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PhD thesis

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THE BIOLOGY OF LARVAL AND JUVENILE  
NEPHROPS NORVEGICUS (L.) IN THE  
FIRTH OF CLYDE

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A Thesis submitted for the degree of  
Doctor of Philosophy in the Faculty of  
Science at the University of Glasgow

University Marine Biological Station Millport

and

Department of Zoology, Glasgow University

May 1987

## DECLARATION

I hereby declare that this thesis represents,  
except where a note is made to the contrary,  
work carried out by myself. It has not been  
previously submitted for any degree.

Ronald Stuart Martin Smith

1st May 1987

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## Summary

In Scotland over the past 30 years the Nephrops fishery has expanded and is now the largest in Europe with landings in 1986 valued at over £29m. Much of the species biology is poorly understood, especially the early life history. The biology of larval and juvenile Nephrops is the subject of this thesis and the work reported has been conducted on Nephrops within the Firth of Clyde.

Because Nephrops incubate their eggs externally (on their pleopods) they suffer progressive egg loss. Creels were set to capture ovigerous females incubating eggs at various stages of development to provide fecundity data and egg samples for a study of the biochemical changes associated with embryonic development. During the incubation period mean egg loss was estimated as 18%. Almost a fifth of the population were judged to have suffered additional egg loss, probably at the moment of spawning. Estimation of the mean number of eggs hatched per female per year took such losses into account and involved the use of length frequency information and a maturity ogive. The best estimate of the fecundity range was 985 - 1115 eggs hatched per female, which includes an allowance for the proportion of the population that may be biennial spawners. Lipids are the principal energy reserve utilized during embryonic development and full development requires about 6 - 7 joules per egg. Most energy is expended during the later stages of development and is associated with a rapid uptake of water and salts.

An aquaculture facility was developed and newly hatched larvae



were reared under different culture conditions. Survival was found to be better in isolation compared to mass rearing conditions and initial periods of starvation in excess of 1 - 2 days led to an increase in mortality. Larvae were reared over a temperature range of 8 - 20°C and relationships were derived between temperature and the rate of larval development for each zoeal stage. The development time increased for successive zoeal stages at each temperature and 16°C seemed to be the overall 'optimum' temperature for development.

A larval survey programme was conducted in the Lower Firth of Clyde (L.F.C.) to investigate temporal and spatial changes in larval abundance. The larval abundance data were used in conjunction with the temperature information obtained in the laboratory study to obtain a seasonal production estimate for the 1st zoeal stage of  $177 \times 10^8$  larvae within the 876 square kilometres sampling area. This value, when used in conjunction with the mean number of eggs hatched per female per year, gave an estimate of  $15.87 \times 10^6$  to  $17.97 \times 10^6$  females for the L.F.C. spawning stock.

Information on the vertical distribution of Nephrops zoeae was obtained over two 24h cycles, one coincided with a spring and the other with a neap tidal period. The distribution of the larvae was related to several environmental variables and compared with the light and pressure responses demonstrated under controlled laboratory conditions. 1st, 2nd and early 3rd stage zoeae demonstrated positive phototaxis and high barokinesis. No dramatic diel changes were detected in the vertical distribution of the larvae. Most remained between 6 to 38m depth during daylight hours. A limited nocturnal ascent resulted in peak larval abundance shifting from 16 to 5m depth.

Shortly after dawn the larvae descended to their daytime distribution. Depth regulation is probably achieved by light and pressure sensitivity and may be influenced by the position of the pycnocline.

During the 3rd zoeal stage the light response changed from photopositive to photonegative and Nephrops may first come into contact with the sea bed towards the end of this stage. Substratum selection and settlement behaviour experiments were conducted with early postlarval stages and these suggested that the 1st postlarval stage is a transitional form between the planktonic and benthic environments. Final recruitment to the benthos may be delayed until sometime after the 1st postlarval stage. Morphological examinations have shown that although most structural changes occur at metamorphosis, the development of some adult features are only completed several moults later. Juveniles beyond the 1st postlarval stage are capable of constructing their own burrows but will enter inhabited adult burrows through surface openings. If the inhabitant does not evict the recruiting juvenile it excavates an adjoining burrow through one of the walls of the adults burrow to form a juvenile / adult association. These associations are known to occur in the field and implications to the fishery are discussed.

## General introduction

Nephrops norvegicus (hereafter referred to by the generic name Nephrops) is an important commercial species with Scottish landings valued at over £29m (DAFS, 1987). Yet, despite the commercial importance of Nephrops much of the species biology is poorly understood. This especially applies to the early life history from the egg hatching through the planktonic larval phase to the eventual recruitment of the juvenile into the fishery. This thesis reports on aspects of the biology of these early stages in the Firth of Clyde. The Nephrops fishery in this region represents over 20% of the total value of Scottish Nephrops landings (Bailey et al., 1986).

It is necessary to review briefly the terminology applied to the early stages of Nephrops since there is some potential for confusion in the literature. Kurian (1956) considered that Nephrops possessed three larval stages which he termed larval stages 1, 2 and 3. Farmer (1975) reported the existence of an initial short-lived non-pelagic larval stage. Farmer (1975) therefore described Nephrops as possessing a prezoeal stage followed by the 3 pelagic larval stages, ie. 1st, 2nd and 3rd zoeae. It was then later reported that there were 4 pelagic larval stages before settlement of the juvenile on to the bottom (Phillips and Sastry, 1980 ; Nichols et al., 1987). Williamson (1982) assigned this 4th pelagic larval stage as a 'megalopa' stage but accepted the term 'decapodid' as proposed by Kaestner (1970) to cover this stage for crustaceans belonging to the Order Decapoda. Although Williamson (1982) assigned the 'megalopa' as a larval phase, 'decapodid' was defined as possessing the full complement of metameres

and appendages of the Order and was considered to be a postlarval form. As this 1st postlarval stage resembles the adult Felder et al. (1985) considered the moult into the 'decapodid' stage as a metamorphosis and this form to be the 1st juvenile stage. A similar conclusion was reached by Anger and Püschel (1986).

Although the swimming function shifts from the thoracic to abdominal appendages during this metamorphosis, and is the reason for the creation of a separate 'megalopa' phase (Williamson, 1982), the 1st postlarval stage still retains residual thoracic exopods which are only completely lost after moulting into the 2nd postlarval stage (Felder, 1985). This moult could therefore be considered as slightly metamorphic. For the purposes of this investigation the larval classification as originally defined by Farmer (1975) will be used : prezoea, 1st, 2nd and 3rd zoeae. The 'decapodid' stage will be referred to as the 1st postlarval stage. The terms postlarva and juvenile are synonymous. The equivalent stages of larval and early postlarval development used by different authors are shown in Table 0.1.

During the period of egg development, lasting about 9 months in Scottish waters (Farmer, 1975), ovigerous Nephrops carry their eggs beneath the abdomen, attached to the pleopods. The biochemical changes associated with embryonic development and the mechanisms which control hatching were investigated, including a description of the initial moult from the short-lived prezoea into the 1st zoea.

Because ovigerous Nephrops incubate their eggs externally they are prone to progressive egg loss during development. A fecundity



Table 0.1

Stages of larval and early postlarval development compared with the equivalents of other authors.

Kurian (1956)	Farmer (1975)	Phillips and Sastry (1980)	Williamson (1982)	Felder et al, (1985)	Nichols et al, (1987)	This study
-	Prezoea	Prezoea	Prezoea	Prezoea	Prezoea	Prezoea
First Larva	First Zoea	First Larva	First Zoea	First Larva	First Larva	First Zoea
Second Larva	Second Zoea	Second Larva	Second Zoea	Second Larva	Second Larva	Second Zoea
Third Larva	Third Zoea	Third Larva	Third Zoea	Third Larva	Third Larva	Third Zoea
First Juvenile	First PL	Fourth Larva	Megalopa	Decapodid/4th Stage First PL/First Juv.	Fourth Larva	First PL
Second Juvenile	Second PL	First PL	First Juvenile	Second PL/Second Juv.	-	Second PL

Juv. = Juvenile, PL = Postlarva.

study on Firth of Clyde Nephrops was conducted and relationships derived between fecundity and carapace length for a range of arbitrarily chosen egg development stages so that the loss of eggs during incubation could be estimated. Earlier studies have indicated that regional differences exist in egg loss for different Nephrops populations (Figueiredo and Nunes, 1965 ; Chapman and Ballantyne, 1980 ; Morizur, 1981). The fecundity information obtained for the Firth of Clyde Nephrops in this study is compared with the results of similar studies for other Nephrops populations. The mean effective fecundity, estimated from ovigerous females with eggs close to hatching, was used in conjunction with size frequency and maturity information to determine the mean number of eggs hatched per female.

In the summer months a comprehensive plankton sampling programme investigated temporal and spatial changes in larval abundance in the Lower Firth of Clyde (L.F.C.). A laboratory based study provided information on the effect of temperature on larval development and this was used to transform the larval abundance data into seasonal production estimates for each zoeal stage. These were then used to estimate stage to stage mortality rates and to predict instantaneous larval production for 1st zoeae. The instantaneous larval production estimate was then combined with the mean fecundity per female to predict the female spawning stock size for the L.F.C..

Little information exists on the depth distribution of Nephrops zoeae. The vertical distribution of these stages was therefore investigated over 24 hours during both a spring and neap tidal period and related to environmental variables (light, pressure, temperature, salinity and tide). The results from this study were interpreted in

conjunction with light and pressure responses shown by larvae under controlled laboratory conditions.

Although it is now generally accepted that metamorphosis in Nephrops occurs during the moult from the 3rd zoeal to the 1st postlarval stage (as defined in this present study, Table 0.1) it was not known whether the young Nephrops first come into contact with the sea bed as 3rd stage zoeae or at some later time, even possibly beyond the 1st postlarval stage. An attempt was made in this study to answer this question.

Several aspects of this research depended upon the development of an aquaculture facility. In contrast to the considerable interest shown in the culture of the closely related Homarus spp. (Provenzano, 1985), few similar studies have been conducted on Nephrops. Several of the husbandry techniques which were developed for Homarus spp. have been applied in the present work on Nephrops.

Some of the ovigerous females captured during the fecundity study, whose eggs were close to hatching, were used as a brood stock. Newly hatched larvae were removed from the brood stock facility and reared under different culturing conditions. This study included investigations into the influence of stocking density, temperature and starvation on larval development. Reliable data on the development rates of Nephrops larvae at different temperatures are required to transform field larval abundance data into larval production estimates. The precision of previous attempts to perform this transformation have been hampered by this lack of information (Garrod and Harding, 1980 ; Nichols et al., 1987). The larvae used in the

behaviour experiments were supplied from the culture facility.

In the past the early postlarval stages of Nephrops have been difficult to obtain from the field. Artificial culture may therefore provide the best method for obtaining these stages in large numbers. Although few Nephrops have achieved metamorphosis under artificial culture conditions in earlier studies the present study successfully provided a large number of postlarvae for substratum selection and settlement behaviour experiments.

Field evidence suggests that most Nephrops settle on the sea bed soon after metamorphosis and remain within the confines of their burrows, which in the past have only been discovered in association with adult burrows. It seems that they do not emerge from their burrows until the end of their first year, when they have reached a carapace length of 10 - 15mm (Chapman, 1980). Attempts were made in this study to examine the field evidence and discover when and how final settlement occurs in juvenile Nephrops. The burrows constructed by the juveniles, both independently and in association with existing adult burrows, are described.

Individuals from the 3 zoeal and early postlarval stages (obtained from the culture facility) were examined using several different techniques and used for a study of morphology and growth. The changes associated with metamorphosis were studied in greatest detail and the adaptational significance of these changes has been discussed. The developmental changes which occurred in the eye were closely examined.



## CHAPTER 1

### A study of Nephrops fecundity in the Firth of Clyde

#### 1.1. Introduction

Fecundity of Nephrops has been estimated by counting the number of oocytes in the ovary (Thomas, 1964 ; Fontaine and Warluzel, 1969), the number of eggs on the pleopods (Farmer, 1975 ; Chapman and Ballantyne, 1980 ; Abelló and Sarda, 1982) or both (Figueiredo and Nunes, 1965 ; Morizur, 1981 ; Figueiredo et al., 1982). A summary of some of these results has been compiled by Chapman (1980).

In earlier studies fecundity determinations were performed on ovigerous females obtained from trawl samples. Thomas (1964) suggested that ovigerous females may suffer egg loss as a result of these fishing operations and suggested that counts of oocytes in the ovary of non-ovigerous females were more reliable. It has been subsequently shown that trawling does indeed cause some egg loss (Chapman and Ballantyne, 1980). As a consequence fecundity estimations on ovigerous Nephrops were made on creel caught individuals in this study. Fecundity based on counts of oocytes in the ovary is likely to be slightly overestimated as not all oocytes are released at egg laying (Farmer, 1975). The proportion of non-released oocytes, which includes the germinal strand, has been found to be small in this and other studies (C.J.Chapman pers. comm.).

In previous investigations lower fecundity was reported for Nephrops in the Firth of Clyde when compared with other Scottish waters (Thomas, 1964 ; Chapman and Ballantyne, 1980). In this present study fecundity determinations were made for both non-ovigerous and ovigerous Nephrops in the Firth of Clyde and for non-ovigerous Nephrops in the Sound of Jura. The fecundity estimates were compared and related to earlier investigations.

The fecundity determinations obtained for all ovigerous females incubating eggs close to hatching were used in conjunction with the instantaneous larval production estimate derived in Chapter 2 to estimate the spawning stock size in Chapter 8.

It is known that a steady loss of eggs occurs during the incubation period (Figueiredo and Nunes, 1965 ; Chapman and Ballantyne, 1980 ; Morizur, 1981 ; Morizur *et al.*, 1981). In addition, some females in this study appeared to have lost an unusually high proportion of their eggs, possibly through their failure to adhere to the pleopods at the moment of spawning. This phenomenon was noted in Nephrops from Loch Torridon (C.J.Chapman, pers. comm.) but otherwise has not been reported previously. Egg and ovary development in ovigerous Clyde Nephrops were investigated in the present study and the results compared with those of Bailey (1984).

It is known from previous studies that the mean diameter of Nephrops eggs increases during development (Figueiredo and Barraca, 1963 ; Farmer, 1975), probably as a result of water uptake (see Chapter 7). From an investigation in the Celtic Sea, Morizur and Rivoalen (1982) suggested that smaller Nephrops produced smaller eggs.

Both of these aspects were examined in Clyde Nephrops during the present study.

## 1.2. Materials and methods

This investigation was divided into 2 phases. The first involved an estimate of potential fecundity based on the number of oocytes in the ovary of mature individuals. This included a comparison between two Nephrops populations off the West Coast of Scotland. The second was restricted to a Nephrops population within a small area of the Firth of Clyde. Fecundity information was obtained from ovigerous females incubating eggs at various stages of development. The results were analysed by the Statistics Department of DAFS in Aberdeen and relationships fitted to the two sets of data.

### 1.2.1. Fecundity study based on oocyte counts

Non-ovigerous females were obtained from trawl catches in the Firth of Clyde (approximately 3km south of Little Cumbrae Island ; 55°41'N, 04°56'W) and in the Sound of Jura (approximately 5km NNW of the Island of Gigha ; 55°46'N, 05°45'W). In an earlier study, Bailey and Chapman (1983) compared density, length composition and growth for Nephrops populations within these two areas. The samples were taken during July 1984 using the Millport Marine Station's research vessel, 'Aora'. The animals were fixed in 4% formalin and returned to the laboratory where they were transferred into 70% ethanol. The use of both preservatives hardened the oocytes and enabled dissection of the intact ovary (Figueiredo and Nunes, 1965).

Specimens with mature ovaries, ie. stages 7 or 8 (see Table 1.1), were selected and their carapace lengths were measured using

Table 1.1

Stages of ovary maturation compared with other authors  
(adapted from Farmer (1975)).

(Stages 1-8 representing increasing stages of maturity,  
stage 9 represents resorption after egg laying).

Stage & duration	Mean (A) diameter oocytes (mm)	Colour		Equivalent stages		
		Fresh	Preserved (B)	Symonds (1972)	Farmer (1975)	Bailey (in press)
1 (C)	< 0.19	transluscent/ white	-	0	-	-
2	0.19	white	white	1	1	0
3	-	-	-	-	-	-
4	0.37	cream	buff	2	2	1
5	-	-	-	-	-	-
6	0.63	pale green	pink	3	3	2
7	-	dark green	orange	4	4	3
8	0.93	dark green & swollen	deep orange	5	-	4
9	0.19-0.93	mottled green/ cream	mottled white/ pink	5R (D)	5 (D)	5 (D)

(A) Developing oocytes only.

(B) In alcohol, formalin or ethylene glycol.

(C) Except immature individuals.

(D) Stage 9 showing resorption or (in this study) partial resorption.



callipers. The dissected ovary was dried at 105°C for 24h allowed to cool and weighed on a Mettler AE 163 digital balance to a precision of 0.01mg. If the number of oocytes did not exceed 1,500 all were counted. In other cases an exact number was counted (always greater than 1,000) and referred to the dry weight of the whole sample to calculate the total number. The maximum error in the calculations was found to be less than 3%.

#### 1.2.2. Fecundity study based on egg counts on the pleopods

Two fleets of 25 Nephrops creels were set in the Firth of Clyde in an area west of the Little Cumbrae (approximately, Lat. 55°/43.00'N Lon. 04°/58.00'W). In October 1984 both fleets of creels were lost due to commercial trawling activity. As a consequence replacement fleets were located closer inshore, approximately 1km further north. The study extended over a period of two years, from mid-January 1984 to mid-January 1986. The creels were examined and re-baited weekly (weather permitting) using the Millport Marine Station's research vessel, 'Aplysia'. Ovigerous female Nephrops in the catches were placed in individual polythene bags and returned to the laboratory for examination.

The eggs were carefully stripped from the pleopods and a subsample of 10 - 20 eggs was examined under a stereomicroscope. The eggs were staged on a development scale of 1 - 9 (see Table 7.1) and the mean diameter was calculated to the nearest 0.01mm using an eye-piece graticule. The carapace lengths of the females were measured to the nearest millimetre using callipers and ovary stage was classified

on a scale of 1 - 9 (see Table 1.1). The eggs were then dried, weighed and counted as described in section 1.2.1..

### 1.3. Results

#### 1.3.1. Fecundity study based on oocyte counts

During this investigation 49 individuals were examined from the Firth of Clyde and 33 individuals from the Sound of Jura. Scatter plots of fecundity against carapace length were made for each area (Figure 1.1). These suggested that a curvilinear relationship would fit the data in the form :

$$Y = a X^b$$

where Y is fecundity, X is carapace length, a, b are constants. A logarithmic transformation was performed on the data to give the linear form which can be fitted by least squares. This gave the following relationships :

$$\text{Clyde} \quad Y = 0.1973 X^{2.635} \text{ (s.e. of power} = 0.0858)$$

$$\text{Sound of Jura} \quad Y = 0.1794 X^{2.678} \text{ (s.e. of power} = 0.1534)$$

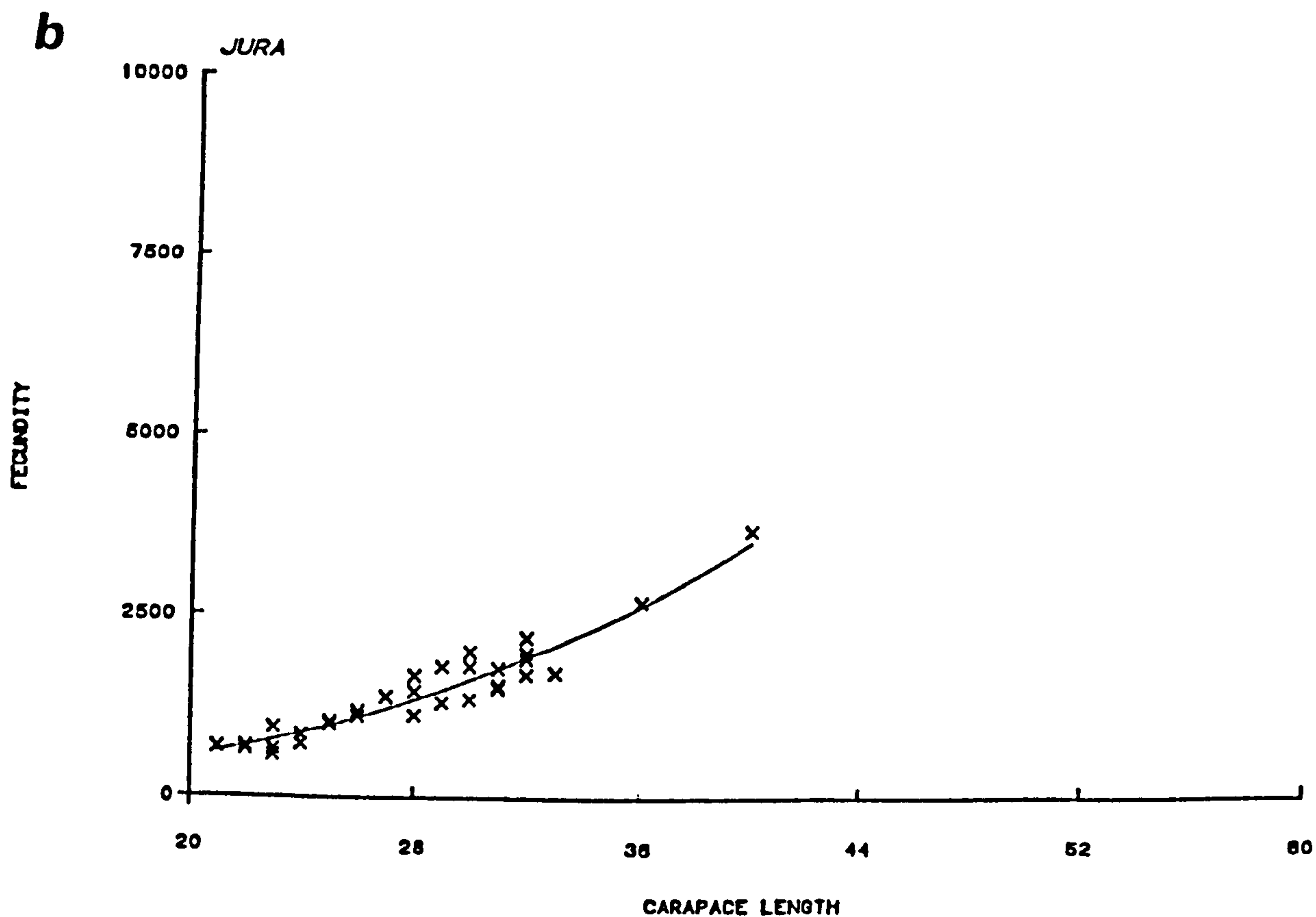
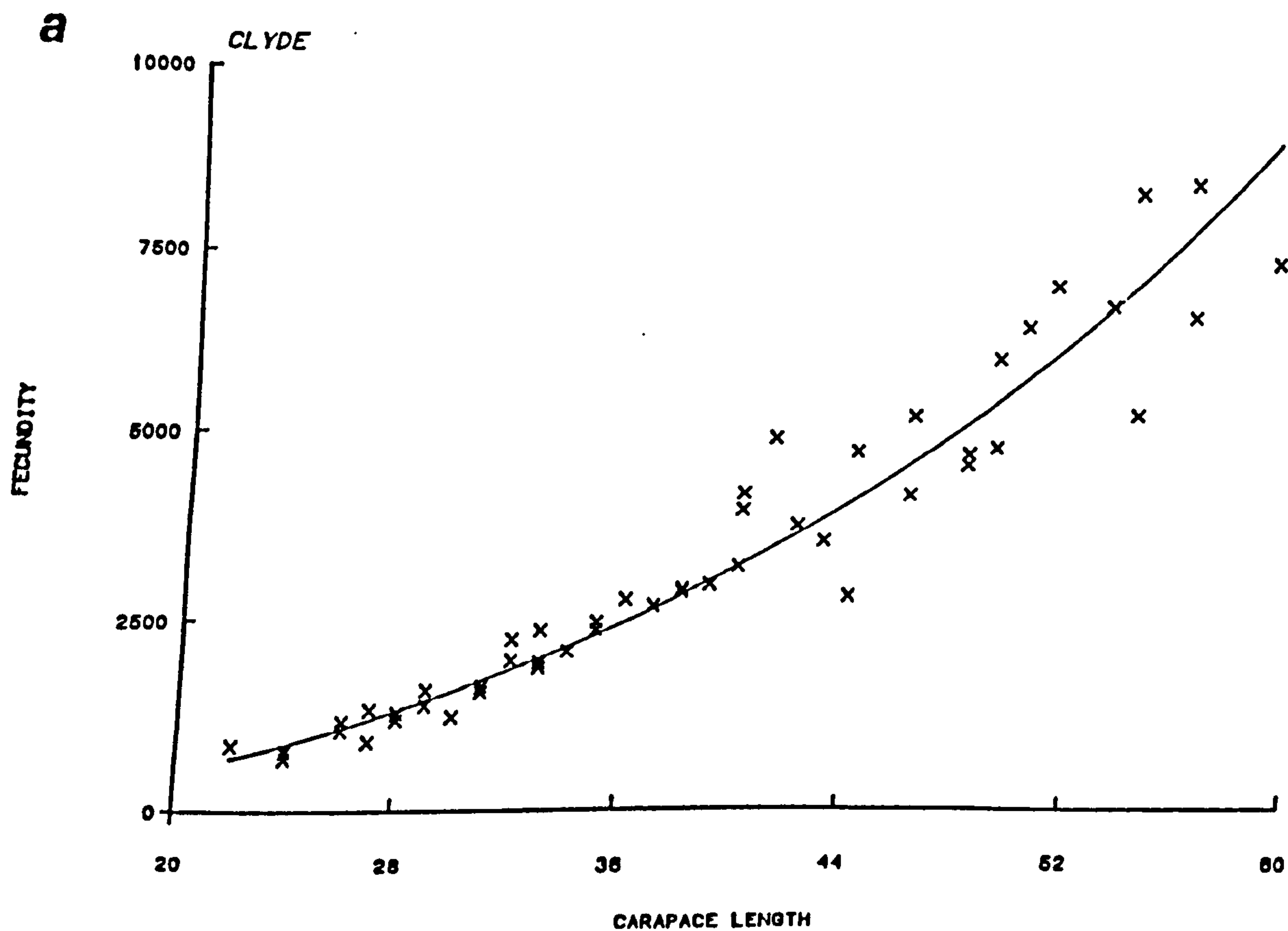
A comparison of these relationships revealed no significant difference between the powers but a difference between constants. A common power of 2.644 was therefore applied to the two sets of data to give :

$$\text{Clyde} \quad Y = 0.1911 X^{2.644}$$

$$\text{Sound of Jura} \quad Y = 0.2014 X^{2.644}$$

These equations were then used to predict the mean fecundities for a range of carapace lengths (Table 1.2). The predicted fecundities for the Sound of Jura were about 5% higher than those for the Firth of Clyde ( $P < 0.01$ ).





**Figure 1.1**

**Relationship between carapace length and number of oocytes in the ovary for mature individuals from the (a) Firth of Clyde and (b) Sound of Jura.**

**Table 1.2**

**The mean fecundities for a range of carapace lengths common to the Firth of Clyde and Sound of Jura.**

Carapace length (mm)	Mean fecundity	
	Firth of Clyde	Sound of Jura
	( $y = 0.1911 \times 2.644$ )	( $y = 0.2014 \times 2.644$ )
25	949	1000
30	1537	1620
35	2311	2435
40	3289	3467

### 1.3.2. Fecundity study based on egg counts on the pleopods

During this investigation 1153 individuals were examined and Table 1.3 shows the breakdown of these by year and month of capture. Nephrops are known to be prone to egg loss during incubation. Attempts were made to determine this loss by formulating relationships between fecundity and carapace length for each egg development stage (Table 7.1). It was evident from scatter plots (Figures 1.2 - 1.7) that many individuals had suffered egg loss. Because Nephrops were only examined during January in 1986 the data were combined with the data for 1985.

A linear plot of egg number against carapace length is shown for all females examined in 1985 that were incubating newly spawned eggs ie. stage 1 (Figure 1.8a). Ovary examination of recently spawned females revealed that only a few oocytes had not spawned (and would therefore be resorbed). If egg loss only resulted from a progressive incubation loss the fecundity data obtained for newly spawned females (Figure 1,8a) would have been expected to be similar to that obtained from the oocyte counts of non-ovigerous Clyde females (Figure 1.1a). The fact that they are not is because of egg loss, possibly due to failure of eggs to adhere to the pleopods. The difference between the two sorts of egg loss is that the possible adhesion failure egg loss is evident at the beginning of development whereas steady loss during incubation is only apparent by the end of development.

There is no simple statistical procedure which will distinguish females subject to egg loss during incubation from those with a particularly low number of eggs due to other factors. The exclusion of individuals which fall into this latter category was therefore a

**Table 1.3**

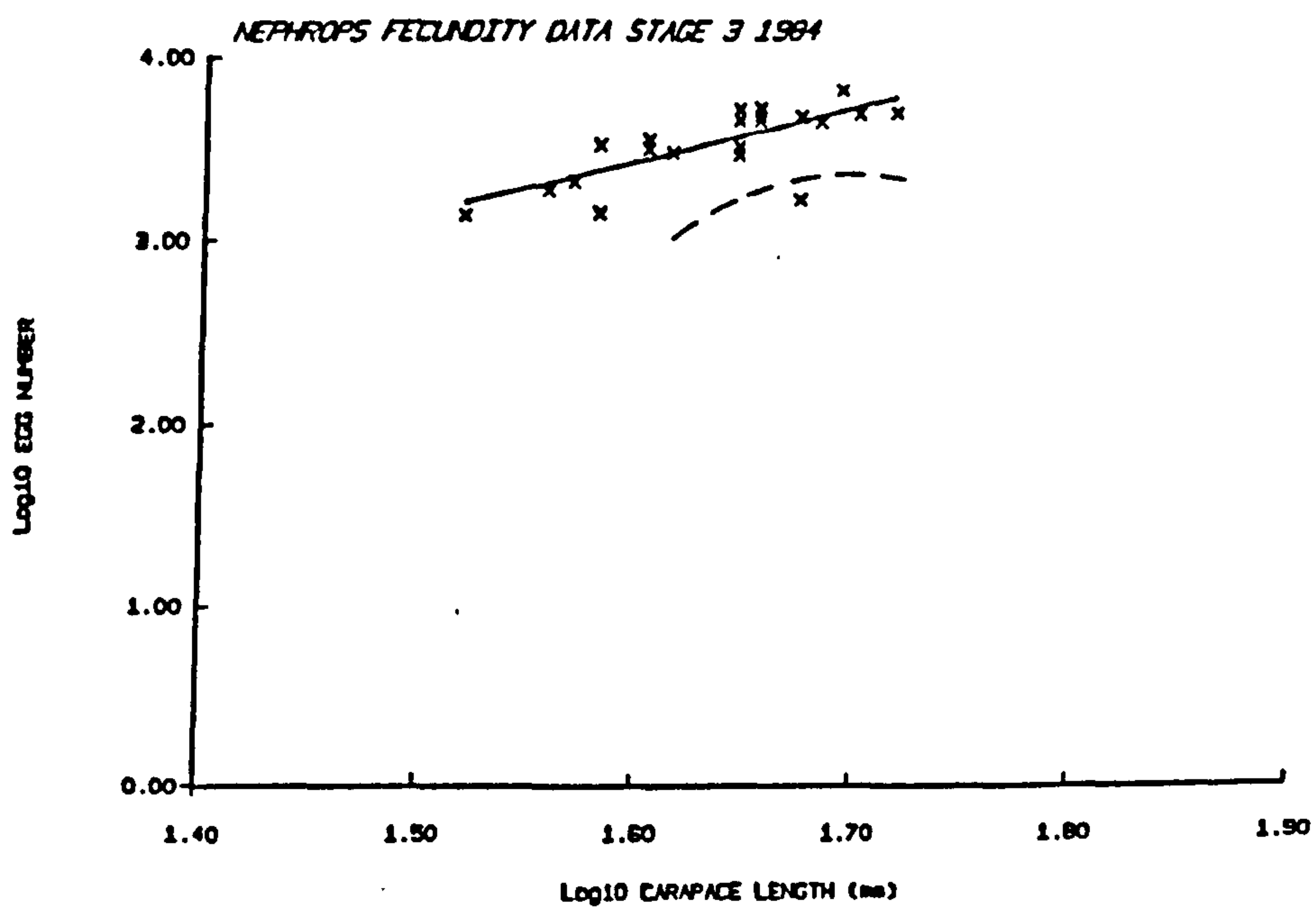
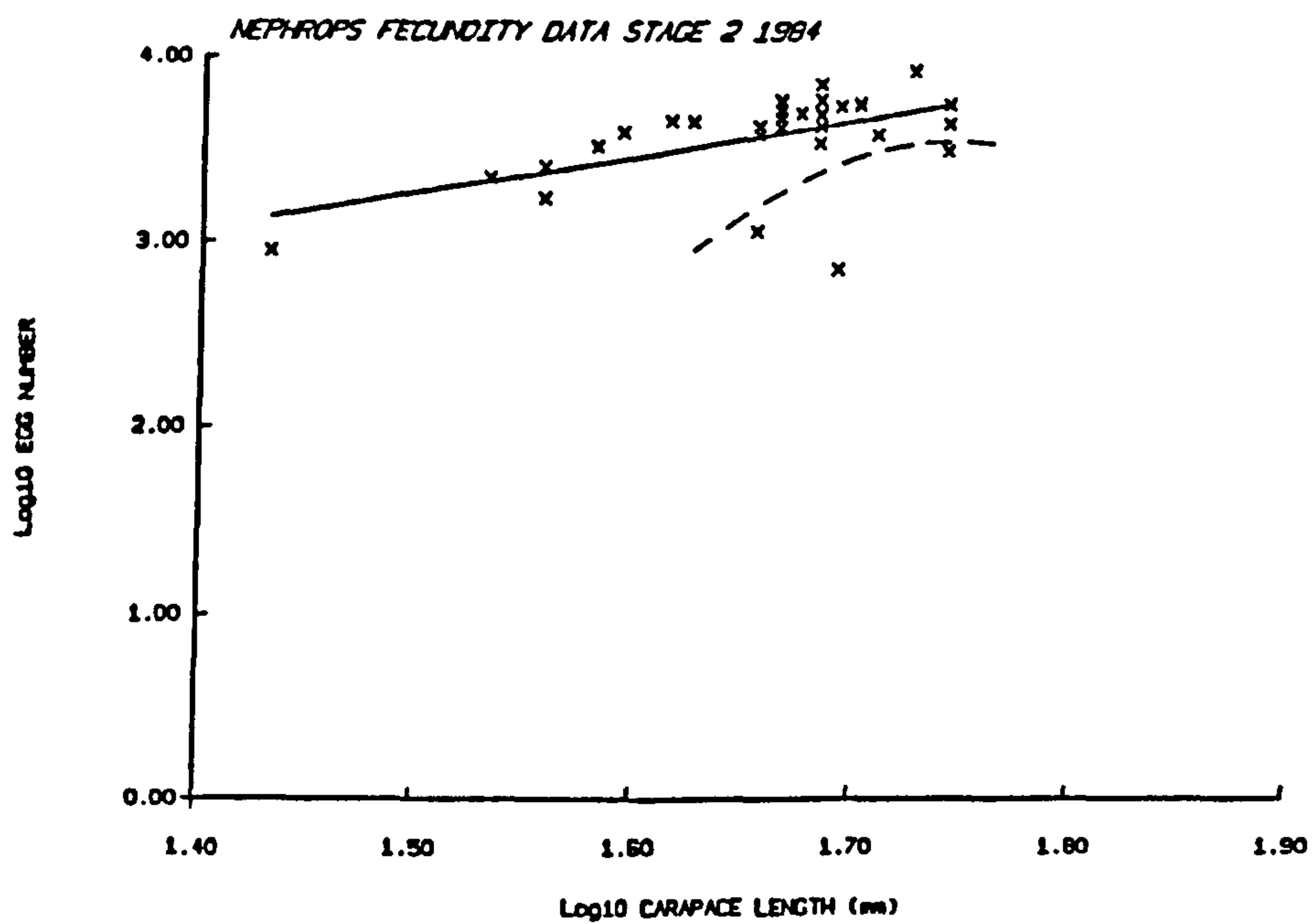
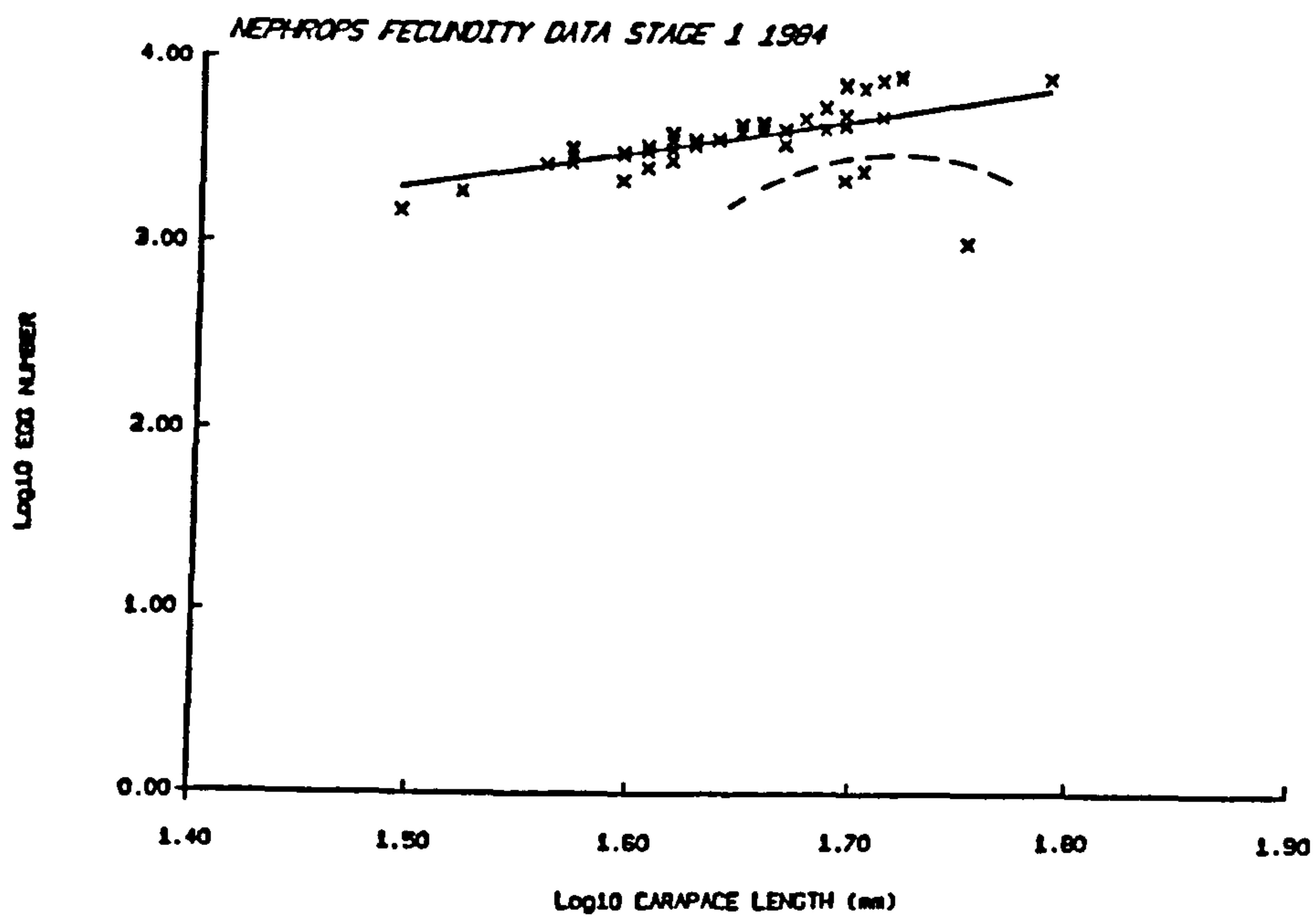
**Number of ovigerous female Nephrops examined each month.**

Year	Month	Number	Year	Month	Number
1984	January	21	1985	January	27
	February	26		February	16
	March	56		March	154
	April	43		April	152
	May	53		May	87
	June	31		June	56
	July	21		July	15
	August	8		August	37
	September	13		September	86
	October	-		October	96
	November	31		November	55
	December	11		December	65
			1986	January	43

Both fleets of creels were lost at the beginning of October 1984 and consequently no data were obtained for this month.

**Figure 1.2**

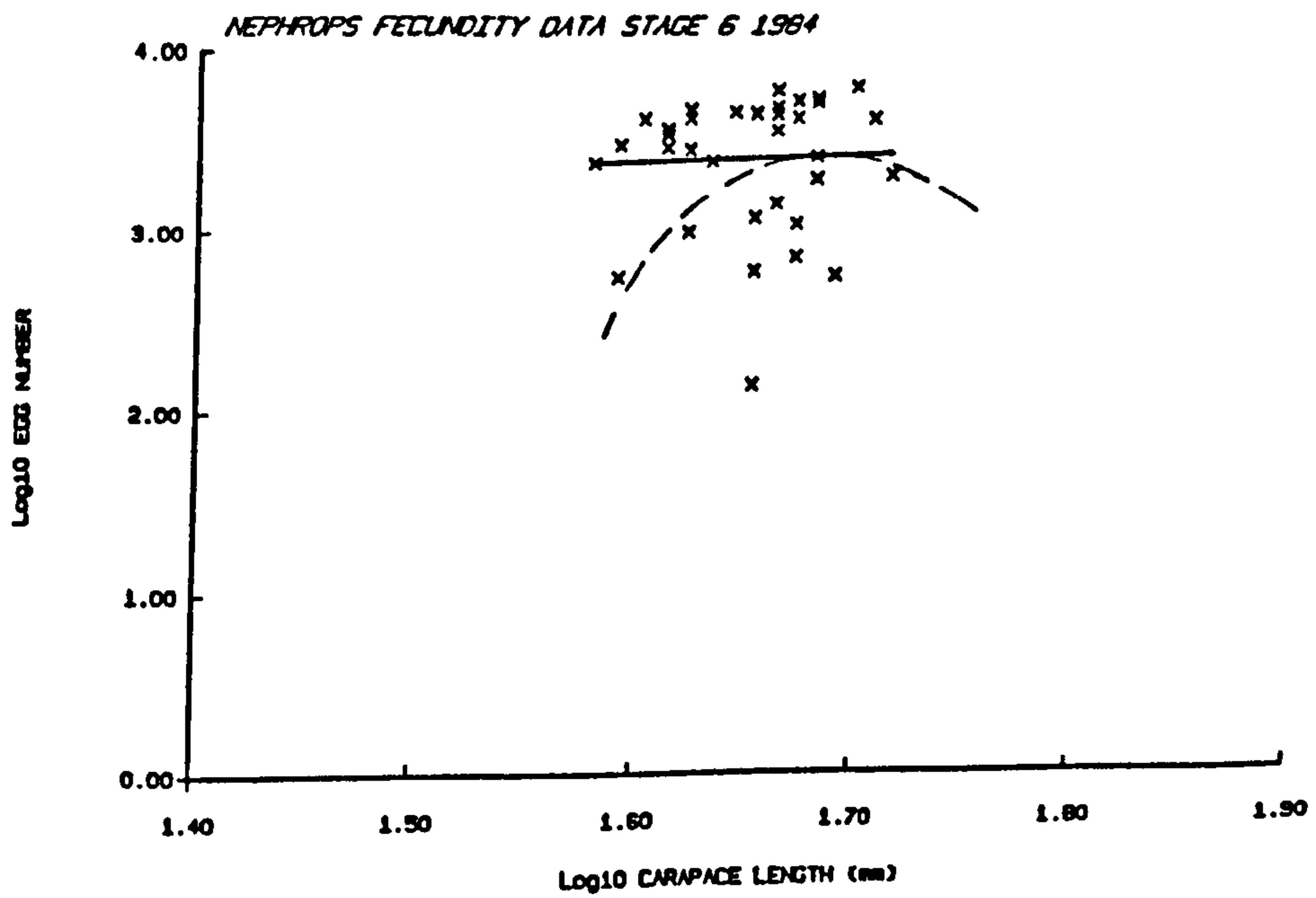
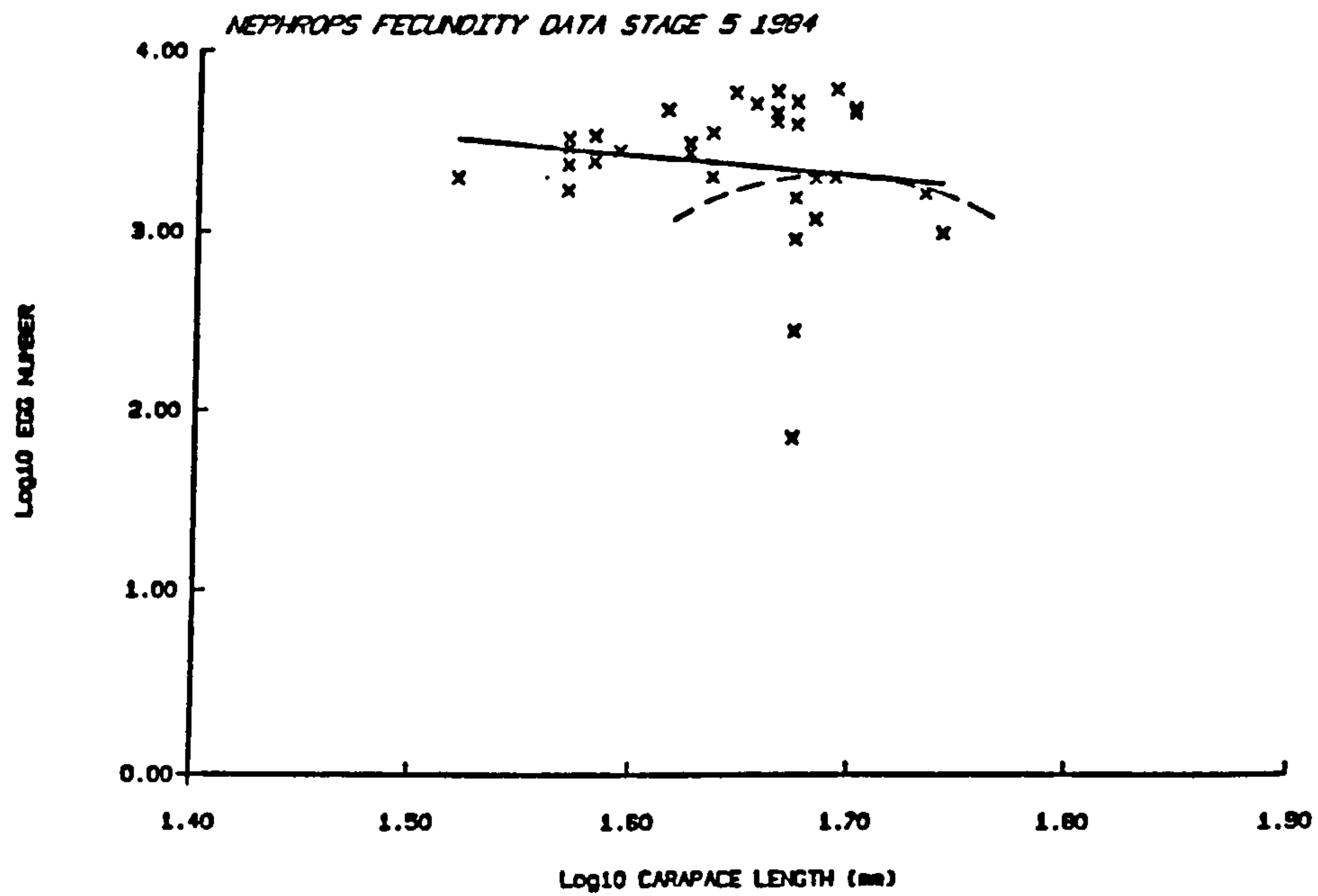
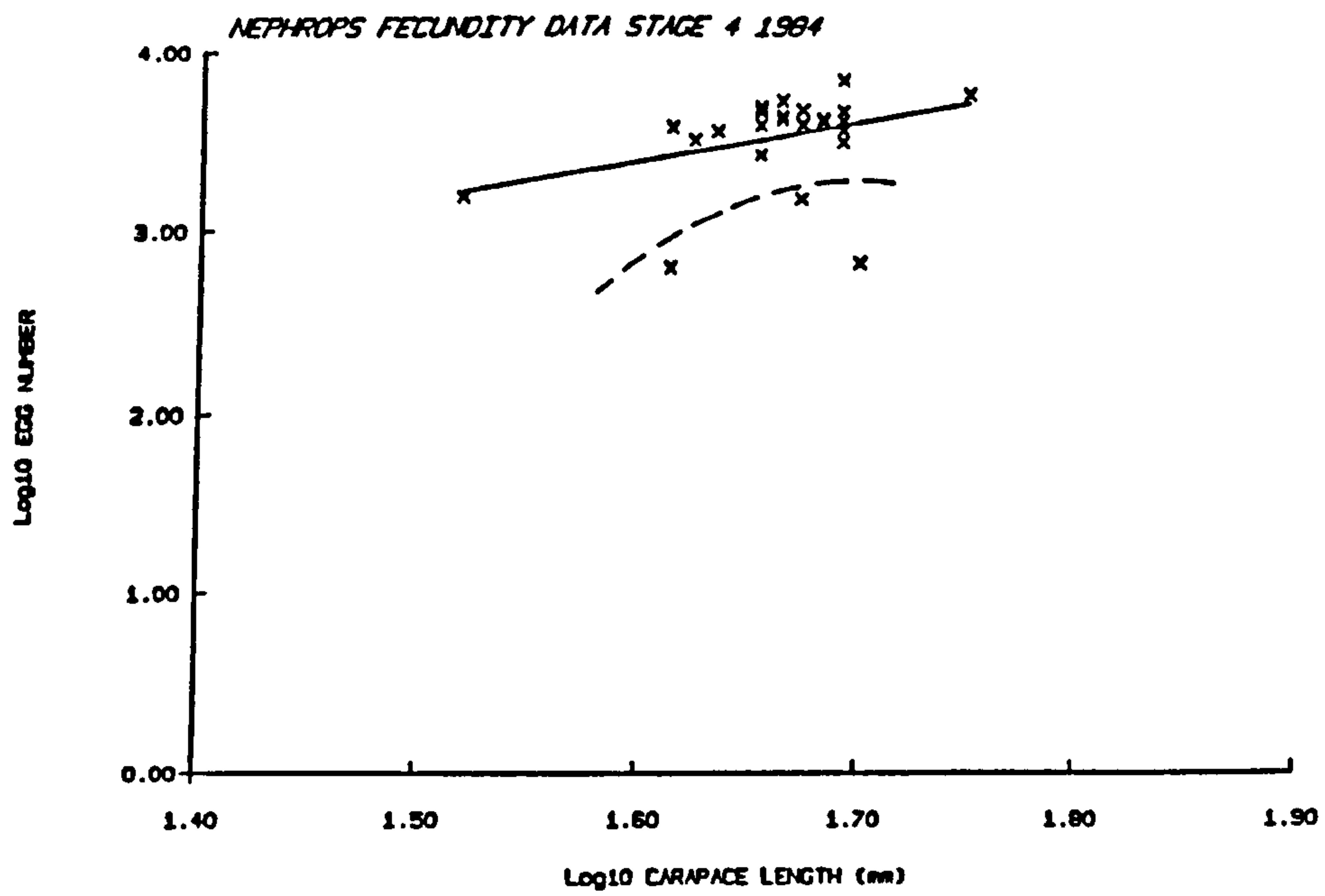
**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1984 that were incubating eggs in the 1st, 2nd and 3rd stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**



**Figure 1.3**

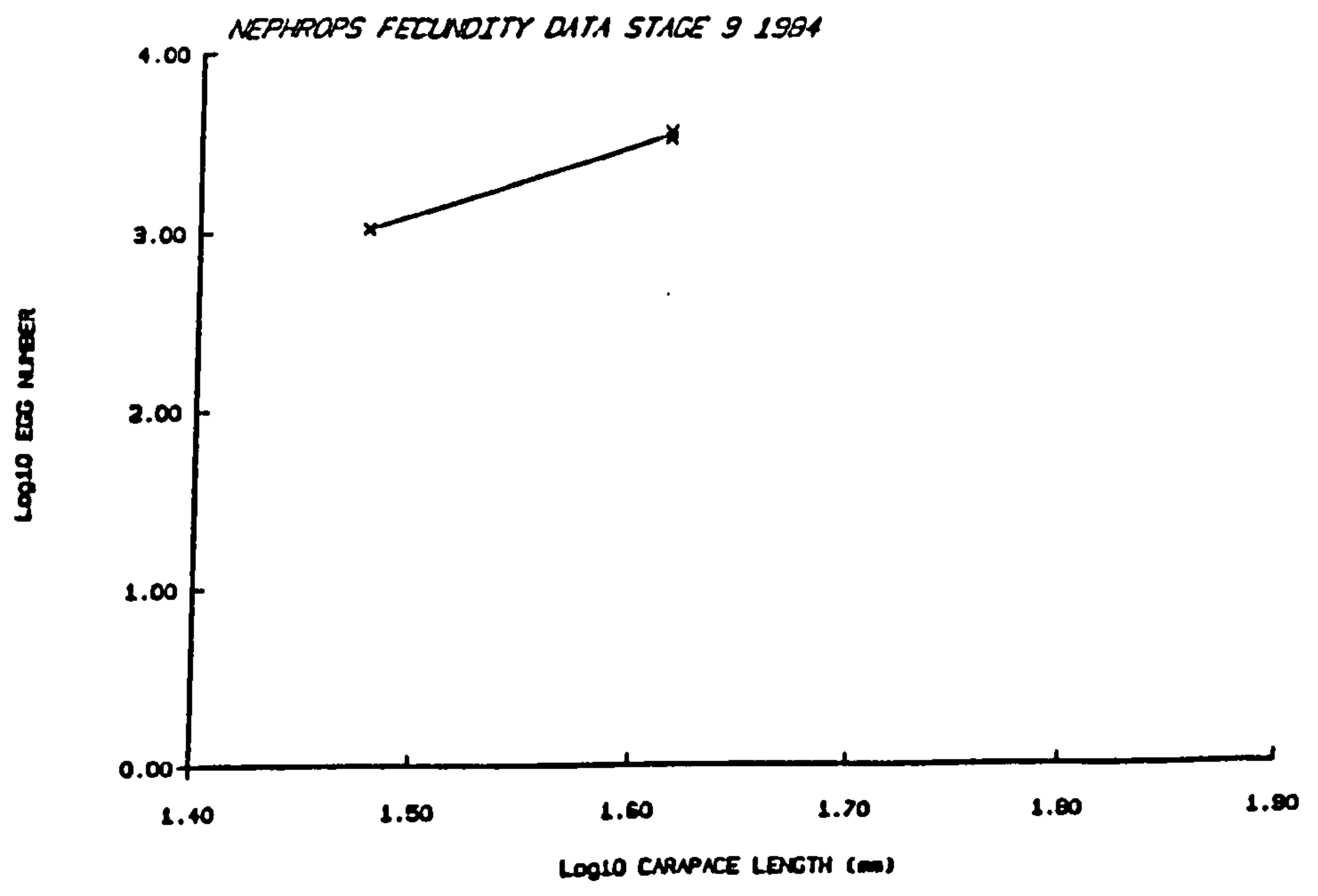
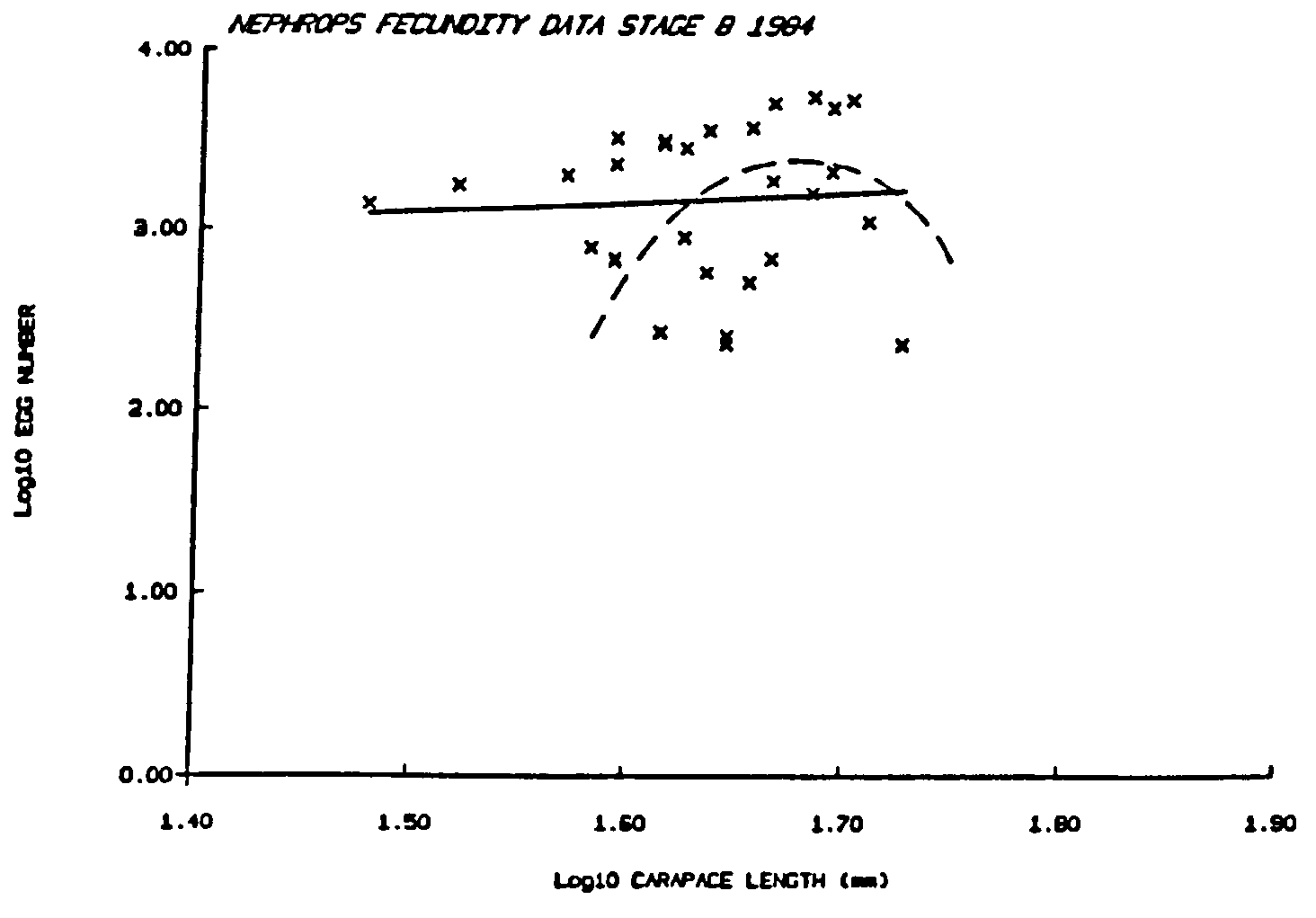
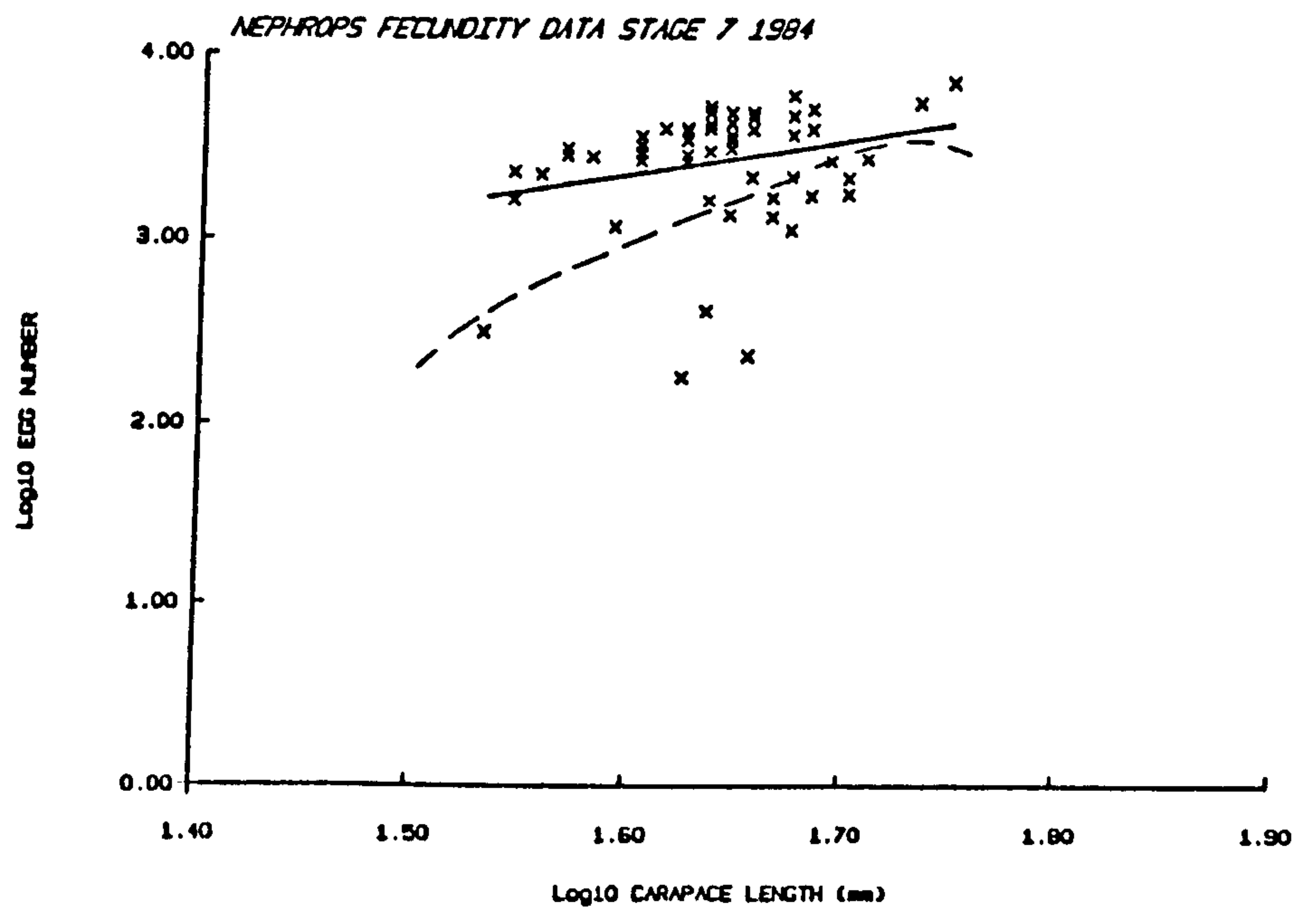
**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1984 that were incubating eggs in the 4th, 5th and 6th stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**





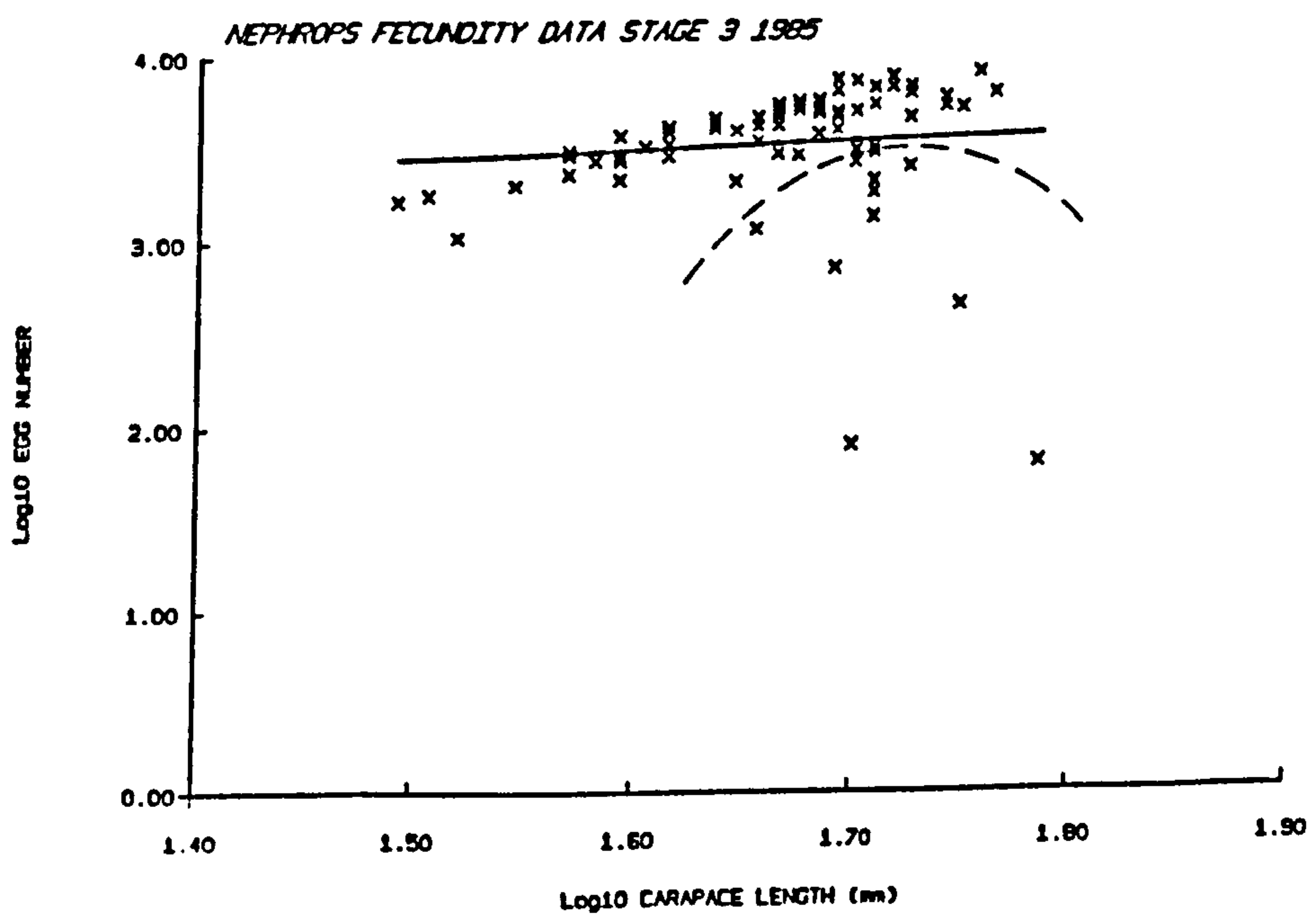
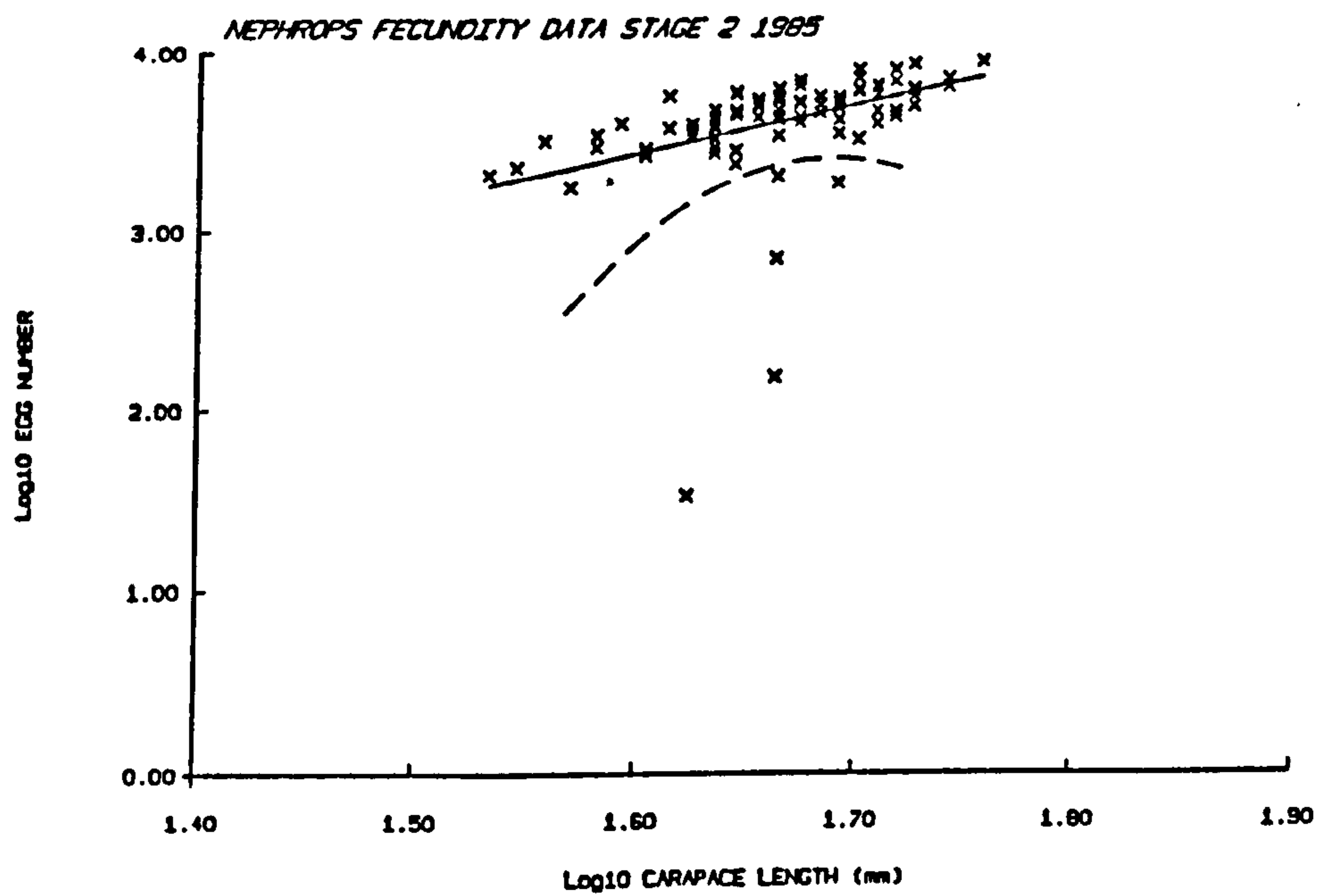
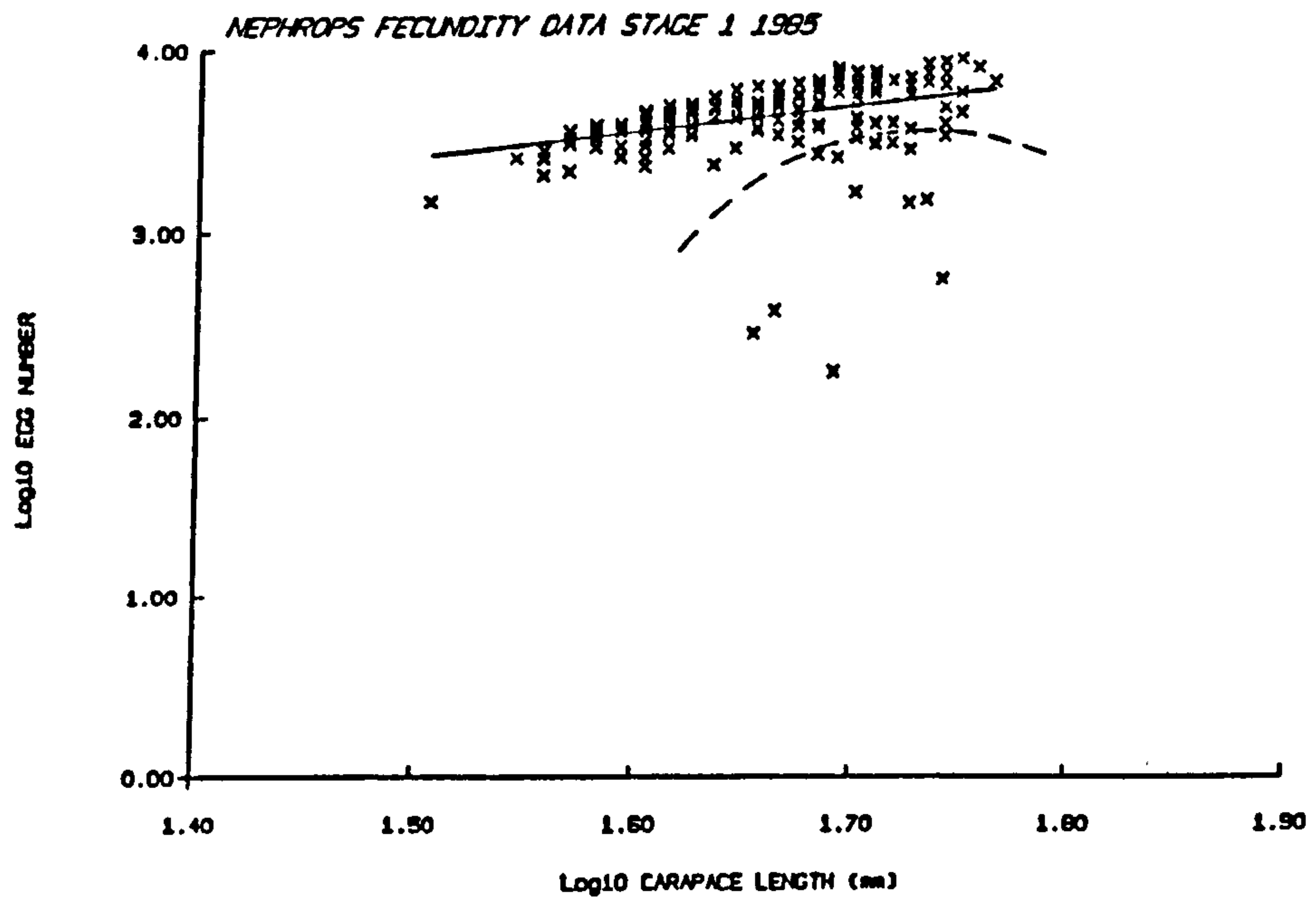
**Figure 1.4**

