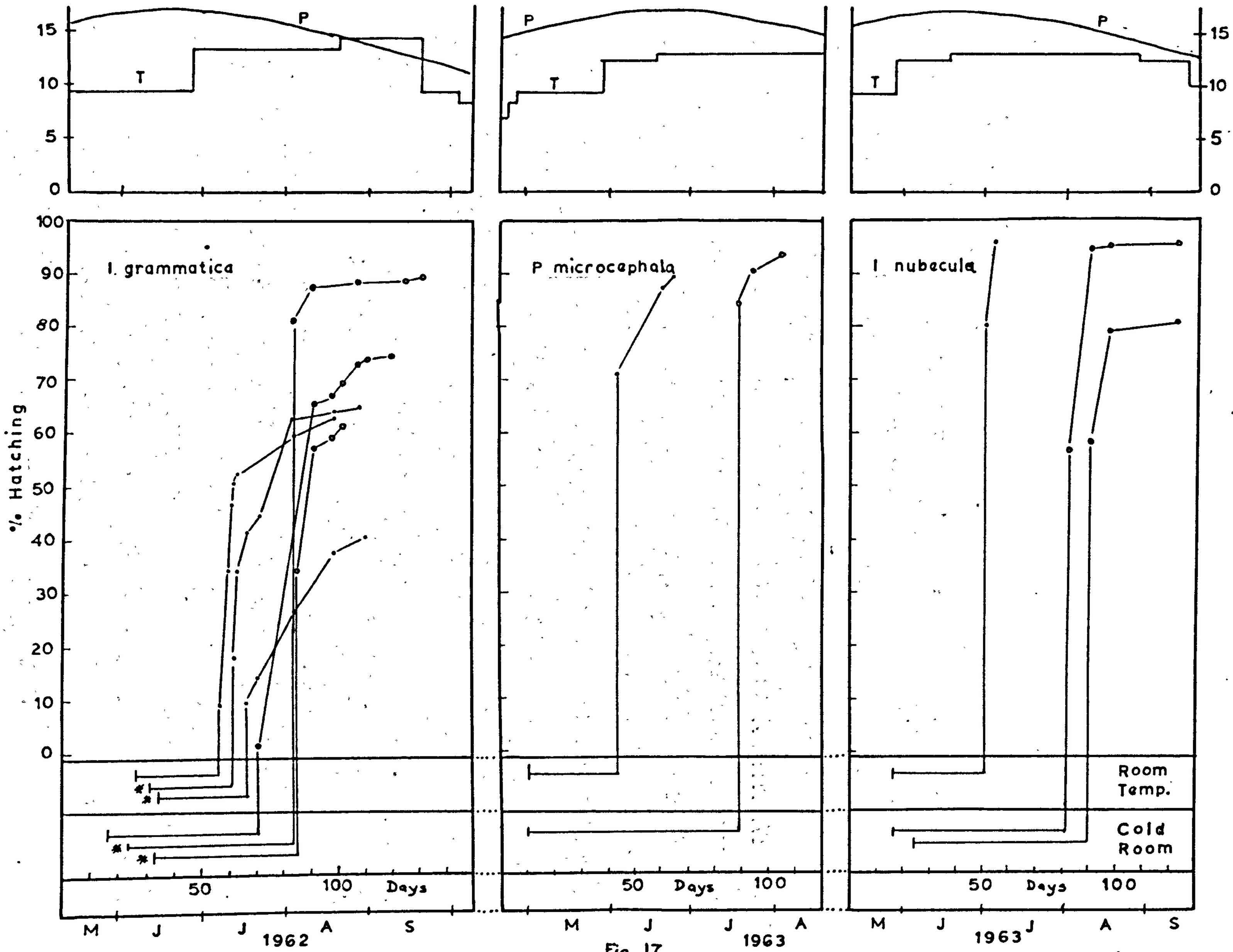

P
T
15
10
5
0
100
90
80
70
60
50
40
30
20
10
0
% Hatching
I. grammatica
P microcephala
I nubecula
Room Temp.
Cold Room
50
100
Days
M
J
J
A
S
1962
1963

Fig. 17

The eggs began to hatch after about 30 days at room temperature and hatching continued for about 45 days. In the cold room, the average incubation period of eggs laid in late May and early June was 54 days and the usual length of hatching period was around 50 days.

A few egg-batches showed nearly 90% hatching but many showed less than 75% hatching. Most of the remaining unhatched eggs were seemingly undeveloped but they could possibly be in diapause at the "germ disc" stage (Stage I C in figure 3). However, these eggs were kept only till early October after which they were discarded when no change was observed.

Perlodes microcephala (Figure 17)

Only 3 egg-batches were studied.

At room temperature, the eggs began to hatch after 33 days and hatching continued for 21 days. The incubation period in the cold room was 78 days. Eggs reared under a constant photoperiod of 12½ hours of light per day and under temperatures varying daily from 1° to 7°C (not shown in figure) began to hatch after 194 days. This long incubation period seems to be due to the low temperatures experienced by the eggs. Percival and Whitehead (1928) recorded an incubation period of 91 days at about 15.5°C.

Isogenus nubecula (Figure 17)

3 egg-batches were studied.

Eggs reared at room temperature began to hatch after 34 days. In the cold room, hatching occurred after 65 days.

Most of the hatching occurred within a short period and the pattern of behaviour was fairly similar to that of P. microcephala.

The present study has shown, for the first time, the occurrence of diapause in the eggs of N. cinerea, A. standfussi, B. risi and D. bicaudata. In those species that did not undergo diapause in the egg-stage, the incubation periods were generally shorter at room temperature than at the lower temperatures in the cold room. The breaking of diapause in N. cinerea, A. standfussi and D. bicaudata seemed to depend upon a fall of temperature but in B. risi this did not appear to be so. Although the fairly high room temperature did not prevent the development of the embryos in B. risi and in the non-diapause eggs of the various species, the successful hatching of these eggs was greatly reduced in several cases. The unfavourable effect of high temperatures on the hatching of the eggs was probably due to the lower oxygen content of the water, which might not be sufficient to meet the needs of the embryos during eclosion.

The occurrence of a delayed hatching of some eggs was shown by only a few species. In L. fusca, L. moselyi and probably also I. grammatica the fairly long hatching period of any egg-batch was due to the irregular development among the eggs. In B. risi and A. standfussi this was the result of a gtadual breaking of diapause. In N. cinerea,

the long hatching period of about 5 months seemed to be due to the irregular development of the eggs and to the ability of the fully-developed embryos to survive within the eggs for long periods at low temperatures. The prolonged hatching period recorded for D. cephalotes seemed to be due partly to the environmental conditions experienced by the eggs. The very long hatching period of the eggs laid by adults of D. bicaudata from Lake Bala differed significantly from the pattern shown by other species in that there was a definite 'break' in the hatching period due to the occurrence of diapause and non-diapause eggs within the same egg-batch.

5. Biology of the Nymphs

(a) Introduction

The number of instars of only 4 species of stoneflies has so far been studied, viz. Nemoura vallicularia Wu (22 instars, recorded by Wu 1923), Perla burmeisteriana Claas. (22 instars, according to Samal 1923), Perla cephalotes Curtis (33 instars in the females and fewer in the males, according to Schoenemund 1925) and Pteronarcys proteus Newm. (13 instars in the females and 12 in the males, according to Holdsworth 1941a, b). Of these 4 species, only N. vallicularia has ever been reared through its life-cycle. Wu (1923) reared the newly-hatched nymphs individually in small vials covered with bolting silk, and these were placed in a shallow tray which was then sunk into the bed of a stream. Various attempts by other workers to rear newly-hatched nymphs in the laboratory have not been successful. Helson (1935) was unable to rear the nymphs of Stenoperla prasina Newm. beyond the first instar. Samal (1923) and Miller (1939), however, reared P. burmeisteriana and P. proteus respectively to the 3rd or 4th instars. The morphology of the early instars has been described by several authors (e.g. Samal 1923, Helson 1935, Miller 1939, Hynes 1941 and Brinck 1949).

During the present studies, the nymphs of several species were reared through their complete life-cycles in the laboratory and of these, the number of instars and the rate of moulting of nine species were successfully followed. Many other species were

	1st		2nd		3rd		4th	
	Cercal	Antennal	Cercal	Antennal	Cercal	Antennal	Cercal	Antennal
(a) FILIPALPIA								
(1) Nemouridae								
Nemoura avicularis	4	9	5	11	♂	14	10(11)	20
N. cambrica	4	9	5	11	7 (♂)	13	9(10)	15
N. cinerea	4	9	6 (5)	11	♂ (9)	14(15)	11(12)	20
Nemurella picteti	4	9	6 (5)	11	♂	14	11	20
Amphinemura sulcicollis	4	9	5	10	6	12	7 (♂)	14
A. standfussi	4	9	5	11	-	-	-	-
Protonemura praecox	4	9	5	10	6 (7)	11	♂ (9)	13
P. meyeri	4	9	5	10	6	11	♂ (9)	13
(2) Leuctridae								
Leuctra fusca	4	9	6	11	♂	13	10(11)	15
L. geniculata	4	9	6	10	9	12	11	15
L. hippopus	4	9	6	10	♂ (7)	12	10 (9)	15
L. inermis	4	9	6	10	7	11	♂	13
L. moselyi	4	9	6	9 (10)	♂ (9)	12	10(11)	15(16)
L. nigra	4	9	6	10	9	12	12	14(15)

(3) Capniidae								
Capnia bifrons	3	9	5 (6)	11	8 (9)	13	10(11)	13
(4) Taeniopterygidae								
Brachyptera risi	4	9	4	9	10	13	17	18
Taeniopteryx nebulosa	3	9	4	10	6 (7)	13	9	16
(b) SETIPALPIA								
(5) Chloroperlidae								
Chloroperla torrentium	3	9	3	9	4	11	5	13
C. tripunctata	3	9	3	9	-	-	-	-
(6) Perlodidae								
Diura bicaudata	3	9	4	9	6	13	8	?
Isogenus nebecula	3	9	4	9	-	-	-	-
Isoperla grammatica	3	9	3	9	4	10	5 (6)	12
Perlodes microcephala	3	9	5	11	7	13	-	-
(7) Perlidae								
Dinocras cephalotes	3	9	3	9	4	11	5	14

Table 4

reared through the early instars, and the morphology and growth of these young stages will first be described.

(b) Morphology and growth of early instars

Samal (1923) and Miller (1939) recorded 3 cercal and 9 antennal segments in the newly-hatched nymphs of P. burmeisteriana and P. proteus respectively. The same number of segments has also been recorded by Hynes (1941) for P. microcephala, D. cephalotes, P. bipunctata and C. torrentium. Brinck (1949) studied the early instars of T. nebulosa, C. bifrons, L. hippopus, N. erratica and N. picteti. He mentions that "the number of antennal segments in the first (and second) instars is always 9, and cercal joints are 3, in Leuctra 4". Nelson (1935) found that the first instars of S. prasina have 5 cercal and 11 antennal segments. The results of the present studies, however, are not in complete agreement with Brinck's statement.

Table 4 shows the number of cercal and antennal segments in the first four instars of 24 species. The data for all of them except T. nebulosa were based on nymphs reared in the cold room. The nymphs of T. nebulosa were reared at room temperature. The figures within brackets indicate the numbers that were occasionally encountered.

From this table, it can be seen that the first instars of all species have 9 antennal segments. However, as regards the cercal segments, the Setipalpia have 3 while the Filipalpia have 4 except C. bifrons and T. nebulosa which have 3. Thus, the

descriptions of the various Setipalpian species by Samal, Miller and Hynes agree with the present observations. The nymphs of N.pictati have 4 cercal segments and the same number seems to occur throughout the Nemouridae which therefore disagrees with Brinck's description of N. pictati and possibly also N. erratica It is perhaps of significance that the first instars of S. prasina, which belongs to the very primitive family Eusthenilidae (sub-order Archiperlaria Illies 1960), should have a higher number of cercal and antennal joints than the numbers of the two other sub-orders, namely, the Setipalpia and the Filipalpia. Further studies on the first instars of other species and families might provide an indication of the evolutionary tendencies within the Plecoptera and to the affinities between the various members of this group.

There is an increase in the number of joints in the second instars of most of the Filipalpian species except B. risi in which the number remains the same as that of the first instars. There is no increase in many of the Setipalpia but in D. bicaudata and I.nubecula there is an increase of one cercal segment even though the number of antennal segments remains the same, and in P. microcephala both the cerci and antennae show an increase of 2 segments each.

After the second instar, the nymphs of all species normally show an increase in the number of joints with each moult but in C. bifrons there is an interesting exception. The nymphs of this species undergo diapause (Chapter IV) usually at the 4th or 5th instar and the diapause stage often does not show an

increase in the number of antennal segments over the previous instar. Thus, a diapause 4th instar nymph has 10 (sometimes 11) cercal and 13 antennal segments while a non-diapause nymph of the same instar has 10 (sometimes 11) cercal and 15 antennal segments. Although there is an increase in the cercal segments in the diapause stage, the cerci are, however, quite different from those of a non-diapause nymph in which there are bristles on every joint. In the diapause nymph these bristles are found only on the few basal segments. Also the cercal segments towards the tip are extremely slender (Figure 50). The post-diapause nymphs have the same or fewer cercal joints than the previous diapause stage but there is an increase of one or two antennal joints. Thus, a post-diapause 5th instar nymph has 9 (sometimes 10) cercal and 14 or 15 antennal segments. The absence of any increase in the cercal segments seems to be due to the breaking off of the slender segments at the tip during moulting.

The increase in the number of joints, particularly of the antennae, seems to be associated with the increase in size of the nymphs. Among the various Leuctra spp., the young nymphs of L. inermis show a relatively smaller increase in size than those of other species (figures 19 to 22) and this is also reflected in the fewer cercal and antennal joints in the early instars of L. inermis. In C. bifrons, nymphs destined for diapause are smaller than non-diapause nymphs of the same instar and it has been found that the former show a lower number of antennal segments than the latter. Within the Nemouridae, the number of joints

in the 3rd and 4th instars of N. cambrica, A. sulcicollis and Protonemura spp. is fewer than that of the corresponding instars of other species. In the above mentioned species, the increase in size of the early instars was observed to be rather small. It has also been found that stunted nymphs of various species (e.g. L. moselyi, L. inermis) reared at room temperature showed a smaller number of joints than the corresponding instars of specimens reared in the cold room.

Gills are present in the form of a tiny stump on the prosternum of the newly-hatched nymphs of Amphinemura spp. and Protonemura spp. In D. cephalotes, the first and second instars possess only anal gills which are present as 4 strands on each side of the body. In T. nebulosa no gills are found during the early instars.

The second instar nymphs are generally much more similar to the later instars than to the first and this is especially obvious in the Leuctridae. In this family, the first instars of the different species are indistinguishable from one another and all have long hairs or bristles on every abdominal segment. However, the second instars of L. moselyi show the occurrence of bristles only on the 10th (sometimes also the 9th) abdominal segment. In L. hippopus the bristles occur on the last 3 or 4 abdominal segments while in L. fusca they are present on the last 5 or 6 segments. In L. inermis, L. nigra and L. geniculata the bristles are found on every segment but in L. inermis they are shorter and less-tapering than in the other two species. In the Setipalpia there does not appear to be any great

difference in the distribution of bristles between the first and later instars, but the bristles on the thoracic nota are much more conspicuous in the latter than in the former. Also the nymphs begin to acquire pigmentation in the second instar.

The first instars of Setipalpia do not feed but in the Filipalpia feeding occurred in the first instar.

Brinck (1949) prefers to regard the first 2 instars as nymphulae since they are very much alike and differ from older nymphs. He mentions too that the nymphulae often survive at seasons adverse to the species as quiescent stages. Brinck's analysis, however, seems to be a misconception which arises from the fact that the second instars of several of the Setipalpian species do not show an increase in the number of cercal and antennal joints. It has now been found that the second instars of many species in fact differ significantly from the first and that they are much more like the later instars. The ability to survive adverse conditions is not restricted to the first two instars but to the young nymphs in general. It was found that the first few instars of many species were able to survive without ill-effects at room temperatures which were unfavourable to the later instars.

The rate of moulting of the early instars of several species that were reared in the cold room is shown in figure 18. The date of occurrence of an instar is indicated by a dot on top of the number of that instar. A cross indicates only the probable date of occurrence since the nymphs of the previous instar died

Figure 18 - Interval between instars of nymphs reared in cold room.

Environmental conditions in cold room:

P - photperiods in hours of light per day.

T - temperature in ^{o}C.

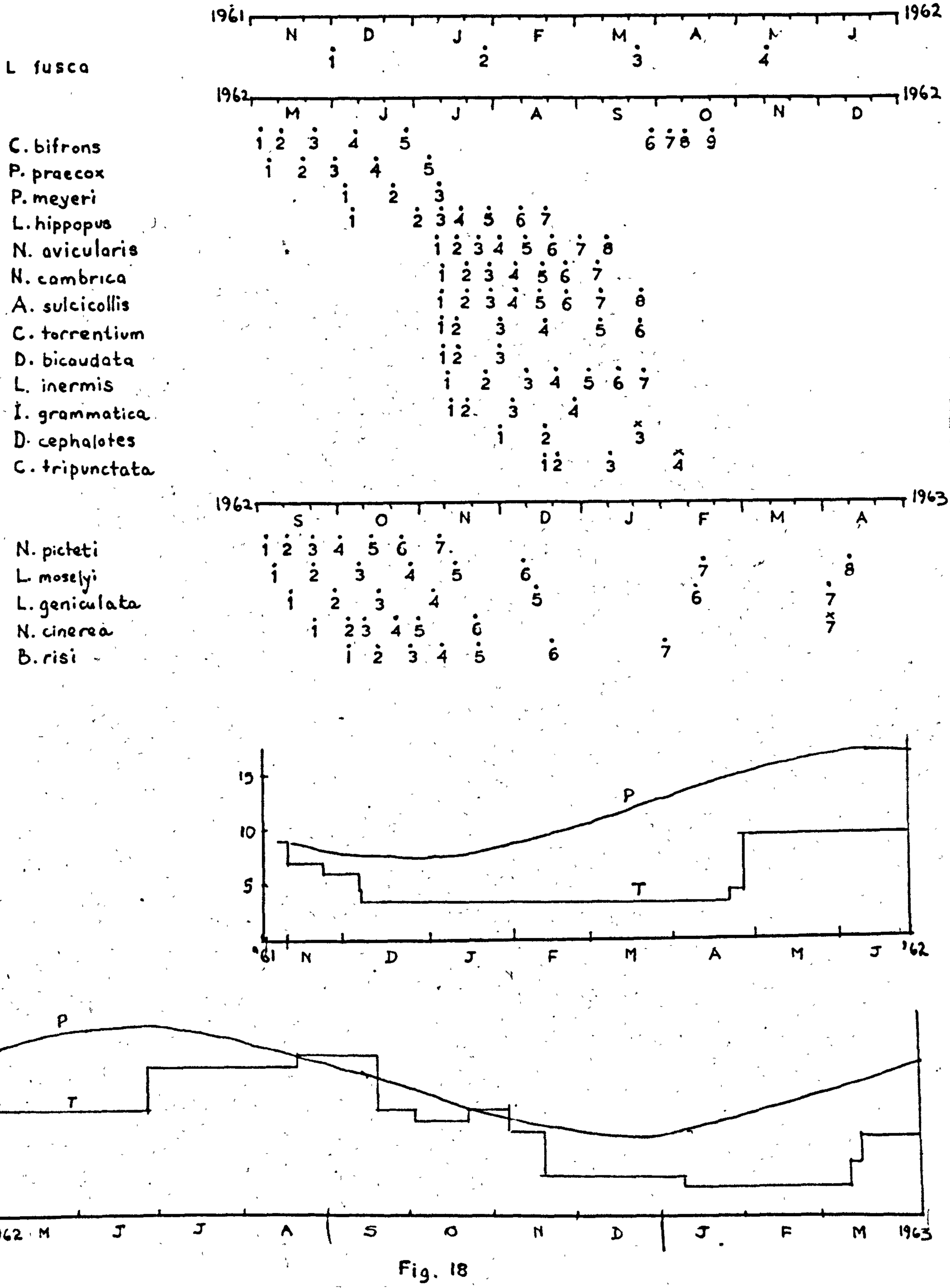

1961
1962
N D J F M A M J
L fusca
1962
1962
M J J A S O N D
C. bifrons
P. praecox
P. meyeri
L. hippopus
N. avicularis
N. cambrica
A. sulcicollis
C. torrentium
D. bicaudata
L. inermis
I. grammatica
D. cephalotes
C. tripunctata
1962
1963
S O N D J F M A
N. picteti
L. moselyi
L. geniculata
N. cinerea
B. risi
15
10
5
0
P
T
'61 N D J F M A M J '62
P
T
1962 M J J A S O N D J F M 1963

Fig. 18

during that period.

In general, it can be said that the rate of moulting is fairly rapid during the warmer months and that there is a retardation during the cold winter period. In C. bifrons, however, the nymphs undergo diapause (see also Chapter IV) during the summer and there is a gradual retardation in the rate of moulting as the nymphs approach the stage of diapause. In P. praecox there appears also to be a slowing-down in the rate of moulting during the summer. Although there is generally a retardation during the winter,,the extent to which the nymphs are affected is quite variable between species. In a cold-water stenotherm such as B. risi the rate of moulting is only slightly retarded but in N. picteti and N. cinerea the nymphs do not seem to moult at all throughout the winter. In N. cinerea, there appears to be some active form of seasonal regulation by the nymphs and the dormancy during winter does not appear to be due only to the cold since nymphs reared at room temperature were also found to show a delayed rate of moulting during the winter period. Some nymphs which hatched in early October were reared at room temperature and by the second half of November they were at the 8th instar. However, no further moults occurred till mid-January. Thus, there was a delay of about 2 months even at room temperature. It is possible that the dormancy in this case may be a case of diapause and that the nymphs may be sensitive to changes of photoperiods. The dormant nymphs of N. cinerea are fairly inactive but they are not completely immobile as are the nymphs of C. bifrons.

In L. fusca and L. moselyi, the fairly long interval between instars in the cold room during the winter months was due to quiescence resulting from the low temperature, and nymphs that were reared at room temperature during this period did not show a significant fall in the rate of moulting.

The first instars of Setipalpian species, (e.g. C. torrentium, C. tripunctata, D. bicaudata, I. grammatica and D. cephalotes) moult much more rapidly than the later instars. In C. torrentium the interval between the first and second instars was 6 days while that between the second and third instars was 16 days at 13°C. Except for the first instars, the interval between moults in the Setipalpia tends to be longer than that of non-diapausing Filipalpian nymphs. In the Filipalpia, the first instars do not normally show any significant difference in the rate of moulting as compared with the later instars reared under fairly similar conditions. The difference in the Setipalpia seems to be associated with the fact that the newly-hatched nymphs do not feed.

(c) Life-cycles and the number of instars

The following species were reared through their complete life-cycles in the cold room: L. fusca, L. geniculata, L. inermis, L. moselyi, L. hippopus, A. sulcicollis, A. standfussi, P. praecox, N. pinteti, N. cambrica, N. avicularis, N. cinerea, C. bifrons, and C. torrentium. A univoltine life-cycle was found in all these species except N. cinerea which has a two-year cycle. N. cinerea

has a long incubation and hatching period (Chapter II, 3e), and nymphs which hatched during October and November of 1962 only began to emerge in April and May of 1964. The young nymphs have been shown to undergo prolonged dormancy during the winter and it has also been found to occur in the older nymphs as well. It was observed that by October 1963 there were several almost full-grown as well as many half-grown nymphs but very little or no growth occurred until the following Spring when the emergence began.

The data for the univoltine species agree with the field investigations made by Hynes (1941, 1961), Brinck (1949) and Rauser (1963), but the first two authors have indicated a one-year life-cycle for N. cinerea. The flight period of this species has been given by Hynes (1958) to be from March to September and by Brinck (1949) to be from April to September. Thus, there is a long flight peropd. From the present laboratory studies it has been found that the hatching of the eggs in dependent upon a fall in temperature and that for any single egg-batch the hatching may continue from late September to February. It is certain therefore that the adults which emerge during the early part of the flight period cannot have come from eggs laid by the last generation since the nymphs undergo prolonged dormancy during the winter and there is only a very short period for growth. However, those adults which emerge at the end of the flight period may possibly complete their development within a year since the nymphs which hatched early would be able to reach a fairly late stage of development before becoming dormant as the winter set

in. There would also be a reasonably long interval, once the dormant period was over, for the nymphs to undergo through the remaining instars before emergence.

There was no opportunity to rear L. nigra through its life-cycle. However, nymphs which hatched in early July 1963 and were reared in the cold room were found to be only 2 to 3 mm. long in late April 1964; in fact, many nymphs were less than 2 mm. Hynes (1958) gave the flight period of this species as April to August but mainly April to May. Brinck (1949) mentioned a univoltine life-cycle for this species and he gave the flight period in Southern Sweden as May to July. The possibility of a 2-year life-cycle for some of the nymphs cannot be excluded since they show so very little growth during the period from July to April, and it seems quite unlikely that the very small nymphs found in April could have completed their development by the end of the flight period. This species seems to show also a prolonged dormancy during the winter period.

The nymphs of D. bicaudata were reared only through their first few instars. Brinck (1949) and Hynes (1961) found a rapid growth rate in the nymphs and had suggested a univoltine life-cycle for this species. Both suggestions were based on the assumption that there was a very short incubation period. This has now been found not to be so. The specimens of D. bicaudata from Afon Hirnant, where Hynes carried out his study, have been found to lay only diapause eggs which have an extremely long incubation period of 9 to 10 months. Thus, from the present information on the

incubation period and from the studies on the growth of the nymphs by Hynes (1961), it appears that the D. bicaudata which hatch from diapause eggs must have a 2-year life-cycle. The specimens of D. bicaudata studied by Brinck (1949) seemed also to belong to the same category. The D. bicaudata from Lake Bala, however, lays both diapause and non-diapause eggs and the incubation period of the latter was about 3 months in the cold room and $1^{2}/_{3}$ months at room temperature. It is possible that nymphs hatched from non-diapause eggs may complete their development within a year, in which case then the species occurring in this locality will have a one-year as well as a two-year life-cycle.

The nymphs of C. tripunctata were reared through their early instars and they seemed to show a longer interval between moults than those of C. torrentium. The former species is the larger of the two and there is no reason to suppose that it undergoes a fewer number of instars than the latter. Although the incubation period was not particularly long it was nevertheless longer than in C. torrentium. The overall slower rate of growth and development in C. tripunctata thus supports the statement by Hynes (1941, 1961) that this species has a two-year life-cycle. The size-frequency histograms given by Hynes (1961) showed the occurrence of half-grown nymphs even after the flight period was over. These nymphs would have been one year old since they could not possibly have grown so rapidly from any recently hatched nymphs. The half-grown nymphs continued their growth and development

until their emergence at the following flight season.

The number of instars of 9 species were successfully followed and the results are shown in Table 5. The figures within brackets indicate the number of nymphs that completed development at a particular instar.

	COLD ROOM		ROOM TEMPERATURE	
	Male	Female	Male	Female
L. fusca	* 12 (1)	* 13 (1)	16 (1)	16 (1)
L. geniculata	12 (3)	12 (1), 13 (1)	++ 14 (1)	
L. hippopus	-	12 (2)	-	-
L. inermis	13 (1), 14 (1)	14 (1), 15 (2)	-	-
L. moselyi	13 (2), 14 (1)	13 (1)	-	-
C. bifrons	14 (2), 15 (1)	15 (1), 16 (1)	-	-
N. avicularis	13 (1)	13 (3)	-	-
N. picteti	-	16 (1)	-	-
C. torrentium	12 (1)	-	-	-

* - First 2 instars reared at room temperature

++ - Died at final instar, sex was not determined

Table 5

From this table, it can be seen that the nymphs of stoneflies go through a fairly large number of instars. The number is variable within a single species and there is a tendency for the females to have a higher number than the males, e.g. L. fusca, L. geniculata, L. inermis and C. bifrons. Nymphs reared at

room temperature also tend to have more moults than those reared in the cold room. It is interesting to point out that only L. fusca and L. geniculata successfully completed their life-cycles at room temperature. Both these species normally grow during late Spring and Summer and as such are tolerant of the high temperatures. The nymphs of L. hirpopus were reared till the 16th (and probably the penultimate) instar at room temperature while in the cold room, emergence occurred at the 12th or 13th instar. L. moselyi and L. inermis were reared at room temperature till the 14th and 15th instars respectively but they were still immature and were less than half the size of mature nymphs reared in the cold room. In N. avicularis the nymphs reared till the 14th instar at room temperature were still immature whereas in the cold room emergence occurred at the 13th instar. Although the number of instars in A. sulcicollis was not successfully followed, it was found that the nymphs reared in the cold room were fairly well developed at the 14th instar, whereas at room temperature a 20th instar nymph was still immature.

(d) Growth and Development

The pattern and rate of growth of 9 species that successfully completed their development in the cold room are shown in figures 19 to 27. Figure 27, however, shows only the rate of moulting of L. fusca as there was no

complete set of exuviae available to show the increase in size of the nymphs. For the purpose of comparison, the growth of some of these species at room temperature is also given in figures 28 to 32. Of these, however, only L. fusca (Figure 28) and L. geniculata (Figure 29) completed their development at room temperature.

Graph A in each of these figures shows the increase in length of the hind tibia at each instar. The tibial lengths were measured from the exuviae of each instar by means of an ocular micrometer placed inside a monocular microscope. The lengths are expressed in units, one unit being equivalent to 17.4 microns. The pattern of growth has also been expressed by plotting the logerithm of the tibial lengths against the number of the instar so as to show the deviations, if any, from the geometric increase that normally occurs in each moult of an insect. Graph B shows the rough increase in size of the nymphs based on measurements of the whole exuviae. Graph C shows the rate of moulting (n) and the rate of increase in the length of hind tibia (x) by plotting the instar number and the tibial length of a particular instar against the date of occurrence of that instar. The temperatures in the cold room have been superimposed on Graph C in figures 19 to 27. The room temperatures are not shown in figures 28 to 32 but the averages of the maximum and minimum temperatures varied from 11° to 21°C.

Figures 19 - 26 - Pattern and rate of growth of nymphs in the cold room.

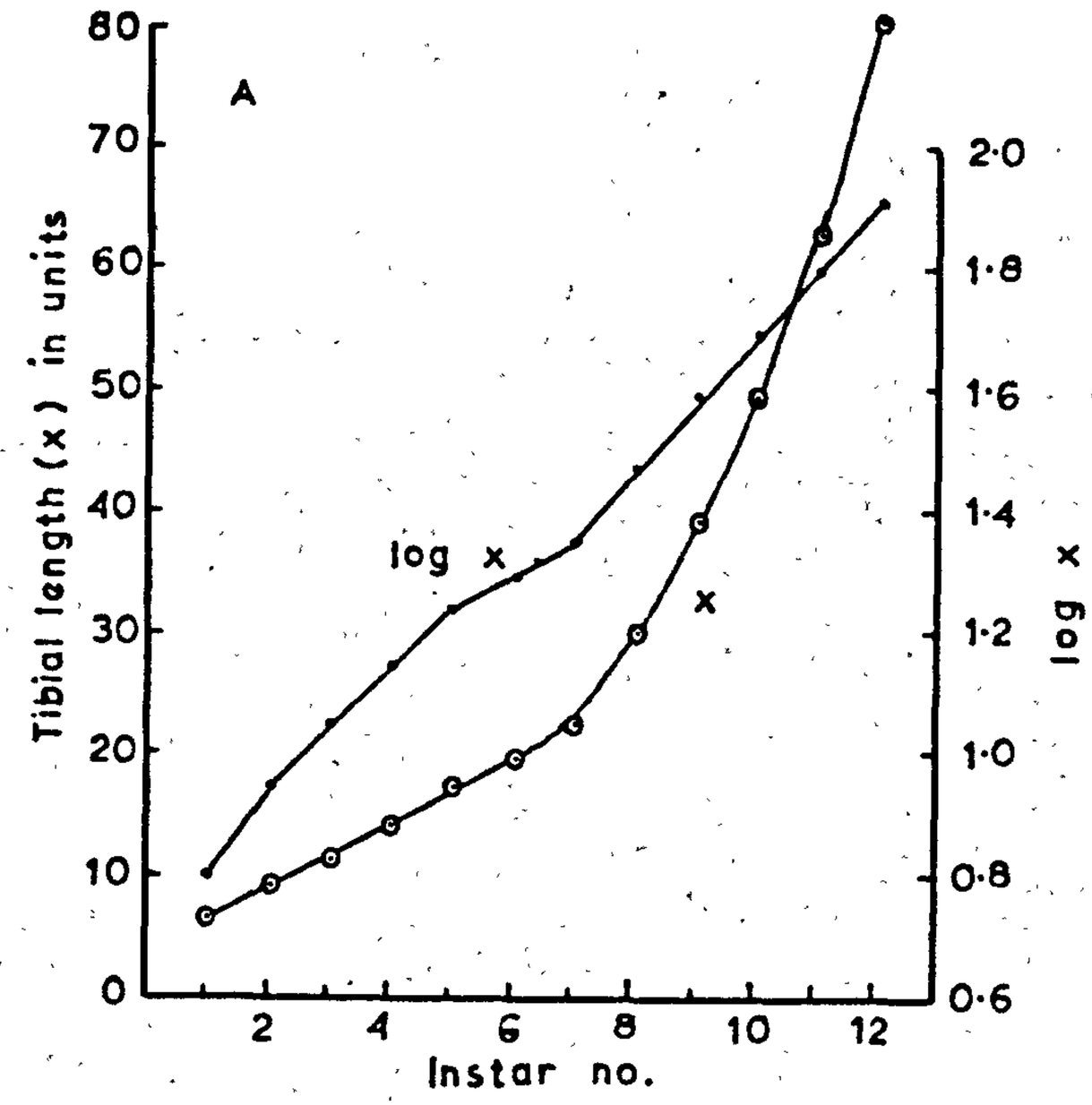
Figure 27 - Rate of moulting of L. fusca in the cold room.

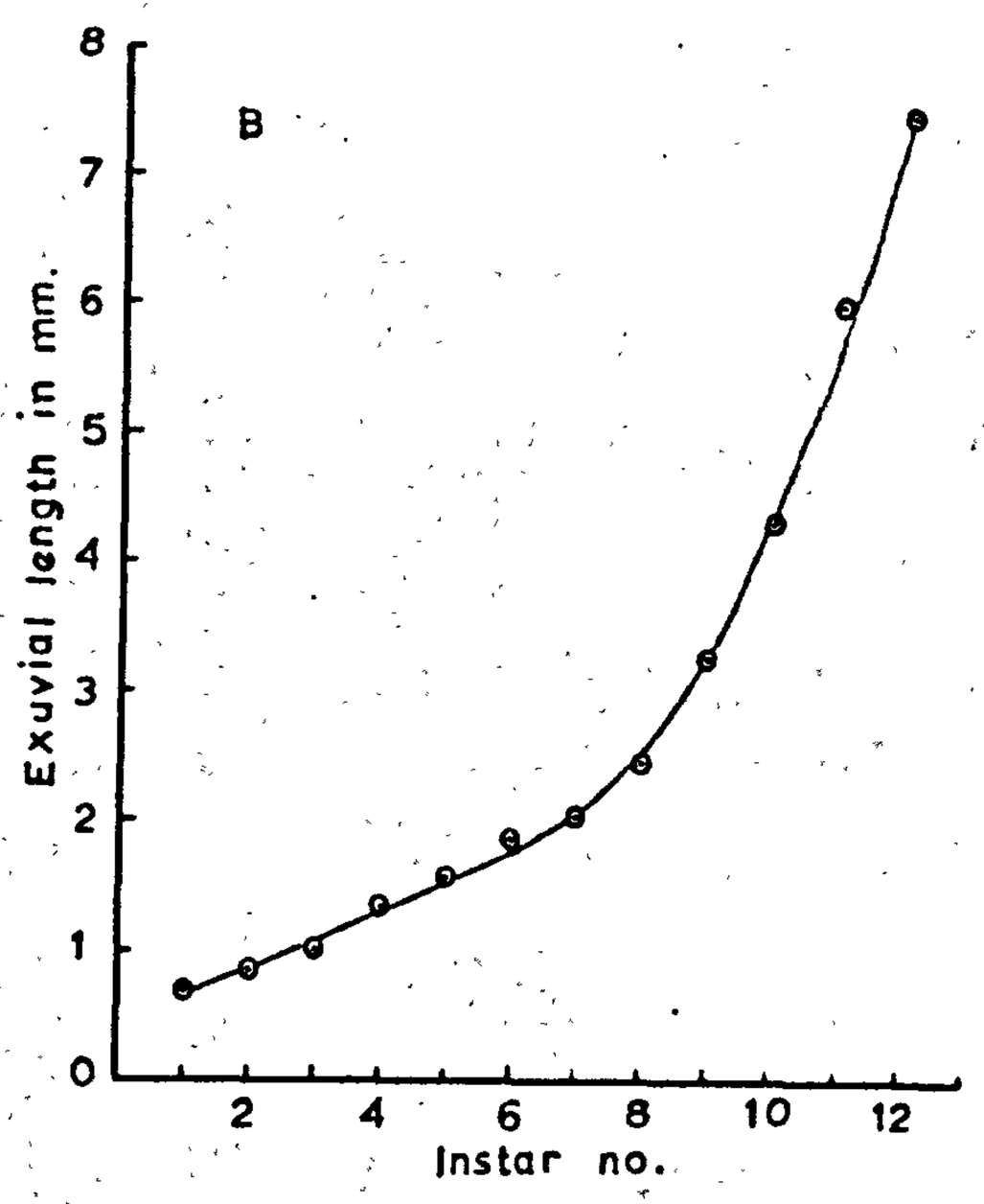
Figures 28 - 32 - Pattern and rate of growth of nymphs reared at room temperature.

Graph A - Logarithmic and linear increase in length of hind tibia at each instar.
Tibial length (1 unit - 17.4 microns)

Graph B - Increase in length of exuviae (in mm.) at each instar.

Graph C - Rate of moulting and of increase in length of tibia.
(Temperature conditions in cold room have been superimposed).