**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1984 that were incubating eggs in the 7th, 8th and 9th stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**



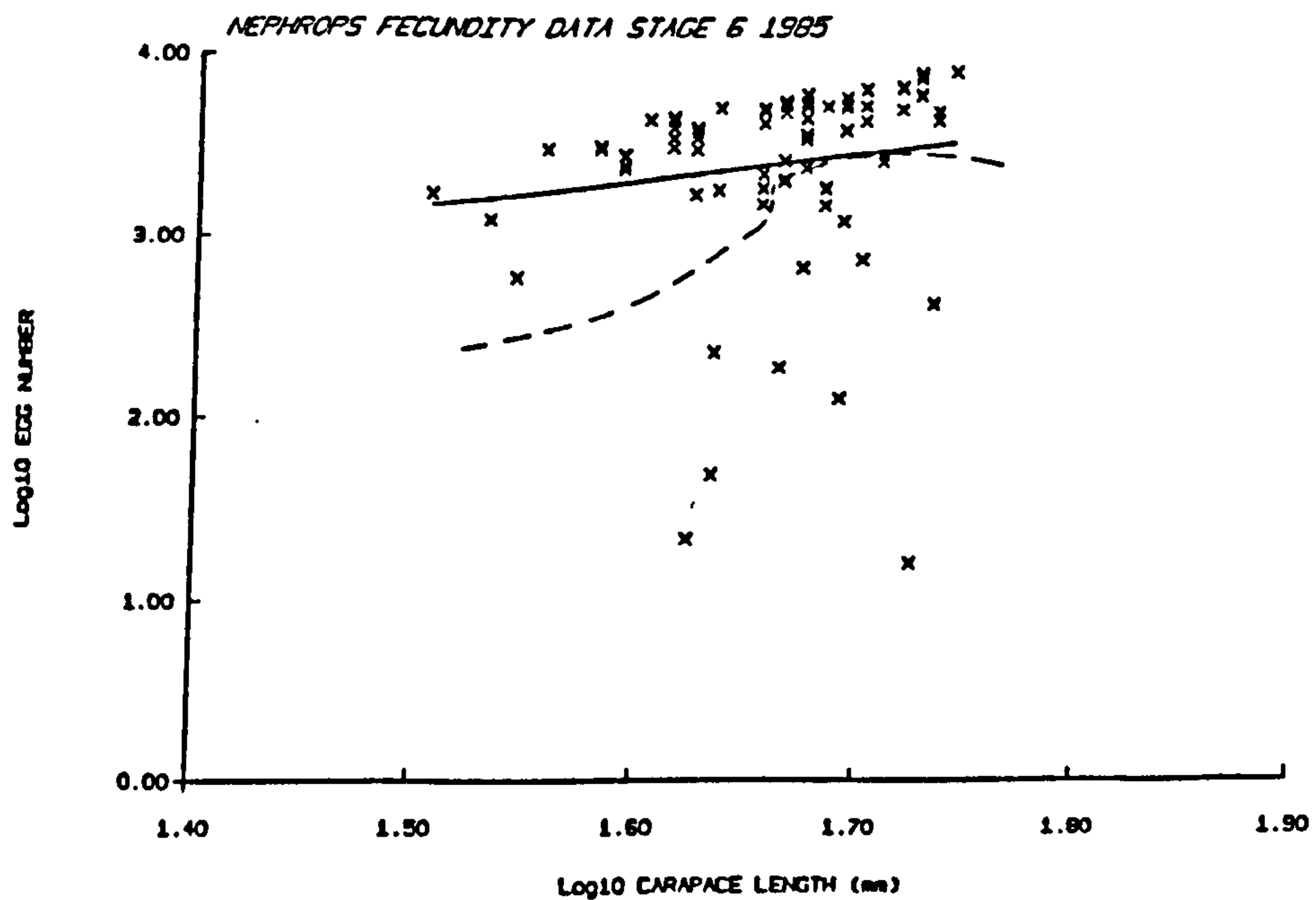
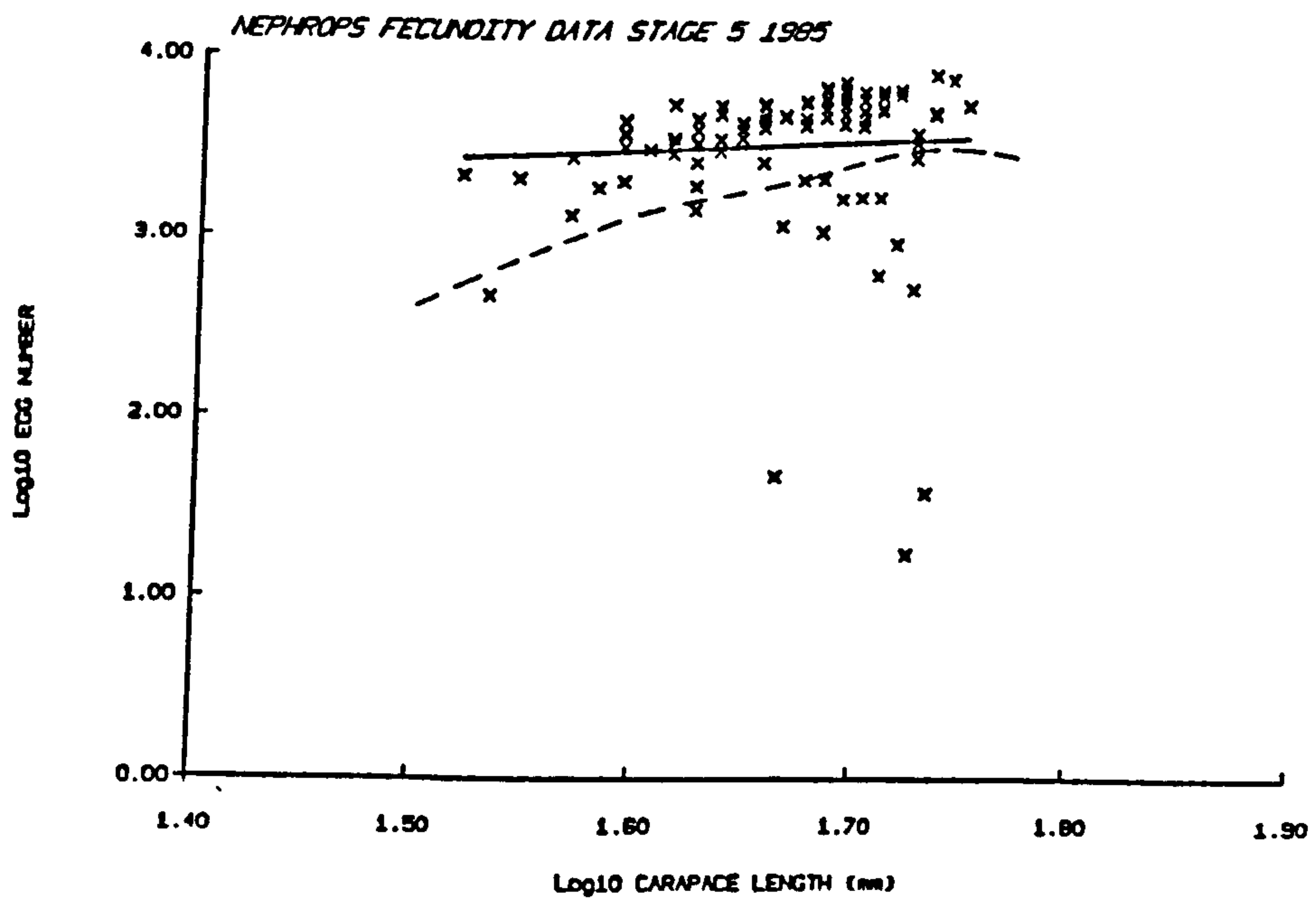
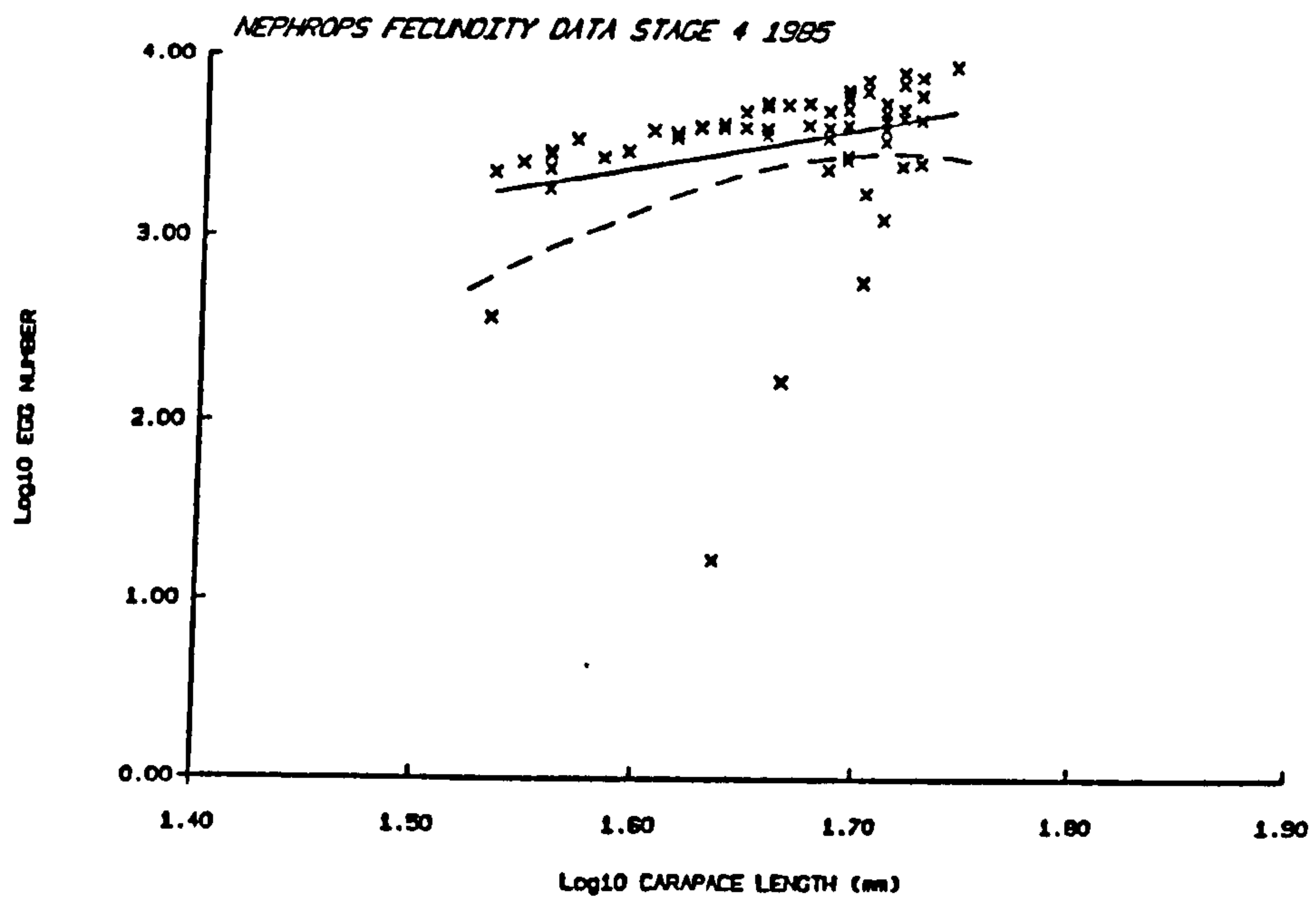
**Figure 1.5**

**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1985 that were incubating eggs in the 1st, 2nd and 3rd stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**



**Figure 1.6**

**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1985 that were incubating eggs in the 4th, 5th and 6th stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**



**Figure 1.7**

**Log-log relationships between carapace length and number of eggs carried on the pleopods for females caught in 1985 that were incubating eggs in the 7th, 8th and 9th stages of development. Data points below the broken line represent individuals which were judged to have suffered abnormal egg loss (see text).**





### Figure 1.8

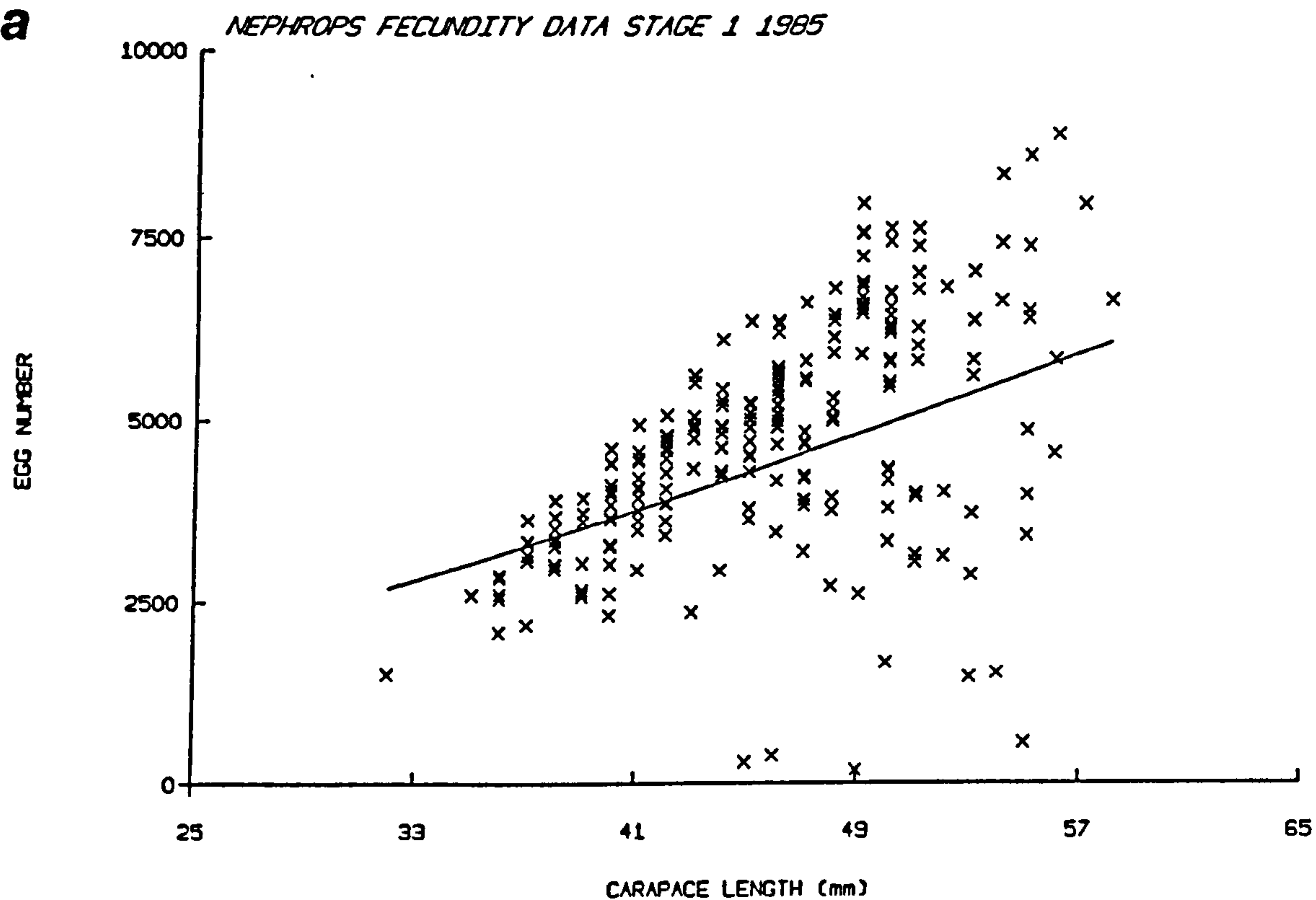
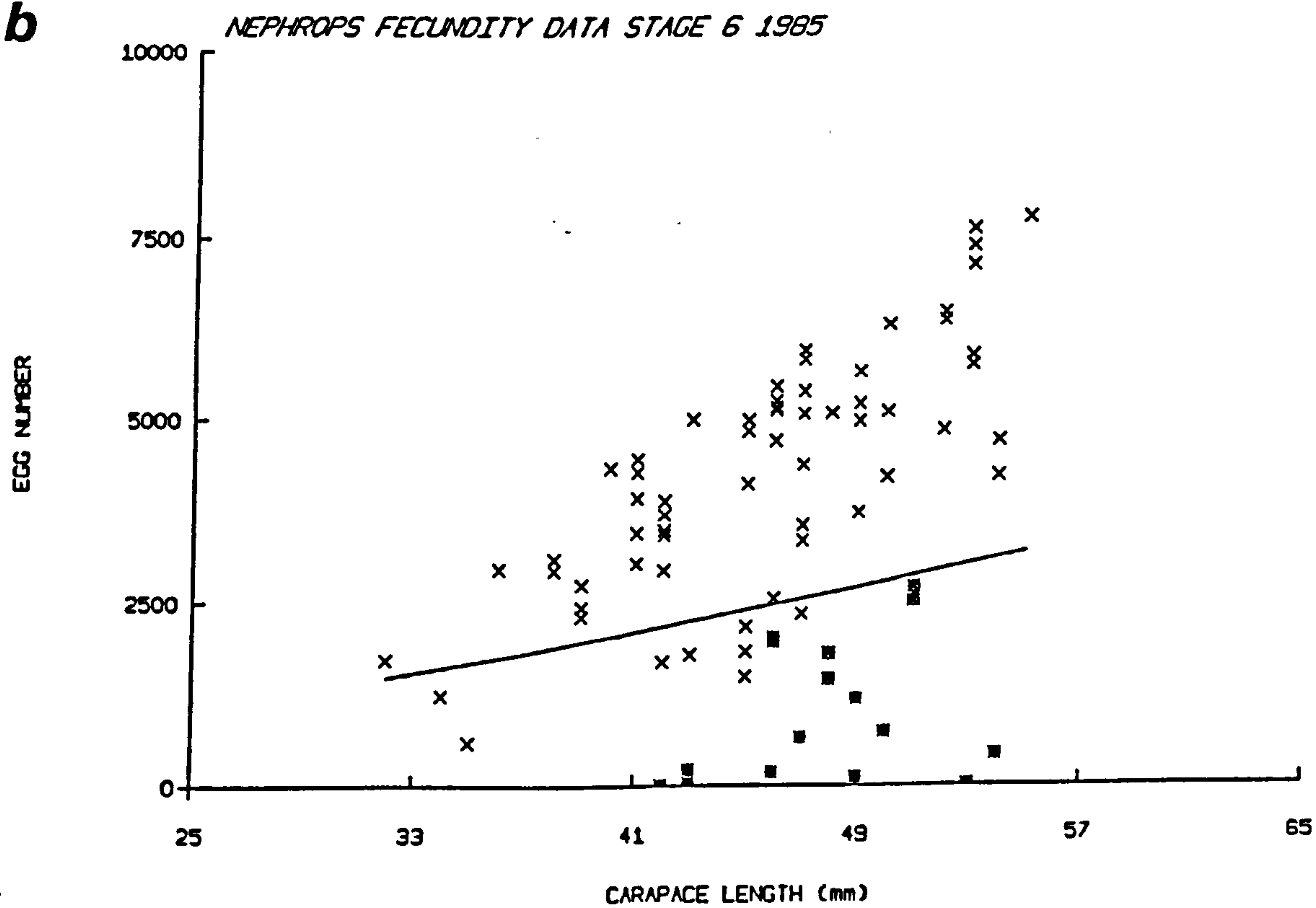
(a) Relationship between carapace length of female and number of recently spawned eggs (stage 1) during 1985.

(b) Relationship between carapace length of female and number of stage 6 eggs during 1985, showing individuals which were judged to have suffered abnormal egg loss.

The individuals that were excluded from the data set on the basis that their fecundity was :

(1) 3 residuals or more below the fitted value   ✖.

(2) the same or lower than any larger individual in any other stage that had already been excluded using method (1)   ✖.

**a****b**

somewhat subjective exercise, using rules derived from a statistical analysis of egg number. A linear relationship (after log transformation was performed on X and Y):  $Y = bX + a$  was first fitted to all the data for each egg stage (Figures 1.2 - 1.7) and the residuals of the observed fecundities from their fitted values were determined. Any values which resulted in a standardized residual of 3 or more below the fitted value was deemed to represent an individual which had suffered abnormal egg loss. The choice of 3 for the standardized residual was arbitrary and represented a probability of 0.05% that the individual belonged to a 'normal' population of individuals. Using this criterion a number of individuals were excluded for any particular stage and the regression was recomputed to determine whether any of the remaining individuals produced a standardized residual of 3 or more below the regression line. This process was repeated until no other low egg counts were identified (Figure 1.8b). Further exclusions were then made by removing any larger individuals from a given stage which had fewer eggs than an already excluded individual in any other stage. This exclusion of females with low egg counts resulted in a considerably reduced set of data (Table 1.4) which was used to calculate linear regressions after logarithmic transformation of the data (Figure 1.9). The parameter estimates are presented in Table 1.5. There was no significant difference between the slopes of the regressions but there were differences in intercepts ( $P < 0.001$ ). A pooled slope of 2.434 was therefore used and the regression recalculated. The predicted fecundity at each stage of egg development for a 'standard' female of 45mm carapace length are given in Table 1.6. This size was within the length range of all the monthly samples and was close to the mean carapace length of all ovigerous females caught. As expected, the

Table 1.4

For each egg development stage the total number of females examined, number excluded due to additional egg loss and the remainder which were used for the estimation of progressive egg loss during the incubation period.

Year	Stage	Total number examined	Number excluded	Remainder
1984	1	47	3	44
	2	30	3	27
	3	24	1	23
	4	26	3	23
	5	32	9	23
	6	37	11	26
	7	58	13	45
	8	30	12	18
	9	3	0	3
1985	1	229	16	213
	2	92	6	86
	3	78	11	67
	4	62	9	53
	5	101	15	86
	6	78	16	62
	7	100	29	71
	8	89	43	46
	9	32	30	2

### **Figure 1.9**

**Log-log relationships between carapace length and number of eggs carried on the pleopods for each development stage during (a) 1984 and (b) 1985 after the exclusion of individuals which had suffered abnormal egg loss. The individuals that were excluded from these relationships are represented in Figure 8.2 (see Chapter 8).**



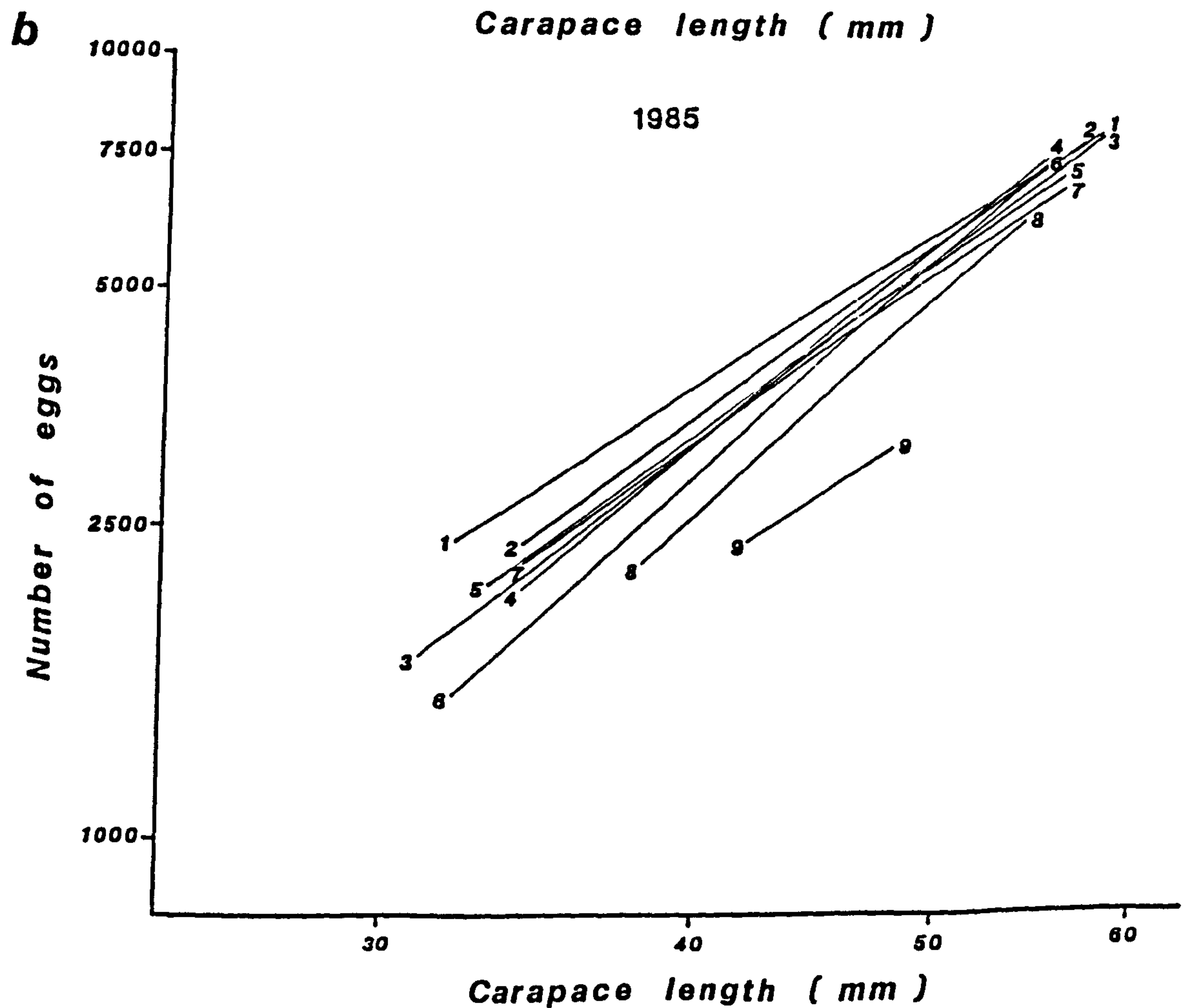
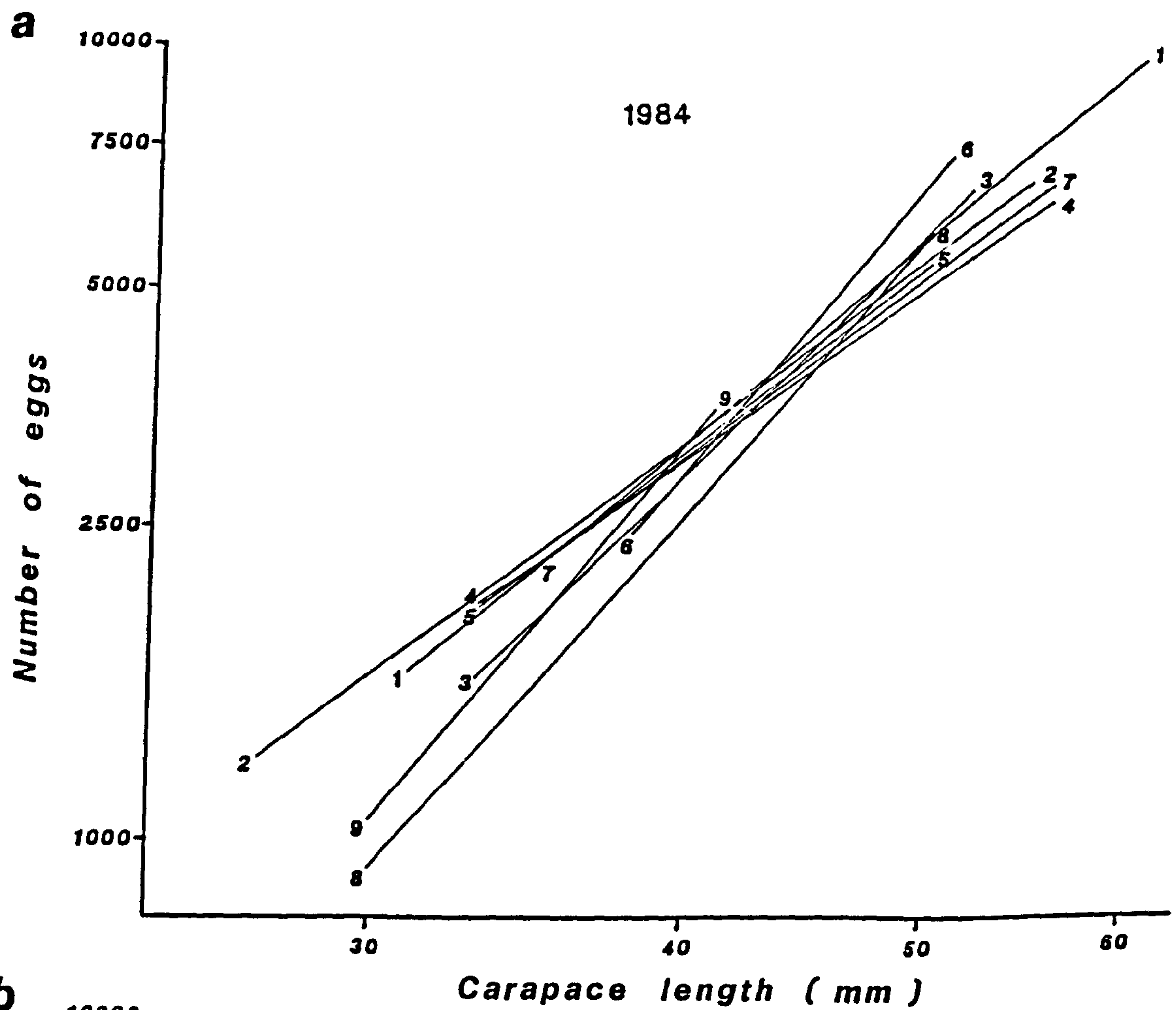


Table 1.5

Constant and power of regressions of egg number and carapace length for each development stage using  $y = ax^b$  where  $y$  is fecundity and  $x$  is carapace length (mm).

Year	Stage	Constant (a)	Power (b)	Significance of regression (P)
1984	1	0.1931	2.633	< 0.001
	2	0.4964	2.381	< 0.001
	3	0.0303	3.112	< 0.001
	4	0.8976	2.205	< 0.001
	5	0.4688	2.388	< 0.001
	6	0.0050	3.604	< 0.010
	7	0.5753	2.327	< 0.001
	8	0.0041	3.623	< 0.010
	9	0.0023	3.835	< 0.050
1985	1	2.1959	2.018	< 0.001
	2	0.7366	2.290	< 0.001
	3	0.3927	2.441	< 0.001
	4	0.1858	2.643	< 0.001
	5	0.7331	2.277	< 0.001
	6	0.0770	2.856	< 0.001
	7	0.8609	2.228	< 0.001
	8	0.0660	2.867	< 0.001
	9	1.2198	2.026	< 0.001

Table 1.6

Fitted fecundity estimates (in ascending order) for  
a female of 45mm carapace length.

Development stage	Year	Fitted egg number	Development stage	Year	Fitted egg number
9	1985	2828	5	1984	4186
8	1984	3251	6	1984	4235
8	1985	3613	5	1985	4250
6	1985	3951	4	1985	4273
9	1984	3971	3	1985	4300
4	1984	3997	2	1984	4312
7	1984	4029	1	1984	4415
7	1985	4110	2	1985	4523
3	1984	4153	1	1985	4829

predicted fecundity decreased during egg development.

The intercept values for each development stage (1984 and 1985) were analysed using a range test. This revealed that for each given development stage there was no significant difference ( $P > 0.05$ ) between the intercept values for the 1984 and 1985 data sets. It therefore seems likely that fecundity did not vary from one year to the next.

Very few samples were used to compute the relationships for egg development stage 9 so a comparison of fecundity at stages 1 and 8 was used to estimate egg loss during the incubation period (Table 1.7). Also, the eggs of females incubating stage 9 eggs may have been in the process of hatching and therefore some larvae may have been liberated. Although no statistical difference was detected between the fecundity estimates for 1984 and 1985, marginal differences may exist. For purposes of estimating the incubation loss comparisons should therefore be made between females contributing to the same brood. The fecundity estimates for females incubating eggs at the beginning (stage 1, 1984) and end (stage 8, 1985) of the same spawning year indicate that during the incubation period egg loss was approximately 18%.

### 1.3.3. The reproductive cycle

Figure 1.10 shows the frequency distribution of egg stage in monthly samples of ovigerous females to illustrate the course of egg development. During the winter months, December to February, there was little change in the composition of the catch which appeared to be

Table 1.7

Estimates of egg loss between development stages 1 and 8.  
(A-B, egg loss during the incubation period, see text).

Carapace length (mm)	Egg Number					
	1984			1985		
	Stage 1	Stage 8	Egg Loss	Stage 1	Stage 8	Egg Loss
	A				B	
30	1647	1212	435	1801	1348	453
35	2396	1764	632	2621	1961	660
40	3315	2441	874	3626	2713	913
45	4415	3251	1164	4829	3613	1216
50	5705	4200	1505	6240	4669	1571
55	7193	5296	1897	7868	5887	1981
60	8889	6544	2345	9722	7274	2448

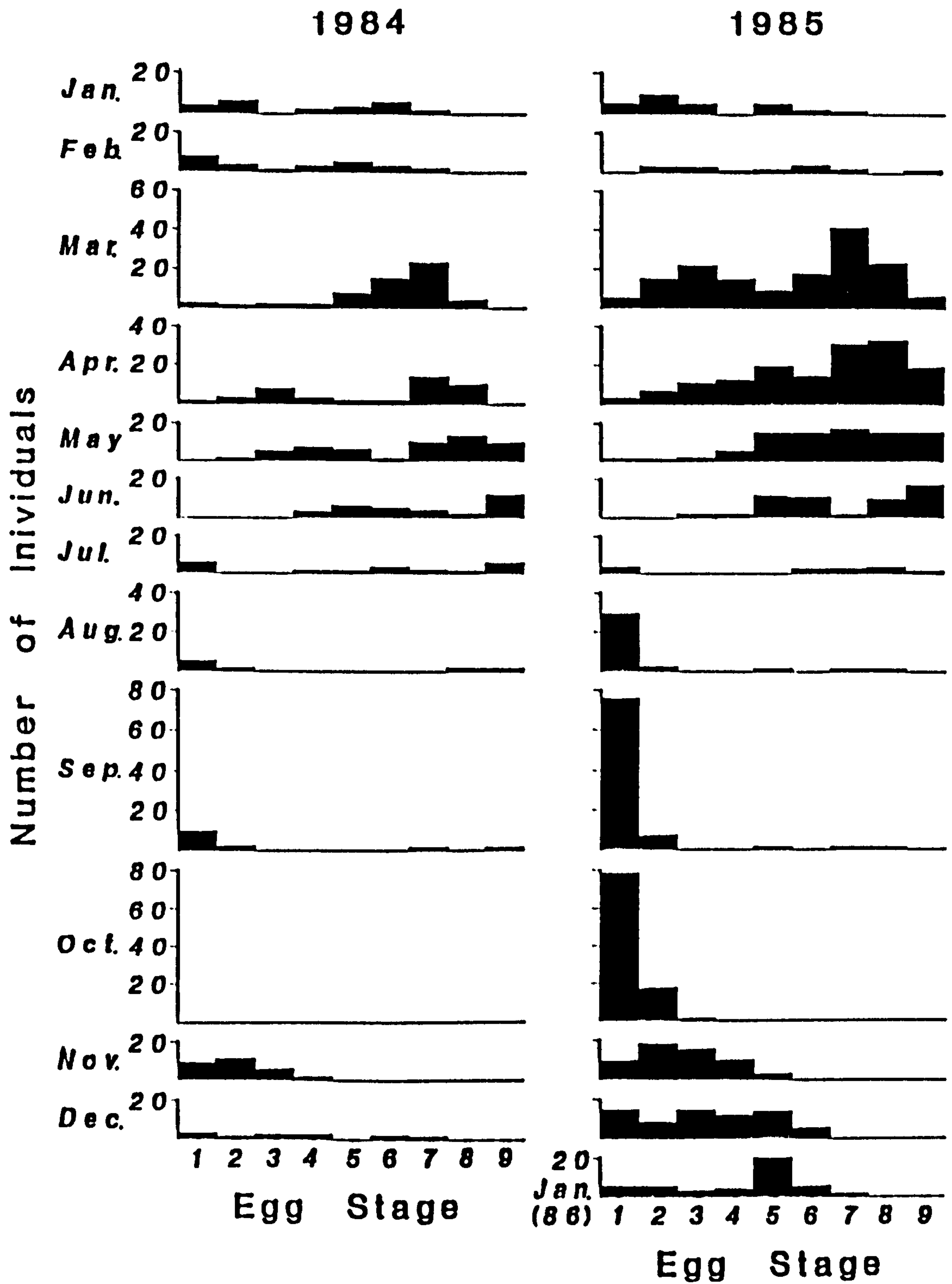
relatively evenly distributed between females incubating eggs in the first 6 development stages. During March and April there was a substantial increase in the number of ovigerous females captured and an increase in the proportion of females with late stage eggs. During May and June most ovigerous females possessed eggs in an advanced stage of development, but there was a progressive reduction in the numbers caught. As fishing effort was relatively constant over these months the reduction in ovigerous females incubating eggs in an advanced stage of development can probably be attributed to hatching activity, which reached a peak at the end of May (see Chapter 2). In July, Nephrops with newly spawned eggs first appeared, though females with eggs in an advanced stage of development were still present in the catches. Most ovigerous females possessed eggs in the early stages of development during August - October showing this to be the new spawning season.

Figure 1.11 shows the frequency distribution of ovary stage in monthly samples of ovigerous females to illustrate the course of ovary development. During the winter months, December to February, most females had ovaries in stages 3 - 5. Over this period development appeared to be slow. Between February and June there was a progressive increase in the proportion of ovigerous females with ovaries in advanced stages of development. Stage 7 was the highest ovary stage observed in ovigerous females and was generally found in females incubating eggs close to hatching. From August to October most females had ovaries in stages 3 - 4, followed by a gradual increase in the proportion of females with stage 5 ovaries from November to December. There was little evidence of a relationship between ovary stage and egg stage. In most months several ovary stages were observed in



**Figure 1.10**

**Frequency distribution of egg stage in monthly samples of ovigerous females to illustrate the seasonal change in egg condition during 1984 and 1985.**

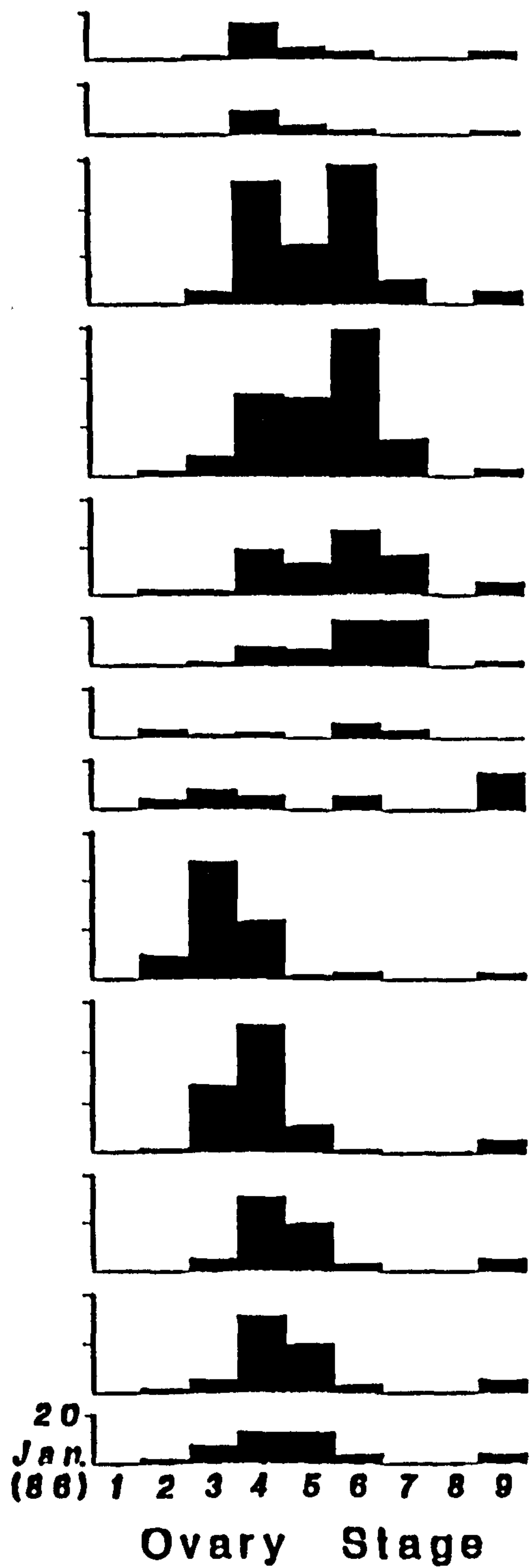
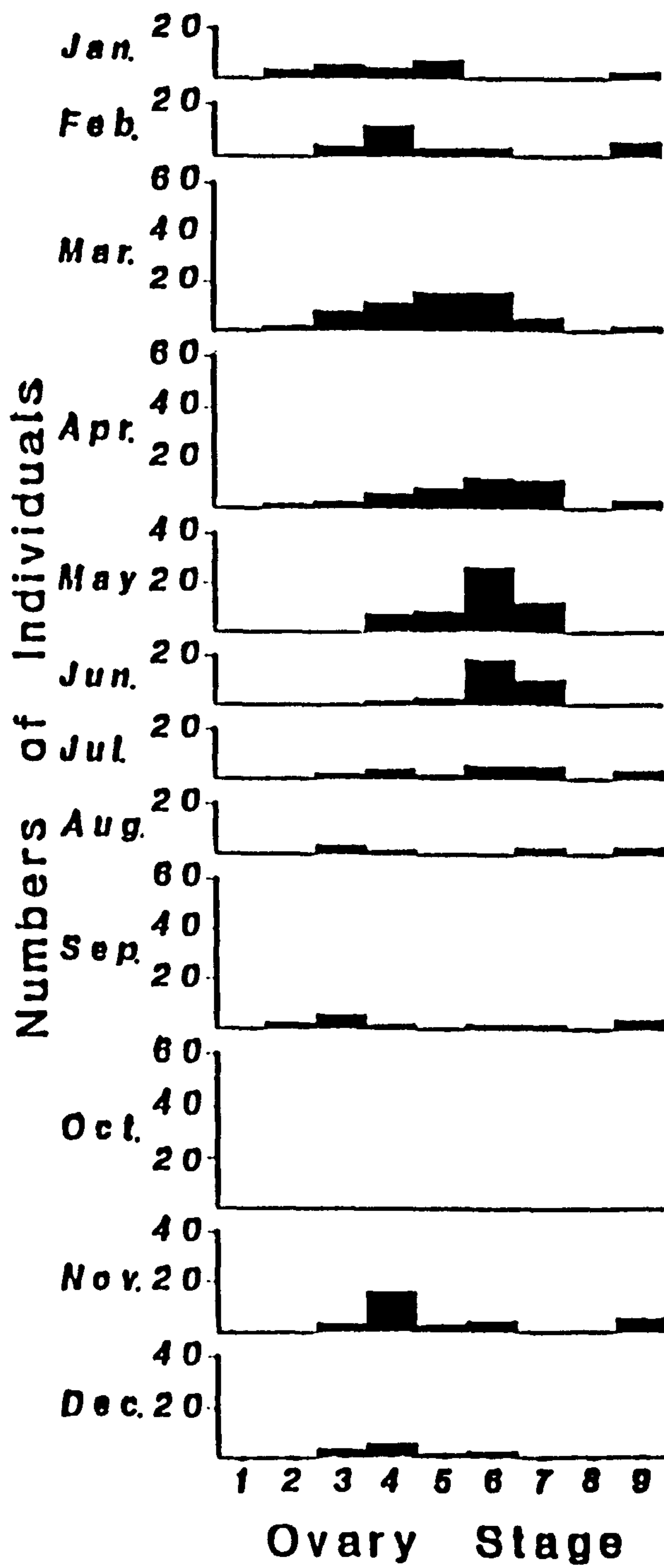


**Figure 1.11**

**Frequency distribution of ovary stage in monthly samples of ovigerous females to illustrate the seasonal change in ovary condition during 1984 and 1985.**

1984

1985



Jan. (86)

Nephrops of the same egg development stage.

#### 1.3.4. Relationship between egg diameter and carapace length

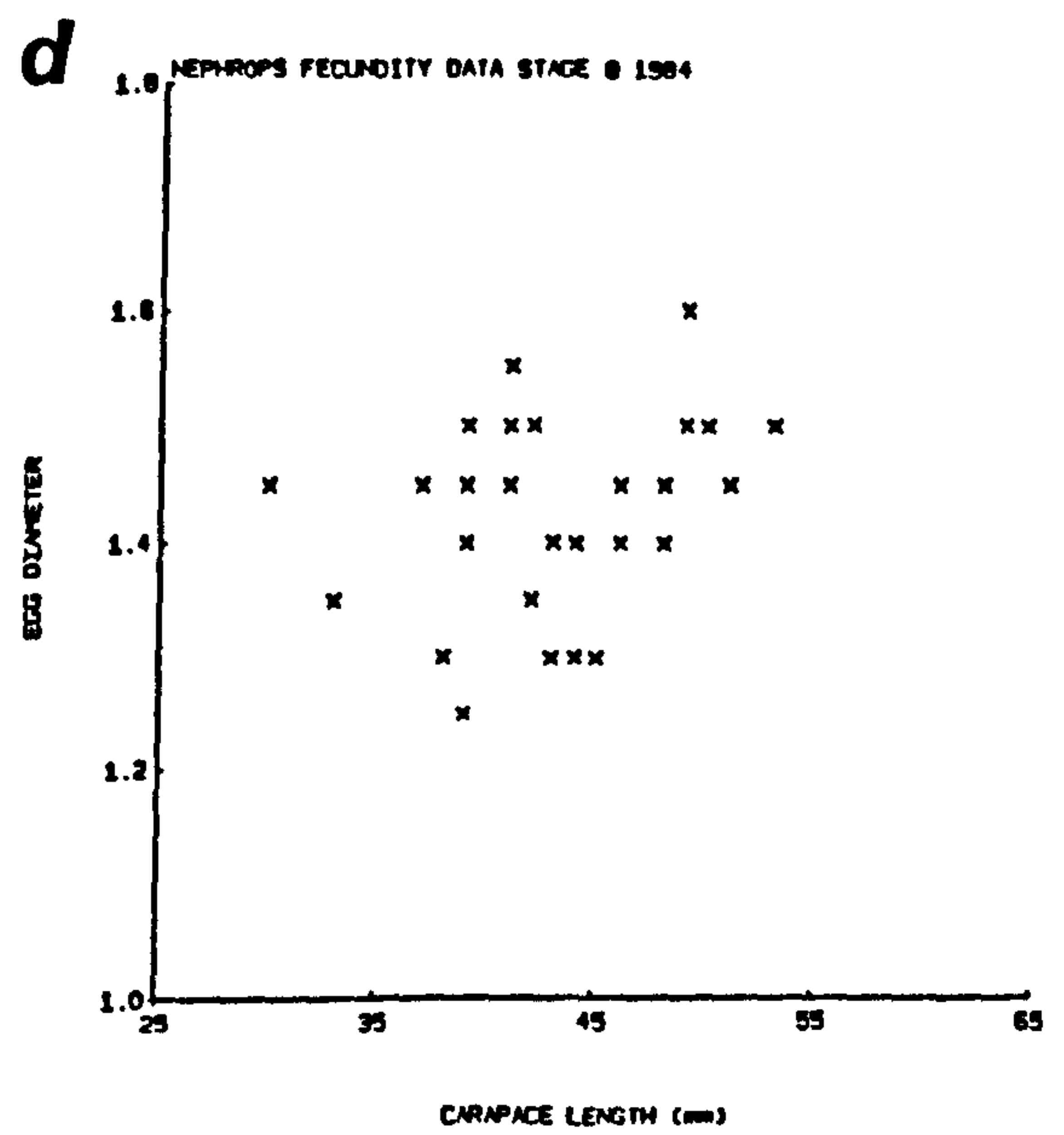
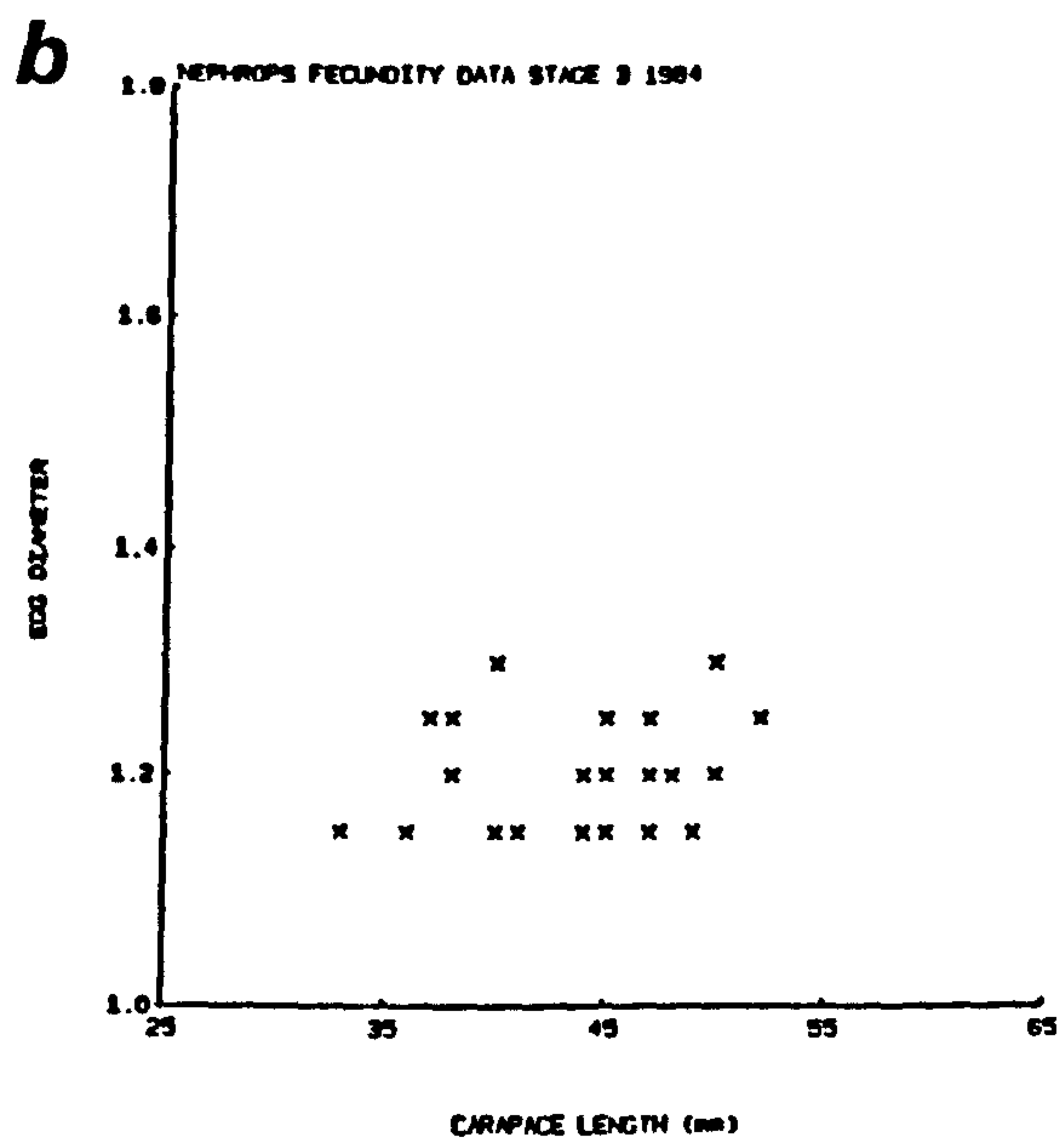
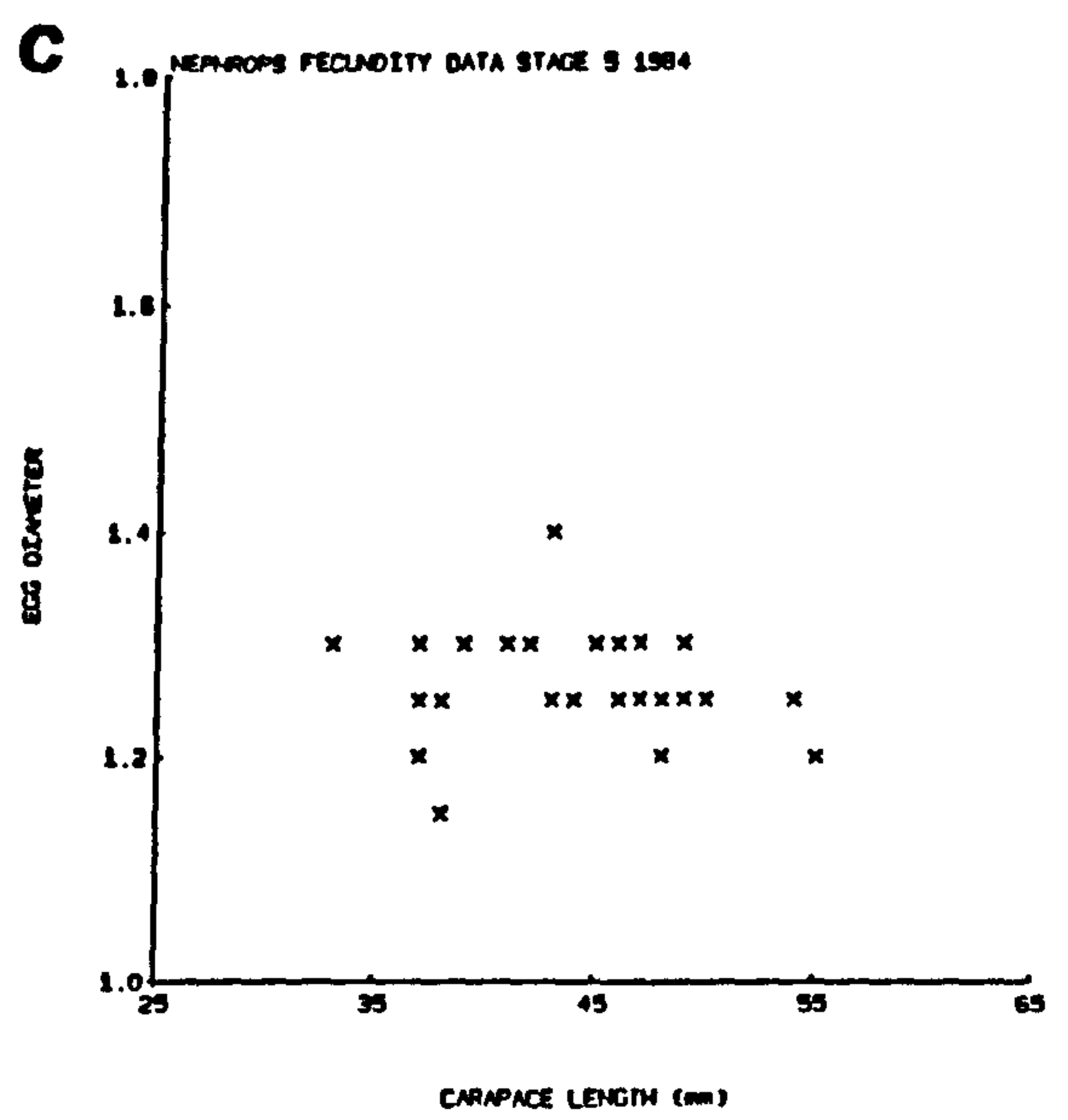
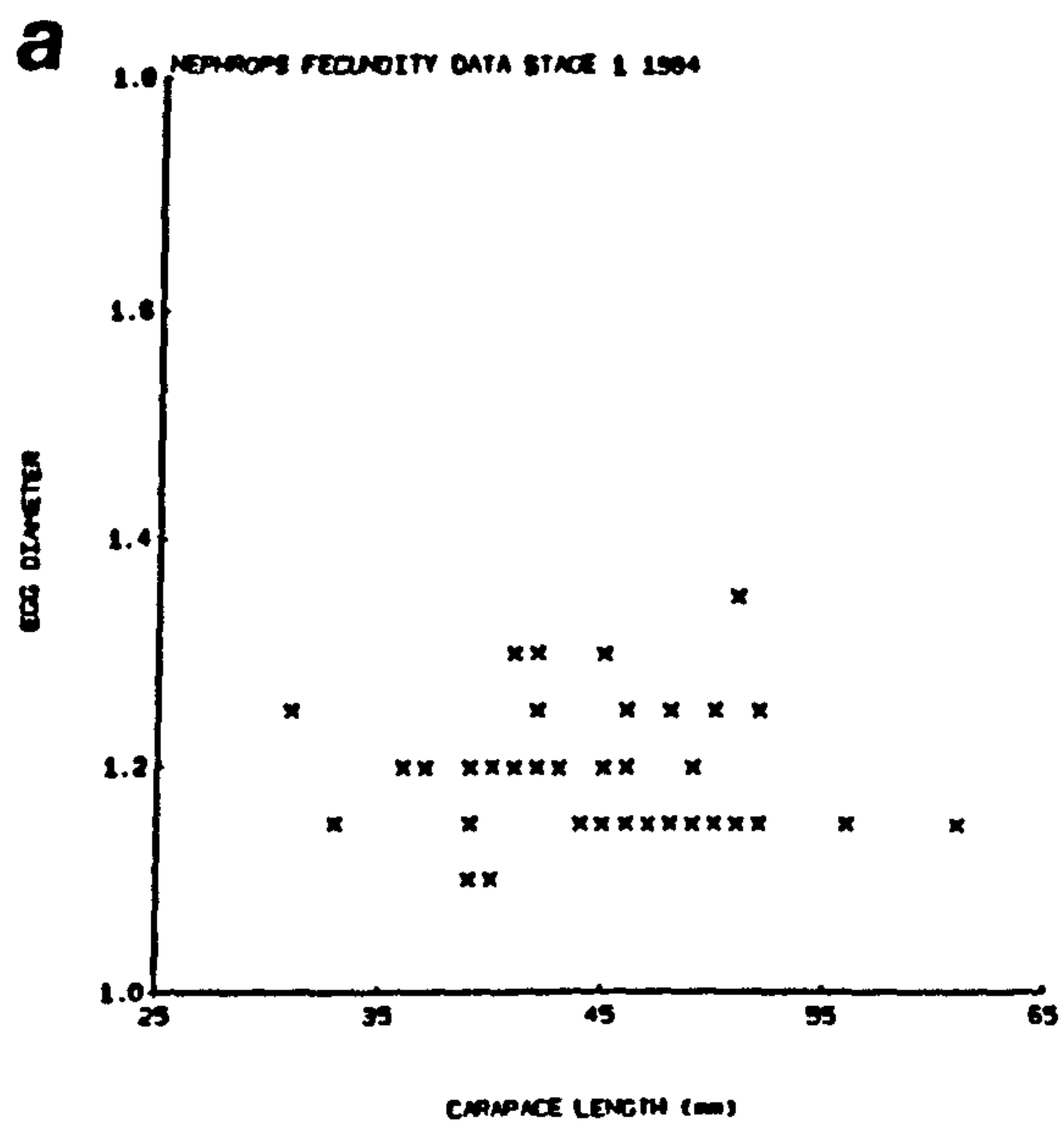
Scatter plots of egg diameter against carapace length of the female were made for each egg stage, some of which are shown in Figure 1.12 and 1.13. From the 1984 data only egg stage 7 produced a significant regression (see Table 1.8a) . In 1985, the results for egg stages 3,6,8 and 9 were not significant but those for the other egg stages were significantly correlated at the 5% level or better (Table 1.8b).

This difference between years was examined further by comparing the mean egg diameter for each egg stage in both years. The variability between samples was fairly consistent so a pooled estimate of variance (with over a thousand degrees of freedom) was used to compute the standard error of the difference between the two sample means. The difference divided by its standard error was then compared with a normal deviate. Except for stages 2 and 7 the mean egg diameter was always smaller in 1984 than in 1985 and the difference was statistically significant for stages 3, 6, 8 and 9.

**Figure 1.12**

**Scatter plots of egg diameter against carapace length of female for development stages (a) 1, (b) 3, (c) 5 and (d) 8 during 1984.**





**Figure 1.13**

Scatter plots of egg diameter against carapace length of female for development stages (a) 1, (b) 3, (c) 5 and (d) 8 during 1985.