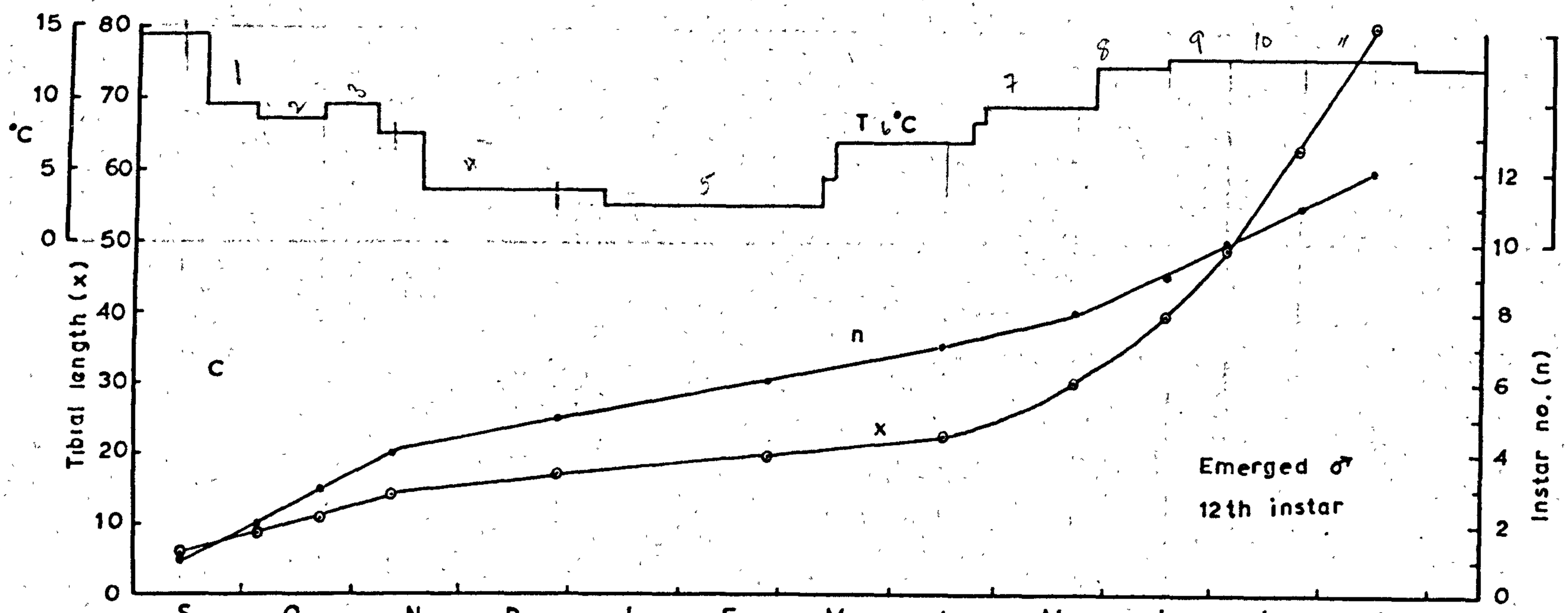
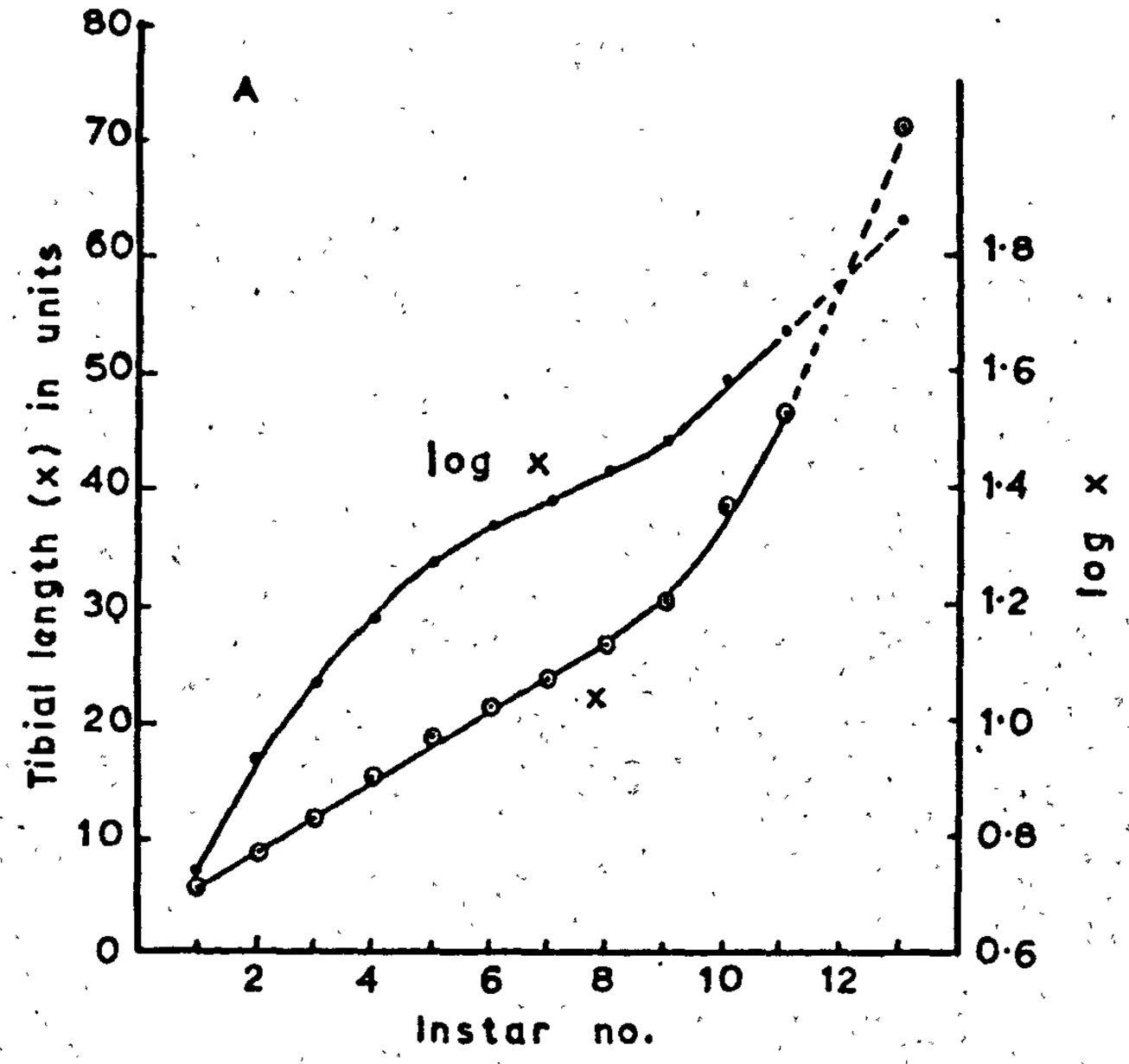
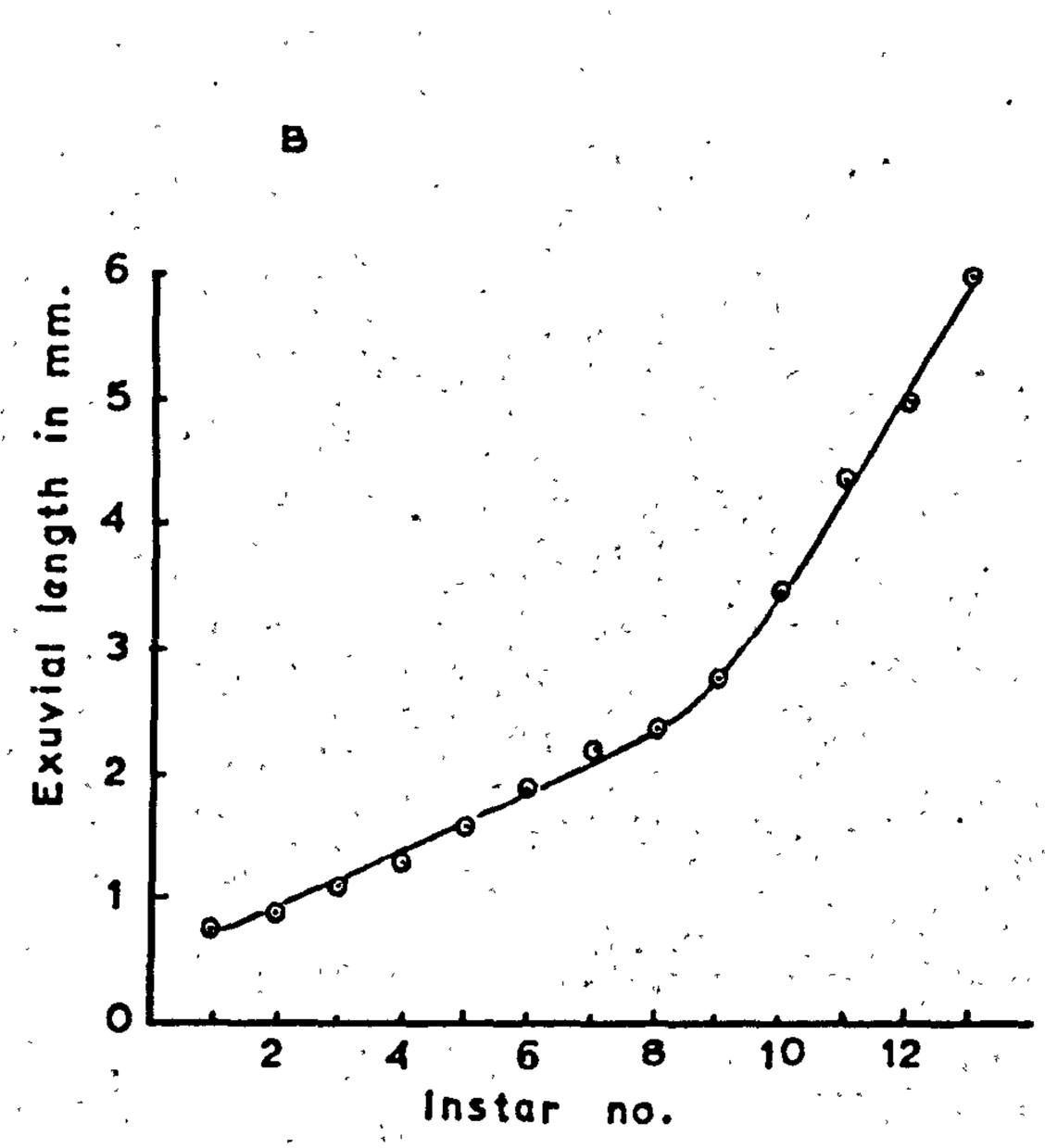


Fig. 19 L. geniculata





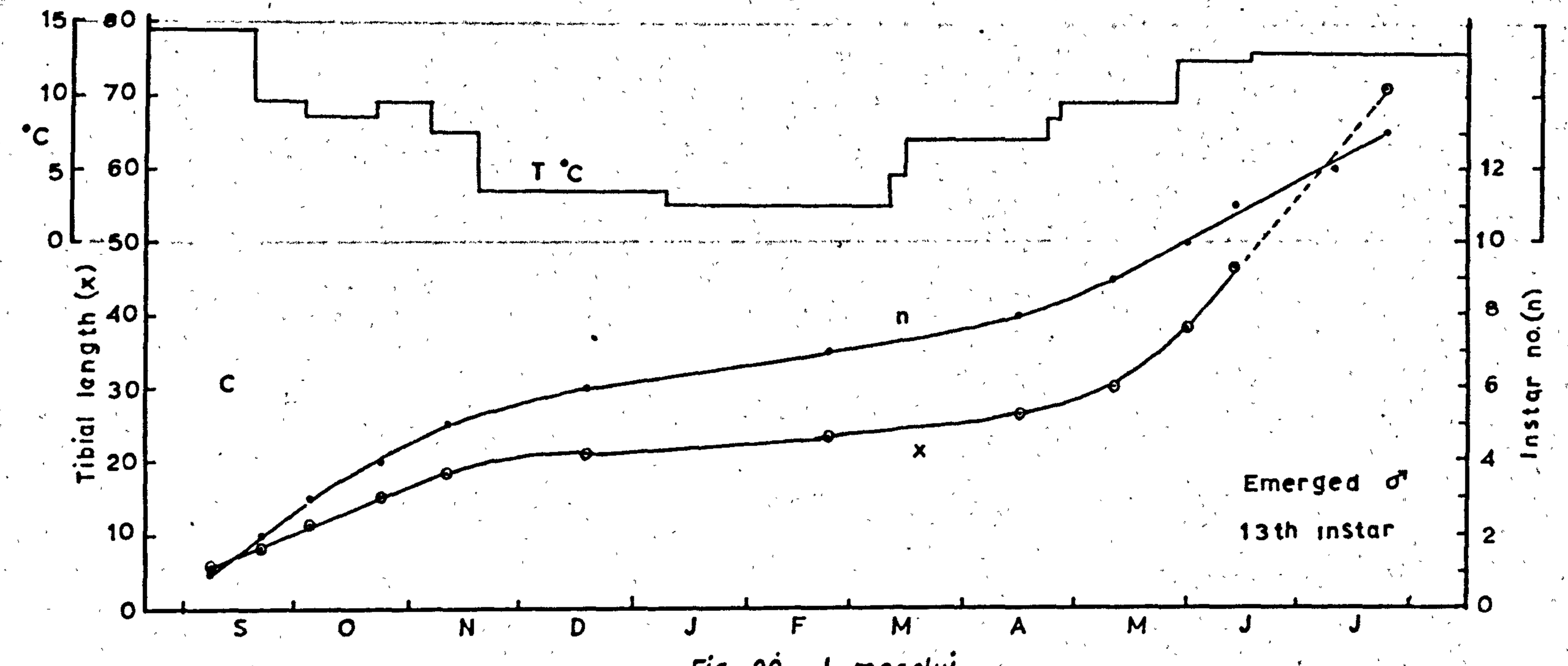


Fig. 20 L. moselyi

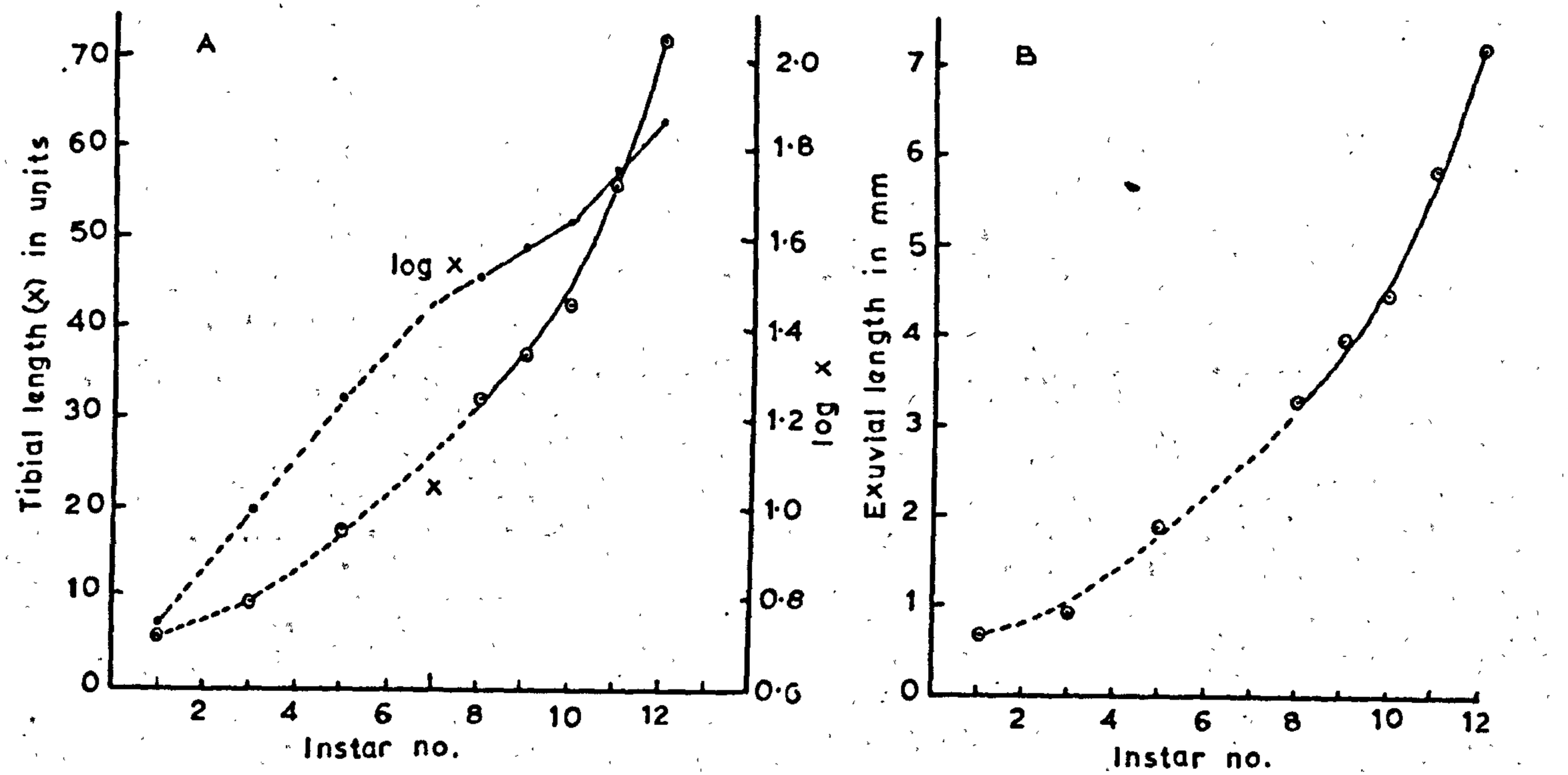


Fig. 21 L. hippopus

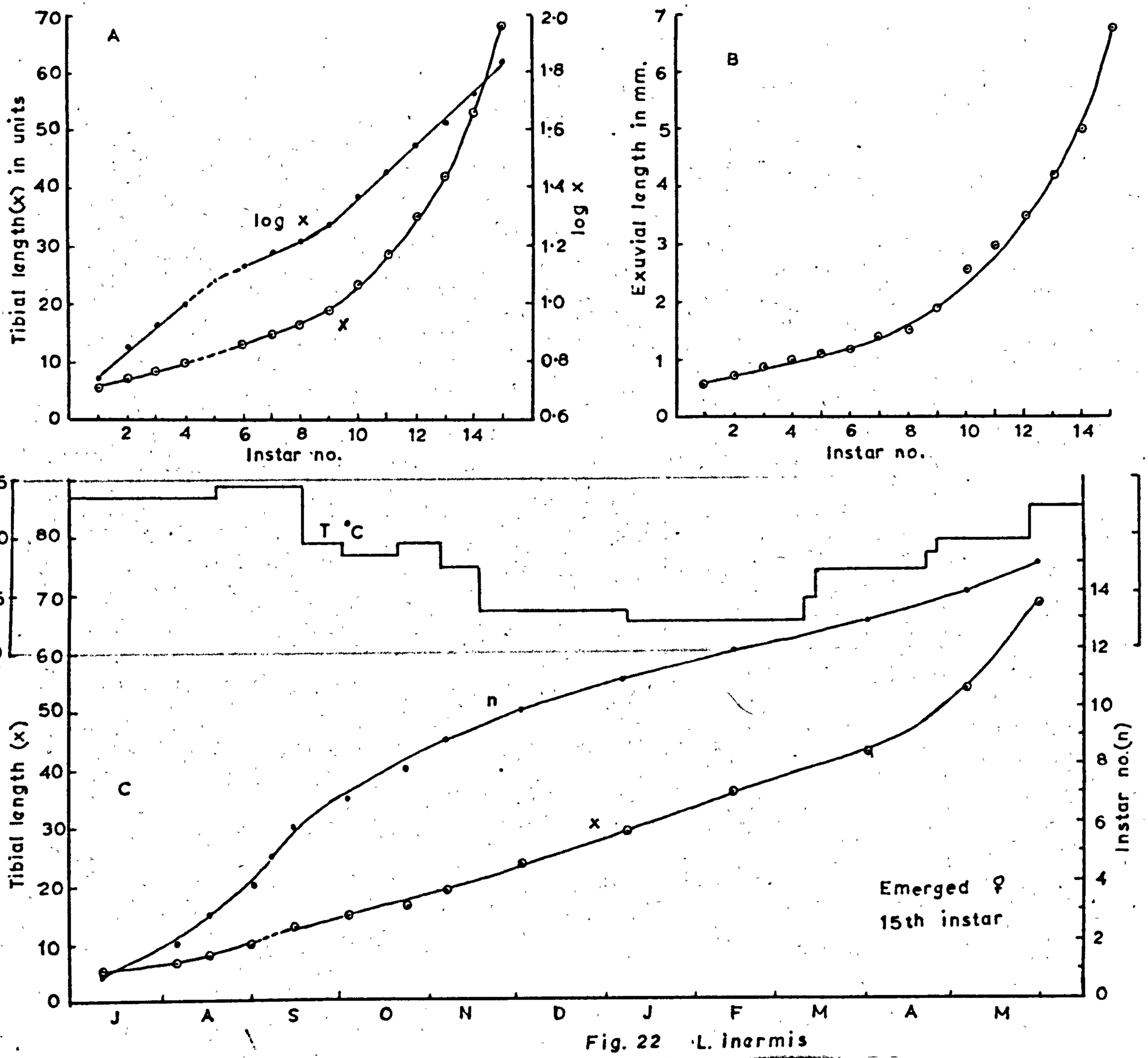
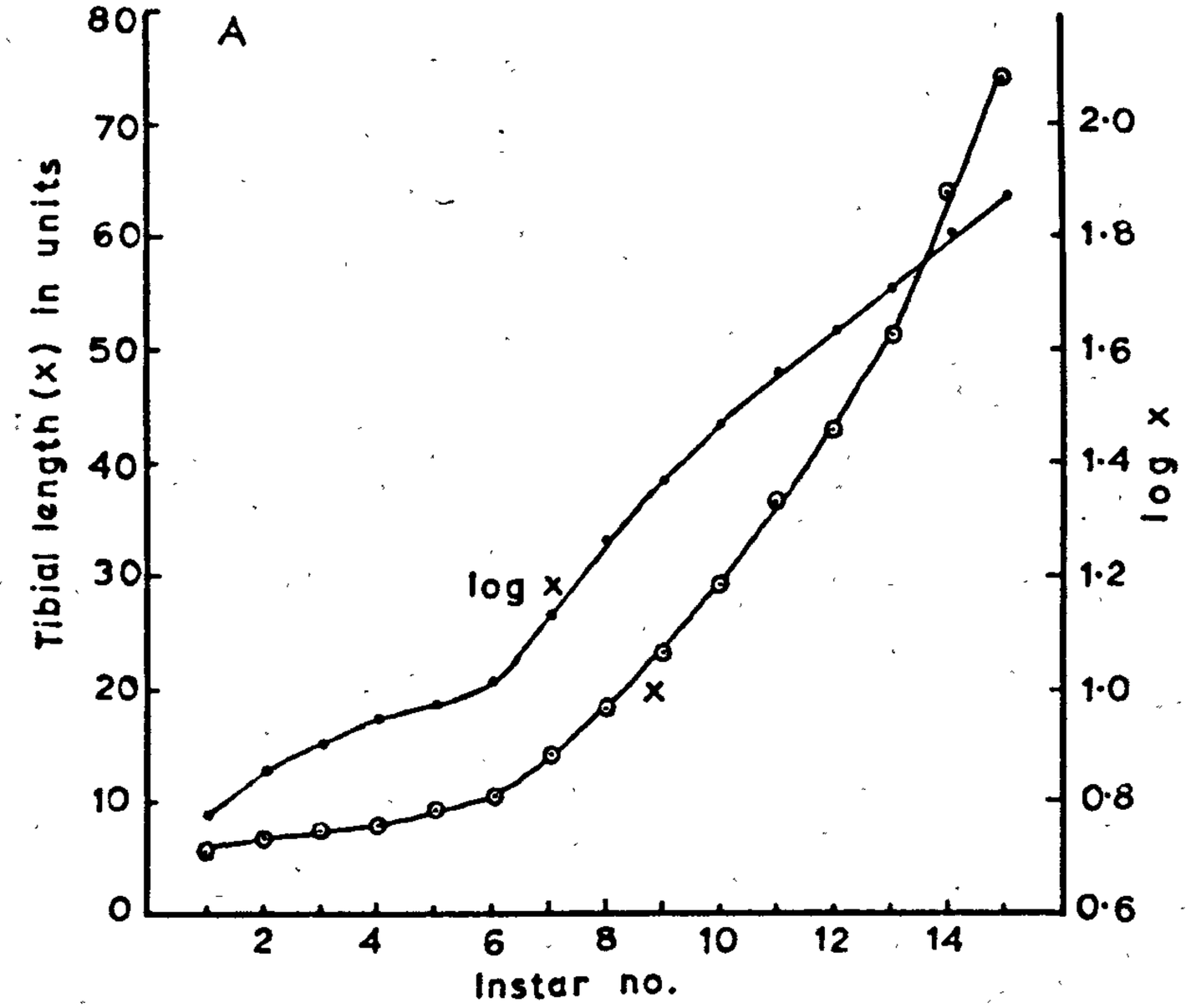
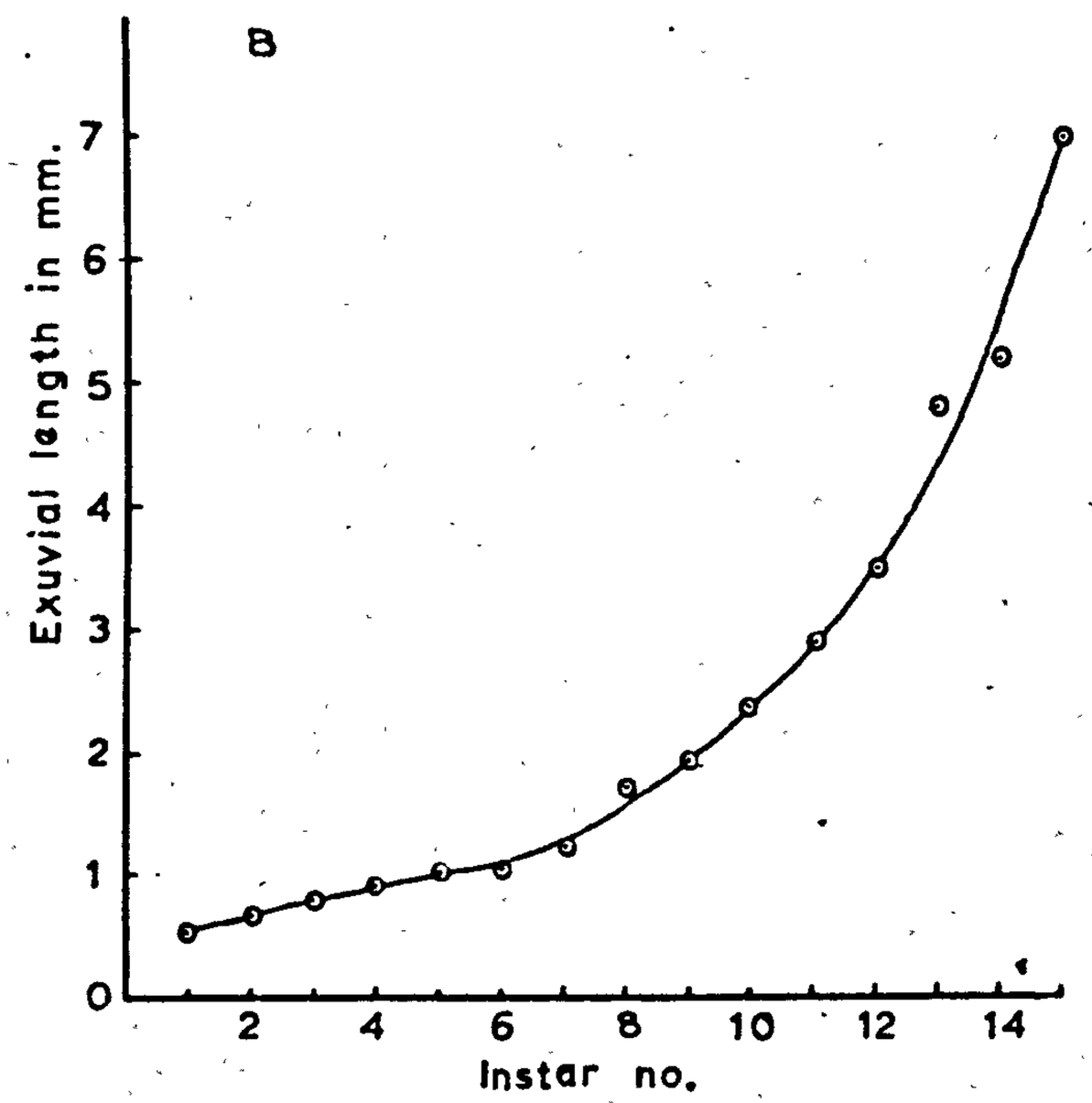


Fig. 22 L. inermis





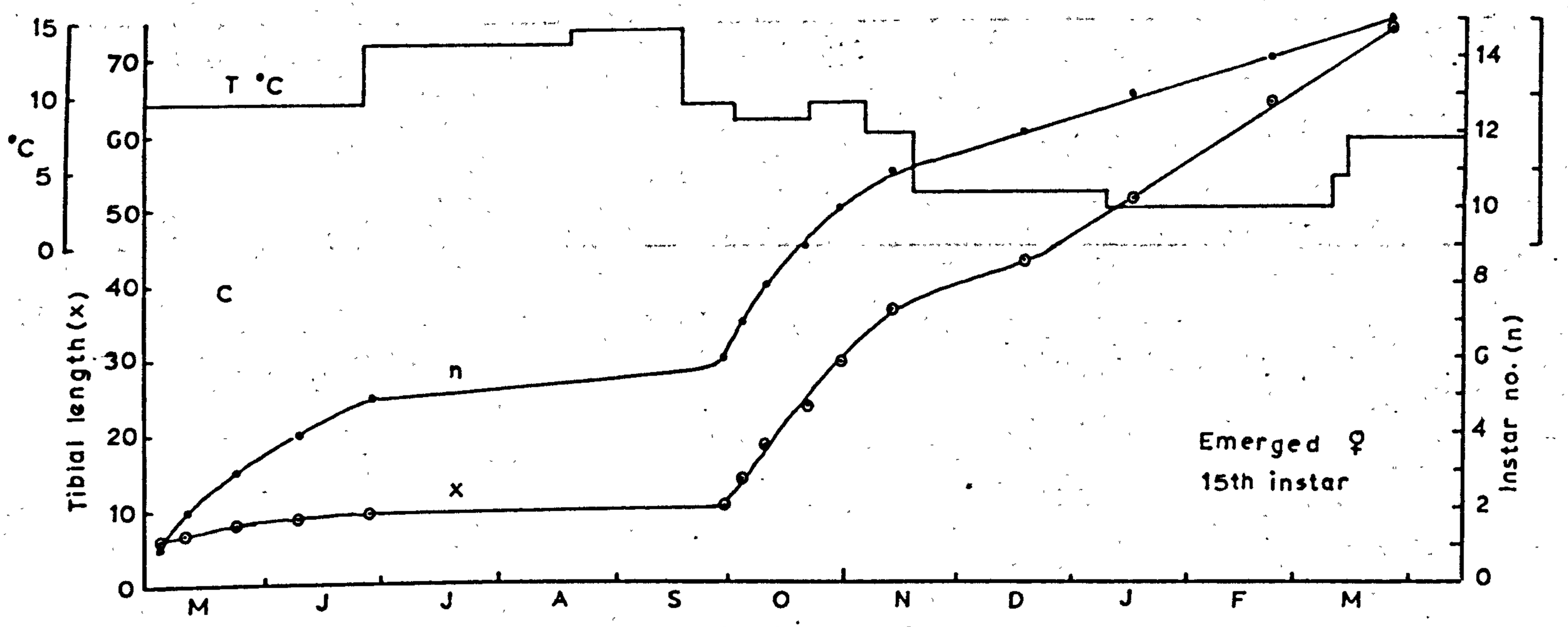


Fig 23 C. bifrons

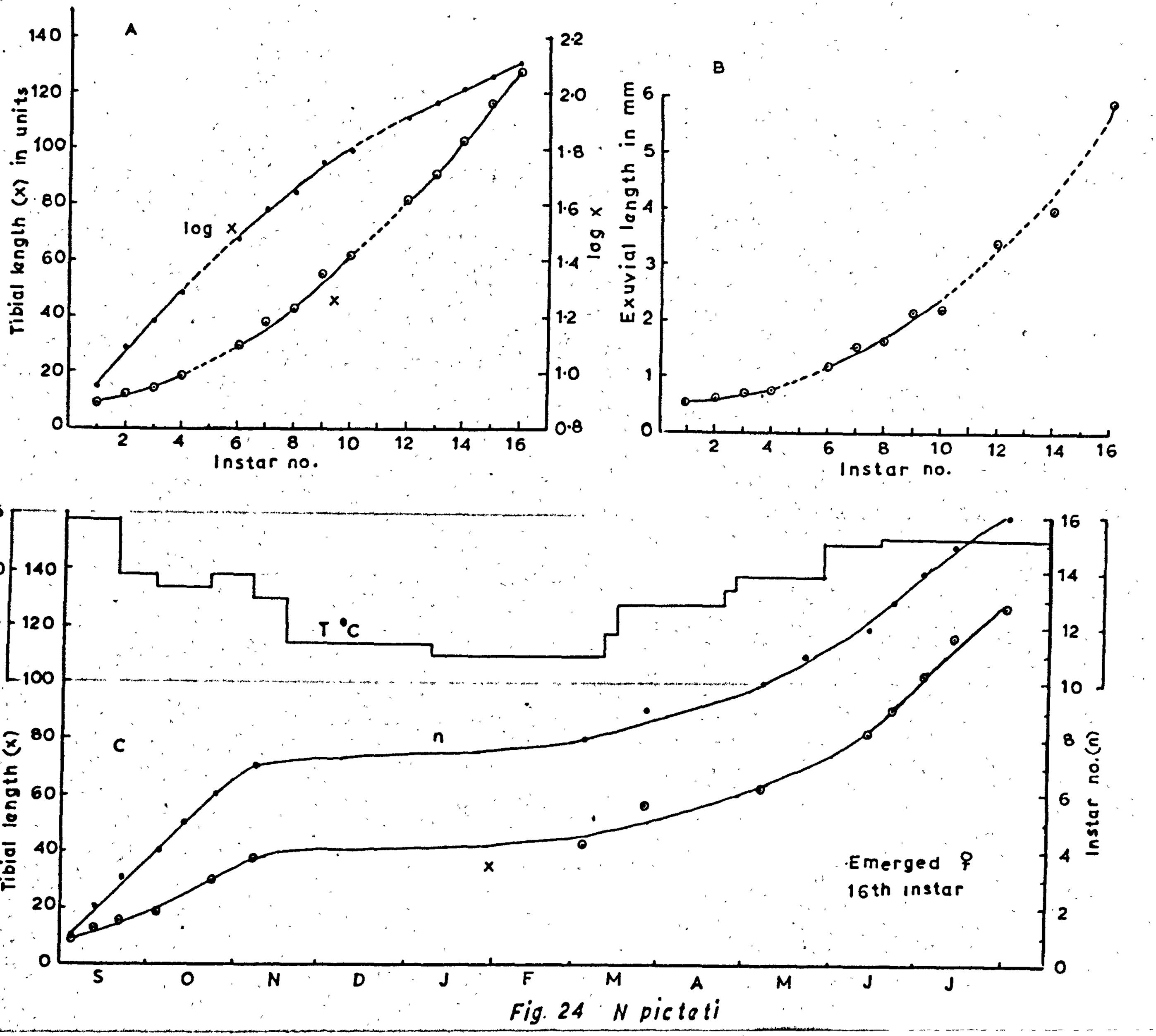


Fig. 24 N pictoti

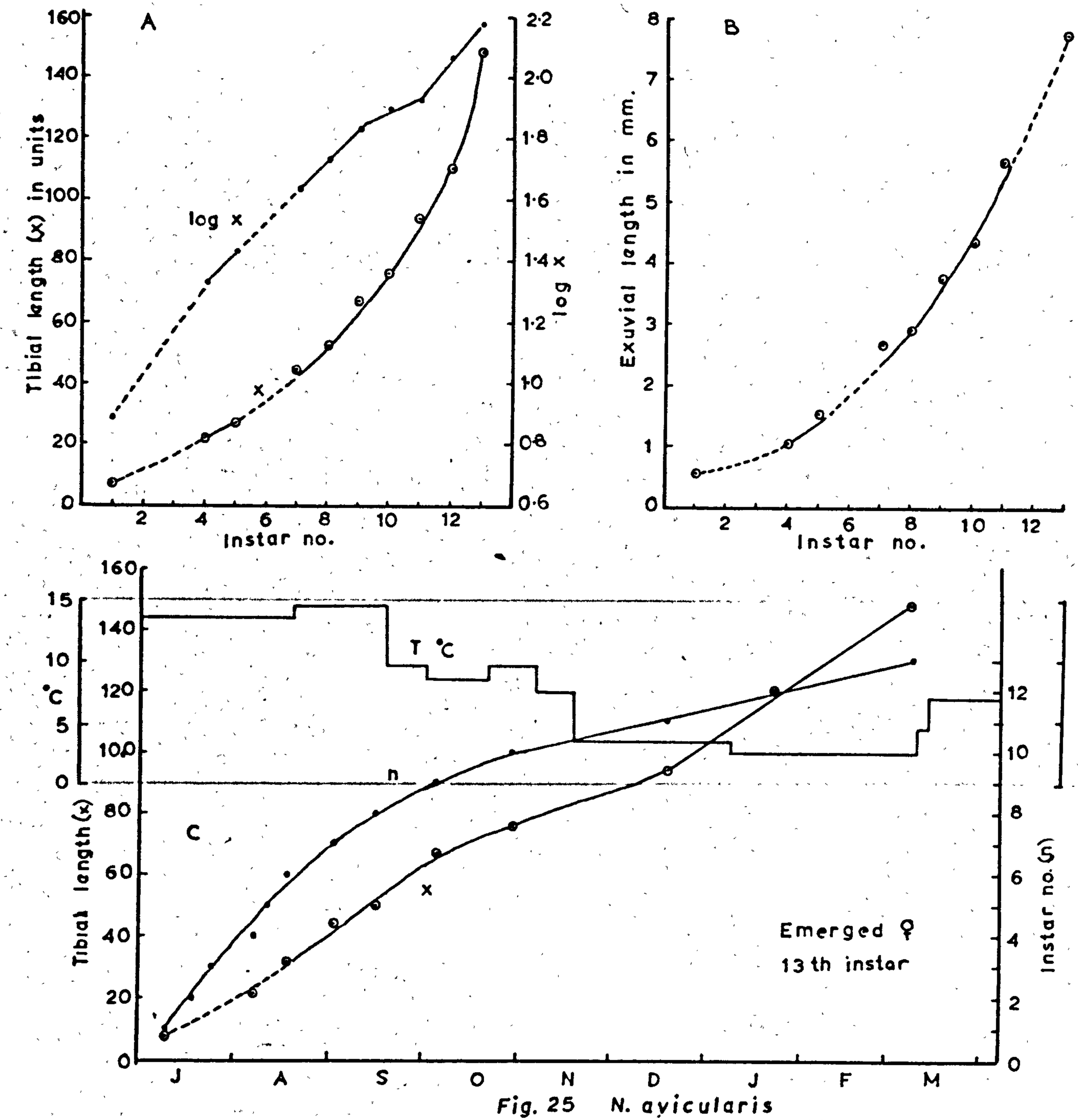
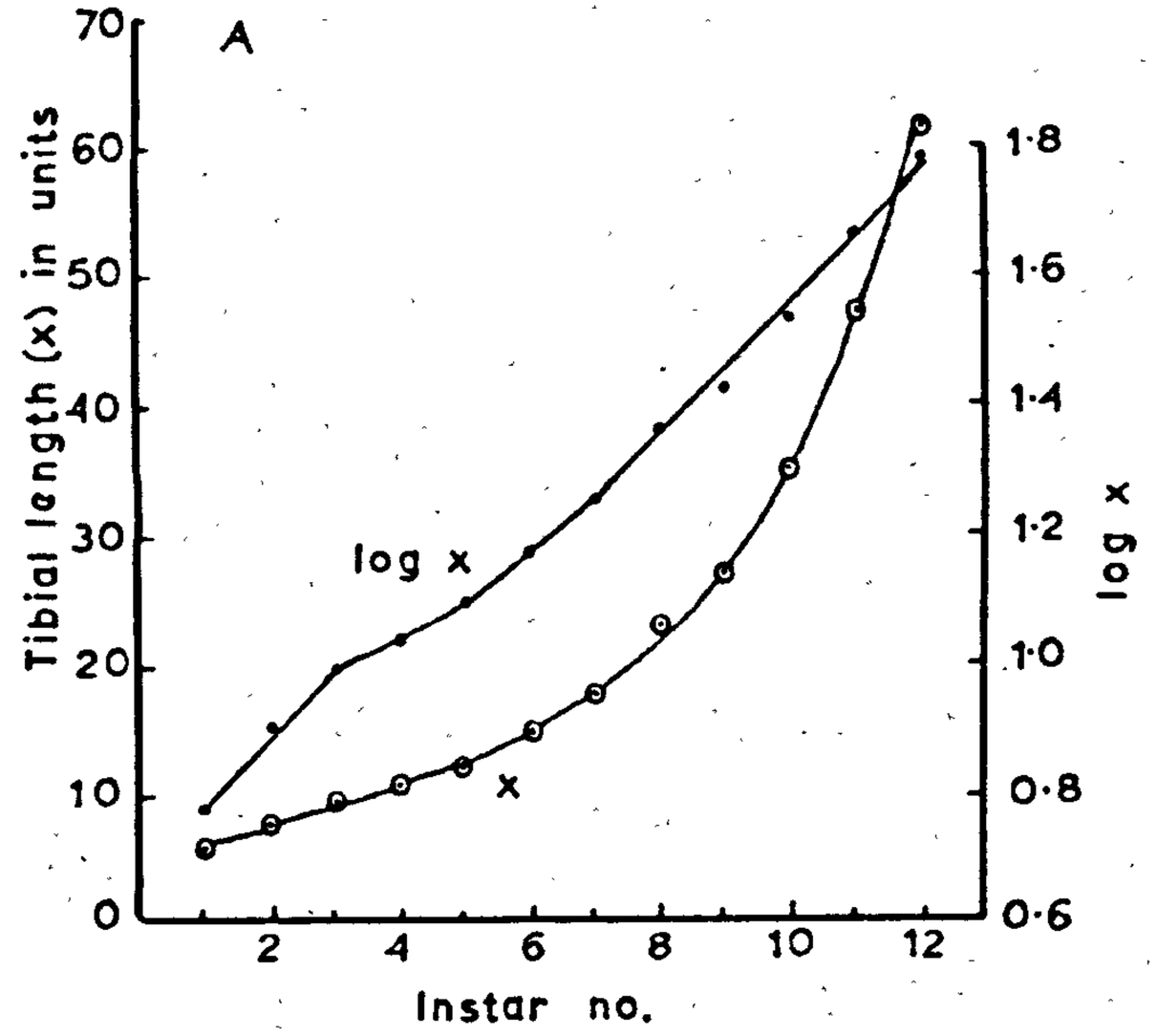
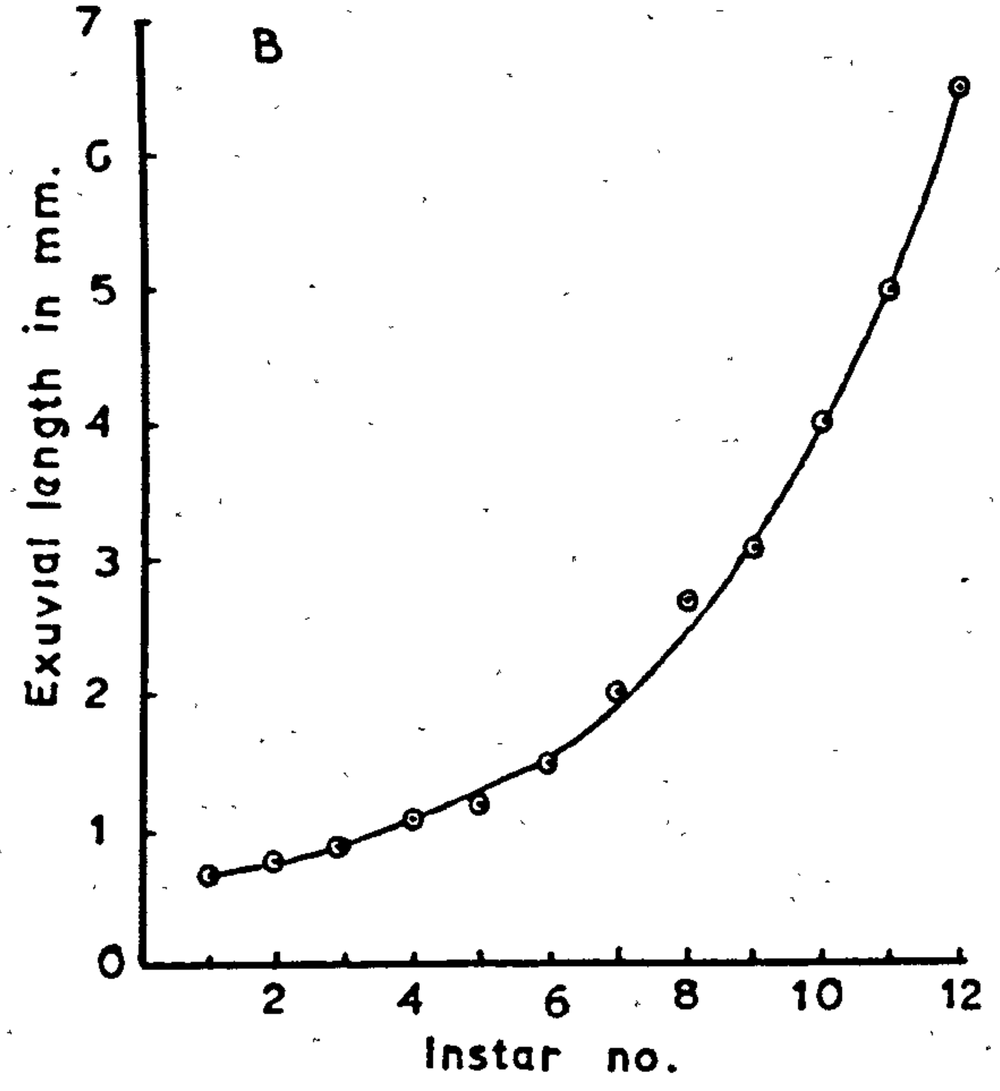


Fig. 25 N. avicularis





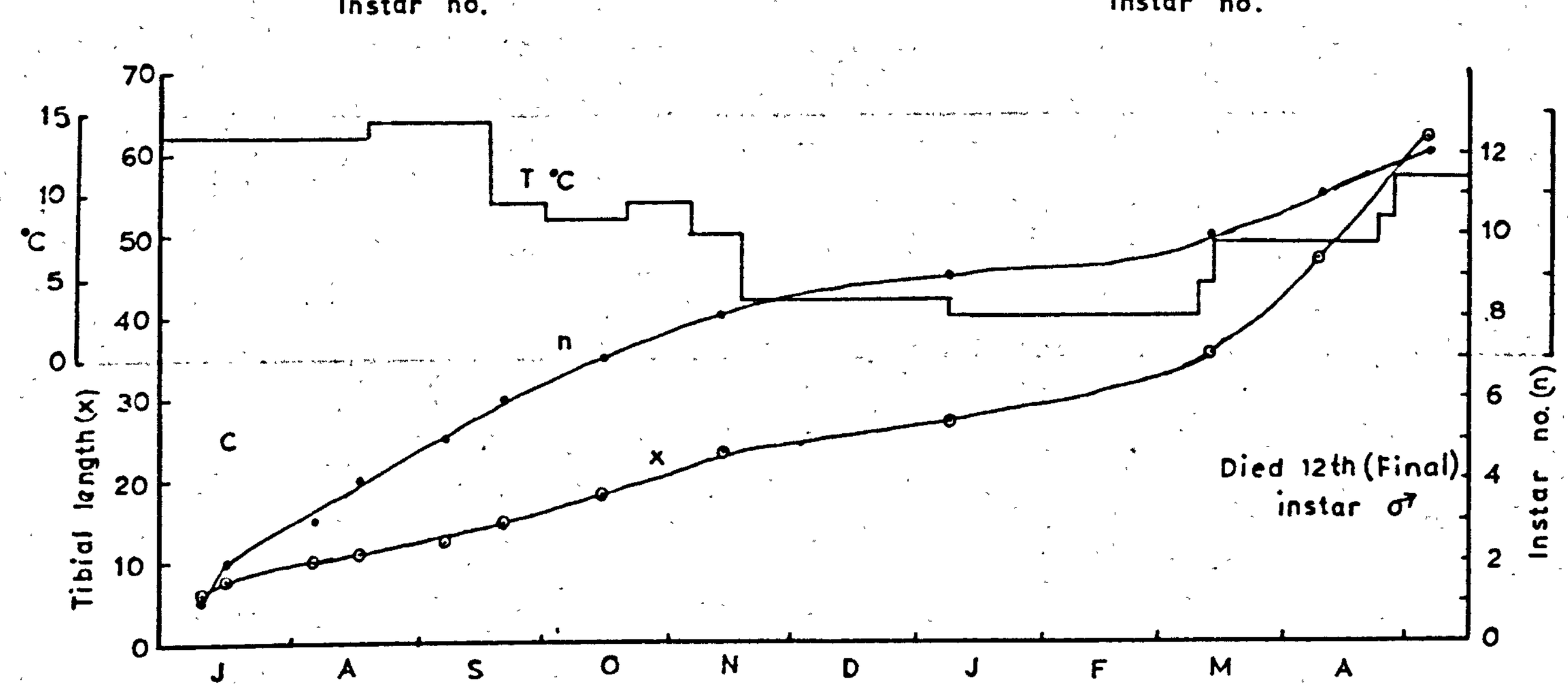
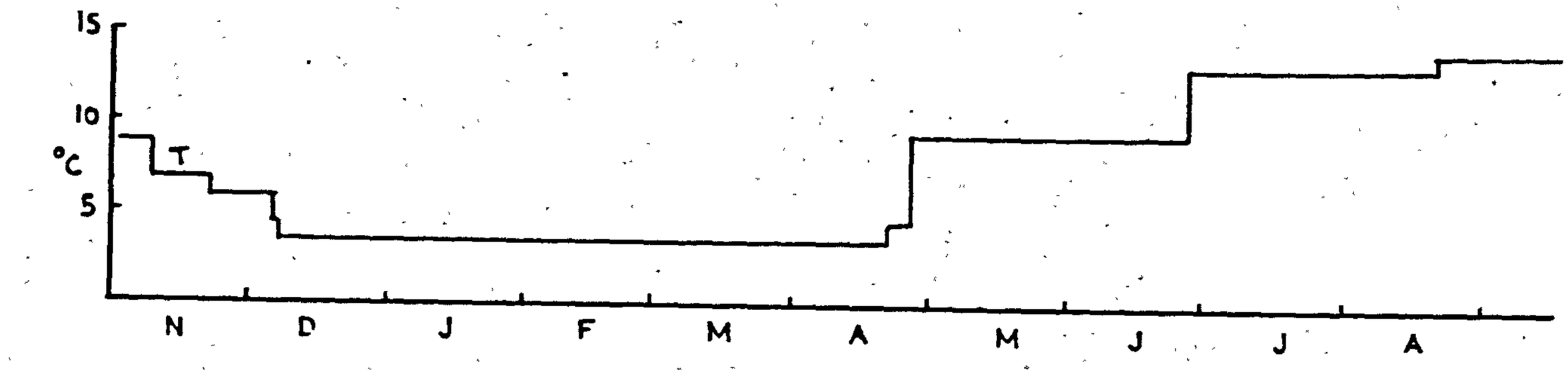


Fig. 26 C torrentium



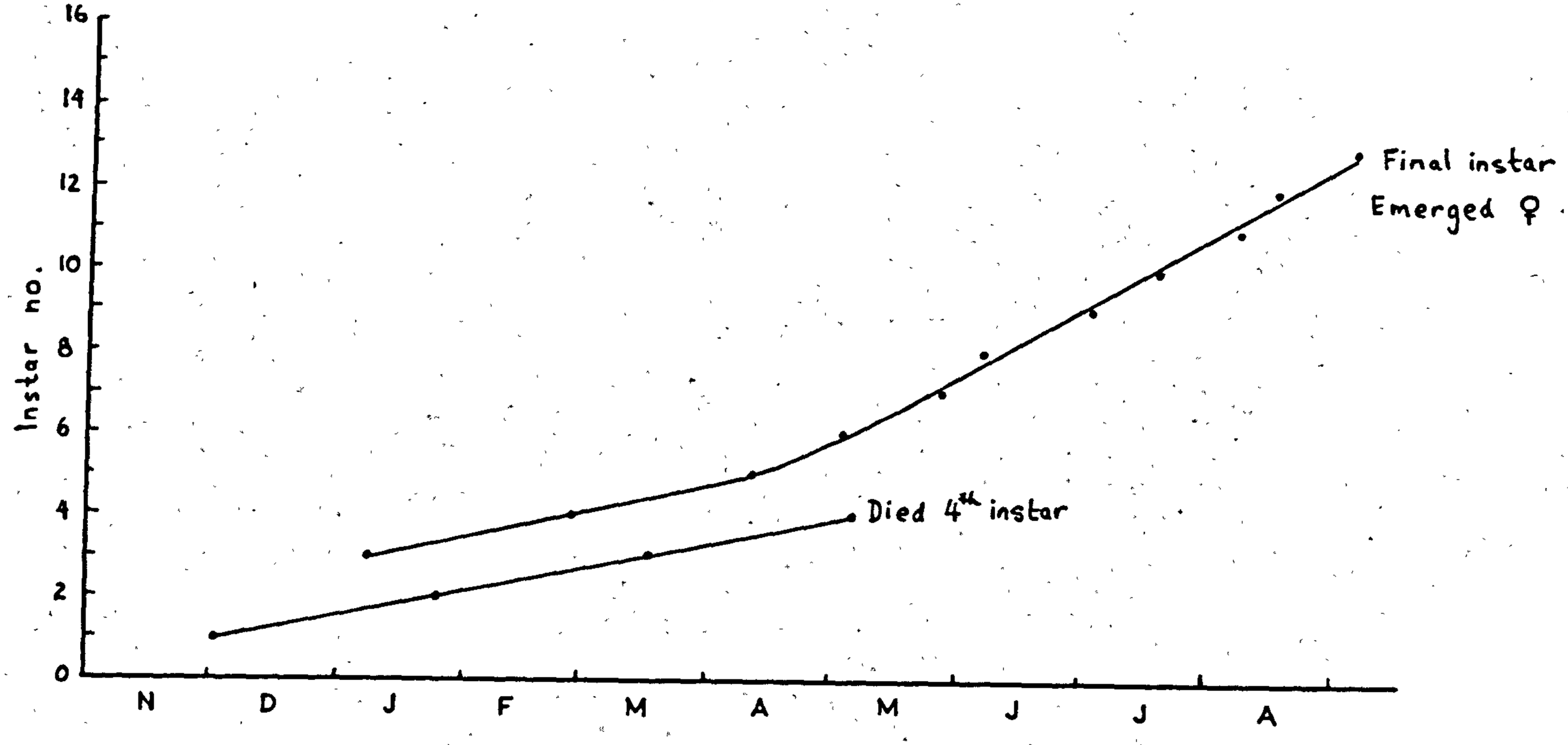


Fig. 27 L. fusca

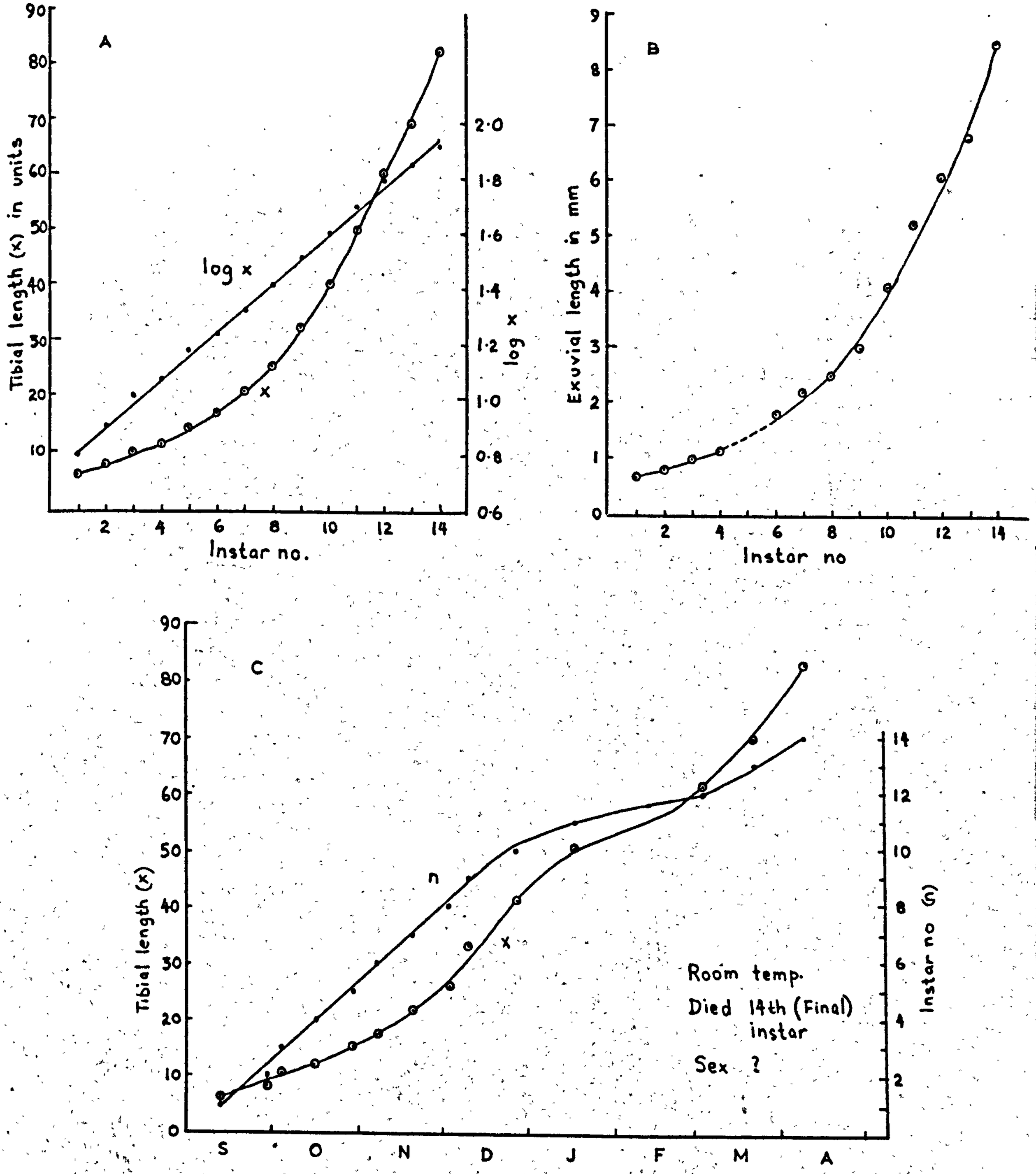
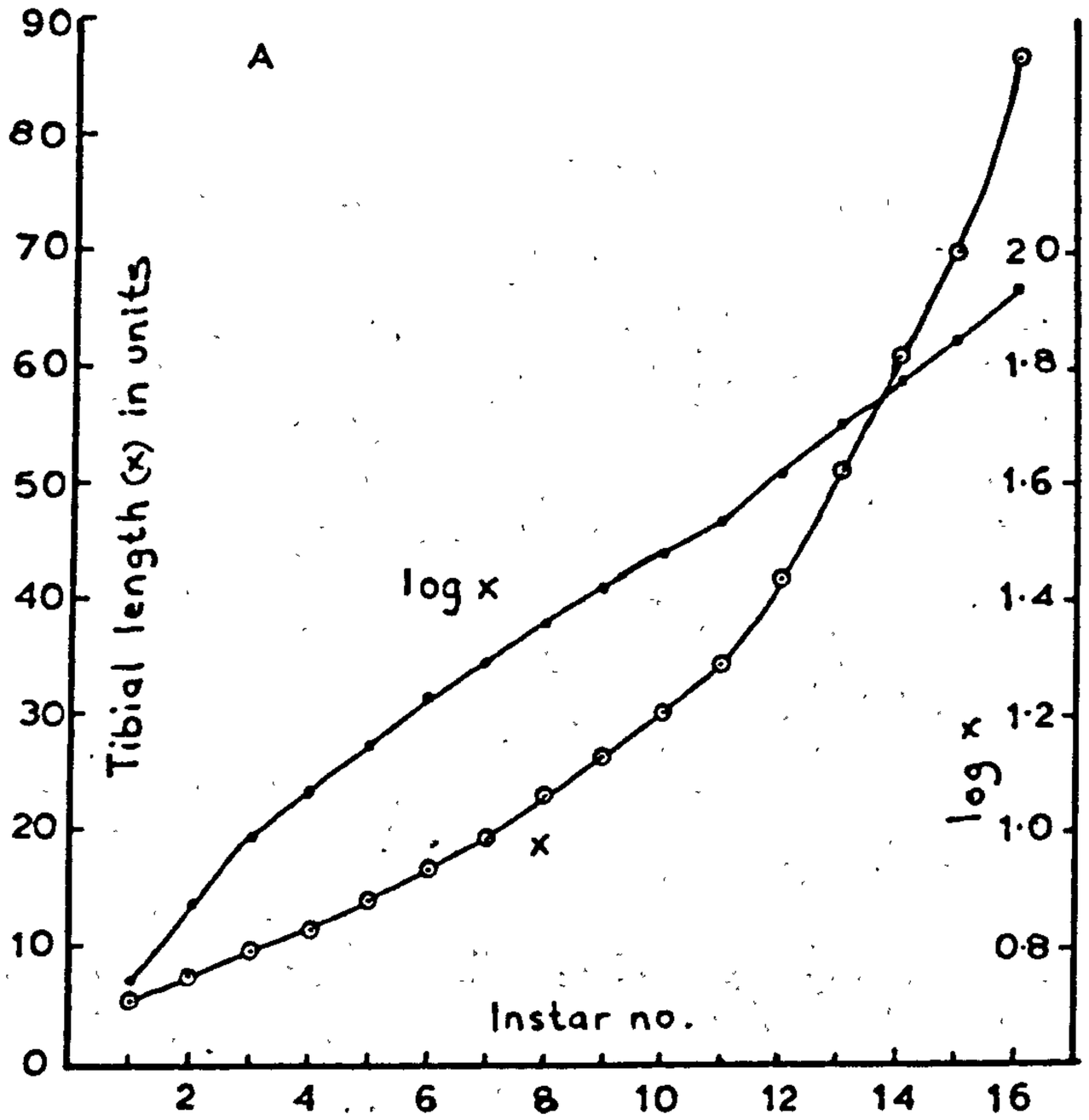
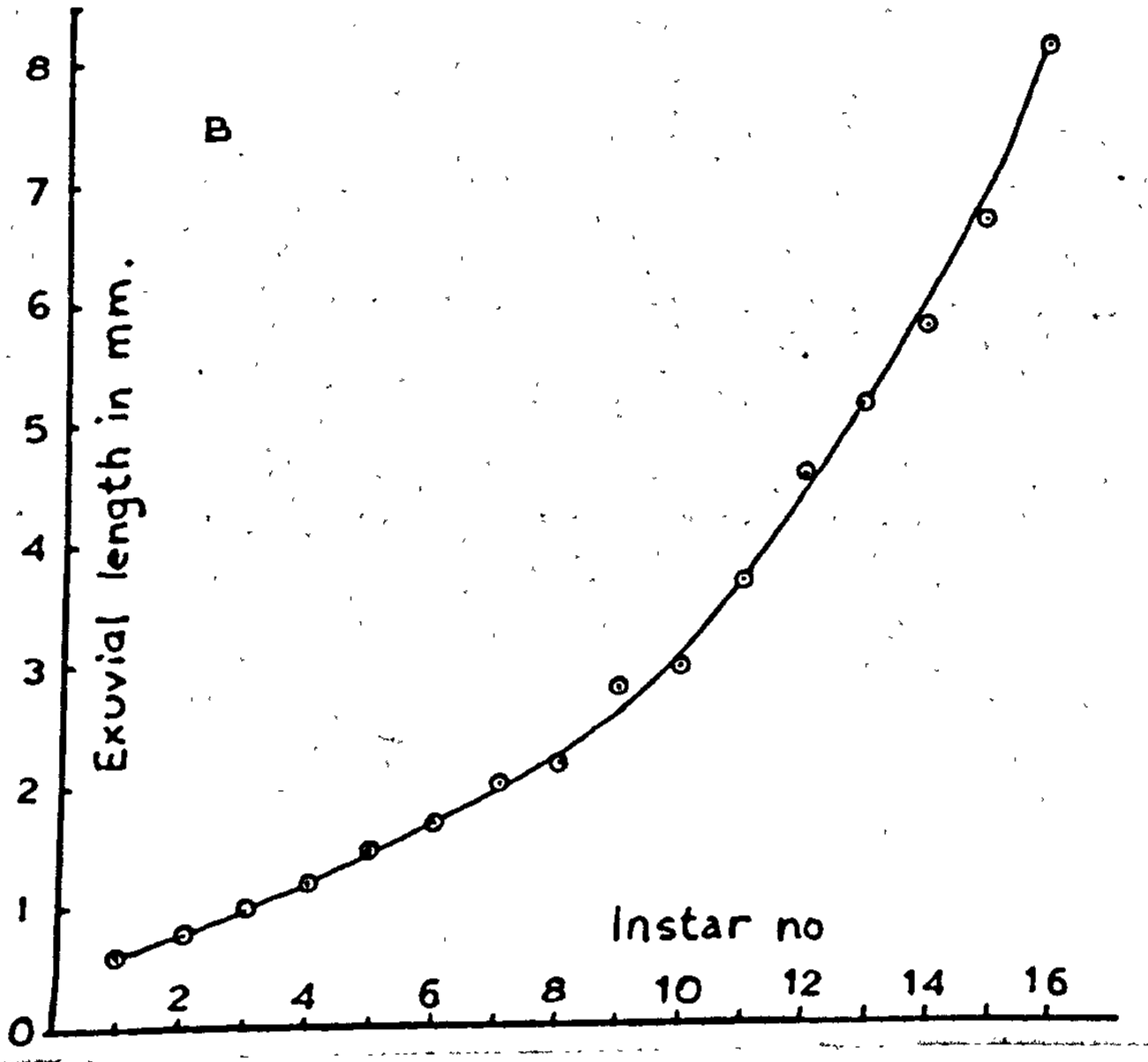
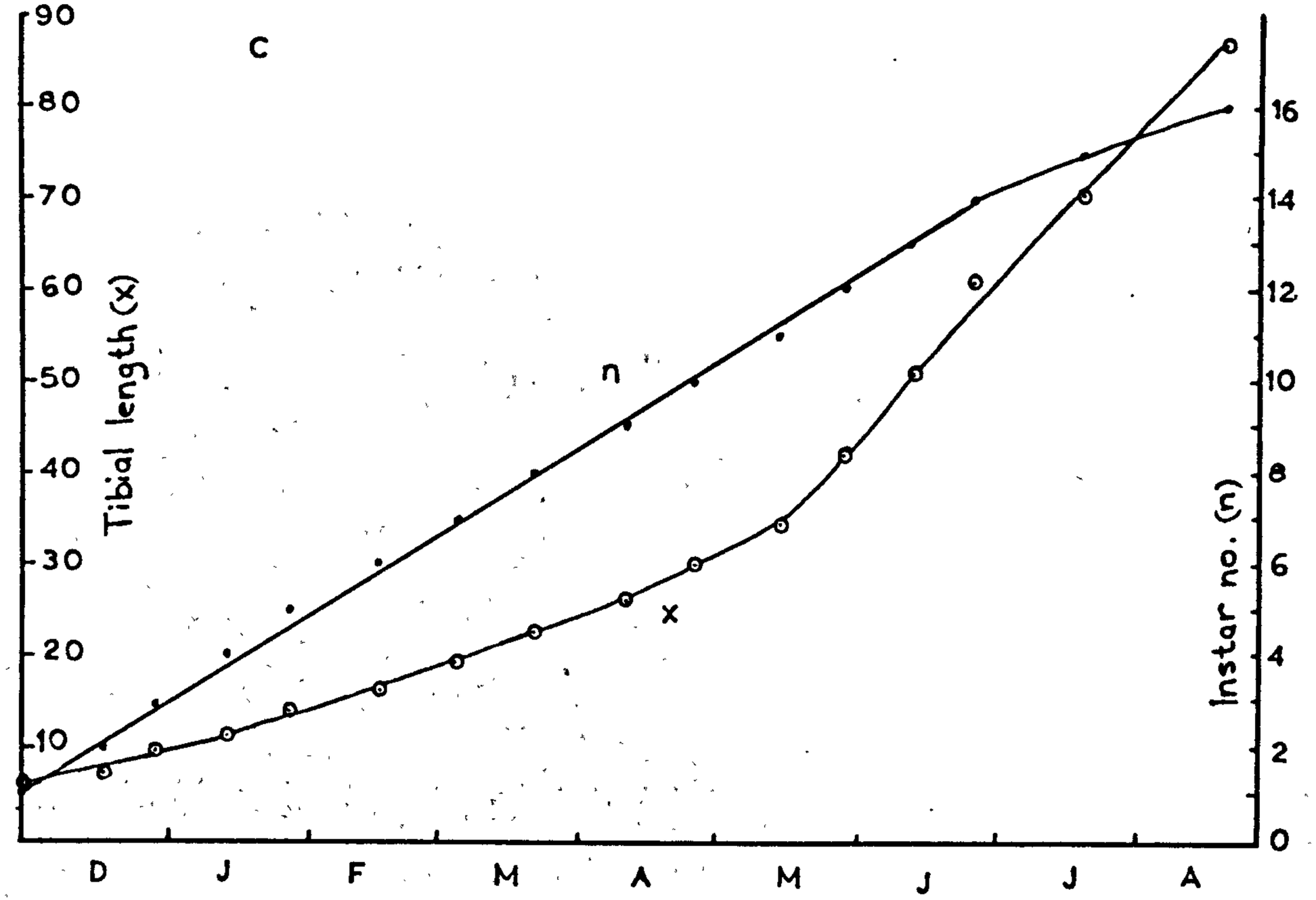