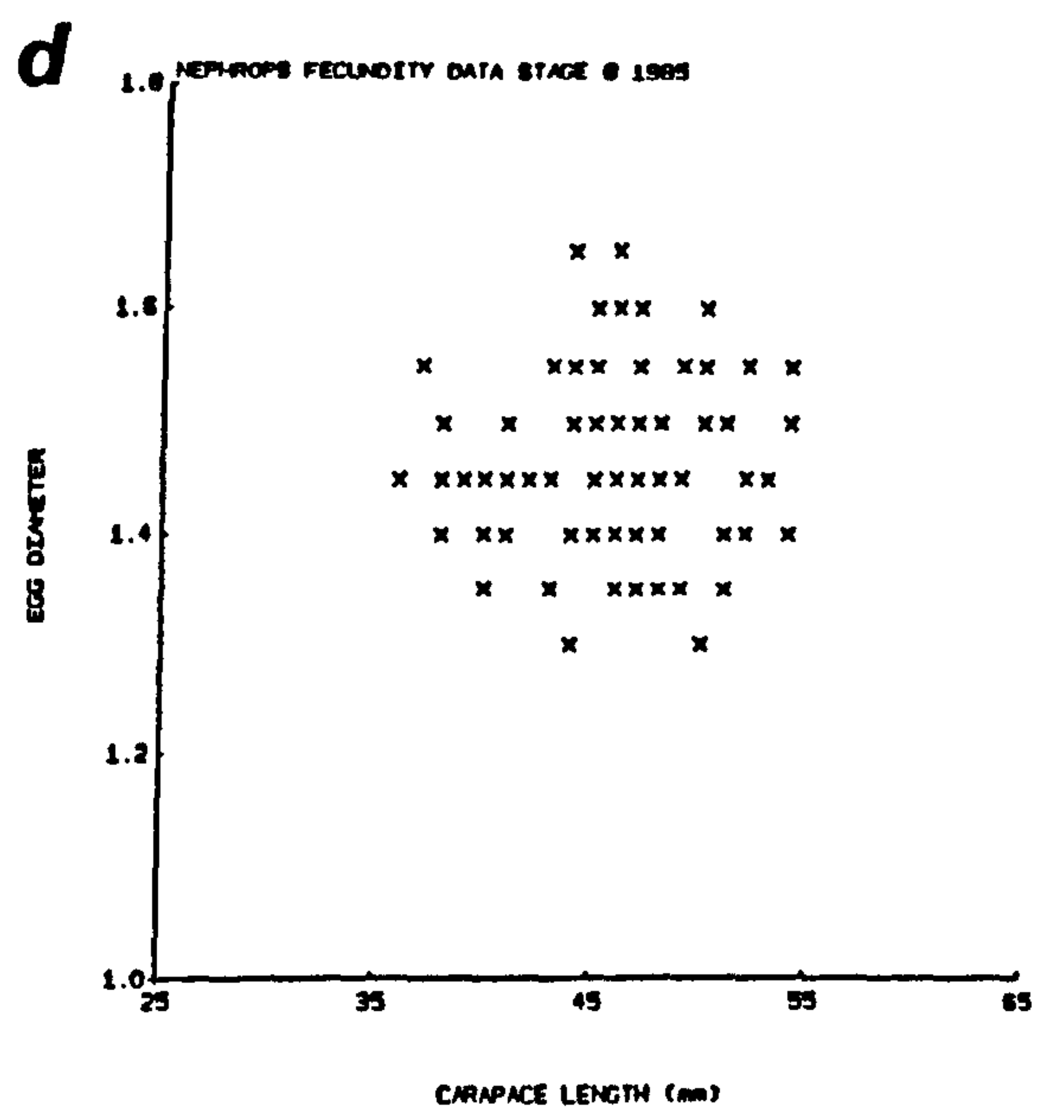
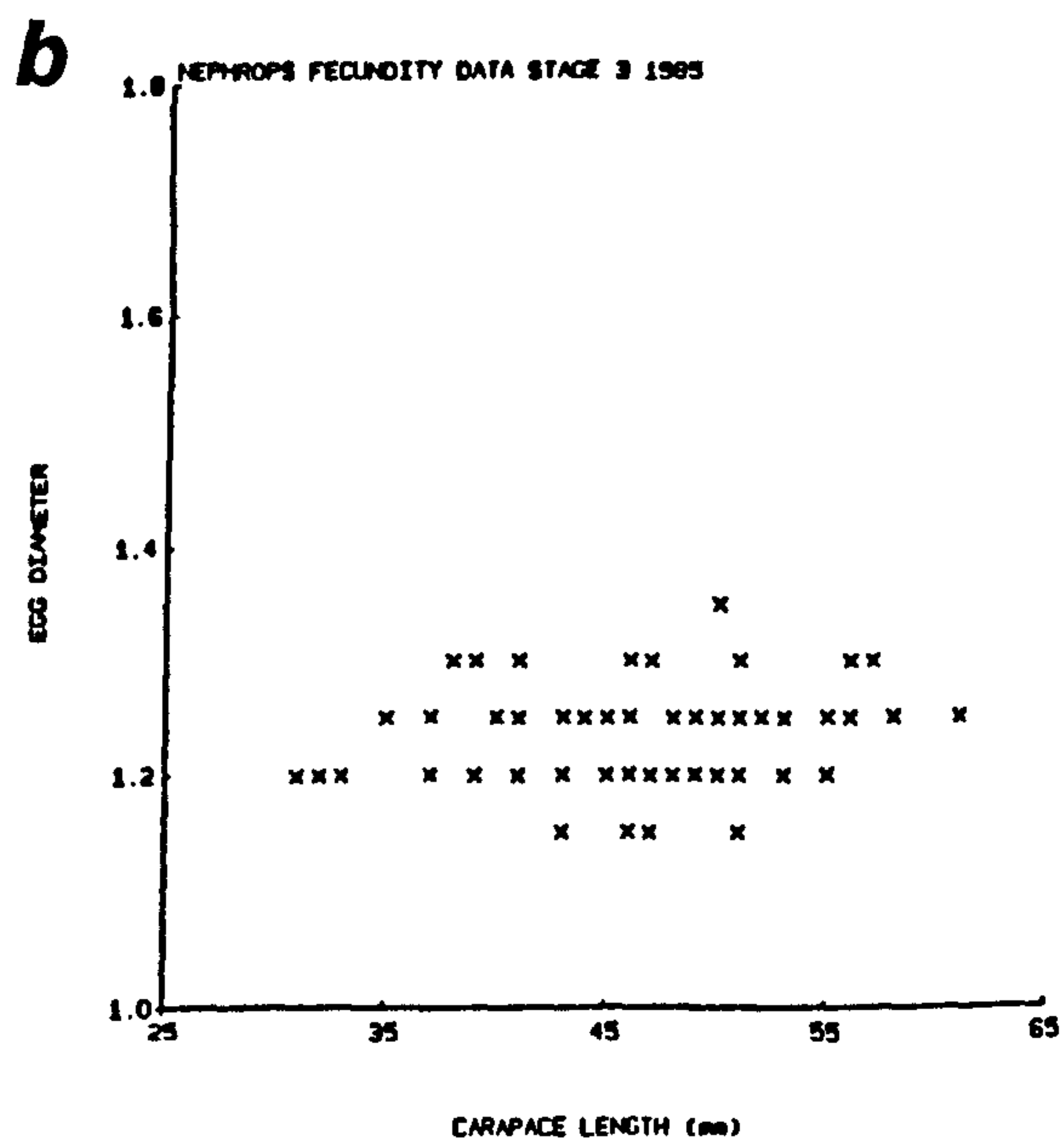
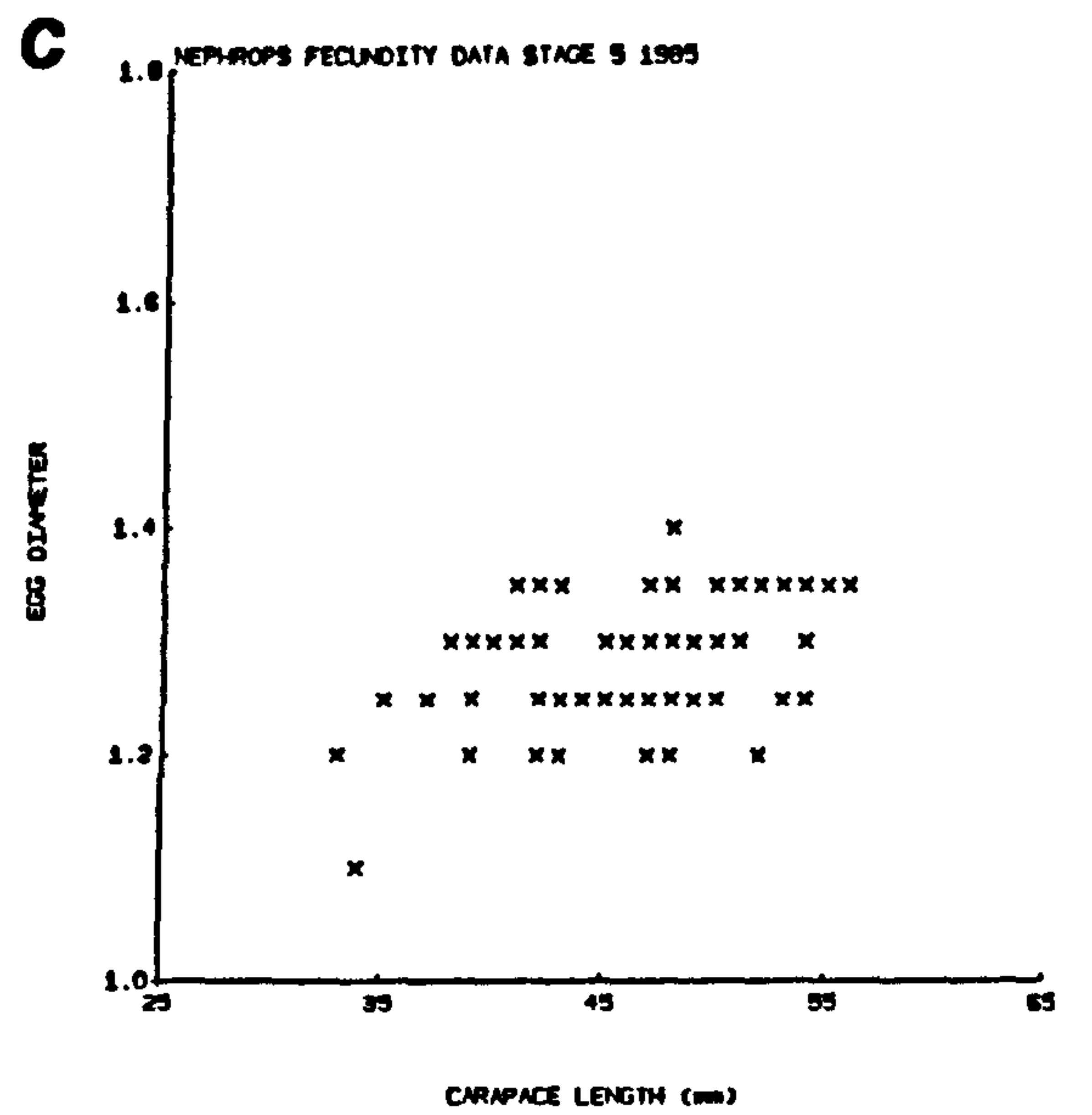
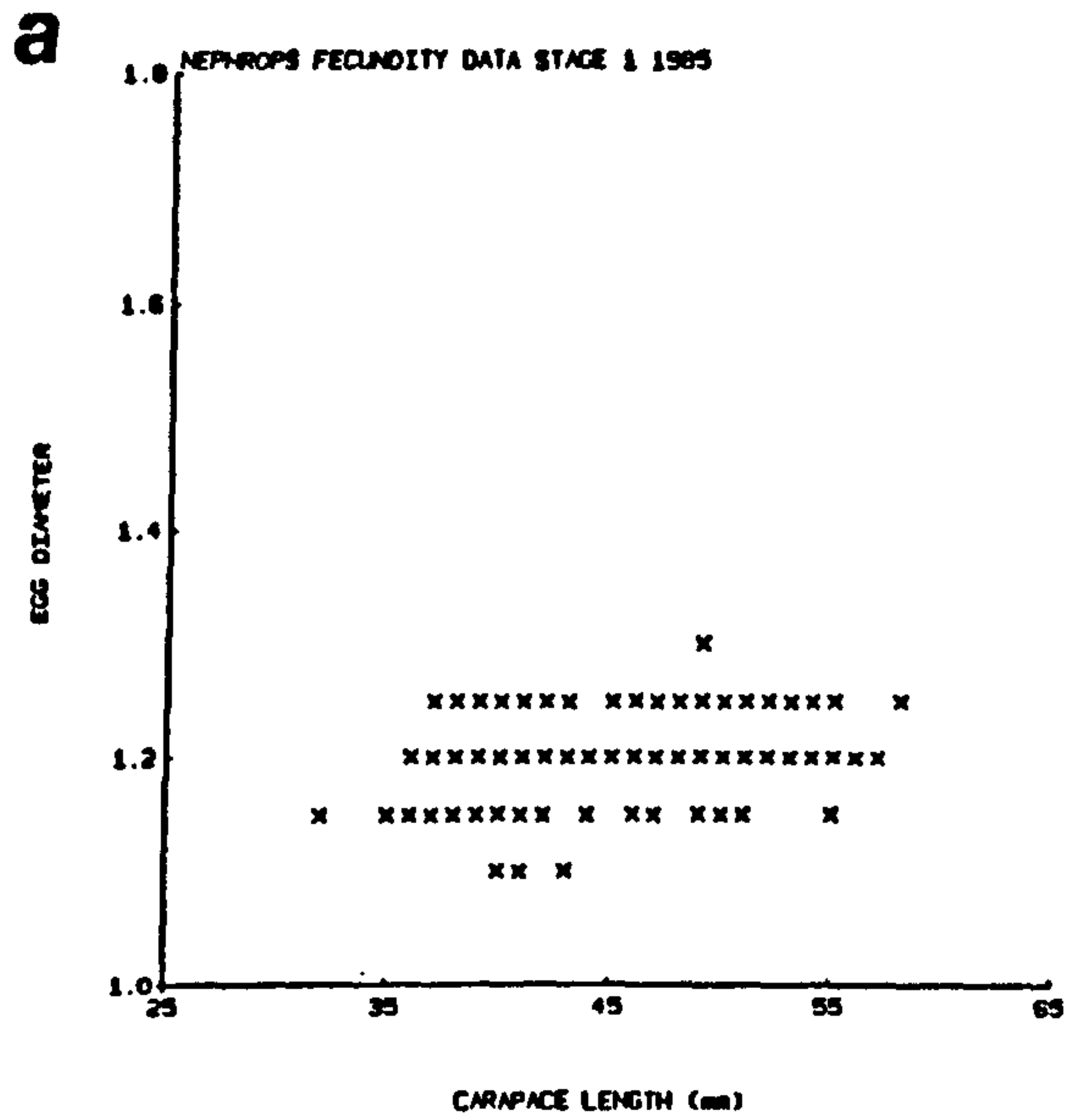


Table 1.8a

Mean egg diameter by egg stage for 1984 and the correlation between egg diameter and carapace length (+ see Figure 1.12).

Egg stage	Number of observations	Mean egg diameter Mean (mm)	S.D.	Correlation coefficient	Significance of regression (P)
1+	47	1.19	0.053	-0.089	n.s.
2	30	1.21	0.046	0.215	n.s.
3+	24	1.20	0.048	0.189	n.s.
4	26	1.24	0.055	0.250	n.s.
5+	32	1.27	0.046	-0.105	n.s.
6	37	1.30	0.068	0.147	n.s.
7	58	1.38	0.088	0.302	< 0.050
8+	30	1.42	0.085	0.235	n.s.
9	3	1.47	0.076	0.189	n.s.

n.s. = not significant

Table 1.8b

Mean egg diameter by egg stage for 1985 and the correlation coefficient between egg diameter and carapace length (+ see Figure 1.13).

Egg stage	Number of observations	Mean egg diameter Mean (mm)	S.D.	Correlation coefficient	Significance of regression (P)
1+	228	1.20	0.032	0.253	< 0.001
2	92	1.21	0.045	0.326	< 0.010
3+	78	1.23	0.042	0.170	n.s.
4	62	1.25	0.034	0.262	< 0.050
5+	101	1.28	0.047	0.353	< 0.001
6	78	1.33	0.052	0.152	n.s.
7	100	1.38	0.059	0.199	< 0.050
8+	89	1.47	0.077	- 0.012	n.s.
9	32	1.56	0.079	0.095	n.s.

n.s. = not significant.

## 1.4. Discussion

### 1.4.1. Fecundity study based on oocyte counts

Previous investigations of both the ovary (Thomas, 1964) and eggs on the pleopods (Chapman and Ballantyne, 1980) have indicated that fecundity is lower in Nephrops from the Firth of Clyde than in those from other Scottish waters. In this study fecundity appeared to be approximately 5% lower in an area of the Firth of Clyde compared to an area in the Sound of Jura.

The fecundity estimates obtained in this investigation are compared with those recorded by Thomas (1964) in Table 1.9. Within the size range of 30 - 40mm carapace length it was clear that the estimated fecundity of Clyde Nephrops in this study were considerably higher than previously recorded by Thomas (1964) in the Clyde and the Minch. The results from the fecundity study based on egg counts on the pleopods indicate that fecundity remains relatively constant between different spawning years. On the assumption, therefore, that Nephrops fecundity in the Firth of Clyde has remained unchanged since 1964, this suggests that there may be local variation in fecundity throughout the Clyde.

It has been suggested that Nephrops growth may be influenced by the composition of the sediment they inhabit (Chapman and Bailey, 1987). It is known that Nephrops can inhabit sediments in which the silt and clay component varies from 30% to almost 100% (Chapman and Bailey, 1987), sand comprising the remaining fraction (see Chapter 6).



**Table 1.9**  
**Fecundity of Nephrops based on the number of oocytes.**

Area	Equation	Estim. fecundity between CL30-40mm	Reference
Firth of Clyde	$F = 0.191L^{2.644}$	1537-3289 }	this study
Sound of Jura	$F = 0.201L^{2.644}$	1620-3467 }	
Firth of Clyde	$F = 0.353L^{2.423}$	1339-2689 }	Thomas (1964)
Minch	$F = 0.808L^{2.204}$	1455-2744 }	
Shetland	$F = 0.128L^{2.758}$	1517-3355 }	
Moray Firth	$F = 0.408L^{2.450}$	1697-3433 }	
Firth of Forth	$F = 0.803L^{2.269}$	1804-3466 }	

F = Fecundity.

Chapman and Bailey (1987) have indicated that faster growth appears to be associated with the finer mud sediments. The mud grounds which support Nephrops populations are often interspersed with sediment types which are unsuitable for burrowing (Chapman and Bailey, 1987). The results from tagging experiments (Chapman and Bailey, 1987) suggest that these regions of 'unsuitable' sediment form boundaries to the dispersal of Nephrops. As a consequence the Nephrops communities within each mud patch may be self-contained forming a separate 'stocklet'. This isolation, however, will probably not be maintained during the pelagic larval phase and there are unlikely to be any genetic differences between adjacent 'stocklets'. Although there is a wide range of mud types inhabited by Nephrops in the Firth of Clyde (Bailey *et al.*, 1986) there is little evidence of other types of sediment within the region and, with the exception of a few localised areas, the mud substratum forms an extensive unbroken continuum (B.G.S., 1985). Early investigations indicated considerable variation in Nephrops density within the Firth of Clyde (Barnes and Bagenal, 1951 ; Bagenal, 1952 ; Thomas, 1965), later confirmed by Bailey *et al.* (1986). Bailey *et al.* (1986) also observed variations in the size composition of Nephrops at different places within the Firth. There is therefore some evidence for the existence of separate 'stocklets' within the Firth of Clyde.

If the sediment does influence the biology of the resident Nephrops 'stocklet', which is as yet unproven, it could account for the different fecundities recorded within the Firth of Clyde and elsewhere. It has already been shown that in comparison with fine mud sediments, Nephrops of the same size supported on coarser sandy-mud sediments tend to be more fecund (Bailey *et al.* 1986). The underlying

reasons for the variations in fecundity remain unknown although these authors suggested that they could be explained by a difference in growth rates.

#### 1.4.2. Fecundity study based on egg counts on the pleopods

Progressive egg loss in ovigerous Nephrops during the incubation period was observed in this study and has been well documented in the literature (Farmer, 1975 ; Chapman and Ballantyne, 1980). The loss was mainly attributed to predation by Morizur (1981). In this present study, however, there was also a proportion of the population which was found to possess very few eggs even at the commencement of development. Egg loss in these individuals was not consistent with a steady loss incurred over the incubation period. It therefore appears that egg loss in these individuals had probably occurred at the time of spawning through failure of the eggs to adhere to the pleopods. These individuals were identified and eliminated from the estimation of egg loss during incubation.

In the present work, egg loss during the incubation period was approximately 18%. In other studies the reported egg loss incurred during the incubation period for Nephrops was considerably higher ; 75% in Portugese waters (Figueiredo and Nunes, 1965) ; 45 - 50% in the Bay of Biscay (Morizur, 1981 ; Morizur et al., 1981) ; 32 - 51% from the Moray Firth (Chapman and Ballantyne, 1980). As suggested by Chapman and Ballantyne ( 1980), the comparatively high egg loss recorded by Figueiredo and Nunes (1965) for Nephrops in Portugese waters (75%) can probably be partly attributed to greater levels of predation. However, the ovigerous Nephrops examined by Figueiredo and

Nunes (1965) were obtained from commercial trawl catches and had also probably suffered an additional egg loss caused by the fishing operations. Chapman and Ballantyne (1980) suggest that trawling causes 11 - 22% loss of eggs.

For reasons stated earlier, creels were used in preference to trawls for the capture of ovigerous females in the present study. Unfortunately creels tend not to capture small Nephrops which are therefore under-represented in the catches. As a result the relationships derived between carapace length and fecundity includes very few small individuals. This may explain why the fecundity estimate for Nephrops of carapace length 30mm based on stage 1 egg counts was slightly higher than the estimate from the oocyte counts. For a female of carapace length 45mm, the potential fecundity predicted from the ovary study (4480 eggs) fell between the 1984 (4415 eggs) and 1985 (4829 eggs) estimates based on recently spawned eggs.

#### 1.4.3. The reproductive cycle

The reproductive cycle of Nephrops in Scottish waters has been described by Thomas (1964) and a detailed study into various aspects of reproduction in Firth of Clyde Nephrops has already been conducted by Bailey (1984). In this study spawning appeared to occur in the late summer and early autumn, after which there was a reduction in the number of ovigerous females caught. This is indicative of the change in behaviour after spawning resulting in most females remaining within their burrows (Bailey et al., 1986). After a period of slow development during the winter months egg and ovary development proceed at a faster rate in the spring as water temperatures begin to rise.



During this period the ovigerous females begin to re-emerge from their burrows and hatching occurs in the late spring and early summer. Peak hatching in the Firth of Clyde occurs at the end of May (see Chapter 2).

Ovary development in Nephrops occurs in two phases, primary and secondary vitellogenesis (Bailey, 1984 ). Primary vitellogenesis is a slow phase and is described by ovary stages 1 - 7. Following stage 7 secondary vitellogenesis occurs. This involves a rapid increase in weight and is described by ovary stage 8. The present results indicate that ovary development in ovigerous females can only proceed as far as primary vitellogenesis. Similar observations were reported by Bailey (1984).

By July the hatching season appeared to be almost completed and most females were in the non-ovigerous condition with ovaries in the final stage of maturation. The dark green colour and increased size of the ovary (which results from secondary vitellogenesis) was clearly visible through the carapace. During this period the female moults and copulates before finally spawning again in the early autumn.

#### 1.4.4. Relationship between egg diameter and carapace length

During egg development there appeared to be a gradual increase in the mean diameter of the egg from approximately 1.20 to 1.55mm. These measurements agree quite closely with those of Figueiredo and Barraca (1963) who recorded 1.05 and 1.55mm for the minimum and maximum diameters respectively. The slightly higher minimum diameter recorded in this study may have been due to the fact that most eggs

representing the earliest stage of development (stage 1) had already attained the gastrula stage (see section 7.4.2.). A detailed account of the biochemical changes associated with egg development is presented in Chapter 7.

In an earlier investigation (Morizur and Rivoalen, 1982) reported a relationship between egg diameter and the size of the incubating female. They suggested that egg diameter was minimal for the smallest females and maximal for the middle sized females (33 - 37mm carapace length). As the middle sized individuals of Morizur and Rivoalen's study correspond to the smallest individuals of this present study it may have been expected that a linear regression with a negative sign to the slope may have adequately described the data. This was clearly not the case with both large and small females incubating eggs at either end of the size range (Figure 1.12 and 1.13). As previously mentioned, however, the smaller individuals were under-represented in this study. This may have prevented the identification of a more obvious relationship between these two variables.

Although there was a significant difference between the egg diameters recorded in 1984 and 1985 for egg stages 3, 6, 8 and 9 the actual size differences between the mean egg diameters for corresponding stages were very small (Table 1.8a,b). These differences may be partly attributable to the problems of using an arbitrarily chosen egg development scale and they are unlikely to have biological significance.



## CHAPTER 2

### Larval distribution and abundance in the Lower Firth of Clyde

#### 2.1. Introduction

Temporal and spatial changes in larval abundance were investigated and field information was obtained on the vertical distribution of the larvae throughout two 24h periods. The results from these studies enabled larval production estimates to be made which were later used to determine spawning stock size in an area known hereafter as the Lower Firth of Clyde (L.F.C.) (see Chapter 8).

The fossorial habit of the postlarval stages of Nephrops (see Farmer (1975) and Chapman (1980) for comprehensive reviews) makes direct sampling for purposes of stock assessment difficult if not impossible. Sampling the pelagic larval stages of Nephrops may therefore offer a convenient method for estimating the size of the adult stock. Although this technique has been widely used for stock estimates of fish species, based on the abundance of their planktonic eggs (Cushing, 1957 ; Southward, 1963 ; Lockwood et al., 1978 ; Southward and Barry, 1983 ; Thompson et al., 1984), it is only comparatively recently that the first attempts were made to apply it to Nephrops (Garrod and Harding, 1980 ; Nichols et al., 1987).

For Nephrops, the lack of information on the fecundity of the spawning stock and on the general ecology of the larval stages has presented serious problems to fisheries management. Work on Homarus

americanus (Scarratt, 1964, 1973) attempted to relate larval abundance to parent stock size, but the results were disappointing. As yet a stock-larval recruitment relationship has not been determined for any of the clawed lobster fisheries (Cobb and Wang, 1985). Greater success has been achieved for the Western Australian rock lobster (Panulirus cygnus) fishery. Morgan et al. (1982) succeeded in describing the relationship between the settlement of the peurulus larval stage and subsequent abundance of the breeding stock using the stock-recruitment model devised by Ricker (1958).

This present study has provided an estimation of larval production. The vertical distribution of the larvae in the water column was determined so that the mean temperature to which the larvae were exposed could be predicted. This was then used in conjunction with the development rates determined in the culture study (Chapter 3) to estimate daily larval production and total larval production over the season. This together with maturity and fecundity data (Chapter 1) is used in Chapter 8 to provide an estimate of spawning stock size.

Although diurnal vertical migration patterns have been well documented for a large number of zooplanktonic organisms (holoplankters and meroplankters) there is considerable variation not only in the extent of the distances travelled (Hopkins and Evans, 1979) but also in the factors which control vertical migration (Naylor and Atkinson, 1972). It was originally considered that these migration patterns were exclusively controlled by light intensity (Russell, 1927). More recent investigations have shown them to be more complex, often involving both an exogenous and endogenous component. Although pressure sensitivity has been shown to be a depth regulatory mechanism

in a variety of marine organisms (see Chapter 5) there is also evidence for its involvement in synchronizing vertical migration cycles (Mauchline and Fisher, 1969). There is also increasing evidence that other exogenous factors may be involved in vertical migration patterns. Williams (1985) reported that the seasonal formation of the thermocline in the Celtic Sea restricted the diurnal vertical migrations of Calanus helgolandicus and Calanus finmarchicus to the regions above and below the pycnocline (density discontinuity resulting from the stratification of the water column through formation of a thermocline and/or halocline).

The typical diel vertical migration pattern involves a series of consecutive steps, a dusk ascent from the day-time depth, night-time dispersion, regathering at the surface at around dawn and a final retreat to the original day-time depth with increasing light intensity (Cushing, 1951). Limited vertical migrations have previously been described for Nephrops zoeae (Fraser, 1965 ; Hillis, 1974). According to Hillis (1974) greatest larval abundance occurs at depths between 19 - 28m during the day and ascends by approximately 10m around dusk, this is followed by a retreat to their original day-time depth shortly after dawn. Unfortunately, the environmental conditions were not closely monitored during his survey and only photometric light intensity (lux) measurements were available. In this present investigation the vertical distribution of Nephrops zoeae was determined over 24 hours during both a spring and neap tidal period and related to the several environmental variables, light, pressure, temperature, salinity and tidal state. The results from this study were then compared with those obtained under controlled laboratory conditions (Chapter 5). This permitted a better understanding of the

exogenous factors which may govern the vertical distribution and modulate the limited vertical migratory behaviour of Nephrops zoeae.



## 2.2. Materials and methods

The investigation was carried out in 2 phases using the University Marine Biological Station's research vessels 'Aplysia' and 'Aora'.

(1) a general survey to determine temporal and spatial changes in larval abundance in the L.F.C. (Figure 2.1) using oblique tows.

(2) vertical distribution throughout the 24h cycle was studied using a series of horizontal tows at a station where large numbers of Nephrops zoeae had been caught in phase (1).

### 2.2.1. Temporal and spatial changes in abundance

Between April and September 1984 a series of five plankton surveys were conducted in the L.F.C. (Figure 2.1). The survey covered an area of approximately 876 square kilometres and extended from the Little Cumbrae down the eastern coast of Arran and across to the Ayrshire coast. 39 sample stations were completed for each survey. Each was at the intersect of grid co-ordinates spaced 5km apart (Figure 2.1). A weekly sampling programme was conducted at stations A1, A2, A3, B1, B2 and B3 (Figure 2.1) before the first survey (20 - 22/4/84) and after the fifth survey (22 - 23/8/84) to determine the beginning and end of the larval season.

A framework was designed and built to house four DAFS WP3 type plankton nets of 0.57m mouth diameter (Plate 2.1a). The nets were made

## **Figure 2.1**

**Sampling grid for the five general surveys conducted to determine temporal and spatial changes in larval abundance in the Lower Firth of Clyde.**



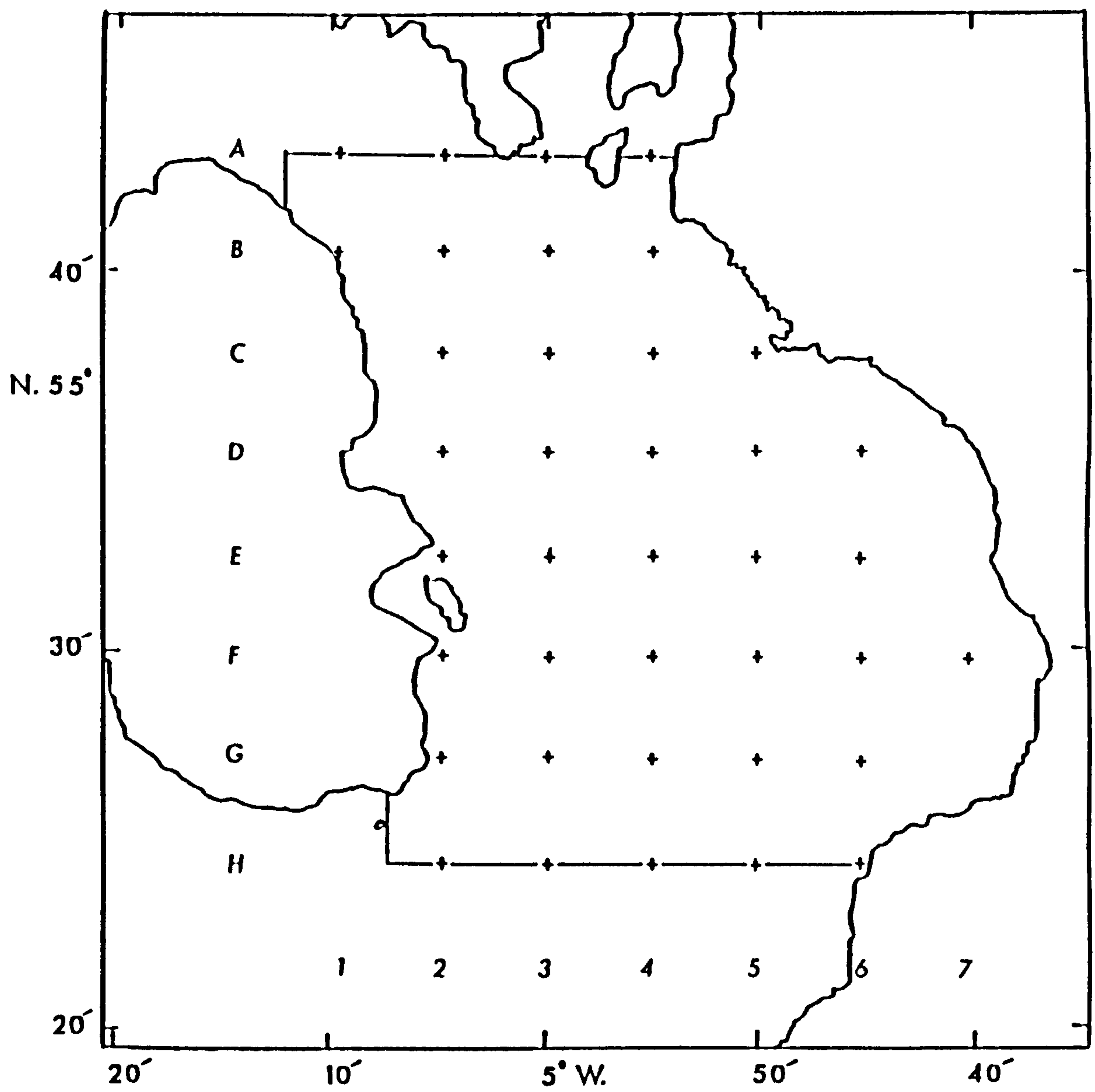
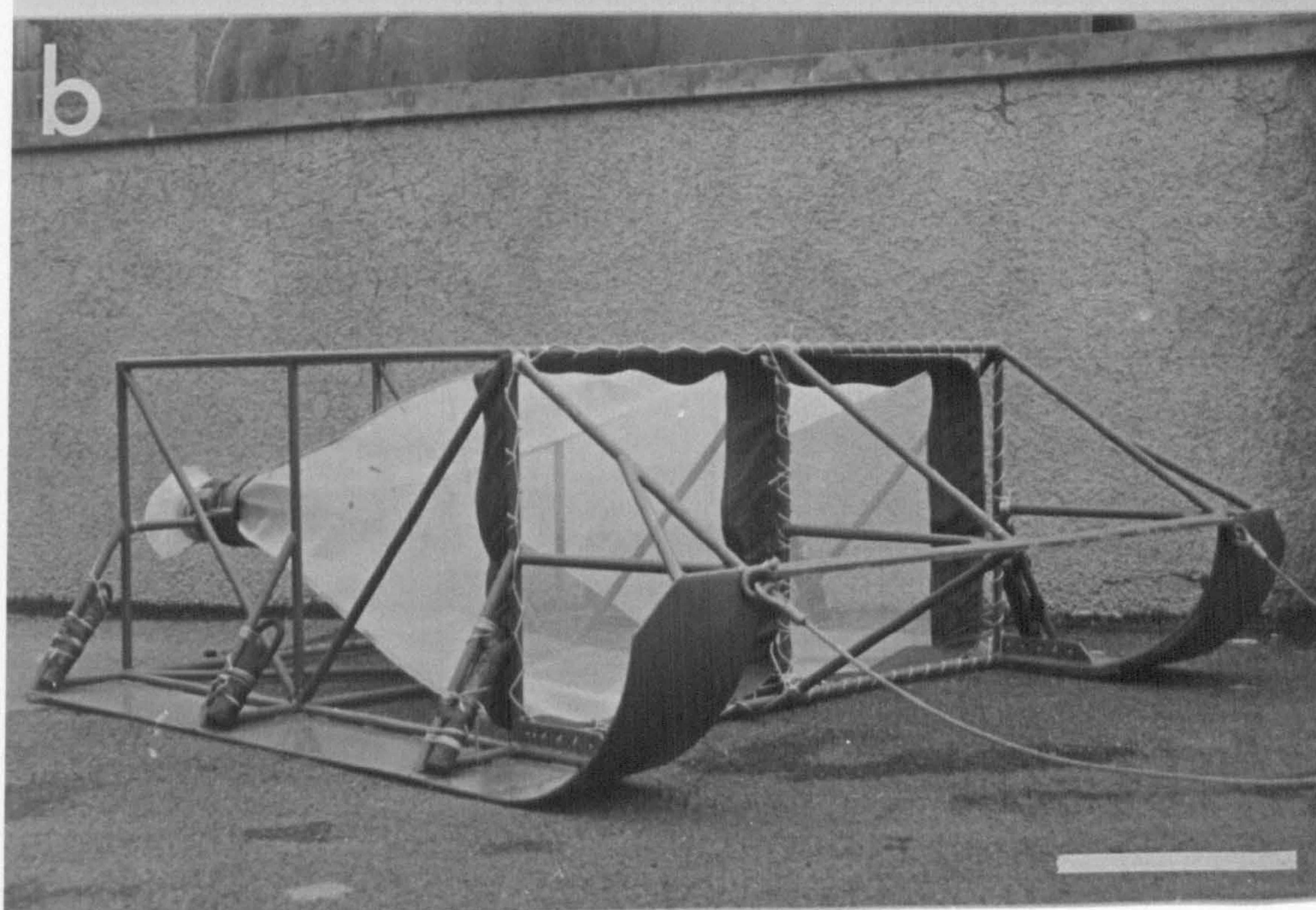
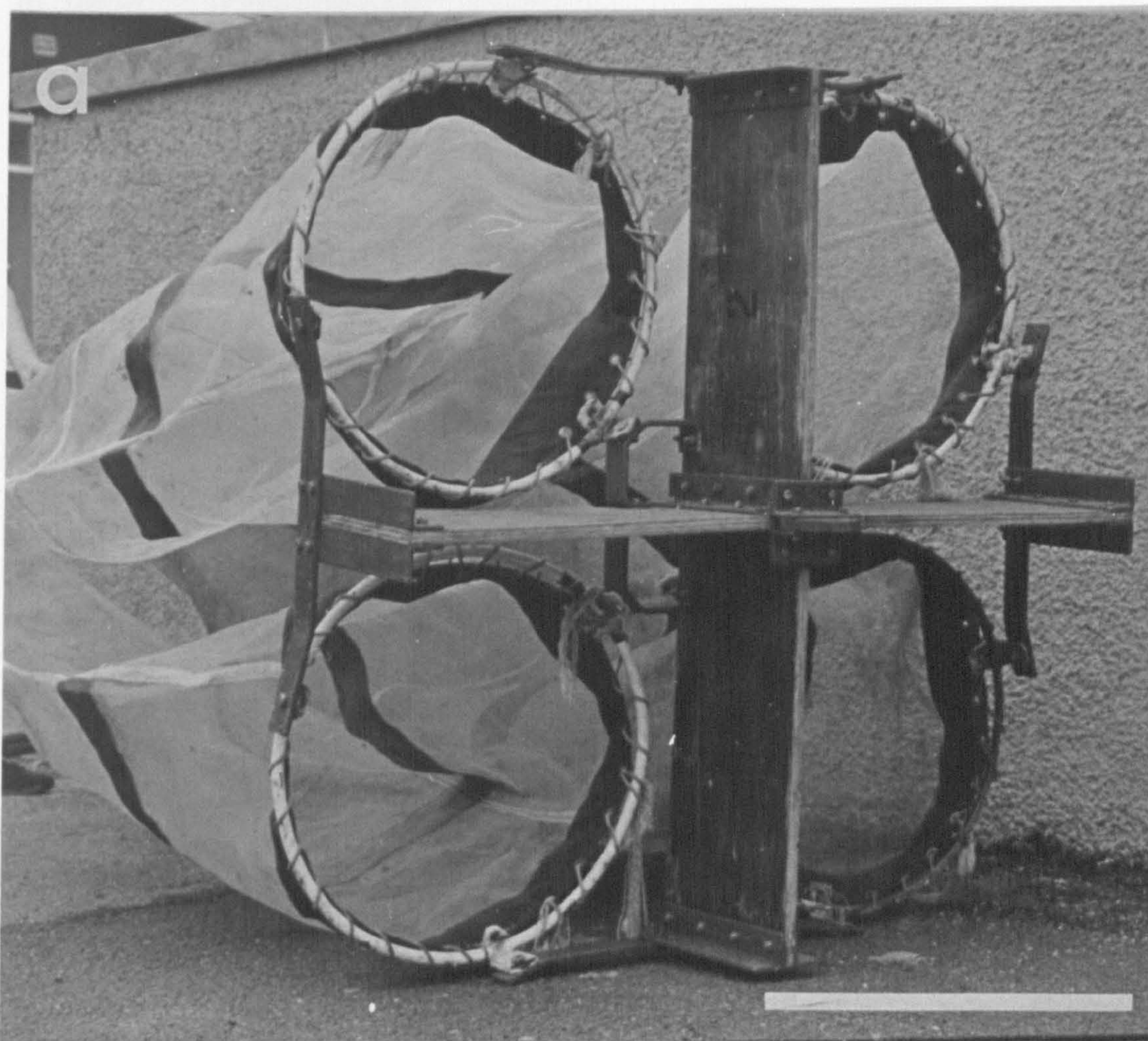


Plate 2.1

(a) The plankton sampler used to determine temporal and spatial changes in larval abundance. Scale bar represents 0.5m.

(b) The benthic sledge used to sample the settling stages of Nephrops. Scale bar represents 0.5m.







of monofilament Nylon mesh with a pore diameter of 1.0mm. The volume of water filtered during each tow was calculated using a TSK flowmeter which was fitted to the mouth of one of the four nets. Using the research vessel's echo sounder to estimate water depth the plankton sampler was shot close to the sea bed. The gear was then hauled to the sea surface on an oblique path at 1.5 - 2.0 knots ( $0.78 - 1.04\text{ms}^{-1}$ ). The duration of each tow was approximately 20min, although this was obviously dependent upon water depth. A recording depth meter (OSK 896, Yanagi Instrument Co. Ltd) was secured to the plankton sampler on each tow to provide an accurate depth profile of each haul. After each tow the plankton samples were fixed in 4% buffered formalin and returned to the laboratory for sorting.

Under low power stereomicroscopic examination the three zoeal stages were separated and counted. A total count for each tow was obtained by adding the catch from the four nets. The flow meter and depth recordings were then used to estimate the number of zoeae under one square metre of sea surface for each stage separately and all stages combined (see appendix, Table A.1).

During the larval season, temperature and salinity depth profiles were obtained using a salinity temperature bridge (Electronic Switch Gear Ltd M.C.5). All measurements were taken from the same area (Figure 2.1 sample station B3 :  $55^{\circ}40.71'\text{N}$  ;  $05^{\circ}00.00'\text{W}$ , the site later selected for the diel surveys). The larval depth distribution (section 2.2.2.) was used in conjunction with the temperature profile to calculate the average temperature appropriate to the larval distribution in each survey.

In a similar study conducted for the Western Irish Sea Nichols et al. (1987) used a high speed plankton sampler to capture Nephrops zoeae. A series of comparative tows were therefore made with a high speed Gulf 3 plankton sampler as a check on the efficiency of the sampling gear utilized in this programme. The net was made of monofilament nylon with an internal mesh size of 0.25mm. The catching efficiency of both plankton samplers appeared to be similar.

A preliminary investigation to determine the level of recruitment of the settling stages to the benthos was carried out using a specially designed benthic sledge (Plate 2.1b). The results were not very promising and it was decided not to pursue this investigation any further.

#### 2.2.2. Diel changes in vertical distribution

As large numbers of larvae had been caught at station B3 during phase (1) (Figure 2.1), phase (2) of the study was conducted at this site. Two diel surveys were conducted, one coinciding with spring tides (4 - 5 June, 1985) and the other with neap tides (11 - 12 June, 1985).

During each survey the water column was sampled at eight depths (0,5,16,27,38,50,63 and 75m) using DAFS WP3 and WP2 plankton nets. The tows were conducted across the tide (to reduce the effect of tidal currents) on a southerly course at 2 hour intervals and each was of 20min duration. The nets were made of monofilament Nylon mesh with a pore diameter of 1.0mm. The surface and bottom nets had a mouth opening of 0.57m diameter (WP3) and those sampling the intermediate

depths were of 1.14m diameter (WP2). Apart from the surface net which was attached to a separate warp and towed from the port side to avoid the wash of the ship, the other six nets were attached to a single warp and towed from the stern. The end of the warp was shackled to a heavily weighted beam trawl frame which ensured that contact was made with the sea bed during each tow.

From a preliminary investigation of the towing wire configuration it was determined that the nets should be fixed at intervals of 32m along the warp to sample the required depths. At these positions snoods were attached to the warp to enable the plankton nets to be quickly snapped on and off during shooting and hauling using 'carabena' clips. On the initial tows of both surveys the depth at which each net was sampling was checked using Kelvin Tubes. On all tows a recording depth meter (OSK 896, Yanagi Instrument Co. Ltd) was fixed to the warp several metres above the beam trawl providing a profile of each tow. This therefore permitted comparisons to be made between the different tows. Figure 2.12 shows a typical depth trace made during one of the tows. The stepwise descent and ascent at either end of the trace illustrates the delay caused by handling each net during shooting and hauling.

During each tow 2 TSK flow meters were used to monitor the volume of water filtered by the plankton nets sampling at a depth of 5m and 63m. The difference between the two values was therefore contributed by water filtered during shooting and hauling. This value was always relatively small but a check was nevertheless made to ensure that the bulk of the catches of each net were made at the final towing depth and not at intermediate depths during shooting and hauling. This check



was made on two occasions by shooting the nets in the usual way and then immediately hauling them to the surface. On both occasions very few larvae were caught so that no corrections were applied to the catch data to compensate for this possible source of error. For each tow the volume of water filtered by each of the 1.14m diameter nets was therefore assumed to be the same as that filtered by the net sampling at 5m depth. The 2 smaller nets (0.57m) filtered approximately a quarter of this volume. So that direct comparisons could be made between tows the numbers of larvae caught by each net were adjusted to a standard volume of 850m<sup>3</sup> of sea-water filtered. The samples were fixed in 4% buffered formalin and returned to the laboratory for future sorting.

Immediately after each tow, light measurements at depth intervals of 5m were made using a photomultiplier tube (EMI 940B) (Figure 2.17 and 2.24). The spectral sensitivity of the instrument extends approximately between 300nm to 620nm with a maximum at around 400nm. In photobiological studies light intensity should be measured as radiant power (Arnold, 1976a). The photomultiplier was consequently re-calibrated using standard lamps from photometric units (lux) into quantum units ( $\mu\text{Es}^{-1}\text{m}^{-2}$ ) at the Lowestoft Fisheries Laboratory using the method described by Arnold (1976b). This calibration was conducted with a Wratten 64 filter fitted to the photomultiplier tube. This filter was considered to most closely represent the spectral sensitivity of the adult Nephrops eye (see Chapter 5) but may not be appropriate to the larval eye. Spectral sensitivity curves are not available for the larval stages at present. Several days before the first survey (30.5.85) a depth profile for light attenuation was measured at the sample station (B3). A Licor quantum light meter (LI -



185B) and underwater sensor (LI - 192SB) with a spectral response in the wavelength band 400 - 700nm was used. The attenuation coefficient  $K$  was calculated. The diffuse attenuation coefficient  $K$  is usually measured using monochromatic light. Sea-water acts as a filter and at depths below 10 - 15m light in coastal waters is monochromatic green (Jerlov, 1968). The attenuation coefficient was therefore measured over the depth range 20 - 30m.

At intervals of 4h during both the spring and neap tide surveys depth profiles for temperature and salinity were obtained to determine the position of the thermocline and halocline over both 24h periods. During both surveys the tidal height was recorded on an Ott manometer guage at Keppel Pier, Millport (Figure 2.13).

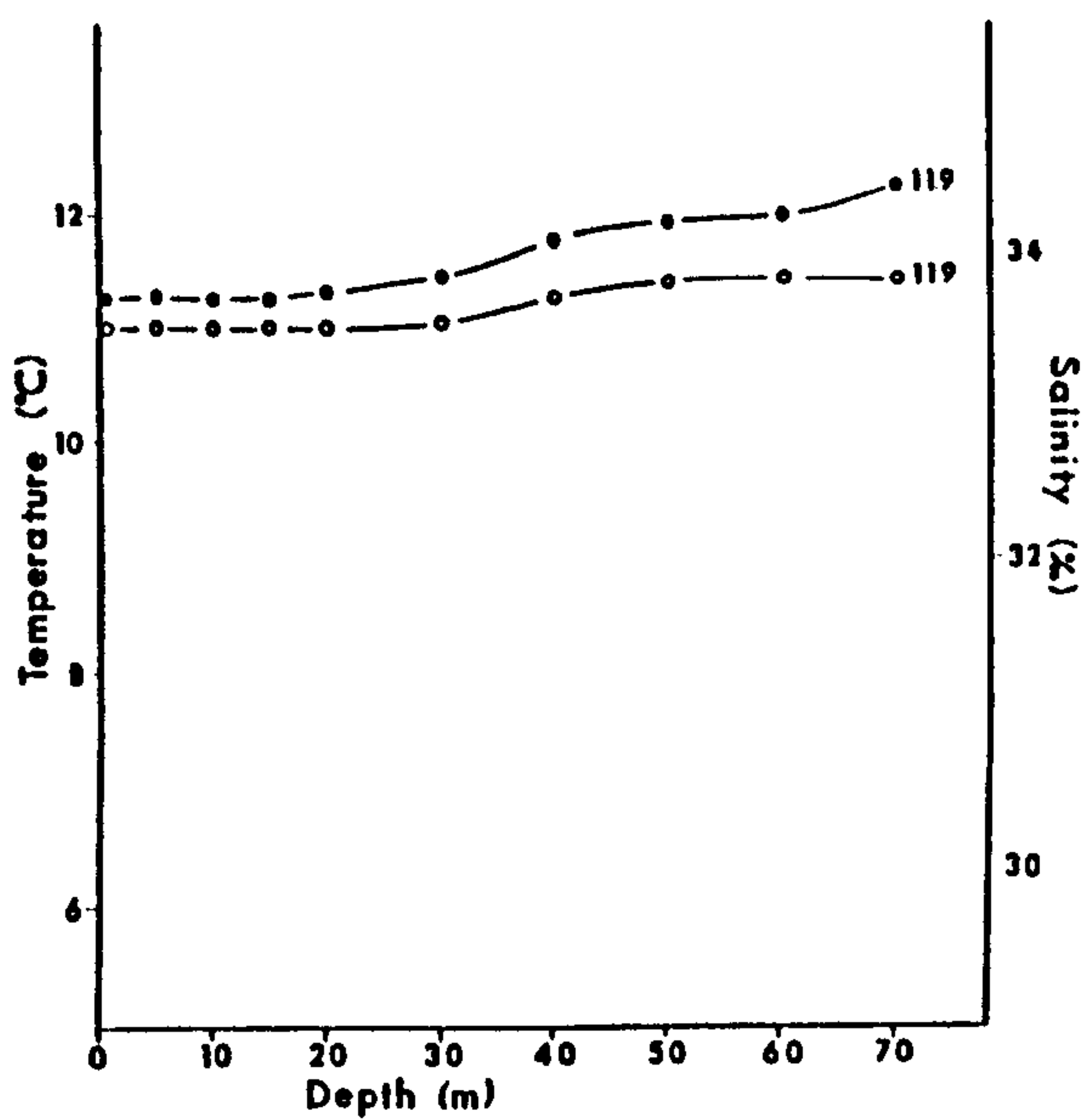
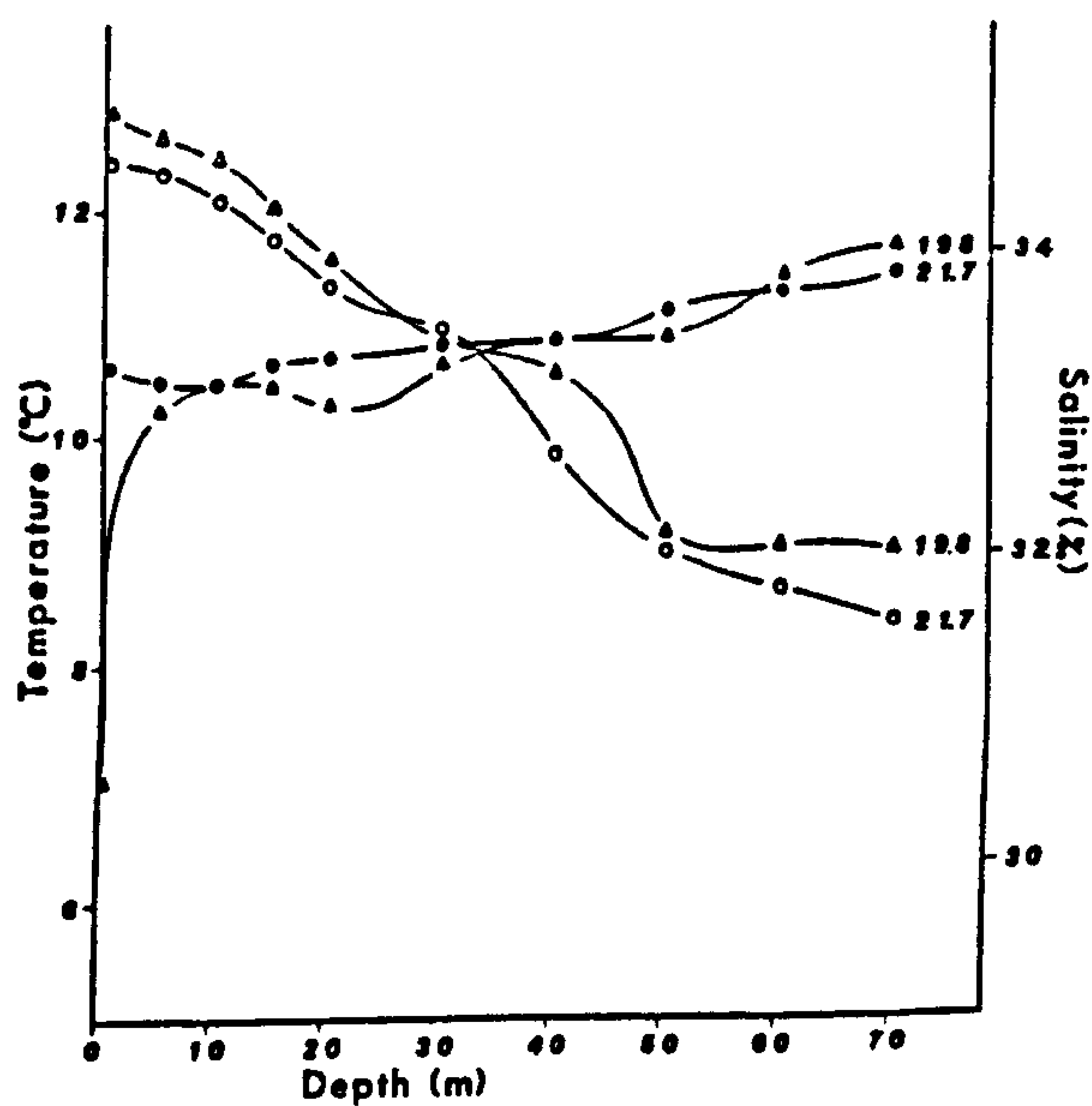
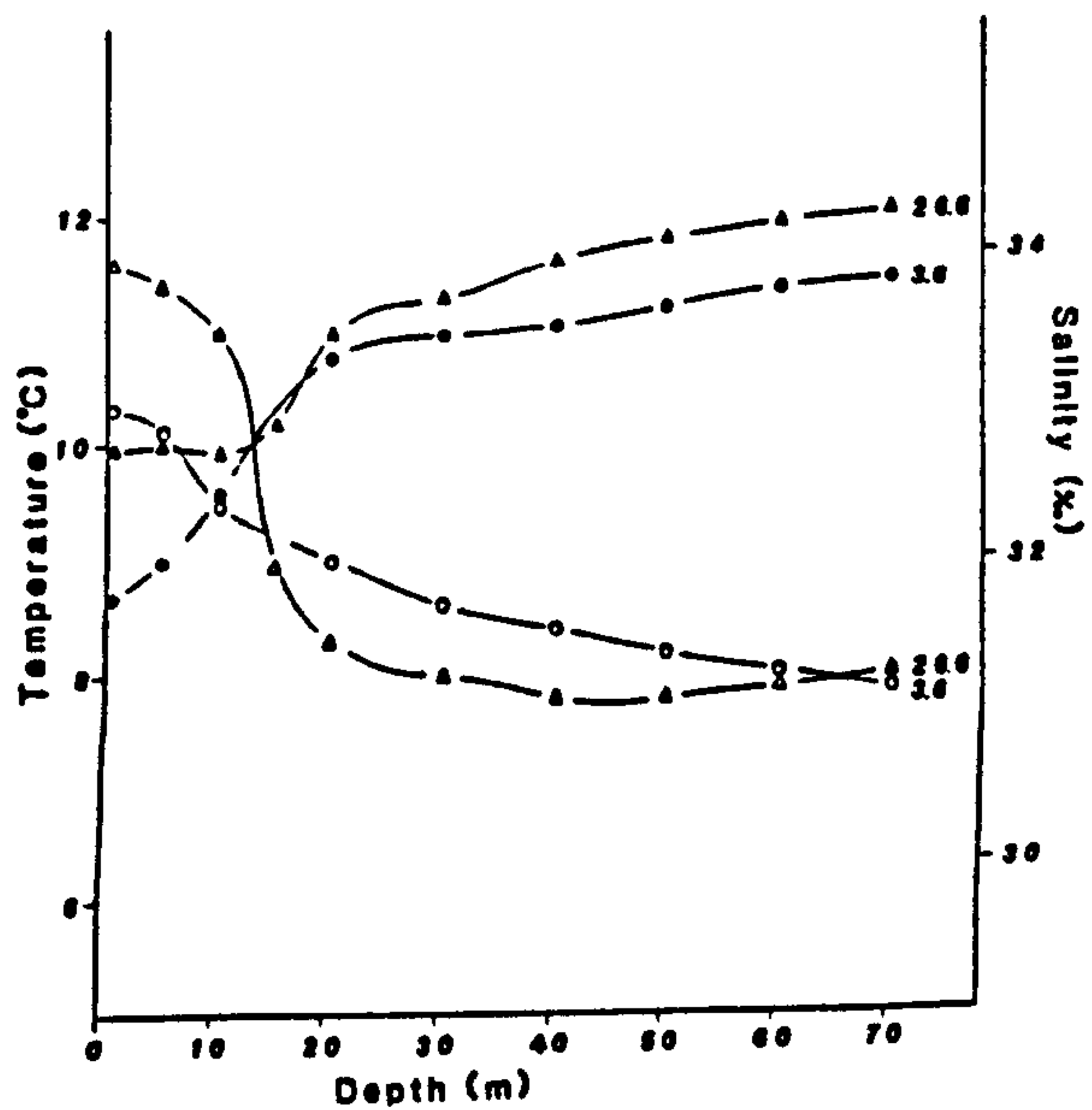
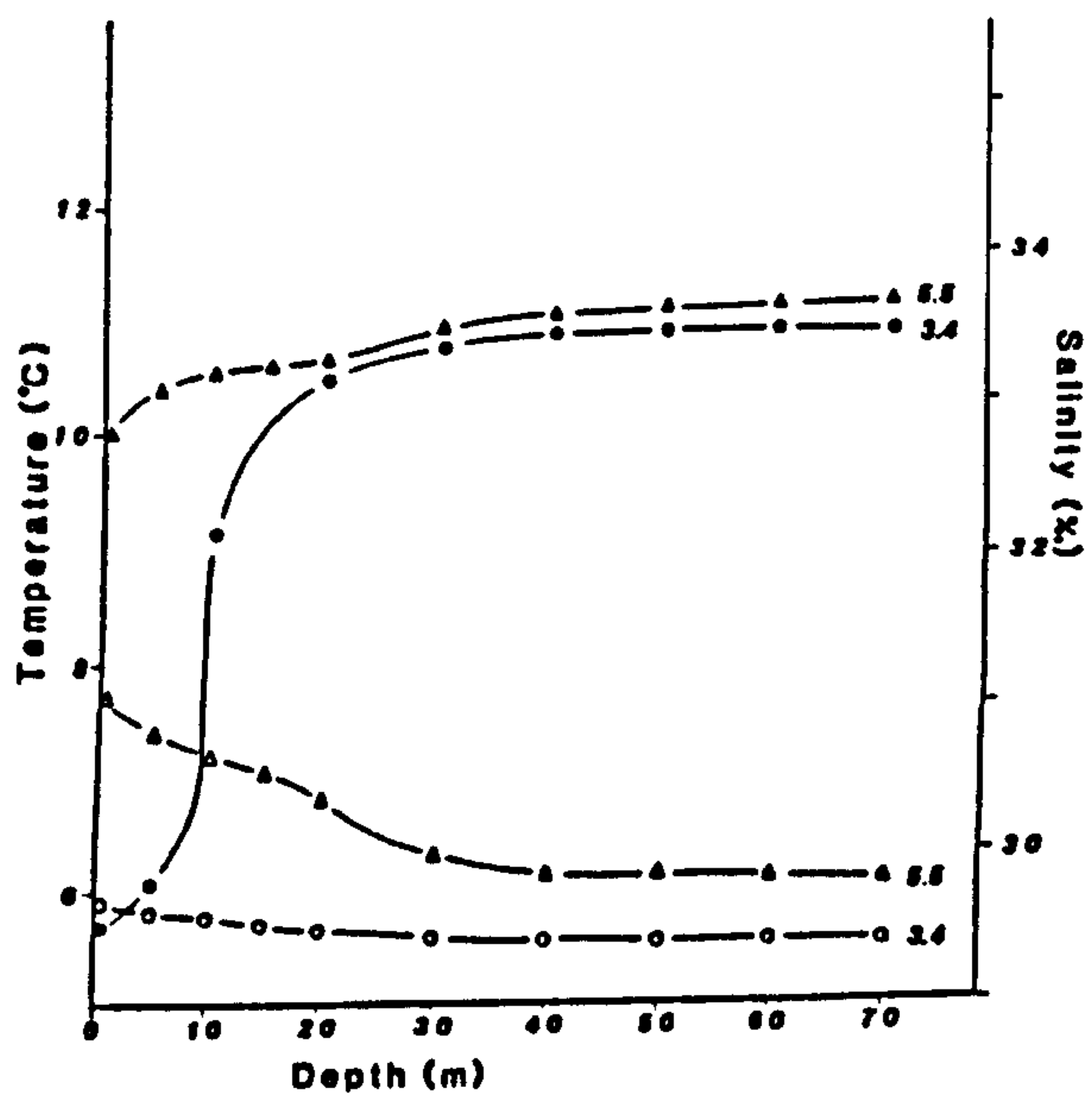
## 2.3. Results

### 2.3.1. Temporal and spatial changes in abundance

The seasonal changes in temperature and salinity depth profiles are illustrated in Figure 2.2 and 2.3. At the beginning of the larval season (early April), although a halocline had formed a thermocline had not and water temperature was relatively uniform (approximately 6°C) throughout depth. From April onwards there was a gradual increase in water temperature with some evidence of a thermocline between 5 and 20m depth (Figure 2.3). Below 20m there was a steady decrease in temperature with depth. At the beginning of June the surface temperature was 10.2°C. This was 2°C warmer than that recorded at a depth of 50m. By the end of June the thermocline had become more clearly defined between 10 and 15m and, as expected, the halocline occurred at approximately the same depth as the thermocline. Thus the water had become stratified into 2 distinct layers ; an upper warm low salinity layer (32.7‰ and 11.6°C at the surface) above a colder more saline layer (34.1‰ and 7.8°C at 50m). During the period 3rd to 26th June there was an increase of approximately 1.4°C in the temperature of the surface waters but a decrease of 0.5°C at a depth of 50m. During the month of July the position of the thermocline appeared to sink and towards the end of the month the warmer surface layer extended to a depth of approximately 30m ; a temperature increase of 3°C was recorded at 20m depth over the period 26 June to 21 July. Over the same period the temperature of the surface water had increased by 0.8°C to 12.4°C and at 50m by 1.2°C to 9.0°C.

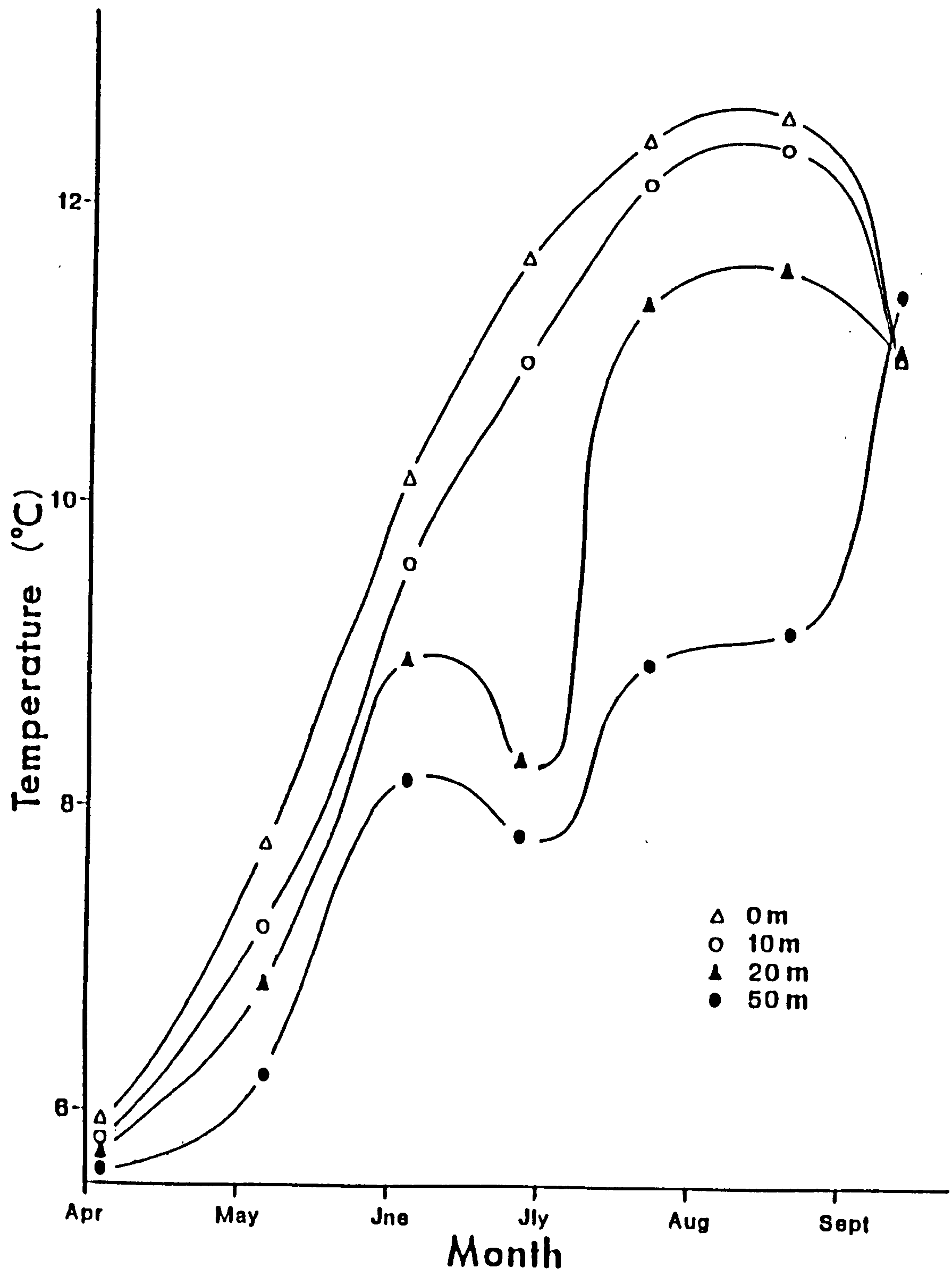
## Figure 2.2

The configuration of the thermocline (open symbols) and halocline (closed symbols) at different times during the larval season. The numbers assigned to each curve denote the date the measurements were taken.



### Figure 2.3

Seasonal changes in temperature at four selected depths 0, 10, 20 and 50m. These curves were constructed from the data shown in Figure 2.2.



From July 21 to August 19 the thermocline continued to sink and by August 19 the warmer surface layer extended to a depth of 40m. There was only a slight increase in the surface water temperature over this period ( $0.3^{\circ}\text{C}$ ) and the seasonal maximum of approximately  $12.8^{\circ}\text{C}$  appears to have occurred at the beginning of August (Figure 2.3). Between August 19 and September 11 the pycnocline was broken up and the water column became relatively uniform throughout. There was a slight increase in temperature with depth ( $0.4^{\circ}\text{C}$ ). This mixing of the water column and the consequential inversion of the temperature profile was probably caused by autumn gales. The surface temperature dropped over this period by  $1.7^{\circ}\text{C}$  to  $11.0^{\circ}\text{C}$  and at 50m it increased by  $2.2^{\circ}\text{C}$  to  $11.4^{\circ}\text{C}$ . During the larval season the temperature at the surface of the water column increased from  $5.9$  to  $12.8^{\circ}\text{C}$  and the bottom temperature (at approximately 75m for this station) rose from  $5.6$  to  $11.4^{\circ}\text{C}$ .

For each survey the numbers of larvae per square metre (ie. beneath a square metre of surface) were plotted and contoured for the 3 zoeal stages and as a cumulative total for all 3 stages (Figures 2.4 - 2.9). After consultation with the Department of Geography at Glasgow University it was considered that linear interpolation between the survey data points, carried out by hand, would provide an adequate method of mapping contour lines. The areas between adjacent contours were then calculated using a computer programme at Glasgow University. The calculated areas multiplied by the arithmetic mean of the observed values within them gave an estimation of the total abundance for each contour level. For contoured areas containing one or no observations a mean of the higher and lower contours was used. The abundance values within each contour were then summed to determine total abundance for



Figure 2.4

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde between 20 - 22.4.84 for (a) 1st stage zoeae, (b) 2nd stage zoeae, (c) 3rd stage zoeae and (d) all 3 zoeal stages combined. Scale bar represents 5km.