Fig. 28 L. geniculata

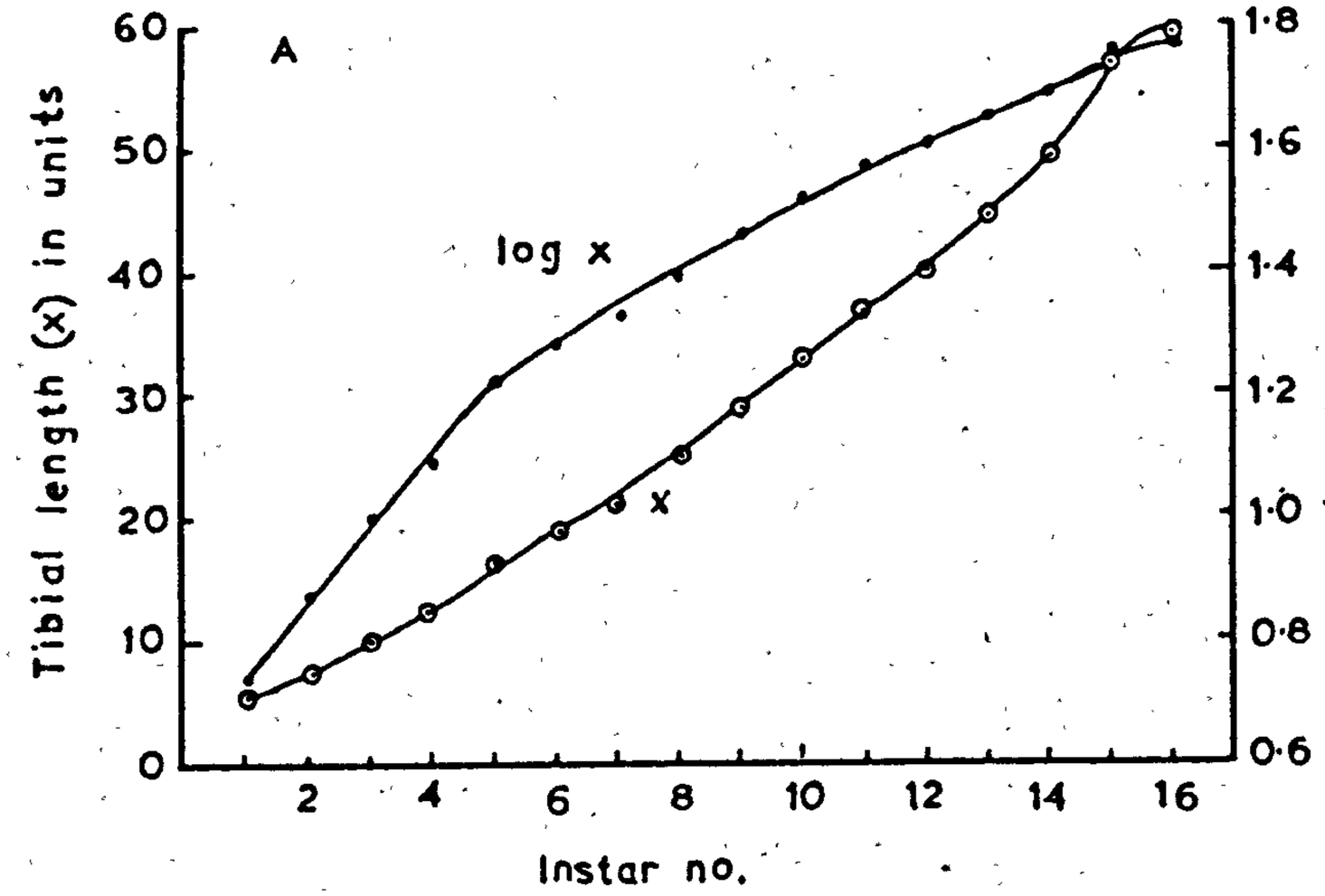


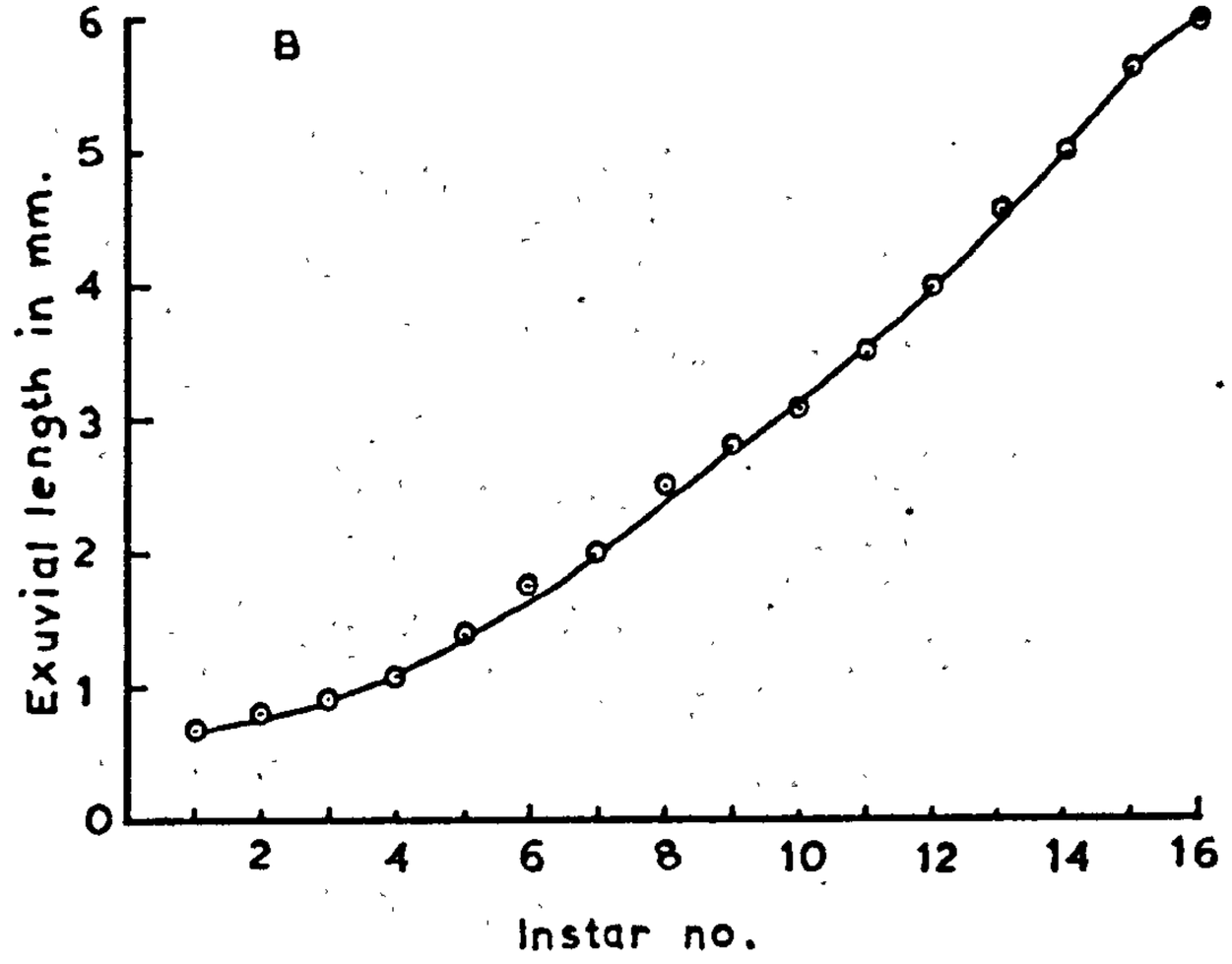




Room temp.
Emerged 16th Instar
Female

Fig. 29 L. fusca





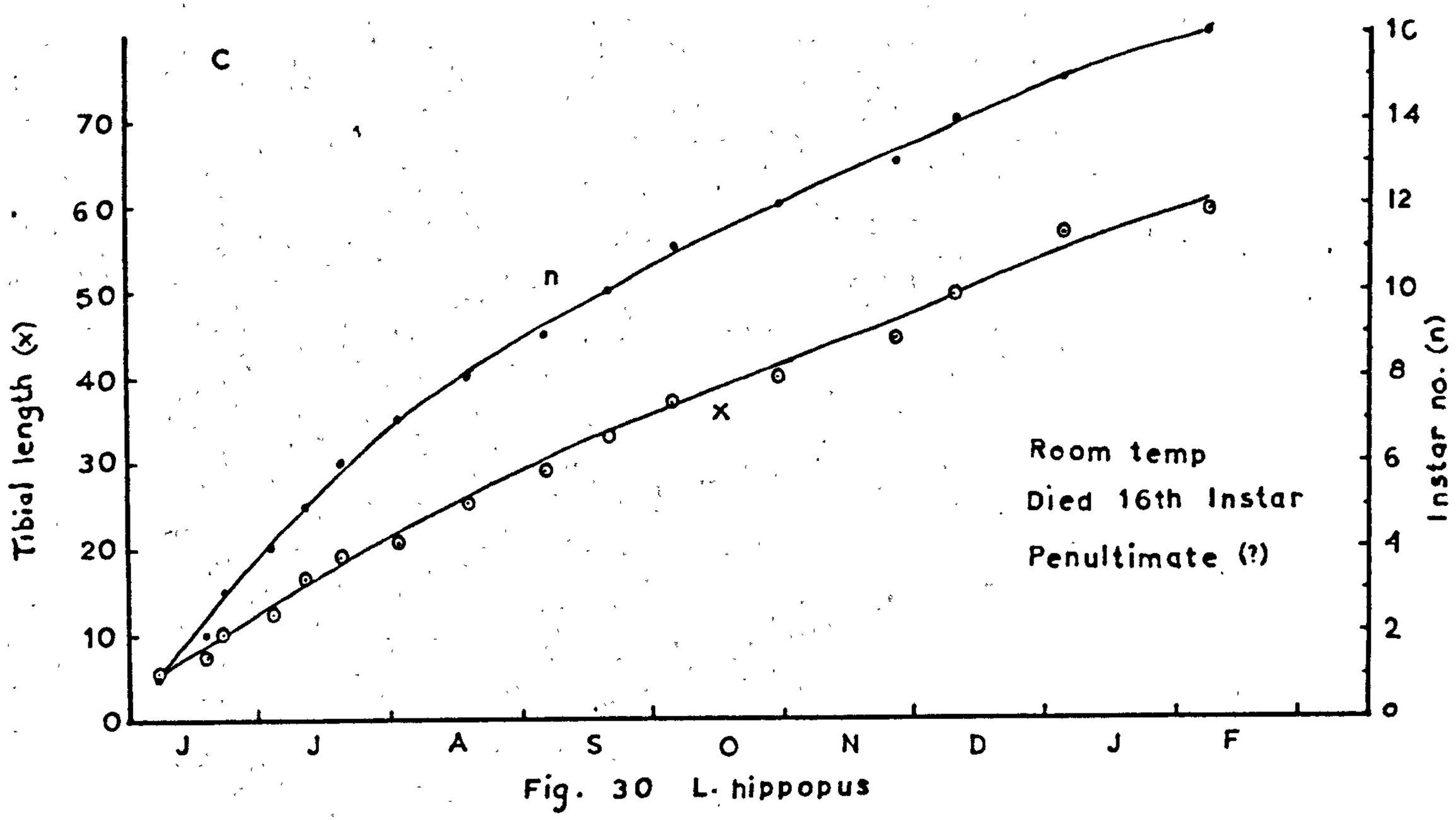
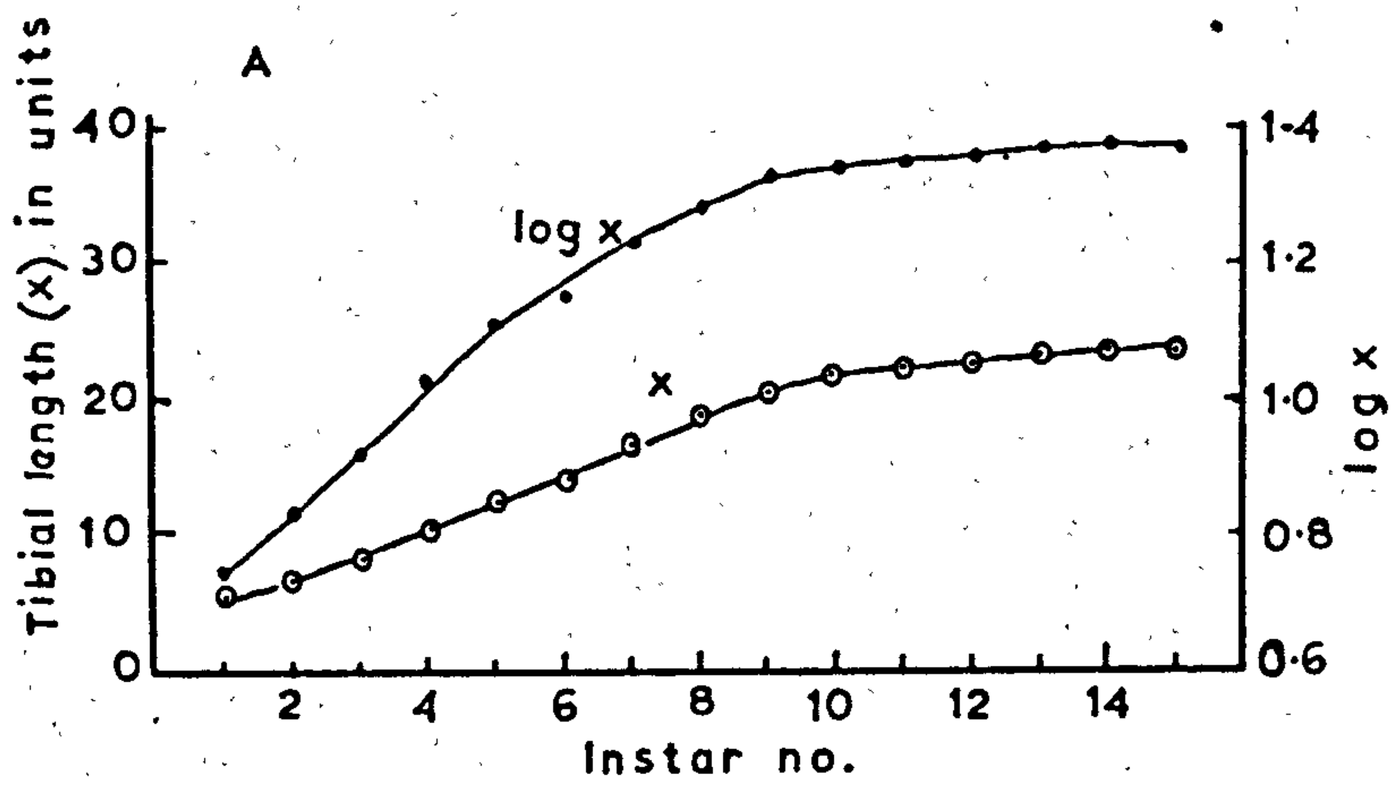
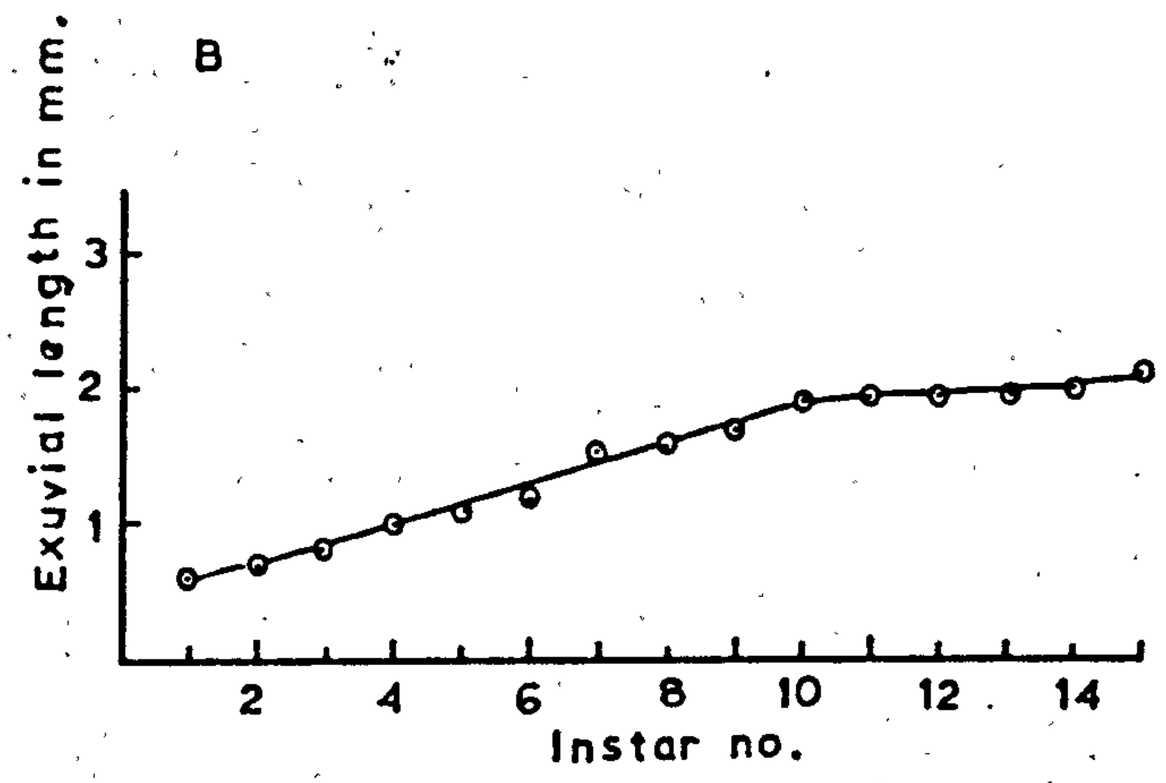


Fig. 30 L. hippopus





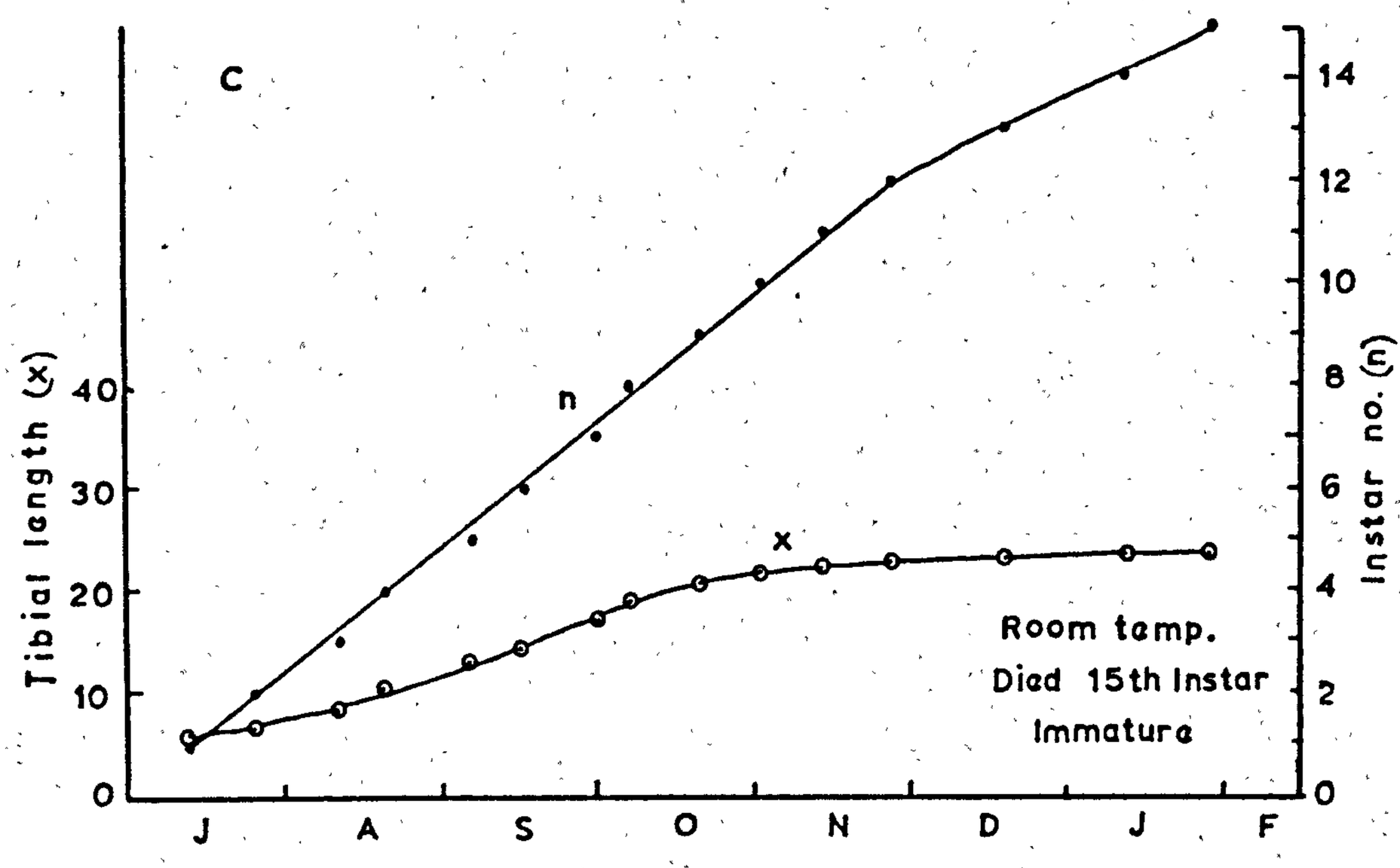


Fig 31 L inermis

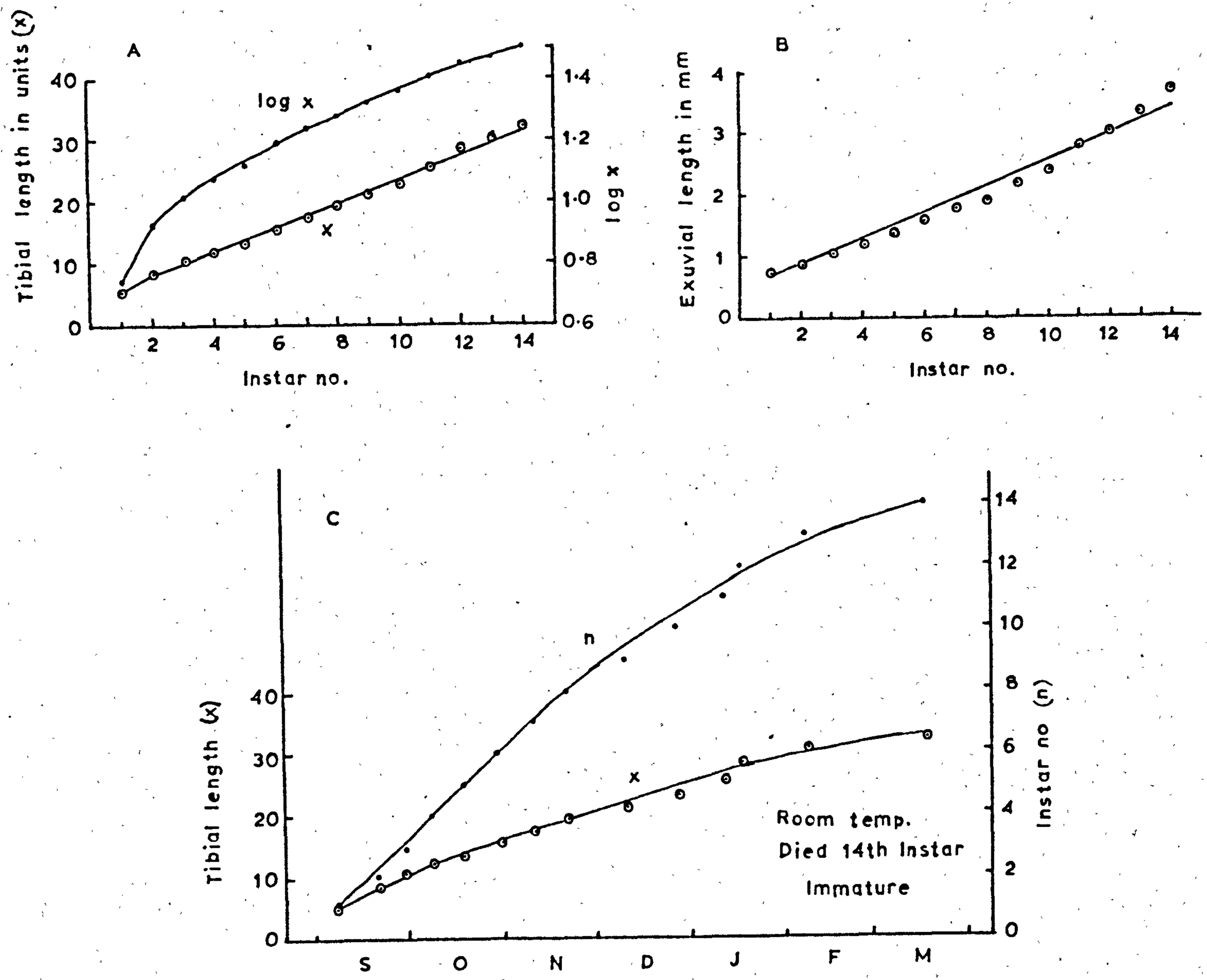


Fig 32 L. moselyi

Except for figures 19 and 25, the graphs for each species were based on a single individual. In figure 19 the results were from the averages of 3 male specimens that emerged at the 13th instar. In figure 25 they were based on two females that emerged at the 13th instar. The increase in tibial lengths of various individuals that emerged at different instars is also given in the Appendix.

From a comparison of graphs A and B in figures 19 to 26, and 28 to 32, it can be seen that the increase in the length of the hind tibia follows a similar pattern to the increase in size of the nymphs. Thus, it is possible to study the pattern of growth in terms of changes in the length of the tibia which could be measured with greater accuracy.

The pattern of growth has been expressed in the form of a logarithmic curve shown in Graph A. If the growth ratio is constant throughout the various instars a straight line would be obtained. Any fall in the growth ratio at a particular instar would be indicated by a fall in the gradient of the logarithmic curve at that instar.

Under conditions in the cold room there is, except for slight deviations, a more or less geometrical increase at each moult. The fall in the growth ratio is usually not permanent and is accompanied by a later recovery which probably indicates a return of optimal environmental conditions for the nymphs. In L. geniculata (figure 19) and L. moselyi (figure 20) the fall in the gradient of the logarithmic curve seemed to occur

during the stages when the nymphs were undergoing quiescence as a result of low temperatures. In L. hippopus (figure 21), L. inermis (figure 22), C. bifrons (figure 23), N. picteti (figure 24) and C. torrentium (figure 26) the fall did not occur during the stages when the nymphs were experiencing low temperatures and this may be due to the different optimum temperature ranges between species or the feeding of the nymphs since it has been shown by Beck (1950) that adverse dietary conditions may cause a fall in the growth ratio. In C. bifrons the fall in the growth ratio occurred from the third to the fifth instar when the nymphs were preparing for diapause but the post-diapause nymphs showed a high growth ratio which was indicated by the steep gradient of the logarithmic curve after the 6th instar. The immediate post-diapause 6th instar, however, did not show a very great increase in the gradient. The differences in the optimum temperature ranges between species and also between the various stages of the same species can be seen in figures 28 to 32 which are of nymphs reared at room temperature. The logarithmic curve for L. geniculata (figure 28) did not show any definite fall in the gradient whereas that for L. fusca (figure 29) showed a slight fall after the 3rd instar. In L. hippopus (figure 30) the fall in the gradient was slightly greater than that in L. fusca and it occurred after the 5th instar. In L. inermis (figure 31) there was a continual fall in the gradient after the 5th instar but in L. moselyi (figure 32) it occurred after the 2nd instar. In L. inermis the fall was

considerable. The fact that the first few instars are able to survive at room temperature without adverse effects on their growths suggests a higher upper limit in the optimum temperature range for the younger nymphs than for the older ones. In L. geniculata the species as a whole is fairly tolerant of high temperature and the growth of the later instars is not adversely affected by it. The degree of tolerance by the later instars of the various Leuctra spp. to high temperature appears to be in the following order:- L. geniculata, L. fusca, L. hippopus, L. moselyi and L. inermis - this being indicated by the extent of the fall, if any, in the gradient of the logarithmic curve.

The influence of environmental conditions on the rate of growth is shown in Graph C. There is generally a rapid rate of moulting at warmer temperatures and a retardation during the cold winter period. However, the rate of increase in tibial length does not necessarily follow the same pattern as the rate of moulting.

In the cold room, the effect of low temperature on the rate of increase in size is influenced partly by the rate of moulting and partly by the stage of development of the nymphs when the winter sets in. In some species such as L. inermis (figure 22) and C. bifrons (figure 23) the rate of moulting was only slightly retarded during the winter months whereas in L. geniculata (figure 19), L. moselyi (figure 20), L. hippopus (figure 21), N. pictati (figure 24), C. torrentium (figure 26) and L. fusca (figure 27) it was very much so. Because of the

more or less geometrical increase between instars, the larger or older nymphs would show a bigger increase in size with each moult than did the smaller nymphs. The occurrence of larger nymphs during the winter would therefore mask the retardation in the rate of moulting and the unfavourable effects of winter temperatures. In L. hippopus, L. inermis, C. bifrons, N. avicularis, and C. torrentium, the nymphs were in a fairly late stage of development during the winter and, therefore, there was no obvious retardation in the rate of increase in size in spite of the retardation in moulting. In L. geniculata, L. moselyi N. pictati (and L. fusca) the nymphs were still in the early instars when the winter set in and in these species the retardation in moulting was accompanied by a definite fall in the rate of increase in size. The nymphs of C. bifrons showed a very different growth pattern from the other species. There was a fall in the rate of moulting and of increase in size during the summer due to the occurrence of diapause. The most favourable period for growth in this species seemed to be during the autumn when there was a rapid rate of moulting as well as a rapid increase in size.

Except for L. geniculata, there was only a slight retardation in moulting at room temperature during the winter months which could be due to changes in room temperature during this period or to the age of the nymphs. In L. geniculata (figure 28), moulting was very much delayed and it may be possible that the nymphs normally undergo dormancy during the winter period

and that the factors influencing this may not necessarily be low temperatures. The nymphs showed a rapid rate of increase in size after the 8th instar but there was a temporary fall during the dormant period. L. fusca (figure 29) showed a fairly constant rate of moulting but there was a rapid rate of increase in size during the later instars. In L. hippopus (figure 30) and L. mosalvi (figure 32) the rate of increase was quite constant throughout but in L. inermis (figure 31) there was a marked fall in the later instars owing to the falling growth ratio. It is interesting to note that these nymphs were able to survive for quite some time in spite of the unfavourable temperatures.

6. Discussion

The study on the hatching of eggs has shown the influence of external and intrinsic factors on the length of incubation period (i.e. interval between oviposition and first hatching of eggs) in the various species. In species which do not undergo diapause, e.g. L. hippopus, L. fusca, the length of incubation is determined mainly by environmental conditions such as temperature, and eggs that are laid during the warm season hatch sooner than those laid during the cold period. In B. risi the eggs undergo obligate diapause and the length of diapause (hence also the incubation period) seems to be determined by the adults. The termination of diapause does not depend upon any environmental stimulus but it can be postponed by very low temperatures. Under normal conditions, the eggs terminate diapause during late Summer, and post-diapause development occurs till early Autumn when hatching begins. This species is a cold-water stenotherm and there is only a fairly short period, from Autumn to early Spring, suitable for growth. It is, therefore, important that the termination of diapause should occur before the cold period sets in. The ability of the eggs to remain in diapause at low temperatures is of significance in the northern latitudes where the summer is fairly short, and it is possible that under these conditions the species may have a two-year life-cycle. The eggs of D. bicaudata from Afon Hirnant show a very long incubation period of 9 to 10 months and this is because they are unable, unlike B. risi, to terminate diapause until after a fairly long period of chilling at winter temperatures (Chapter IV).

In A. standfussi the termination of diapause is also dependent upon chilling. The flight period of N. cinerea is from Spring to Summer, and the eggs laid at the end of the flight period show a relatively shorter incubation period due to the correspondingly shorter length of diapause. The diapause seems to be facultative and can be prevented or terminated by fairly cool temperatures.

Delayed hatching of some eggs occurs in all the species which undergo diapause and in only a few of those which do not. In most species, the delay is only slight. In the non-diapause eggs of L. fusca, L. moselyi and I. grammatica this is the result of an irregular rate of embryonic development while in B. risi, D. bicaudata (from Afon Hirnant) and A. standfussi it is due to differences in the period of termination of diapause. In L. fusca and L. moselyi very little growth of the nymphs occurs until Spring arrives, and the variability in the rate of embryonic development may provide a basis upon which the diapause character may eventually develop. This is because it would be advantageous for the species if the eggs could remain unhatched until the arrival of favourable conditions for growth. In N. cinerea there is a long hatching period of about 5 months. In contrast to other species, the fully-developed embryos can survive within the eggs for long periods at low temperatures. In species which lay only one type of egg, the hatching period is continuous. In D. bicaudata from Lake Bala there are, however, two distinct hatching periods, due to the occurrence of diapause and non-diapause eggs. The differences in the type of eggs laid by Afon Hirnant and Lake Bala adults are discussed in Chapter IV. It has been

suggested by Macan (1958b) that delayed hatching of eggs is advantageous in that it enables a species to exploit the resources of an environment more fully through changes in the food habits of the small and large nymphs. Hynes (1961) has pointed out that there is no evidence of a change in the size of food eaten by herbivores as they grow, and the present studies on the rearing of the nymphs through their complete life-cycles have shown that this is in fact so. He believes that a long hatching period is advantageous to many species as a mechanism for drought resistance as well as a means of avoiding competition for living space. The close association between the occurrence of diapause and delayed hatching would suggest that the latter phenomenon is an adaptation to ensure the survival of the species, should the environmental conditions be unfavourable, when some of the hatching occurs. This explanation would be applicable to the variations in the period when the nymphs of C. bifrons terminate diapause.

The growth of the young nymphs of most species is not adversely effected by fairly high temperatures which may be unfavourable for the growth of later instars. In these species, therefore, it is possible for the early instars to grow during the warm summer months when the conditions are favourable for rapid growth. Since the flight periods are usually between Spring and Summer, the absence of a diapause in the egg-stage is therefore advantageous for these species. The life-cycle of N. cinerea, however, does not appear to fit in with the environmental conditions as a result of diapause in the egg-stage. The nymphs grow rapidly at warm

temperatures and they undergo dormancy during the Autumn and Winter despite the fact that termination of diapause is associated with falling temperatures. It is possible that the developmental cycle of this species may be adpated to a very cold climate where the occurrence of diapause may previously have been a device to survive very severe winters. The occurrence of dormancy in the nymphs would too appear to have the same effect. The life-cycle of the species in Britain seems to be in a state of flux, and although the nymphs which hatch in October and November have been found to have a two-ygar life-cycle there seems to be a possibility that some nymphs which hatch earlier may complete their development within a year. That the developmental cycle of a species may be a reflection of its past history has been shown to occur in the caddis fly, Apatidae muliebris (Nielsen 1950) and also in the present study of D. bicaudata (Chapter IV).

Besides the occurrence of diapause in the egg-stage (e.g. B. risi, A. standfussi) or in the nymphal stage (C. bifrons) there is also the possibility that some species may be able to regulate their flight periods by variations in the number of instars preceding emergence. This positive means of regulating the flight period seems to occur in C. bifrons which respond to increasingly long photoperiods by the differentiation of adult characters in half-grown nymphs.

III - FIELD INVESTIGATIONS

1. Introduction

The field investigations were aimed at studying the detailed life-cycles of the commoner species of stoneflies occurring in River Terrig in North Wales. Much work on the rate of growth and the life-histories of many species has been done by Hynes (1941, 1961, 1962), Brinck (1949), Illies (1952 and Rauser (1962, 1963). There has been, however, very little information on the early stages of the life-cycles. Brinck (1949) found that the young nymphs of L. fusca do not grow till Spring arrives and Hynes (1961, 1962) showed that, in many species, the period during which very small specimens can be found was much longer than the flight period and he suggested a delayed hatching of some eggs. In the present study, emphasis has been placed on the early stages of the life-cycles and the very small nymphs have been followed in greater detail than has been done previously. It is now possible to interpret fairly accurately the field data in the light of information obtained from laboratory investigations.

2. Methods and Description of Stream

River Terrig is a fairly fast-flowing stream which forms part of the Alun-Dee drainage system and it has a rich and varied stonefly fauna. After a preliminary study of the distribution of the various species in the middle and upper stretches of the stream, two stations were selected for regular monthly sampling in 1962.

(a) Station 1

(b) Station 2

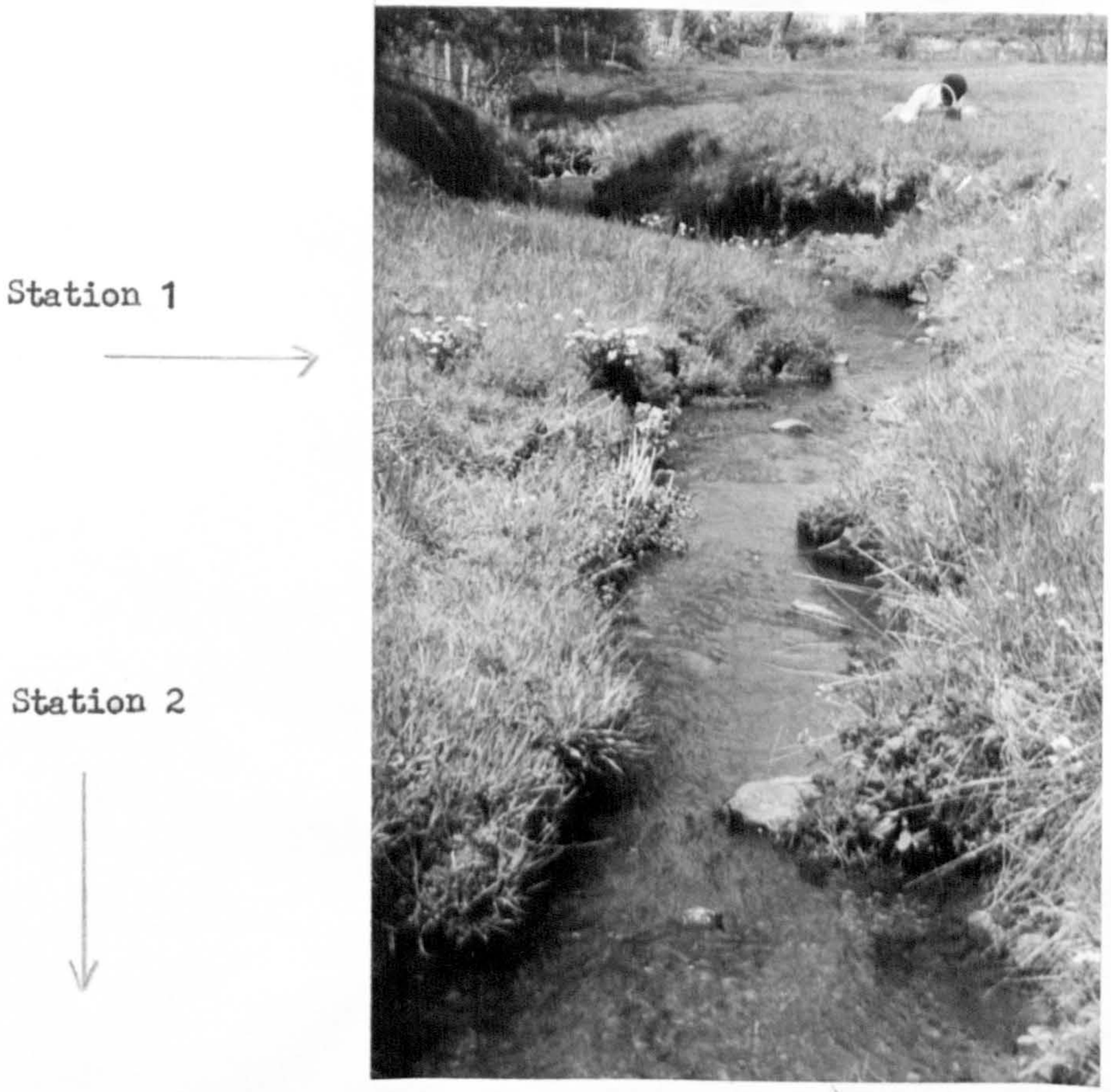


Figure 33

Station 1 (figure 33a) is situated at the upper reaches of the stream near Rhydtalog (Nat. Grid map-reference SJ 234548). The altitude here is about 300 metres and the stream flows through open moorland and upland pastures. The stream is about 1 - 2 metres wide and reaches a depth of about 30 cm. in some places. The substratum is generally stony and there is very little accumulation of decaying leaves of higher plants even during the autumn. There is, however, a fair amount of grass trailing along the edges of the stream. The stones in some places are thickly covered with liverworts belonging to the genus Chiloscyphus.

Station 2 (figure 33b) is situated near Caegwydd (Nat. Grid map-reference SJ 235576), about 3½ kilometres downstream from Station 1. The altitude is about 210 metres. For most of the distance between the stations, the stream is very much shaded by trees. The width of the stream is about 6 metres and the substratum is generally stony, with some boulders. The depth of water is fairly similar to that of Station 1 but during dry periods half the area of the stream bed may lie exposed. Sycamore (Acer sp.) leaves contribute mainly to the accumulated vegetable debris at this station.

From January to December 1962 regular monthly samples were collected with a stramin net (Mesh-size: 7 threads per cm.) as well as a fine net of bolting silk (Mesh-size: 40 threads per cm.) at each end of the two stations. The sampling method employed was fairly consistent throughout, and each net sample

consisted of collections made at 6 spots in the stream. The collecting procedure was carried out by holding a triangular hand-net vertically against the stream bed and then kicking vigorously the area immediately upstream of it. The cloud of detritus that had been stirred up was then scooped with the net. All samples were preserved immediately in formalin and they were later sorted in the laboratory, using a concentrated solution of calcium chloride (details of the method are given by Hynes 1961). After sorting, they were preserved in 70% alcohol and were examined in the following year after laboratory studies had enabled the identification of tiny nymphs.

The samples were examined by placing the material onto a flat petri-dish, to the bottom of which was attached a millimetre graph paper that had been surfaced over by a thin layer of wax. The nymphs were then identified and measured underneath a binocular microscope. The very young stages had to be identified under a monocular microscope and the stages of development were noted. The nymphs were placed in millimetre size-groups, e.g. 1 = 0 - 1.5 mm., 2 = 1.5 - 2.5 mm. and so on. However, the 1 mm. group was further divided into 3 sub-groups, namely 0 - 0.5 mm., 0.5 - 1.0 mm. and 1.0 - 1.5 mm.

During the periods of sampling, the stream temperatures at both stations were taken and adults collected. In between these periods, visits were also made to the stream to collect adults and nymphs for the laboratory investivations and temperatures were also taken.

3. Stream temperatures and composition of stonefly fauna

The water temperatures taken at the two stations at the times of sampling are shown in Table 6. They are only useful

Station	18 Jan.	22 Feb.	22 Mar.	26 Apr.	21 May	25 Jun.	20 Jul.	20 Aug.	19 Sep.	22 Oct.	19 Nov.	17 Dec.
1	3	4	4½	13	12	14	15	14	10	10	2½	4
2	3½	3½	4	8½	10	12	12	15	10	9	2½	4½

Table 6 - The water temperature in °C to the nearest half degree.

in indicating the seasonal changes in the temperature of the stream and Macan (1958a) has shown that very little reliance can be placed on such isolated temperature records. Station 1 is very much exposed and the diurnal changes in water temperature would be greater than at Station 2 where the stream is well shaded for a greater part of the distance above and at this point.

Table 7 shows the percentage composition of the stonefly fauna collected at the two stations, from January to December 1962. *C. tripunctata*, which is not indicated in the table, was collected in small numbers in between the two stations. There is a distinct distribution of the various species in the stream. *A.sulcicollis*, *B. risi*, *I. grammatica* and the various *Leuctra* species except *L. nigra* were less common at Station 1 than at Station 2. The reverse was found to occur for *A. standfussi*, *N. cambrica*, *C. bifrons* and *L. nigra*. *C. torrentium* was fairly common at both stations while *P. mayeri* and the larger Setipalpian

		Station	
		1	2
1.	A. sulcicollis	0.6	18.6
2.	A. standfussi	2.3	0.3
3.	N. cambrica	19.9	4.0
4.	N. cinerea	+	+
5.	N. erratica	+	0.1
6.	P. praecox	+	0.3
7.	P. meyeri	-	+
8.	C. bifrons	5.0	0.3
9.	Leuctra spp. (1st instar)	1.0	2.9
10.	L. fusca	1.5	12.4
11.	L. geniculata	+	4.2
12.	L. hippopus	2.2	10.7
13.	L. inermis	0.4	1.7
14.	L. moselyi	+	1.0
15.	L. nigra	2.0	0.3
16.	B. risi	0.1	0.5
17.	P. microcephala	-	+
18.	C. torrentium	2.5	2.3
19.	I. grammatica	0.7	1.9
20.	P. bipunctata	-	+
	Percentage total	38.2	61.5
	Total no. collected in 1962	29715	

- absent; + less than 0.1%

Table 7 - Percentage composition of the various species collected in 1962.

species such as P. microcephala and P. bipunctata were completely absent from Station 1. The absence of the larger Setipalpia from Station 1 may be due to the lack of a suitable substratum of large stones and boulders at this part of the stream. The scarcity of decaying leaves of higher plants at Station 1 may possibly explain the small numbers of some of the species while it would appear that A. standfussi, N. cambrica C. bifrons and L. nigra may not be so dependent on such material for food or shelter and the lack of competition may have contributed towards the success of these species. A. standfussi is found commonly among vegetation growing in the stream. It is interesting to note that C. torrentium is fairly common in the stream in spite of the fact that it has a very low reproductive capacity. This seems to indicate a very low mortality rate in the nymphal stage.

4. Life-cycles and growth

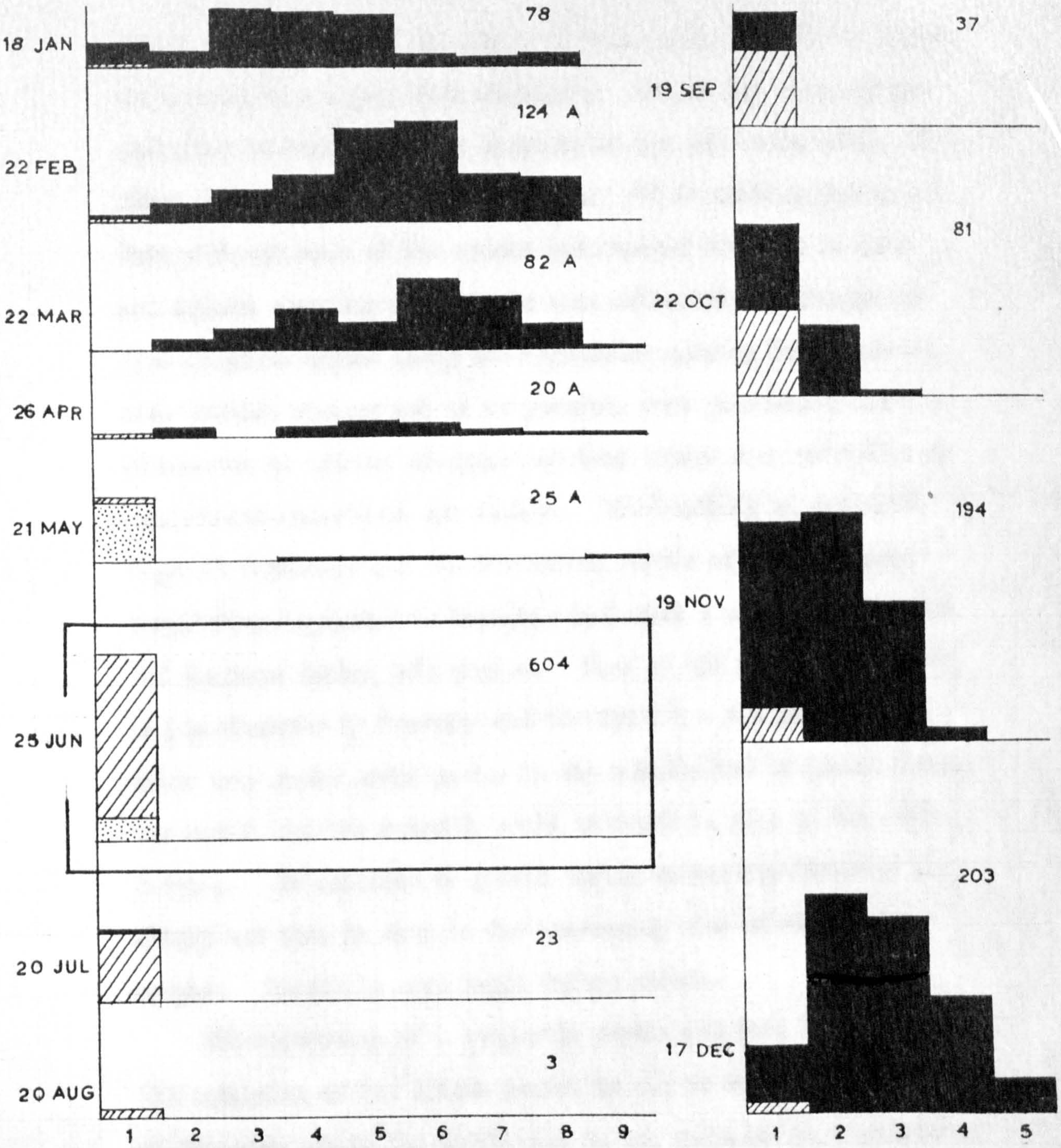
The life-cycles of 13 species are shown in figures 34 - 47. The histograms are based on data from the station where the species is most common. Only the results of fine net samples have been used for L. fusca, L. hippopus, N. cambrica and A. sulcicollis while the other species have been based on both fine and coarse net samples. The numbers collected are shown on the top right of each histogram and the occurrence of adults is indicated by the symbol 'A'. The histograms are calculated according to numbers and the same scale has been used for each

month. In C. bifrons, however, the number of nymphs collected in June was very large and the histogram for this month is given on a scale which is one-tenth of that of other months. Within the 1 mm. size-group, the histograms for 0 - 0.5 mm. are stippled, for 0.5 - 1.00 mm. shaded by oblique lines and for 1.0 - 1.5 mm. completely black. It was not possible to identify the first instars of the different Leuctra species and they are not represented in figures 41 - 46. They are, however, shown on the left column in figure 47 in which the histograms for the various Leuctra spp. have been repeated but the details of the 1 mm. group have not been indicated.

C. bifrons (Figure 34)

The life-history of this species has previously been studied by Hynes (1941) and Brinck (1949). Both workers have shown that the eggs hatch immediately after oviposition. Although the adults are known to occur from February to May, the young nymphs have never been previously collected before September or October.

Figure 34 shows a clearly univoltine life-cycle and a flight period from February to May. Egg-laying (and hatching) occurred during May and June when first instar nymphs were found. Some nymphs began to undergo diapause in June when 3 diapause nymphs, two in the 4th and one in the 5th instar were collected. The rapid fall in the number of nymphs in July and August indicates that most of them had entered diapause during these months. In July, only 23 nymphs were collected and of these, three were 4th



C. bifrons

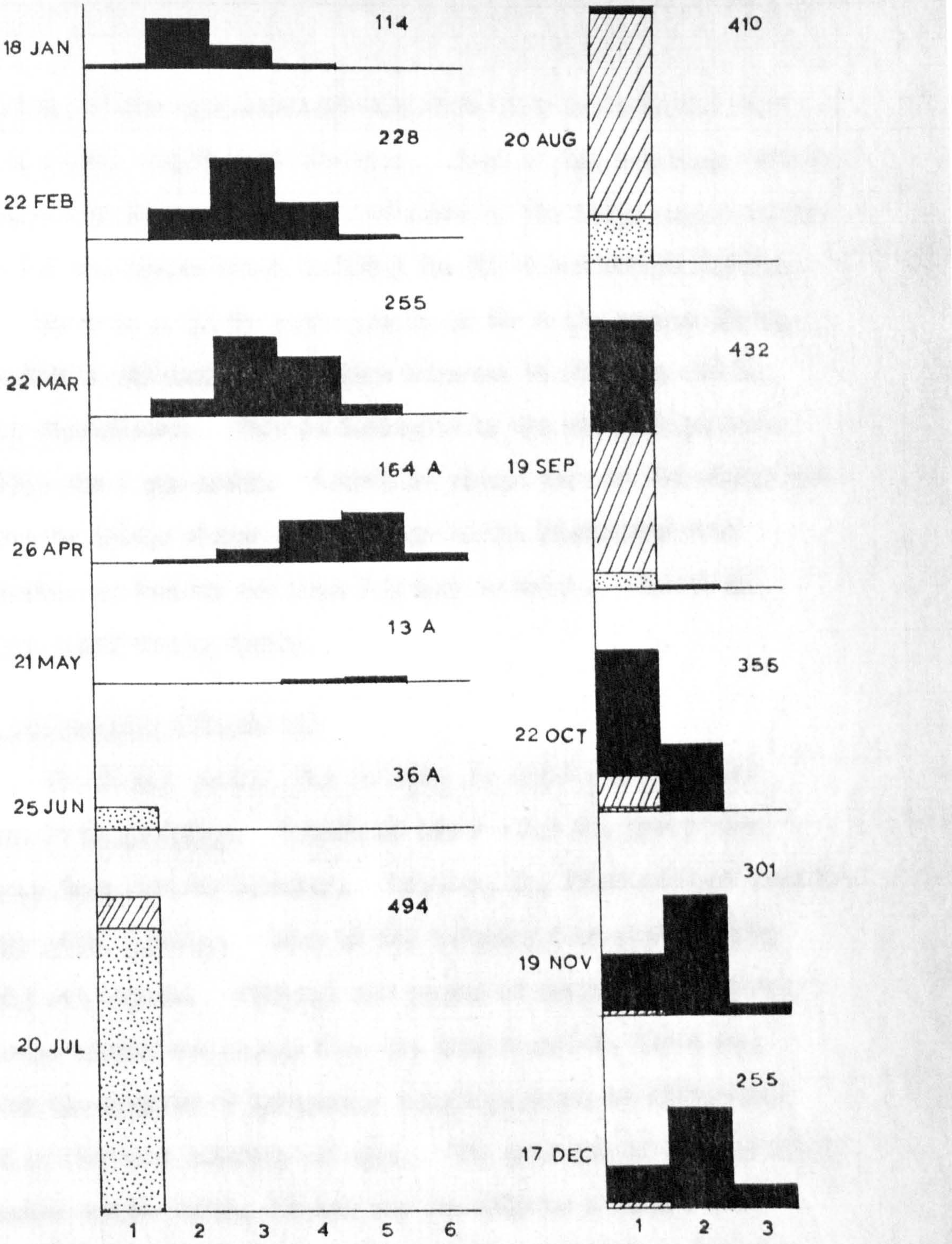
Fig. 34

instar diapause nymphs and the rest were mainly 3rd instar nymphs which had not yet gone into diapause. Two of the three nymphs collected in August were in diapause at the 4th instar while the other was an active 5th instar nymph. It is worth pointing out that although most of the nymphs had entered diapause in July and August, very few such nymphs were collected. Attempts to find diapause nymphs among the vegetation growing in the stream were without success and it is possible that they burrow into the substratum to undergo diapause and thus escape from predation by carnivorous insects in the stream. The breaking of diapause began in September and the increasing number of 1 mm. nymphs caught from September to November indicates a continual breaking of diapause during this period. Most of the nymphs would have broken diapause by November and the few 0.5 - 1.0 mm. nymphs after this month could be due to the retardation of growth during the winter and the normally small increase in size of the early instars. Retardation of growth during winter is, however, only slight and this is seen in the increasing size of the larger nymphs. Growth is most rapid during autumn.

The occurrence of a composite population just before and at the beginning of the flight period is due to the gradual breaking of diapause and to the difference in the increase in size between instars of the young and old nymphs.

N. cambrica (Figure 35)

The flight period is from April to June. Although nymphs of the 0 - 0.5 mm. group were found from June to October,



N cambrica

Fig. 35

hatching of the eggs occurred only from June to September when first instar nymphs were observed. Most of the hatching, however, occurred in July and this is indicated by the large number of the 0 - 0.5 mm. nymphs which included the first and second instars.

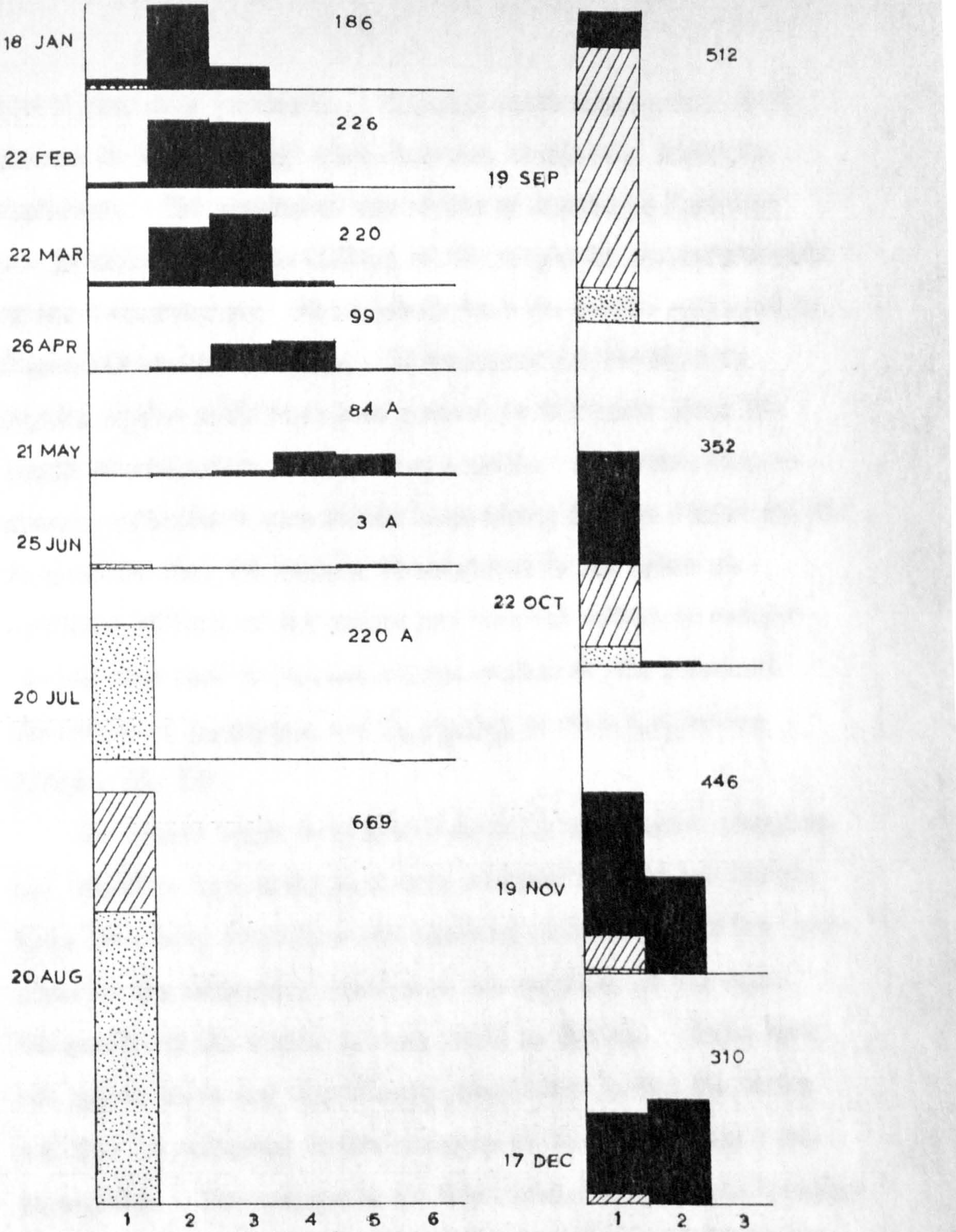
There is a fairly rapid growth of the early stages during the Summer although the absolute increase in size may not be very significant. This is indicated by the changing pattern within the 1 mm. group. Growth is slowed down in the winter and there is little change in the shape of the histograms from November to January and from February to March. Growth is again rapid during Spring.

A. sulcicollis (Figure 36)

The flight period, May to July, is about a month later than in N. cambtica. Nymphs of the 0 - 0.5 mm. group were found from June to November. However, the first instars occurred only until October. Most of the hatching took place during July and August. Although the period of occurrence of first instar nymphs was longer than the flight period, there was, from the results of laboratory investigations, no indication of any delayed hatching of eggs. The presence of the few first instar nymphs during October may possibly be a result of a delayed rate of moulting. The pattern of growth is fairly similar to that of N. cambrica.

A. standfussi (Figure 37)

This species has a univoltine life-cycle with a short flight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19 NOV
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17 DEC
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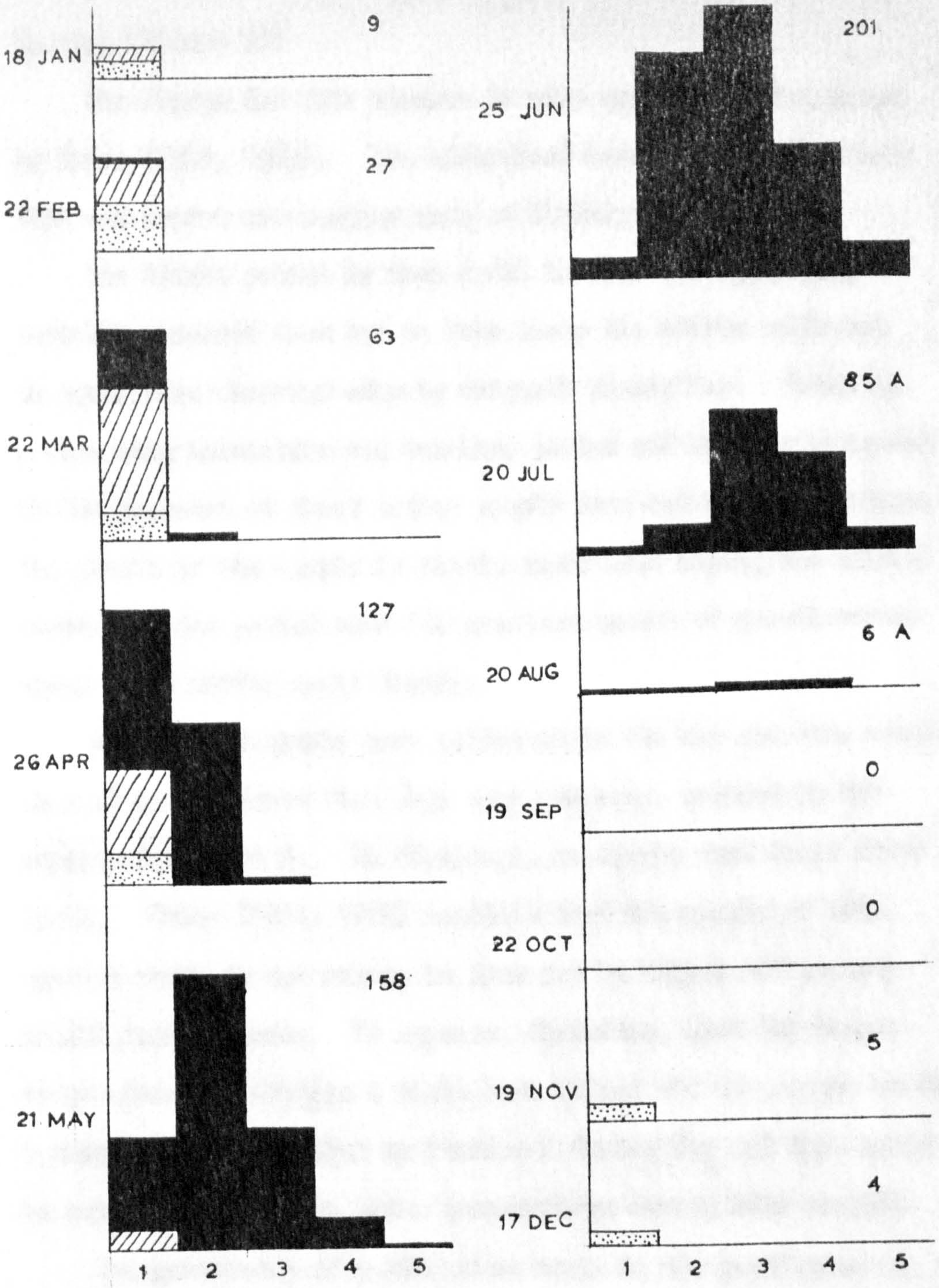
1 2 3
A sulcicollis

Fig. 36

period from July to August. Although small nymphs were still present in August, they were, however, completely absent in September. The absence of any adults or nymphs in September was possibly due to the killing of the nymphs by the unfavourable summer temperatures. It is likely that the adults collected in August had emerged in July. If emergence had occurred in August, adults would have been present in September since the length of adult-life is more than a month. The water temperatures at Station 1 were fairly high during June to August and it is possible that the adverse temperatures do not cause an immediate killing of the nymphs but that the effect is delayed as has been seen in the laboratory studies on the growth of the nymphs of L. inermis and L. moselyi at room temperature (Chapter II, 5d).

The adults begin to oviposit about 1½ weeks after emergence and therefore egg-laying must have occurred in July and August. There is a long incubation and hatching period and this has been shown in the laboratory studies on the hatching of the eggs. The growth of the nymphs is very rapid in Spring. There does not appear to be any significant retardation during the winter and this is reflected in the changing pattern within the 1 mm. size-group. The occurrence of first instar nymphs from November to April must therefore be the result of a delayed hatching of some eggs since there is no delayed growth of the nymphs during winter.



A. standfussi

Fig. 37

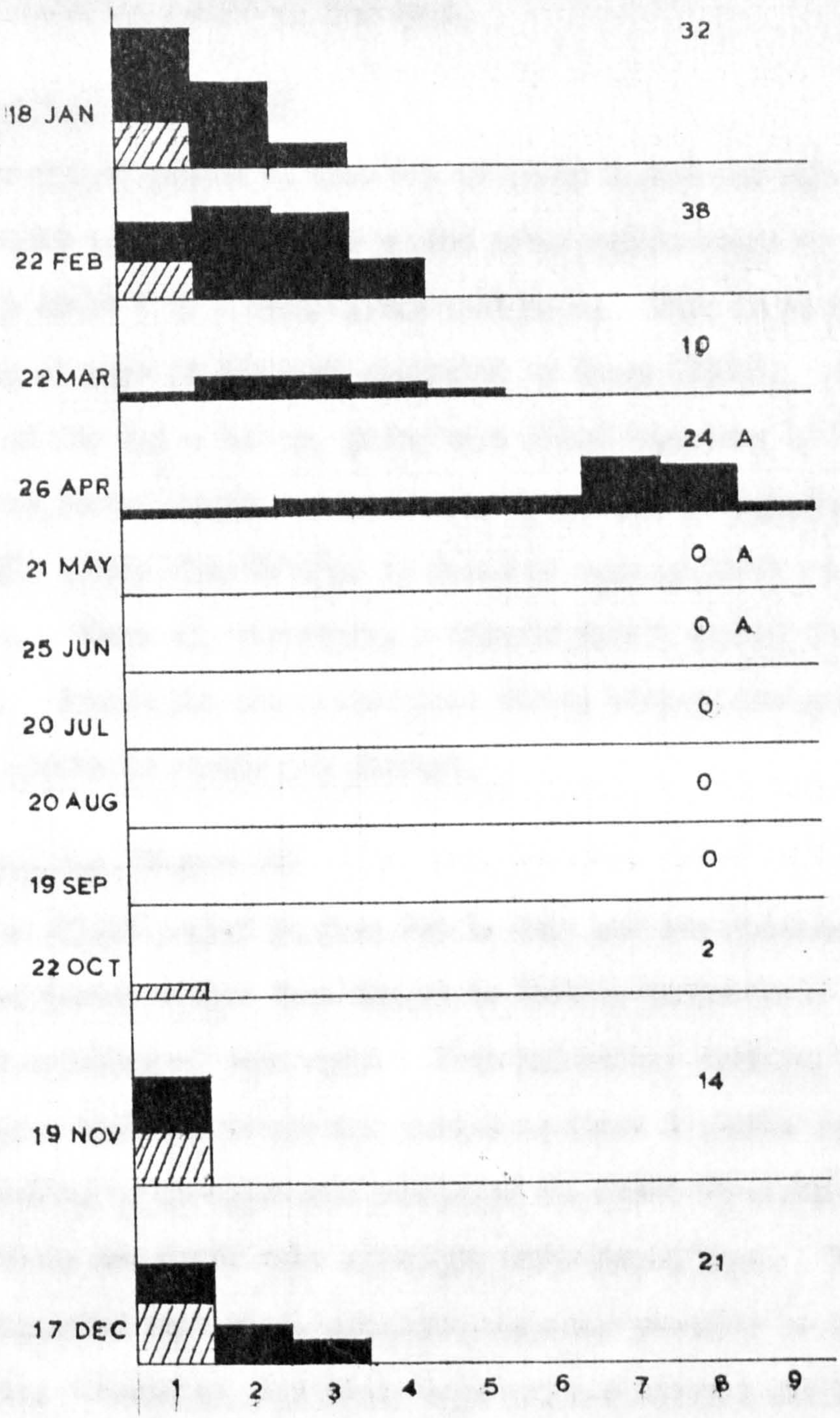
B. risi (Figure 38)

The figure for this species is very similar to that given by Hynes (1961, 1962). The histograms have been based on both fine and coarse net samples made at Station 2.

The flight period is from April to June and egg-laying probably occurred from May to June since the adults collected in April were observed only to oviposit during May. There is a very long incubation and hatching period and this is indicated by the presence of first instar nymphs from October to February. The growth of the nymphs is fairly rapid even during the coldest months but the period when the greatest amount of growth occurs seems to be during early Spring.

Although no nymphs were collected in the May and June samples, it must be mentioned that they were, however, present in the stream at Station 2. In Station 1, no nymphs were found after April. Hynes (1961, 1962) mentions that the nymphs of this species which do not emerge in time may be killed off by heat in all normal years. It appears, therefore, that the higher temperatures at Station 1 might have killed off the nymphs at Station 1 whereas their survival at Station 2 during May and June could be associated with the lower temperatures during this period.

The occurrence of a wide size range in the population is due to the long hatching period, the rapid growth of the nymphs throughout the winter and the geometric increase in size between instars. Brinck (1949) has shown that this species may sometimes produce a very uniform population. This may possibly be due to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B. risi

Fig. 38

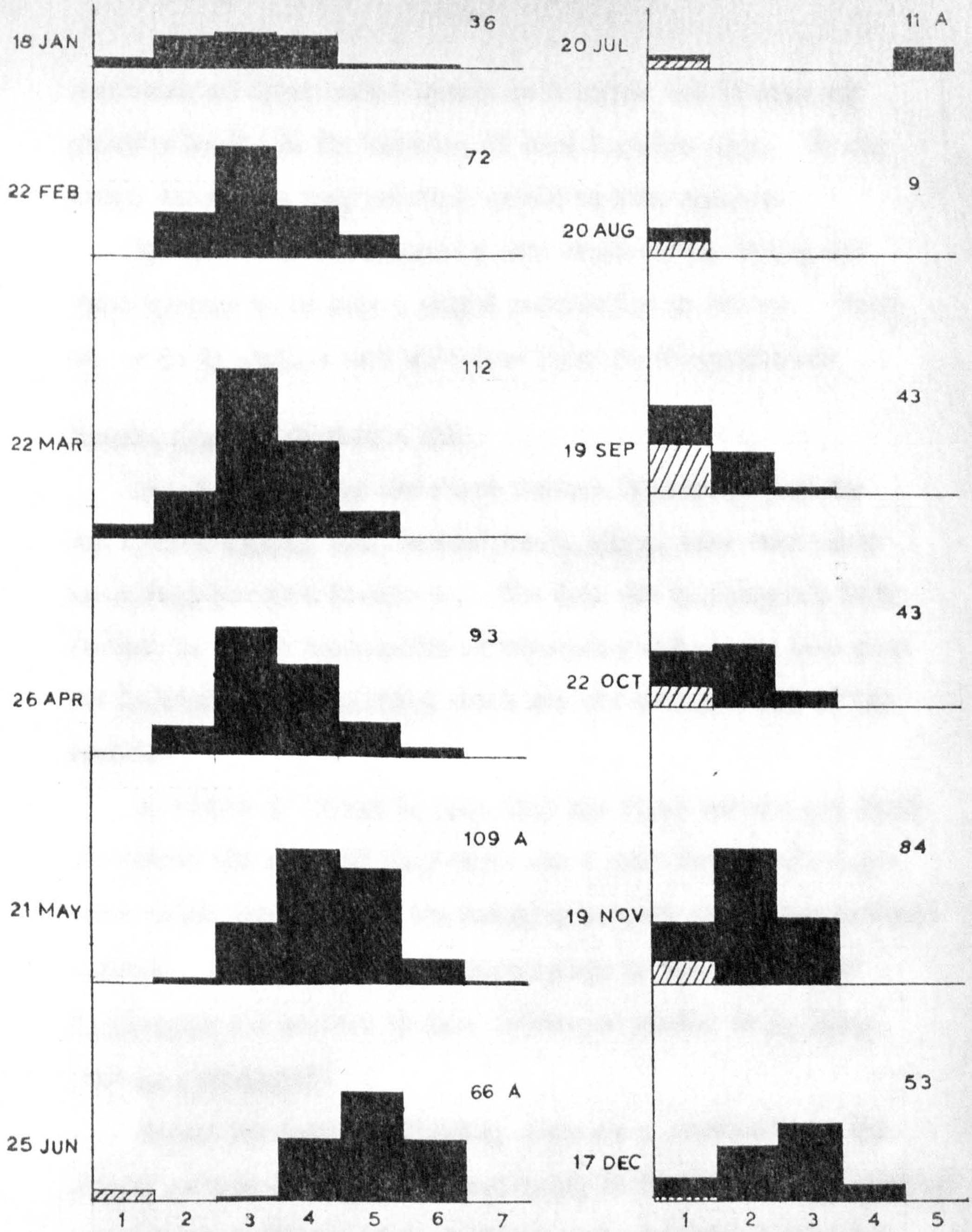
a short hatching period of the eggs.

C. torrentium (Figure 39)

The flight period is from May to early August and egg-laying must occur during this period since adults begin to oviposit about 1 to 2 weeks after emergence. This is no delayed hatching of eggs as has been suggested by Hynes (1962). Although nymphs of the 0.5 - 1.0 mm. group were found from June to December, the first instar nymphs occurred only from June to September; the small nymphs from October to December were in their second instars. There is, therefore, a delayed growth during the cold period. Except for the retardation during winter, the growth of the nymphs is steady and gradual.

I. grammatica (Figure 40)

The flight period is from May to July and the occurrence of first instar nymphs from August to January indicates a delayed hatching of some eggs. From laboratory studies, it has been shown that the incubation period is about 2 months and that the hatching of an egg-batch continues for about 1½ months after which there are still some seemingly undeveloped eggs. It has been suggested that these remaining eggs may possibly be in diapause. Assuming that these eggs are undeveloped and that the hatching period of any one batch of eggs is 1½ months, then one would expect the hatching period to be from August to November. The first instars of the Setipalpia do not feed and they moult fairly soon after hatching. It appears, therefore, that the



C. torrentium

Fig. 39

occurrence of first instar nymphs in December and January may possibly be due to the hatching of some diapause eggs. In any event, there is a long hatching period in this species.

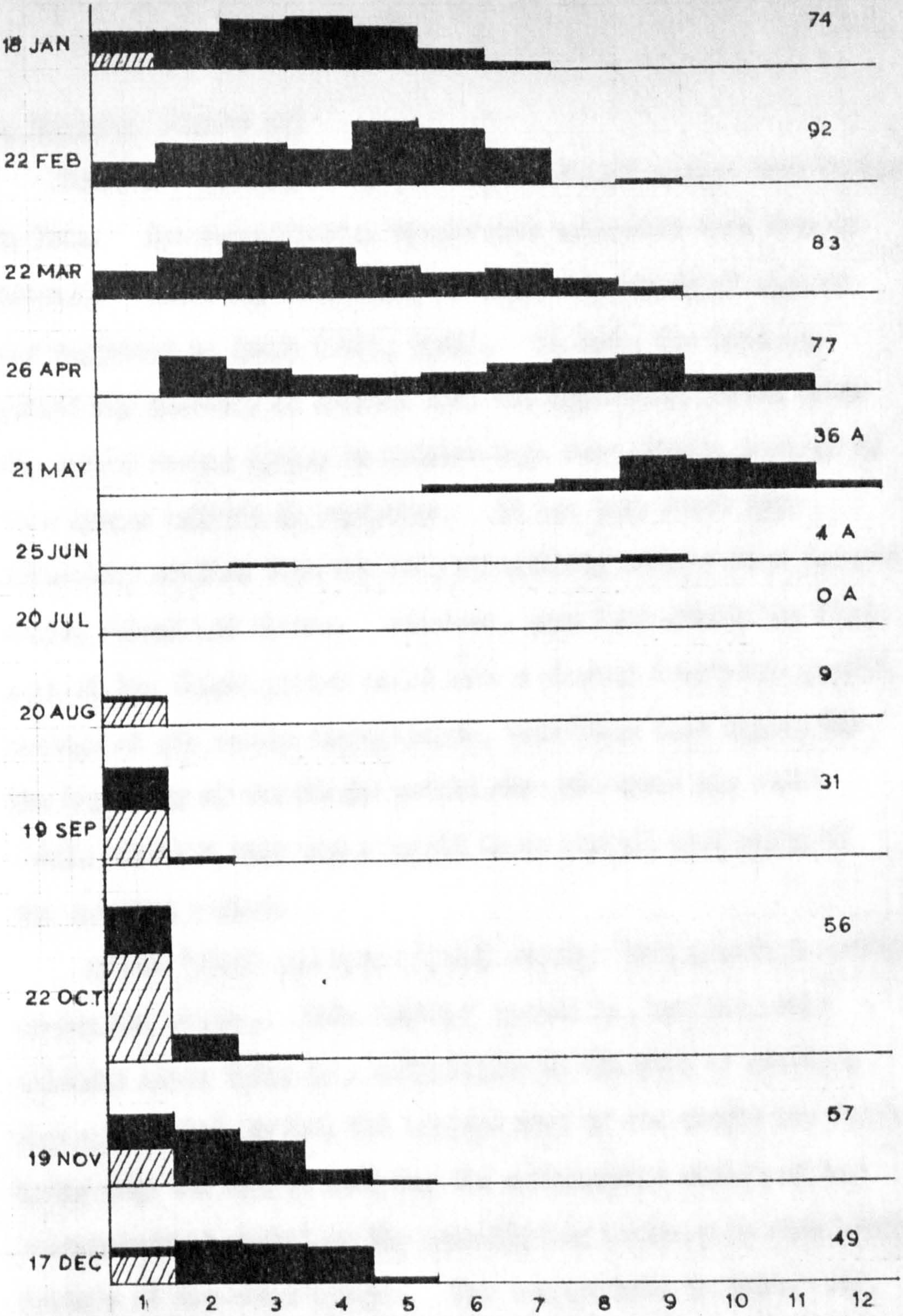
The growth of the nymphs is very rapid during Spring and there appears to be only a slight retardation in winter. There is, as in B. risi, a very wide-size range in the population.

Leuctra spp. (Figures 41 - 47)

The histograms for the first instars (figure 47) and for the various Leuctra spp., except for L. nigra, have been based on collections from Station 2. The data for L. nigra are from Station 1. Only the results of fine net samples have been used for L. hippopus and L. fusca which are the most abundant of the species.

In figure 47 it can be seen that the first instars are found throughout the year and that there are 2 peak periods of occurrence which correspond to the hatching periods of the two dominant species. One peak in Summer corresponds to the hatching of L. hippopus and another in late Autumn and winter to L. fusca (and L. geniculata).

Except for some overlapping, there is a succession in the flight periods of the species occurring in Station 2. L. hippopus emerged the earliest and is followed by L. inermis, L. moselyi and L. fusca. The two dominant species have fairly long flight periods while in the other species the flight periods are relatively short.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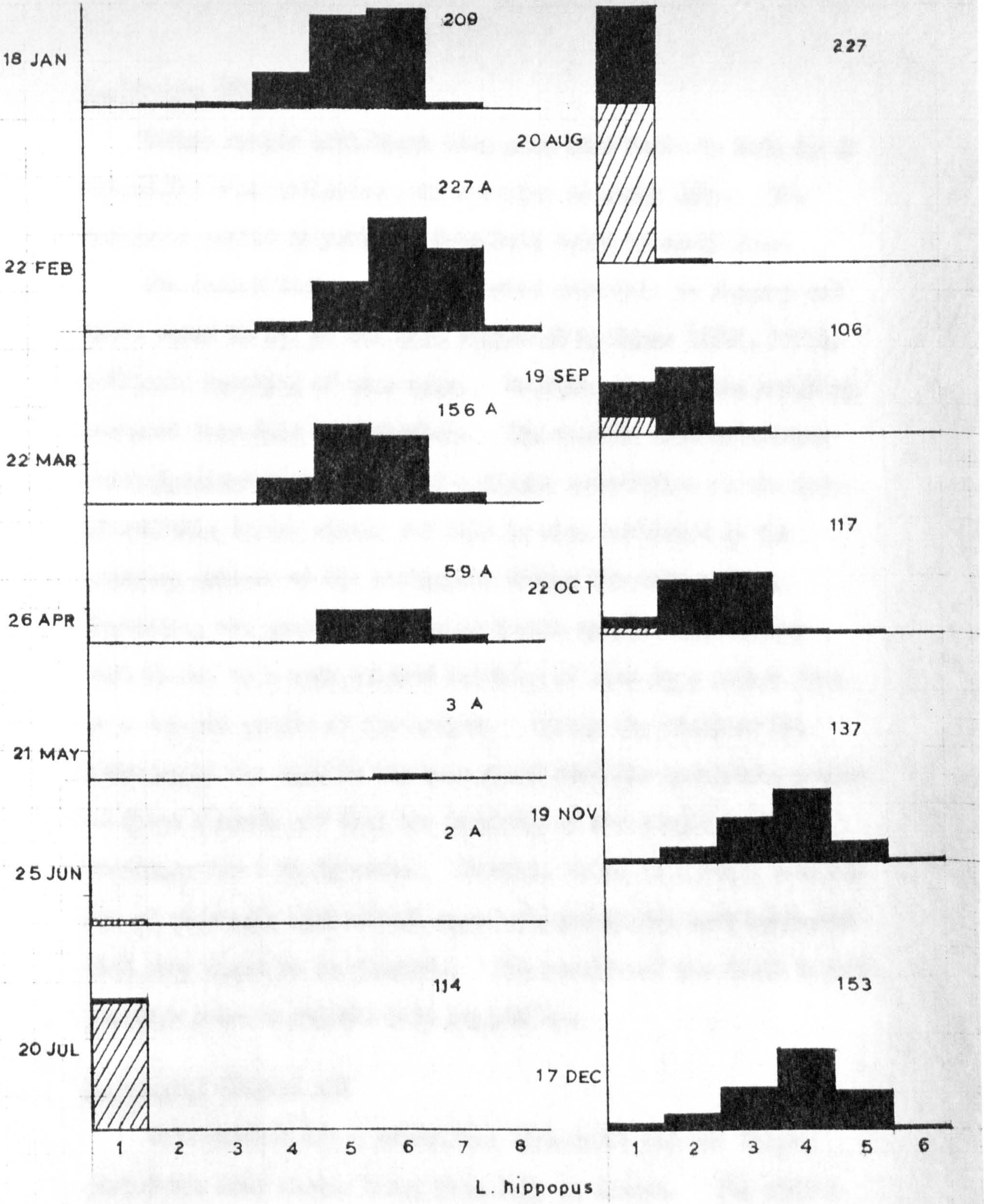
49
1 2 3 4 5 6 7 8 9 10 11 12
l. grammatica

Fig. 40

L. hippopus (Figure 41)

This is a univoltine species with a flight period from February to June. The second instar nymphs were collected from June to October. There is, therefore, no delayed hatching of eggs as was suggested by Hynes (1961, 1962). In fact, the hatching period may possibly be shorter than the egg-laying period since the second instar nymphs in October may, very likely, have grown from nymphs hatched in September. It has been found from laboratory studies that the rate of moulting is very much delayed during Autumn and Winter. Moreover, eggs laid during the later part of the flight period would have a shorter incubation period, because of the warmer temperatures, than those laid during the the beginning of the flight period when the water was still fairly cold and this would result in an overall shortening of the hatching period.

Brinck (1949) and Hynes (1962) mention that growth is active during the winter. This 'active' growth is, however, only illusory since there is a retardation in the rate of moulting during the cold period, but because many of the nymphs are fairly large when the winter sets in, the unfavourable effect of low temperature is masked by the normally big increase in size between instars of the older nymphs. The temperatures in Summer are, in fact, favourable for the growth of the species even though the increase in size of the small nymphs during this period may not be significant.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17 DEC
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1 2 3 4 5 6 7 8
1 2 3 4 5 6
L. hippopus

Fig. 41

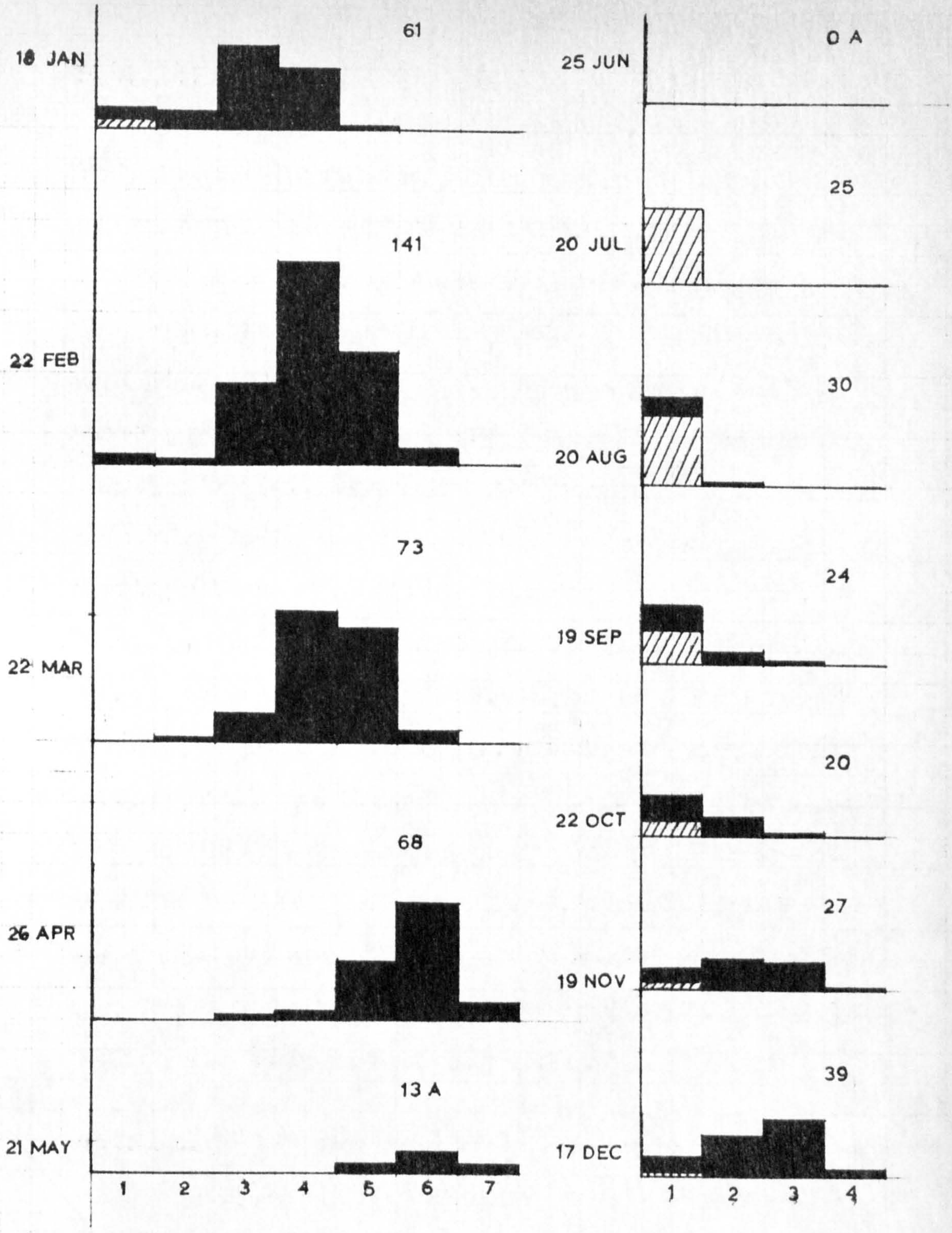
L. inermis (Figure 42)

Mature nymphs with black wing pads were found in late April but adults were collected only from May to early July. The emergence period is probably from late April to early June.