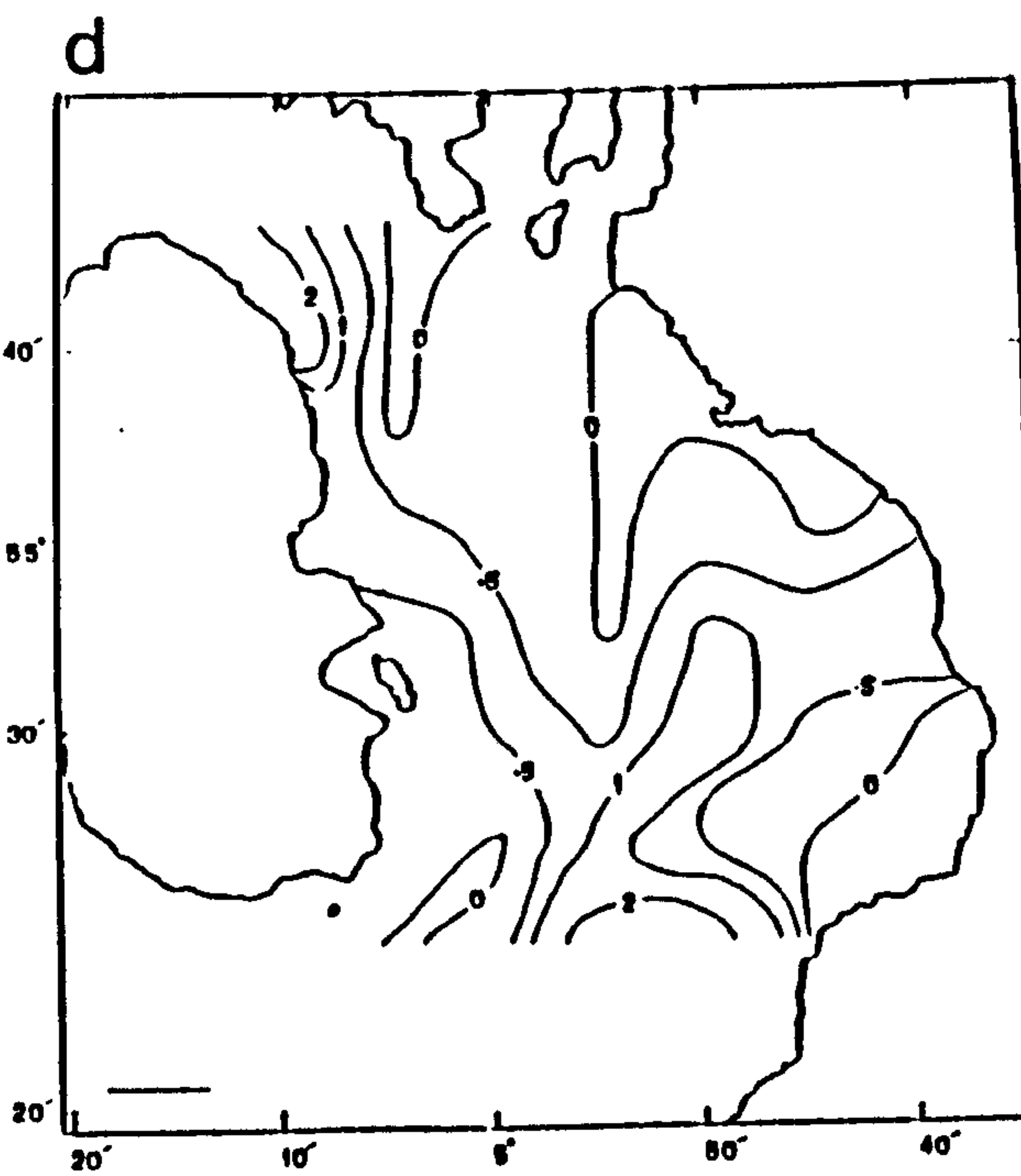
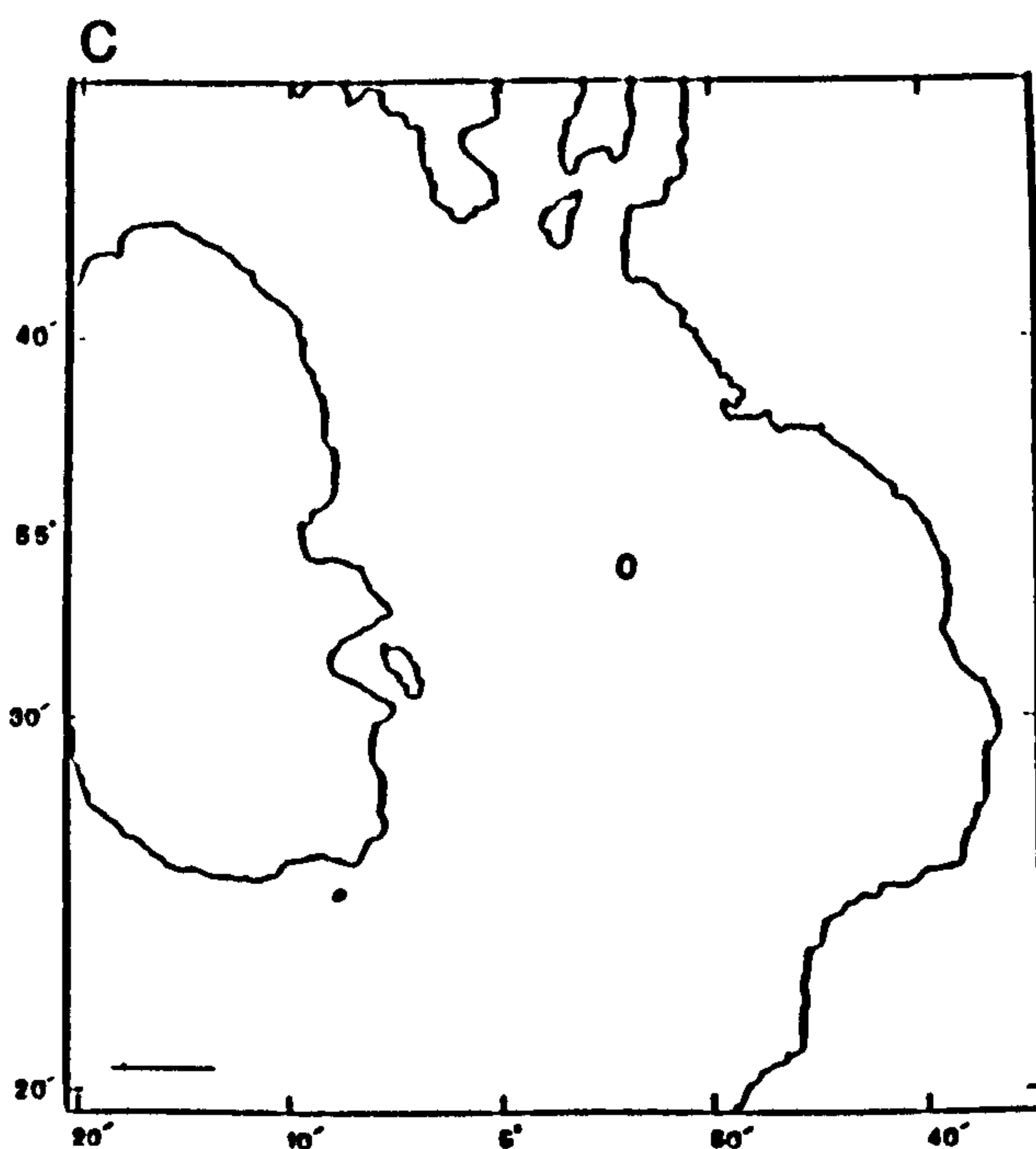
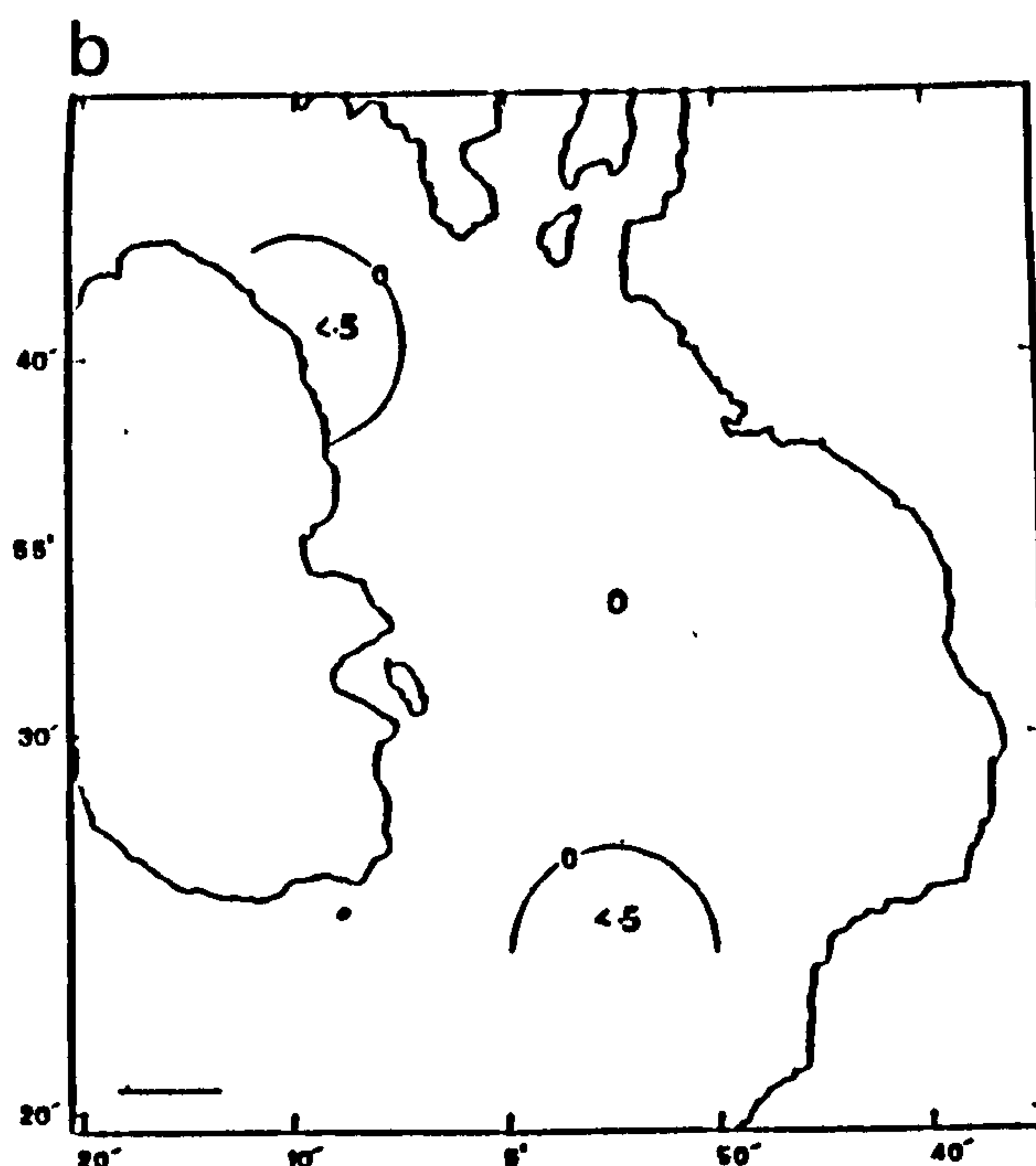
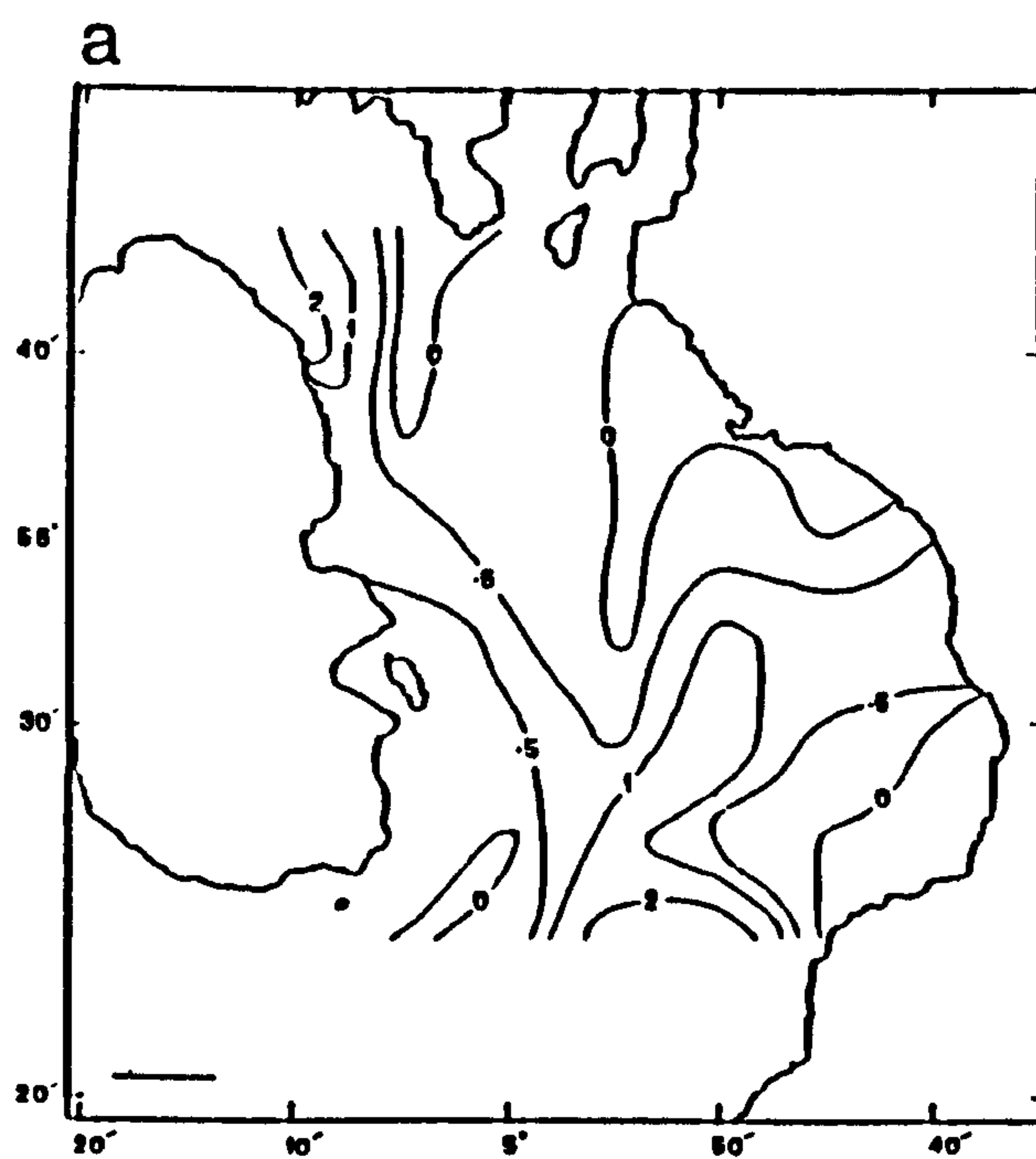


Figure 2.5

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde between 30.5 – 1.6.84 for (a) 1st stage zoeae, (b) 2nd stage zoeae, (c) 3rd stage zoeae and (d) all 3 zoeal stages combined.

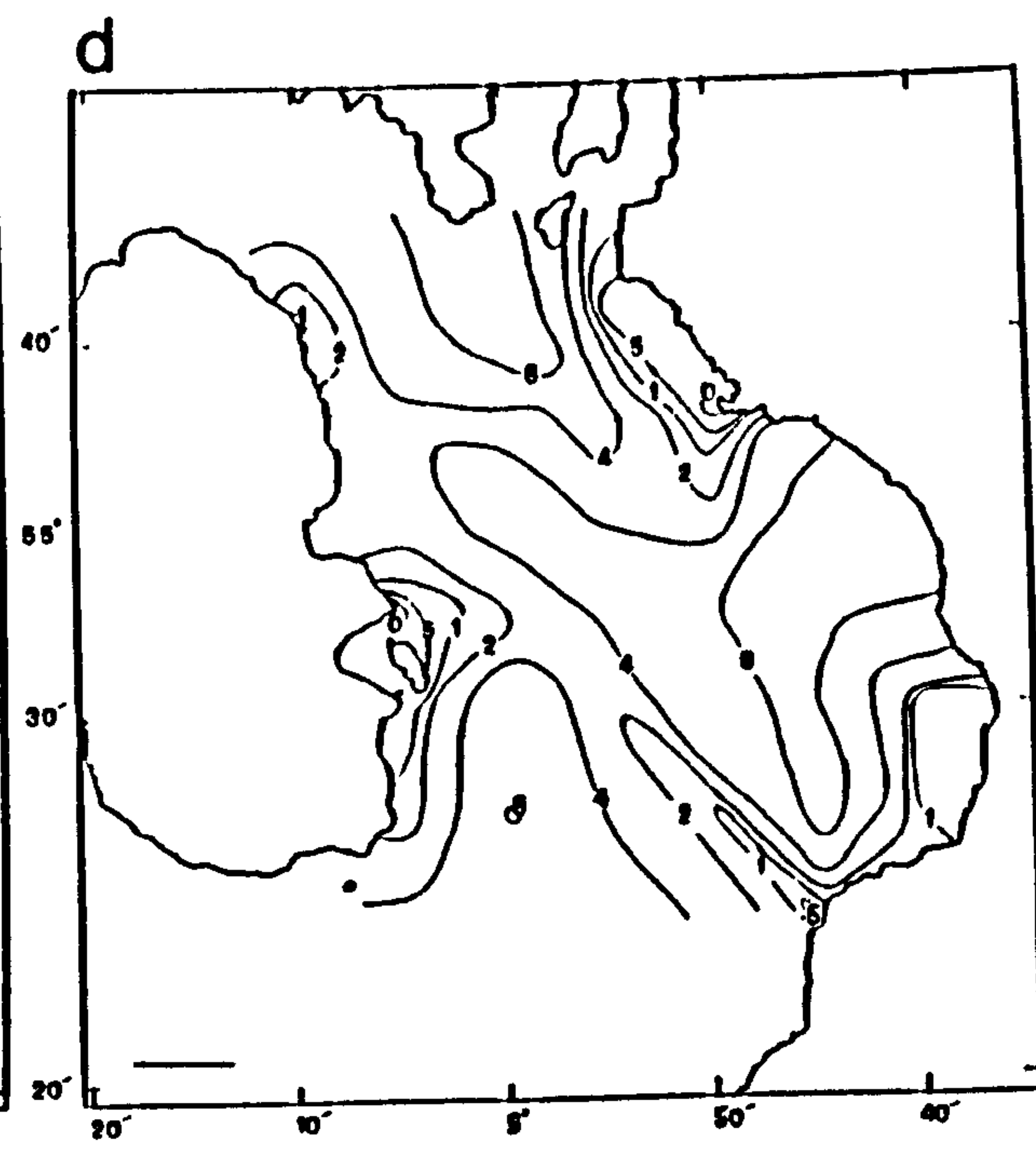
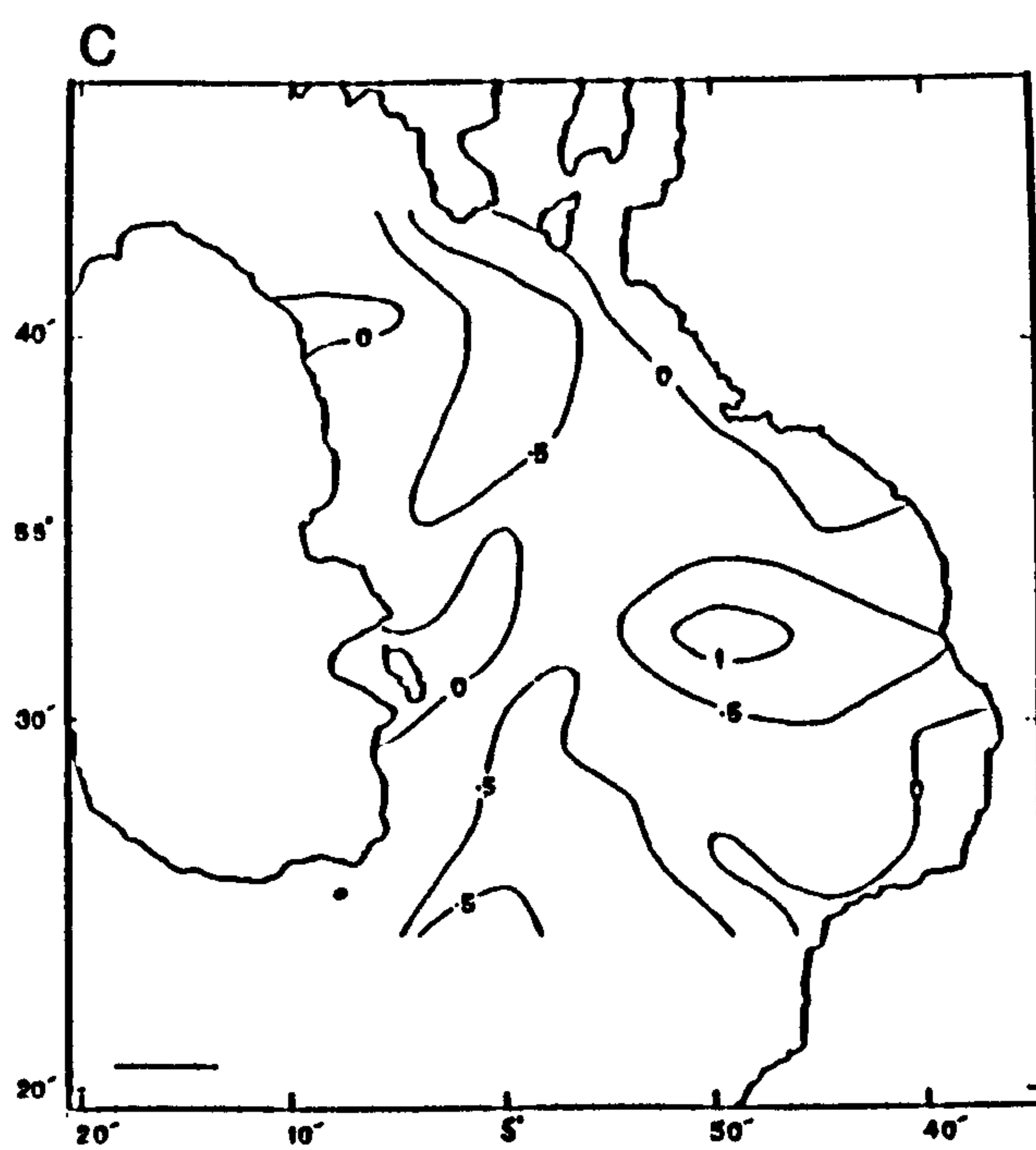
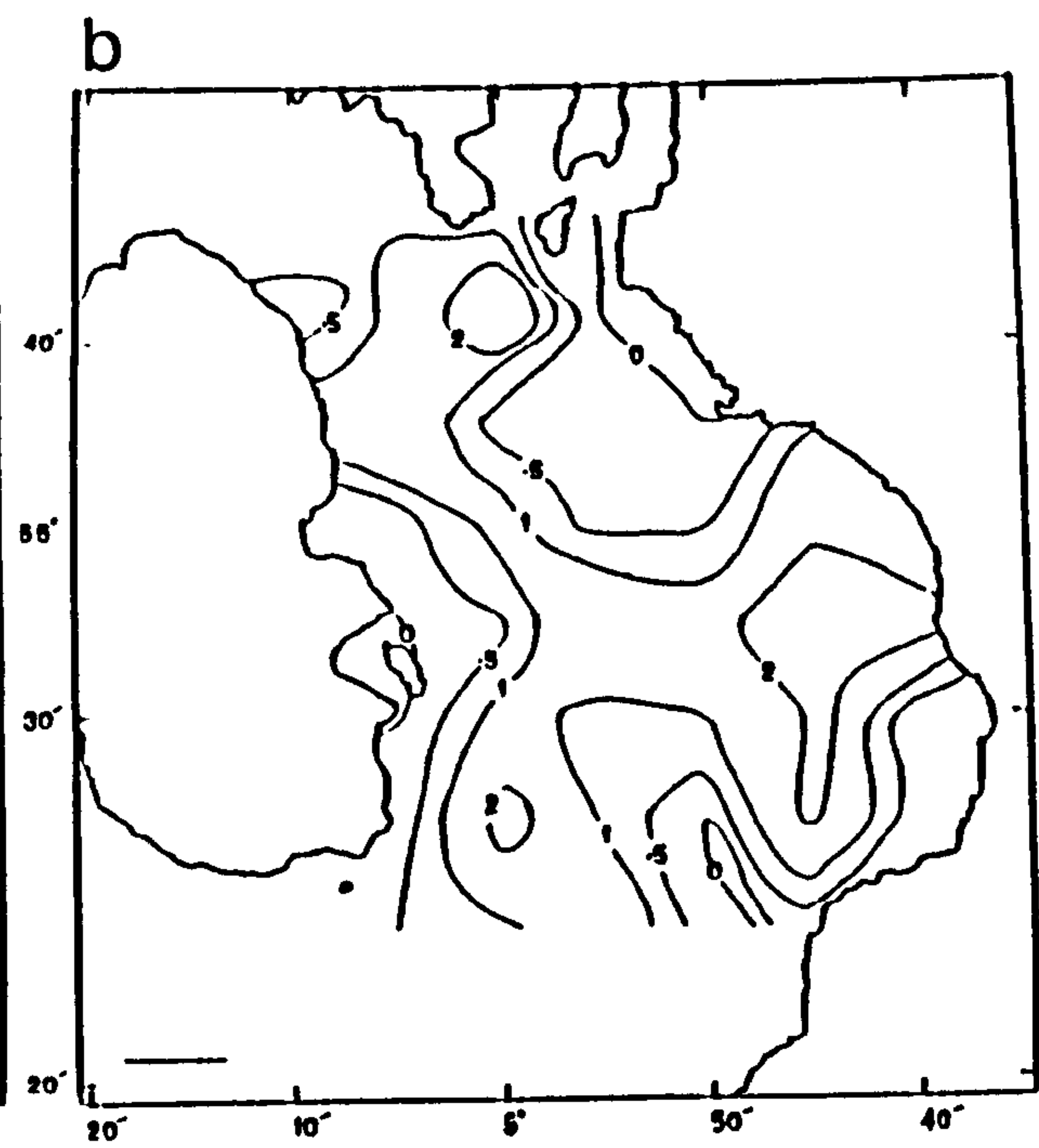
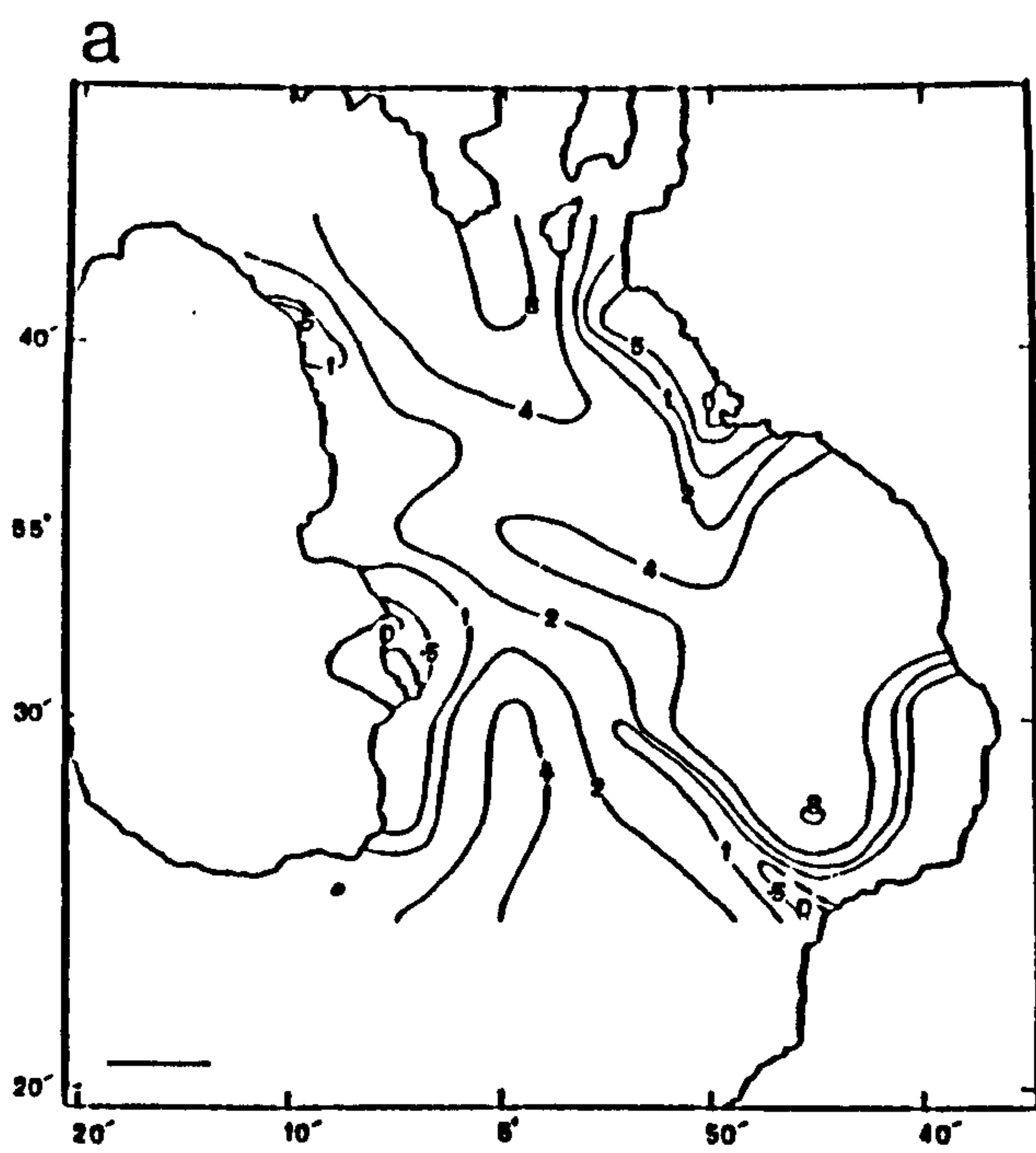


Figure 2.6

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde between 27 - 28.6.84 for (a) 1st stage zoeae, (b) 2nd stage zoeae, (c) 3rd stage zoeae and (d) all 3 zoeal stages combined.

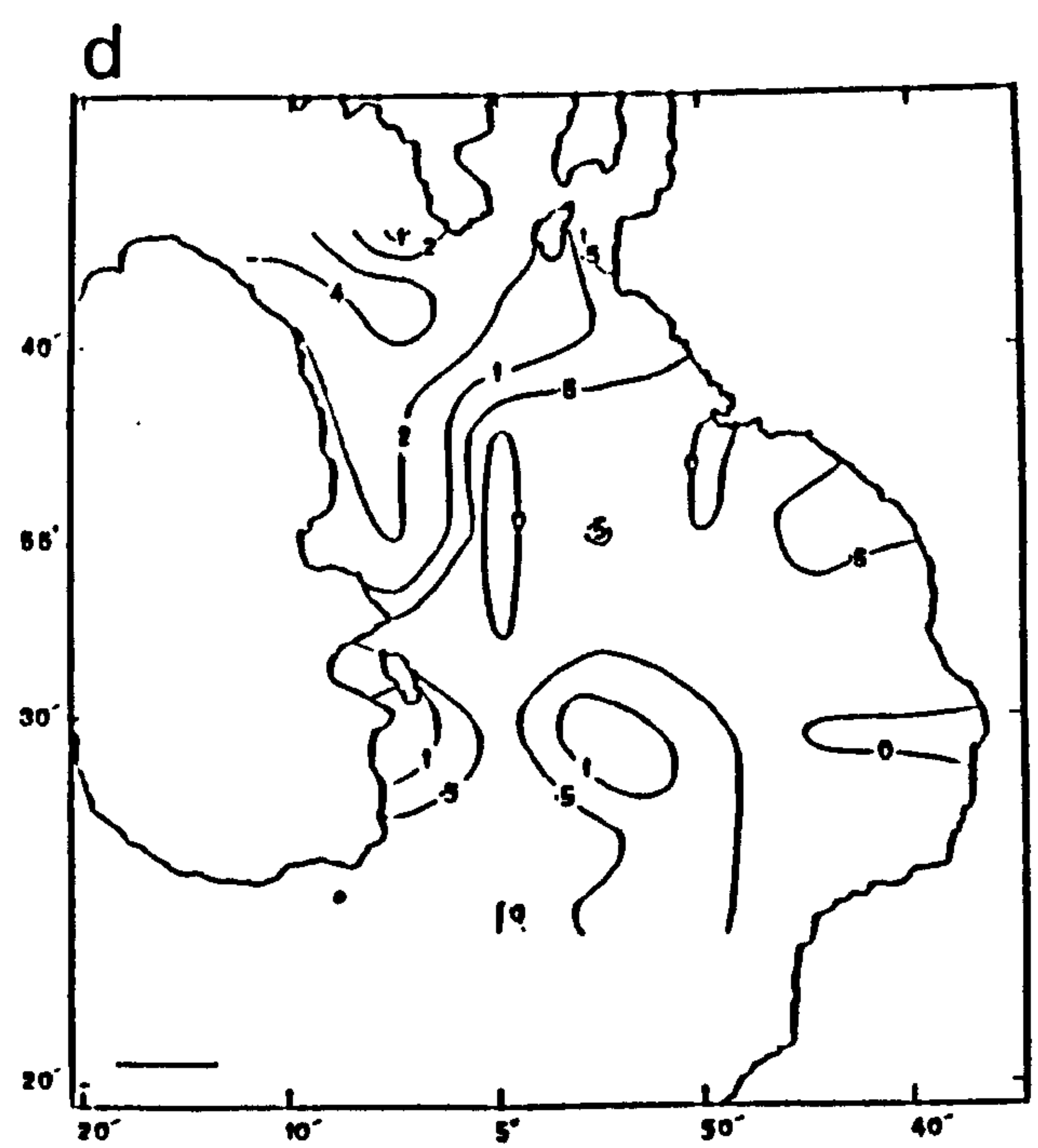
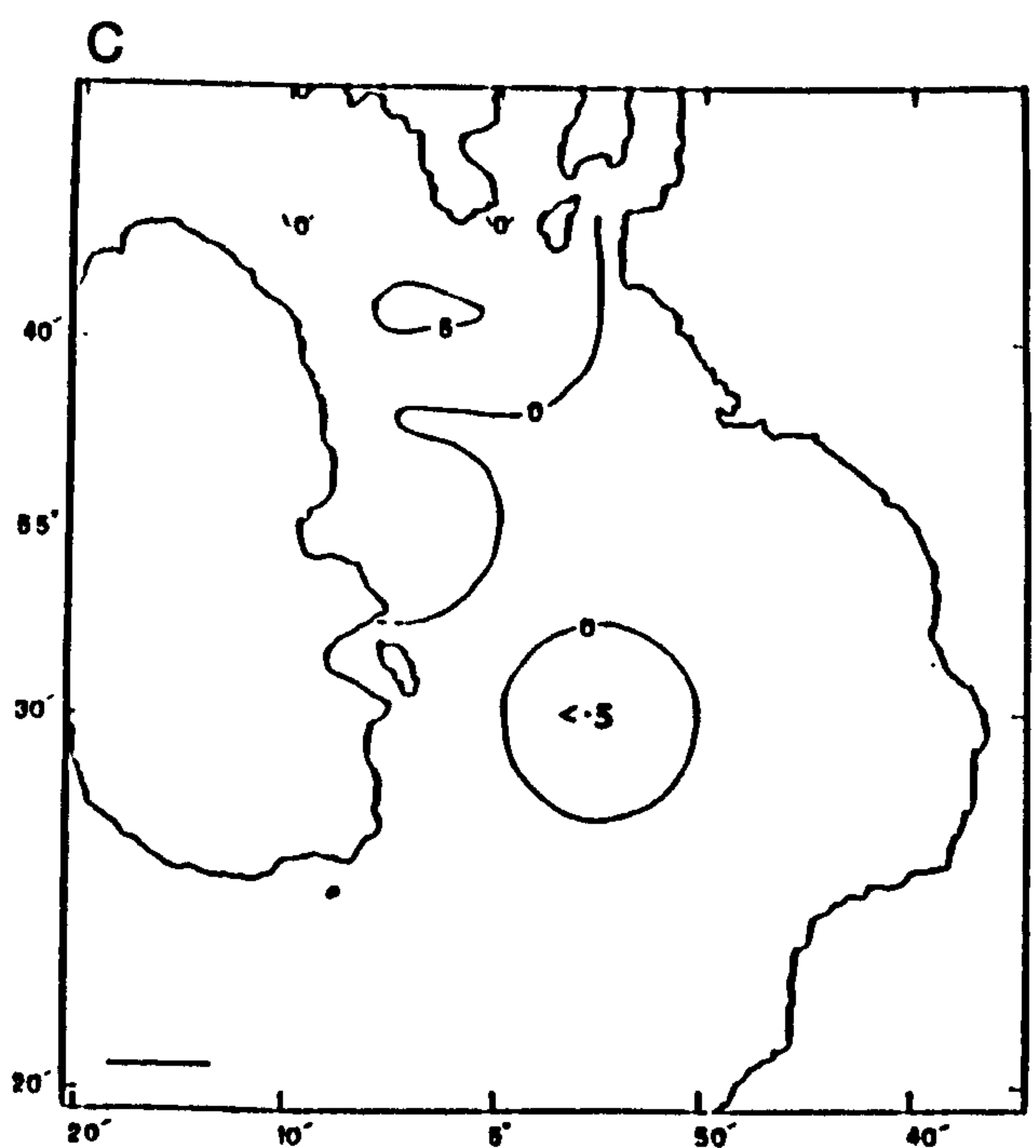
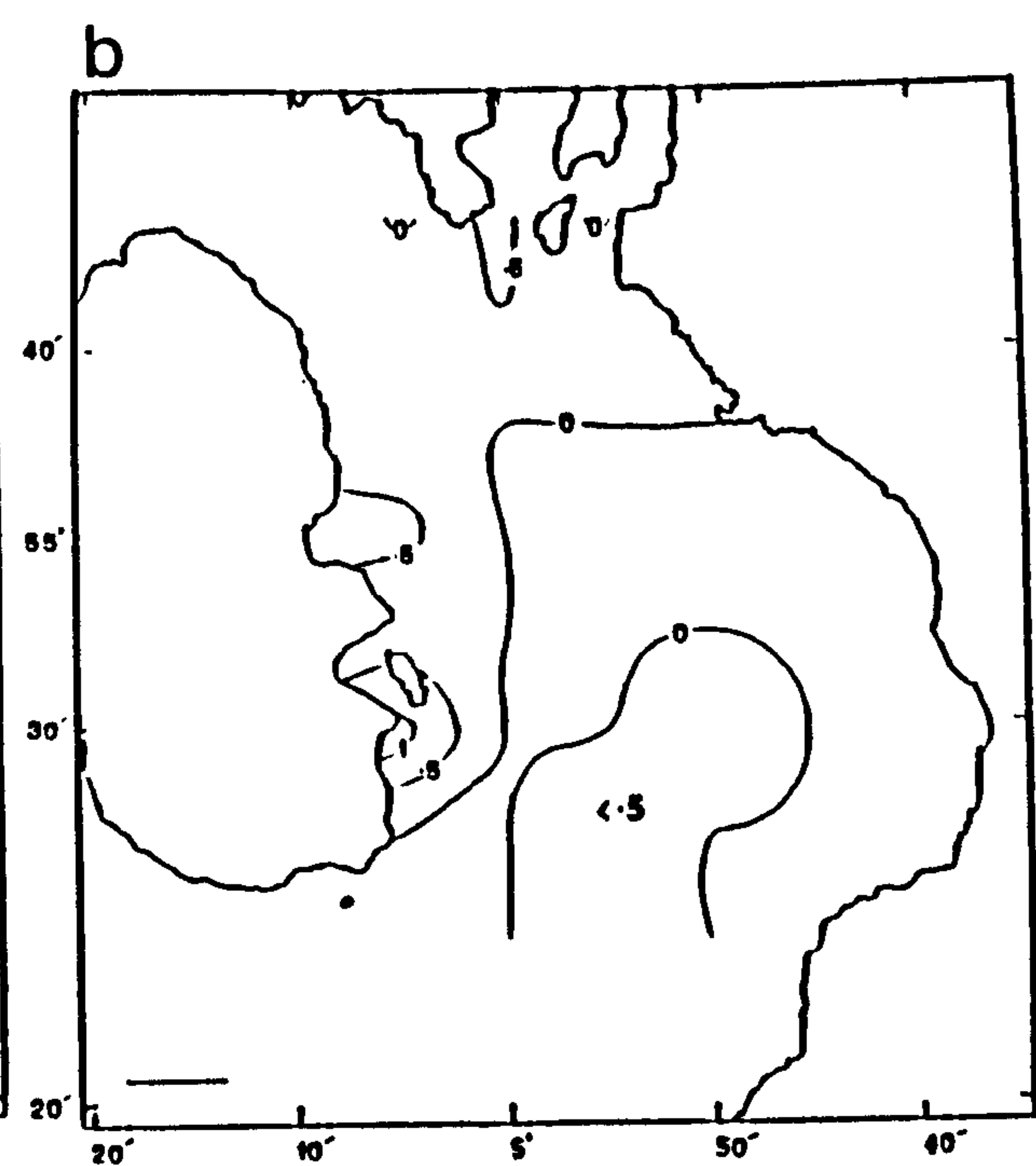
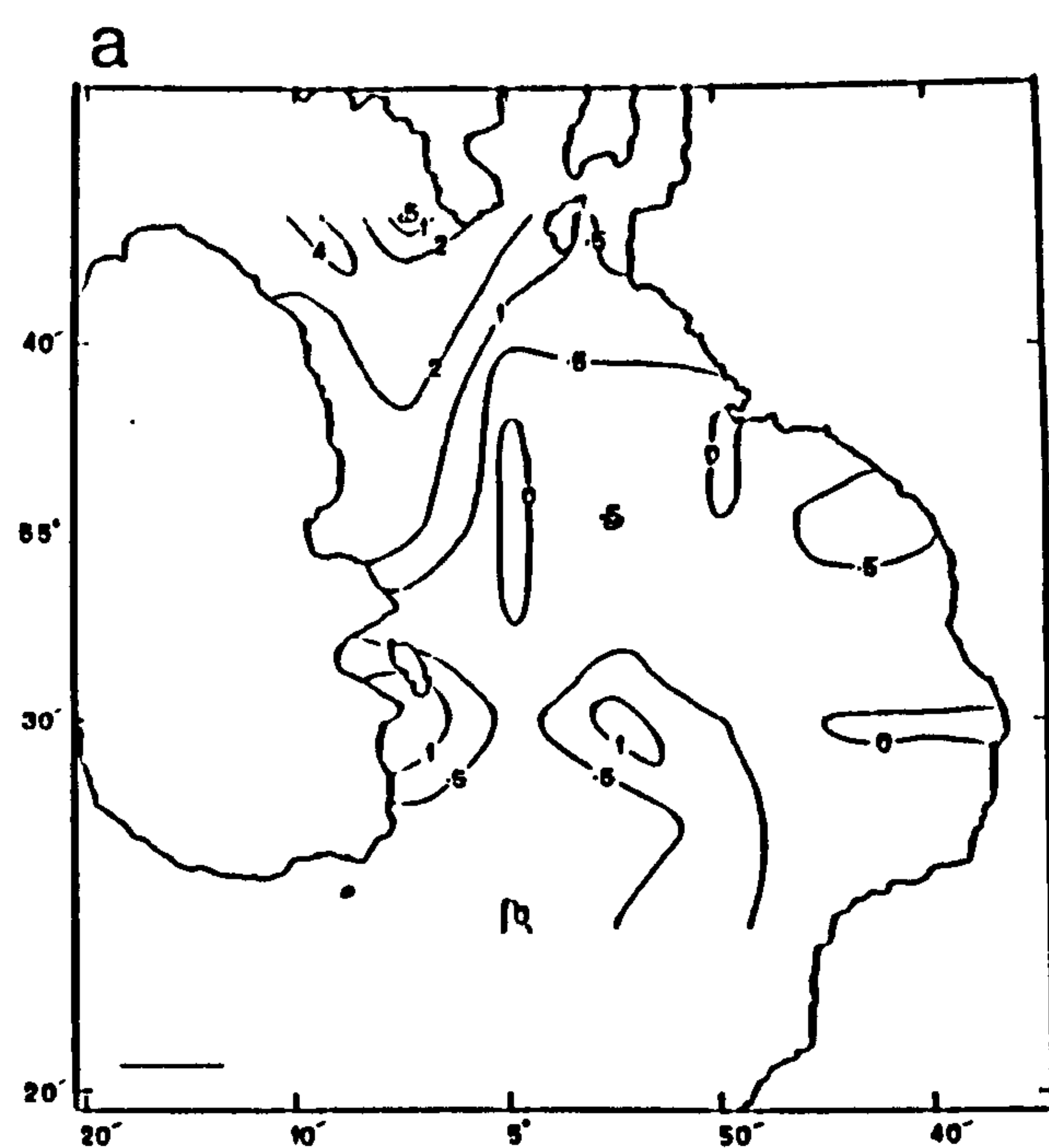




Figure 2.7

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde between 24 - 25.7.84 for (a) 1st stage zoeae, (b) 2nd stage zoeae, (c) 3rd stage zoeae and (d) all 3 zoeal stages combined.

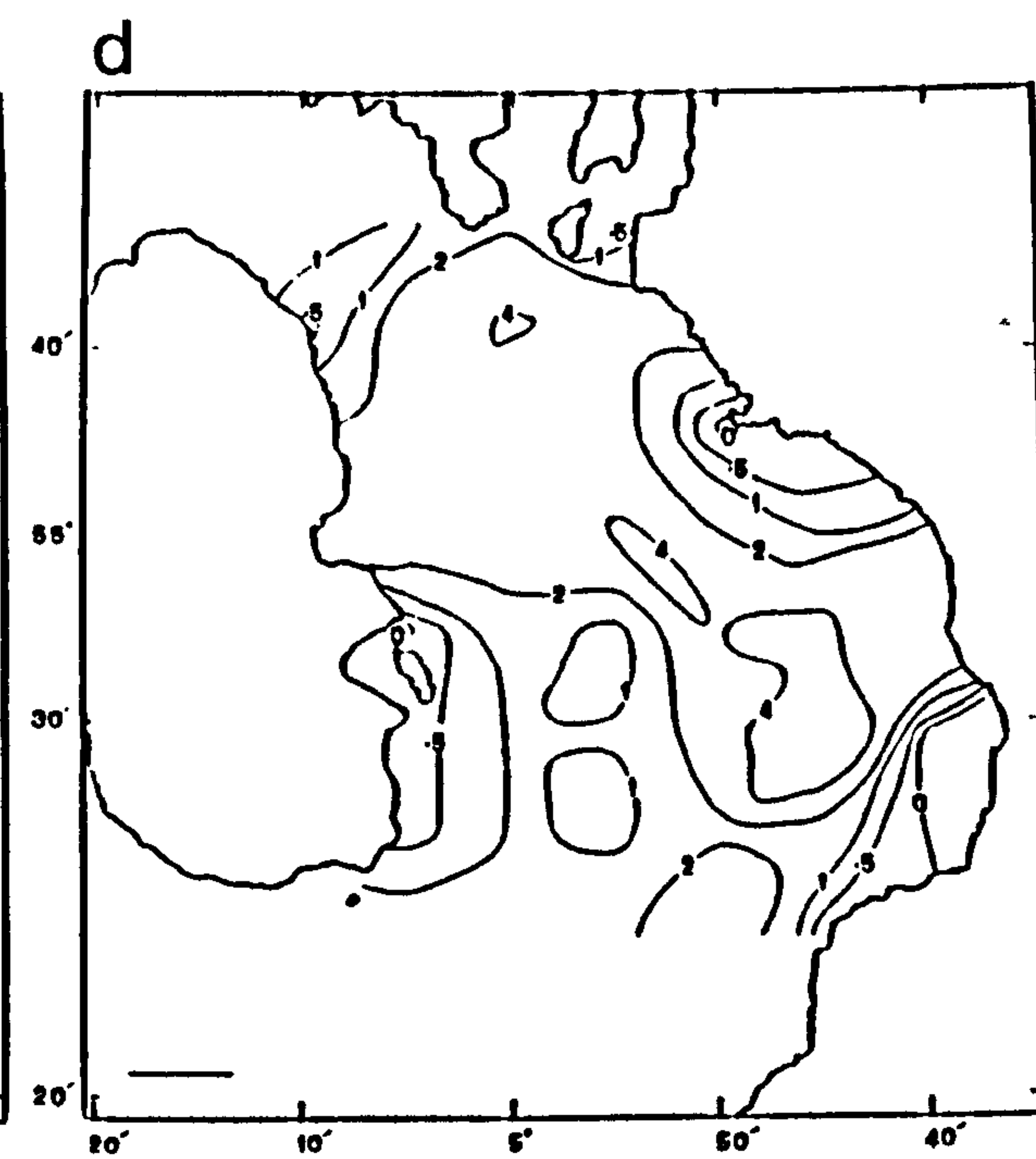
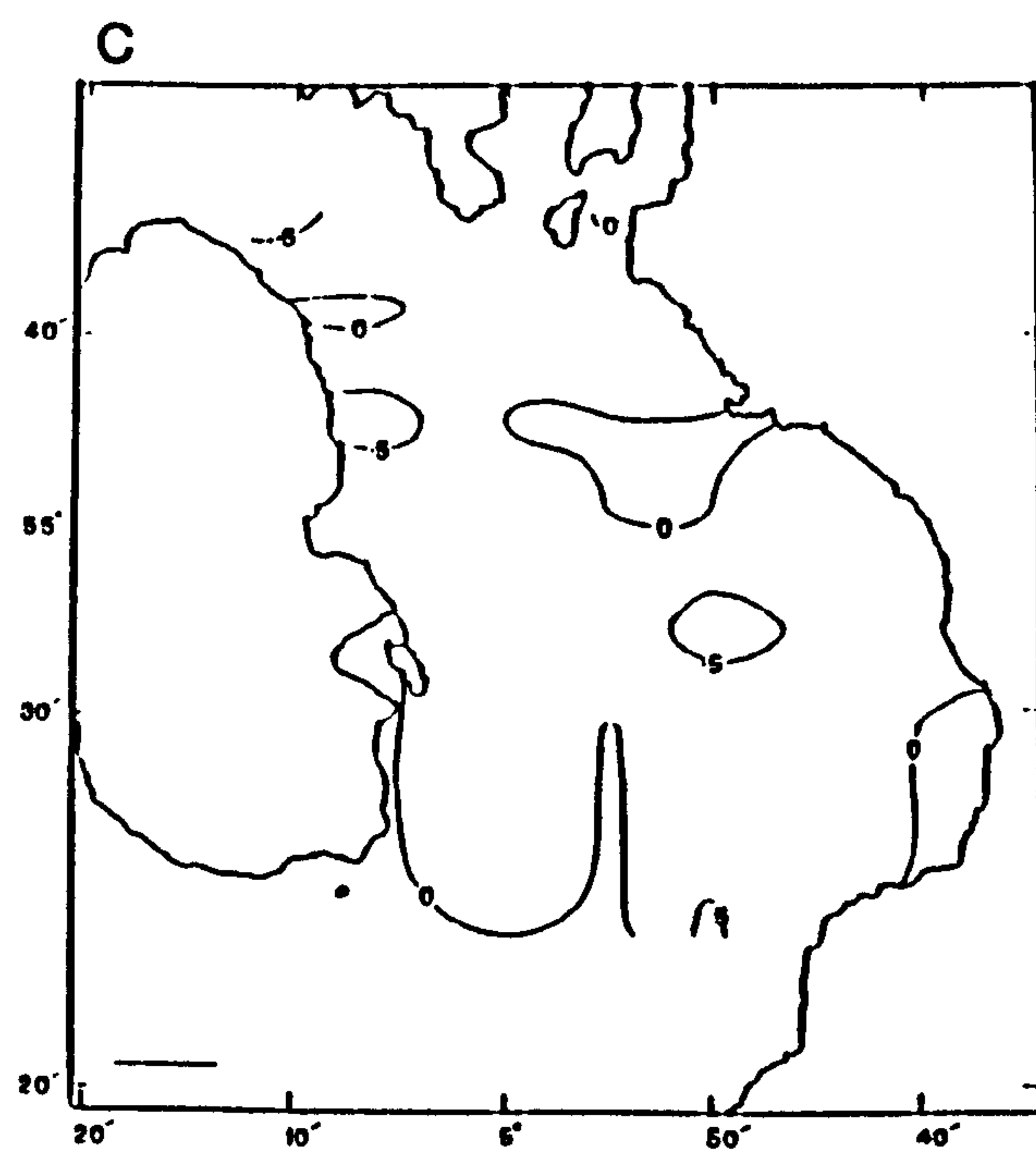
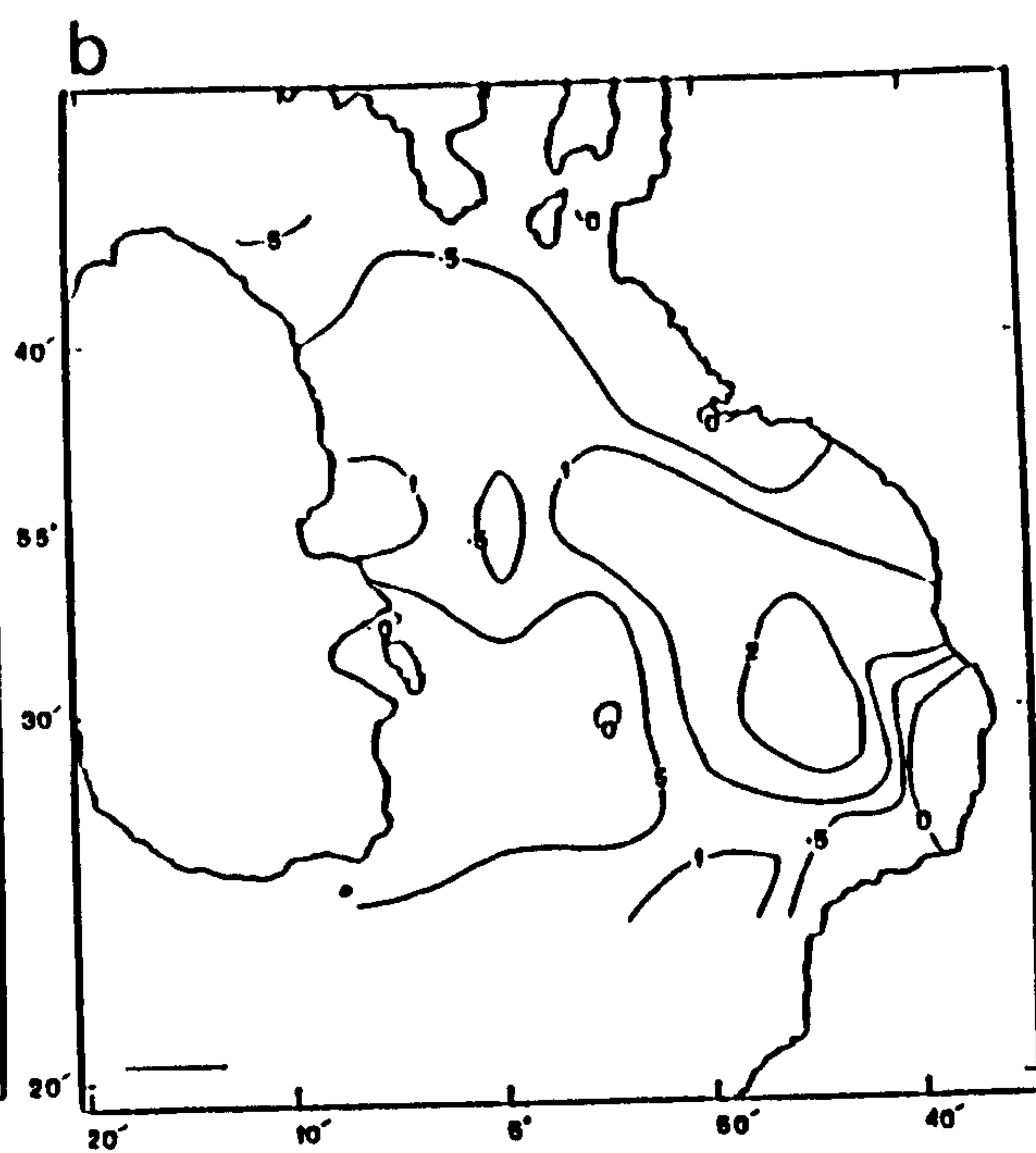
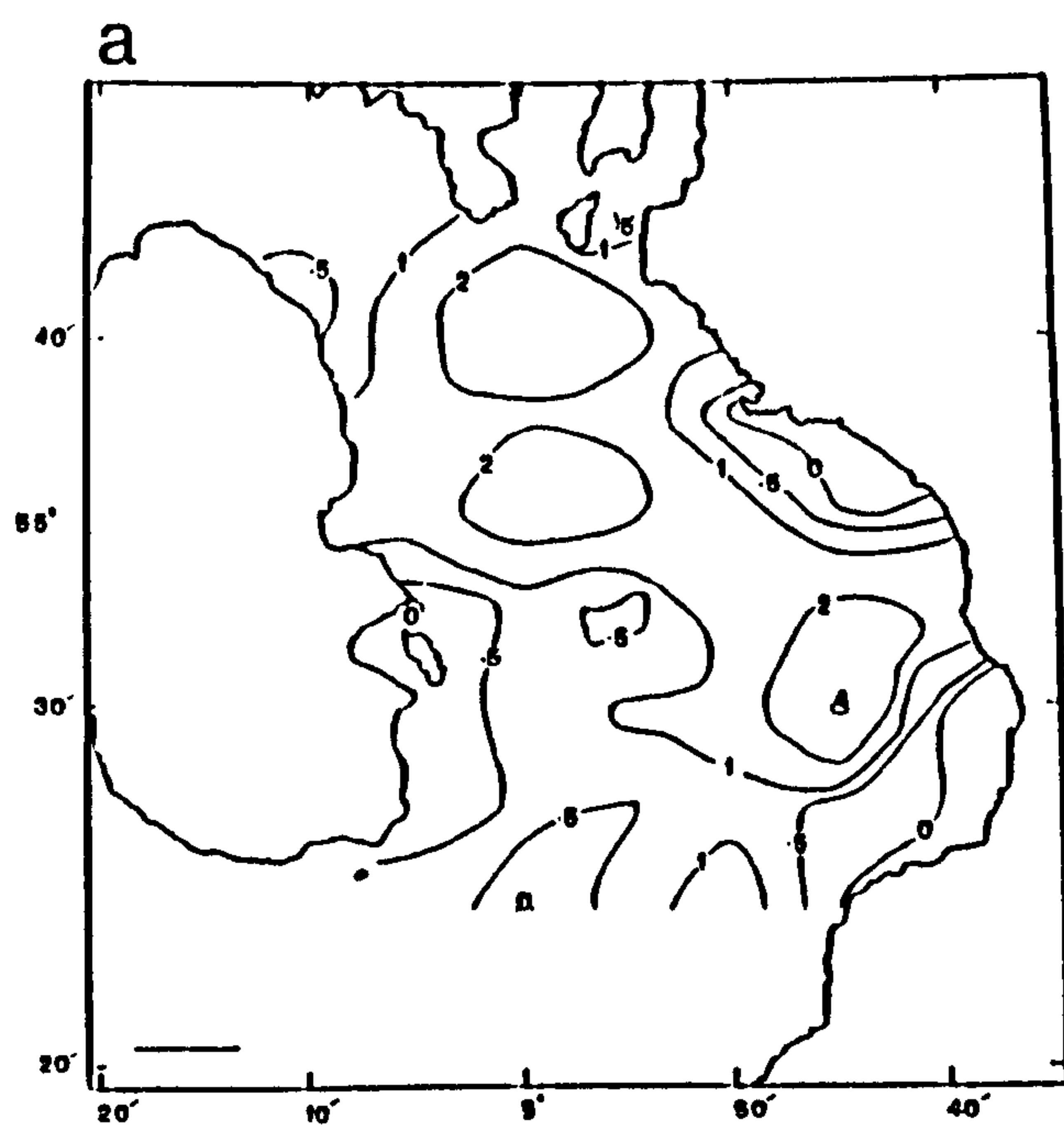


Figure 2.8

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde between 22 - 23.8.84 for (a) 1st stage zoeae, (b) 2nd stage zoeae, (c) 3rd stage zoeae and (d) all 3 zoeal stages combined.