The second instar nymphs occurred from July to January and there seems to be, as has been suggested by Hynes (1961, 1962), a delayed hatching of some eggs. However, most of the hatching occurred from July to September. The results from laboratory investigations have shown only a slight retardation in the rate of moulting during winter and this is also reflected in the changing pattern of the histograms during the cold months. Therefore, the presence of second instar nymphs till January must be due to a very delayed hatching of some eggs rather than to a delayed growth of the nymphs. During the study on the hatching of the eggs it has been found that the incubation period is about a month and that the hatching of any single egg-batch continues for 1 to 2½ weeks. However, there is a small percentage of seemingly undeveloped eggs left and it has been indicated that they might be in diapause. The results of the field investigations seem to support this suggestion.

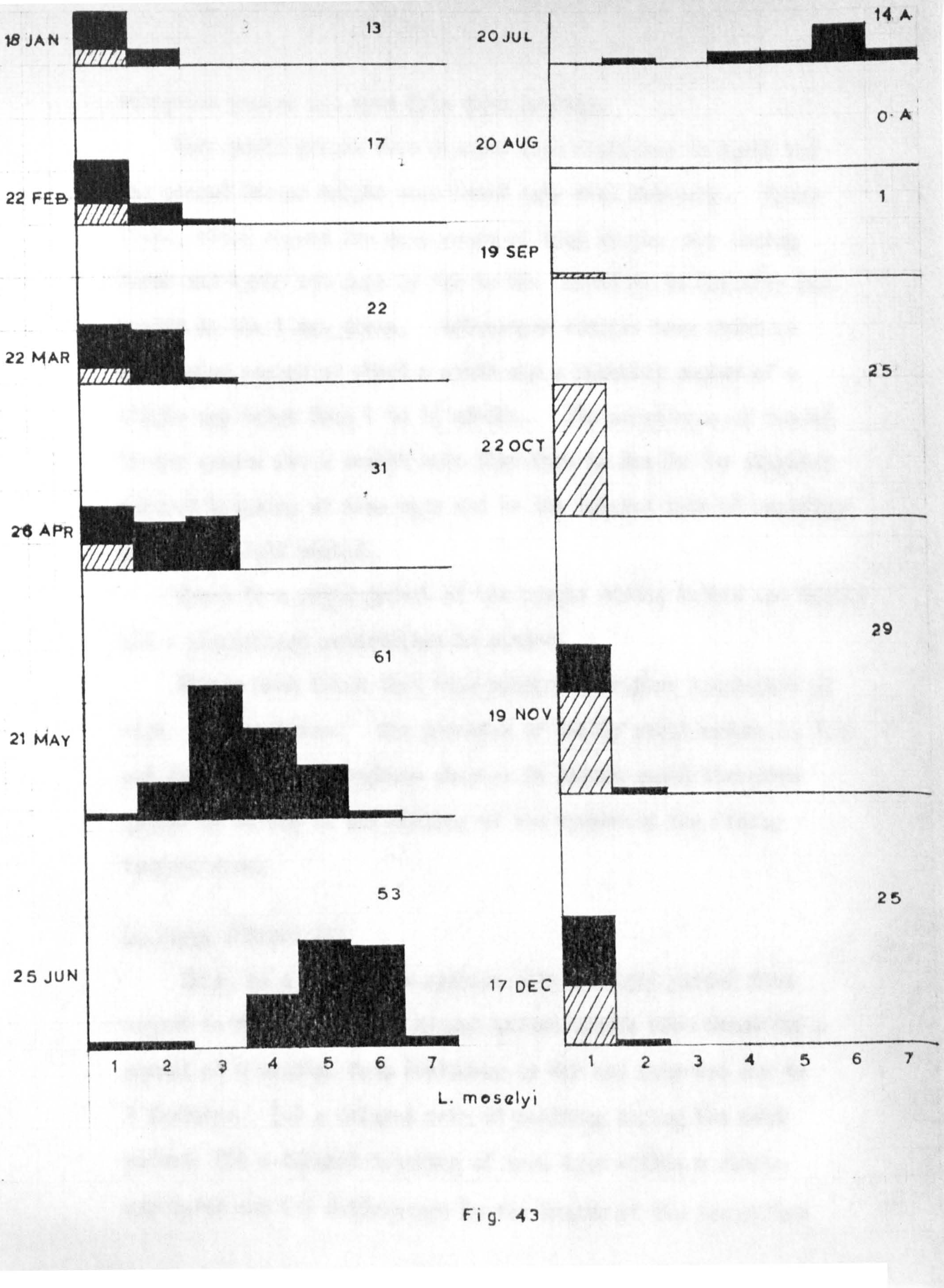
L. moselyi (Figure 43)

This species has a univoltine life-cycle and the flight period was very short, being from July to August. The adults occurring in August probably emerged in July. Although no adults were collected in June, mature nymphs with black wing pads were found during this period and it is possible that the



L inermis

Fig. 42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25
25 JUN
17 DEC
1 2 3 4 5 6 7
1 2 3 4 5 6 7
L. moselyi

Fig. 43

emergence period was from late June to July.

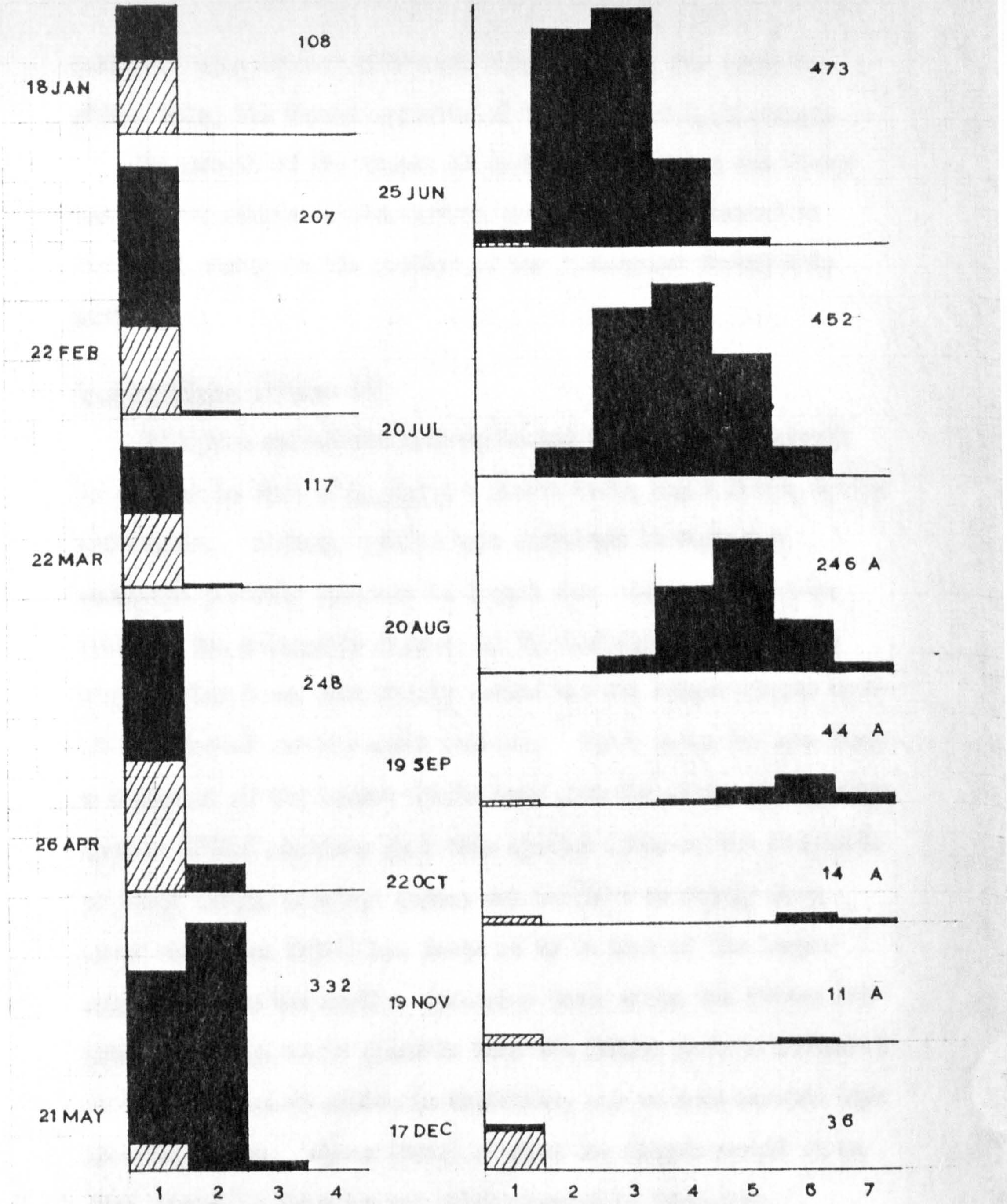
Very small nymphs were present from September to April but the second instar nymphs were found only till February. Hynes (1961, 1962) showed the occurrence of tiny nymphs only during March and April but this is due to the inability to identify the nymphs in the 1 mm. group. Laboratory studies have shown an incubation period of about a month and a hatching period of a single egg-batch from 1 to 1½ months. The occurrence of second instar nymphs for 6 months must therefore be due to the slightly delayed hatching of some eggs and to the delayed rate of moulting during the cold period.

There is a rapid growth of the nymphs during Autumn and Spring and a significant retardation in winter.

It has been found that this species is rather intolerant of high temperatures. The presence of fairly small nymphs in June and July and their complete absence in August would therefore appear to be due to the killing of the nymphs by the rising temperatures.

L. fusca (Figure 44)

This is a univoltine species with a flight period from August to November. The second instar nymphs were found for a period of 9 months, from September to May and this was due to 3 factors:- (a) a delayed rate of moulting during the cold period, (b) a delayed hatching of some eggs within a single egg-batch and (c) differences in the length of the incubation



L. fusca

Fig. 44

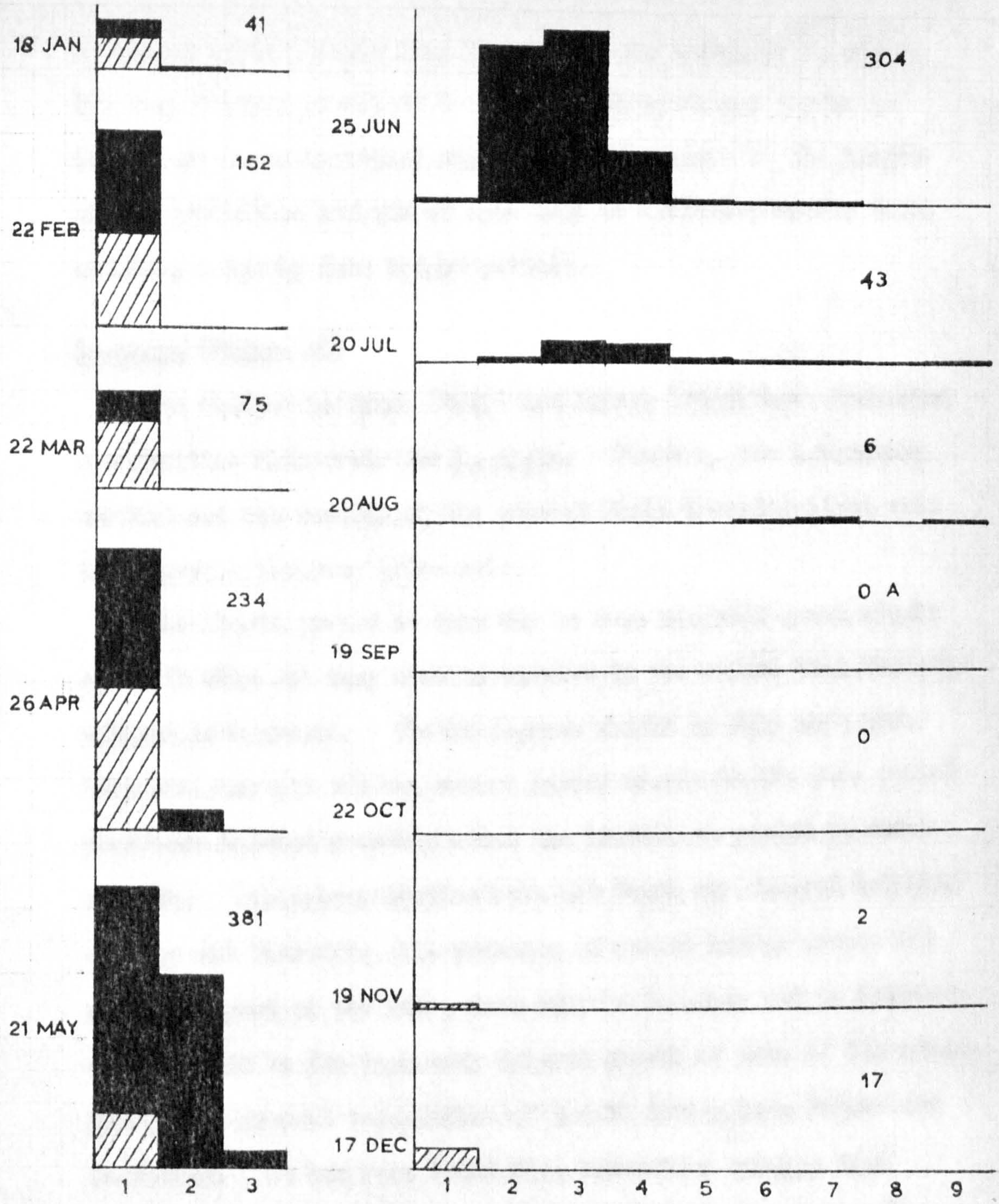
period of eggs laid at different times of year, the overall effect being the direct opposite of that seen in L. hippopus.

The growth of the nymphs is rapid during Spring and Summer and the retardation during Autumn and Winter is reflected in the small change in the pattern of the histograms during this period.

L. geniculata (Figure 45)

This is a univoltine life-cycle and the pattern of growth is similar to that of L. fusca - growth being rapid during Spring and Summer. Although adults were collected in September emergence probably occurred in August when mature nymphs were found. One noticeable feature in the histograms is that the nymphs below 5 mm. were fairly common but the larger nymphs were only collected in very small numbers. There seems to have been a migration of the larger nymphs away from the areas of sampling. Lestage (1920) mentions that this species lives on the underside of large deeply embedded stones and boulders in fairly deep water and Hynes (1941) has found it to be true of the larger nymphs whereas the smaller specimens occur among the stones and gravel. Thus, it is possible that the flight period, indicated by the presence of adults in September, may be much shorter than it actually is. Hynes (1958) records the flight period to be from August to November but mainly August to September.

The laboratory studies have not indicated any delayed hatching of the eggs of any one batch and therefore, the presence



L. geniculata

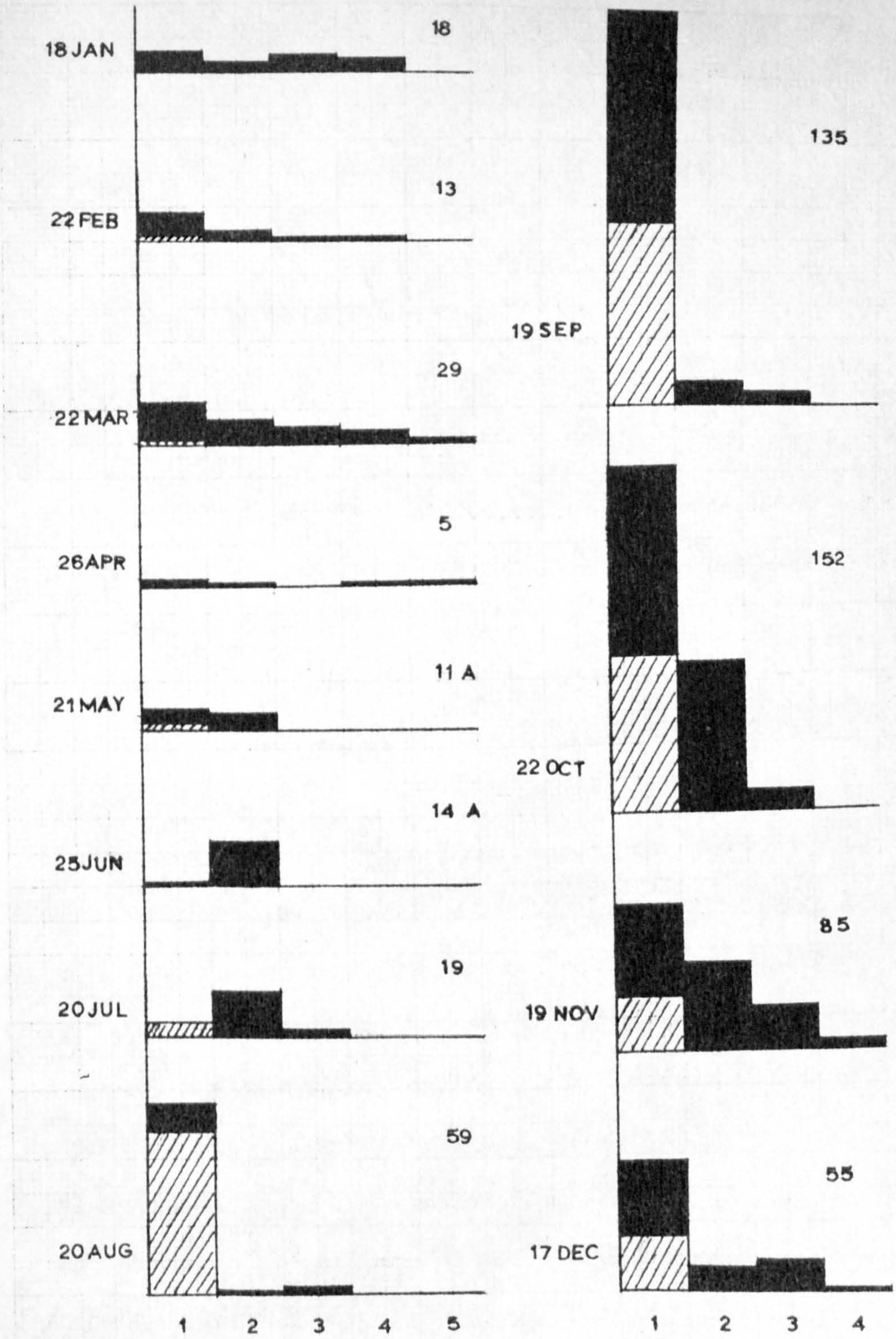
Fig. 45

of second instar nymphs from November to May seems to be due to the very delayed growth of the nymphs during Winter (shown in laboratory investigations) and to the differences in the length of the incubation periods of eggs laid at different months (i.e. assuming a fairly long flight period).

L. nigra (Figure 46)

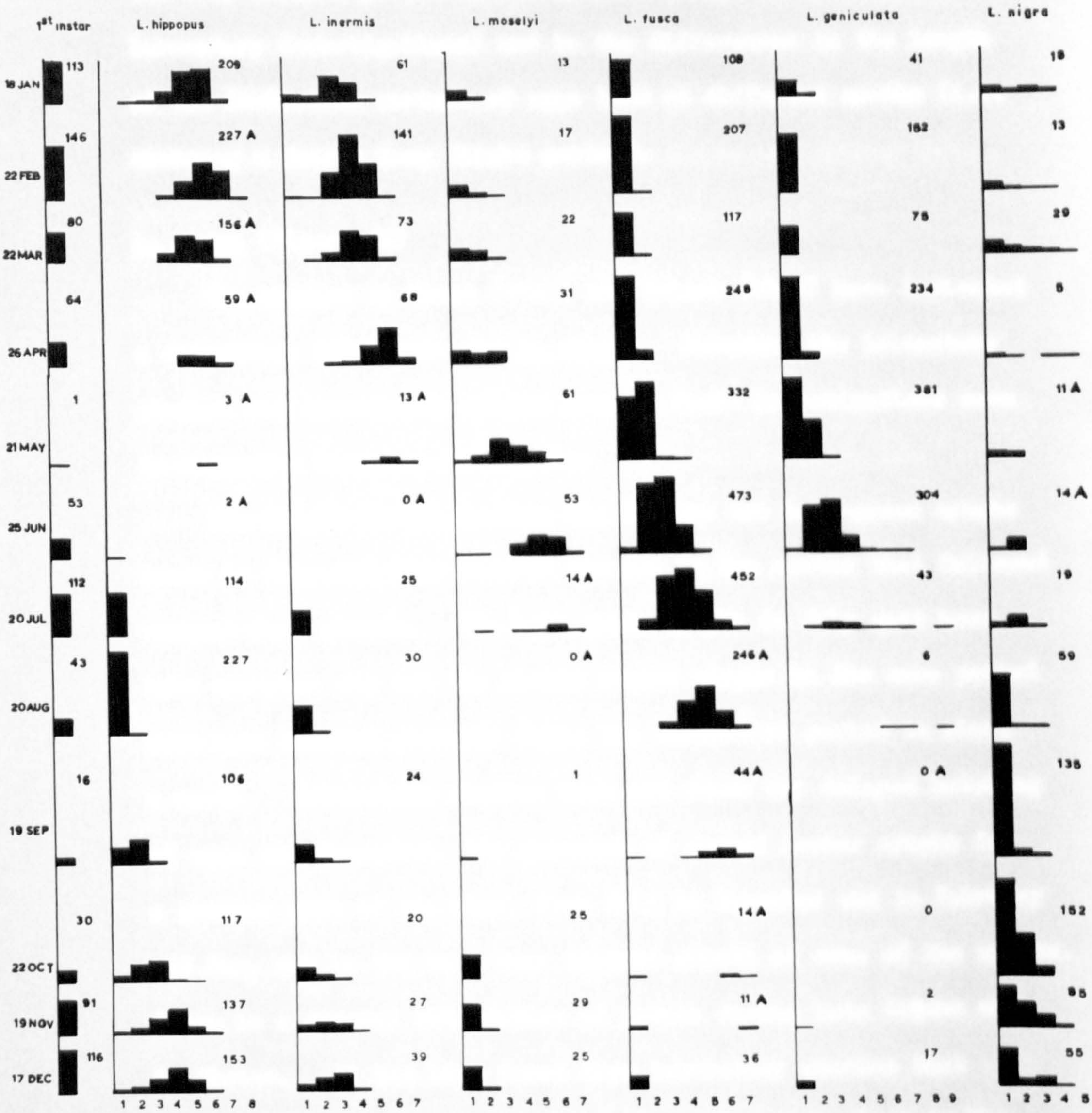
The studies by Hynes (1941) and Brinck (1949) have indicated a univoltine life-cycle for L. nigra. However, the laboratory studies and the results of the present field investigations seem to suggest a two-year life-cycle.

The flight period is from May to June but half-grown nymphs occur in July and they seem to survive in the months that followed without much growth. The half-grown nymphs in July must have been one-year old but the second instar nymphs in the same period must have hatched recently since the incubation period is about a month. Laboratory studies have not shown any delayed hatching of eggs and therefore, the presence of second instar nymphs for a greater part of the year, from July to December and in February and May, may be due to a very delayed growth of some of the nymphs. There is a general retardation of growth during late Autumn and in Winter. It has been found from laboratory studies that nymphs hatched in July were mostly less than 2 mm. long in May of the following year and this seems to support the interpretation of a two-year life-cycle in L. nigra.



L. nigra

Fig. 46


1st instar
L. hippopus
L. inermis
L. moselyi
L. fusca
L. geniculata
L. nigra
18 JAN
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21 MAY
25 JUN
20 JUL
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19 SEP
22 OCT
19 NOV
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113 209 61 13 108 41 18
146 227 A 141 17 207 182 13
80 156 A 73 22 117 75 29
64 59 A 68 31 248 234 8
1 3 A 13 A 61 332 381 11 A
53 2 A 0 A 53 473 304 14 A
112 114 25 14 A 452 43 19
43 227 30 0 A 246 A 6 59
16 106 24 1 44 A 0 A 135
30 117 20 25 14 A 0 152
91 137 27 29 11 A 2 85
116 153 39 25 36 17 55
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7
1 2 3 4 5 6 7 8 9
1 2 3 4 5

Fig. 47

5. Discussion

Of the thirteen species, only one, L. nigra, has a two-year life-cycle; the rest are all univoltine.

From the results of this study, it can be seen that the interpretation of field data must necessarily be cautious and that a knowledge of the biology of the eggs and of the nymphs is essential before anydefinite conclusions can be drawn.

The rate of growth of many species, based on the increase in the average size of nymphs in successive months, has been studied by Hynes (1941), Brinck (1949), Illies (1952) and Rauser (1961, 1963). Brinck (1949) points out that this method is applicable only if there is no long hatching period or retardation in the growth of the young nymphs. Macan (1960) has indicated other sources of error such as the stopping of growth of mature nymphs and the migration of nymphs into the sampling area. Another criticism of this method is that the geometric increase between instars would tend to mask the effect of environmental conditions on the growth of the nymphs. Macan (1960) has devised a method of assessing the amount of growth by superimposing the histograms for successive months in pairs, one on the other. It appears, however, that the standard method of placing the specimens into equal linear size-groups is inappropriate because the young nymphs would tend to show a slower rate of increase than the older nymphs. A suitable method probably would be to place the nymphs into equal logarithmic rather than equal linear size-groups. In the present study, however, the measurements of the nymphs has

been done in the standard manner but comments regarding the growth of the small nymphs have been based on the observations regarding the stages of development of the nymphs and on the changing pattern within the 1 mm. size-group.

Macan (1957) and Hynes (1961, 1962) have shown that in many species of mayflies and stoneflies respectively, the period during which small specimens can be found is very much longer than the flight period and have suggested a delayed hatching of some eggs. Macan has also pointed out the possibility of a delayed growth of young nymphs as has been previously shown by Brinck (1949) for L. fusca. Besides the possibility of a delayed growth as a result of unfavourable conditions there is also the normal small increase in size between instars in the young nymphs. Thus it is important to be able to recognise the stages of development of the small specimens. In order to distinguish between a long hatching period and a delayed growth of the nymphs, it is necessary to take into account the period of occurrence of the very young nymphs since there is generally a retardation of growth and development during the winter. In B. risi, A. standfussi, C. bifrons, L. inermis and I. grammatica this retardation is relatively slight whereas in the other species growth appears to be slowed down considerably during the cold period. In some species such as C. bifrons growth is stopped completely during the Summer because of the occurrence of diapause in the early instars. In A. sulcicollis and L. nigra the long period during which the newly-hatched or tiny nymphs are found is due to delayed growth of the

nymphs. In L. moselyi, L. fusca and L. geniculata it is the result of a long hatching period as well as a delayed nymphal growth while in A. standfussi, B. risi, I. grammatica and L. inermis it seems to be due to a long hatching period of the eggs.

A long hatching period may be due to intrinsic factors within the eggs or to the influence of environmental conditions on the length of the incubation periods or to both. In L. moselyi, A.standfussi, B. risi, I. grammatica and L. inermis the flight (and egg-laying) periods are relatively short and do not spread over seasons when there are great changes in temperature. Hence, the long hatching periods are due to delayed hatching of some eggs with a singe egg-batch that is, a result of intrinsic factors. In L. fusca there is a delayed hatching of 1½ months for any singe egg-batch but in this species, however, the effect of external factors is particularly important, since the flight period extends from Summer to late Autumn. The eggs that are laid in Summer hatch sonner, because of the warmer temperatures, than those that are laid in late Autumn so that there is an overall extension of the hatching period.

In L. hipporus, the hatching period is shorter than the flight period which extends from February to June. In this species, the overall effect of the long flight period is the reverse of that in L. fusca. A short hatching period is also seen in C. bifrons but the effect of environmental factors is on the interval between emergence and egg-laying. Adults which emerge early in the year, because of the lower air temperatures,

show a longer interval between emergence and egg-laying than those which emerge later. The eggs are normally fully-developed when they are laid and hatching is immediate.

The occurrence of a short emergence period is found in several species but in some species such as L. hippopus and L. fusca, which are able to tolerate a wide range of temperature, the flight period is fairly long. In A. standfussi, L. moselyi and B. risi, which are quite intolerant of high temperature, it appears that the short emergence period may be due to the killing of the nymphs by the rising temperatures. The importance of temperatures as a factor in the restriction of flight periods has been discussed by Macan (1958b). High temperatures need not necessarily result in the immediate death of the nymphs but instead, the effect may be delayed as has been shown from the laboratory studies on the rearing of L. moselyi and L. inermis. There is a different degree of tolerance to high temperatures between the young and old nymphs. The early instars of many species are able to develop without much ill-effects at temperatures which are unfavourable for the later instars. This clearly suggests that the effect of temperature isthrough the medium of oxygen, as has been pointed out by Pleskot (1953). At high temperatures, the amount of oxygen available maymnot be sufficient for the increasing demands of the larger nymphs. Besides the negative aspect of unfavourable environmental conditions in preventing a species from extending its flight period there is also the possibility that some species may actively restrict the flight period by emerging after a

smaller number of instars, since the instar number for any species is quite variable. In C. bifrons (Chapter II, 3c) it has been shown that adults which emerge towards the end of the flight period tend to be smaller than those which emerge earlier and there are indications, in this species, that increasingly long photoperiods may induce differentiation of adult characters in half-grown nymphs.

IV. STUDIES ON DIAPAUSE

1. Introduction

The interpretation of diapause as used here follows the definition given by Andrewartha (1952) and Lees (1955) and is distinct from quiescence in which the condition of arrested growth is directly controlled by unfavourable conditions which when removed will cause an immediate resumption of growth.

There has been, previously, no definite record of diapause in stoneflies. Miller (1939) mentions that the development of the embryo of P. proteus is complete in about 5½ months, but under natural conditions the naiad remains dormant over winter and hatches about 10 months after oviposition. Although the dormancy has been referred to as diapause by Miller, it seems likely that this is a case of quiescence since eggs that were maintained for about 5½ months under natural conditions began to hatch 8 days later when transferred to a temperature of 22°C.

From the laboratory investigations on the biology of the eggs and nymphs, it has been shown that several species exhibit this phenomenon of diapause. In B. risi, A. standfussi, N. cinerea and D. bicaudata diapause occurs in the egg-stage, while in C. bifrons it is the nymphs which undergo diapause. The possibility of diapuase occurring in some of the eggs of I. grammatica and L. inermis and in the nymphs of N. cinerea has also been indicated.

During the investigations, a study was made of the factors influencing diapause in some of the species, namely, C. bifrons,

D. bicaudata and B. risi. Time has been a great limiting factor and the investigations were carried out in 1963, in the midst of other studies on the biology of stoneflies. The experiments had to be designed partly to fit in with the existing facilities that were also being used for other purposes during that period. The conclusions that can be drawn are therefore limited but nevertheless they should narrow the scope for further analyses on diapause in these insects.

2. Experimental conditions

The following experimental conditions were employed:-

A_1 - cold room. Normal photoperiods and temperatures simulating the conditions in R. Terrig.

A_2 - Box at room temperature. Normal photoperiods as in A_1. Average temperature 20.5°C, the range was 15 to 26°C.

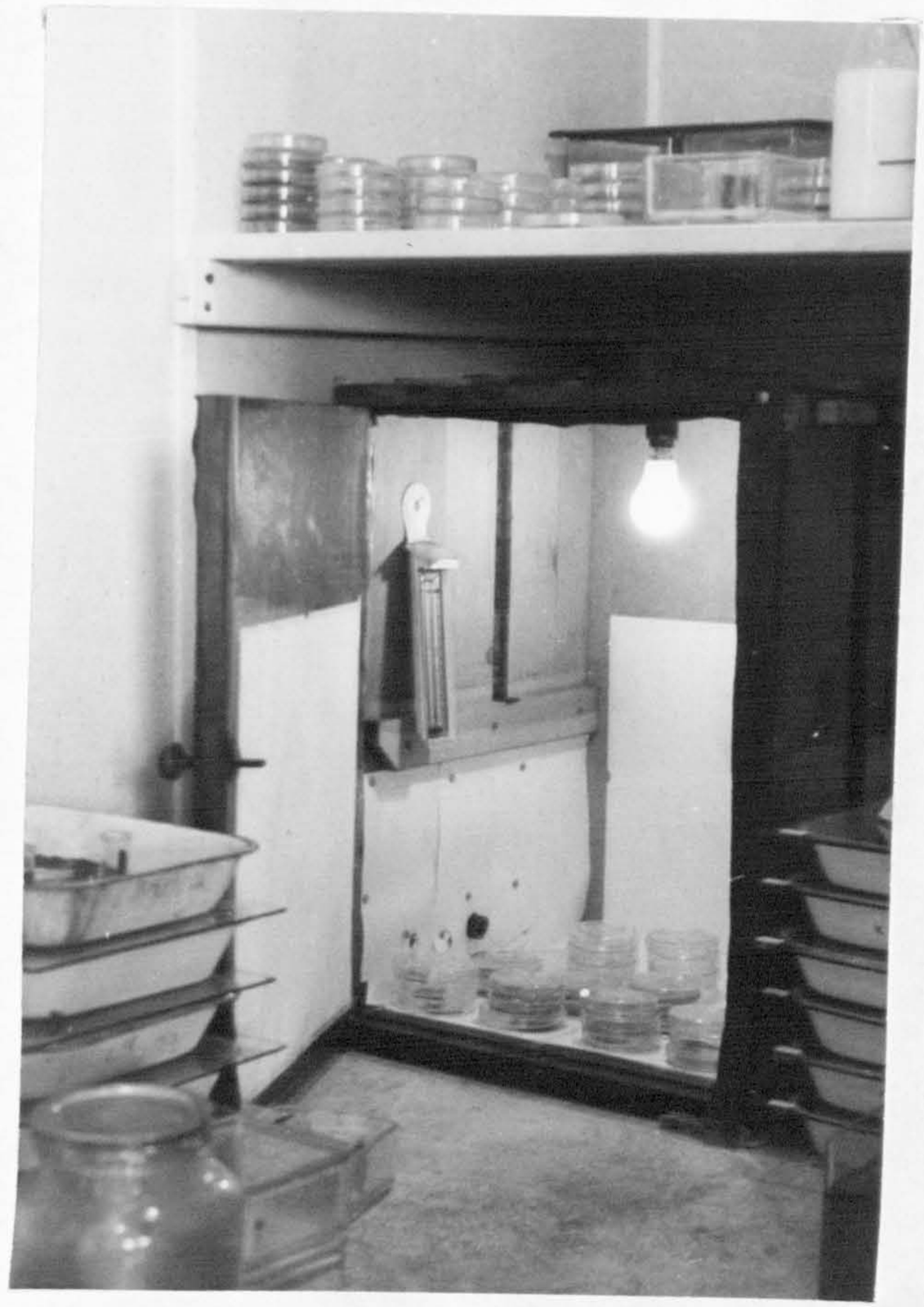
B_1 - Box inside cold room. Photoperiods about 4½ months ahead of normal photoperiodic regime. Temperatures varied from that of A_1 to about 2°C higher: this was caused by the lamp.

B_2 - Box at room temperature. Photoperiods as in B_1. Average temperature 20.5°C, range 15 to 26°C.

C_1 - Refrigerator. Constant photoperiod of 12¼ hours of light per day. Temperatures varied daily from 1 - 7.5°C.