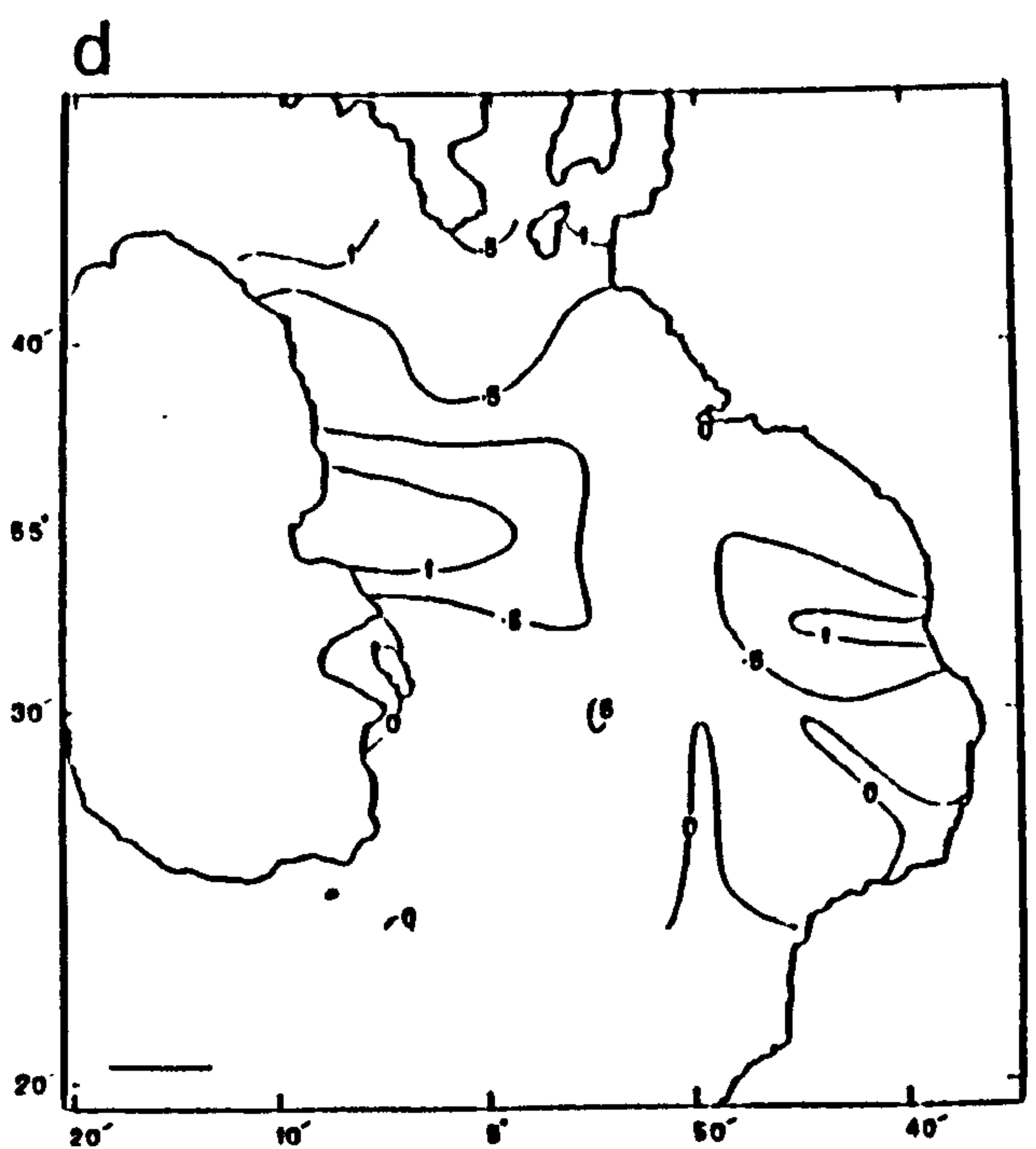
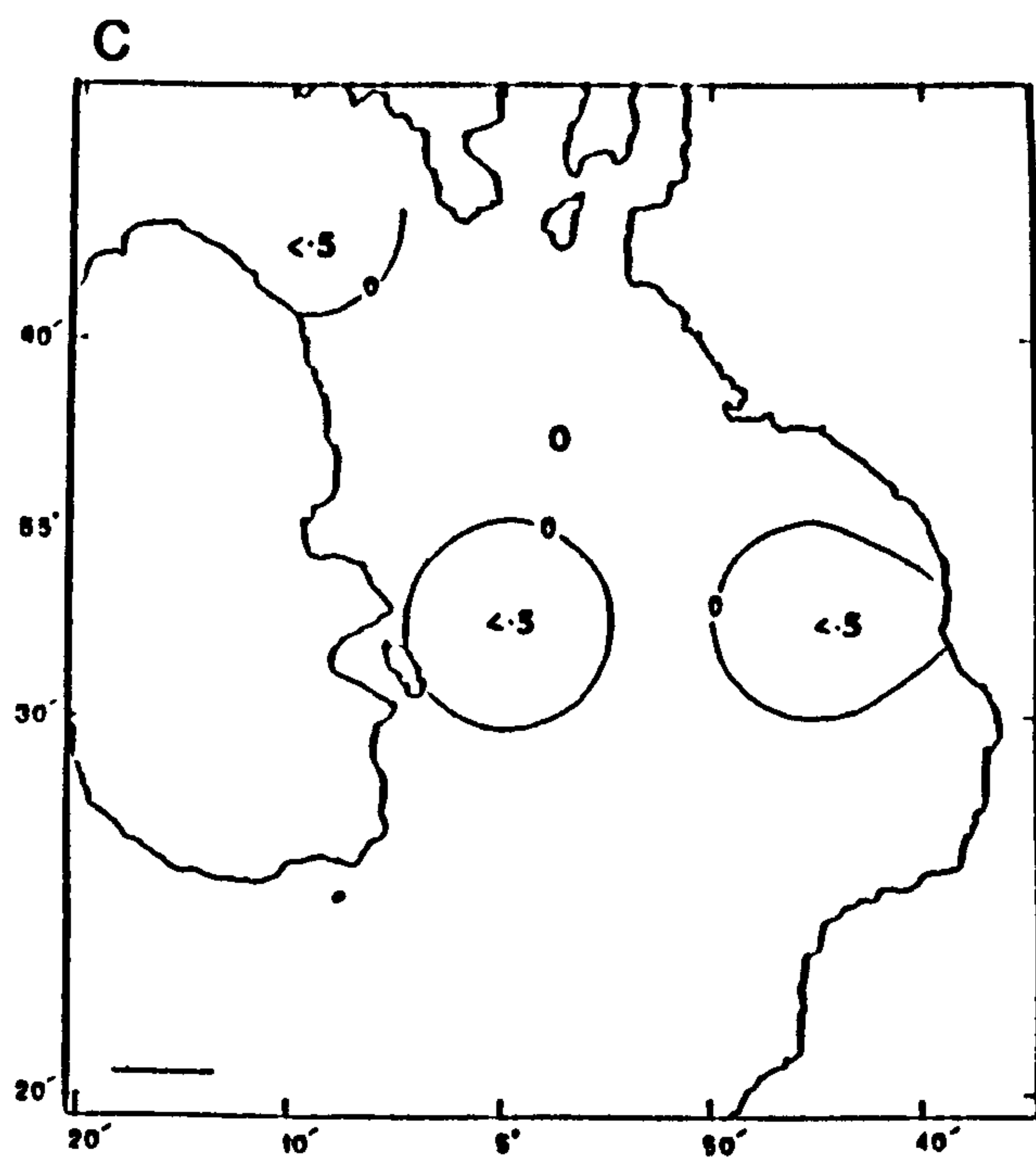
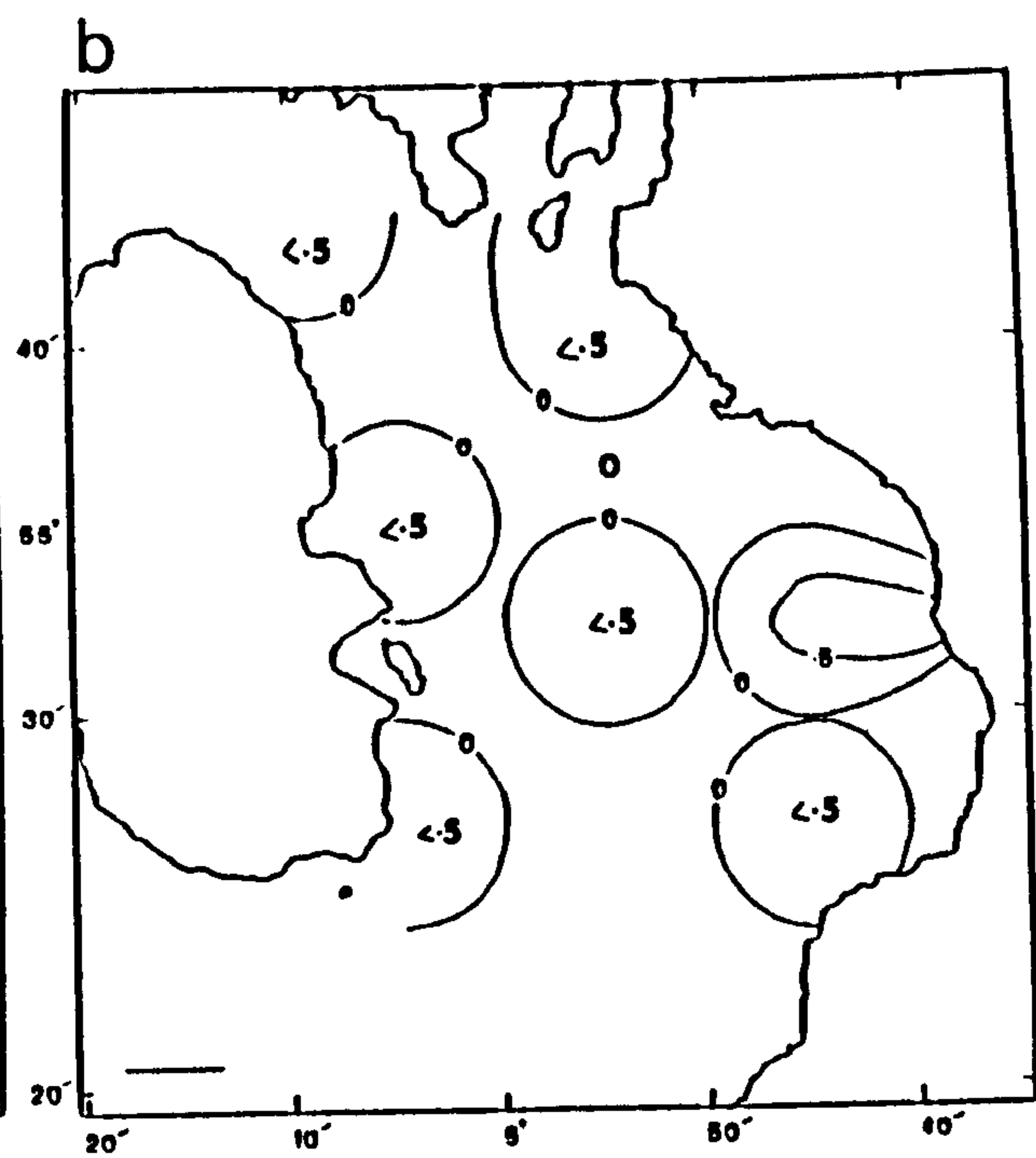
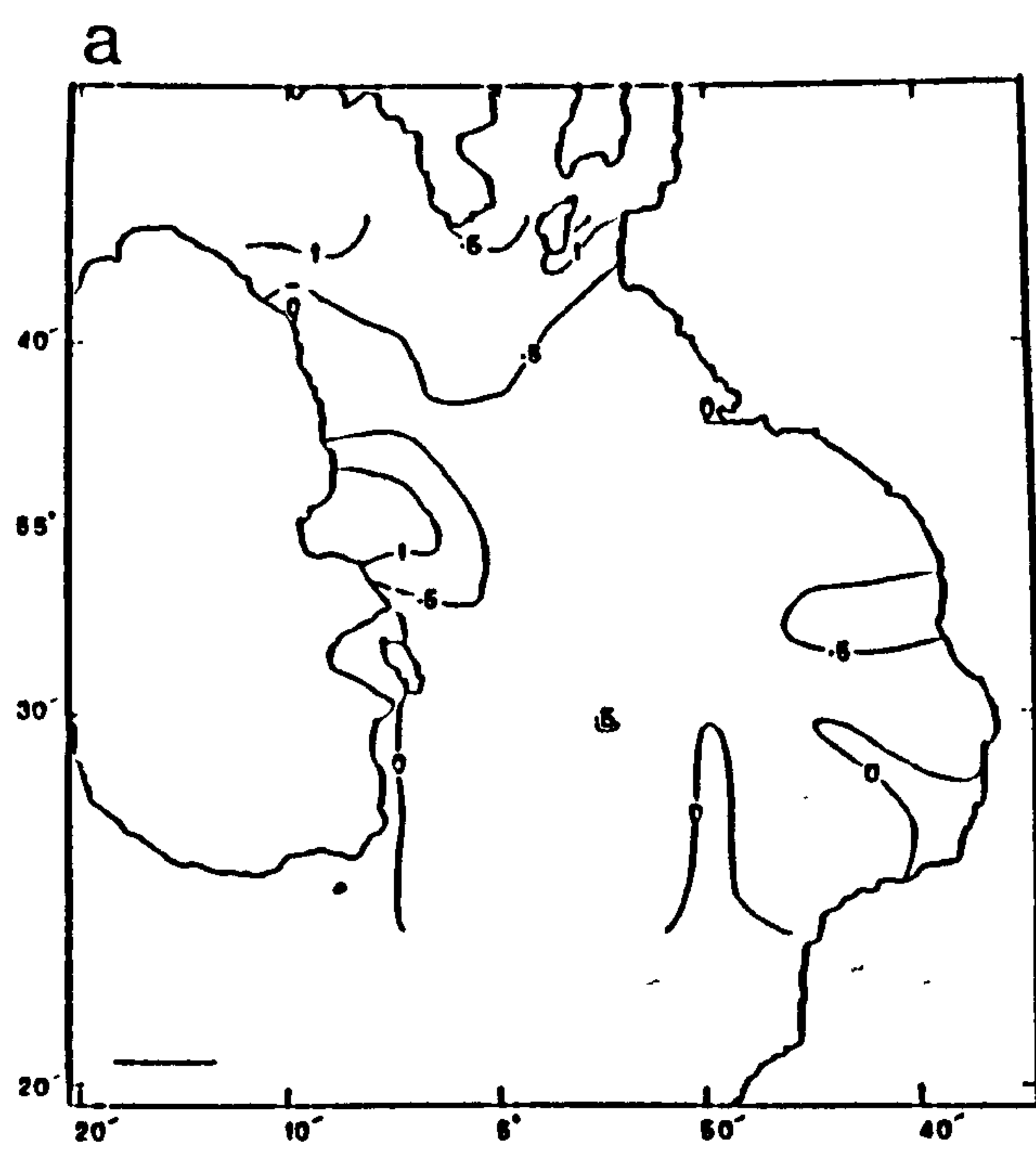
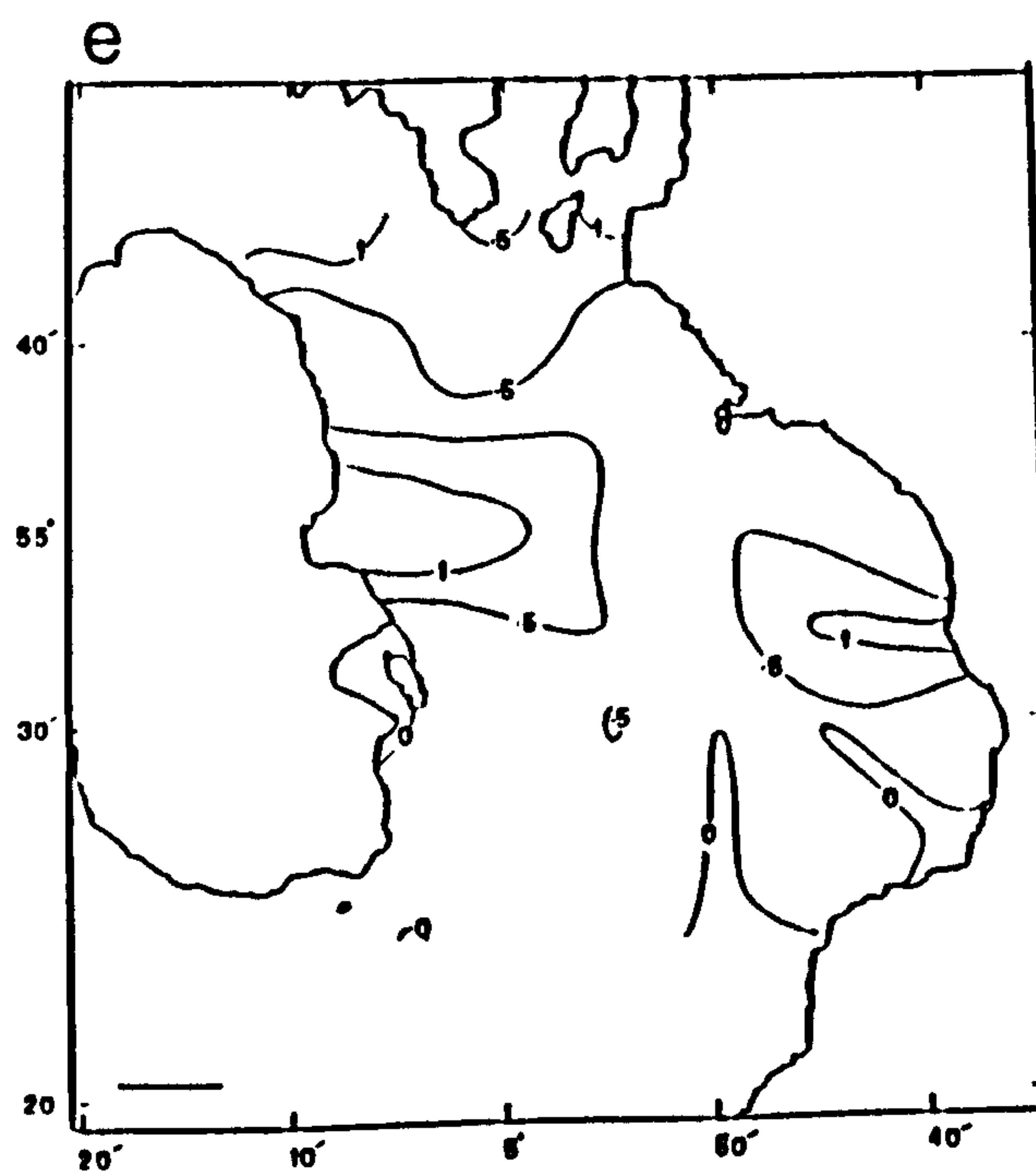
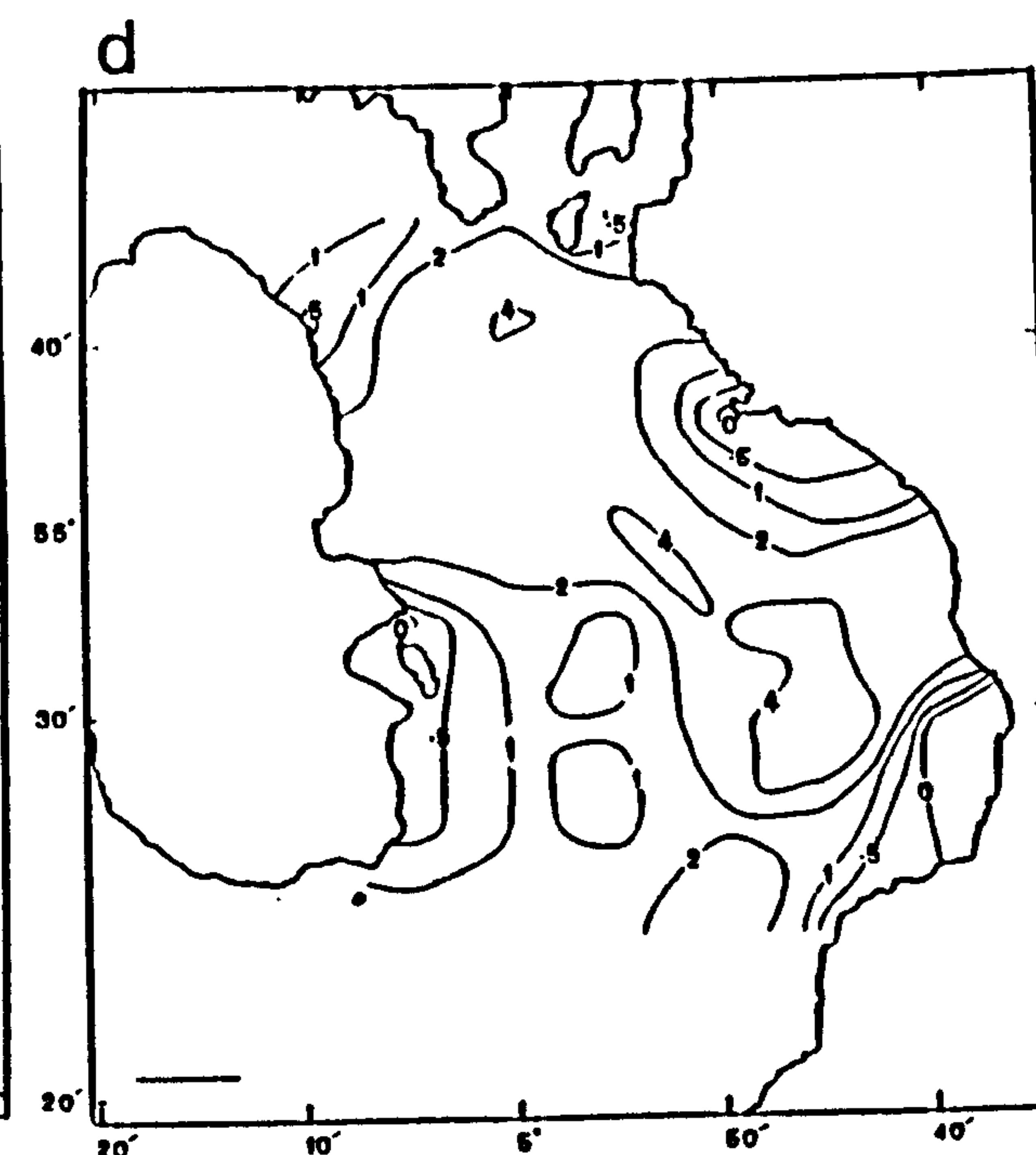
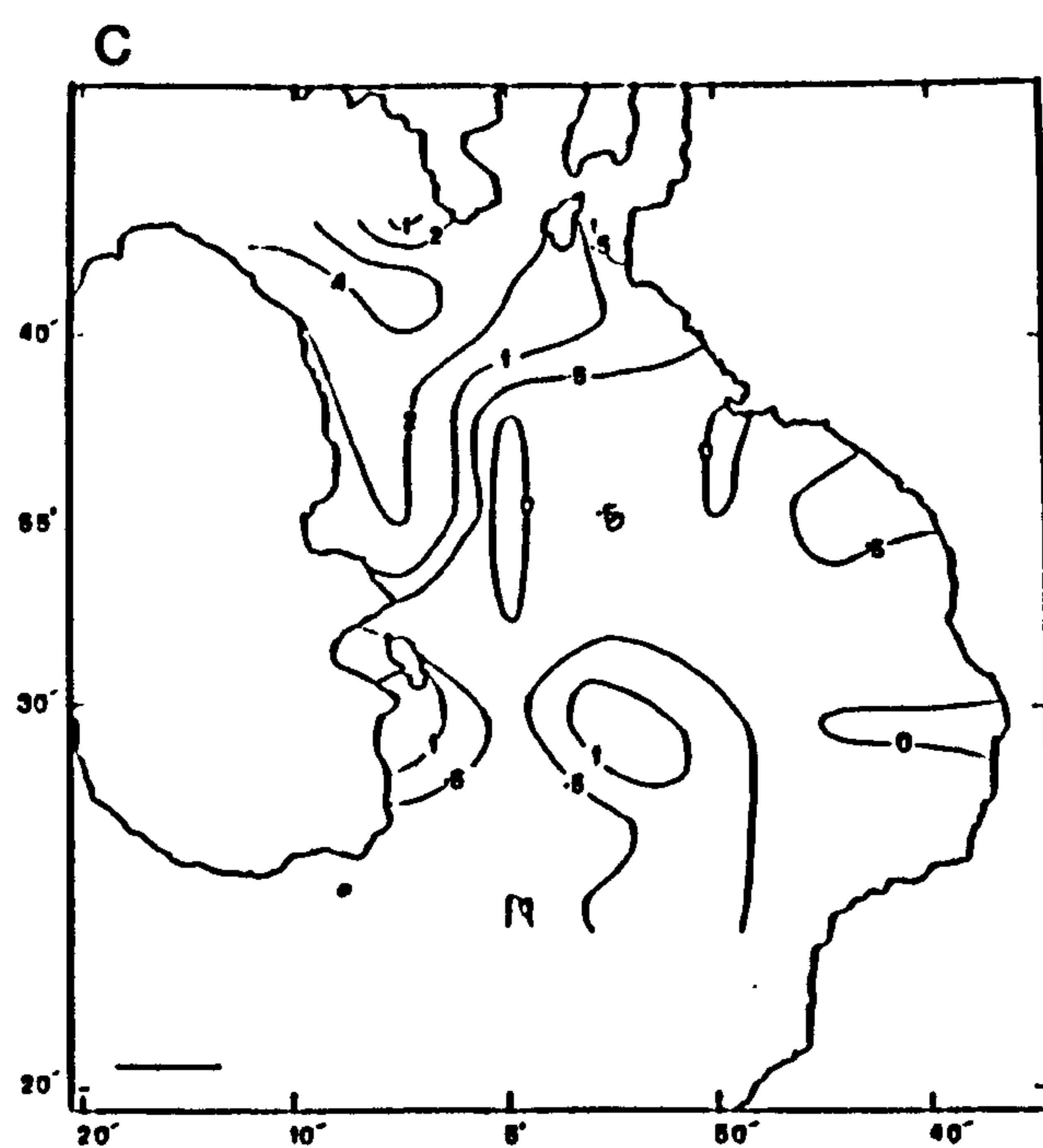
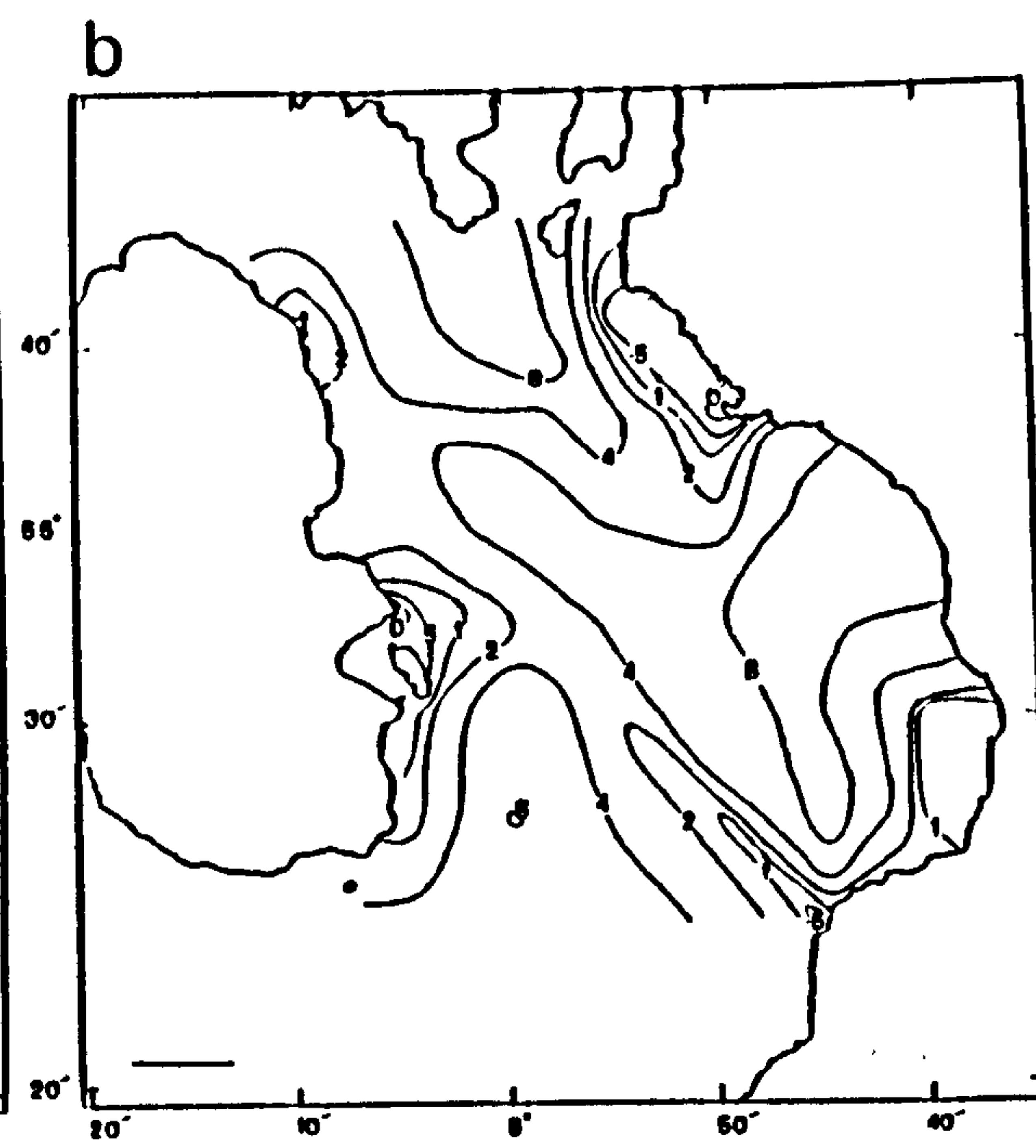
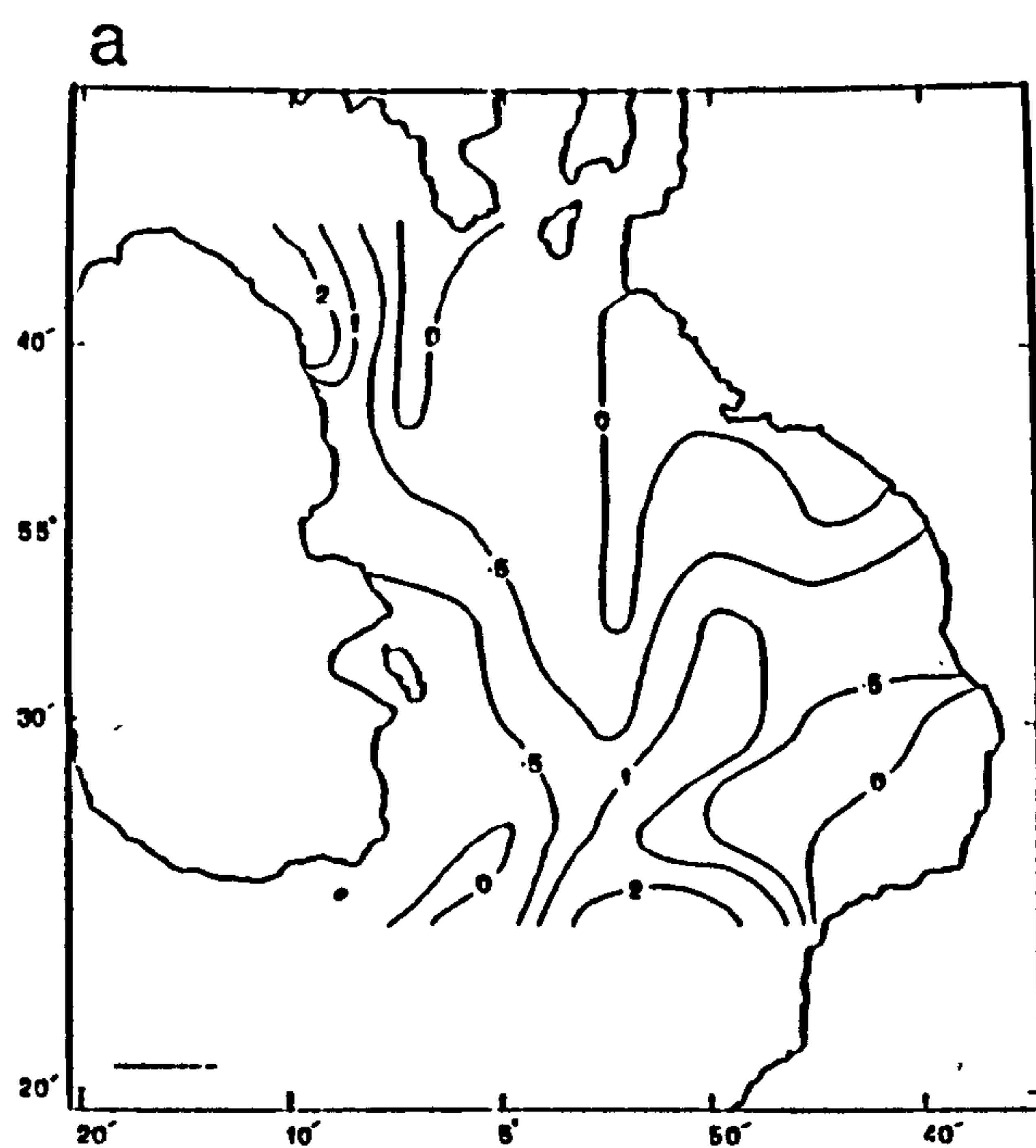


Figure 2.9

The distribution of Nephrops zoeae as numbers  $m^{-2}$  in the Lower Firth of Clyde for all 3 zoeal stages between (a) 20 - 22.4.84, (b) 30.5 - 1.6.84, (c) 27 - 28.6.84, (d) 24 - 25.7.84 and (e) 22 - 23.8.84.





each zoeal stage and for all 3 zoeal stages (see Table 2.1). Using this contour method 2 estimates were therefore derived for total larval abundance. One by adding the separate abundance values calculated for the 3 zoeal stages and the other from the values calculated for all 3 zoeal stages (Figure 2.9). These results were then compared to the abundance values obtained by direct calculation from the data presented in Table A.1 (see appendix). In this method the abundance values were calculated from the product of the mean number of zoeae per square metre and the total survey area (876km<sup>2</sup>). The larval abundance estimates derived by direct calculation are generally lower than those computed by the contouring method. This is not surprising since they fail to take account of local regions of high abundance. All further treatment and analysis of the abundance estimates was therefore performed on the integrated values derived from the contoured assessment.

Without a detailed knowledge of the hydrographic conditions within the L.F.C. it was not possible to equate localised regions of larval abundance with those of larval production. On the first survey, which was conducted towards the end of April, although the abundance of 1st stage zoeae was reasonably high ( $4.6 \times 10^8$ ) only two 2nd stage zoeae were caught. This therefore indicated that hatching had only just begun, as predicted from the pre-survey sampling programme. The areas of highest abundance appeared to occur at the north western and south eastern regions of the L.F.C. (Figure 2.4). During the next few weeks total abundance for the 3 stages increased by almost an order of magnitude with peak abundance recorded during the 2nd survey ( $40.9 \times 10^8$ ) (end of May and beginning of June). The results from this survey show that the spatial distribution of the zoeae was fairly even

Table 2.1

Larval abundance estimates derived using 2 different methods, contouring and by direct calculation from the results presented in Table A1.  
( $\Sigma$  contour refers to the estimates obtained from the total abundance contours).

Survey Date	Zoeal Stage	Larval Abundance (x 10 <sup>6</sup> )		Σ Contour
		Contouring	Direct Calculation	
20-22 April 1984	1	464.600	456.598	475.957
	2	31.185	17.677	
	3	-	-	
	Σ	495.785	474.275	
30 May - 1 June 1984	1	2811.975	2879.389	4281.644
	2	949.072	786.289	
	3	332.862	276.883	
	Σ	4093.909	3942.561	
27-28 June 1984	1	581.443	577.913	717.550
	2	137.341	89.397	
	3	83.650	45.664	
	Σ	802.434	712.974	
24-25 July 1984	1	995.297	925.775	1868.004
	2	698.575	614.278	
	3	180.398	134.881	
	Σ	1874.270	1672.934	
22-23 August 1984	1	302.203	266.237	393.448
	2	91.800	41.105	
	3	29.740	10.871	
	Σ	423.743	318.313	
Σ = Total				

throughout the study area, although localised areas of higher abundance have been identified at the northern and eastern regions (Figure 2.5). The zoeae of all 3 stages appear to have been similarly distributed.

By the end of June (3rd survey) there was a reduction in total larval abundance ( $8.0 \times 10^8$ ), to approximately a fifth of that recorded at the beginning of the month. This level of reduction was similar for all 3 stages. The spatial distribution of the zoeae appears to have changed during June with greatest larval abundance recorded at the north-western region of the L.F.C. (Figure 2.9b,c).

The results of the 4th survey show that an increase in larval abundance had occurred during the month of July (Table 2.1). Total abundance for all 3 zoeal stages was  $18.7 \times 10^8$ , 2.3X greater than the previous estimate at the end of June. The spatial distribution of the 3 stages appears to have been relatively even throughout the study area (Figure 2.7). The proportion of zoeae representing the 2nd and 3rd stages during this survey was much higher than that recorded during all previous surveys (Table 2.1). This indicated that the hatching season was relatively far advanced. This was verified by the results of the final survey (end of August), total larval abundance having been reduced to less than a quarter of that recorded during the previous survey (Figure 2.8).

The log-log linear regression equations of development time against temperature calculated during the culture programme (see Chapter 3, Table 3.4) were applied to determine the development time for each stage during each survey (Table 2.2). The corresponding

larval abundance was then divided by this value to determine daily larval production for the 3 zoeal stages on each of the 5 surveys. These values were then plotted on a time scale at the midpoint of the survey date to give a larval production curve for each zoeal stage (Figure 2.10). The area under each curve was then calculated to provide the total production for each stage during the hatching season (Table 2.2).

For each zoeal stage the mean sea temperature for the larval season was calculated. This was accomplished by integrating the proportion of the total abundance value captured during each survey, for each stage, with the corresponding mean sea temperature (Table 2.2). From the estimation of the mean sea temperature the mean stage duration was calculated for each stage (Table 2.3) using the log-log linear regression equations of development time against temperature.

The starting point for the stage 1 seasonal production curve (1 April) was based on the capture of few stage 2 zoeae during the first survey. This date was calculated from the expected development time for these zoeae to achieve the 2nd stage at the temperature prevailing on the first survey. The end point for all 3 production curves was calculated from sampling surveys conducted over a limited region of the L.F.C. after the last full survey.

The instantaneous coefficient of mortality per day ( $Z$ ) was then calculated from the equation :

$$N_t / N_0 = \exp.(-Zt)$$

where  $N_0$  is the initial numbers of larvae and  $N_t$  is the numbers surviving to the next stage in time  $t$  (days), the mean stage duration

**Figure 2.10**

**The seasonal production curves for zoeal stages 1 (Z1), 2 (Z2) and 3(Z3) in the Lower Firth of Clyde in 1984.**



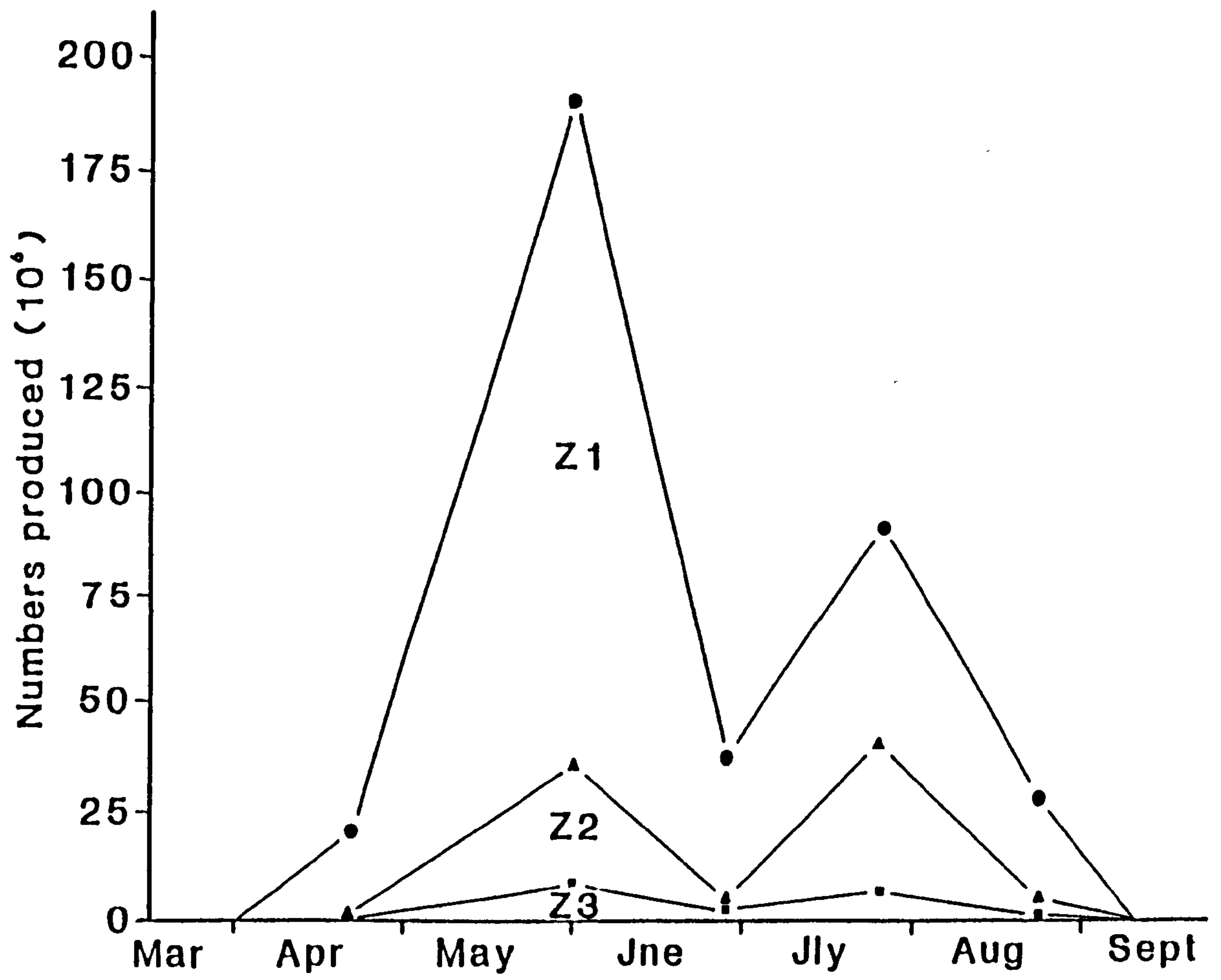


Table 2.2

Larval production as numbers produced per day ( $10^6$ ) for all 3 zoeal stages. The mean sea-water temperature was calculated for each survey and used to determine the inter-stage development time by applying the regression equations derived in Chapter 3.

Survey Dates	Mean Sea Temp. (°C)	Zoeal Stage	Develop. Time (Days) (T)	Larval Abund. ( $10^6$ )(A)	Daily Prodn. ( $10^6$ )(A/T)
20-22 April 1984	6.51	1	22.9	464.600	20.288
		2	53.5	31.185	0.583
		3	74.6	-	-
30 May-1 June 1984	8.92	1	14.8	2811.975	189.998
		2	26.8	949.072	35.413
		3	37.8	332.862	8.806
27-28 June 1984	8.74	1	15.0	581.443	38.763
		2	28.1	137.341	4.888
		3	39.5	83.650	2.118
24-25 July 1984	10.87	1	10.9	995.297	91.312
		2	17.4	698.575	40.148
		3	24.6	180.398	7.333
22-23 August 1984	11.12	1	10.5	302.203	28.781
		2	16.6	91.800	5.530
		3	23.5	29.740	1.266

for the season (Nichols et al., 1987). From these results it appears that percentage daily mortality over the larval season between stages 1 to 2, 2 to 3 and 1 to 3 averaged 10.0, 6.3 and 7.7% respectively (Table 2.3).

Based on the estimation of mean stage duration the first stage zoeae would have been between 0 – 14.5 days old when caught. The total production of stage 1 zoeae calculated from the production curve ( $111.14 \times 10^8$ ) will therefore underestimate the instantaneous production at the time of hatching. A survival curve was therefore constructed from the total numbers of zoeae produced for each stage using the midpoint of the mean stage duration for the 1st stage zoeae (Figure 2.11). The mortality regression was then used to estimate the intercept value for instantaneous production at zero time before any losses occurred. This was accomplished by extending the line formed between the production estimates for 1st and 2nd stage zoeae to 0 days from hatching.

A value of  $177.00 \times 10^8$  was estimated for instantaneous larval production which was later used to estimate the number of spawning females within the L.F.C. contributing to the hatch. The predicted mortality between 1st and 2nd and the 1st and 3rd zoeal stages increases from 76.6 to 85.3% and from 94.7 to 96.6% if the instantaneous production figure is used instead of the value obtained from the production curve.

### 2.3.2. Depth distribution of larvae

The total number of each zoeal stage caught at each depth (Table

Table 2.3

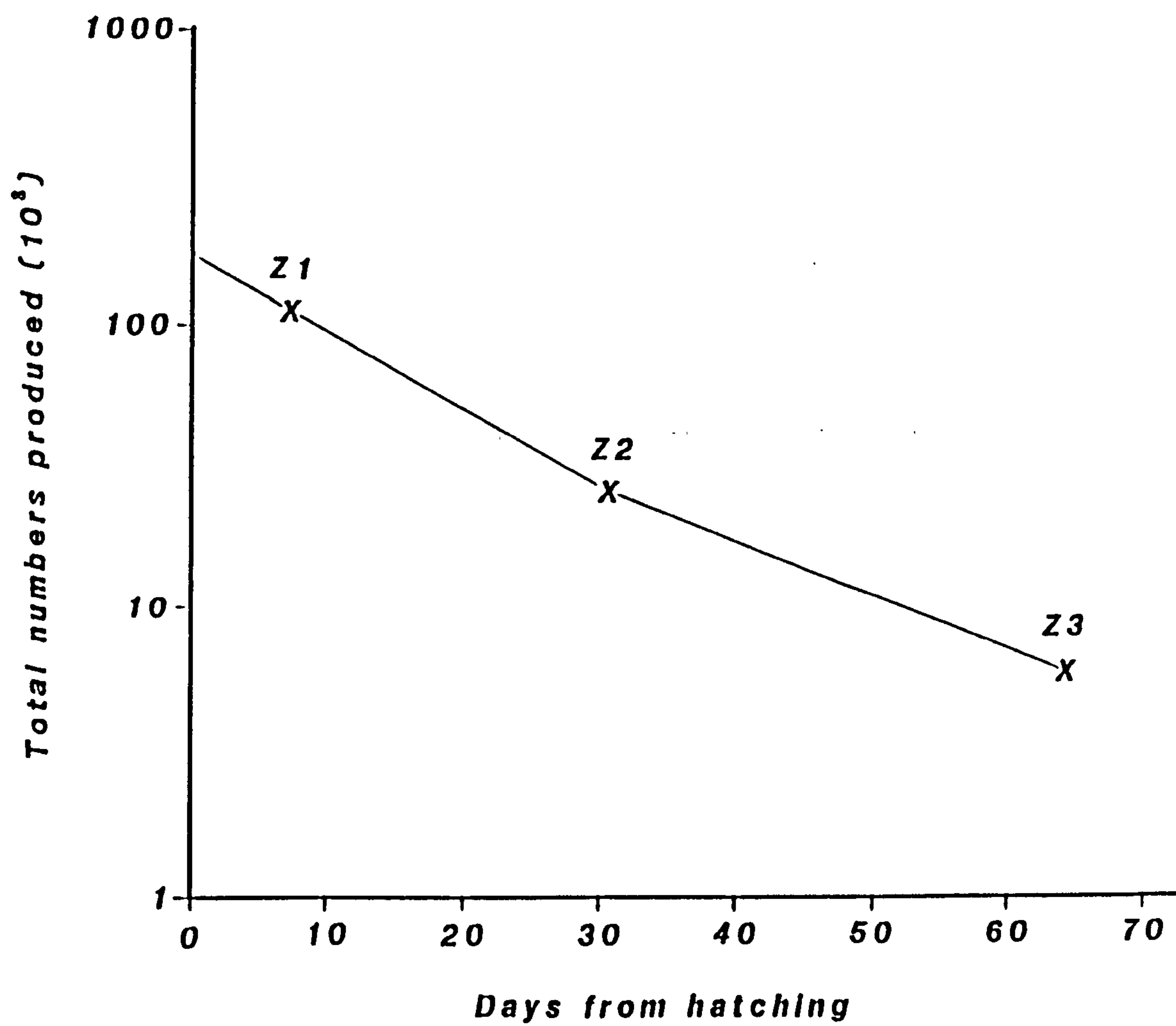
Seasonal production and mortality for the 3 zoeal stages.  
Z, the instantaneous coefficient of loss per day, was calculated from the equation presented in Section 2.2.1. Mean stage duration was calculated for the 3 zoeal stages by integrating the abundance estimates obtained for the 5 surveys with the corresponding mean sea temperature.

Zoeal Stage	Nos. produced (10 <sup>8</sup> )	% loss	Mean stage duration in days	Z	% Daily mortality
1	111.14	-	14.5	0.100	10.0
2	26.04	76.6	23.4	0.063	6.3
3	5.91	77.3	33.6	-	-
1 - 3	-	94.7	-	0.077	7.7

Figure 2.11

The survival curve for Nephrops zoeae in the Lower Firth of Clyde in 1984.





2.4) was converted to a percentage depth distribution curve over both 24h periods (Figure 2.14). For each 24h period the mean temperature and salinity readings were calculated for each depth and used to construct an 'average' thermocline and an 'average' halocline. As the depth distribution of the 1st and 2nd stage zoeae appeared similar and only two 3rd stage zoeae were caught the data for all 3 stages were combined for further analysis.

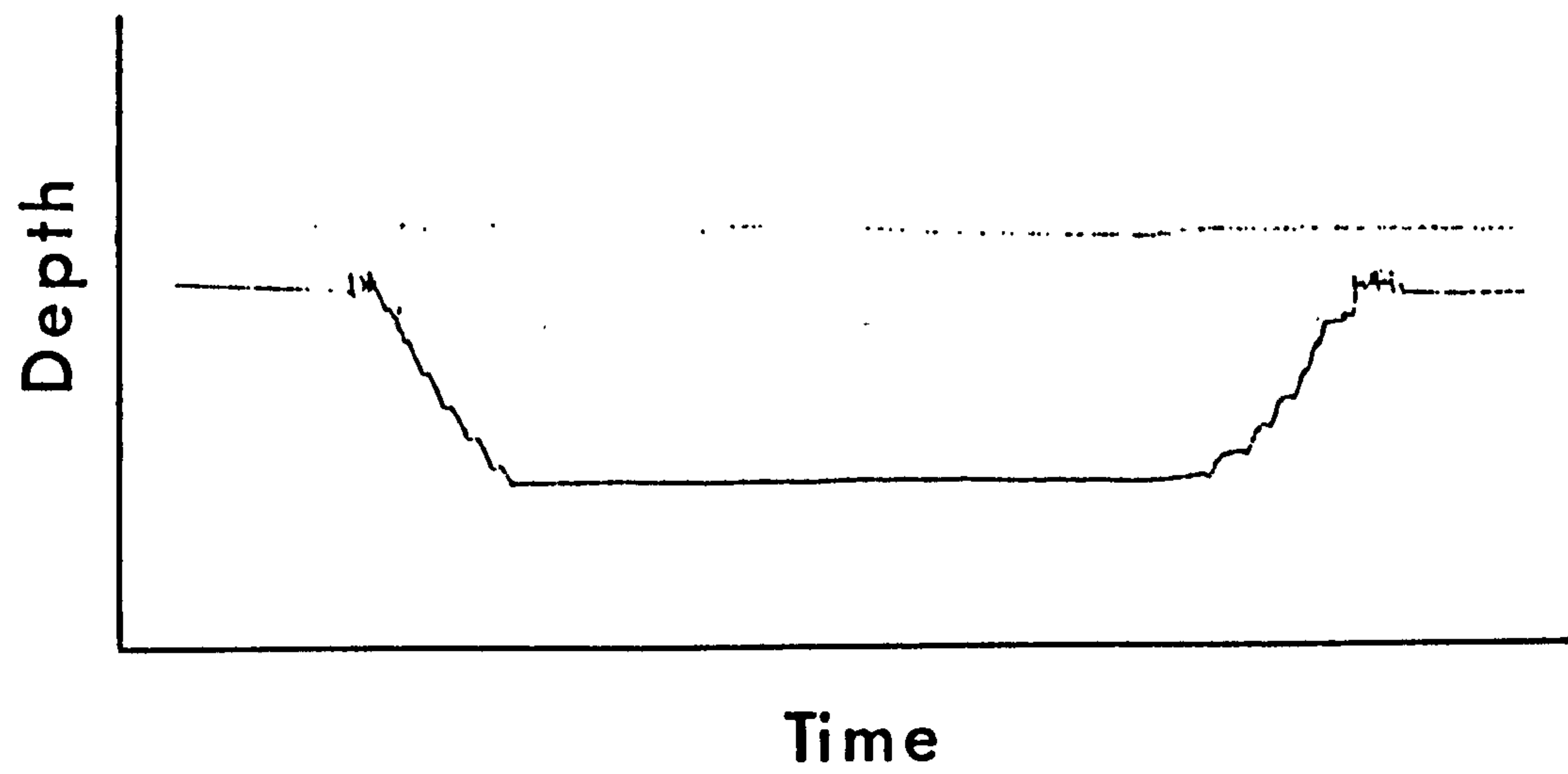
During both 24h periods very few larvae were captured at the surface, but a small proportion of the catch was taken at a depth of 5m (9.5%). The bulk of the larvae were caught by the nets sampling at 16 and 27m depth (57.8%). Below 38m the larvae were fairly evenly distributed with depth. The dominant depth preference therefore appears to have occurred immediately below the pycnocline (Figure 2.14).

Although the number of larvae caught for each tow was standardized for 850m<sup>3</sup> of filtered sea-water there was considerable variation between the total number of larvae captured at each sampling time (Figure 2.15). These changes do not appear to have been correlated with the tidal cycle (Figure 2.13) which suggests that the spatial distribution of the larvae in the vicinity of the sampling station was very patchy.

The depth profile of light attenuation measured on position (station B3) using the Licor sensor is illustrated in Figure 2.16. The light curve most closely resembles Jerlov's coastal water type 3 (Jerlov, 1968, 1976), the curve characterising the second clearest of the 5 coastal water types described by Jerlov. The light curve will

**Figure 2.12**

**Magnified trace of a typical recording made by the depth meter during the course of one complete tow.**



**Figure 2.13**

**The shape of the tidal curve corresponding to the (a) spring and (b) neap tide surveys. (Om = Chart Datum).**



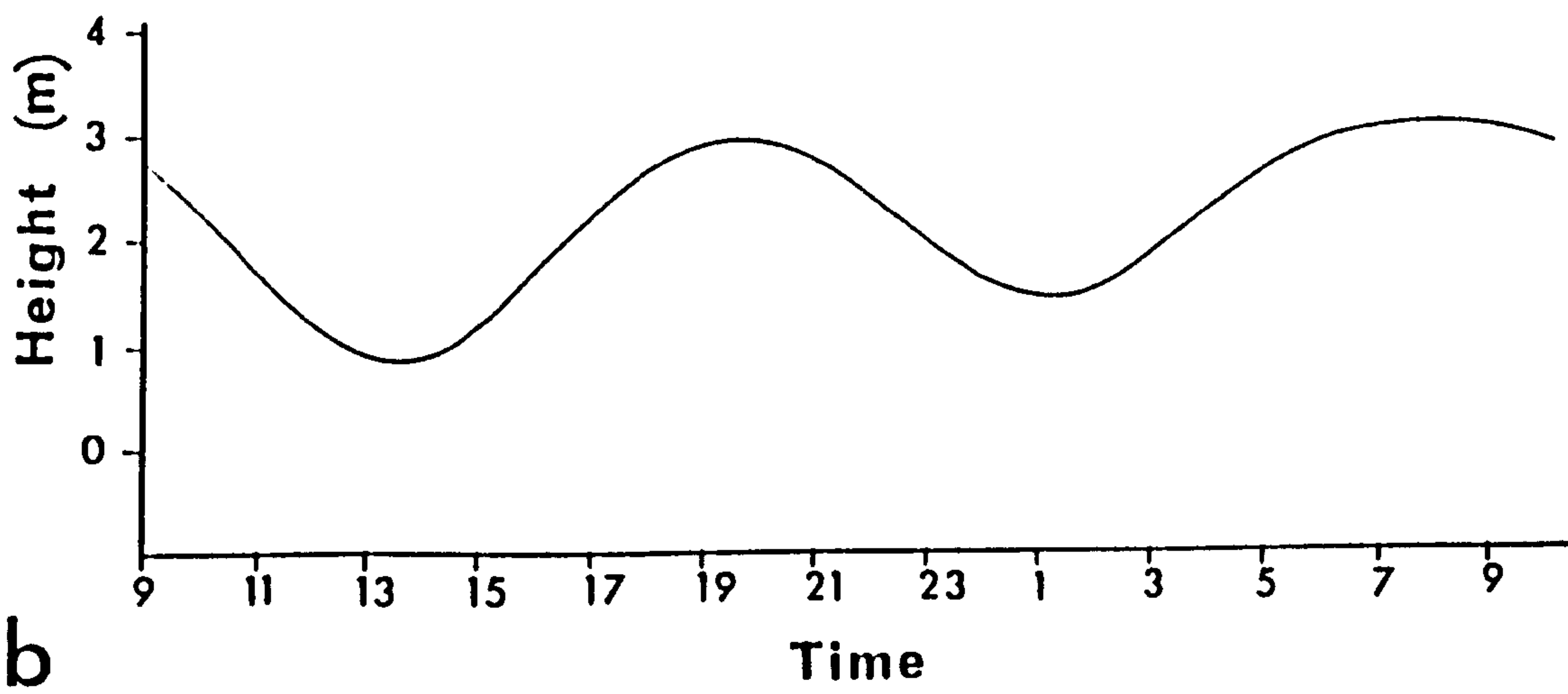
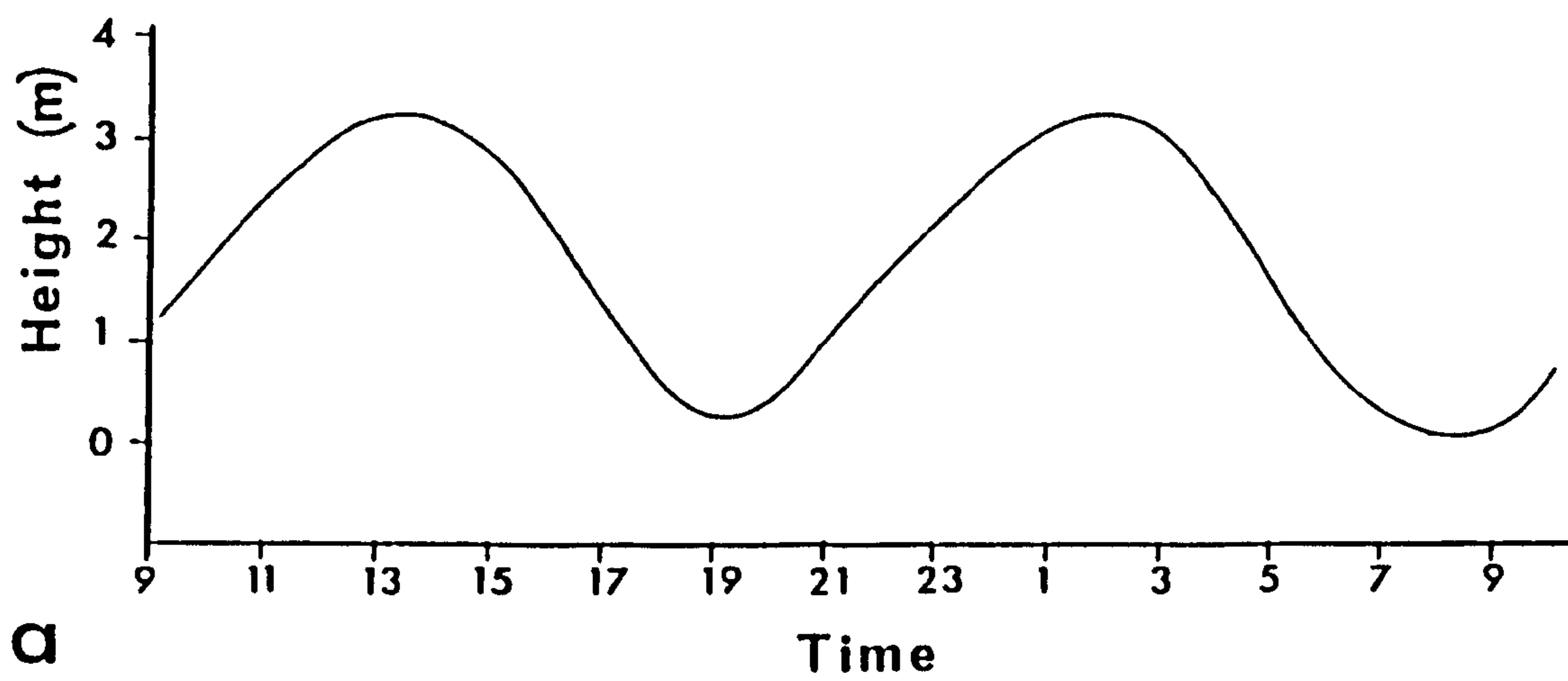


Table 2.4

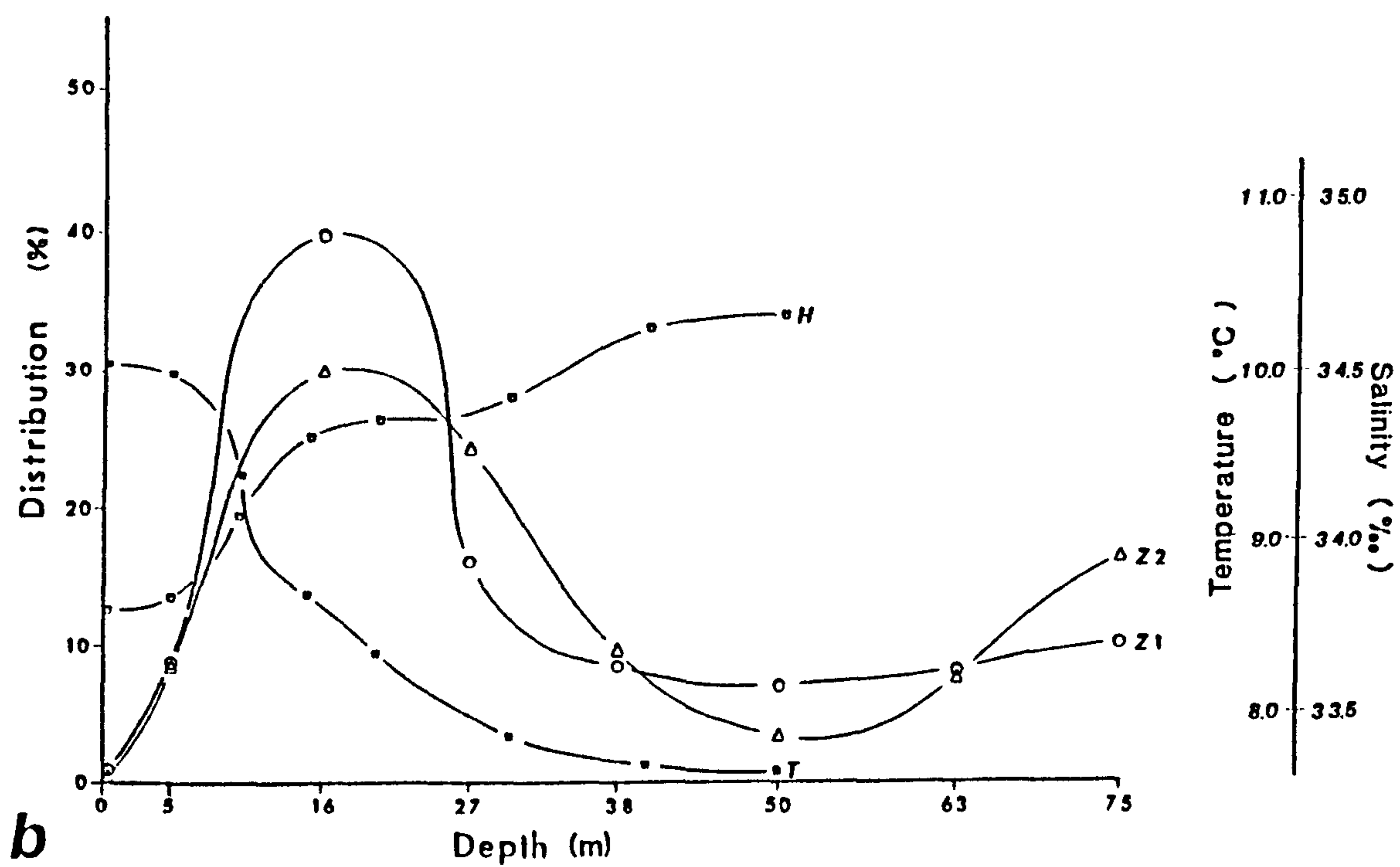
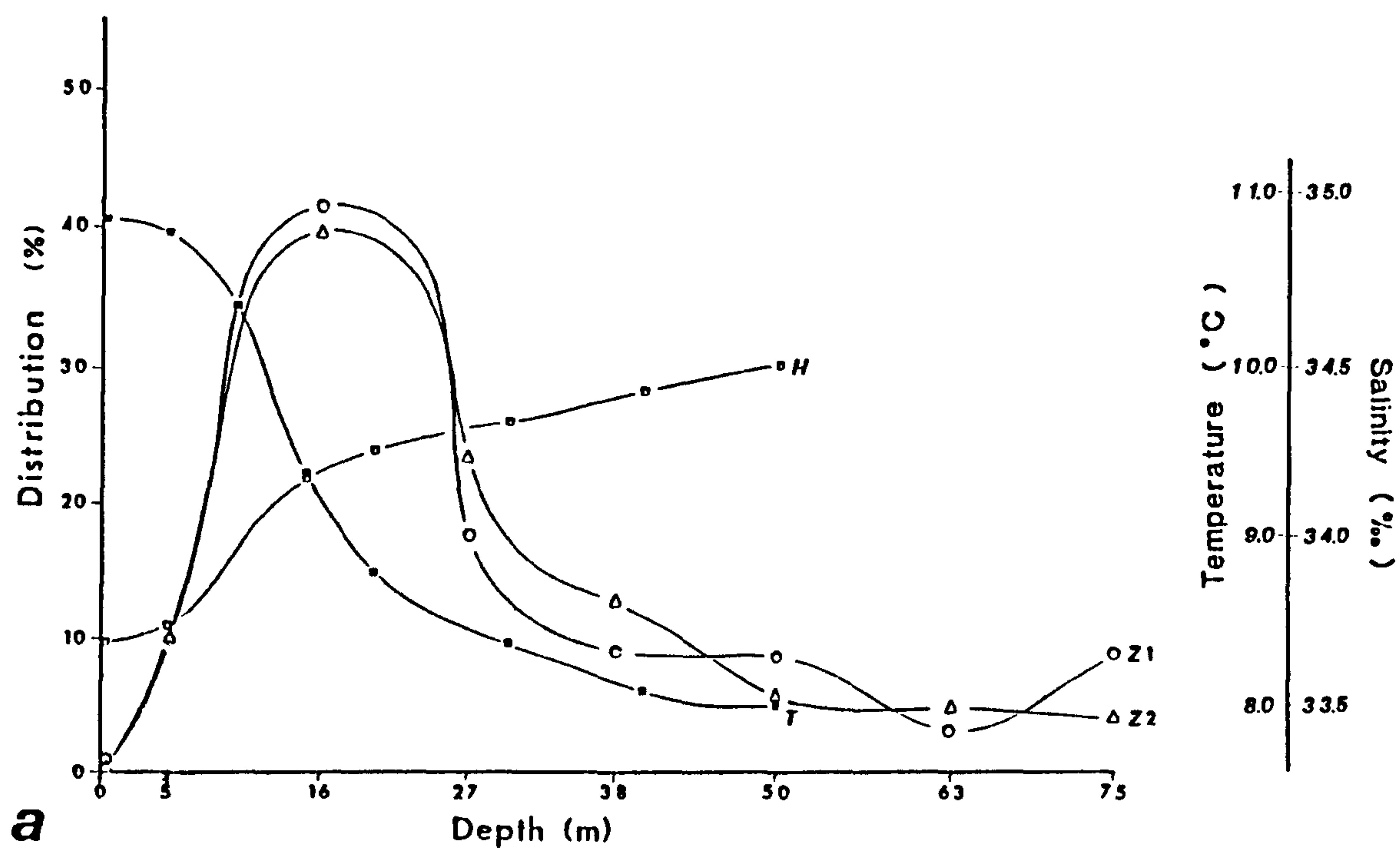
The cumulative number and percentage distribution of *Mephrops* zoeae captured at each sampling depth during the spring and neap 2<sup>nd</sup> surveys. Combined total values have also been calculated from the two surveys. Only 2 stage 3 zoeae were caught and are excluded from the Table.

Depth	Spring Tide (4-5/6/85)						Neap Tide (11-12/6/85)						Combined Total	
	Zoea 1		Zoea 2		Total		Zoea 1		Zoea 2		Total			
	No.	% Distn.	No.	% Distn.	No.	% Distn.	No.	% Distn.	No.	% Dist.	No.	% Distn.	No.	% Distn.
0	8	0.53	0	0	8	0.49	12	0.74	0	0	12	0.69	20	0.60
5	151	9.96	10	10.00	161	9.96	146	9.06	10	8.33	156	9.00	317	9.47
16	633	41.75	40	40.00	673	41.65	646	40.11	36	30.00	683	39.41	1356	40.49
27	273	18.01	23	23.00	296	18.32	253	15.70	29	24.17	282	16.27	578	17.26
38	139	9.17	13	13.00	152	9.41	137	8.50	12	10.00	149	8.60	301	8.99
50	132	8.71	5	5.00	137	8.48	115	7.14	4	3.33	119	6.87	256	7.64
63	46	3.03	5	5.00	51	3.16	134	8.32	9	7.50	144	8.31	195	5.82
75	134	8.84	4	4.00	138	8.53	168	10.43	20	16.67	188	10.85	326	9.73
Σ	1516		100		1616		1611		120		1733		3349	

Σ = Total.

**Figure 2.14**

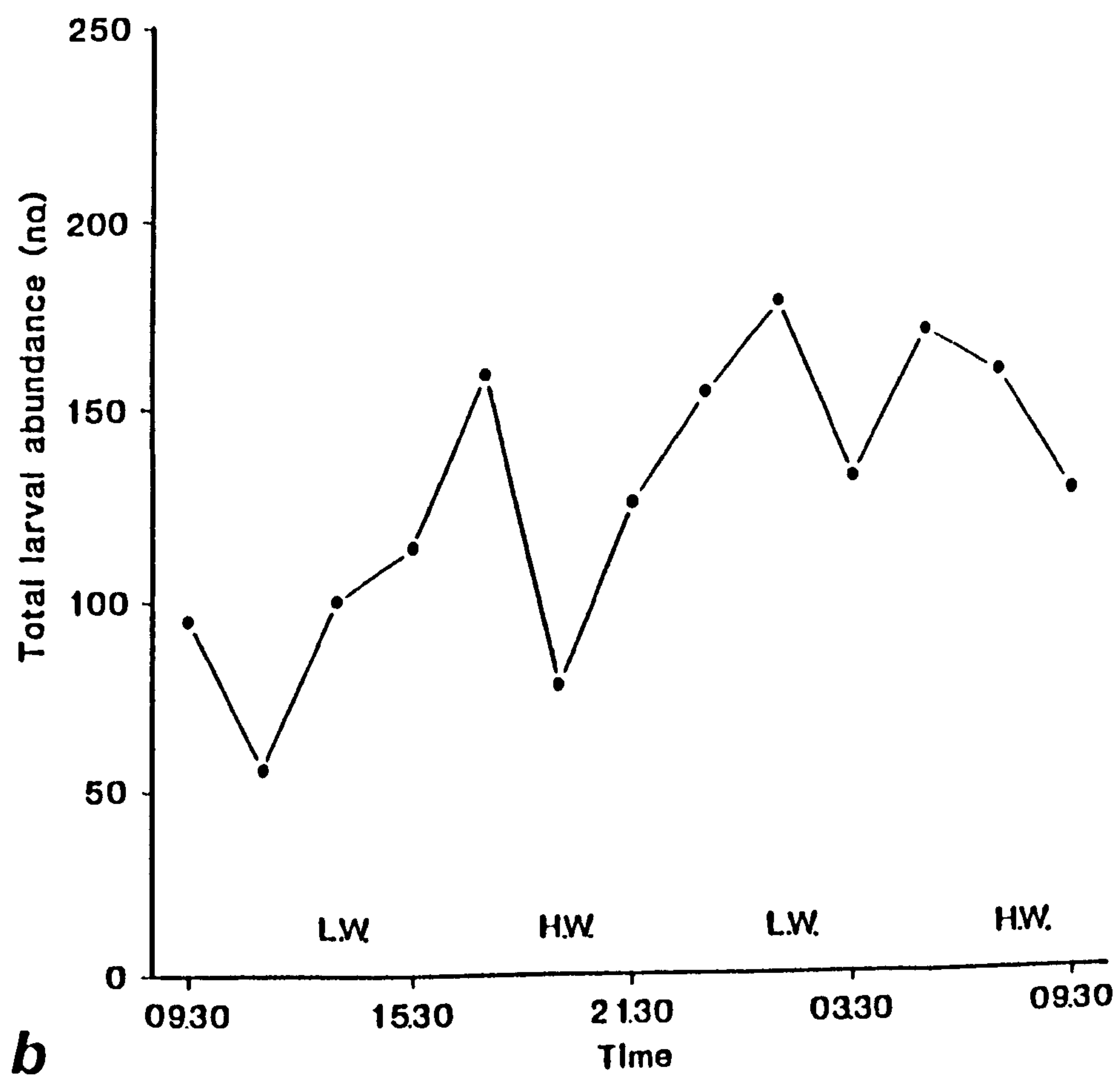
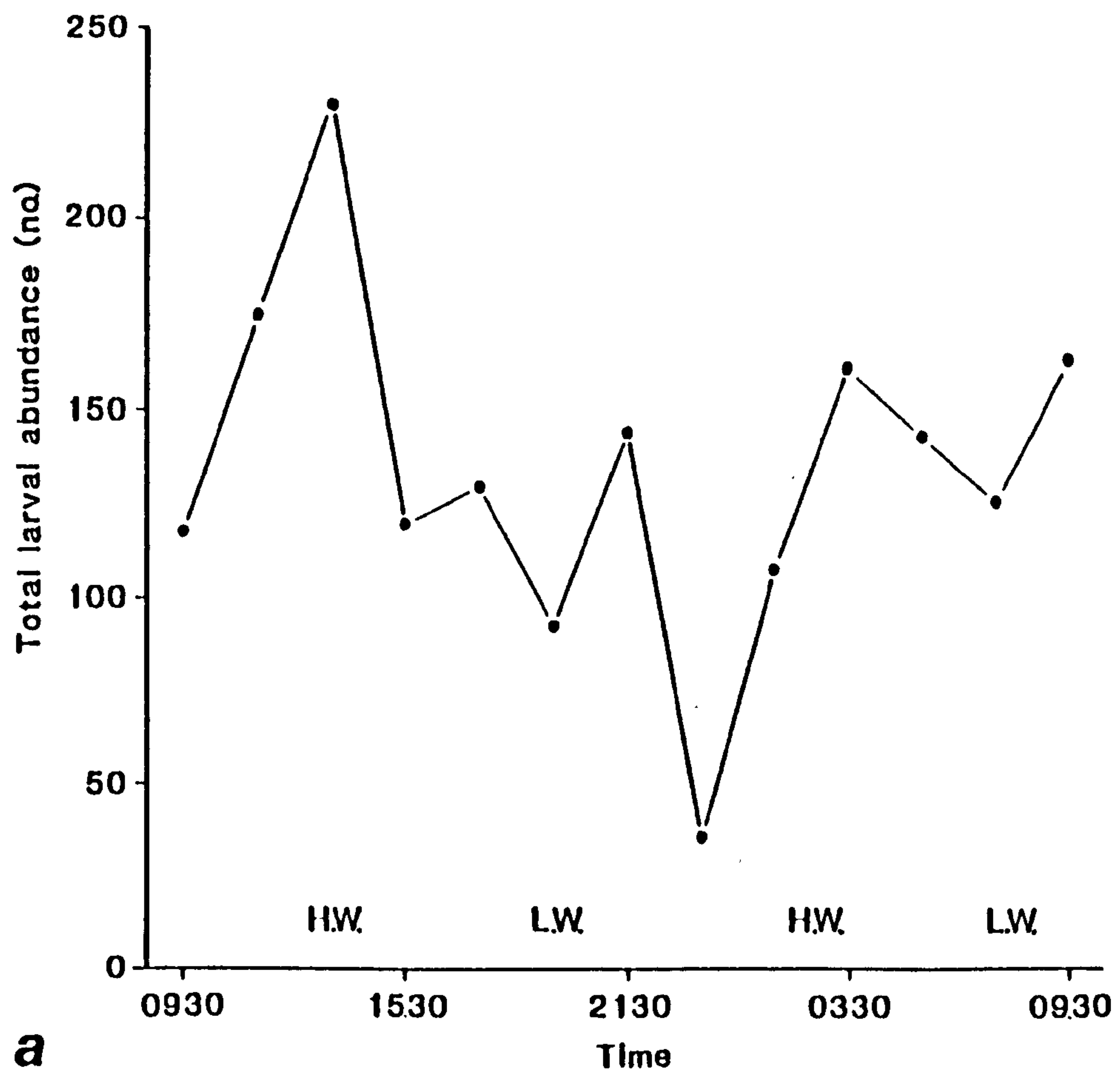
**Percentage depth distribution of zoeal stages 1 (Z1) and 2 (Z2) integrated over all sampling periods for the (a) spring and (b) neap tide surveys. The mean temperature and salinity readings were calculated for each depth for the two 24h periods and used to construct an 'average' thermocline (T) and halocline (H) for the spring and neap tidal periods.**



### Figure 2.15

Total larval abundance for all depths at each sampling time during the (a) spring and (b) neap tide surveys after the number of larvae caught during each haul was standardized for 850m<sup>3</sup> of filtered sea-water.

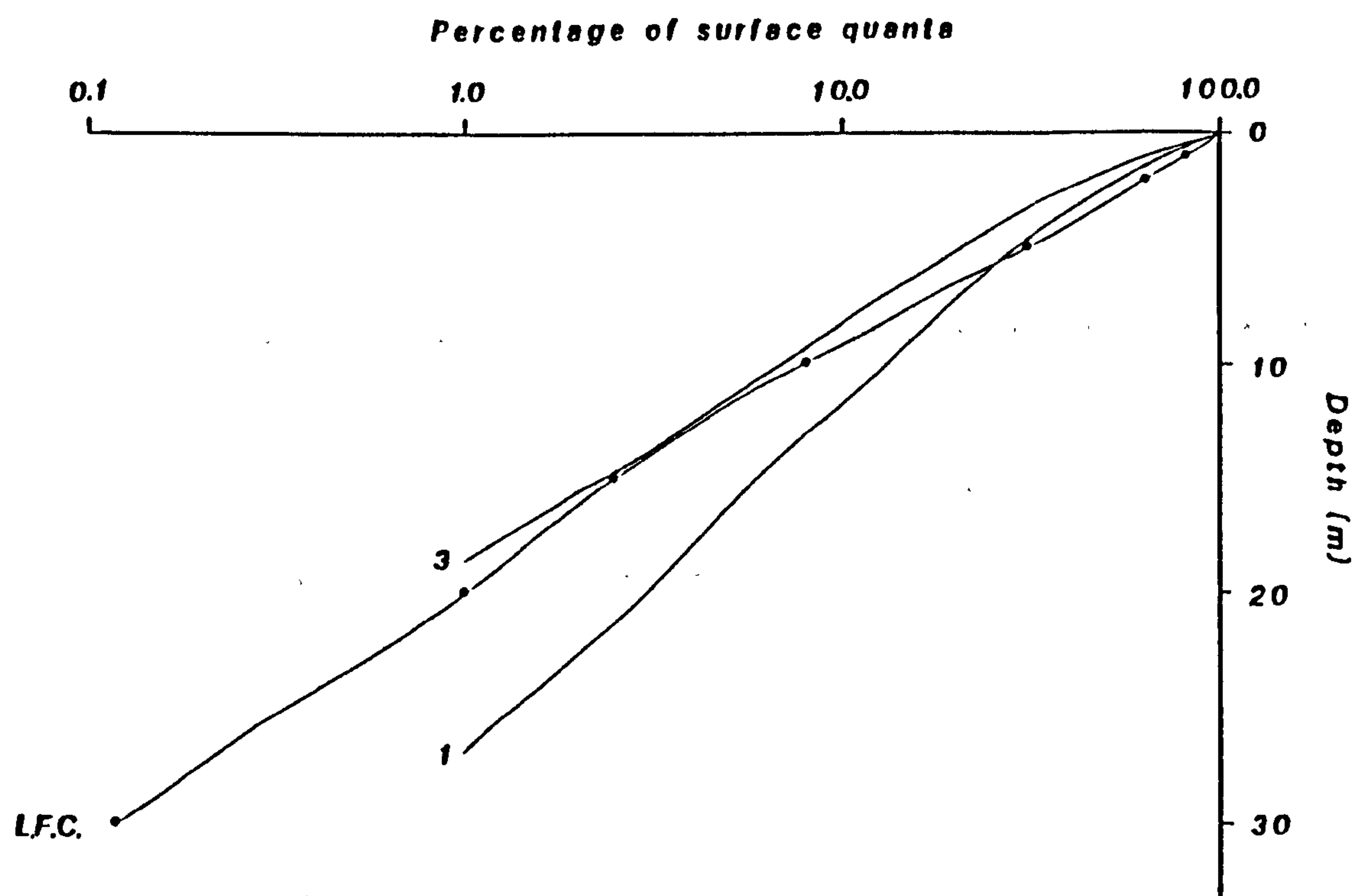
H.W. = High Water, L.W. = Low Water





**Figure 2.16**

Depth profiles of quanta irradiance as percent of surface value for the Lower Firth of Clyde and Jerlov's coastal water types 1 and 3, taken from Jerlov (1976). The profile constructed for the Lower Firth of Clyde was derived from measurements taken over the wavelength band 400 - 700nm whereas those formed by Jerlov (1976) were over the wavelength band 350 - 700nm.



show some seasonal variation depending upon particulate and dissolved substances in the water. The change in attenuation will tend to be greatest over the shortest wavelengths (Jerlov, 1968). Light is best transmitted within the wavelength band 400 - 600nm for coastal water. The attenuation coefficient  $K$  over the depth range 20 - 30m was  $0.206 \text{ m}^{-1}$ .

For both the spring and neap surveys, light, temperature and salinity curves were constructed from the measurements taken over each 24h period at selected depths (0,10,20 and 50m) to provide an indication of the diel changes occurring throughout the water column (Figures 2.19 and 2.26 respectively).