The cold room, 6 x 6 x 7 feet, was illuminated by an ordinary 100W

a



b

Figure 48

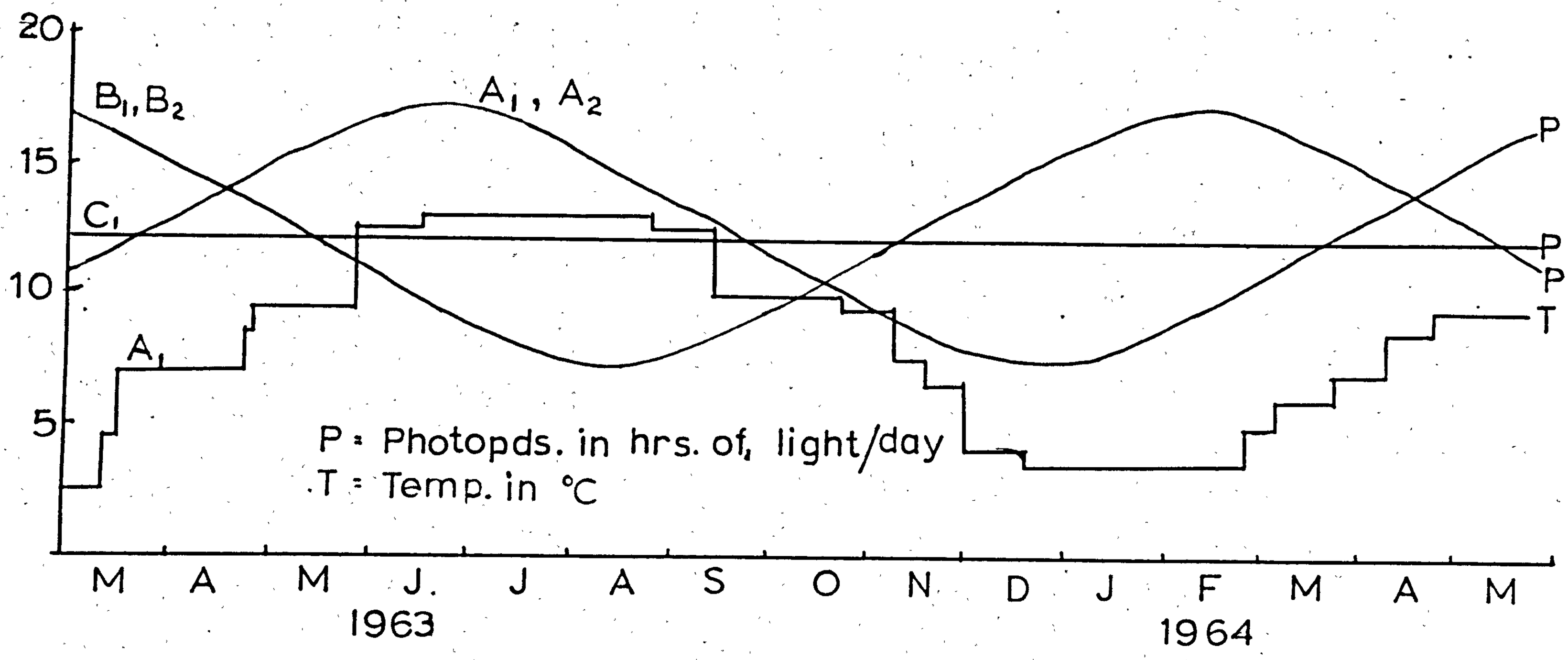

20
15
10
5
B_1, B_2
A_1, A_2
C_1
A_1
P
P
P
T
P = Photopds. in hrs. of, light/day
T = Temp. in °C
M A M J. J A S O N D J F M A M
1963
1964

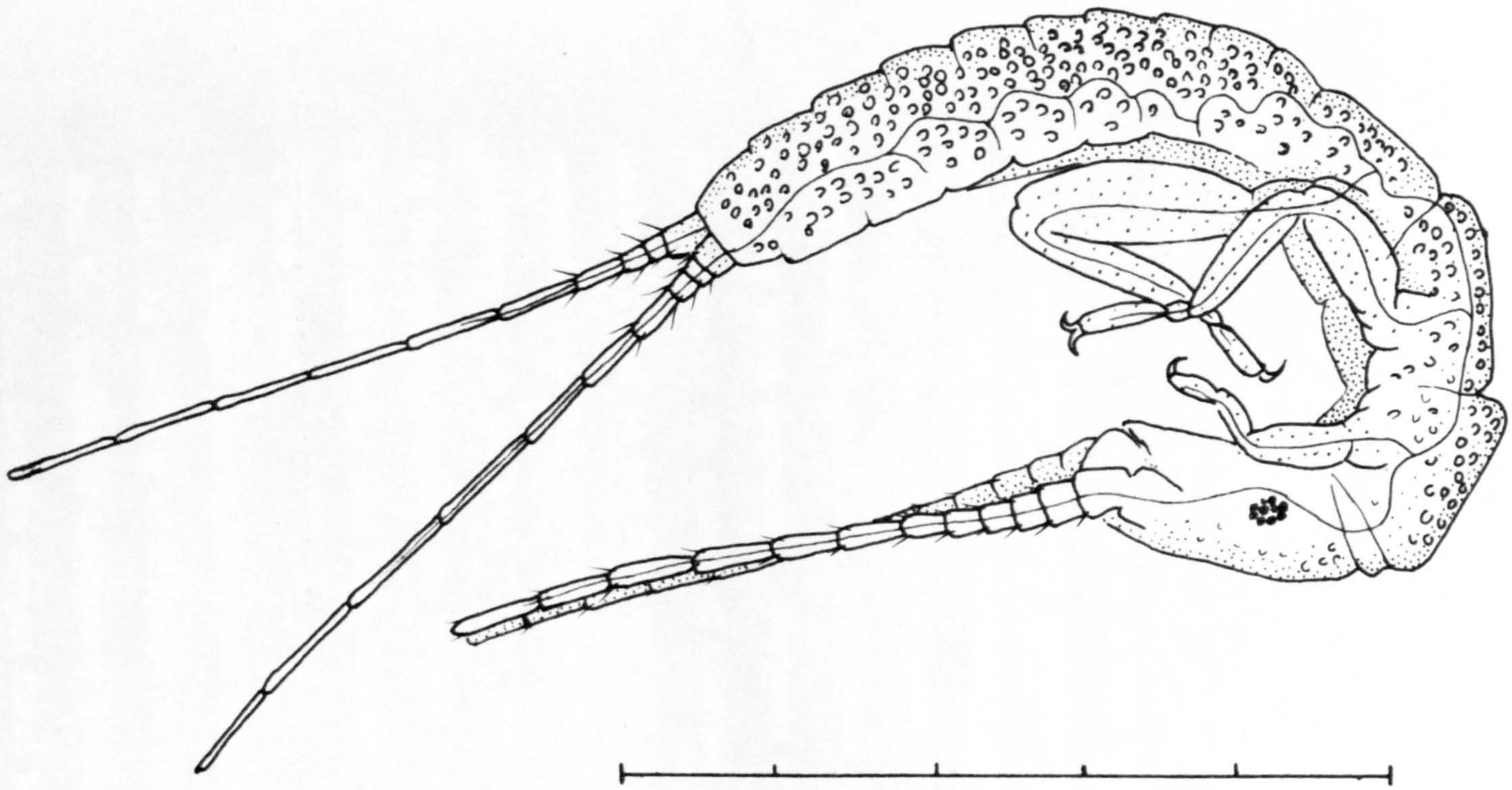
Fig 49

lamp. The temperature was adjustable and was thermostatically controlled, with an accuracy of ± 0.5°C. The size of boxes A_2, B_1 and B_2 was $1\frac{3}{4}$ x $1\frac{3}{4}$ x 2 feet and the source of lighting in them and in the refrigerator C_1 was a 15 W lamp. There was no facility for maintaining temperatures independent of photoperiods in the refrigerator or in the boxes. However, to prevent excessive rise of temperature in the boxes 2 one-inch holes were drilled at the sides,(one at the lower and another at the upper end) to allow convection currents to occur. A foot length of rubber tubing was fitted to each of these openings and it was bent away from the external source of light. The lamps in A_1, A_2 and B_1, B_2 were connected to 2 time-switches and the photoperiods were adjusted once a week. C_1 was connected to a separate time-switch and the photoperiod was kept constant.

Figure 48a shows the cold room A_1 with the box B_1 inside it. A close-up view of B_1 is shown in figure 48b. The three photoperiodic regimes and the temperature conditions in A_1 are indicated in figure 49.

3. Diapause in Capnia bifrons

C. bifrons is a univoltine species with an emergence period from February to May in River Terrig. The females are ovoviviparous and each adult lays only a single batch of eggs. In nature, the egg-laying period is from May to June. The interval between emergence and egg-laying is influenced by temperature and it is possible to induce early oviposition by



0·5 mm

DIAPAUSE NYMPH (4th instar) of

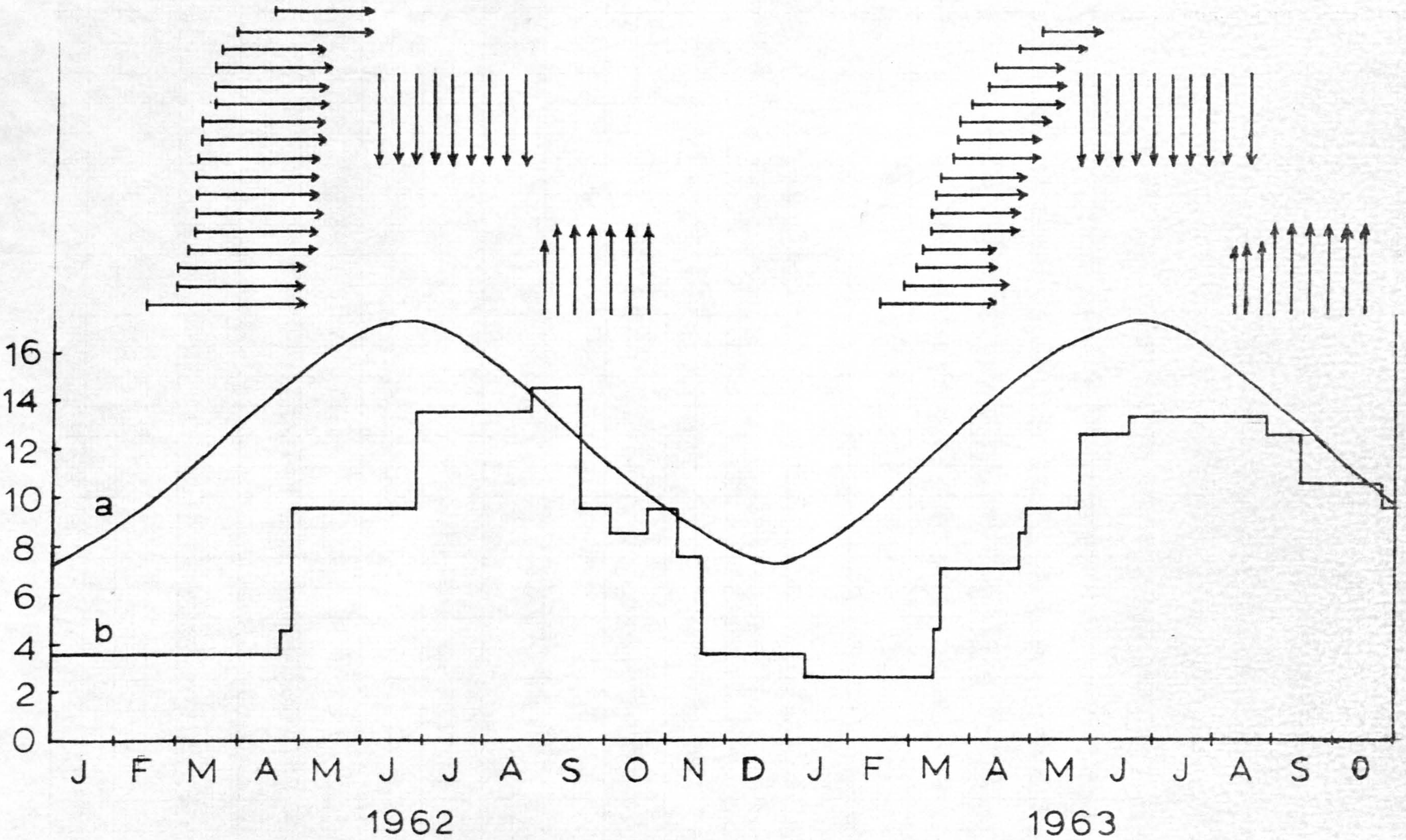
CAPNIA BIFRONS

Fig. 50

maintaining the adults at warm temperatures.

The eggs hatch immediately after oviposition and about a month after hatching, the nymphs begin to undergo diapause. Diapause occurs usually in the 4th or 5th instar. Occasionally some nymphs undergo diapause in the 3rd or 6th or very rarely in the 7th instar. Diapause nymphs are morphologically very distinct from non-diapause nymphs. In general appearance they are opaque and whitish in colour due to a huge accumulation of fat globules. The tracheal tubes are very conspicuous and the segments towards the tip of the cerci are extremely slender. In the non-diapause nymphs (i.e. pre- and post-diapause instars) bristles occur on every cercal segment but in diapause nymphs they are found only on a few basal segments. Diapause nymphs usually do not show an increase in the number of antennal segments over the previous instar. They do not feed and are at first sluggish in their movements but they soon become immobile in a characteristic position, with the head flexed towards the body (Figure 50). At the end of diapause the nymphs become active again but they do not feed until they have moulted, which is a few days to about a week later.

In River Terrig, diapause nymphs occur from June to August while the breaking of diapause is from September to November. From the results of laboratory studies in 1962 (See figure 51) it was found that the nymphs first began to enter diapause in June when the temperature was 9.5°C. Some nymphs went into diapause during August when the temperature was 13.5°C.



⟶ Interval between emergence and egg-laying

↓ Beginning of diapause

↑ Breaking of diapause

a Photoperiod in hours per day

b Temperature in °C

Fig. 51

Although the majority of the nymphs broke diapause in the second half of September and in October, the breaking of diapause began in late August and in early September before any fall in temperature had occurred. It appeared from this study, that the breaking of diapause is not dependent upon a fall in temperature and that the factor responsible for the induction of diapause may be photoperiods above a certain critical length. In 1963 a series of experiments was carried out to study the influence of environmental factors on diapause and the critical stage of development when such factors become effective. At the same time, nymphs were also reared in the cold room and the results in figure 51 again showed the breaking of diapause in August, before any fall in the temperature had occurred.

The newly-hatched nymphs were reared in petri-dishes (details of rearing are given in Chapter II, 2) and they were obtained from eggs laid by adults that emerged in the cold room, from fairly mature nymphs collected from R. Terrig.

Experiment 1

Aims:

To determine the influence of differing conditions of photoperiod and temperature on the induction and termination of diapause and on the stage during which diapause occurs.

Materials and Methods:

Two similar sets of experiments were carried out at different periods. The newly-hatched nymphs were reared

in petri-dishes in 5 different conditions:-

A_1 - normal photoperiods and temperatures,

A_2 - normal photoperiods; average temperature 20.5°C,

B_1 - Photoperiods about 4½ months ahead of normal regime; temperature varied from that of A_1 to about 2°C higher.

B_2 - Photoperiods as in B_1; average temperature 20.5°C,

C_1 - Constant photoperiod of 12¼ hours per day; daily range of temperature 1 - 7.5°C.

Experiment 1a began on 11.4.63 when the temperature in A_1 was 7°C (in B_1 between 7 and 9°C). The photoperiod in A_1 and A_2 was increasing from 13¾ hours per day and in B_1 and B_2 decreasing from 14¼ hours per day. 30 nymphs were kept in each set of conditions. Experiment 1b began on 27.5.63 when the temperature in A_1 was 12.5°C (in B_1 between 12.5 and 14.5°C). The photoperiod in A_1 and A_2 was increasing from 16½ hours per day and in B_1 and B_2 decreasing from 11 hours per day. 10 nymphs were used in each set of conditions.

Results:

A summary of the results is shown in Table 8.

Date of commencement	A_1	A_2	B_1	B_2	C_1
11.4.63	Diapause (5) - iv, v	Diapause (4) - iv, v, vi	Diapause (4) Non-diapause (1) v	Diapause (10) - v, vi	- Non-diapause (7)
27.5.63	Diapause (2) - iv	- -	Diapause (8) - iv	- -	Diapause (3) Non-diapause (5) iv, v

The figures within brackets indicate the number of diapause or non-diapause nymphs that survived the experiment. The Roman numerals indicate the stage of diapause.

Table 8 - Experiment 1 - The occurrence of diapause and non-diapause nymphs under various conditions.

Experiment 1a

A_1 - The nymphs entered diapause during late May and early June at the 4th or 5th instars. The breaking of diapause occurred in September.

A_2 - Diapause occurred in May and early June at the 4th, 5th or 6th instars. Only 1 nymph succeeded in breaking diapause in late August, the rest died between September and October.

B_1 - Four out of the five nymphs that survived the experiment entered diapause at the 5th instar, during late May and in June. The breaking of diapause occurred from late

August to September. The single nymph that did not undergo diapause was found to be fully developed in late October.

B_2 - Ten nymphs entered diapause at the 5th or 6th instars during May. None broke diapause and the nymphs died between late August to October.

C_1 - Seven nymphs were left by the end of August but none entered diapause. One was in the 4th, two were in the 5th and the rest were in the 7th, 8th and 9th instars. One nymph emerged in late February.

Experiment 1b

The nymphs in A_2 and B_2 died during the early stages of development.

A_1 - Two nymphs were left and both entered diapause in early July. They broke diapause in late September.

B_1 - Eight nymphs were left and all were in diapause in early July. The breaking of diapause occurred from October to November.

C_1 - Three nymphs entered diapause at the 4th or 5th instars at the end of August while five (one 4th, two 5th and two 7th instars) remained active. The breaking of diapause occurred in October and December. Two of the active nymphs were in the 12th instar in early November.

In spite of the different photoperiodic regimes in A_1, A_2 and B_1 and B_2. The dates of occurrence of diapause were fairly similar. In C_1 (Expt. 1b) the nymphs entered diapause after

a longer period because of the slower rate of growth at lower temperatures.

Diapause occurred mainly at the 4th or 5th instars. At the higher temperatures in A_2 and B_2 some nymphs underwent diapause in the 6th instar.

Most of the diapause nymphs in A_2 and B_2 died without breaking diapause. It is possible that diapause development was completed between late August and October but the high temperatures probably killed off most of the nymphs that were ready to break diapause.

In B_1, all the nymphs which hatched on 27.5.63 were of the diapause type whereas one of the nymphs which hatched on 11.4.63 failed to enter diapause. This may be associated with the lower temperatures that the first instars were experiencing in the latter case.

<u>Conclusions</u>

1. The stage at which diapause occurs is variable but it is usually in the 4th or 5th instar. The average period of diapause under normal stream temperatures is about 3 to 4 months.
2. Diapause is induced by temperatures above 12.5°C irrespective of increasing or decreasing photoperiods (A_2, B_2 in Expt.1a and A_1, B_1 in expt. 1b).
3. Increasing photoperiods from 13¾ hours of light per day and rising temperatures from 7 to 12.5°C will induce diapause in all nymphs (A_1 in Expt. 1a) whereas decreasing

photoperiods from 14¼ hours per day and rising temperatures from 7 to 14.5°C may result in a few nymphs failing to enter diapause (B_1 in Expt.1a). A clearer understanding of this conclusion can be obtained once the critical stage during which diapausing factors are effective and also the sensitivity of different instars to different enviornmental factors have been determined (See Experiment 2).

4. There is a tendency for nymphs not to enter diapause at temperatures of 1 to 7.5°C and at constant photoperiod of 12¼ hours per day (C_1).
5. The breaking of diapause can occur independently of photoperiod and temperature. However, the successful termination of diapause may be impaired by temperatures of 15 - 26°C (A_2, B_2).
6. The length of diapause in C_1 is about 2 to 4 months.

<u>Experiment 2</u>

Aims:

To determine the critical stage of development during which diapausing factors are effective and the sensitivity of different instars to photoperiods and temperatures.

Materials and Methods:

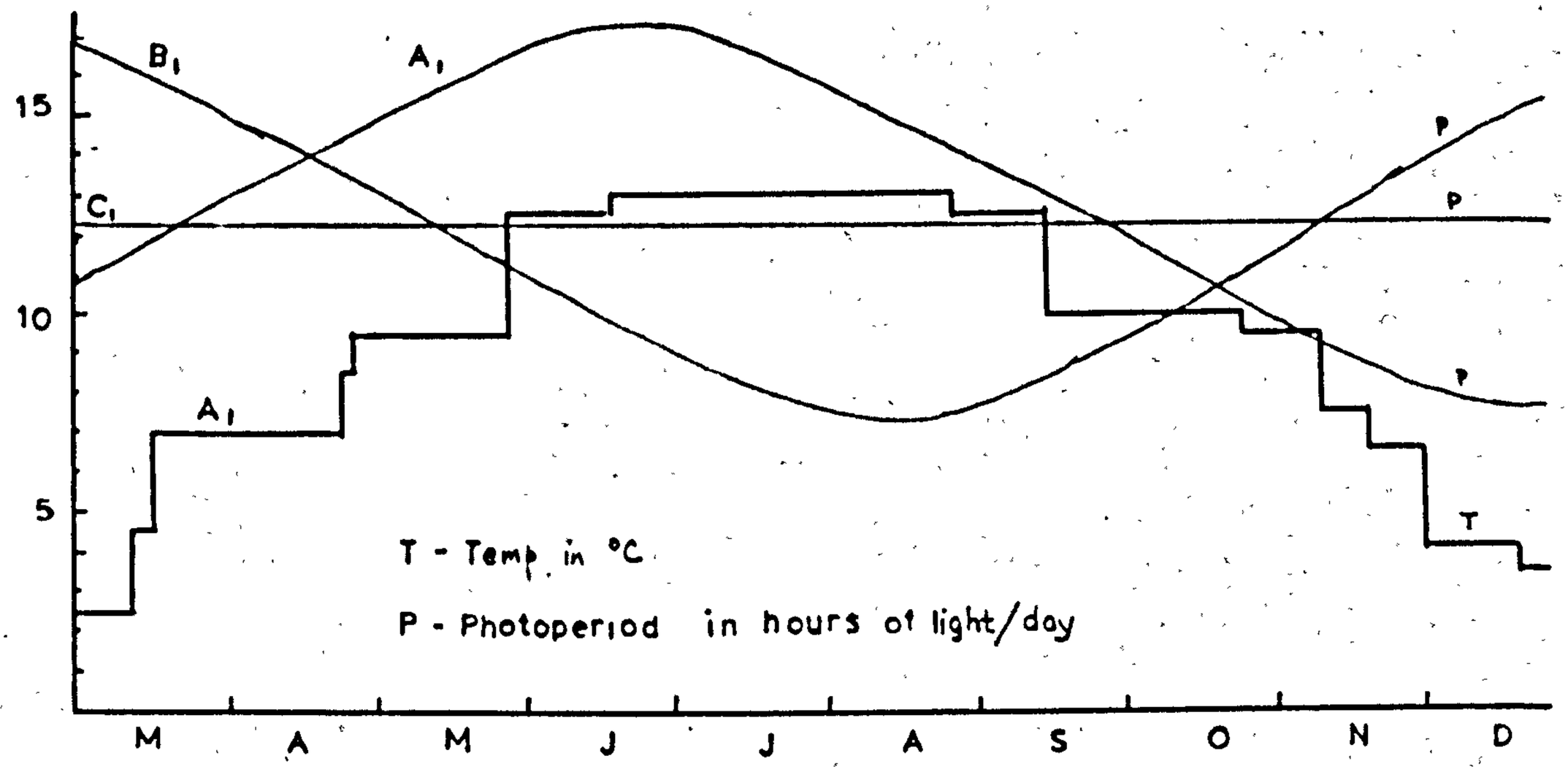
Newly-hatched nymphs were placed in A_1 and B_1 on 14.5.63. Ten nymphs were each transferred from A_1 to B_1 and C_1 and from B_1 to A_1 and C_1 at the 2nd, 3rd and 4th instars. The nymphs were transferred as soon as that particular instar

was reached. 10 other nymphs were also separated at the 2nd instar and kept as controls under experimental conditions (A_1 or B_1) in which they had started. Newly-hatched nymphs were also placed in C_1 on 11.4.63 and 4.6.63 and 5 nymphs each were transferred to A_1 and B_1 at the 2nd, 3rd, 4th and 5th instars. 5 other nymphs were kept as control in C_1. In A_1 and B_1 it was possible to begin with a large number of nymphs and so a sufficient number was always available for transfer as soon as a particular instar was reached. Unfortunately, many fewer were available to start their lives in C_1, so the numbers transferred from, and retained in, these conditions were small. Also, some nymphs of a particular instar had to remain in C_1 for some time before there were sufficient nymphs of that instar to be transferred together to A_1 and B_1.

Results:

The results are summarised in figure 52 in which the environmental conditions experienced by the various instars are indicated. The date of transfer of a particular instar to a different environmental condition is given at the bottom of the column for that instar. The number of nymphs that survived the experiment, the percentage of these nymphs that underwent diapause and the stage of diapause are shown in the figure.

A 100% diapause was obtained from nymphs reared throughout in A_1 and B_1. None of the nymphs kept as control



Instar no I II III IV	No.	% Diapause	Stage of Diapause
A_1 A_1 A_1 A_1	9	100	IV V
A_1 B_1 B_1 B_1	9	100	IV V
A_1 A_1 B_1 B_1	8	100	IV V
A_1 A_1 A_1 B_1	C	100	V
A_1 C_1 C_1 C_1	8	75	IV V
A_1 A_1 C_1 C_1	10	100	IV V
A_1 A_1 A_1 C_1	10	90	V

Instar no. I II III IV V	No.	% Diapause	Stage of Diapause
C_1 C_1 C_1 C_1 C_1	5	0	
C_1 A_1 A_1 A_1 A_1	4	100	IV V
C_1 C_1 A_1 A_1 A_1	5	100	V VI
C_1 C_1 C_1 A_1 A_1	5	100	V VI VII VIII
C_1 C_1 C_1 C_1 A_1	5	100	VI VII IX

B_1 B_1 B_1 B_1	9	100	IV V
B_1 A_1 A_1 A_1	9	100	IV
B_1 B_1 A_1 A_1	9	100	IV V
B_1 B_1 B_1 A_1	9	100	V
B_1 C_1 C_1 C_1	8	87·5	IV
B_1 B_1 C_1 C_1	9	100	IV
B_1 B_1 B_1 C_1	8	100	V

C_1 B_1 B_1 B_1 B_1	4	100	IV
C_1 C_1 B_1 B_1 B_1	4	100	IV V VI
C_1 C_1 C_1 B_1 B_1	5	60	V IX
C_1 C_1 C_1 C_1 B_1	5	40	VIII

				Date of Transfer
14.5	18.5	31.5	18.6	
4.6	1.7			
11.4	8.6	8.6	26.6	

Fig. 52

throughout in C_1 entered diapause. However, a few nymphs (not used as control) were found to undergo diapause in C_1. The above results do not contradict the conclusions drawn in Experiment 1.

A 100% diapause was also obtained from nymphs transferred from A_1 to B_1 and vice versa at the various instars.

Transferance of nymphs from A_1 and B_1 at the 2nd instar to conditions in C_1, which by themselves did not induce or only rarely induced diapause, resulted in 75% and 87.5% of the nymphs respectively undergoing diapause. Transferance from A_1 to C_1 at the 3rd and from B_1 to C_1 at the 3rd and 4th instars resulted in a 100% diapause. However, only 90% of the nymphs underwent when they were transferred from A_1 to C_1 at the 4th instar. The single nymph that did not enter diapause may have been aberrant.

All the nymphs that were transferred from C_1 to B_1 at the 2nd and 3rd instars and from C_1 to A_1 at the 2nd, 3rd, 4th and 5th instars entered diapause. Only 60% and 40% of the nymphs underwent diapause when they were transferred from C_1 to B_1 at the 4th and 5th instars respectively. The nymphs which did not enter diapause emerged in early September and in October (very much earlier than the normal emergence period).

There was a tendency for the nymphs which were transferred from C_1 to A_1 or B_1 at the later instars (e.g. 4th and 5th) to undergo diapause at a fairly

late stage of development. A few nymphs, however, entered diapause at the 5th or 6th instar, quite soon after they were transferred. These nymphs were possibly already destined for diapause while in C_1 whereas those which underwent diapause at the later instars (e.g. 8th or 9th) were possibly destined for diapause while in A_1 or B_1.

Conclusions:

1. The first five instars are sensitive to diapause-inducing factors but the sensitivity and response to certain factors are decreased ih the later instars. The first three instars are sensitive to temperatures of 9 to 15.0°C irrespective of photoperiods (cf. transfer of 2nd and 3rd instars from A_1 and B_1 to C_1 and from C_1 to A_1 and B_1). The sensitivity and response to these temperatures in the 4th and 5th instars are decreased under decreasing photoperiods (cf. transfer of 4th and 5th instars from C_1 to B_1). The response is, however, unimpaired under long photoperiods of about 17 hours /day, (cf. transfer of 4th and 5th instars from C_1 to A_1). To summarise, the first five instars are sensitive to temperatures and photoperiods. In the first three instars, diapause is induced over a wide range of temperature (9 - 15°C) irrespective of photoperiods. In the 4th and 5th the sensitivity to temperature decreases, and photoperiods begin to assume an important role in the induction of diapause.

2. A large percentage of the nymphs are destined for diapause during the first instar and by the second instar all or virtually all the nymphs are destined for diapause (cf. transfer from A_1 and B_1 to C_1).
3. Diapause cannot be prevented once the fate of the nymphs has been determined (cf. transfer from A_1 and B_1 to C_1).
4. The onset of diapause occurs several instars after the prospective fate has been determined. Thus, nymphs which are destined for diapause during the first or second instar usually enter diapause at the 4th or 5th instar while those which are destined during the fourth or fifth instar tend to undergo diapause at the 8th or 9th instar.
5. Non-diapause nymphs are able to develop fairly rapidly and emerge successfully under decreasing photoperiods and at temperatures of 12.5 - 15°C.

Experiment 3

Aims:

To determine the influence of environmental factors on the termination of diapause.

Materials and Methods:

Newly-hatched nymphs were placed in A_1 and A_2 on 11.4.63 and 10.5.63 respectively and were kept there until they had been in diapause for about 3 weeks. Five nymphs each were then removed on 3.7.63 and were transferred to B_1, B_2, C_1 and A_1 or A_2 with similar numbers remaining as

controls. The breaking of diapause, i.e. when the nymphs became active again, was then observed.

Results:

The results are shown in Tables 9 and 10.

Condition	Remarks	Length of Diapause (Days)	
		Range	Average
A_1 (Control)	Broke diapause between 26.8.63 and 5.9.63	75 - 85	80
A_2	Only 1 broke diapause on 15.10.63. Rest died during October	125	125
B_1	3 broke diapause between 20.9.63 and 3.10.63. 2 died on 30.9.63	100 - 113	106.5
B_2	2 broke diapause on 15.10.63. Rest died during October	125	125
C_1	Broke diapause between 22.7.63 and 29.7.63	40 - 47	43.5

Table 9 - Expt. 3 - Total length of diapause in days.

Nymphs in diapause for 3 weeks in A_1 before being transferred on 3.7.63.

Condition	Remarks	Length of Diapause (Days)	
		Range	Average
A_1	2 broke diapause on 8.8.63 and 5.9.63	57 - 85	71
A_2 (Control)	All died during October	-	-
B_1	2 broke diapause on 22.8.63 and 10.9.63	71 - 90	80.5
B_2	All died during September and October	-	-
C_1	Broke diapause between 29.7.63 and 15.8.63	47 - 64	55.5

Table 10 - Expt. 3 - Total length of diapause in days. Nymphs in diapause for 3 weeks in A_2 before being transferred on 3.7.63.

Table 9 - Only a few nymphs succeeded in breaking diapause at temperatures of 15 - 26°C (A_2, B_2). The high temperatures probably killed off most of the nymphs that had completed diapause development.

The length of diapause of nymphs transferred to C_1 (temperatures 1 - 7.5°C) was shorter than that of nymphs remaining in A_1 (temperatures 12.5 - 13°C) or transferred to B_1 (temperatures 12.5 - 15°C) and the latter was shorter than that of nymphs transferred to A_2 or B_2 (temperatures 15 - 26°C). The length of diapause in A_1 was slightly shorter than in B_1 and this seems to be associated with the lower temperatures in A_1.

Table 10 - Nymphs transferred from A_1 to C_1 showed a

shorter period of diapause than those transferred to A_1 or B_1. It is worth noting that nymphs remaining in A_1 or transferred from A_1 to B_1 (Table 9) showed a longer length of diapause than nymphs transferred from A_2 to A_1 or B_1 (Table 10).

Conclusions:

1. Diapause development can occur independently of temperatures and photoperiods.
2. High temperatures of 15 - 26°C tend to prevent the successful breaking of diapause.
3. The period of diapause development is shortened when nymphs are transferred to temperatures that are lower and is prolonged when transferred to temperatures that are higher than those which they were previously experiencing.

Miscellaneous observations:

Under natural conditions in A_1 the nymphs underwent diapause only once in their life-cycle, at either the 3rd, 4th, 5th, 6th or 7th instar. The usual stage of diapause was at the 4th or 5th instar. However, the nymphs may be induced to undergo diapause twice as can be seen in the following experiment.

Nymphs hatched on 10.5.63 and placed in B_2 (Average temperature 20.5°C; decreasing photoperiods from 12¼ to 10½ hours of light per day) were transferred at the 4th instar (active stage) on 5.6.63 to C_1 (Constant photo-

period of 12¼ hours per day and temperatures 1 - 7.5°C). Some 4th instar nymphs which had just reached the stage of diapause but had not yet become dormant were also transferred to C_1 on the same date.

The results are shown in Table 11. The first diapause occurred at the 4th or 5th instars while the second diapause occurred at the 7th or 8th instars. The length of the second diapause (about 4 - 5 months) was much longer than that of the first (about 1½ - 2 months). The first diapause was determined while the nymphs were in B_2 and the shorter diapause period in this instance may be the result of the transfer to the lower temperatures in C_1. The second diapause was probably induced while the nymphs were in C_1 and the increase in photoperiod, resulting from the transfer from B_2 to C_1 could have been responsible for the induction. The photoperiod in B_2 at the date of transfer was 10½ hours of light per day while in C_1 the photoperiod was 12¼ hours per day.

Stage of transfer	Stage & date of 1st Diapause	Termination of 1st Diapause	Stage & date of 2nd Diapause	Termination of 2nd Diapause
iv (Active)	v 20.6.63 - 3.7.63	- 8.8.63	vii, viii 28.10.63	- 27.2.64 - 27.3.64
iv (Ready for diapause)	iv 12.6.63	- 6.8.63	vii 28.10.63	- 27.2.64 - 27.3.64

The Roman numerals indicate the instar number.

Table 11 - Nymphs transferred from B_2 to C_1 on 5.6.63.

Discussion

The results of this study have shown that, under normal conditions, the nymphs of C. bifrons undergo diapause only once, and in any of the instars, from the 3rd to the 7th; the usual stage of diapause is however, in the 4th or 5th instar. Under certain environmental conditions the range can be extended to the 9th instar and diapause can be induced to occur twice during the life-cycle.

The onset of diapause occurs several instars after the prospective fate has been determined. Nymphs that are destined for diapause show a progressively longer interval between instars as they approach the stage of diapause. They show also a greater accumulation of fat gobules and a smaller increase in size than nymphs which are not destined for diapause. The diapause stage itself is characterised not only by a long period of arrest as in Anax imperator (Corbet 1956) but also by very distinct morphological features such as the immense accumulation of fat globules, the smaller size of the nymphs, the fewer antennal segments, the slender cercal segments and the absence of bristles on the segments towards the tip of the cerci. All these observations seem to suggest that a different pattern of metabolic activity is invoked in nymphs destined for diapause so that most of the food ingested is accumulated in the form of fat-globules and very little is used for growth and development. Lees (1954) mentions that the "inception of diapause is commonly associated with the temporary failure of some

component of the endrocrine system, usually the neurosecretory cells of the brain". Andrewartha (1952) believes that the general cause of diapause is the accumulation of "intractable food reserves" and that they may be used only after an adequate exposure to low temperature, or in certain instances in response to some other appropriate stimulus from the environment. He suggests that the neurosecretory cells are stimulated after the breaking down proccesses have reached a certain threshold value.

In C. bifrons the termination of diapause can occur irrespective of the environmental conditions but the period of diapause development is shortened when the nymphs are transferred to temperatures that are lower and is prolonged when they are transferred to temperatures that are higher than those which they were previously experiencing. This clearly suggests that the neurosecretory cells are not completely inactivated during the period of diapause. It is possible that the increasing accumulation of fat globules tends progressively to decrease the activity of the neurosecretory cells so that there is an increasingly longer interval between moults as the nymphs approach the stage of diapause. The period of arrest is much longer during the diapause stage probably because of the immense accumulation of fat globules. The fact that the length of diapause is shorter when the nymphs are transferred from a higher to a lower temperature than when they are reared throughout under the latter condition suggests that the activity of the neurosecretory cells of nymphs in diapause does not depend

upon the breaking down of the intractable food reserves but instead is associated with the change in temperatures during transfer. It appears that rising temperatures during the period of diapause tend to decrease the activity of the neurosecretory cells and hence prolong the diapause while falling temperatures result in the opposite effect. The active stages of insects normally show a faster rate of moulting at warmer than at colder temperatures and this would imply a greater activity of the neurosecretory cells. However, the physiological mechanisms that operate in the diapausing insect are different from those of the active stages (Lees 1954) and it is therefore not unusual that the stimulus for the neurosecretory cells in the diapause and active stages should be quite different. It is possible that in the active stages the stimulus for the secretion of the growth and moulting hormones may come from the breaking down processes during metabolism as has been suggested by Andrewartha (1952).

The environmental factors influencing the induction of diapause in C. bifrons are temperature and photoperiod. The sensitive stage is not localised in one particular instar but extends over the first five instars. Diapause is induced in a large percentage of the nymphs after the first instar and by the end of the second instar all or virtually all the nymphs are destined for diapause. Diapause cannot be prevented once the process of determination is completed. The first three instars are sensitive over a wide temperature range of 9 - 20.5°C

and diapause is induced irrespective of photoperiods. (As no study was made on the response of individual instars it is, therefore, really not possible to say for certain whether the 2nd or 3rd instars are sensitive to the lower limit of the temperature range, but nevertheless the assumption is made here). At temperatures which will induce diapause, the influence of photoperiods is not evident.. However, at temperatures below 9°C, there are indications that increasingly long photoperiods are important in the induction of diapause (Expt. 1 - Conclusion 3). The significance of increasingly long photoperiods is also apparent in the later instars when the sensitivity and response to temperature are progressively decreased. At temperature range of 12.5 - 15°C and under long photoperiods of about 17 hours per day diapause was induced in all the nymphs during the 4th and 5th instars whereas under decreasing photoperiods some nymphs failed to be induced for diapause (Expt. 2 - Conclusion 1).

It is not possible, from the present investigations, to determine whether the influence of photoperiods in the induction of diapause is due to photoperiods above a critical length or to the increasing daylength or to both. At temperatures of 1 - 7.5°C and under constant photoperiod of 12¼ hours per day, there is a tendency for nymphs to fail to undergo diapause. However, if nymphs that had been previously induced for diapause are transferred just before the period of dormancy from a photoperiod 10½ hours per day into a photoperiod of 12¼ hours per day, diapause could be induced a second time (See Miscellaneous

observations). It appears that increase in photoperiods or constant photoperiods of above 12½ hours per day will induce diapause. In any case, however, the early instars of C. bifrons are sensitive to such a wide temperature range that photoperiods play a relatively insignificant role in the induction of diapause.

In nature, the first instar nymphs are found in May and June. During this period the factors influencing diapause are already in operation and all the nymphs are therefore destined for diapause after the first or second instars. They enter diapause at the 4th or 5th instars during June to August and the termination of diapause occurs from September to about November. The growth of the nymphs is thus restricted to Autumn and Winter and emergence occurs from late Winter to Spring. Although increasingly long photoperiods will induce diapause in the early instars, its effect on the later post-diapause instars results in the differentiation of adult characters in apparently still immature nymphs (Chapter II, 3c). This species, therefore, has a strictly univoltine life-cycle and with a photoperiodically controlled flight period.

4. Diapause in Diura bicaudata

It has been found from the laboratory investigations in 1962 (Chapter II, 4e), that some adults of D. bicaudata lay only diapause eggs while others lay both diapause and non-diapause eggs. The incubation periods of non-diapause eggs vary between $1^{2}/_{3}$ to about 3 months, depending on the temperature. The hatching of diapause eggs occurs about 9 to 11 months after oviposition, under conditions of temperatures and photoperiods which simulate those in R. Terrig. These eggs are, however, unable to break diapause at room temperatures.

Diapause occurs at the stage prior to the revolution of the embryo (Figure 6 - A) when the eyes and head are facing the ventral side of the egg. The termination of diapause occurs when the embryo starts to undergo revolution but the process of revolution is fairly rapid and the intermediate stage (Figure 6 - B) was not often observed. The breaking of diapause is therefore indicated by the stage (Figure 6 - C, D) when the revolution of the embryo is completed and the eyes and head are dorsal in position.

During the studies in 1962, the nymphs collected from two different localities, Afon Hirnant and Lake Bala, were reared together and the exact origins of the adults were unfortunately not determined. The aims of the present investigation are (i) to determine whether the different type of eggs may be linked to the source of origin and (ii) to study the influence of environmental factors on the termination of

diapause. The experimental conditions used are the same as those employed for the study on diapause in C. bifrons.

Experiment 1

Aims:

To determine the type of eggs laid by adults from A. Hi Hirnant and Lake Bala and to study the hatching of these eggs at different temperatures.

Materials and Methods:

Fairly mature nymphs collected from Afon Hirnant and Lake Bala during April and May 1963 were reared in separate enamel dishes in the cold room, A_1. Adults emerging from nymphs belonging to the same locality were allowed to mate and were kept in A_1. The eggs laid were kept in petri-dishes in A_1 (normal photoperiods and temperatures) and A_2 (normal photoperiods; average temperature 20.5°C, range 15 - 26°C). The development and hatching of the eggs were observed. A total of 22 egg-batches laid between 13.5.63 and 11.6.63 by 10 females from Afon Hirnant and 15 egg-batches laid between 21.5.63 and 5.6.63 by 5 females from Lake Bala were studied.

Results:

All the eggs from Afon Hirnant were of the diapause type.

The adults from Lake Bala laid both diapause and non-diapause eggs (Table 12) and the percentage of diapause eggs varied between 2 and 33.5%.

There was no correlation between the date of oviposition

and the type of eggs, as can be seen in Table 13 showing the hatching of the eggs kept under the same conditions in A_1 and laid on the same dates by adults from Afon Hirnant and Lake Bala.

Under normal stream temperatures in A_1, the eggs from Lake Bala showed 2 distinct hatching periods - one in summer and another in Spring. The eggs from Afon Hirnant showed only a hatching period in Spring. There was a tendency for eggs from Afon Hirnant to hatch slightly earlier than the diapause eggs from Lake Bala (Table 13). This was associated with earlier breaking of diapause (during February) among afon Hirnant eggs than among the eggs of Lake Bala in which the breaking of diapause occurred during March.

The non-diapause eggs from Lake Bala hatched after a shorter incubation at the warmer temperatures in A_2 than at the normal stream temperatures in A_1. The diapause eggs, however, did not hatch in A_2 and were unable to break diapause (Table 14).

Besides the occurrence of diapause in the eggs from Afon Hirnant there was also a generally slower rate of development than the non-diapause eggs from Lake Balal Under normal conditions in A_1 the eggs from A. Hirnant reached the stage of diapause in late September whereas the non-diapause eggs from L. Bala began to hatch in August.

Adult	Date of oviposition	Size of egg-batch	Conditions	Non-diapause (%)	Diapause (%)
1	28.5.63	296	A_1	97	3
	30.5.63	247	A_1	98	2
2	21.5.63	246	A_1	97	3
	24.5.63	152	A_1	98	2
	28.5.63	105	A_1	91.5	8.5
	30.5.63	45	A_1	84.7	15.3
3	24.5.63	292	A_1	97	3
	28.5.63	259	A_2	96.6	3.4
	30.5.63	167	A_2	67.6	32.4
	3.6.63	133	A_1	* 94.7	5.3
	5.6.63	87	A_1	* 66.7	33.5
4	31.5.63	176	A_1	* 87.5	12.5
	3.6.63	130	A_1	- 66.6	33.4
	4.6.63	148	A_2	92.5	7.5
5	30.6.63	213	A_1	72.8	27.2

* includes a high proportion of degenerate eggs.

A_1 and A_2 indicate environmental conditions in which the eggs were kept.

Table 12 - Experiment 1 - Percentage of diapause and non-diapause eggs laid by adults from Lake Bala.

Origin	Condn.	Date of oviposition	Non-diapause	Diapause
L. Bala	A_1	28.5.63	97% (12.8.63-4.10.63)	3% (3.4.64-16.4.64)
		3.6.63	66.6% (16.8.63-30.9.63)	33.4%(27.3.64-7.5.64)
		3.6.63	72.8% (16.8.63-10.9.63)	27.2%(11.4.64-15.5.64)
A. Hirnant	A_1	28.5.63	-	100% (26.2.64-27.3.64)
		3.6.63	-	100% (26.2.64-3.4.64)
		3.6.63	-	100% (2.3.64-27.3.64)

The dates within brackets indicate the period of hatching.

Table 13 - Experiment 1 - Hatching of eggs from L. Bala and A.Hirnant under the same environmental conditions in A_1.

Origin	Condn.	Date of oviposition	Non-diapause	Diapause
L. Bala	A_1	22.5.63	91.5% (12.8.63-30.9.63)	8.5% (27.3.64-24.4.64)
	A_2	22.5.63	96.6% (11.7.63-19.7.63)	3.4% (None hatched)
L. Hirnant	A_1	28.5.63	-	100% (26.2.64-27.3.64)
	A_2	28.5.63	-	100% (None hatched)

The dates within brackets indicate the period of hatching.

Table 14 - Experiment 1 - Hatching of eggs from L. Bala and A. Hirnant under different conditions of temperature in A_1 and A_2.

Conclusions:

1. The type of eggs that are laid is linked with the origin of the adults. Adults from Afon Hirnant lay only diapause eggs while those from Lake Bala lay both diapause and non-diapause eggs, with a higher percentage of the latter.
2. At temperatures of 15 - 26°C in A_2, the non-diapause eggs are able to develop and hatch successfully after about $1^2/_3$ months while the diapause eggs are unable to develop beyond the stage prior to the revolution of the embryo.
3. In the normal temperatures in A_1, the stage of diapause is reached in September, about 4 months after oviposition. The termination of diapause occurs in February and March.
4. The incubation period of diapause eggs from Lake Bala is slightly longer than that from Afon Hirnant, due to a later termination of diapause in the former.

<u>Experiment 2</u>

Aims:

To determine the type of eggs laid after crossing adults from Afon Hirnant with those from Lake Bala.

Materials and Methods:

One female from Lake Bala was allowed to mate with a male from Afon Hirnant and vice versa. The eggs laid were kept in A_1 and the development and hatching of the eggs were studied.

Results:

The results of the crosses are shown in Table 15. The

Condn.	Origin	Date of oviposition	Undeveloped & degenerate	Non-diapause	Diapause
A_1	L. Bala ♀ x A. Hirnant ♂	13.6.63	74.2%	-	25.8% (23.3.64-24.4.64)
		17.6.63	96.1%	-	3.9% (27.3.64-16.4.64)
		20.6.63	77.5%	-	22.5% (20.3.64-16.4.64)
	A. Hirnant ♀ x L. Bala ♂	17.6.63	-	-	100% (23.3.64-11.4.64)
		20.6.63	-	-	100% (20.3.64-11.4.64)

The dates within brackets indicate the period of hatching.

Table 15 - Experiment 2 - Hatching of eggs obtained by crossing adults from L. Bala and A. Hirnant.

percentage of diapause eggs that are laid is associated with source of origin of the female. Thus the result of the cross between an A. Hirnant female and a Lake Bala male showed the usual 100% diapause eggs that is characteristic of A. Hirnant. The cross between a Lake Bala female and an A. Hirnant male resulted in the normal percentage of diapause eggs that is usual of the Lake Bala type. However, the remaining eggs, instead of being non-diapause, were undeveloped and they degenerated fairly soon after oviposition. The period of hatching of the eggs from these two crosses is intermediate between that of the diapause eggs from Lake Bala and Afon Hirnant.

Conclusions:

As only simple crosses were made and also only of each, the conclusions that are drawn must be regarded as provisional.

1. The character for diapause or non-diapause probably is genetically controlled.
2. The type of eggs that are laid is determined by the females. However, the manifestation of a particular character is dependent upon the presence of that factor in the male parent. This conclusion is based on the assumption that the undeveloped and degenerate eggs were determined for non-diapause and that the non-diapause factor is absent from the Afon Hirnant population.
3. Only eggs determined for diapause are able to survive from the crosses between the adults for L. Bala and A. Hirnant.

Experiment 3

Aims:

To study the influence of different environmental factors on the development and hatching of the eggs from Afon Hirnant.

Materials and Methods:

A batch of 355 eggs laid on 13.5.63 by an Afon Hirnant female that had mated with a male from the same locality was divided into 6 groups which were kept under the following conditions:

A_1 - normal photoperiods and temperatures,

A_2 - normal photoperiods; average temperature 20.5°C,

B_1 - photoperiods about 4½ months ahead of normal regime; temperature varied from that of A_1 to about 2°C higher,

B_2 - photoperiod as in B_1; average temperature 20.5°C,

C_1 - constant photoperiod of 12¼ hours/day; temperatures varied daily from 1 - 7.5°C,

D_1 - complete darkness, temperature as in A_1.

Results:

Figure 53a shows the period of diapause and the hatching of the eggs kept under the various conditions.

Except in C_1, the stage of diapause was reached in late September. In C_1 the rate of development was rather slow because of the lower temperatures and the stage of diapause was not reached until late December.

The breaking of diapause in A_1, B_1, and D_1 occurred in mid-February; in C_1 it occurred in mid-March. The eggs in A_2 and B_2 were unable to break diapause.

The period of diapause was longer in A_1, B_1 and D_1 than in C_1 and this seemed to be correlated with the fact that the temperatures in A_1, B_1 and D_1 did not fall to a fairly low level (below 6.5°C) until the beginning of December.

The post-diapause development is quite short, about 2/3 months.

Conclusions:

1. The termination of diapause and the hatching of the eggs are independent of photoperiods,
2. The termination of diapause is associated with low temperatures.
3. At temperatures of about 20.5°C the eggs are able to develop only till the stage of diapause.

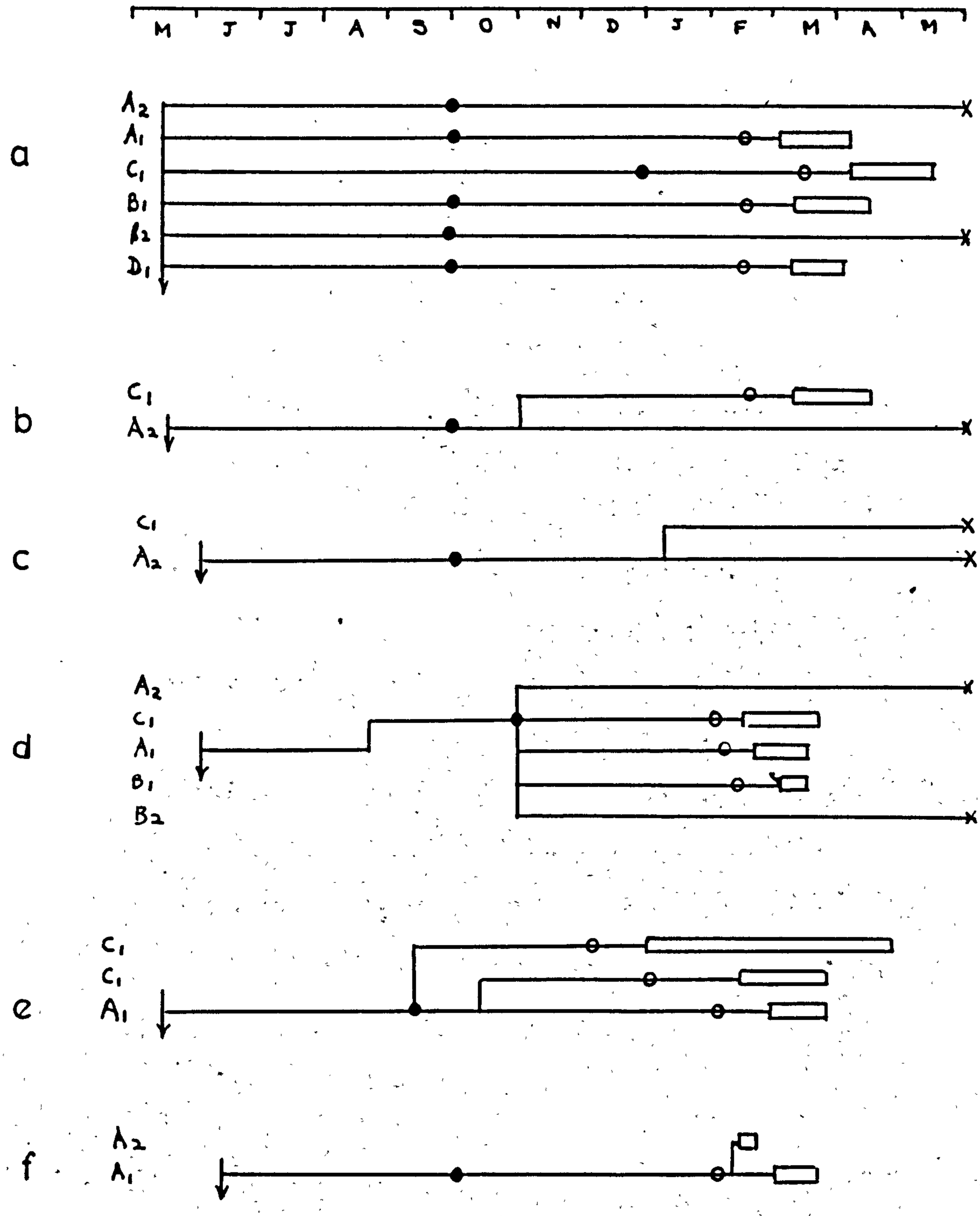

M J J A S O N D J F M A M
a
A2
A1
C1
B1
B2
D1
b
C1
A2
c
C1
A2
d
A2
C1
A1
B1
B2
e
C1
C1
A1
f
A2
A1
Beginning of Diapause
Termination of Diapause
Date of oviposition
Period of Hatching
X Failed to hatch

Fig. 53

Experiment 4

This consists of a series of minor experiments involving several egg-batches laid by adults from Afon Hirnant at various periods.

Aims:

To study the effect of chilling of the eggs at various stages of development.

Methods and Results:

Figure 53b

Eggs were kept in A_2 and about a month after the stage of diapause was reached, some eggs were transferred to C_1 while the rest were left in A_2.

The termination of diapause occurred after $3\frac{1}{2}$ months in C_1.

None of the eggs in A_2 terminated diapause.

Figure 53 c

Some eggs, kept in A_2, were transferred to C_1 about $3^1/_3$ months after the stage of diapause was reached.

None of the eggs terminated diapause.

Figure 53 d

Eggs were kept in A_1 for about $2\frac{1}{2}$ months after which they were chilled in C_1 for 2 months till the stage of diapause was reached. The eggs were then transferred in October to A_1, A_2, B_1 and B_2 while some were kept in C_1.

None of the eggs in A_2 or B_2 terminated diapause.

The termination of diapause in A_1 and B_1 is associated to the low temperatures innWinter.

Figure 53 e

A batch of eggs laid in mid-May was kept in A_1. One group was transferred to C_1 in September as soon as the diapause stage was reached while another was transferred a month later. The rest of the eggs remained in A_1.

The length of diapause of eggs transferred to C_1 in September and October was about $2\frac{1}{2}$ months and $3\frac{1}{2}$ months respectively while eggs remaining in A_1 showed a diapause period of about $4\frac{1}{2}$ months. This indicates that little or no diapause development occurred in A_1 from September to early November.

Figure 53 f

Eggs laid in June were kept in A_1. Soon after the termination of diapause in February a batch of eggs was transferred to A_2.

In A_2 the hatching of the eggs occurred successfully and the length of post-diapause development was shorter than in A_1. A few eggs that had not yet terminated diapause when they were transferred to A_2 on February did not hatch at all.

Conclusions:

1. Eggs are unable to survive in diapause for long periods at average temperatures of 20.5°C. They are still viable after a month but they are killed off after about $3^1/_3$ months.
2. The length of diapause under normal conditions in A_1 is about 4 to $4\frac{1}{2}$ months.
3. At temperatures which vary daily from 1 - 7.5°C, diapause

development is completed after about 2½ months.

In nature , diapause development propably occurs only during the winter.

4. Chilling of the eggs is effective only after the stage of diapause has been reached.

5. Successful hatching of eggs can occur at temperatures of about 20.5°C only after the termination of diapause.

6. Post-diapause development is rapid at temperatures of about 20.5°C.

Discussion:

The adults of D. bicaudata from A. Hirnant lay only diapause eggs while those from L. Bala lay both diapause and non-diapause eggs, with a high percentage of the non-diapause type. The character of the eggs is genetically controlled. The type of eggs that are laid seems to be determined by the females but the manifestation of a particular character is dependent upon the presence of that character in the male parent. In the A. Hirnant population the character for non-diapause does not appear to be present and when a Lake Bala female is crossed with an A Hirnant male only the normal percentage of diapause eggs characteristic of the L. Bala population are viable, and none of non-diapause eggs are able to survive. There is, therefore, a sufficient degree of incompatibility between the two populations, as far as the none-diapause character is concerned, for them to be regarded as belonging to two sub-species.

The non-diapaused eggs develop and hatch fairly rapidly

at warm temperatures while the diapause eggs show a slower rate of development and are unable to break diapause under such conditions. The termination of diapause is dependent upon a fairly long period of chilling. The length of diapause development is about 2½ months at temperatures which vary daily from 1 to 7.5°C. The chilling of the eggs prior to the stage of diapause does not effect the later diapause development.

D. bicaudata has an essentially northern distribution in Eurasia and North America, and its occurrence in the mountainous areas of central Europe and in Britain has been regarded by Brinck (1949) as indicating that it is a glacial relict. It is possible that the eggs laid by this species in the northern latitudes are all of the diapause type. The flight period in Britain is from April to June (Hynes 1958) and in northern Sweden it is from June to August (Brinck 1949). The diapause character seems to be an adaptation to a very cold climate where the short Summer and relatively low temperatures do not allow a rapid rate of embryonic development to result in an early hatching of the eggs before the winter sets in. The occurrence of diapause would thus enable the eggs to survive the period of extreme cold and to allow the nymphs to hatch during the season when there would probably be more food available. The population in Afron Hirnant could be regarded as a true glacial relict in which the pattern of development of the eggs has remained unchanged. Although the environmental conditions in A.Hirnant

are not particularly severe during the winter, the temperatures are generally low throughout the year (Hynes 1961) and as such would be of no great selective value for any change that might occur. In Lake Bala, however, the conditions are quite different. The water temperatures at the surface (hence also near the shore where the species occurs) do reach a fairly high level during the Summer (Dunn 1961) and the favourable temperatures for growth would be of great selective value in the evolution of a non-diapause character in the eggs. Lake Bala lies in a glacial valley and it is possible that the present population may have been derived from the original relicts that were left behind when the ice retreated. The present predominance of non-diapause over diapause eggs may be an example of natural selection in action which in course of time could probably eliminate the diapause character from this population and thus result in a distinct species. Although several streams like the A. Hirnant flow into Lake Bala there is, however, a certain amount of ecological isolation between the populations in these two areas. In A. Hirnant, the species occurs essentially near the source at an altitude of about 440 metres and lower downstream it is replaced by Perlodes microcephala (Hynes 1961). The absence of D. bicaudata in running waters at lower altitudes is believed to be due to the inability of the species to compete with P. microcephala (Hynes 1952). That there has been little or no intermixing between the two populations is seen in the predominance of non-diapause eggs in the L. Bala population

since the crosses between adults of both populations would result only in the survival of diapause eggs. The present discussion on D. bicaudata is very similar to the study by Nielsen (1950) on the problem of speciation in the caddis fly, Apatidea muliebris, which is also believed to be a glacial relict. This species shows a life-cycle adapted to an arctic climate but which seems to be inappropriate to the springs where they occur. In other springs this species has given rise to two mutant forms, A. cimbrica and A. intermedia, which have a life-cycle clearly adapted to their present environment. However, unlike in the Apatidea spp. which are parthenogenetic, the populations of D. bicaudata in A. Hirnant and L. Bala have yet to evolve into two separate species.

5. Diapause in Brachyptera risi

This is a univoltine species with a flight period from April to June in River Terrig. The eggs are laid in May and June but no hatching occurs until October. The eggs undergo diapause a few days after oviposition and they remain in diapause for several months. Diapause occurs, probably, at the germ disc stage and its termination is indicated by the invagination of the germ disc to form an early embryo (Chapter II, 4d and figure 3).

It was found from the laboratory studies in 1962 (Chapter II, 4e) that the eggs laid by an adult (collected from Horseshoe Pass in N. Wales) kept at room temperature showed a shorter period of diapause than the eggs laid by adults (collected from River Terrig) kept in the cold room.

It had been the intention here to study the influence of environmental conditions experienced by the adults on the length of diapause in the eggs but unfortunately none of the adults which emerged in the laboratory succeeded in laying eggs.

The following experiment is based on eggs laid by an adult collected from River Terrig.

Experiment

Aims:

To study the influence of different environmental conditions on the hatching of the eggs.

Materials and Methods:

An adult collected from River Terrig was kept in the cold

room, A_1. Eggs laid on 7.5.63 were divided into 6 batches and were kept under the various conditions:-

A_1 - normal photoperiods and temperatures,

A_2 - normal photoperiods; average temperature 20.5°C,

B_1 - photoperiods about 4½ months ahead of normal regime; temperatures varied from A_1 to about 2°C higher,

B_2 - photoperiod as in B_1; average temperature 20.5°C,

C_1 - constant photoperiod of 12¼ hours/day; temperatures varied daily from 1 - 7.5°C,

D_1 - complete darkness, temperatures as in A_1.

Results:

The results are given in Table 16.

Condition	Period of diapause	Date of first hatching
A_1	15.5.63 - 22.8.63 (99)	28.10.63
A_2	11.5.63 - 22.8.63 (103)	*
B_1	15.5.63 - 22.8.63 (99)	20.10.63
B_2	11.5.63 - 1.9.63 (103)	*
C_1	18.5.63 - 1.9.63 (106)	4.12.63
D_1	15.5.63 - 22.8.63 (99)	28.10.63

* - Embryos fully-developed on 4.10.63 but died without hatching.

Figures within brackets indicate length of diapause in days.

Table 16 - Eggs of B. risi, laid on 7.5.63.

The termination of diapause occurred in all the experimental conditions and there was no significant difference in the

length of diapause. The post-diapause development was completed much earlier in A_2 and B_2 but the embryos were unable to hatch and they died soon afterwards.

Conclusions:

1. The breaking of diapause is not dependent upon any fall of temperature and is independent of photoperiods. It has, however, been found (Chapter II, 4e) that the length of diapause can be prolonged by subjecting the eggs during diapause to low temperatures of about 3 - 5°C.
2. The post-diapause development is faster at the higher temperatures in A_2 and B_2 than at the lower temperatures in A_1, B_1, C_1 and D_1. The embryos are however unable to hatch in A_2 and B_2.
3. The onset of diapause is not determined by immediate environmental conditions experienced by the eggs.

Discussion:

B. risi is a cold-water stenotherm in which growth occurs from Autumn to early Spring when emergence occurs. The occurrence of diapause thus allows the species to remain in the egg-stage during the unfavourable period of the year.

The onset or termination of diapause does not depend upon any environmental stimulus and it can occur even at a high average temperature of 20.5°C. The length of diapause seems to be influenced by the maternal physiology (Chapter II, 4e) and this may be determined by the environmental conditions

experienced by the adults. In contrast to most other examples where chilling is often beneficial or essential for diapause development, the effect of low temperatures on the eggs of B. risi results in a prolongation of the diapause period. The unusual effect of chilling seems to be associated with the fact that diapause in this species is an adaptation to survive unfavourable summer temperatures rather than severe winter conditions as in the case in D. bicaudata. This effect of chilling may be of some significance in the northern latitudes where some of the eggs may not have sufficient time for the completion of diapause development before the winter sets in. Under these circumstances the winter temperatures would enable the eggs to remain in diapause until the following year and the species may this have a two-year life-cycle under some conditions.

REFERENCES

ANDREWARTHA, H.G., 1952 - Diapause in relation to the ecology of insects,
- Biol. Rev.,27, 50 - 107.

BECK, S.D., 1950 - Nutrition of the European corn borer, Pyrausta nubilalis (Hbn.). (i) Some effects of diet on larval growth characteristics
- Physiol. Zool.,23, 352 - 61.

BRINCK, P., 1949 - Studies on Swedish stoneflies
- Opusc. ent. suppl., 11, 1 - 250.

BRINCK, P., 1955 - Repropductive system and mating in Plecoptera,
- Opusc. ent. suppl., 21, 57 - 127.

CORBET, P.S., 1954 - Seasonal regulation in British dragonflies,
- Nature, London, 174, 655.

CORBET, P.S. 1956 - Environmental factors influencing the induction and termination of diapause in the Emperor Dragonfly, Anax imperator Leach (Odonata, Aeshnidae),
- J. exp. Biol., 33, 1 - 14.

DUNN, D.R., 1961 - The Bottom Fauna of Llyn Tegid (Lake Bala), Merionethshire,
- J. Anim. Ecol., 30, 267 - 81.

FRISON, T.H., 1929 - Fall and winter stoneflies or Plecoptera,
- Illinois Nat. Hist. Surv., 18, 345 - 409.

FRISON, T.H., 1935 - The stoneflies or Plecoptera of Illinois,
- Illinois Nat. Hist. Surv., 20, 281 - 471.

HELSON, G.A.H., 1935 - The Hatching and early instars of Stenoperla prasina Newman,
- Trans. Roy. Soc., New Zealand, 65, 11-16.

HOLDSWORTH, R.P., 1941a - The life history and growth of Pteronarcys proteus Newman,
- Ann. Ent. Soc. Amer., 34, 495 - 502.

HOLDSWORTH, R.P., 1941b - Additional information and a correction concerning the growth of Pteronarcys proteus Newman,
- Ann. Ent. Soc. Amer., 34, 714 - 15.

HYNES, H.B.N., 1941 - The taxonomy and ecology of the nymphs of British Plecoptera with notes on the adults and eggs,
- Trans. R. ent. Soc., London, 91, 459 - 557.

HYNES, H.B.N., 1942 - A study of the feeding of adult stoneflies (Plecoptera),
- Proc. R. ent. Soc., London, A 17, 81-82.

HYNES, H.B.N., 1952 - The Plecoptera of the Isle of Man,
- Proc. R. ent. Soc., London, A 27, 71-76.

HYNES, H.B.N., 1958a - A key to the adults and nymphs of British stoneflies (Plecoptera), - Sci. Pub. freshwat. biol. Assoc., 17, 1 - 87.

HYNES, H.B.N., 1958b - The effect of drought on the fauna of a small mountain stream in Wales, - Verh. int. Ver. Limnol., 13, 326 - 33.

HYNES, H.B.N., 1961 - The invertebrate fauna of a Welsh mountain stream, - Arch. Hydrobiol., 57, 344 - 88

HYNES, H.B.N.,1962 - The hatching and growth of the nymphs of several species of Plecoptera, - IXth Int. Congress of Ent., 3, 271 - 73.

HYNES, H.B.N.,1963 - Isogenus nubecula Newman in Britain (Plecoptera : Perlodidae), - Proc. R. ent. Soc. London, A 38, 12-14.

HYNES, H.B.N.,1963 - The Gill-less Nemourid Nymphs of Britain (Plecoptera), - Proc. R. ent. Soc., London, A 38, 70-6.

IDE, F.P., 1935 - The effect of temperature on the distribution of the mayfly fauna of a stream, - Univ. Toronto Stud. biol. No. 39 (Publ. Ont. Fish. Res. Lab. No. 50), 1 - 76.

ILLIES, J., 1952 - Die Plecopteren und das Monardshe Prinzip.,
- Ber. Limnol. Flussst. Freudenthal, 3, 53 - 69.

ILLIES, J., 1959 - Retardierte Schlupfzeiten von Baetis-Gelegen (Ins. Ephem.),
- Naturwissenschaften, 46, 119 - 120.

ILLIES, J., 1960 - Archiperlaria, eine neue Unterordnung der Plecopteren (Revision der Familien Eusthenilidae und Diamphipnoidae),
- Beit. Ent., 10, 661 - 697.

JOHANNSEN, O.A. and BUTT, F.H., 1941 - Embryology of Insects and Myriapods,
- McGraw-Hill Book Co., 1941, 453 pp.

KÜHTREIBER, J., 1934 - Die Plecopteren fauna Nordtirols,
- Ber. naturw.-med. Ver. Innsbruck, 44, 1 - 219.

LEES, A.D., 1955 - The Physiology of Diapause in Arthropods,
- Cambridge Univ. Press, 1955, 151 pp.

LESTAGE, J.A., 1920 - Etudes sur la biologie des Plecoptéres I. La larve de Leuctra geniculata Stephens
- Ann. Biol. lacust., 9, 257 - 268.

MACAN, T.T. 1957 - The life histories and migrations of the Ephemeroptera in a stony stream,
- Trans. Soc. Brit. Ent., 12, 129 - 156.

MACAN, T.T., 1958a - The temperature of a small stony stream,
- Hydrobiologia, 12, 89 - 106.

MACAN, T.T., 1958b - Causes and effects of short emergence periods in insects,
- Ver. internat. Ver. Limnol XII, 84 - 89.

MACAN, T.T., 1960 - The effect of temperature on Rhithrogena semicolorata (Ephem.),
- Int. Revue ges. Hydrobiol., 45, 197 - 201.

McLACHLAN, R., 1864 - Note on the manner in which the females of the genus Leuctra carry their eggs,
- Ent. mon. Mag., 1, 216.

NIELSEN, A., 1951 - Spring fauna and speciation,
- Verh. int. Ver. Limnol., 11, 261 - 63.

NEERACHER, F., 1910 - Die Insektenfauna des Rheins und seiner Zuflusse bei Basel,
- Rev. suisse Zool., 18, 497 - 589.

NEWCOMER, E.J., 1918 - Some stoneflies injurious to vegetation,
- J. agric. Res., 1, 37 - 42.

PERCIVAL, E. and WHITEHEAD, H., 1928 - Observations on the ova and oviposition of certain Ephemeroptera and Plecoptera.
- Proc. Leeds Phil. Soc. (Sci.Sect.), 1, 271 - 288.

PLESKOT, G., 1953 - Zur Ökologie der Leptophlebiiden (Ins., Ephemeroptera),
- Öst. zool. Z., 4, 45 - 107.

RAUŠER, J., 1962 - K. zoogeografii severomoravských druhù rodu Protonemura Kyn. a Leuctra Steph. (Plecoptera) (Přispevek k poznání vlivu pleistocénu na stredoevropskou biotu),
- Přirodovédný Časopsis Slezsky XXIII 2, 175 - 92.

RAUŠER, J., 1963 - Contribution à la connaissance des larves du genre Amphinemura de la Tchecoslovaquie (Plecoptera),
- Čas. Čs. Spol. ent. (Acta Soc. ent. Čechoslov.), Tom 60, No. 1 - 2, 32 - 54.

SAMAL, J., 1923 - Etude morphologique et biologique de Perla abdominalis Burm.
- Ann. Biol. lacust., 12, 229 - 73.

SCHOENEMUND, E., 1924 - Plecoptera - Biologie die Tiere
- Deutschlands, 32, 1 - 34.

SCHOENEMUND, E., 1925 - Beitrage zur Biologie der Plekopteren larven, mit besonderer Berucksichtung der Atmung,
- Arch. Hydrobiol., 15, 339 - 69.

SMITH, L.C., 1913 - The biology of Perla immarginata
- Ann. Ent. Soc. Amer., 6, 203 - 11.

WHEELER, W.M., 1893 - Contribution to insect embryology,
- J. Morph., 8, 1 - 160.

WU, C.F., 1923 - Morphology, anatomy and ethology of Nemoura,
- Lloyd Libr. Bul., 23, Ent.Ser.No. 3, 1-81.

* MILLER, A., 1939 - The egg and early development of the stonefly, Pternonarcys proteus, - Journ. Morph., 64, 555 - 609.

APPENDIX

Changes in the room temperatures

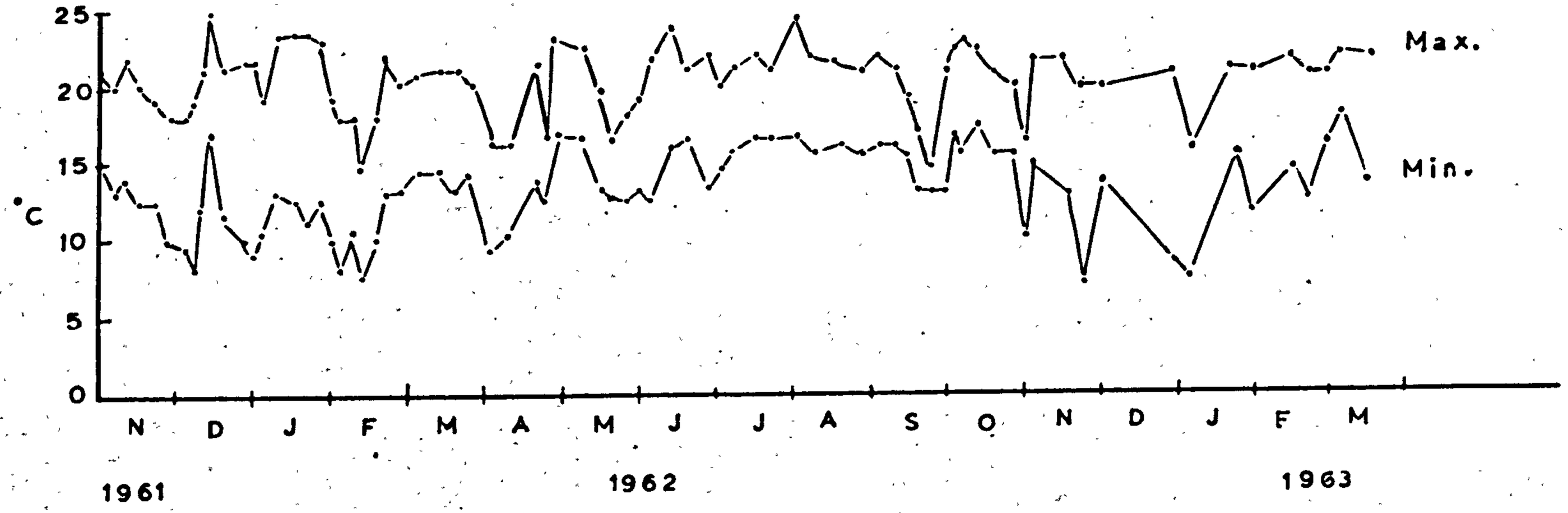


FIG. I

Instar no.	L. fusca Room temp.		N. picteti Cold room	C. torrentium Cold room
	Male	Female	Female	Male
1	5.5	5.5	9.0	5.8
2	8.0	7.5	12.2	8.0
3	10.8	9.7	15.0	10.0
4	12.8	11.5	19.0	11.2
5	14.9	14.0	?	12.3
6	17.2	16.8	30.0	15.0
7	20.1	19.5	38.0	18.0
8	23.5	23.0	43.5	23.5
9	26.5	26.3	56.0	27.0
10	30.5	30.2	62.0	35.5
11	32.5	34.5	?	47.0
12	40.5	42.0	82.0	62.0
13	51.5	51.0	91.0	↓
14	58.0	61.0	103.0	
15	67.0	70.0	116.0	
16	82.0	87.0	128.0	
Remarks	Emerged 31.8.62	Emerged 14.9.62	Emerged 19.8.63	Final instar Died 6.6.63.

Tibial length in units (1 unit - 17.4 μ)

Leuctra geniculata

Instar no.	Room temp. Sex (?)	Cold room				
		Male			Female	
1	6.3	6.3	6.3	6.3	6.3	6.3
2	8.2	9.0	9.0	9.0	9.0	9.0
3	10.5	11.2	11.0	11.0	11.2	11.0
4	12.0	14.0	14.0	14.0	14.3	14.3
5	15.0	17.0	17.5	17.3	17.0	17.2
6.	17.5	19.0	19.2	20.5	20.4	20.5
7	21.5	21.0	22.5	23.5	?	24.0
8	26.0	31.0	29.0	30.0	?	31.2
9	33.0	41.5	38.0	38.5	36.5	38.0
10	41.0	?	51.0	47.5	47.0	48.2
11	50.5	65.0	65.0	59.0	?	61.5
12	61.0	80.2	80.0	81.5	75.0	79.5
13	70.0	↓	↓	↓	?	↓
14	83.0	↓	↓	↓	↓	↓
Remarks	Final instar(?) died on 11.5.63	Emerged 19.9.63	Emerged 30.9.63	Emerged 11.9.63	Emerged 15.10.63	Emerged 15.10.63

Tibial length in units (1 unit - 17.4 μ)

Leuctra moselyi

Instar no.	Room temp. Sex (?)	Cold room Male			Female
1	5.5	5.5	5.5	5.5	5.5
2	8.5	8.5	?	8.5	8.5
3	10.5	11.5	?	11.3	11.0
4	12.0	15.0	13.0	14.6	14.0
5	13.2	18.5	16.0	17.5	17.5
6	15.5	21.0	18.5	21.6	20.1
7	17.5	23.3	19.6	23.2	22.3
8	19.2	26.5	23.2	24.0	26.0
9	21.0	30.0	29.5	28.0	31.0
10	23.0	38.2	35.0	32.0	39.0
11	25.5	46.5	42.5	38.0	47.0
12	28.5	?	55.0	47.4	57.0
13	30.0	71.0	66.0	59.0	71.0
14	32.0	↓	↓	71	↓
Remarks	Immature Died on 18.3.63.	Emerged 24.8.63	Emerged 10.9.63	Emerged 19.8.63	Emerged 1.9.63.

Tibial length in units (1 unit - 17.4 μ)

Leuctra hippopus

Instar no.	Room temp.	Cold room		
	Sex (?)	Female		Sex (?)
1	5.5	5.5	5.5	5.5
2	7.5	?	?	?
3	10.0	9.8	?	11.0
4	12.5	?	?	?
5	16.5	17.5	?	18.5
6	19.0	?	?	23.0
7	21.0	?	23.5	27.0
8	25.0	32	?	32.0
9	29.0	37	35.2	?
10	33.0	42.5	40.7	44.0
11	37.0	56.0	53.5	54.0
12	40.0	72.0	?	63
13	44.5	↓	↓	↓
14	49.5			
15	57.0			
16	59.5			
Remarks	Penultimate instar (?) Died on 17.2.63.	Emerged 31.5.63	Emerged 21.5.63	Final instar Died on 2.4.63.

Tibial length in units (1 unit - 17.4μ)

Leuctra inermis

Instar no.	Room temp.	Cold room				
	Sex (?)	Male		Female		
1	5.5	5.5	5.5	5.5	5.5	5.5
2	6.8	7.0	7.2	7.2	7.0	7.0
3	8.3	8.5	?	9.5	8.5	8.5
4	10.8	10.0	?	11.5	10.8	10.0
5	13.0	12.5	12.5	13.0	12.5	?
6	14.3	14.7	15.2	15.5	14.0	13.5
7	17.0	18.5	16.5	?	16.0	15.0
8	19.0	23.0	?	21.5	17.2	16.5
9	20.8	27.5	22.0	26.0	20.5	19.0
10	22.0	32.5	?	29.2	?	23.5
11	22.5	?	?	37.0	?	28.8
12	22.8	51.5	35.5	44.0	36.5	35.0
13	23.3	62.0	42.0	49.5	42.0	42.0
14	23.6	↓	54.5	63.5	52.0	53.0
15	23.6	↓	↓	↓	67.0	68.0
Remarks	Immature Died on 11.2.63.	Emerged 13.6.63	Emerged 25.6.63	Emerged 20.6.63	Emerged 20.6.63	Emerged 4.7.63.

Tibial length in units (1 unit - 17.4 μ)

Capnia bifrons

Instar no.	Cold room				
	Male			Female	
1	6.0	6.0	6.0	6.0	6.0
2	7.2	7.2	7.2	7.2	7.2
3	8.0	8.0	7.8	8.0	8.0
4	9.0	D 8.5	D 8.0	D 8.5	9.0
5	D 9.5	10.0	9.0	10.8	D 9.5
6	10.5	?	11.5	12.5	10.5
7	12.0	16.0	15.0	15.5	14.0
8	15.0	20.0	17.5	18.8	18.5
9	16.5	23.5	23.0	21.0	23.5
10	18.0	27.0	26.0	24.5	29.5
11	21.0	32.0	32.0	28.0	36.5
12	31.5	40.0	38.0	33.0	43.0
13	41.0	47.0	49.0	35.0	51.5
14	49.0	?	?	44.0	64.0
15	?	↓	↓	55.0	74.0
16	↓			?	↓
Remarks	Final instar Died 16.5.63	Final instar Died 6.6.63.	Emerged 17.5.63	Final instar Died 4.7.63.	Emerged 30.4.63

D - stage of Diapause

Tibial length in units (1 unit - 17.4μ)

Nemoura avicularis

Instar no.	Room temp.	Cold room			
	Sex (?)	Male	Female		
1	7.8	7.8	7.8	7.8	7.8
2	10.0	?	?	?	?
3	12.0	?	?	?	?
4	19.0	?	21.5	21.5	?
5	?	?	27.0	?	?
6	31.0	32.0	?	?	?
7	41.0	?	44.0	?	44.0
8	50.5	?	53.5	?	54.5
9	61.0	?	68.0	67.0	?
10	76.0	77.0	77.5	76.0	80.5
11	88.0	?	?	94.0	99.5
12	98.0	?	?	120.0	?
13	110.0	119.0	?	149.0	135.0
14	123.0	↓	↓	↓	↓
Remarks	Immature Died on 22.3.63	Emerged 31.5.63	Emerged 31.5.63	Emerged 28.4.63	Emerged 13.6.63

Tibial length in units (1 unit - 17.4 μ)