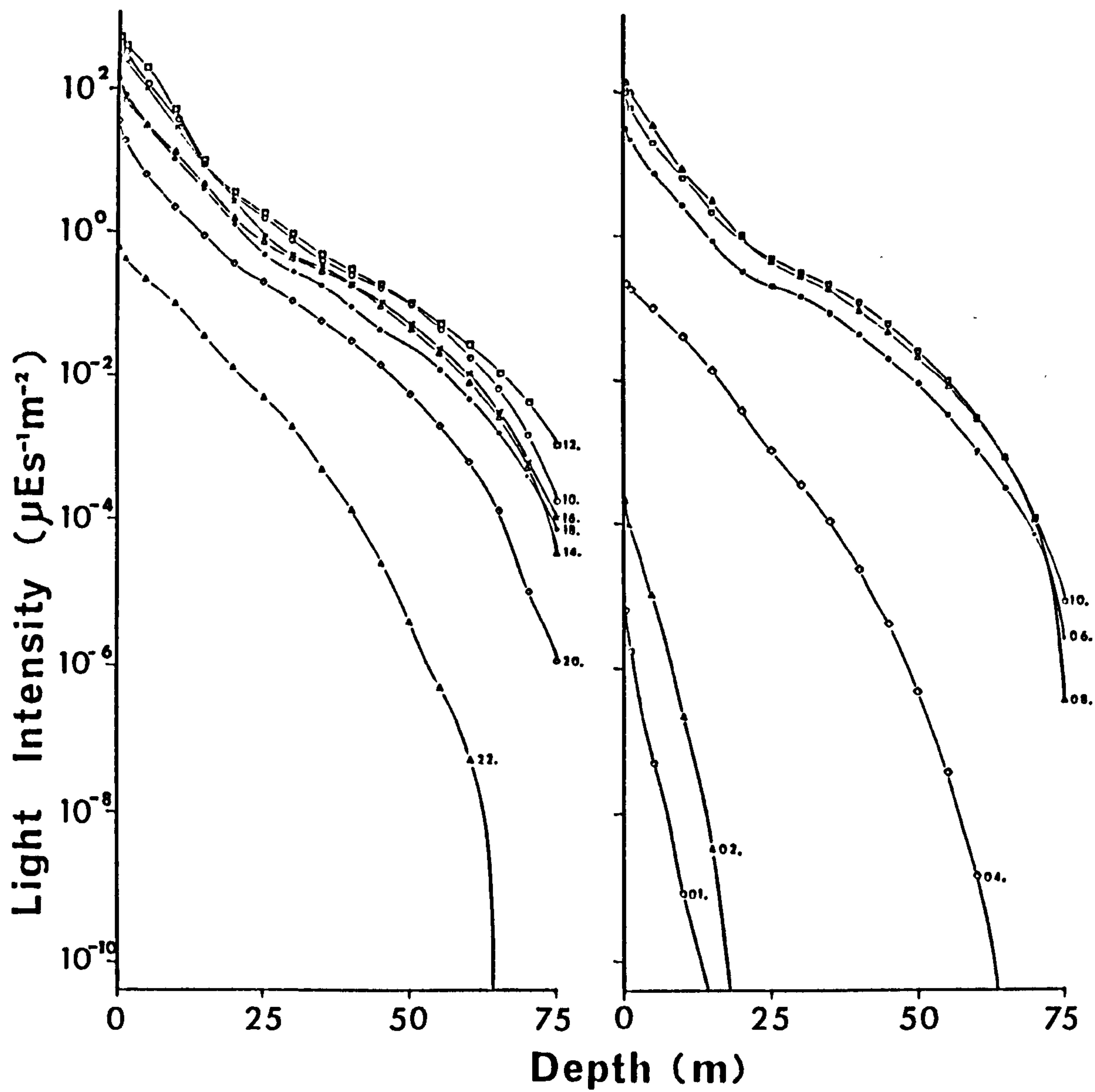
### 2.3.3. Diel survey during spring tides

#### Environmental conditions

As expected, the shape of the light curves constructed from the attenuation profiles (Figure 2.17) were similar at 4 selected depths (Figure 2.19). The light levels (measured with a photomultiplier tube and fitted Wratten 64 filter) at each depth (0, 10, 20 and 50m) remained fairly constant between 1000 and 1800h, approximately 200, 40, 3 and  $0.06 \mu\text{Es}^{-1}\text{m}^{-2}$  respectively. The Licor quantum meter, which measures high levels of light intensity more accurately than the photomultiplier tube, was used to determine light intensity at the surface. During this period of the survey  $1,420 \mu\text{Es}^{-1}\text{m}^{-2}$  was recorded. This was within the expected intensity range for a bright sunny day (Shelton et al., 1985). Sunset occurred at 2150h and a minimum light intensity was recorded at approximately 2400h (surface intensity =

**Figure 2.17**

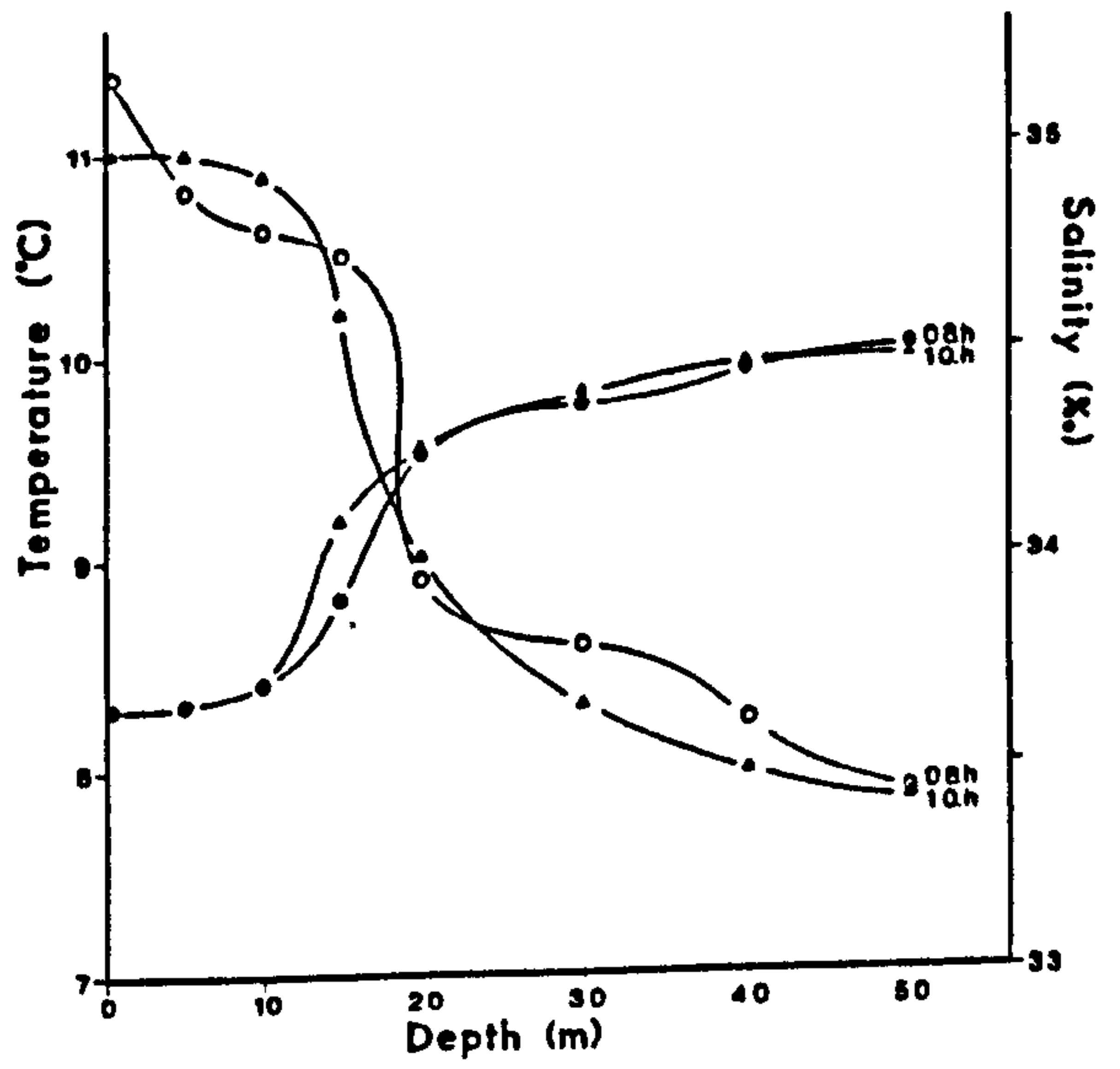
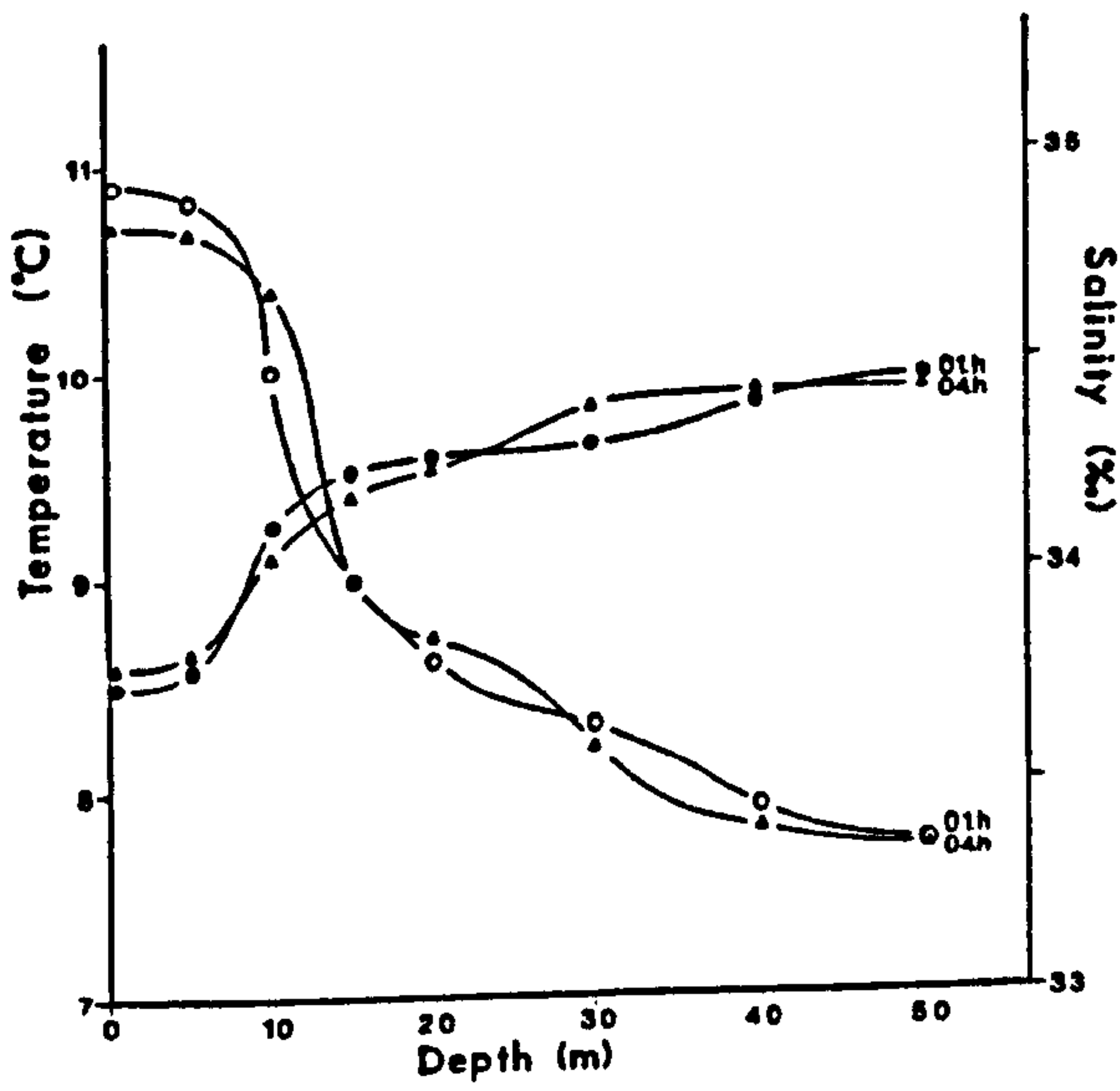
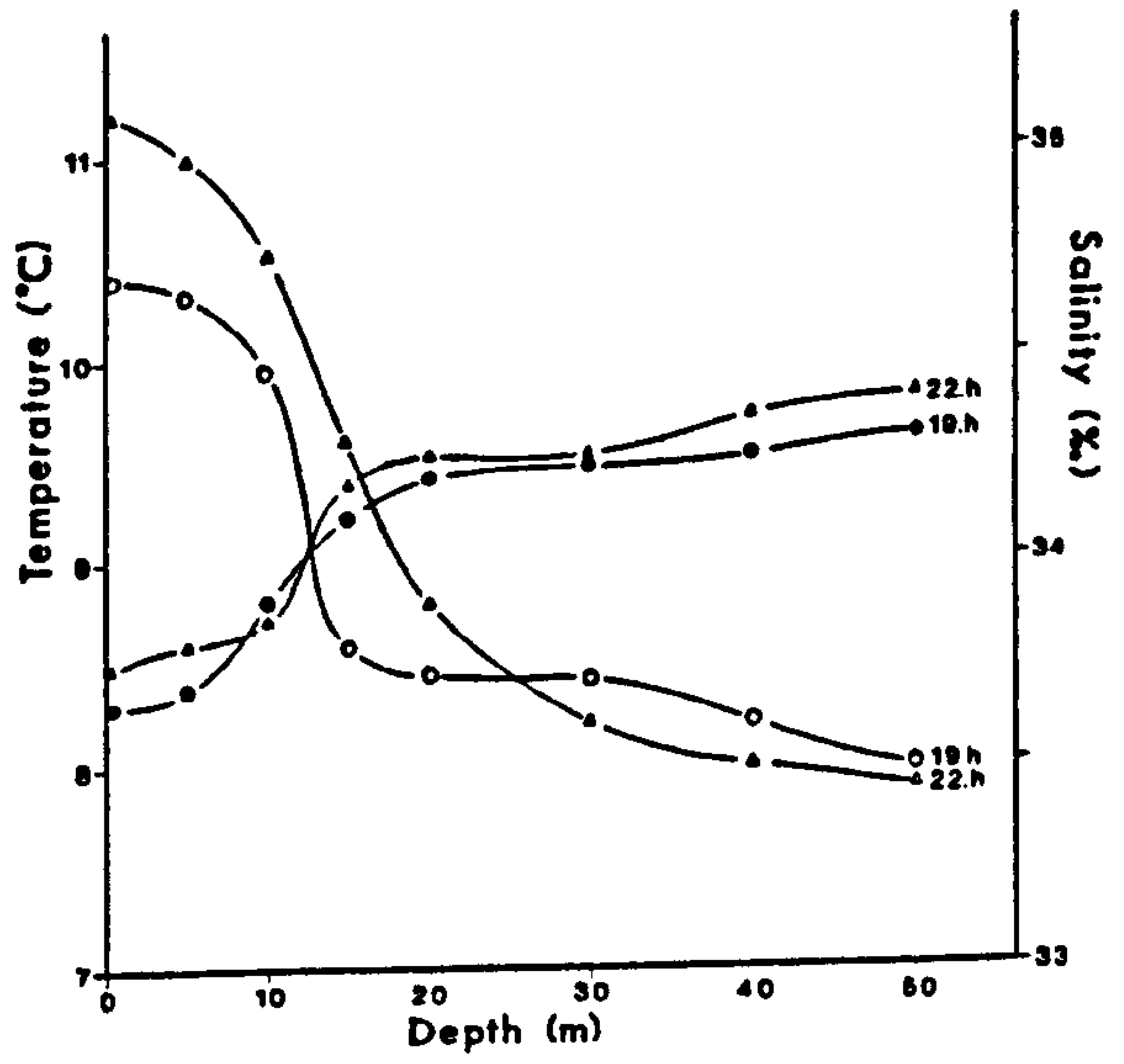
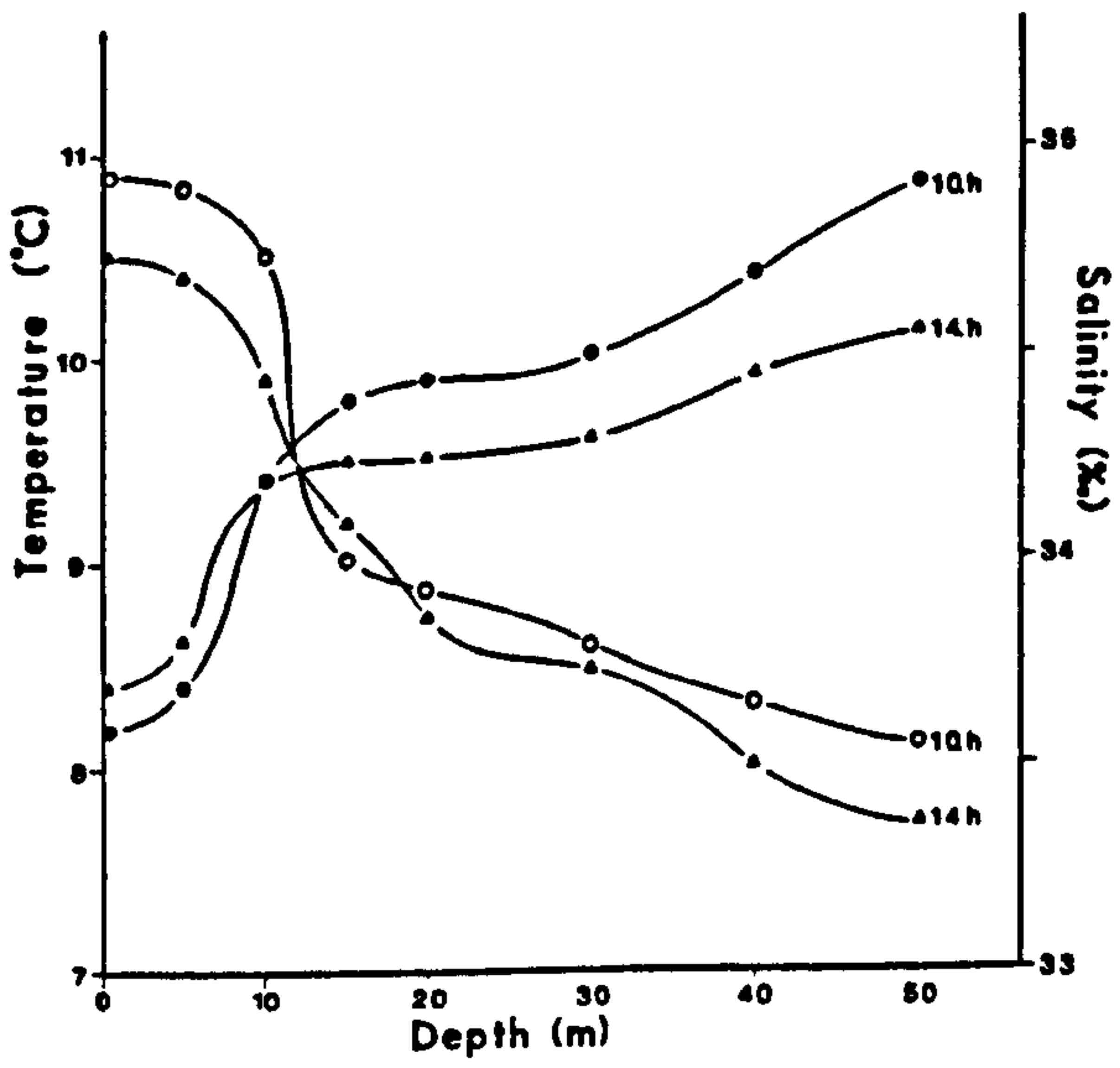
**Light attenuation profiles at different times during the spring tide survey. The numbers assigned to each curve denote the time (hour) at which the measurements were taken.**



**Figure 2.18**

The position of the thermocline (open symbols) and halocline (closed symbols) at different times during the spring tide survey. The numbers assigned to each curve denote the time (hour) at which the measurements were taken.

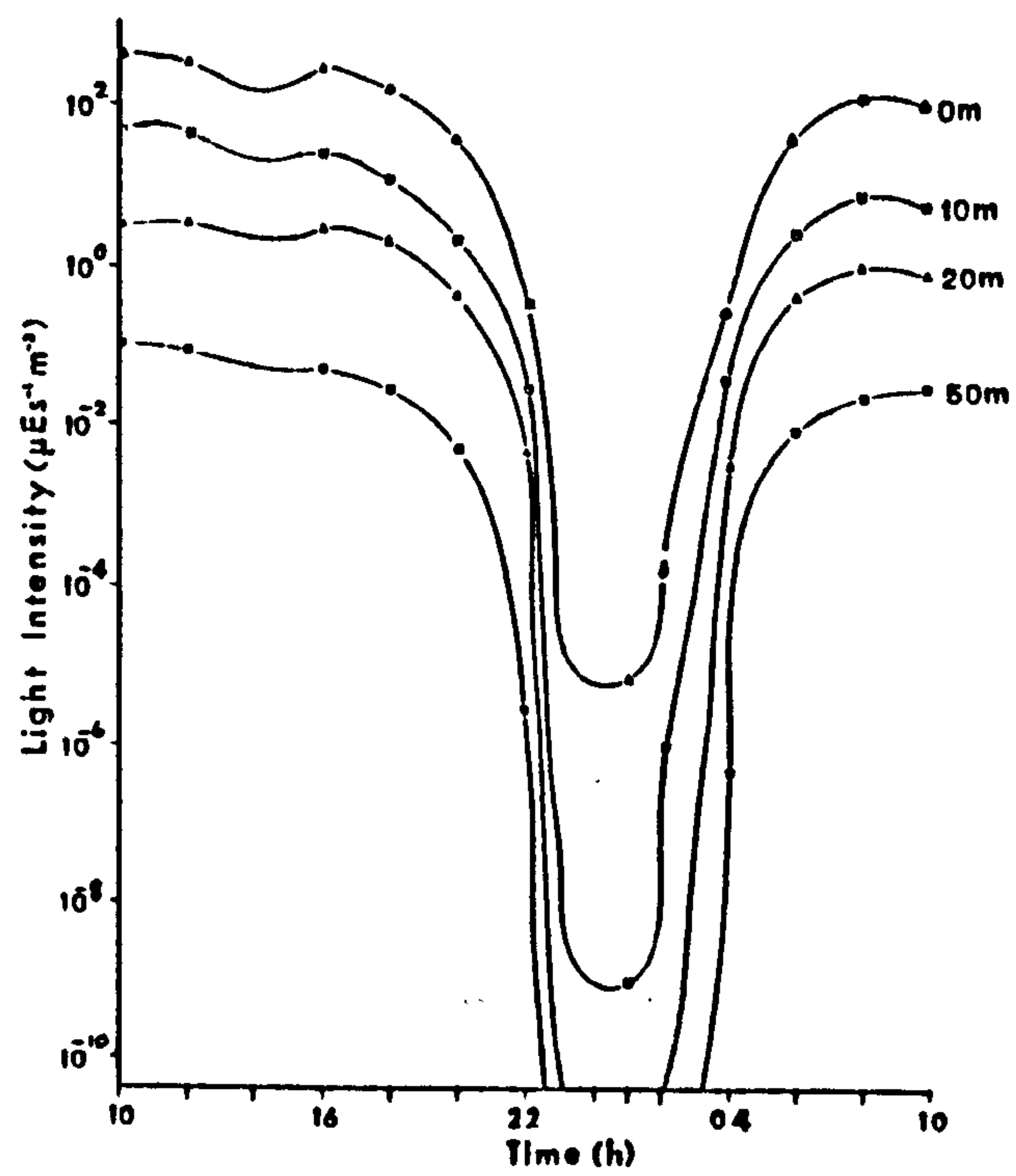




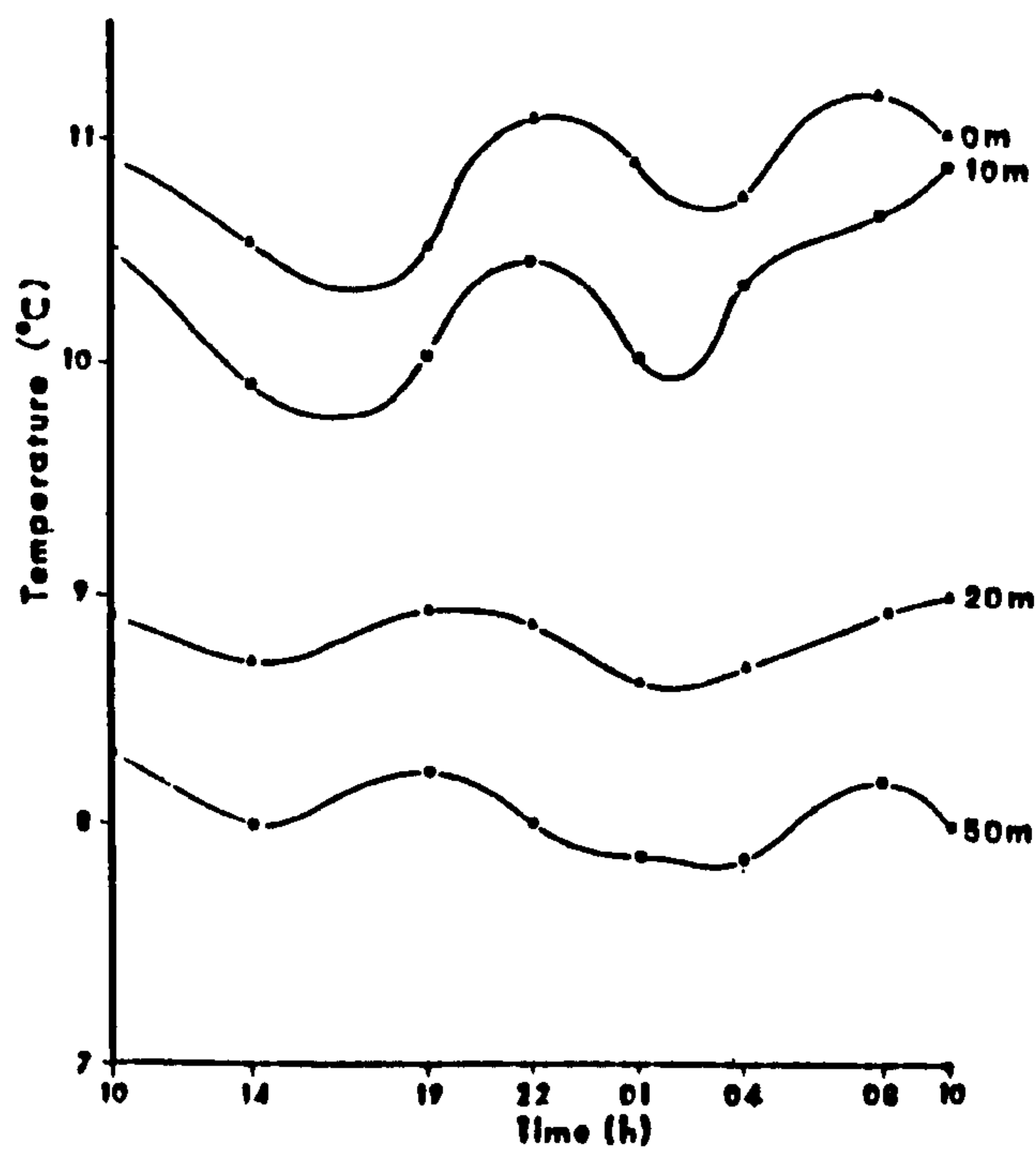
**Figure 2.19**

**The (a) light intensity, (b) temperature and (c) salinity at 0, 10, 20 and 50m depth during the spring tide survey.**

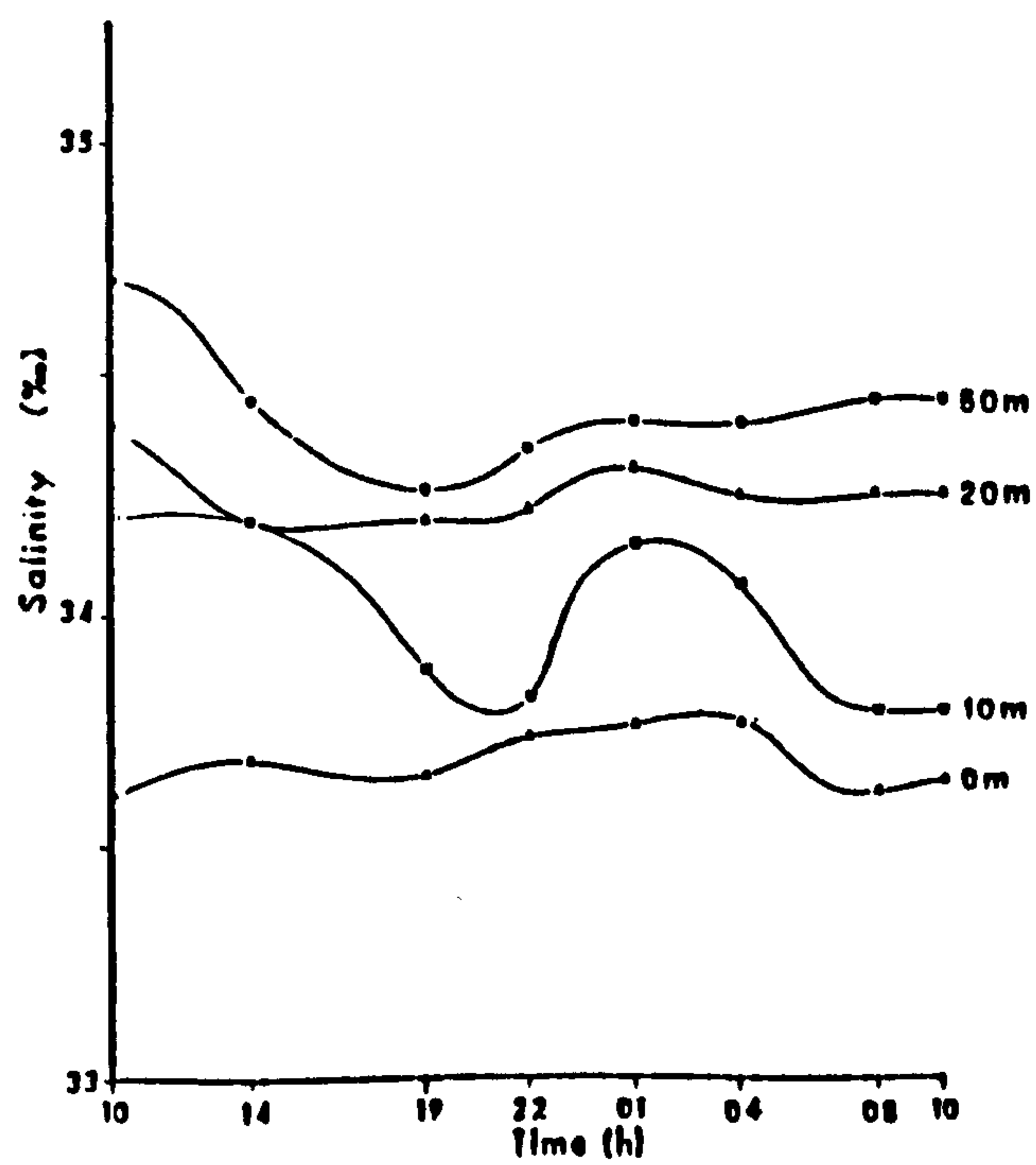
a



b



c



$8.0 \times 10^{-6} \mu\text{Es}^{-1}\text{m}^{-2}$ ). Sunrise occurred at 0427h and by 1000h light intensity had returned to its original level, completing the symmetrical shape of the light curves.

The halocline occurred between 5 and 20m depth (Figure 2.18) and the cyclical changes which were recorded at 10m depth (Figure 2.19c) appear to be correlated with the tidal trace (Figure 2.13a). Greatest salinity was recorded during both periods of high water (1330 and 0200h) and lowest salinity during both periods of low water (1930 and 0800h).

As expected, the thermocline occurred at approximately the same position as the halocline (5 - 20m) (Figure 2.18). Greatest temperature fluctuations occurred in the upper regions of the water column (0 and 10m) (Figure 2.19b). The temperature of the water below the thermocline (20 and 50m) was colder and more stable. The cyclical changes in temperature which occurred in the upper regions of the water column, like those of salinity, can be correlated with the tidal trace (Figure 2.13a). Warmest temperatures were recorded during both periods of low water (1930 and 0800h) and coldest temperatures during both periods of high water (1330 and 0200h). It therefore appears that the temperature changes in the upper regions of the water column (above the thermocline) were not caused by short-term diel changes in the ambient air temperature but by the tidal movement of water masses of differing temperatures.

#### Larval abundance

The mean depth of the larvae (for all depths combined) at each

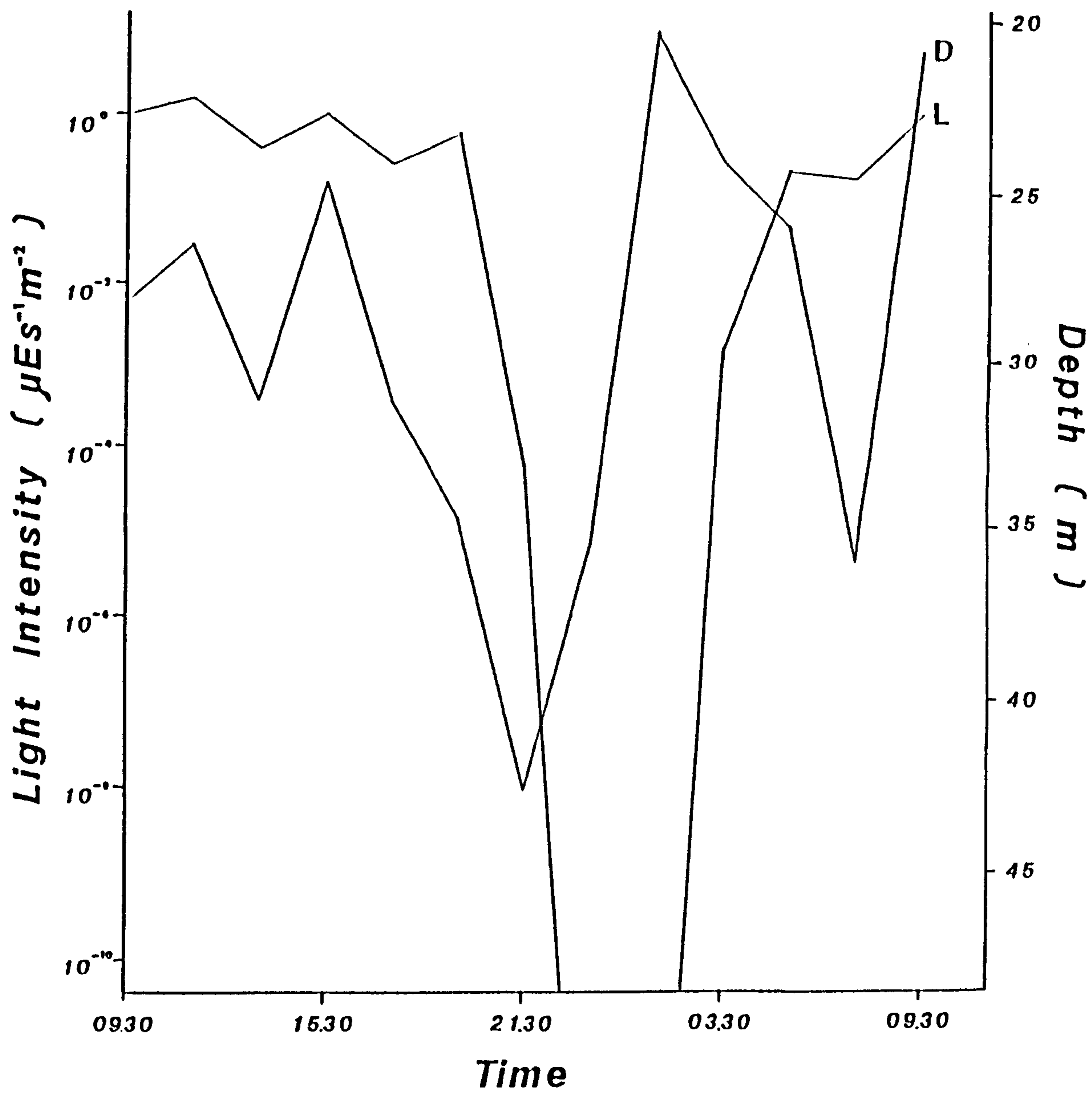
sampling time throughout the 24h period and, the corresponding light intensity at each mean depth, is shown in Figure 2.20. During the daylight hours (0930 - 1930h and 0530 - 0930h) the mean larval depth fluctuated between 20 - 35m. This corresponded to a light intensity range of  $0.06 - 1.60 \mu\text{Es}^{-1}\text{m}^{-2}$ . The variability in the position of the mean larval depth over this period does not appear to be attributable to light intensity and bears no discernible relationship to the tidal cycle. It therefore seems most likely that these fluctuations have resulted from sampling variability. Although depth monitoring indicated that the nets were consistently fished at a similar position in the water column some variability will have arisen, including that caused by the tidal variables. The patchy distribution of the larvae (Figure 2.15a) and relatively large sampling interval of the nets (11 - 12m) would tend to create artificial differences between the larval distributions. The reduction in light intensity in the evening coincided with an increase in the mean larval depth, which occurred at 42.6m at 2130h. During the hours of darkness (2130 - 0130h) there was a decrease in the mean larval depth.

Larval abundance is presented as a function of time and depth in Figure 2.21 and 2.22 respectively. These results have also been compounded to form a 3 dimensional representation for larval abundance against time and depth in Figure 2.23. Between 0930 - 1730h peak larval abundance remained at around 16m depth. Most larvae were located between 6 and 37m depth (ie. the majority of the larvae were caught by the nets fishing at 16 and 27m depth) and the highest concentration of larvae seemed to occur immediately below the pycnocline. Between 1730 to 1930h there was a slight reduction in light intensity (Figure 2.19) and weak evidence of a change in

**Figure 2.20**

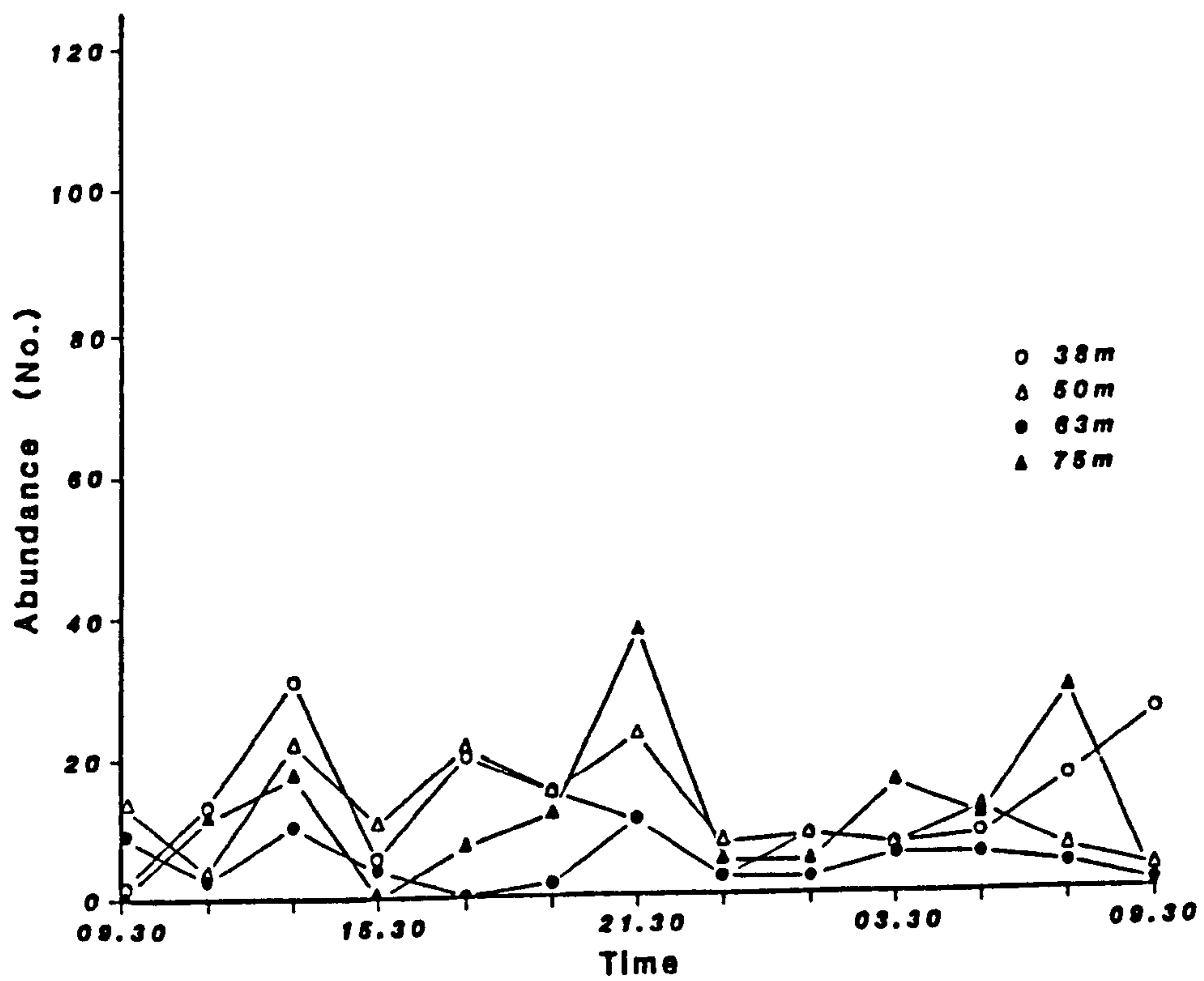
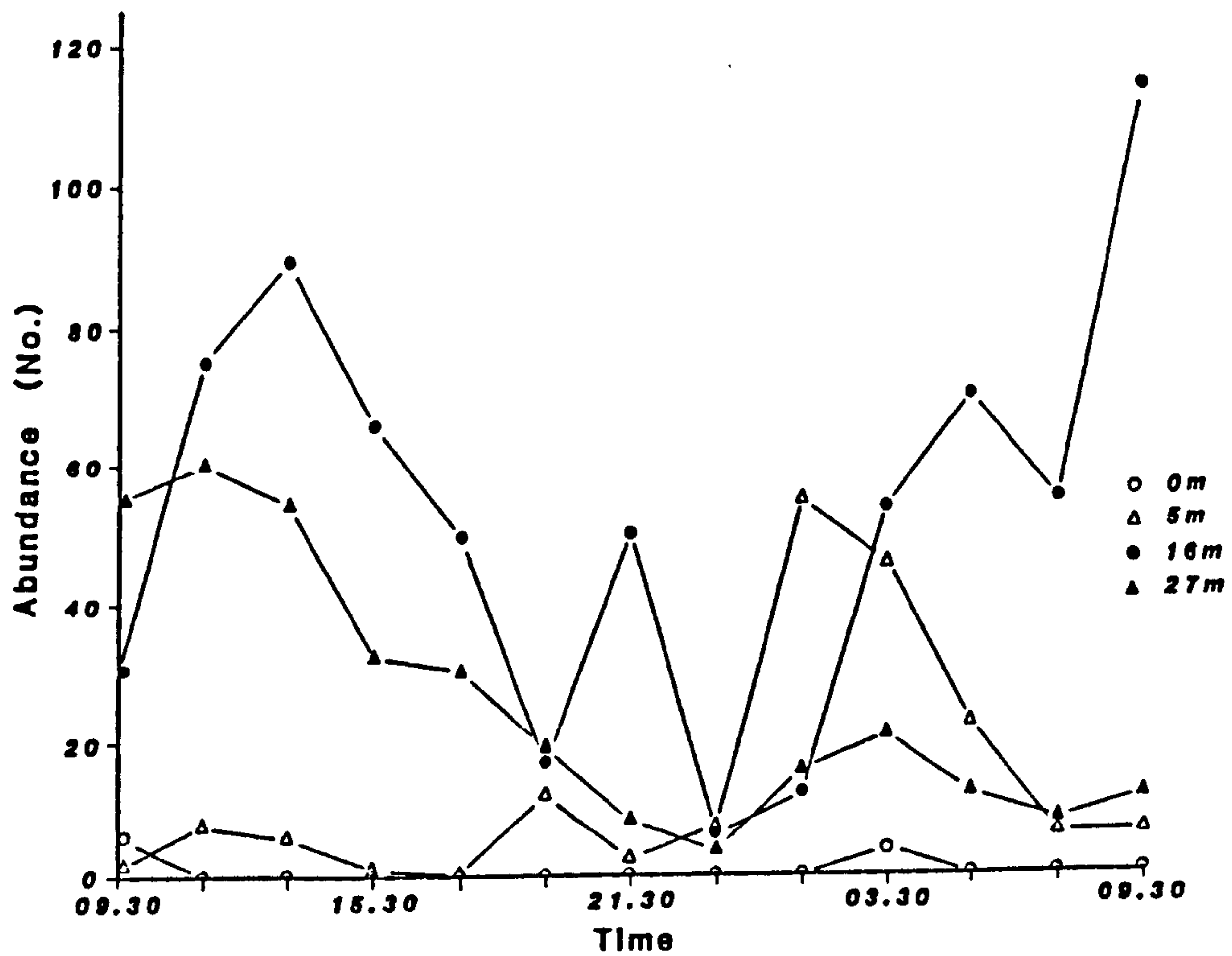
**The mean larval depth (integrated over all sampling depths) calculated for each sampling time during the spring tide survey (D). The corresponding light intensity for the mean larval depth at each sampling time is also shown (L).**





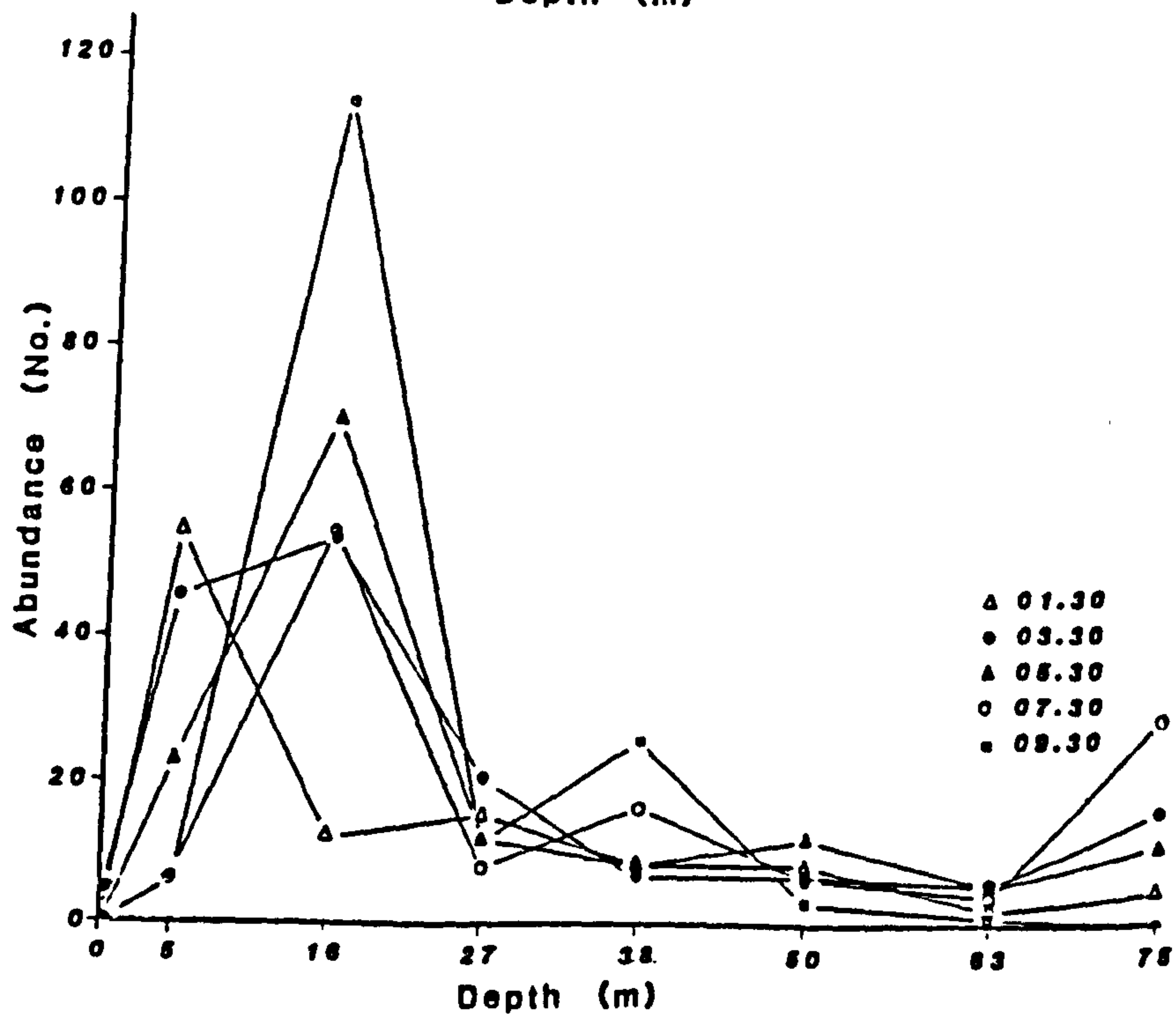
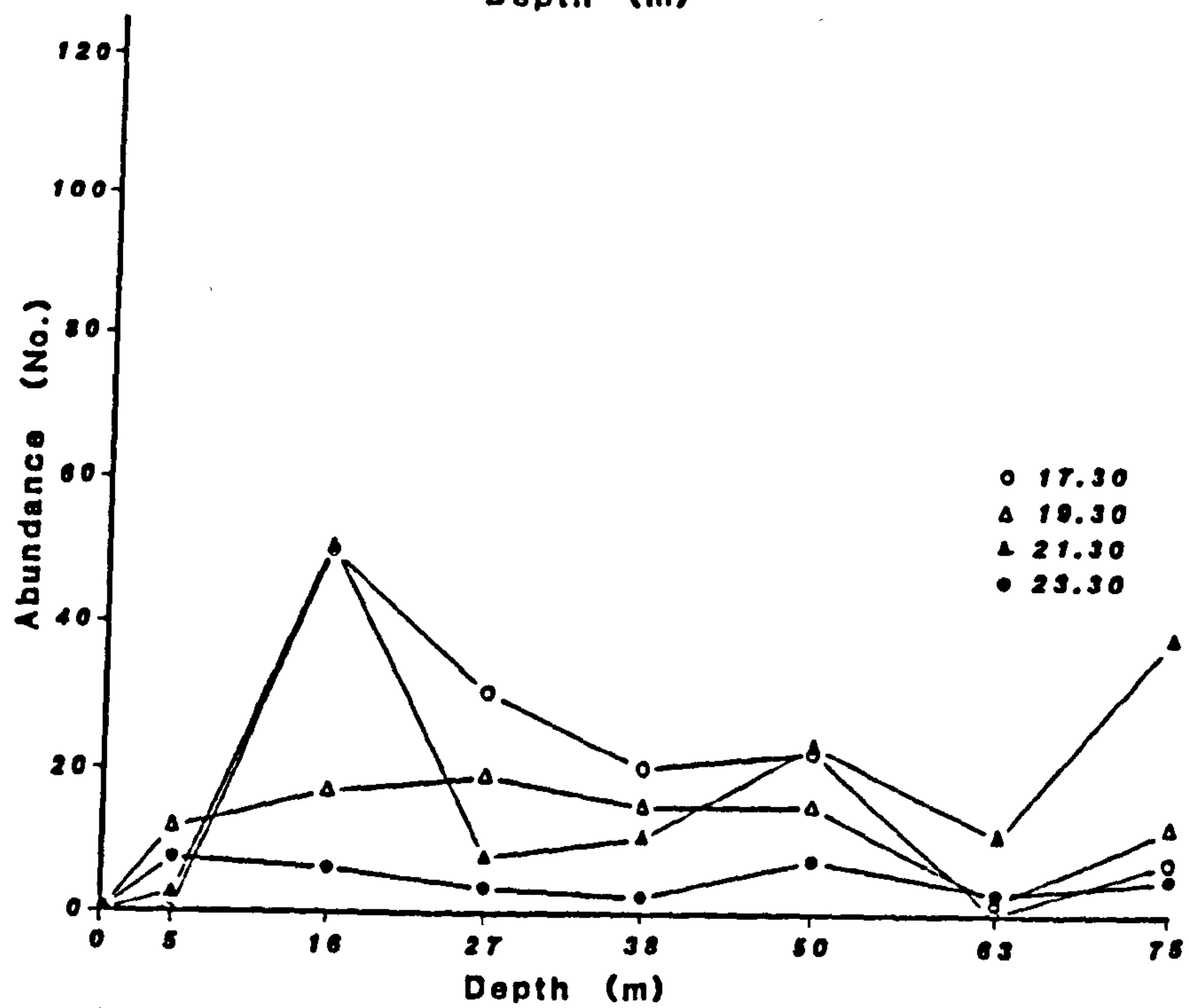
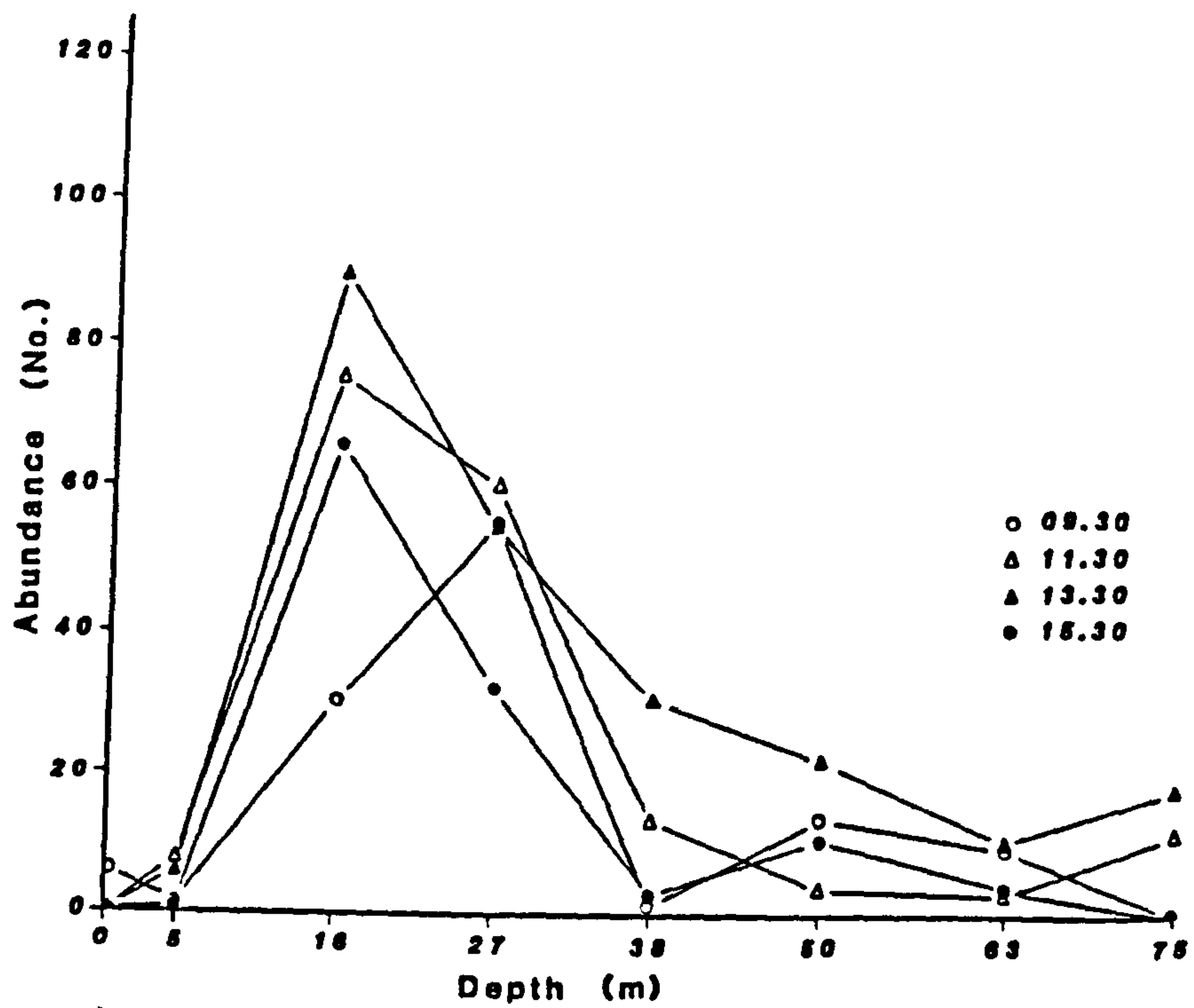
**Figure 2.21**

**Larval abundance during the spring tide survey at the eight different sampling depths.**



**Figure 2.22**

**Larval abundance at the eight different sampling depths during the spring tide survey.**



distribution with a small proportion of the larvae ascending to 5m depth and the larvae becoming generally more evenly distributed between 5 - 50m depth.

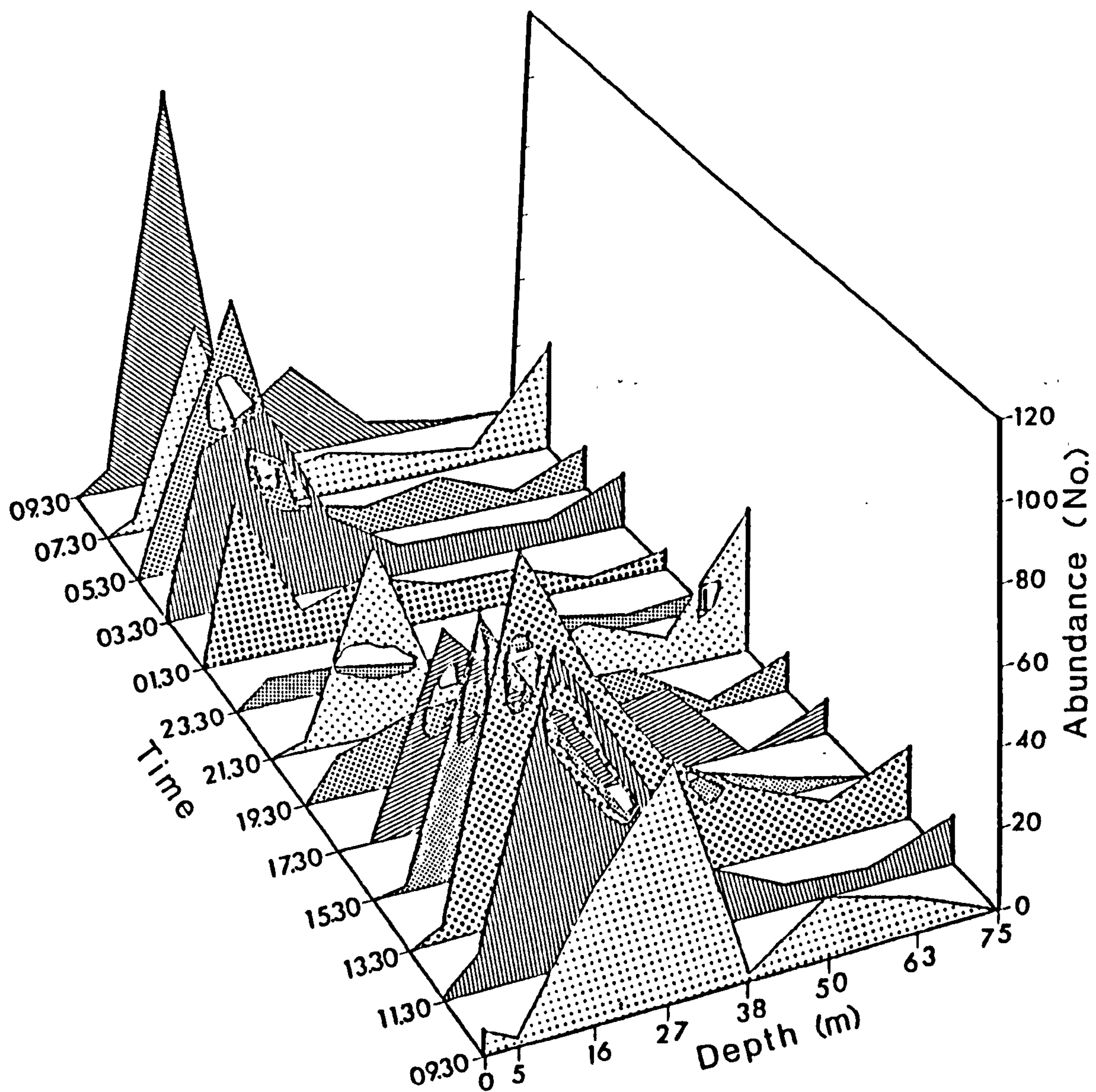
During the following 2h there was a sharp reduction in light intensity (Figure 2.17 and 2.19a). By 2130h a distinct peak was again evident at 16m depth. There (also, however,) appeared to be evidence of a concentration of larvae at the bottom of the water column at this time. The concentration of larvae near the sea bed caused the mean larval depth to be registered at its deepest position for the 24h period (Figure 2.20). Hatching activity in Nephrops occurs at night (Moller and Branford, 1979) and this time of the season corresponds to peak hatching. The concentration of larvae at the bottom of the water column may therefore have resulted from synchronised hatching activity rather than a downward movement of larvae from the upper regions of the water column.

Between 2130 and 2330h the sharp reduction in light energy levels continued (surface minimum of approximately  $8.0 \times 10^{-6} \mu\text{Es}^{-1}\text{m}^{-2}$ ). Relatively few larvae were caught in the 2330h sample and there is limited evidence of a change in distribution. The larvae seemed to be fairly evenly distributed throughout the water column which indicates that some sinking may have occurred between 2130 and 2330h. During the following 2h there was little change in light intensity but by 0130h some upward migration appeared to have occurred. Peak larval abundance was recorded at 5m, a depth at which relatively few larvae had been caught during the earlier hauls. Larval distribution was concentrated around this depth with few caught at any other region of the water column.



**Figure 2.23**

**A three dimensional representation of larval distribution and abundance during the spring tide survey.**



Over the next 2h (0130 - 0330h) there was a sharp increase in light energy levels (Figure 2.17 and 2.19a). By 0330h the larval peak had reformed at its 'normal' daytime position of 16m and most larvae were distributed between 6 - 37m depth. During the final 6h of the survey (0330 - 0930h) there was little evidence of any change in larval distribution and any apparent variability between hauls probably resulted from sampling error and/or the patchy distribution of the larvae. However, there was some evidence of a second concentration of larvae at the bottom of the water column at 0730h which may indicate further larval hatching. The variation between the two larval abundance curves recorded for 0930h (beginning and end of the survey), which resulted in an 8m difference in the position of the mean depth of the larvae (Figure 2.20), is further evidence of sampling variability. However, the general larval distribution for the two 0930h hauls was similar (Figure 2.23), with the highest concentration of larvae occurring between c. 10 - 30m depth.

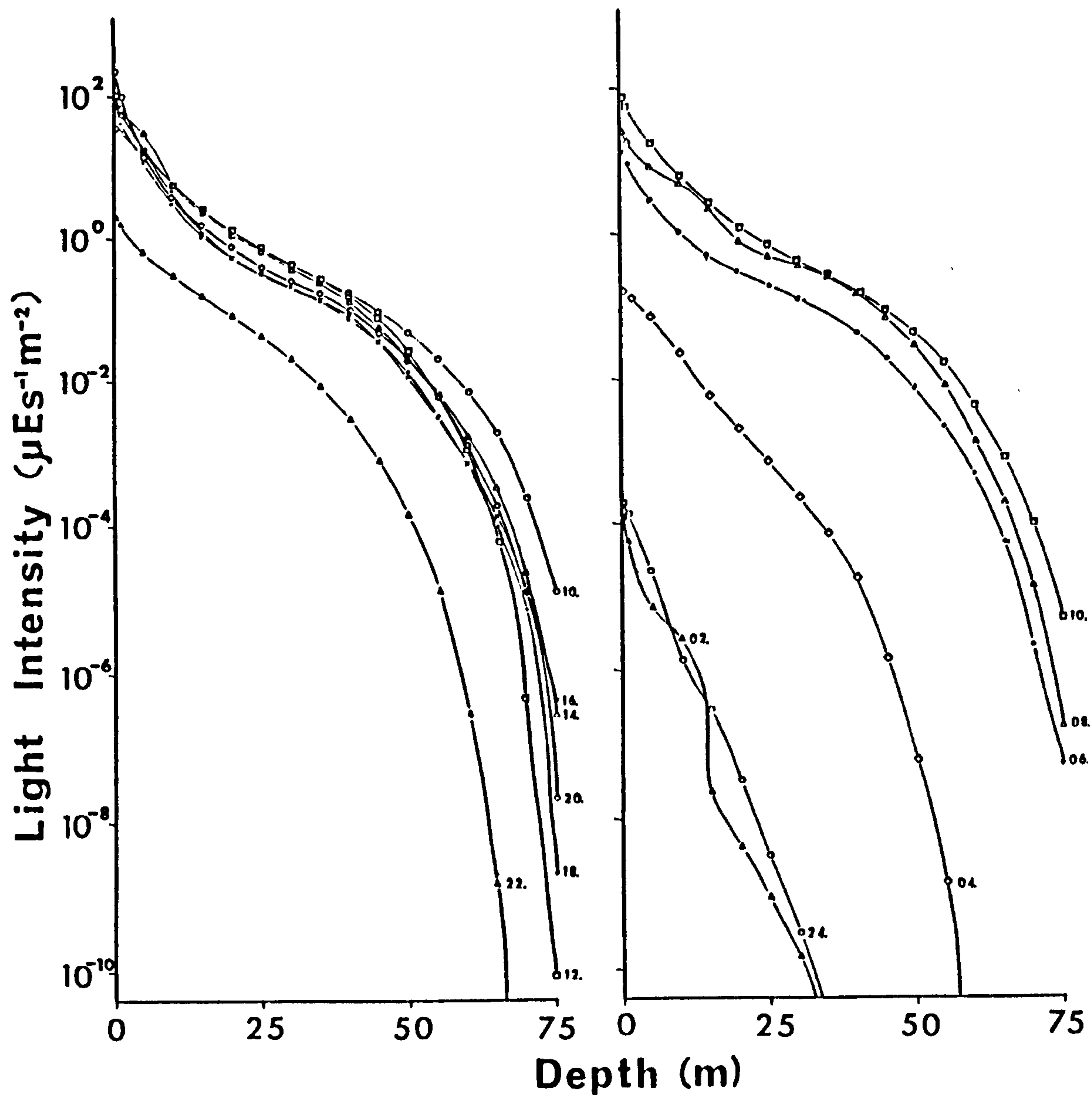
#### 2.3.4. Diel survey during neap tides

##### Environmental conditions

As expected, the shape of the light curves constructed from the attenuation profiles (Figure 2.24) was similar at 4 selected depths (Figure 2.26). Although the general shape of the light curves was similar to those recorded during the spring survey, weather conditions were more overcast during the neap tide survey. The values recorded during the neap tide survey were lower between 1000 - 1800h, higher between 2000 - 0100h and slightly lower between 0200 and 0600h than those recorded during the spring tide survey. The slightly higher

**Figure 2.24**

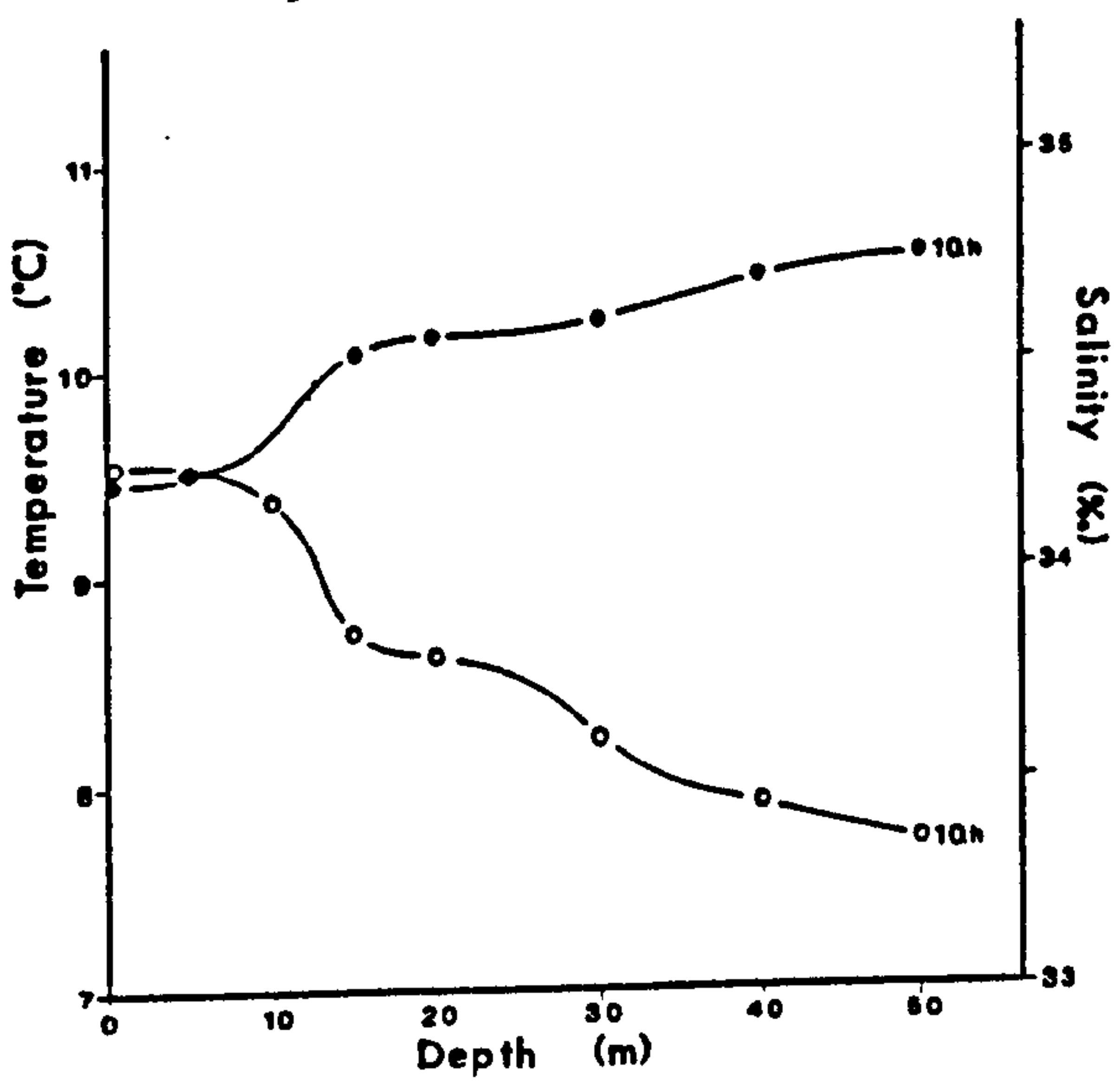
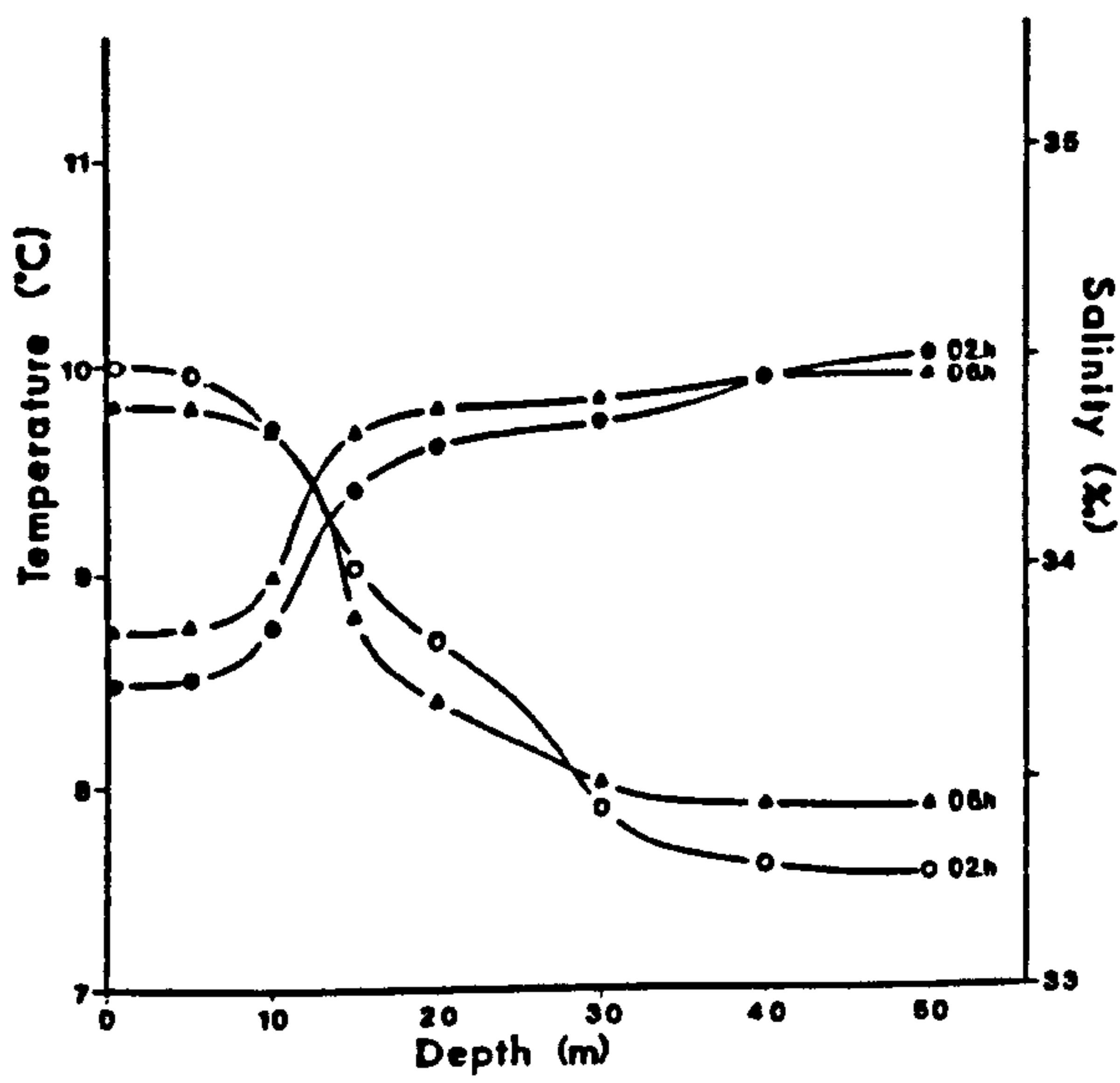
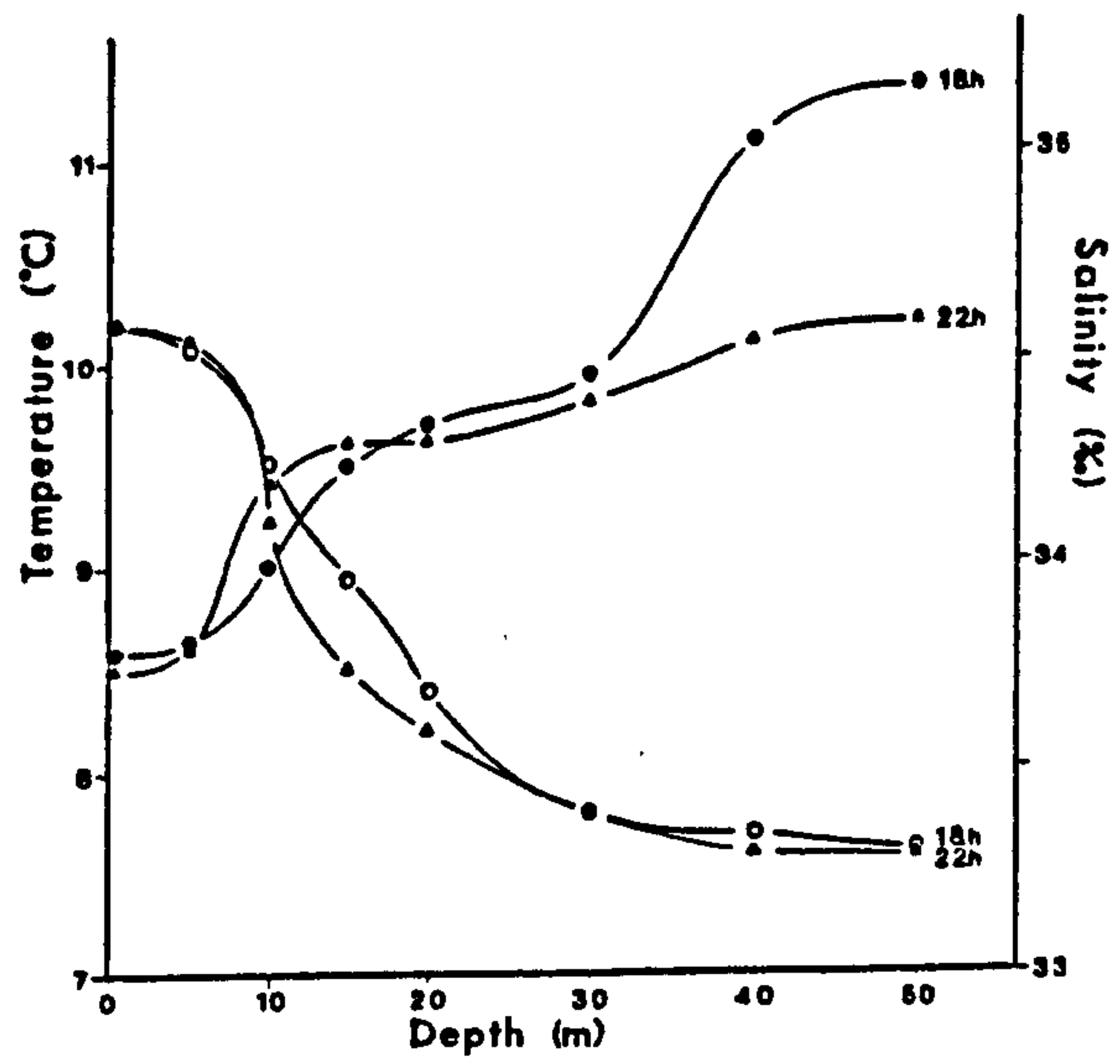
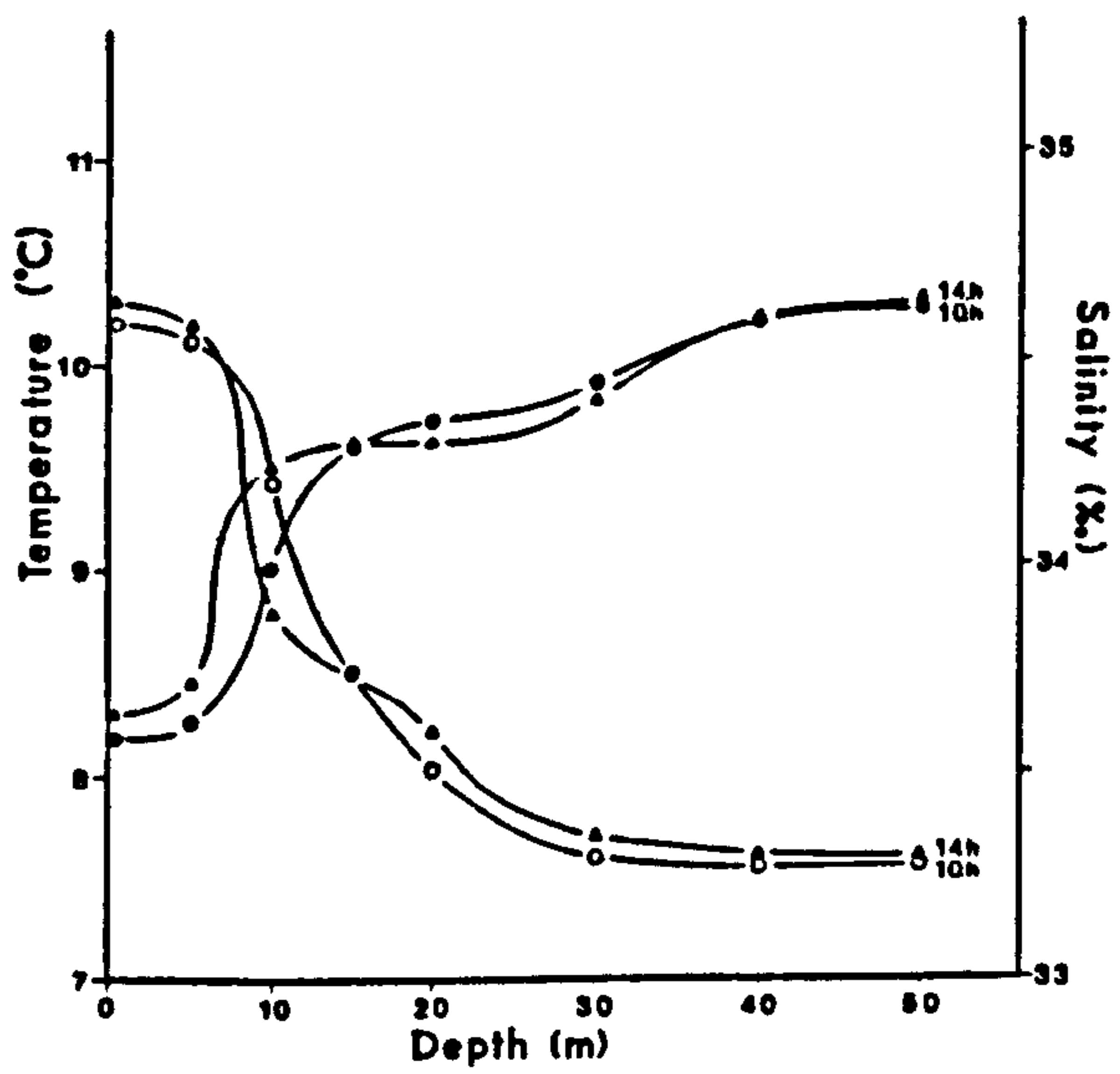
**Light attenuation profiles at different times during the neap tide survey. The numbers assigned to each curve denote the time (hour) at which the measurements were taken.**



**Figure 2.25**

The position of the thermocline (open symbols) and halocline (closed symbols) at different times during the neap tide survey. The numbers assigned to each curve denote the time (hour) at which the measurements were taken.

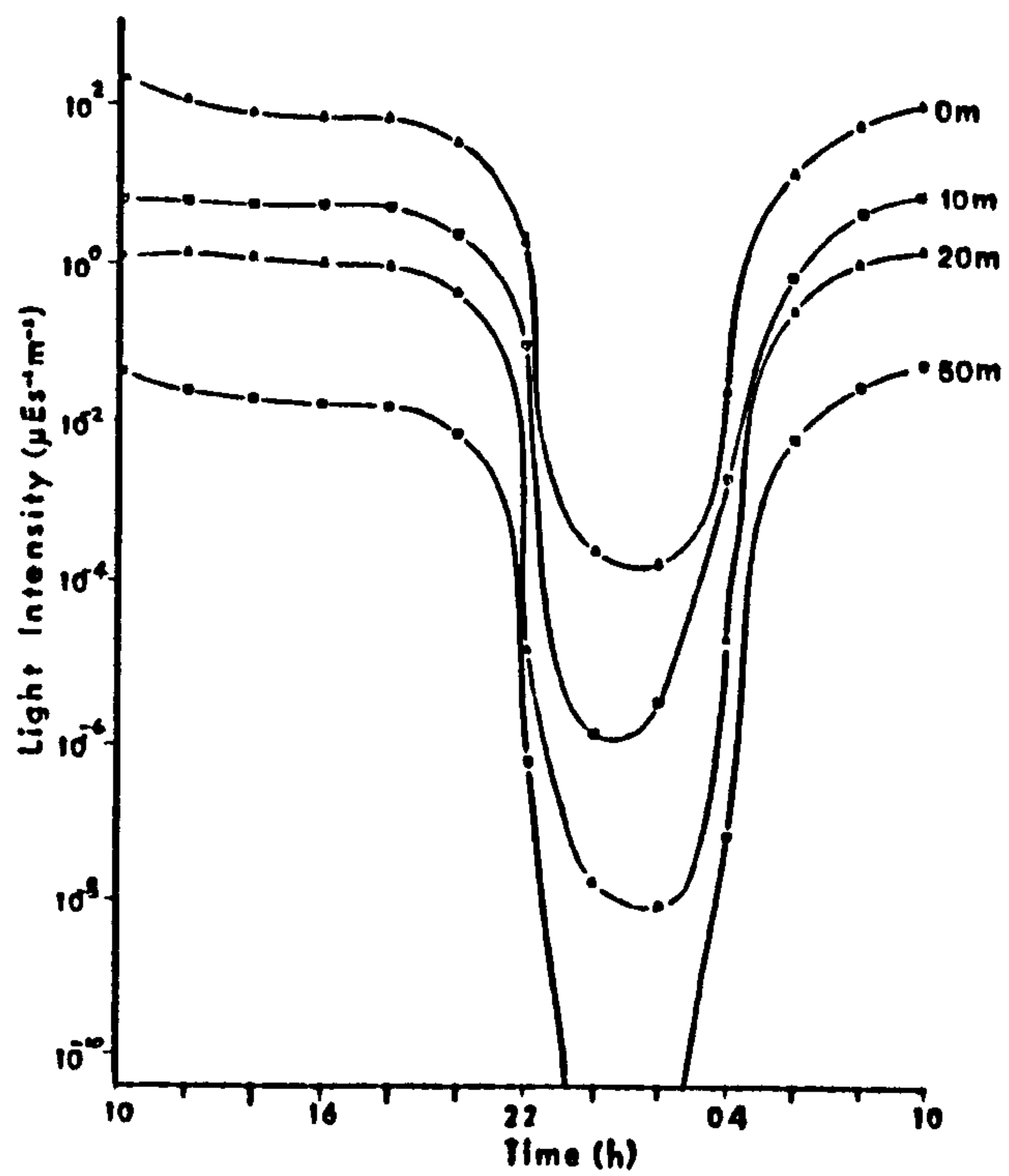




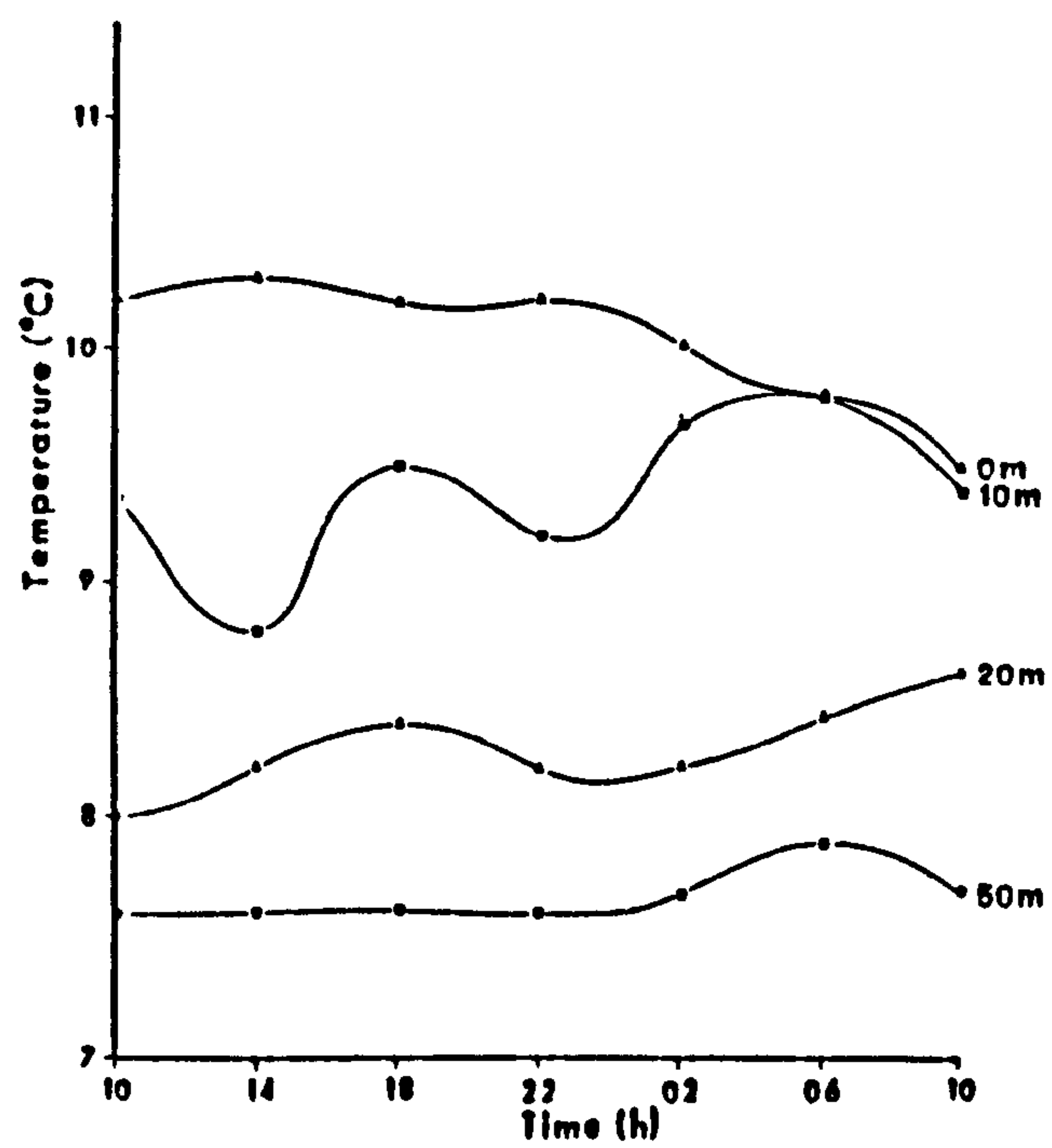
**Figure 2.26**

**The (a) light intensity, (b) temperature and (c) salinity at 0, 10, 20 and 50m depth during the neap tide survey.**

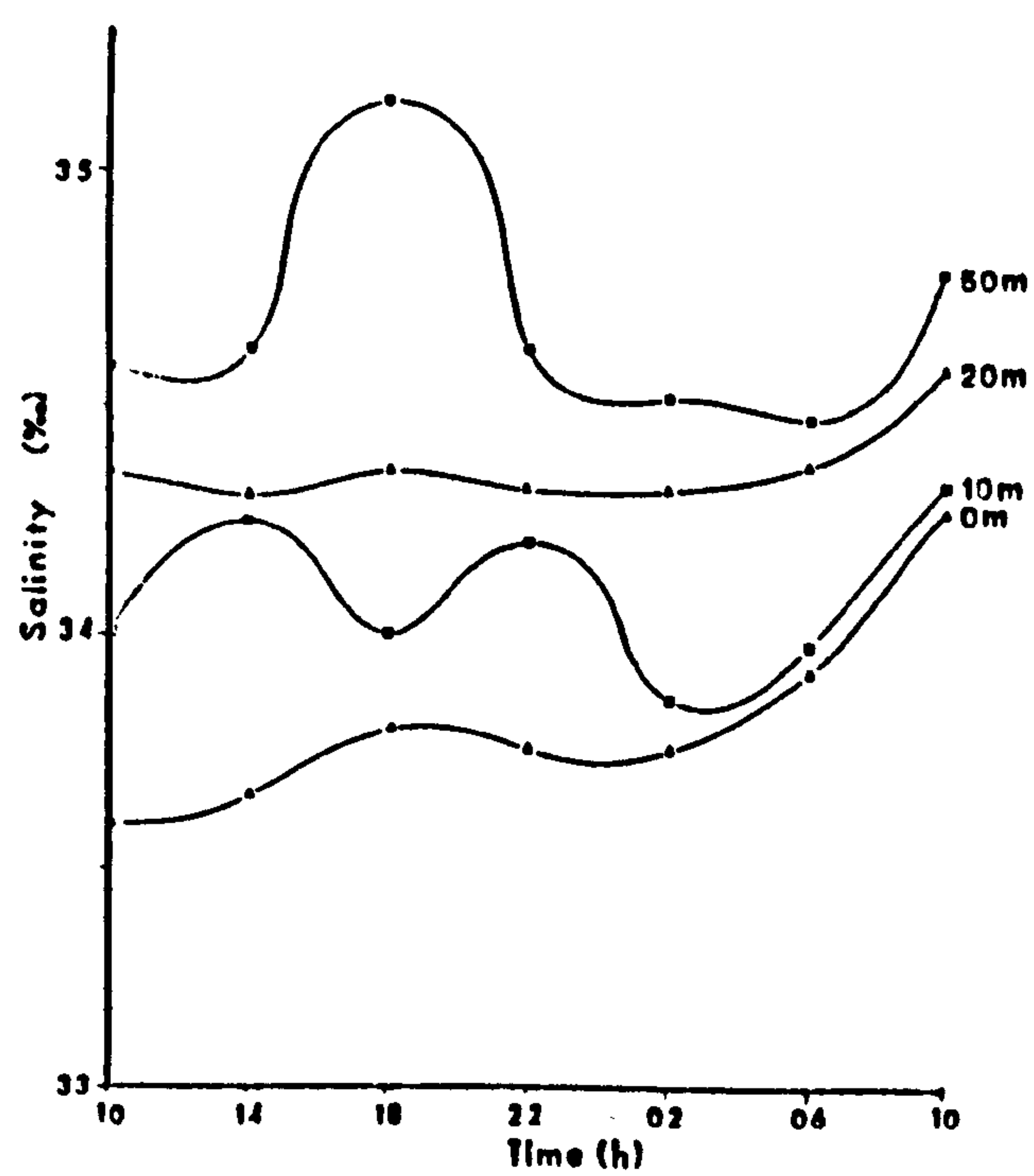
a



b



c



light intensity levels recorded during the spring than during the neap tide survey between 0200 - 0600h can probably be attributed to the full moon, moonrise occurred at 0013h and moonset occurred at 0600h. The moon was within the its final quarter during the neap tide survey.

At each selected depth (0, 10, 20 and 50m) light intensity remained relatively constant between 1000 and 1800h, approximately 100, 5, 1 and  $0.02\mu\text{Es}^{-1}\text{m}^{-2}$  respectively. Total light intensity at the surface over this period (measured with the Licor quantum meter) was approximately  $1,000\mu\text{Es}^{-1}\text{m}^{-2}$ . Sunset occurred at 2202h and a minimum light intensity was recorded at approximately 0030h (surface intensity =  $1.6 \times 10^{-4}\mu\text{Es}^{-1}\text{m}^{-2}$ ). This surface minimum was more than an order of magnitude greater than the corresponding value recorded during the spring tide survey. Sunrise occurred at 0423h and by 1000h light intensity had returned to its original daytime level, completing the symmetrical shape of the light curves.

During the final few hours of the survey (0600 - 1000h) the wind force increased. This caused turbulence in the upper regions of the water column and seems to have caused some mixing between the water masses on either side of the pycnocline (Figure 2.25).

The halocline occurred between 5 and 20m depth (Figure 2.25). The general shape of the salinity curves constructed for the selected depths (0, 10, 20 and 50m) are similar to the corresponding curves of the spring tide survey. Greatest fluctuation occurred at 10m depth, the middle of the halocline. The sample station (B3) is within close proximity of a major sewage sludge dumping site on which dumping

occurs daily, in the late afternoon. Apparently the sewage is less saline than seawater but may have caused the conductivity meter to malfunction due to redox effects and brought about the unusually high salinity reading recorded at 1800h (Strathclyde Sewage Department, pers. comm.). The cyclical changes in salinity which occurred within the region of the halocline (10m) did not appear to be as closely correlated with tidal variables as found during the survey coincident with spring tides. During the neap tidal survey greatest salinity readings were recorded at low water (1400h) and while the tide was ebbing (2200h). Lowest salinity readings were recorded while the tide was flooding (1800h) and just after low water (0200h) (Figure 2.13b and 2.26c).

As expected, the thermocline occurred at approximately the same position as the halocline (5 - 20m) (Figure 2.25). Greatest fluctuation in the water temperature occurred in the upper regions of the water column (0 - 10m) (Figure 2.26b). The temperature of the water below the thermocline (20 - 50m) was colder and more stable. Unlike the spring tide survey the cyclical changes in temperature which occurred within the region of the thermocline (10m), as in the case of salinity, did not appear to be closely correlated with neap tidal variables (Figure 2.13b).

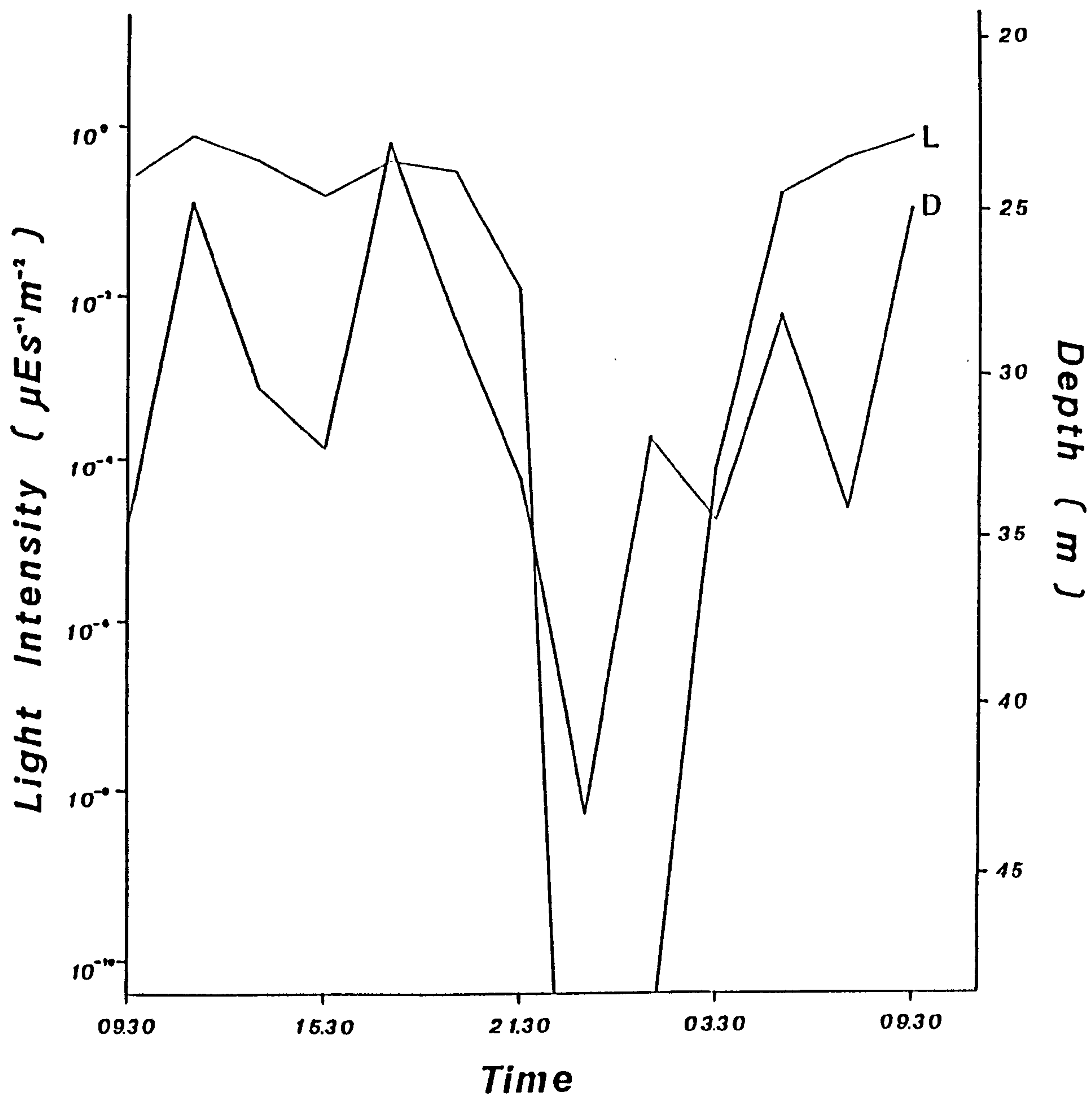
#### Larval abundance

The mean depth of the larvae (for all depths combined) at each sampling time throughout the 24h period and, the corresponding light intensity at each mean depth, is shown in Figure 2.27. During the hours of daylight (0930 - 1930h and 0530 - 0930h) the mean larval

### Figure 2.27

The mean larval depth (integrated over all sampling depths) calculated for each sampling time during the neap tide survey (D). The corresponding light intensity for the mean larval depth at each sampling time is also shown (L).



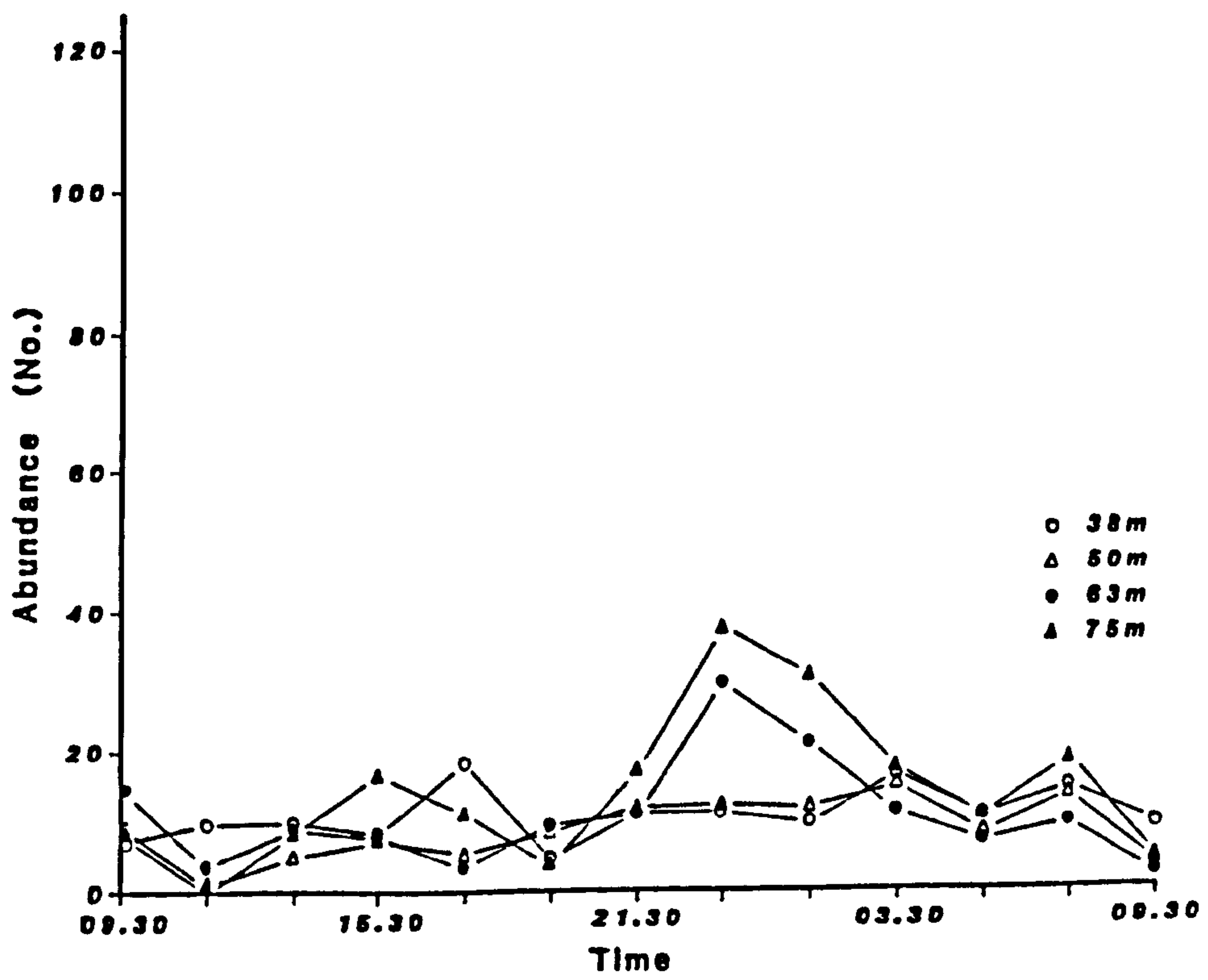
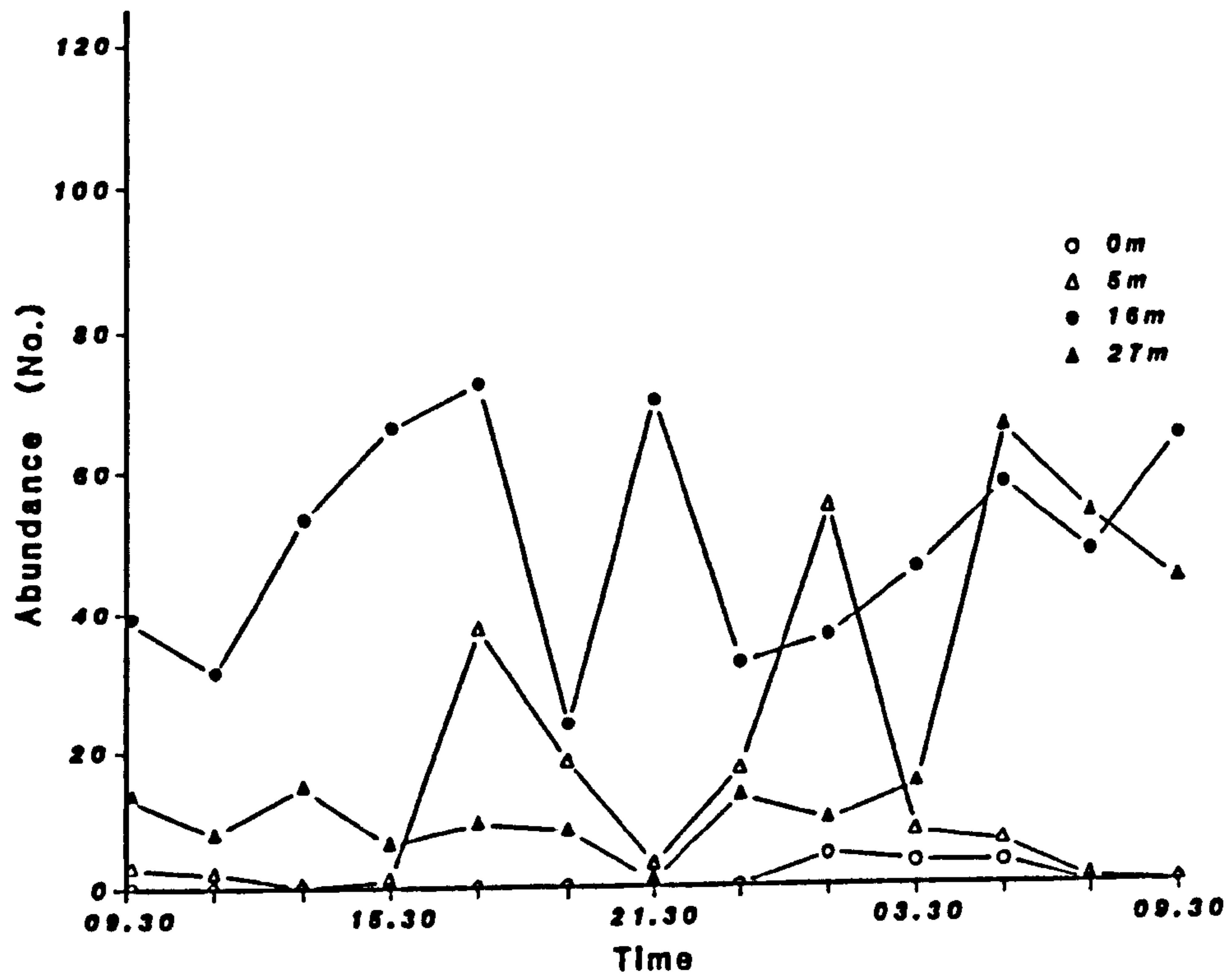


depth fluctuated between 20 - 35m. This corresponds to a light intensity range of  $0.16 - 0.75 \mu\text{Es}^{-1}\text{m}^{-2}$ . In common with the spring tide survey, this variability does not appear to be attributable to light intensity and shows no apparent relationship to the tidal cycle (Figure 2.13b). It therefore seems most likely that the daytime differences recorded between the position of the mean larval depths was an artifact caused by sampling variability. The increase in the mean depth of the larvae at 2330h (43.2m) appears to correspond to a decrease in light intensity. This deeper value for the mean larval depth appears to occur 2h later than a similar change recorded in the larval distribution for the spring tide survey. This difference may have resulted from the comparatively higher light intensity levels during this period (2000 - 0100h) for the neap tide survey or may be a feature of sampling variability. At 2130h (the time when an increase was recorded in the mean larval depth during the spring tide survey) the light intensity at the mean larval depth was almost 3 log divisions higher during the neap tide survey ( $1.2 \times 10^{-2} \mu\text{Es}^{-1}\text{m}^{-2}$ ) than for the spring tide survey ( $5.5 \times 10^{-5} \mu\text{Es}^{-1}\text{m}^{-2}$ ). For the neap tide survey, during the period of darkness (2330 - 0130h) there was a decrease in the mean larval depth.

Larval abundance is presented as a function of time and depth in Figure 2.28 and 2.29. These results have been compounded to form a 3 dimensional representation for larval abundance against time and depth in Figure 2.30. Between 0930 - 1530h peak larval abundance was recorded at around 16m depth. Most larvae were located between 6 and 37m depth and the highest concentration of larvae seemed to occur immediately below the pycnocline. Between 1530 to 1930h there is weak evidence of a change in distribution with a small proportion of the

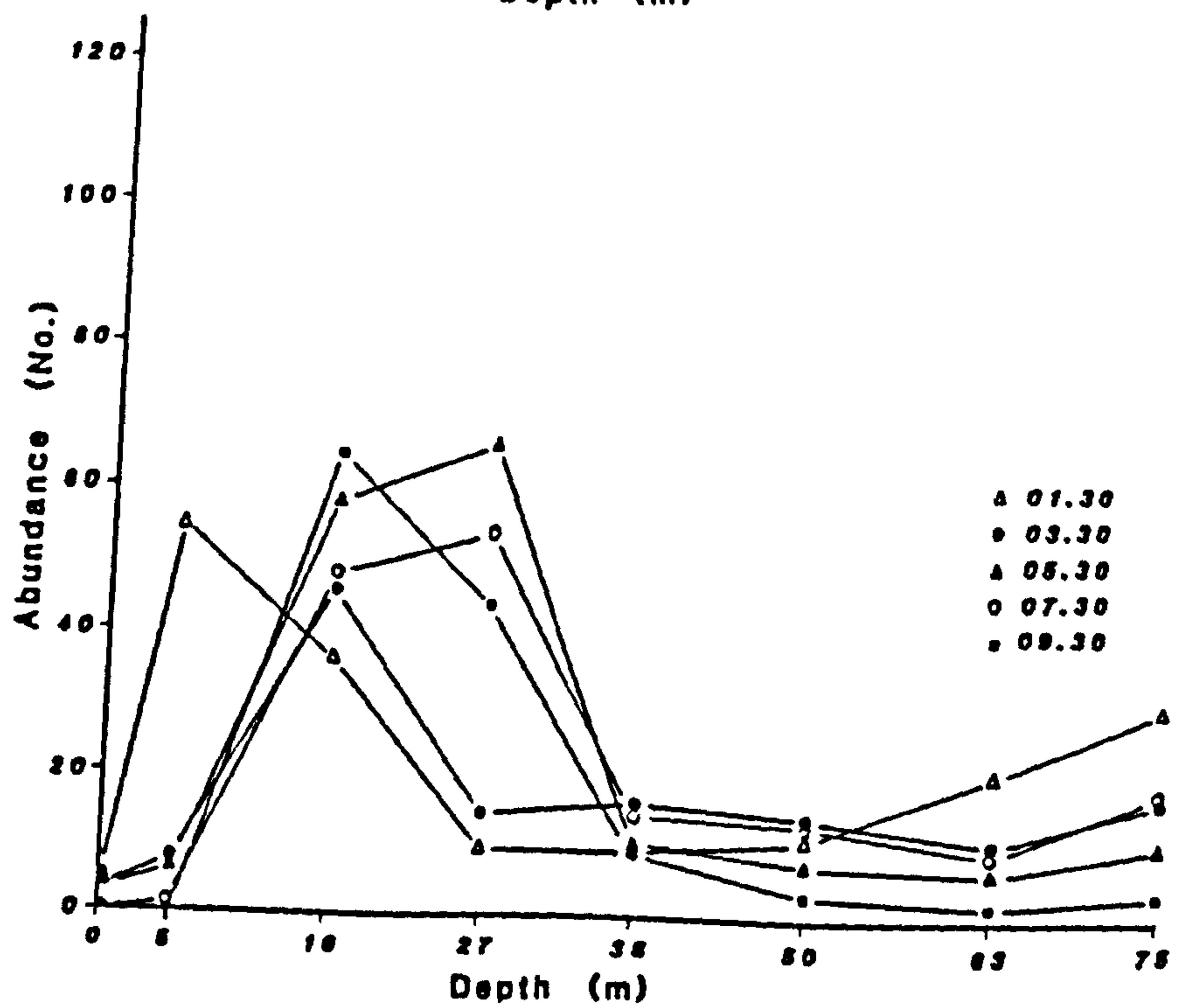
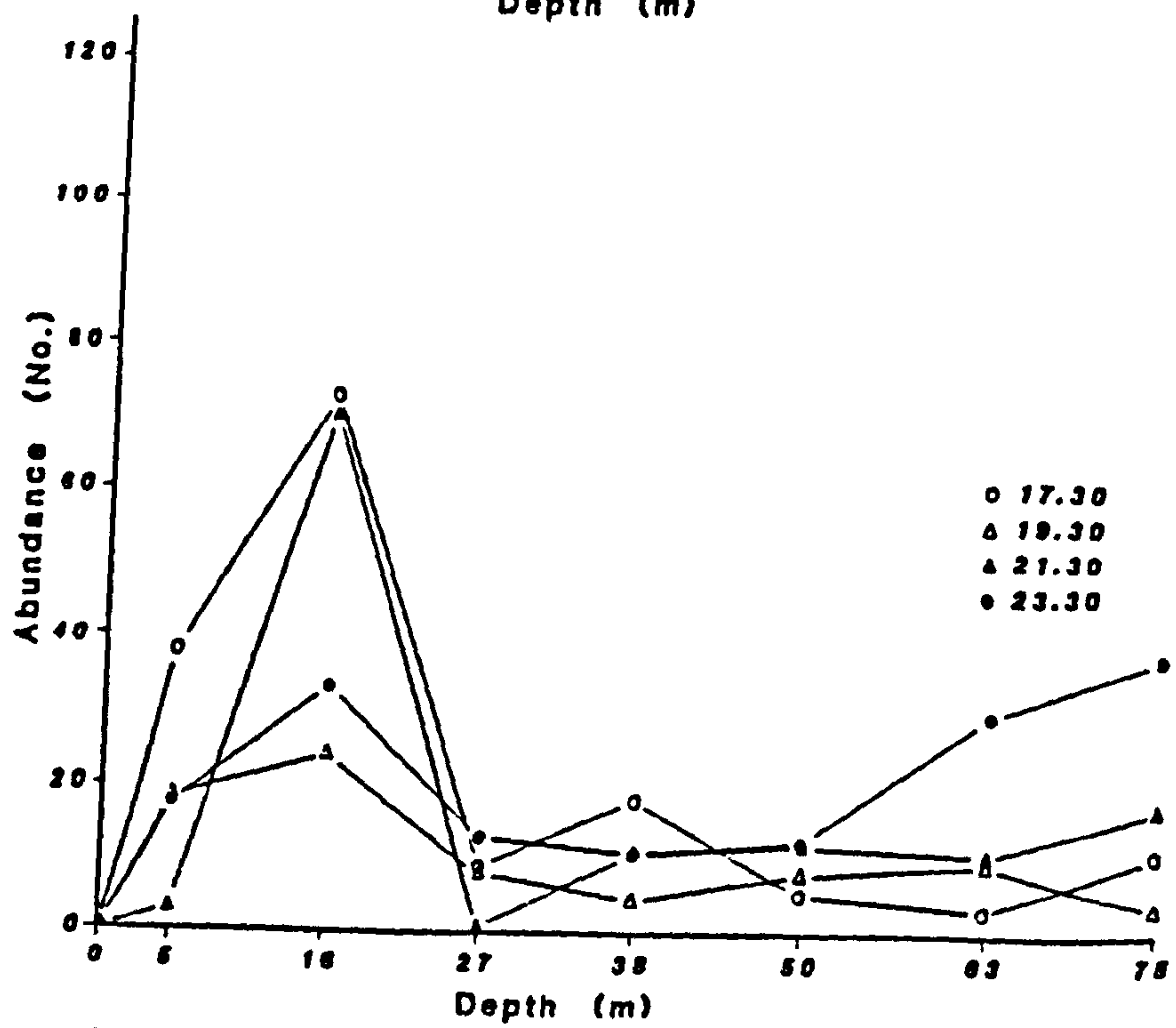
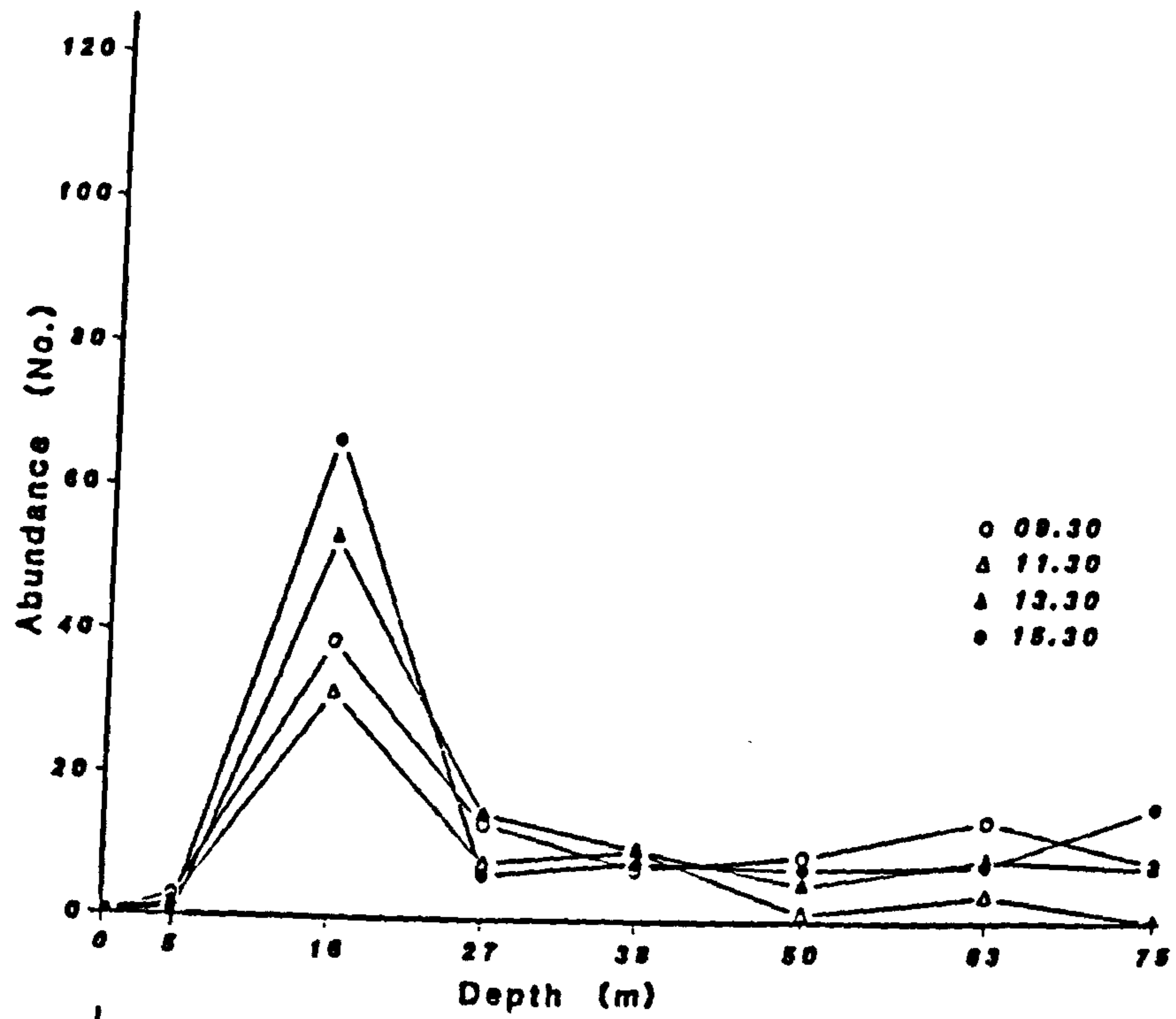
**Figure 2.28**

**Larval abundance during the neap tide survey at the eight different sampling depths.**



**Figure 2.29**

**Larval abundance at the eight different sampling depths during the neap tide survey.**





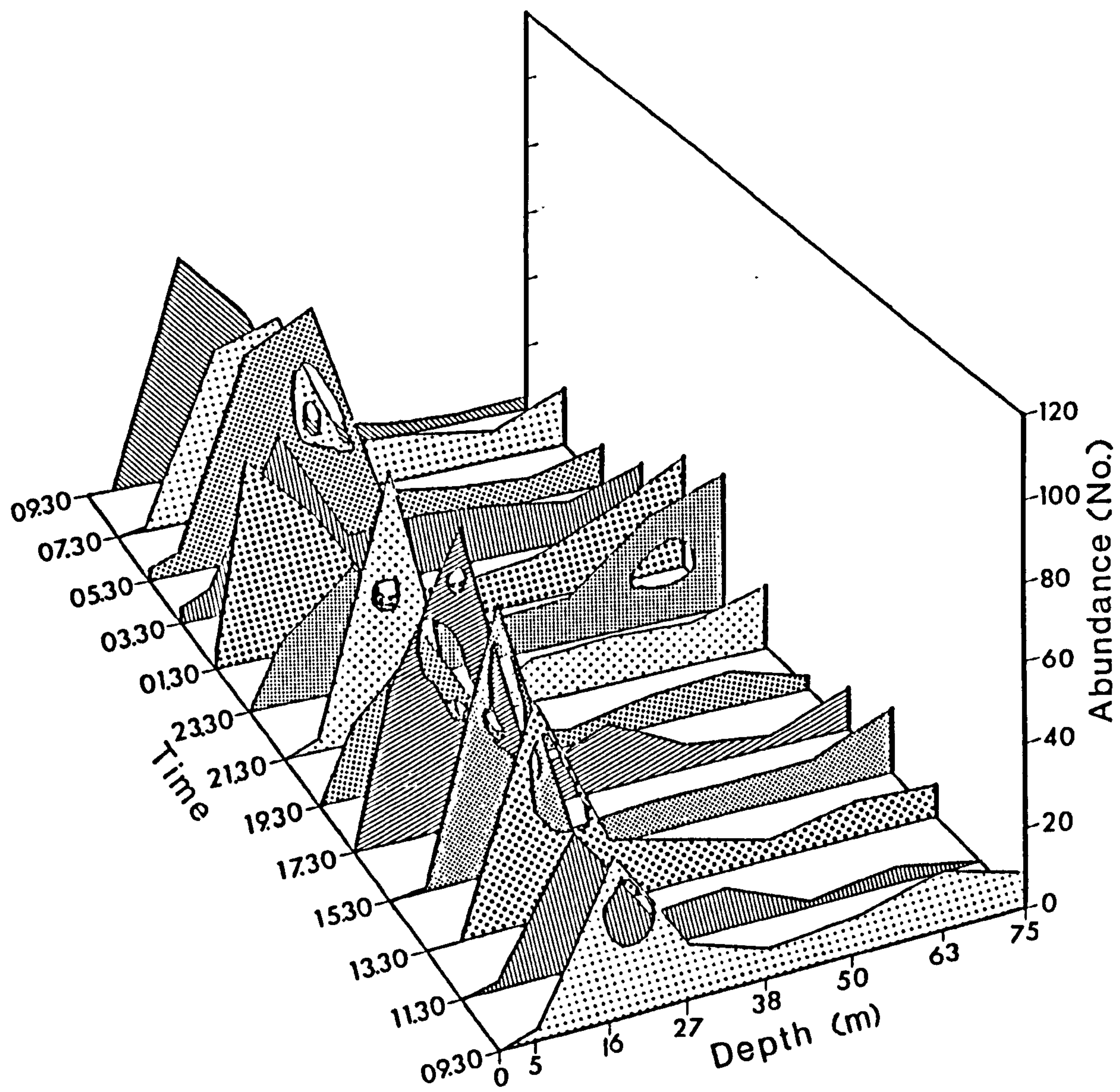
larvae ascending to 5m depth. Over the following 2h a slight descent may have occurred and the shape of the larval distribution curve has returned to its earlier configuration (ie. 0930 - 1530h). It is uncertain whether these apparent up and down movements were real or merely an artifact of sampling variability.

During the following 2h sunset occurred and light intensity became greatly reduced. Over this period (2130 - 2330h) there appeared to have been a change in larval distribution. There is limited evidence that the peak in larval abundance, which was still located at 16m depth, had become less pronounced and the larvae appeared to have become more evenly distributed in the upper regions of the water column (5 - 27m). However, the most obvious change in the larval distribution was the apparent formation of a second concentration of larvae at the bottom of the water column (below 50m). For the same reasons given for the spring tide survey it seems likely that this concentration of larvae near the sea bed consists of newly hatched larvae. In the spring tide survey evidence of larval hatching occurred 2h earlier (ie. at 2130h). This can probably be attributed to the relatively weaker lighting conditions which prevailed during the evening of the spring tide survey.

Between 2300 - 0130h there was a slight reduction in light intensity (surface minimum of  $1.6 \times 10^{-4} \mu\text{Es}^{-1}\text{m}^{-2}$ ) and some upward migration appeared to have occurred from both the upper and lower regions of the water column. At 0130h peak larval abundance was recorded at 5m depth, the shallowest depth it reached throughout the survey. Over the next 2h (0130 - 0330h) there was a sharp increase in light energy levels (Figure 2.24 and 2.26a). By 0330h the larval peak

**Figure 2.30**

**A three dimensional representation of larval distribution and abundance during the neap tide survey.**



had reformed at its normal daytime depth of 16m. The larval concentration at the lower region of the water column had become reduced to its residual daytime level and most larvae were distributed between 6 - 37m depth.

The variation between the 2 larval abundance curves for 0930h (beginning and end of survey), which resulted in a difference of almost 10m between the position of the mean depth of the larvae (Figure 2.27), is further evidence of sampling variability. However, the general distribution was similar (Figure 2.30), with the highest concentration of larvae occurring between c. 10 - 30m depth.

## 2.4. Discussion

### 2.4.1. Temporal and spatial changes in abundance

Previous investigations have shown that a muddy substratum extends throughout most of the L.F.C. (see Chapter 6). This provides a suitable habitat for Nephrops when they become benthic (see Chapter 6). It can be expected therefore that larval production will occur throughout this area. The results of this investigation tend to confirm this.

Earlier attempts to transform larval abundance data to estimates of production have been impaired by the lack of information on the effect of temperature upon development (Garrod and Harding, 1980 ; Nichols et al., 1983, 1987). In the present work a culture programme was devised to provide this information (Chapter 3) and hopefully to improve upon the accuracy of larval production estimates.

The start of larval hatching in the L.F.C. area at the beginning of April confirms the results of earlier investigations conducted by Thomas (1954). A thermocline had not yet developed and the sea temperature was approximately 6°C at all depths. The culture experiments suggest that larval development would have been very slow at this temperature, the time for full development through all 3 zoeal stages would be approximately 180 days. Previous field studies have shown that Nephrops larvae can survive temperatures as low as 4°C (Poulsen, 1946). As the season progressed a temperature gradient was formed between the upper and lower regions of the water column



exposing the larvae to different temperatures and giving rise to different development rates depending upon their depth distribution. It was therefore important to determine the vertical distribution of the larvae so that a mean temperature could be estimated and used to calculate the expected development time.

The diel surveys showed that larvae were distributed throughout the water column indicating that all depths must be sampled for accurate estimates of larval abundance. In this investigation the vertical distribution of the larvae was assumed to remain unchanged from that recorded during the 24h surveys (Figure 2.14). Hillis (1974) detected a general sinking in the depth distribution of the zoeae as the season progressed and a tendency for the older zoeae to occupy a lower position in the water column. If this seasonal change in distribution does occur the mean temperatures derived for this present study would have been too high which would consequently have caused larval production to be overestimated.

During April and May the water temperature gradually increased as a thermocline began to develop and was associated with high larval production reaching a peak at the end of May. In the Western Irish Sea peak larval production for Nephrops was recorded several weeks earlier, at the beginning of May (Nichols et al., 1987), reflecting the earlier warming of the water in this region. The rapid decline in larval production during June in the present study was similar to that recorded in the Western Irish Sea (Nichols et al., 1987). For the L.F.C. this period corresponded to the development of a well defined thermocline between 10 and 20m depth and a drop in the temperature of water below the thermocline (Figure 2.3).



In the L.F.C. there is evidence that the full reproductive cycle for large female Nephrops may take longer than 1 year. Bailey (1984) developed a model for the reproductive cycle of female Nephrops in the Firth of Clyde. It predicted that the post-hatching summer moult tended to occur later in large Nephrops. Consequently they spawned later in the year with the result that hatching the following year occurred later still. Eventually a point could be reached where the delay to the reproductive cycle resulted in resorption of the ovary and the female missed out a year of egg carrying. This would allow faster ovary development and a 'resetting of the clock' so that the next spawning occurred earlier in the year. The late hatching of eggs from the larger more fecund females may explain the second peak of larval production (Figure 2.10). Interestingly there was no evidence of a second peak in larval production for the Western Irish Sea (Nichols et al., 1987) where females are thought to reproduce every year (Farmer, 1975) but a second peak in stage 1 zoeal production was observed off the north-east coast of England (Garrod and Harding, 1980) where a part of the adult female population are believed to spawn in alternate years (Storow, 1912, 1913 ; Symonds, 1972).

Another possible explanation for the apparent rapid decline in larval production during June could be the dispersal of larvae outside the study area. A recent study of the Clyde Sea hydrography (which includes the Clyde estuary, the fjordic sea lochs and the Outer Firth) has shown that movement in the surface waters is partly dissociated from the deeper waters (Edwards et al., 1986). The deeper waters remain relatively stagnant during the summer months (Craig, 1959 ; Edwards et al., 1986) and for the L.F.C. occur at depths below 50m (Edwards et al., 1986). Most Nephrops zoeae would therefore have been

located in the surface waters (ie. above 50m depth, Figure 2.14) where there is free connection throughout and water movement is dominated by pressure gradients set up by tide, wind and fresh water outflows (Edwards et al., 1986). These authors predict a residence time of approximately 2 months for the exchange of surface water between the Outer Firth of Clyde and the seaward North Channel. The survey area in this present study (L.F.C.) mostly corresponds to the region defined as the 'Arran Deep' by Edwards et al. (1986), which borders the Outer Firth at its northern limit. The L.F.C (Arran Deep) and adjacent waters and the Outer Firth are of a similar size which suggests that the residence time for surface water within the study area used in this present work may also be around 2 months (A.Edwards, pers. comm.). There is no equivalent larval abundance information for water north of the L.F.C., which flows into the study area to replace the southerly drift into the Outer Firth. The inability to determine whether a net export or import of larvae had occurred in the study area during the season is a serious potential source of error and may have resulted in production being considerably underestimated or overestimated.

Between the end of May and the end of July, the period when the two maxima were recorded in the larval production estimates (Figure 2.10), the proportion of older zoeae (stages 2 and 3) contributing to the total production estimate increased from 19 - 34%, indicating that the hatching season was well advanced. This was verified by the further decline in production during August. The mean temperature to which the larvae were exposed increased from 8.9 to 10.9°C between the first and second production peaks. This will have caused a reduction in total development time by approximately 4 weeks. These warmer

temperatures may have promoted survival to the older zoeal stages (see Chapter 3). This may consequently have resulted in improved recruitment to the benthic population, compared to that which occurred at colder temperatures earlier in the season.

Throughout the summer months (May - October) the composition of the zooplankton community (on which Nephrops zoeae feed) is always changing (Russell and Yonge, 1975). Towards the end of the larval season water temperatures were beginning to fall but were still considerably warmer than those recorded during the period of greatest production. As growth is dependent upon temperature and food availability it therefore seems likely that the seasonal change in the composition of the food organisms available to Nephrops zoeae may be the most important factor in determining the extent of the larval season. From the creel catches (see Chapter 1) it was found that by the middle of August most ovigerous females had recently spawned and only a small proportion of the previous years spawners were incubating eggs at the hatching stage. It therefore appears that in the L.F.C. the incubation period requires spawning to have occurred by late November. This confirms the results of previous investigations conducted within the study area (Bailey, 1984), during which an increase was recorded in the number of non-ovigerous females with resorbing ovaries at the beginning of the winter.

It was evident from the results of the 24h surveys that within a given area there are considerable short term fluctuations in larval abundance. Consequently no attempt was made to apply confidence limits to the larval production estimates. Another potential source of error in estimating larval abundance was the sampling efficiency of the



gear, although this was checked against a high speed Gulf 3 plankton sampler.

The instantaneous daily mortality rate between stages 1 and 2 of 10.0% per day was much higher than that found by Nichols *et al.* (1987) for the Western Irish Sea (2.8%) and by Garrod and Harding (1980) off the north-east coast of England (3.2%). In the present study mortality between the 1st and 2nd stages and the 2nd and 3rd stages was very similar (approximately 77%), with an overall mortality rate for all 3 zoeal stages of 7.7% per day. Mortality between stages 2 and 3 for larvae off the north-east coast of England (Garrod and Harding, 1980) was estimated to be 80% and the overall mortality rate through all 3 zoeal stages was 7.2% per day, which is similar to the estimate for the L.F.C.. The overall mortality rate in the Western Irish Sea (Nichols *et al.*, 1987) was almost half this value (4.2%). These authors suggested that the mortality rate recorded by Garrod and Harding (1980) may have been an overestimation caused by inadequate sampling of the later larval stages, a consequence of poor temporal coverage. This argument does not appear to apply to the present investigation which extended from April to September.

The mean stage durations recorded in this present study for zoeal stages 1, 2 and 3 were 14.5, 23.4 and 33.6 days respectively. These figures were calculated from the mean sea-water temperatures to which each stage was exposed during the larval season. These temperatures were 8.9, 9.5 and 9.4°C respectively. From the available literature Nichols *et al.* (1987) derived regression equations relating development time to temperature for each stage. Using these relationships they estimated that mean stage duration for zoeal stages

1, 2 and 3 in the Western Irish Sea were 15.8, 12.9 and 17.7 days respectively. Temperatures over the periods of larval development in the L.F.C. and the Western Irish Sea were similar. The disagreement in the predicted development times has therefore been caused by differences between the equations used in the 2 surveys relating development time to temperature. If development of 2nd and 3rd stage zoeae at these mean temperatures is considerably slower than predicted by Nichols et al. (1987) then larval production for both stages will have been overestimated and their mortality underestimated in the Western Irish Sea. Conversely, if my regression equations overestimated larval development times larval production for stages 2 and 3 would have been underestimated and mortality overestimated in the L.F.C..

An accurate estimate of instantaneous larval production, derived from the mortality regression, depends upon adequate sampling of the later larval stages. As oblique hauls were made any stage dependent variation in depth distribution will not alter the catch-ability of the sampling gear. Laboratory based studies indicated that swimming speed was not markedly different between stages (see Chapter 5). It therefore appears unlikely that the older zoeae were better able to avoid the sampling gear. It is therefore assumed that the production estimates from which the mortality regression was derived have permitted instantaneous production to be calculated reasonably accurately. This value was then used in conjunction with the fecundity estimates (Chapter 1 ) to calculate spawning stock size for the L.F.C. (see Chapter 8 ).

#### 2.4.2. Diel changes in vertical distribution

Diel vertical migrations amongst the marine organisms of the zooplankton has been well documented (Russell, 1927 ; Kituchi, 1930 ; Cushing, 1951). According to Cushing (1951) the typical diel vertical migration pattern involves 5 parts : (1) ascent towards the surface from the day depth at around dusk in response to the rising isolume, (2) slow sinking at or before midnight due to reduced activity when the animals are no longer able to perceive the light stimulus, (3) a return to the surface at around dawn with the gradual increase in light intensity, (4) descent to the daytime depth with sunrise as light intensity rapidly increases and (5) the variable day depth. Vertical migrations were originally considered to be governed entirely by a light response, with the organism following an optimum intensity (Russell, 1927). It is now accepted that an endogenous component as well as other external factors, such as pressure, are probably involved (Naylor and Atkinson, 1972) (see Chapter 5). In this study attempts were made to discover any diel changes in the vertical distribution of Nephrops zoeae and interpret observed changes in relation to corresponding changes in the environmental conditions.

Although previous attempts have been made to determine the depth distribution of Nephrops zoeae (Poulsen, 1946 ; Thomas, 1954 ; Williamson, 1956 ; Fraser, 1965) the only previous diel investigation was conducted by Hillis (1974). The results of that study provided evidence that Nephrops zoeae undertake a limited ascent around dusk. However, as the environmental conditions had not been closely monitored and light intensity had been measured as a photometric quantity (lux) rather than radiant power (Arnold, 1976a), any



interpretation of these results would be based on an incomplete picture of the prevailing conditions.

In a previous study, Thomas (1954) recorded no discernible change in larval distribution at depths down to 90m. The evidence from this investigation clearly contradicts these findings, the highest concentration of larvae occurring between 10 and 30m depth during daylight hours. This is similar to the daytime depth distribution recorded by Hillis (1974). He found very few larvae below 38m until later in the season. In the present work almost a third of the larvae were caught at or below 38m. As non-closing nets were used in my study a proportion of the catch may have been caught above the desired sampling depth during shooting and hauling the gear. This potential source of error would obviously have been greater for samples from the greatest depths. However, the evidence from the flow meter recordings and the control samples suggest that this error was negligible. It had been suggested by Nichols et al. (1987) that for estimations of larval abundance a more comprehensive knowledge of the vertical distribution of the larval stages may lead to a reduction in sampling time. Although the vertical distribution of the eggs and postlarval stages of mackerel permits sampling to be restricted to the region of the water column above the thermocline (Southward and Bary, 1980 ; Thompson et al., 1984), the evidence from the present investigation indicates that, in Nephrops larval surveys, the entire water column must be sampled.

No dramatic changes were detected in the vertical distribution of Nephrops zoeae over the two 24h periods and any changes which did occur were subtle. During the hours of day-light most larvae were

located between 6 - 37m depth and the highest concentration of larvae seemed to occur immediately below the pycnocline (ie. at approximately 20m depth). Although short-term fluctuations in the day depth have been reported for other members of the zooplankton that demonstrate night-time vertical migration rhythms (Cushing, 1951) in this present study any apparent difference between the daytime larval distributions can probably be attributed to sampling variability. However, between sunset and sunrise certain trends were demonstrated in both sets of data (spring and neap tide surveys) which indicated that during this period there may have been some genuine subtle changes in the larval distribution.

Although there is weak evidence of a slight pre-dusk rise (1530 to 1930h) by 2130h the depth distribution had returned to its earlier configuration (ie. before 1530h). It seems likely that this apparent movement was merely an artifact of sampling variability. During the period of this study dusk occurred between 2130 and 2330h and between these times the maximum available light energy (surface value) decreased by approximately 5 log divisions during the spring tidal survey ( $7.0 \times 10^{-1}$  to  $8.0 \times 10^{-6} \mu\text{Es}^{-1}\text{m}^{-2}$ ) and by approximately 3 log divisions during the neap tidal survey ( $1.6 \times 10^{-1}$  to  $2.2 \times 10^{-4} \mu\text{Es}^{-1}\text{m}^{-2}$ ). From laboratory results (Chapter 5) it appeared that Nephrops zoeae were unable to respond phototactically at light intensities below  $4.0 \times 10^{-3} \mu\text{Es}^{-1}\text{m}^{-2}$ . The dusk ascent that was described by Hillis (1974) for Nephrops zoeae was not identified in this present study. In Hillis's work the distinct peak in larval abundance (at 9 - 10m depth) which occurred as a result of the dusk ascent became gradually more diffuse and less marked between dusk and night-time. In this present study there is weak evidence of a slight dispersion between 2130 and

2330h in the upper regions of the water column (5 - 27m). This movement would therefore be similar to the post-dusk dispersion reported by Hillis (1974) if the dusk ascent had been missed in this present study. It seems likely that if dispersion did occur between 2130 and 2330h it would have been caused by the reduction in light intensity, disorientation resulting from the removal of the light stimulus. However, evidence for a change in distribution in the upper regions of the water column during this period is very weak and is difficult to separate from the 'noise' of sampling variability.

There is some evidence of an increase in the number of larvae at the bottom of the water column at night. This may either have resulted from a downward dispersion or hatching activity. Although the laboratory based studies (see Chapter 5) indicate that the sinking rates attained during passive descent could have caused larval displacement from the day-time optimum depth (16m) to the bottom of the water column (75m) in 2h (sampling interval) these studies were conducted under static water conditions and may have little relevance for the field. Also, there is no evidence of a descent to the bottom of the water column. It therefore seems more likely that the concentration of larvae at the bottom of the water column was associated with the synchronization of hatching activity by the onset of darkness (Moller and Branford, 1979) and the release of larvae into the bottom regions of the water column. If the concentration of larvae at the bottom of the water column was caused by night-time hatching it seems likely that the relatively weaker lighting conditions which prevailed during the evening of the spring tide survey were responsible for the earlier appearance of larvae at the bottom of the water column during the spring (2130h) than during the neap (2330h)



tide survey.

During the following two hours (2330 to 0130h), while light intensities were still well below the predicted threshold level ( $4.0 \times 10^{-3} \mu\text{Es}^{-1}\text{m}^{-2}$ ), there is evidence of a nocturnal ascent with the formation of a distinct peak in larval abundance at 5m depth (Figure 2.23 and 2.30). Although Hillis (1974) reported a dusk ascent for Nephrops zoeae the study from which he obtained evidence for this was not performed over a 24h period, the last sample was taken before midnight. The results he obtained from a later, overnight study, which he generally discounted because relatively few larvae were caught, produced no evidence of a dusk ascent but did indicate that a nocturnal ascent had occurred (between 2330 to 0230h). Peak larval abundance in Hillis's overnight study occurred at approximately 5m depth at 0230h. The trend demonstrated in the data obtained by Hillis in his overnight study was therefore similar to that shown in this present study.

In this present study the nocturnal ascent may have involved both the newly hatched and older larvae. The swimming speeds recorded under the laboratory conditions in Chapter 5 indicate that Nephrops zoeae are capable of swimming the length of the water column (75m) in 2h. Other laboratory experiments (see Chapter 5) have shown that under conditions of darkness an increase in pressure caused an increase in swimming activity. Pressure sensitivity is known to be a common depth regulatory mechanism in planktonic animals (Knight-Jones and Morgan, 1966 ; Knight-Jones and Qasim, 1967 ; Rice, 1964, 1967 ; Ennis, 1973a). It therefore seems likely that in the absence of a light stimulus high barokinesis in response to increased pressure

encountered when sinking, induced a response to swim upwards and involved migration through the region of the pycnocline, which occurred over the depth range 5 - 20m. Therefore, if there is evidence that the initial removal of the light stimulus at the onset of darkness (2130 to 2330h) caused some dispersion and limited sinking, which is not at all certain, the night-time rise seems to have over compensated for the earlier sinking. Alternatively, if a dusk ascent had occurred and sampling error during both surveys at 2330h had failed to detect the change in distribution, the 0130h peak in larval abundance at 5m depth may be the after effect of this earlier upward migration. This would fit with the dusk ascent and subsequent night-time distribution described by Hillis (1974) for Nephrops zoeae.

Dawn occurred between 0130 and 0330h and by 0330h the surface light intensity (maximum light available) exceeded the predicted threshold for larval perception ( $4 \times 10^{-3} \mu\text{Es}^{-1}\text{m}^{-2}$ ). The increase in light intensity was accompanied by a downward migration and a shift in the position of the larval maximum, which returned to its daytime depth of approximately 16m. This appears to have occurred as a direct result of the increase in light intensity.

The results from the laboratory based study suggested that there was no reversal of the phototactic response from photopositive to photonegative at higher light intensities in Nephrops zoeae. Similar results were also obtained for the larval stages of Homarus gammarus (Ennis, 1973a). As Nephrops larvae were only very rarely caught in the surface waters depth regulation during daylight hours does not appear to be entirely controlled by a light response. Pressure sensitivity (which has already been implicated in the night-time ascent) and the

position of the pycnocline (5 - 20m) may also influence the daytime depth distribution.

During daylight hours most Nephrops zoeae seemed to remain in the lower regions of the discontinuity layer (pycnocline) (Figure 2.14). The discontinuity layer is known to form an impenetrable boundary to the upward migration of many zooplankters (Russell and Yonge, 1975). However, if this layer restricts upward migration during the day the situation has changed at night when a large proportion of the Nephrops zoeae migrate up through the discontinuity layer. At this relatively early stage in the season (beginning of June) the discontinuity layer was not fully defined. It may therefore provide a more formidable barrier to the ascent of the Nephrops zoeae later in the season. If the pycnocline does influence the depth distribution of the larvae the seasonal descent in the position of the thermocline may cause a corresponding change in the depth distribution of the larvae (see Chapter 5). From field observations in the Western Irish Sea Hillis (1974) reported a seasonal increase in the depth at which Nephrops zoeae are distributed.

Alternatively, the non-reversal of the phototactic response demonstrated by Nephrops zoeae under the laboratory conditions may have been an artifact of the artificial conditions. In the sea high light intensities may repel the larvae from the surface waters. In the field study  $1.6\mu\text{Es}^{-1}\text{m}^{-2}$  was the highest light intensity at which the mean larval depth was recorded (both 24h studies combined). The avoidance of high light intensities may therefore provide a possible explanation for the night-time ascent and the subsequent dawn descent.



Although there is considerable variation in the vertical distances travelled by different members of the zooplankton (Wickstead, 1976) it is generally accepted that diel vertical migrations are involved with feeding (Rebach, 1983). Most members of the zooplankton only migrate into the productive surface waters of the euphotic zone during the hours of darkness (Russell and Yonge, 1975). Nephrops larvae are carnivorous and probably opportunistic in their feeding habits. Their diet is likely to include decapod larvae (including other Nephrops zoeae, see Chapter 3), copepods and small mysids (Farmer, 1975). It is uncertain what the natural predators of Nephrops zoeae are but they probably included ctenophores, medusae, plankton feeding fish and other Nephrops larvae (Farmer, 1975). These predators are also likely to undergo diel vertical migrations. Whether there is any adaptive significance to the subtle diel vertical migrations undertaken by Nephrops zoeae for either predator avoidance or improved feeding is unclear (see Chapter 5).

Throughout both 24h periods the zoeae may have been exposed to temperature changes of up to 3°C. This is further evidence of the tolerance of Nephrops zoeae to temperature (Poulsen, 1946).

There did not appear to be any significant difference between the diel vertical migration patterns demonstrated during the spring and neap tidal surveys. This suggests that the daily vertical distribution of Nephrops zoeae was principally governed by a diel rhythm of response to light and hydrostatic pressure and did not seem to be significantly influenced by tidal variables.

Before this study there was a lack of reliable information on the

vertical distribution of the Nephrops zoeae. It was therefore necessary to sample the entire water column. As a result the sampling interval was relatively large (11 - 12m). Consequently, small changes in the larval distribution may have been missed. A future study on the vertical distribution of Nephrops zoeae involving concentrated sampling over the depth range 5 - 30m would provide a useful extension of this present work.

## CHAPTER 3

**A culture facility for determining the effect of temperature, starvation and stocking density upon larval development**

### 3.1. Introduction

The purpose of this investigation was to promote a greater understanding of the biology of the 3 zoeal and the early postlarval stages through the application of culture techniques. This study has relevance for the development of better fisheries management and the advancement of Nephrops culture.

Because of the economic importance of the Nephrops fishery (valued at £29m in 1986, DAFS, 1987) an extensive literature exists on the adult stages. By comparison information concerning the larvae and juveniles is very sparse. As juveniles have proved difficult to obtain from the field (Chapman, 1980), any laboratory based study will depend upon successful culture through the larval stages. Due to a change in the emergence behaviour of ovigerous females towards the end of the hatching period and their increased availability to creel fishing gear (see Chapter 1), ripe eggs are relatively easily obtained for subsequent laboratory hatching.

In view of this and the fact that the pelagic larval phase is potentially the most hazardous (Thorson, 1964) and probably therefore most vulnerable to environmental perturbations the lack of previous research involving these stages is surprising. Annual larval

recruitment to the benthic population will have important implications for the strength of future year classes. Newly settled juvenile Nephrops do not normally become available to the fishery until 2 - 3 years of age when they would be expected to have attained a carapace length of approximately 20mm (Farmer, 1975). The accuracy of previous attempts to estimate the stock size of adult Nephrops from larval surveys has been adversely effected by the lack of data on larval development rates (Nichols et al., 1983, 1987).

The adoption of an aquatic species for commercial culture depends upon a detailed knowledge of its biology (Van Olst et al., 1980). Attempts to artificially propagate nephropid lobsters have been mainly restricted to species within the genus Homarus (Provenzano, 1985). Initial attempts to culture both the European (H. gammarus) and American (H. americanus) lobsters were begun independently over 100 years ago (Van Olst et al., 1980). It was not until the early 1970's that the first efforts were made to culture Nephrops (Figueiredo, 1971). The only other subsequent laboratory studies involving the culture of the larval stages were conducted by Farmer (1972), Figueiredo and Vilela (1972), Hillis (1972), Figueiredo (1979) and Anger and Püschel (1986). It is not surprising, therefore, that much more is known about the biology of the early stages of the Homarus spp.. Although commercial culture of any of the Homarus spp. is not as yet economically feasible the development of new technology and improved husbandry practices suggest that this goal may be achieved in the near future (Provenzano, 1985).

The ability of the Homarus spp. to survive and grow at greatly accelerated rates when maintained under temperatures much higher than



those they normally experience in their natural habitat has been a major reason for the continued research and anticipated suitability of these lobsters for aquaculture. H.americanus can be reared to commercial size (454g) in approximately 2 years from metamorphosis, as opposed to 5 - 8 years in the field (Hughes et al., 1972). 22°C has been determined as the optimum rearing temperature for both growth and survival of the species (Van Olst et al., 1980).

Most of the available literature concerning the effect of temperature on growth for the zoeal stages of Nephrops has been summarized by Farmer (1975). Although these preliminary investigations suggest that development is faster at higher temperatures, it has been reported that temperatures in the region of 11 - 14°C are the most suitable for both growth and survival (Figueiredo and Vilela, 1972). With the development of improved culturing techniques it may now be possible to achieve a higher optimum temperature by improving survival at warmer temperatures. The rate of development over the temperature range 13 - 17°C has been reported to be twice as fast as that achieved at 11 - 14°C (Farmer, 1975). In a recent study Anger and Püschel (1986) reported faster development at 12°C for all 3 zoeal stages of Nephrops than had previously been achieved at this temperature and suggested that 12°C may be close to the optimum temperature for growth.

For Nephrops Figueiredo (1979) compared survival through each zoeal stage under conditions of both mass and individual rearing. She concluded that survival through the full larval phase was considerably higher under conditions of individual rearing. Anger and Püschel (1986) achieved over 90% survival for both 1st and 2nd stage zoeae

when reared separately. Improved survival under conditions of individual rearing has also been reported for the larval and juvenile stages of H. americanus (Van Olst et al., 1980). The present investigation attempts to develop the research work of Figueiredo (1979) and to compare survival and growth under conditions of different initial stocking densities.

It is known that communal culture of lobsters leads to intraspecific aggressive behaviour and mortality (Cobb and Tamm, 1974). These authors reported that the full repertoire of agonistic behaviour was not established in H. americanus until the 7th stage (4th postlarval stage). In the present work attempts were made to determine the influence of social behaviour upon development in the early stages of Nephrops when reared in groups.

It is known that crustacean larvae can only survive for a 'critical period' of initial starvation before a deterioration in their general condition prevents the onset of feeding and leads to eventual mortality (Sastry, 1983). The effect of variable periods of initial starvation upon future development in newly hatched larvae were investigated in this study. The results are used to interpret the likely effects of starvation upon development in the field.



## 3.2. Materials and methods

### 3.2.1. Development of the aquaculture facility

#### 3.2.1.1. Water treatment and supply

All experiments were conducted at the University Marine Biological Station, Millport. Water was pumped directly from the sea (Firth of Clyde) into an elevated storage settlement tank, from where it passed, by gravity, to the culture system. Before reaching the various culture units the water first passed through a sand/gravel filtration unit built into the existing water supply system. The sand/gravel filtration unit ensured an adequate water supply for use in both open and static culture systems. When both of these systems were concurrently in operation, however, the combined demand on the water supply was such that no further filtration was possible. The only subsequent pre-treatment of the water was U.V. sterilization (Hanovia 102. 450 l capacity).

The open system was eventually abandoned in favour of a totally static culture system where the water volume requirements were much reduced. In this case, the water was first subject to sand/gravel filtration and then passed through a graded series of three paper filter tubes (Whatman Gamma 12) before U.V. sterilization. The filter tubes removed particulate matter from the water down to a size of  $2\mu\text{m}$ . Having passed through the treatment stages, the water was either supplied directly to the open system or collected in reservoir tanks for future distribution to the static culture units.

### 3.2.1.2. Disease prevention

On removal from the brood stock tanks (see section 3.2.2.) larvae were initially introduced straight into the culturing units. After fungal infestations (Plate 3.1) had led to heavy larval mortalities it was decided to administer preventative treatment before transferring the larvae into the culturing system. The newly hatched larvae were immersed for periods of 5min in 0.5 ppm malachite green solution and this treatment was repeated thereafter twice weekly throughout larval development. Fisher et al., (1976) showed that survival of H. americanus larvae was adversely effected when treated with malachite green at concentrations above 8ppm for 16min. 'Safe levels' have not been determined for Nephrops larvae but since 0.5ppm had no adverse effects on the larvae and was successful in controlling the infection, it was decided to use this concentration throughout.

### 3.2.1.3. Food supply

The brine shrimp, Artemia salina, (obtained from Willowbank, Aquarium Products), was cultured as the principal food organism for the Nephrops larvae. Initial concentrations of approximately  $10^3$  eggs per litre were incubated in 1 or 2 l flasks of sterilized, well-aerated seawater at 27°C. After 24h the aerators were removed to facilitate separation of the newly hatched nauplii (which settled to the bottom) from their empty egg cases. The nauplii were then siphoned off and either removed to an on-growing culture bin or used directly as a food source. Each day a new batch of Artemia eggs were incubated to provide a continuous supply of fresh food.

**Plate 3.1**

**(a) Light microscope photograph of a living 3rd stage zoea in a diseased condition. Scale bar represents 1.00mm.**

**(b), (c) Scanning Electron Microscope (S.E.M.) photographs of diseased zoeae. Scale bar represents (b) 0.50mm and (c) 0.05mm.**







Newly hatched Artemia nauplii were found to be suitable food organisms for all three zoeal stages of Nephrops but was often supplemented with later moult stage Artemia which had been on-grown to provide a choice of food sizes for the larger stage 2 and 3 zoeae and the initial postlarval stages.

The alga Tetraselmis suecica was chosen initially as the food source for the Artemia and was cultured in two 10 l flasks under conditions of high illumination and vigorous aeration. Using a haemocytometer, algal concentrations were calculated and aliquots taken to provide the Artemia with concentrations of between 50 - 450 algal cells per  $\mu$ l. The algal cultures were maintained in a viable and healthy condition by frequent sub-culturing and careful nutrient additions (Walne, 1974).

Although Artemia readily accepted T.suecica as a food source, the algal culture operation proved too time consuming and was eventually discontinued in preference for a readily available commercial feed (Aquabiofood A, ab-aquatechnik).

The Artemia were reared for a period of up to 4 days by which time their mean length was approximately 2mm (Sorgeloos, 1973) which was still small enough to be readily accessible to the Nephrops.

Since Artemia was a potential disease carrier and therefore a health hazard to the larval and juvenile Nephrops, before being given as food, all Artemia were immersed in malachite green under the same conditions as those applied to Nephrops zoeae. Newly hatched stage 1 Nephrops zoeae, when available, were used to supplement the live

portion of the diet for the later larval and early postlarval stages. Nephrops eggs were also provided but were not as readily accepted as a food source as either the newly hatched larvae or Artemia. The later juvenile and adult Nephrops were fed a diet of frozen mysids (species unknown) and brown shrimps (Crangon crangon). These older Nephrops were maintained under flow-through conditions and therefore did not suffer a deterioration of water quality upon food administration, as would have occurred under static culturing conditions.

All larvae and juveniles were fed to excess through monitoring food availability and consumption, with the larvae fed twice daily and the juveniles fed once a day.

### 3.2.2. Brood stock

Ovigerous female Nephrops were caught in baited creels and taken back to the laboratory where their eggs were examined under a stereomicroscope. Those with eggs approaching the ninth and final stage of development before hatching (Plate 4.1a) were maintained as a brood stock. They were held individually in tanks (1.0 x 0.5 x 0.5m) in a flow-through system. The water depth was maintained at approximately 260mm with a flow rate in the region of 4 lmin<sup>-1</sup>. Air stones were used to ensure adequate oxygenation of the water. Mesh gauze was placed over the stand pipe to retain any newly hatched larvae. The Nephrops were provided with shelter (plastic piping) but were not fed during their period of incubation. The territorial nature and intraspecific aggressive behaviour of brood stock animals necessitated their isolation. Individual containment also allowed individual assessments of fecundity, hatching times and numbers of



spawned larvae (see Chapter 7). The tanks were examined three times a day for newly hatched larvae which were removed by hand net to the culturing facility. Large age differences between larvae usually resulted in increased cannibalism ; therefore each rearing bin was stocked with larvae hatched over a period of no more than two days.

### 3.2.3. Larval culture techniques

#### 3.2.3.1. Open flow-through system

The first attempts to culture Nephrops larvae were made using modified white plastic 'Hughes kreisels' (Hughes et al., 1974). Although the basic design of the bins was unchanged a smaller version of 10 - 15 l capacity was used, the volume depending on the setting of the overflow pipe. The mesh size of the screen surrounding the stand pipe was necessarily finer than that used by Hughes et al. (1974) since the food source to be retained for Nephrops larvae was much smaller than that used for H.americanus. It was eventually found that sterilized nylon stockings provided an adequate mesh screen, with a pore diameter of approximately 0.5mm. Water was supplied at a rate of 1.5 - 2.0 lmin<sup>-1</sup> having been passed through the pre-treatment system. Unfortunately, it was found that the water flow rate was liable to fluctuation due to other sources tapping into the supply or pipe blockages. Consequently the open flow-through system was abandoned and replaced by a static larval culture system. Nevertheless it had been clearly demonstrated that the former system could have been successful had the water supply been more reliable.

### 3.2.3.2. Static larval culture systems

Acrylic bowls of 4 l capacity were used in this system with initial stocking densities of 10 larvae l<sup>-1</sup>. An air stone was used to provide aeration and generate water movement within each bowl. The terminology for the description of this system was borrowed from Jamieson *et al.* (1976), although technically, with the provision of aeration, conditions were not in fact static. Aeration caused uniform dispersal of both the larvae and the food organisms. This method also reduced the number of contacts between larvae, thus reducing the incidence of cannibalism. All culture water, which was supplied from the reservoir tanks, was changed every second day. The temperature of new water was adjusted to within 0.5°C of that which it was replacing.

The larvae were never subjected to direct sunlight and all culture units were maintained under conditions of dimmed laboratory lighting at levels of between 2 - 5  $\mu\text{Es}^{-1}\text{m}^{-2}$ .

### 3.2.4. Juvenile culturing techniques

#### 3.2.4.1. Tray system

This culture unit was designed to operate as an open flow through system and was based on the experimental culture system devised by Richards and Wickins (1979). Two size choices were available with individual containers being made up in groups for ease of construction and handling. The smaller containers (75 x 50 x 40mm) were made from clear acrylic in units of 3 x 3 with 1mm mesh bases. They were supported on blocks to raise them 10mm off the bottom of the tray.

This allowed removal of waste products and food remains which settled to the bottom of the tray. The larger containers (330 x 180 x 100mm) were made from polyethylene and were subdivided into units of 3 (180 x 110 x 100mm) by the insertion of removable partitions. These units were also provided with 1 mm mesh bases but supported on blocks to a height of 20mm off the tray base. Mesh sheeting of variable mesh size (depending on postlarval stage) was then secured above the cubicles to maintain isolation and prevent escape. The groups of cubicles were then placed in trays which were supplied directly with treated water. Water levels were maintained 4 to 5cm above the height of the cubicles with flow rates of between 1 - 2 lmin<sup>-1</sup>. As with the larval culturing system, water flow fluctuations eventually resulted in this system being replaced by a static culture system.

#### 3.2.4.2. Static single-cell system

All juveniles were maintained individually in polypropylene bowls of between 0.5 - 4.0 l capacity. The water, which was supplied from the reservoir tanks, was changed every second day, temperature being adjusted to within 0.5°C of that which it was replacing. Each bowl was provided with a shelter and maintained under conditions of dim green illumination (1 - 1.4  $\mu\text{Es}^{-1}\text{m}^{-2}$ , spectral composition given in section 6.2.2.). Although the precise spectral response curve for these early postlarval stages was unknown, it was considered that these light levels would not induce retinal damage (Shelton *et al.*, 1985), though this is unlikely in the larvae (see Chapter 5).

### 3.2.5. Larval survival and growth rates

For each experiment newly hatched larvae were obtained on the same day and from the same ovigerous female in order to minimise the variability of larval quality. These larvae were reared under conditions of mass and individual culture and fed to excess.

The mass culture experiments were conducted in acrylic bowls of 4 l capacity whereas beakers of volumes 200, 400 and 500ml were used for the individual rearing of stage 1, 2 and 3 zoeae, respectively. All trials were conducted under conditions of static culture, with air stones provided in the mass culture units only. Temperature and salinity readings were taken twice daily. Throughout all growth and survival experiments salinity was maintained at between 32 - 34‰. Any mortalities were removed during daily inspections and survival and growth were recorded daily throughout each experiment.

#### 3.2.5.1. Stocking density

This experiment was carried out in two parts. In the first, zoeae were reared under conditions of mass culture at initial stocking densities of 3, 7, 10, 14 and 23 zoeae  $l^{-1}$ . Each density level was duplicated and the zoeae were maintained under conditions of room temperature (13 - 15°C). In the second part, two groups of 100 zoeae were compared under conditions of individual rearing and mass culture. For the latter, initial stocking densities of 10 zoeae  $l^{-1}$  were used in 3 bins, 2 of which contained 35 larvae in 3.5 l of water and one contained 30 larvae in 3 l of water. The zoeae within this trial were maintained under slightly warmer ambient temperatures (15 - 17°C) than



those participating in the first trial.

#### 3.2.5.2. Temperature

Using water baths and coolers, zoeae of all three stages were incubated at temperatures of 8, 10, 12, 14, 16, 18 and 20 °C. The newly hatched stage 1 zoeae were reared under conditions of mass culture, with initial stocking densities of 10 zoeae l<sup>-1</sup>. The older stages (2 and 3) were cultured individually.

#### 3.2.5.3. Starvation

Newly hatched zoeae were held individually to eliminate cannibalism. They were kept at ambient temperatures (13 - 15°C) and starved for periods of between 1 and 7 days. A control was also conducted with larvae which were fed immediately after hatching. After the initial period of starvation food was administered twice daily. The effect of the initial period of starvation upon future development was monitored through each zoeal stage.

### 3.3. Results

#### 3.3.1. Stocking density

Figure 3.1a and 3.1b compare the survival of newly hatched 1st stage zoeae from initial stocking densities of 3, 7, 10, 14 and 23 newly hatched zoeae per litre. There was no significant difference between the intermoult periods recorded for development from 1st to 2nd stage zoeae (approximately 9 days) or from 2nd to 3rd stage zoeae (approximately 11 days) at the different initial stocking densities. The mortality of zoeae was low for the first 2 days but increased thereafter. Mortality was greatest at the highest stocking densities. After 1 week the proportion of surviving zoeae at the lowest density was more than twice that at the highest. The continuing high mortality of zoeae reared at high densities eventually led to similar numbers of larvae in each culture bin ; by the 13 to 14th day all culture bins contained around 0.4 - 0.5 larvae  $l^{-1}$  (Figure 3.1b).

The effect of initial density upon survival and development through the first 3 zoeal stages is presented in Table 3.1. The proportion of stage 1 zoeae reaching stage 2 was greatest at the lowest density (42%). At higher densities the proportion of zoeae reaching stage 2 was between 13 - 24%. No more than a single zoea reached stage 3 for each initial stocking density.

In a second experiment the mass rearing of larvae at a density of 10 larvae  $l^{-1}$  was compared with individual rearing. The results are presented in Table 3.2. The survival under the two sets of conditions



**Figure 3.1**

**(a) The influence of different initial stocking densities of newly hatched 1st stage zoeae on their future survival.**

**(b) Changes in the mean stocking density for each initial stocking density of newly hatched 1st stage zoeae.**

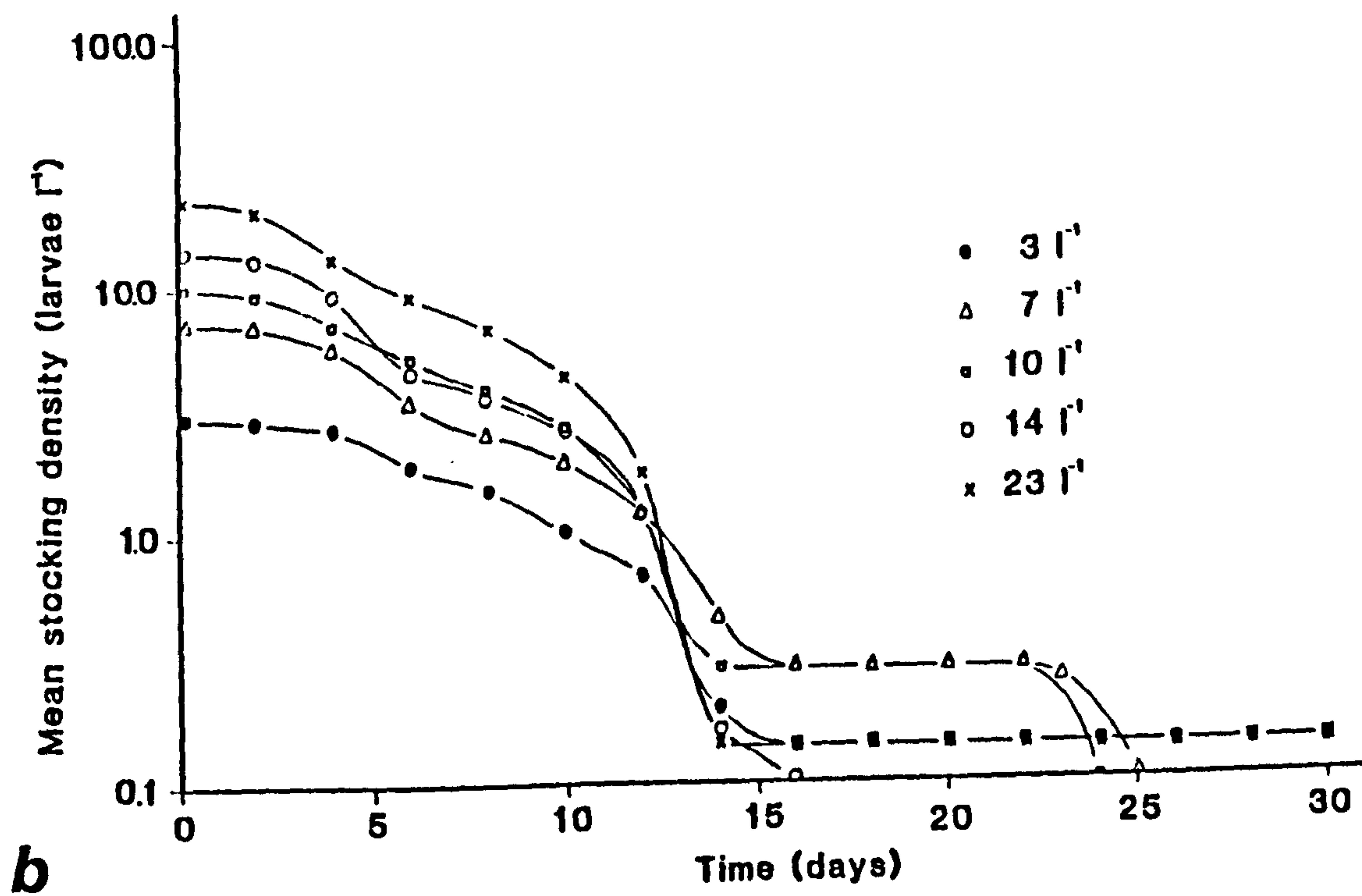
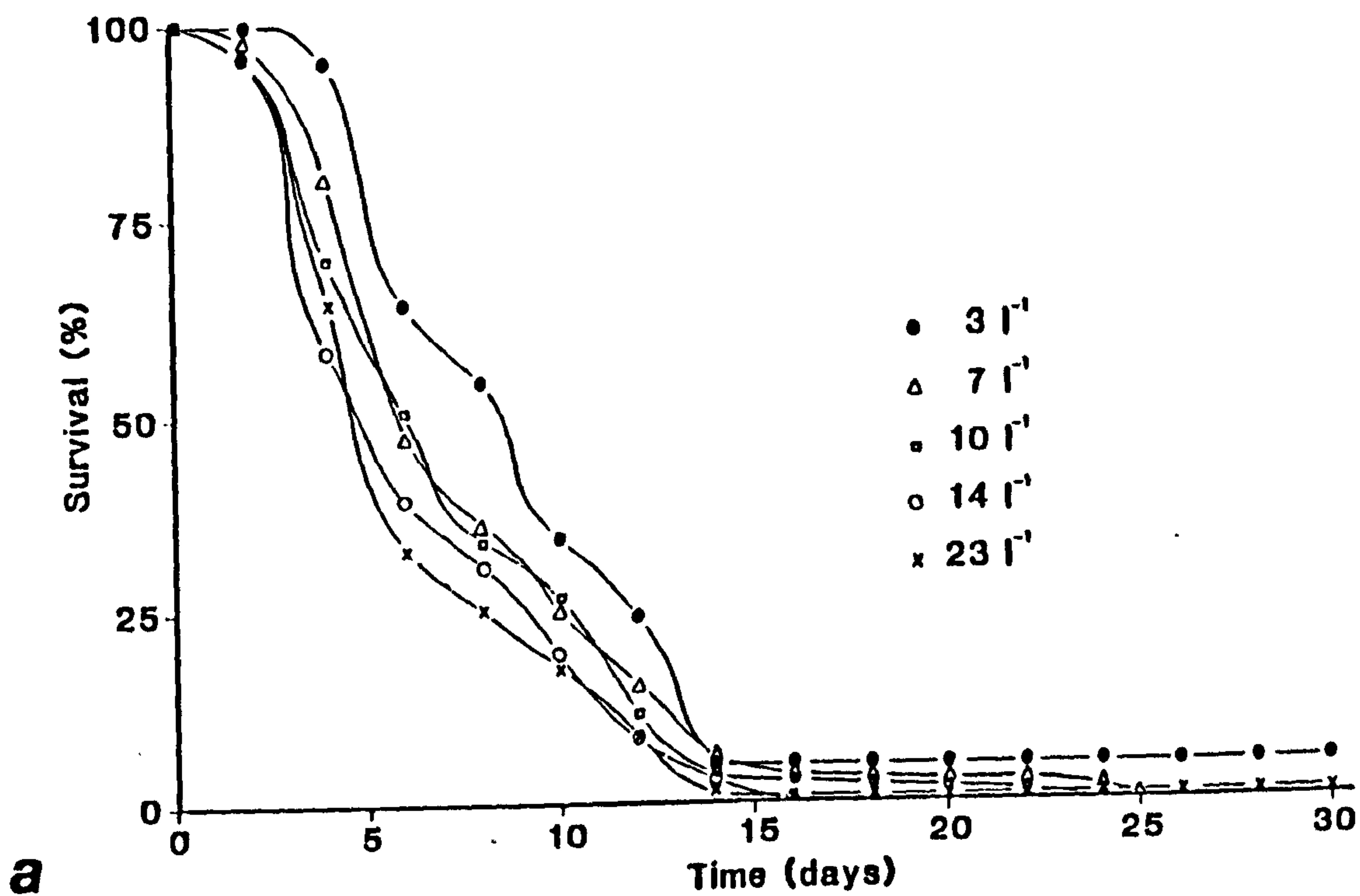


Table 3.1

The number and proportion of first stage zoeae reaching the 2nd & 3rd stages from different initial stocking densities.

Stocking density (No1-1)	Zoea 1	Zoea 2		Zoea 3	
	Initial number of Z1	Number reaching Z2	Proportion stage Z1 - Z2	Number reaching Z3	Proportion stage Z1 - Z3
3	24	10	0.42	1	0.04
7	56	13	0.23	1	0.02
10	80	19	0.24	1	0.01
14	112	15	0.13	0	0
23	184	28	0.15	1	0.01

Z = Zoea.

is illustrated for all zoeal stages combined in Figure 3.2 and for each stage separately in Figure 3.3. Mortality was greater throughout larval development in the mass culturing units and, as shown in the first experiment, greatest mortality occurred during the first few days when densities were at their highest. After 8 days the levels of survival under mass and individual culture were 42 and 79% respectively (Figure 3.2). Table 3.2a shows that development into the 2nd zoeal stage was approximately 2.3 times greater in individual as compared with mass culture. The higher level of mortality recorded under the mass culture conditions continued throughout the 2nd zoeal stage. After 2 weeks only 21 of the initial population of 100 stage 1 zoeae survived in mass culture compared to 70 larvae in the individual rearing units and after a further week the numbers were down to 6 and 56 zoeae respectively (Figure 3.2). The number of stage 1 zoeae which achieved metamorphosis into the first postlarval stage under conditions of individual rearing was nearly 8 times greater than was achieved in mass culture.

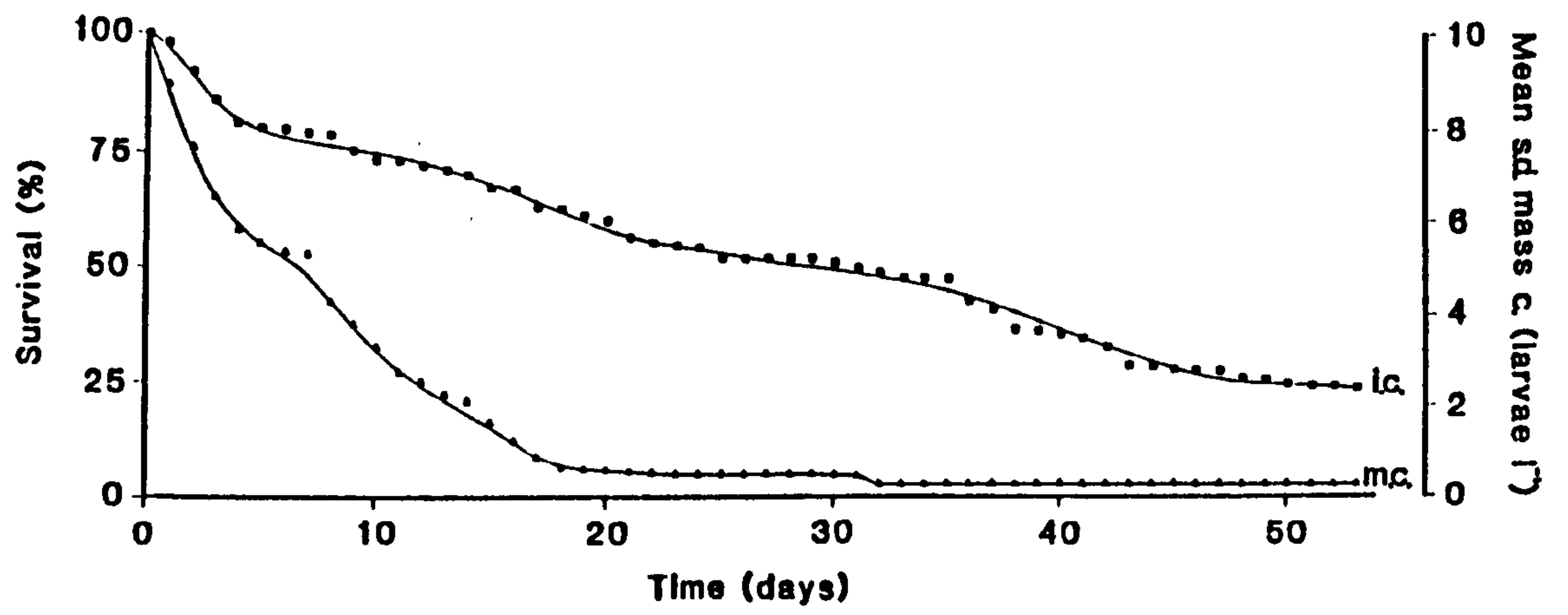
Under mass culture the rate of mortality decreased after 18 days when the larval population had been reduced to 6 stage 3 zoeae, corresponding to 2 individuals per bowl. Only one of these achieved the metamorphic moult to the postlarval stage.

Survival under conditions of individual rearing appeared to be similar over the 1st and 2nd zoeal stages. 73% of the stage 1 zoeae completed ecdysis into the 2nd zoeal stage and of these 75% completed ecdysis into the 3rd zoeal stage. The mortality appeared to be higher during the final zoeal stage and only 40% of them reached the postlarval stage.

**Figure 3.2**

**The survival record for zoeae reared under conditions of individual (ic) and mass (mc) culture. Changes in the mean stocking density (s.d.) for larvae reared in the mass culture units is also indicated.**

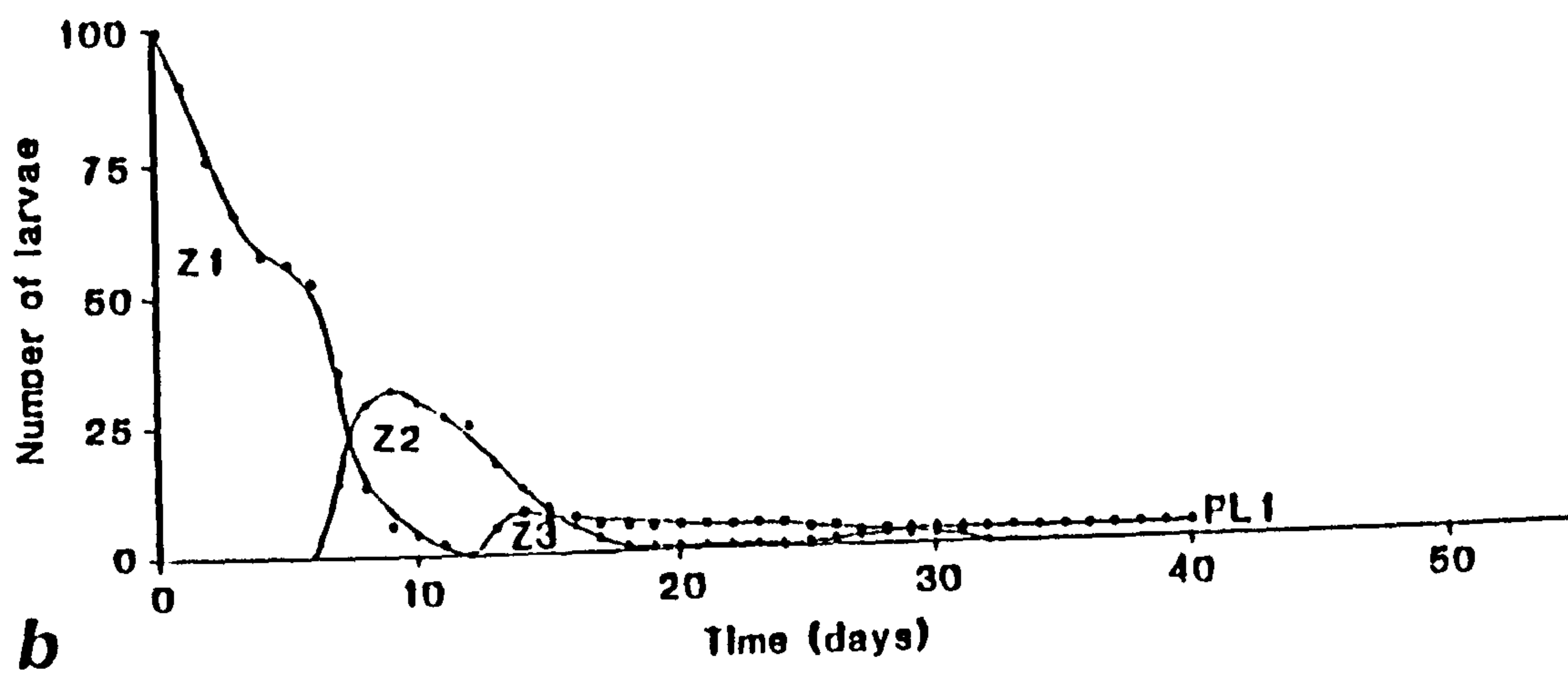
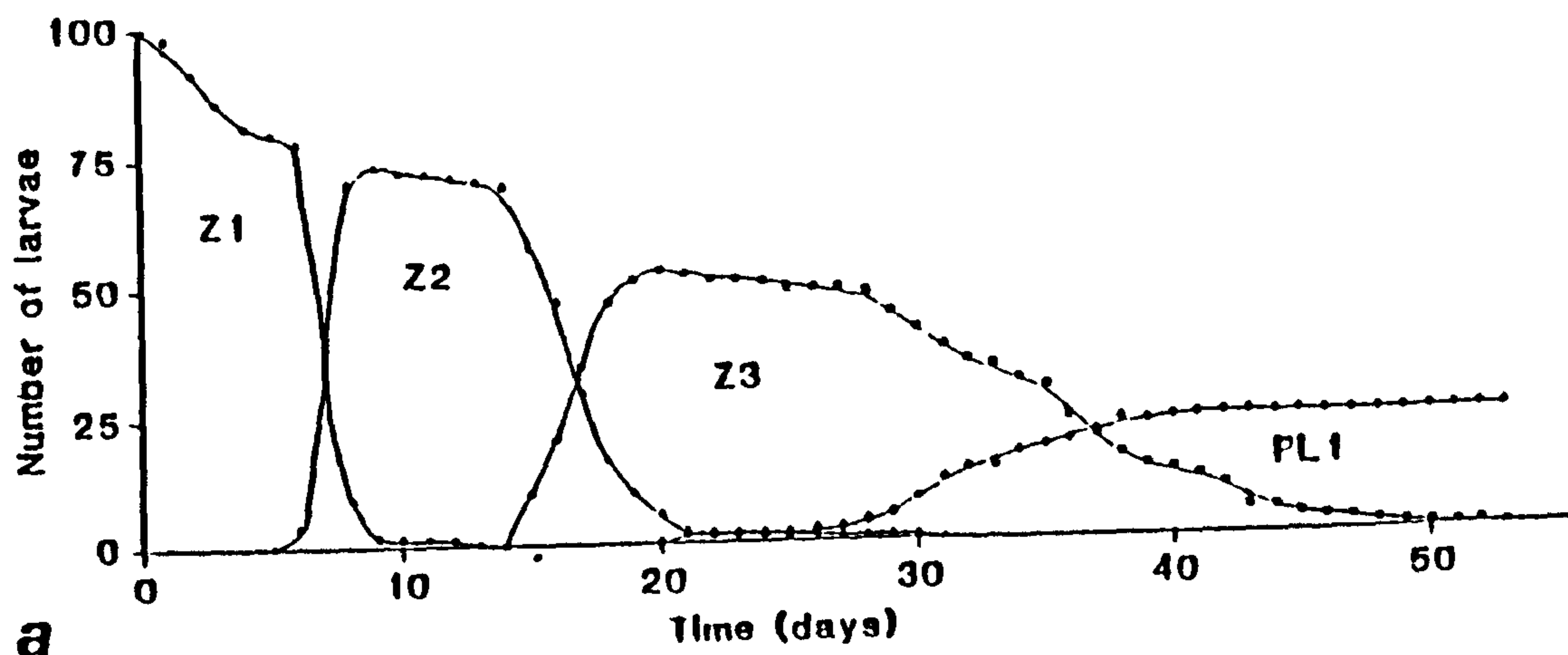




### **Figure 3.3**

**Rearing record for the number of larvae surviving/stage/day under conditions of (a) individual and (b) mass culture.**

**Z = Zoea, PL = Postlarva.**



**Table 3.2a**

**The number and porportion of zoeae reaching the 2nd & 3rd zoeal and 1st postlarval stages under different rearing conditions.**

Culture condition	Zoea 1			Zoea 2		Zoea 3		All 3 stages
	Initial no. Z1	Number reaching Z2	Propn. Z1-Z2	Number reaching Z3	Propn. Z2-Z3	Number reaching PL1	Propn. Z3-PL1	Propn. Z1-PL1
Mass	100	32	0.32	8	0.25	3	0.38	0.03
Individual	100	73	0.73	55	0.75	22	0.40	0.22

**Z = Zoea, PL = Postlarva.**

Although individual rearing has been shown to enhance larval survival it does not appear to accelerate the development time. Indeed, shorter intermoult periods were recorded between the 2nd and 3rd zoeal stages and between the 3rd zoeal stage and the 1st postlarval stage under conditions of mass culture (Table 3.2b). There was no significant difference ( $P > 0.05$ ) between the mean stage duration of 1st zoeae under the individual and mass culturing conditions. The development time was, however, significantly faster ( $P < 0.05$ ) for attainment of the 3rd zoeal stage (stage 1 to stage 3 zoea) under conditions of mass culture but there was no significant difference ( $P > 0.05$ ) between the total development time to the 1st postlarval stage under the two conditions of culture. As only 3 individuals reached the postlarval stage under the mass rearing conditions it is not surprising that no significant difference was recorded between the mass and individual total development times.

### 3.3.2. Temperature

The results of the investigation into the influence of temperature upon larval development are summarised in Table 3.3. The effect of temperature upon intermoult period for the 3 zoeal stages has been computed and described by 'best-fit' curves after logarithmic transformation and linear regression (Figure 3.4, Table 3.4). For the same reasons as proposed by McLaren (1963), Hartnoll (1982) favoured the use of the Bělehrádek (1935) temperature function  $V = a(T + \alpha)^b$  where  $V$  is velocity,  $T$  is temperature and  $a$ ,  $b$  and  $\alpha$  are constants. Alpha is a scale correction ( $\alpha$ ), the 'biological zero', at which  $V = 0$ . However, Hartnoll (1982) concluded that the scale correction was usually small and could be safely ignored. Bělehrádek's equation



### Stage durations for zoeae under different rearing conditions

PL = Postlarva.

**Table 3.3a**

The number and proportion of zoeae reaching the subsequent moult stage for the 3 stages when reared at different temperatures.

Zoea 1			Zoea 2			Zoea 3			
Temp. (°C)	Initial number Z1	Number reaching Z2	Propn. Z1-Z2	Initial number Z2	Number reaching Z3	Propn. Z2-Z3	Initial number Z3	Number reaching PL 1	Propn. Z3-PL1
8	100	10	0.10	21	0	0	4	0	0
10	100	13	0.13	18	5	0.28	13	0	0
12	100	28	0.28	19	9	0.47	13	5	0.38
14	100	26	0.26	50	12	0.24	12	4	0.33
16	100	34	0.34	34	7	0.21	7	4	0.57
18	80	24	0.30	24	13	0.54	13	9	0.69
20	80	9	0.11	13	1	0.08	3	1	0.33

Z = Zoea, PL = Postlarva.

Table 3.3b

Stage durations for zoeae reared at different temperatures.

Temp (°C)	Stage durations (days)									Overall average development time (days)
	Zoea 1			Zoea 2			Zoea 3			
	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	
										Zoea 1 - PL1
8	21.80	2.25	18-25	-	-	-	-	-	-	-
10	11.69	1.32	10-14	21.80	4.44	18-28	-	-	-	-
12	9.1	0.98	7-12	15.33	2.78	13-21	21.60	2.51	18-25	46.11
14	6.96	0.82	6-10	9.33	1.23	8-12	12.75	1.71	11-15	29.04
16	5.24	0.70	4-6	6.71	0.76	6-8	9.50	1.29	8-11	21.45
18	6.04	0.81	5-8	6.00	0.82	5-8	8.67	1.22	7-11	20.71
20	6.11	0.60	5-7	7.00	-	-	7.00	-	-	20.11

PL = Postlarva.

Figure 3.4

The log-log relationships describing the effect of temperature on the intermoult period for the 3 zoeal stages. The equations of the lines are shown in Table 3.4.

Z = Zoea.

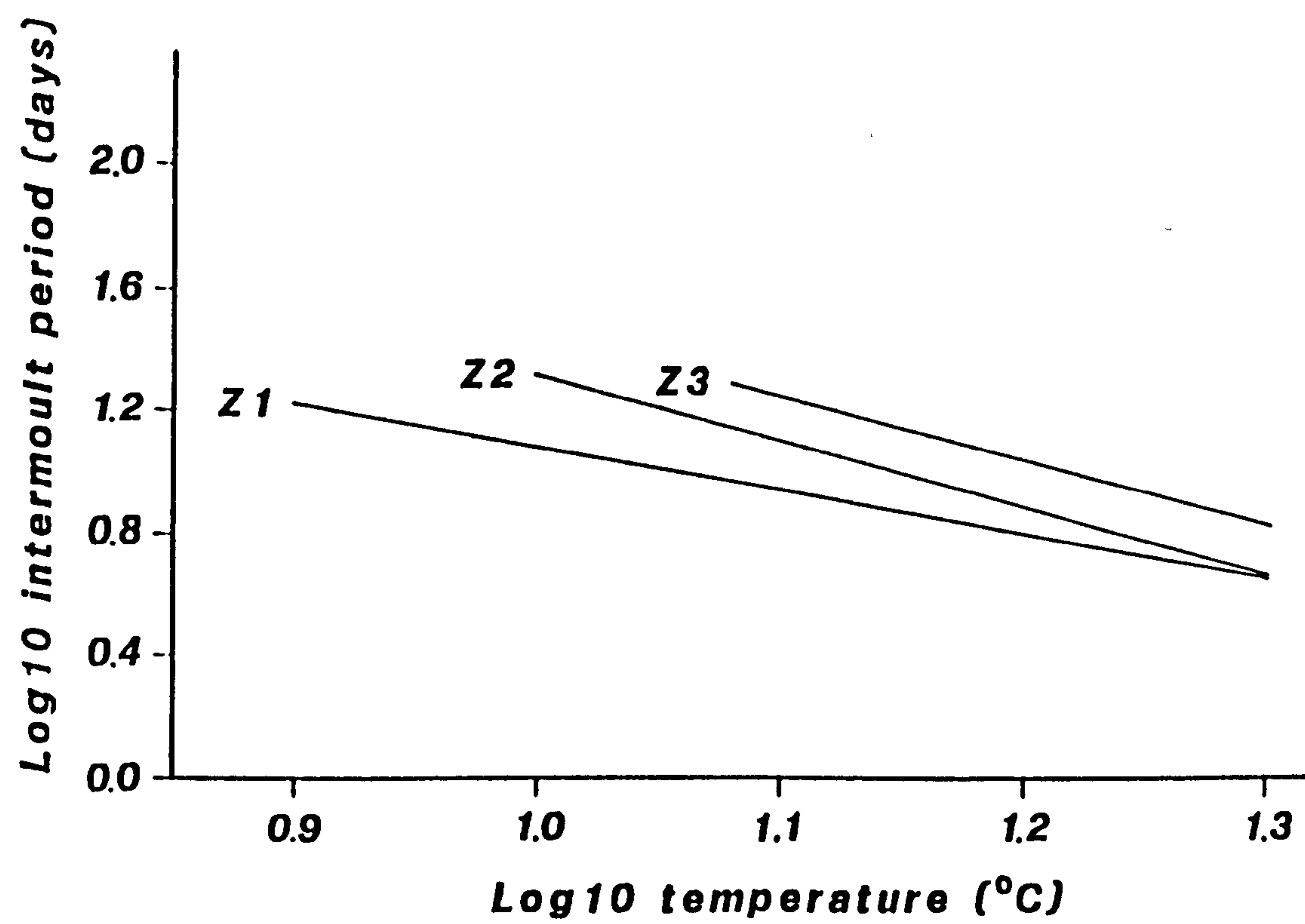




Table 3.4

Log-log regression equations relating intermoult period (Y) to incubation temperature (X). The correlation coefficients are given.

Development stage	Equation of the line	Correlation coefficient	Significance of regression (P)
Zoea 1 - 2	$\text{Log } Y = 2.54 - 1.45 \text{ Log } X$	0.881	< 0.001
Zoea 2 - 3	$\text{Log } Y = 3.51 - 2.19 \text{ Log } X$	0.934	< 0.001
Zoea 3 - PL 1	$\text{Log } Y = 3.63 - 2.16 \text{ Log } X$	0.925	< 0.001

PL = Postlarva.

therefore reduces to the simple power function  $V = aT^b$ , used in this study.

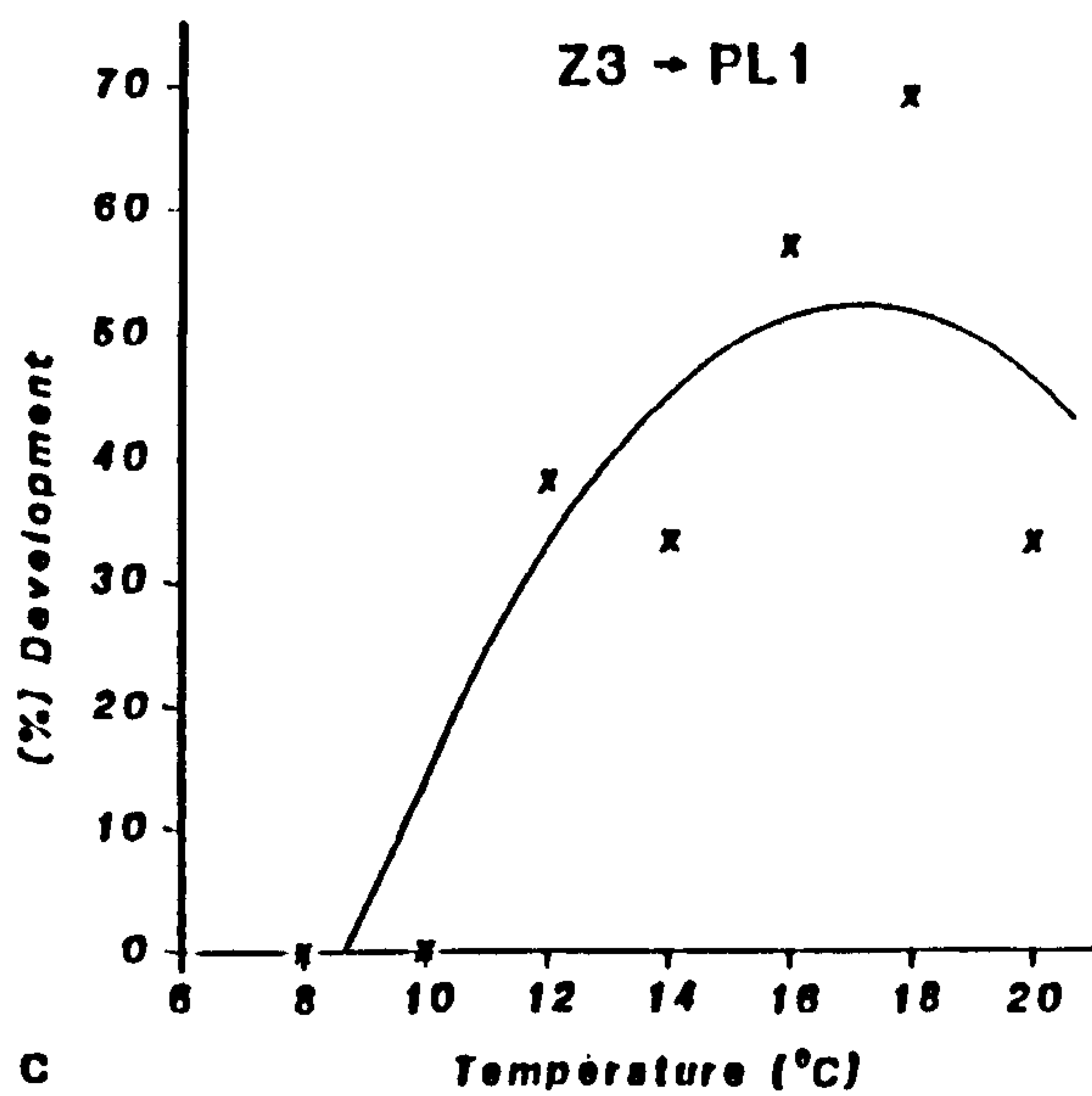
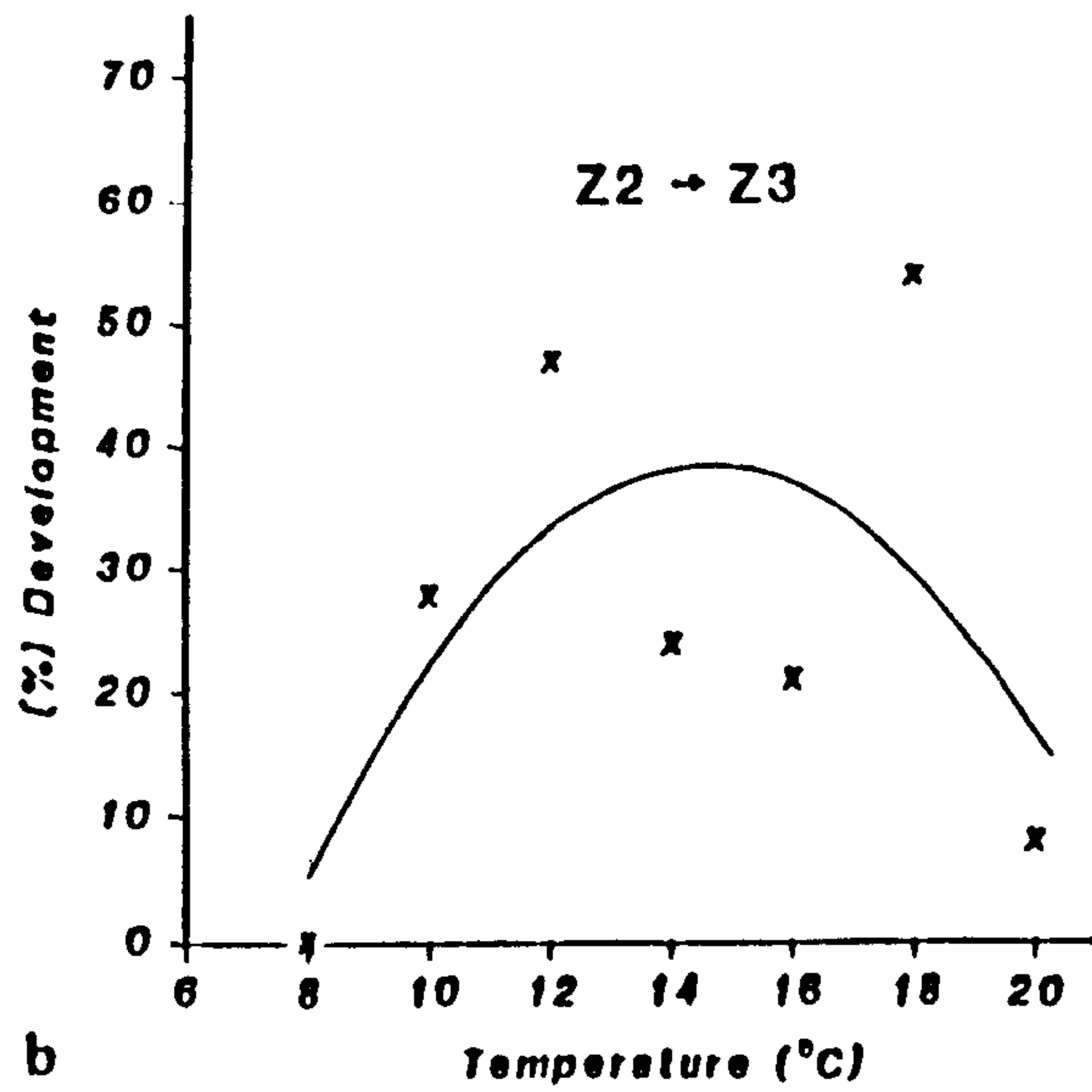
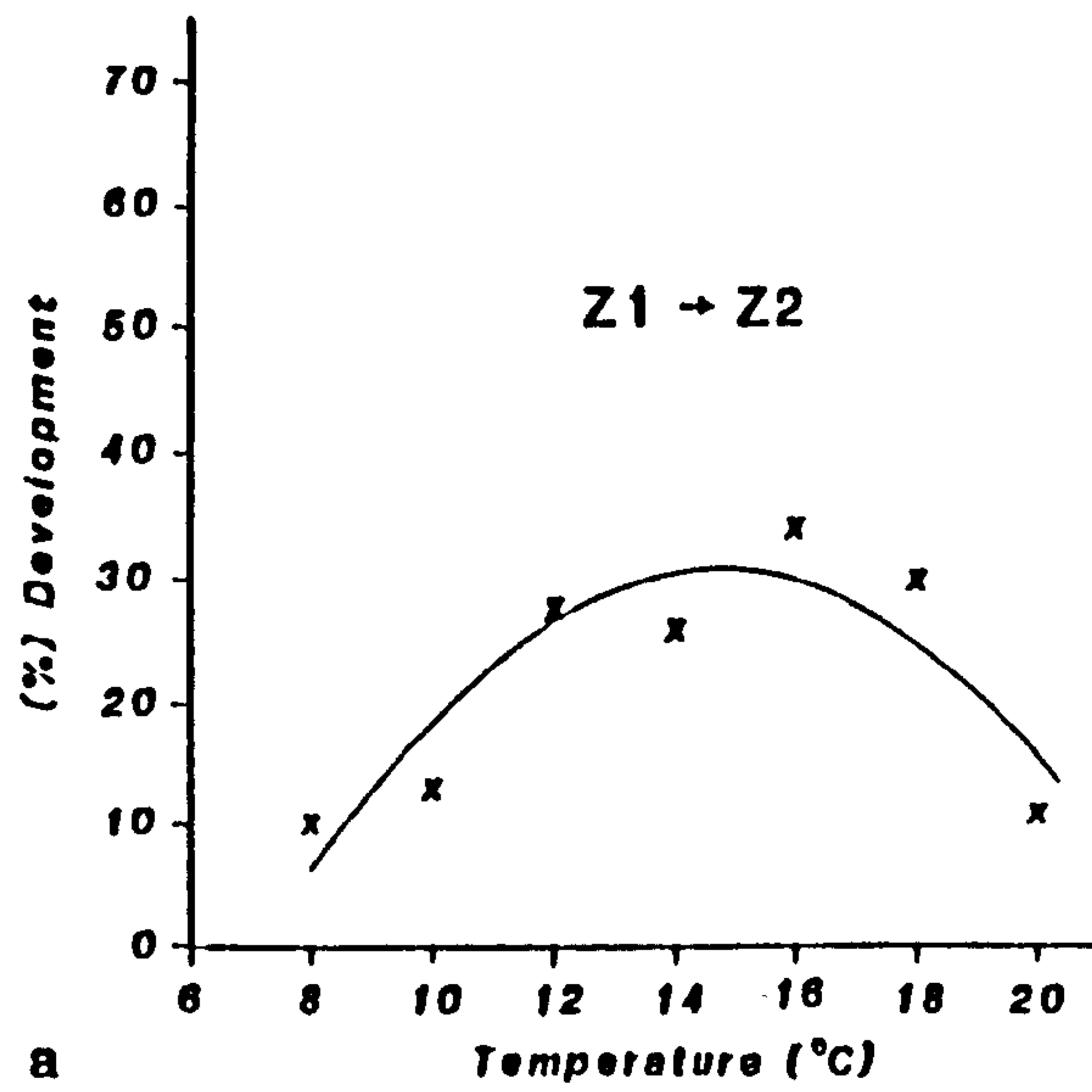
There was a progressive reduction in development time for all 3 zoeal stages with increasing temperature in the range 8 - 16°C and an increase in the intermoult period at each temperature for successive zoeal stages. At 8°C development was very slow, taking more than 4 times longer for the completion of the first moult than at 16°C. At 8°C there was no development beyond the first moult and 12°C was the lowest temperature at which complete development to the 1st postlarval stage was obtained. The slope (b) of the regression line for 1st stage zoeae is significantly different ( $P < 0.05$ ) from those for 2nd and 3rd stage zoeae which do not themselves differ significantly. According to Hartnoll (1982) a steeper slope to the regression indicates greater sensitivity to temperature. It therefore seems that 2nd and 3rd stage zoeae are more sensitive to temperature than 1st stage zoeae. Although the mean period for complete development through all 3 zoeal stages was reduced from 46 to 20 days with an increase in temperature from 12 - 20°C, above 16°C this reduction was very slight (21.45 - 20.11 days). There seems to be a slight increase in the temperature corresponding to the fastest rate of development (ie. shortest intermoult period) for successive stages (16, 18 and 20 °C for stages 1, 2 and 3 respectively).

Scatter plots describing the effect of temperature upon the proportion of zoeae which successfully moult to the next stage are shown in Figure 3.5. The plots indicate that within the temperature range of the experiment (8 - 20°C) the highest proportion of zoeae succeeded in moulting to the next stage at an intermediate

Figure 3.5

Scatter plots and best fit polynomial curves describing the effect of temperature upon the proportion of (a) 1st stage zoeae, (b) 2nd stage zoeae and (c) 3rd stage zoeae which succeed in moulting into the next stage. The equations of the curves are shown in Table 3.5.

Z = Zoea.



temperature. This therefore suggests polynomial curves may best describe the relationship between the variables. Computer analysis revealed that development of the polynomial beyond the second degree did not explain significantly more of the variability. Best-fit quadratic equations were therefore derived to describe the data (Table 3.5). The data for 1st stage zoeae was adequately fitted by a second degree curve ( $P < 0.05$ ). This relationship predicts best survival into the 2nd zoeal stage at approximately 14.5°C (31%). The variability within the data for 2nd and 3rd stage zoeae was not adequately explained by the polynomial curves. The high  $R^2$  value obtained for 3rd stage zoeae was due to the relatively large proportion of the total variability within the data which was explained by the linear term (57.4%). The 2nd degree polynomial does not explain significantly more of the variability ( $P > 0.05$ , ie. 18.4%). However, the shape of the 2nd degree curves constructed for 2nd and 3rd stage zoeae indicate that if more data had been available best survival from the 2nd to the 3rd zoeae would have occurred at approximately 14.5°C (38%) and best survival from the 3rd zoeae to the 1st postlarvae would have occurred at approximately 17.0°C (52%). From the culture records it was observed that the highest proportion of stage 2 zoeae moulted into the 3rd zoeal stage at 18°C and the highest proportion of stage 3 zoeae moulted into the 1st postlarval stage (ie. metamorphosis) at 18°C (69%). The effect of temperature upon the proportion of 1st stage zoeae which successfully completed ecdysis into the next stage cannot be directly compared with the corresponding data for 2nd and 3rd stage zoeae as the method of culture was changed from mass (10 larvae  $l^{-1}$ ) to individual rearing after ecdysis into 2nd zoeae.



Table 3.5

Quadratic equations describing the effect of temperature (X) upon the proportion of zoeae (Y) which successfully moult to the next stage.

Development stage	Equation	R <sup>2</sup>	Significance of regression (P)
Zoea 1 - 2	$Y = -88.0 + 16.2 x - 0.551 X^2$	78.2%	< 0.05
Zoea 2 - 3	$Y = -126.0 + 22.6 x - 0.774 X^2$	39.6%	n.s.
Zoea 3 - PL1	$Y = -166.0 + 25.6 x - 0.750 X^2$	76.0%	n.s.

PL = Postlarva.

### 3.3.3. Starvation

The results of the investigation into the effects of initial periods of starvation on future development in newly hatched larvae are presented in Table 3.6. The 'knock-on' effects of different initial periods of starvation upon the intermoult period between zoeal stages and for complete development through all three stages are illustrated in Figure 3.6. The equations of the computed regression lines are presented in Table 3.7. The slope of the regression line is steepest for the 1st zoeal stage, decreasing progressively over the two subsequent larval stages. For zoeal stages 1, 2 and 3 the slopes were 1.510, 0.874 and 0.184 respectively.

Although the linear regression lines could only be fitted to derived mean values for stage duration in groups of larvae the high 'r' value recorded for the 1st and 2nd zoeal stages is highly significant ( $P < 0.001$ ). It therefore appears that for each day the newly hatched larvae were starved the intermoult period was extended by approximately 1.5 and 0.9 days during the 1st and 2nd zoeal stages respectively. For the final larval stage the slope of the regression line was not significantly different from zero ( $P > 0.1$ ). It therefore appears that the effect of initial periods of starvation upon intermoult period declines progressively through development and after the 3rd zoeae it is no longer evident. Nevertheless, the duration of larval development over all three stages was significantly correlated with the initial starvation period (Table 3.7,  $P < 0.001$ ). It therefore appears that the larval phase was extended by approximately 2.4 days for each day the newly hatched larvae were initially starved.

Table 3.6a

The number and proportion of zoeae reaching the 2nd & 3rd zoeal and 1st postlarval stages after different initial periods of starvation.

Days starvation	Zoea 1			Zoea 2		Zoea 3		All 3 stages
	Initial number Z1	Number reaching Z2	Propn. Z1 - Z2	Number reaching Z3	Propn. Z2 - Z3	Number reaching PL1	Propn. Z3 - PL1	Propn. Z1 - PL1
0	30	23	0.77	23	1.00	21	0.91	0.70
1	30	25	0.83	24	0.96	21	0.88	0.70
2	37	27	0.73	24	0.89	18	0.75	0.49
3	44	32	0.73	32	1.00	20	0.63	0.45
4	40	25	0.63	19	0.76	9	0.47	0.23
5	40	28	0.70	19	0.68	4	0.21	0.10
6	40	10	0.25	6	0.60	2	0.33	0.05
7	40	4	0.10	2	0.50	0	0.00	0.00

Z = Zoea, PL = Postlarva.

Table 3.6b  
Stage durations for zoeae reared after different initial periods of starvation.

Days starvation	Development										time (days)			Calculated average development time (days)		
	Zoea 1			Zoea 1 - 3			Zoea 1 - PL 1									
	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range							
0	8.13	0.76	7-9	17.39	0.94	17-20	34.62	3.06	31-40	9.26		17.23				
1	9.76	0.52	9-11	18.79	0.88	17-21	34.52	3.09	29-42	9.03		15.73				
2	10.15	0.66	9-11	20.67	1.66	19-25	37.72	3.29	33-46	10.52		17.05				
3	11.53	0.72	11-13	22.91	2.10	20-28	40.55	5.12	33-58	11.38		17.64				
4	13.12	0.78	12-15	25.32	2.43	21-29	40.89	2.85	38-48	12.20		15.57				
5	14.57	0.89	14-16	27.79	2.76	23-35	44.25	2.99	41-48	13.04		16.46				
6	16.90	1.20	15-19	30.90	0.75	25-27	49.73	4.24	42-48	14.00		18.83				
7	19.00	2.45	17-22	34.00	1.41	33-35	-	-	-	15.00		-				

PL = Postlarva.

**Figure 3.6**

The relationships between the mean intermoult period for development from 1st to 2nd stage zoeae (Z1), 2nd to 3rd stage zoeae (Z2), 3rd stage zoeae to 1st stage postlarvae (Z3) and for development through all 3 zoeal stages (Total) after exposing newly hatched 1st stage zoeae to different initial periods of starvation.



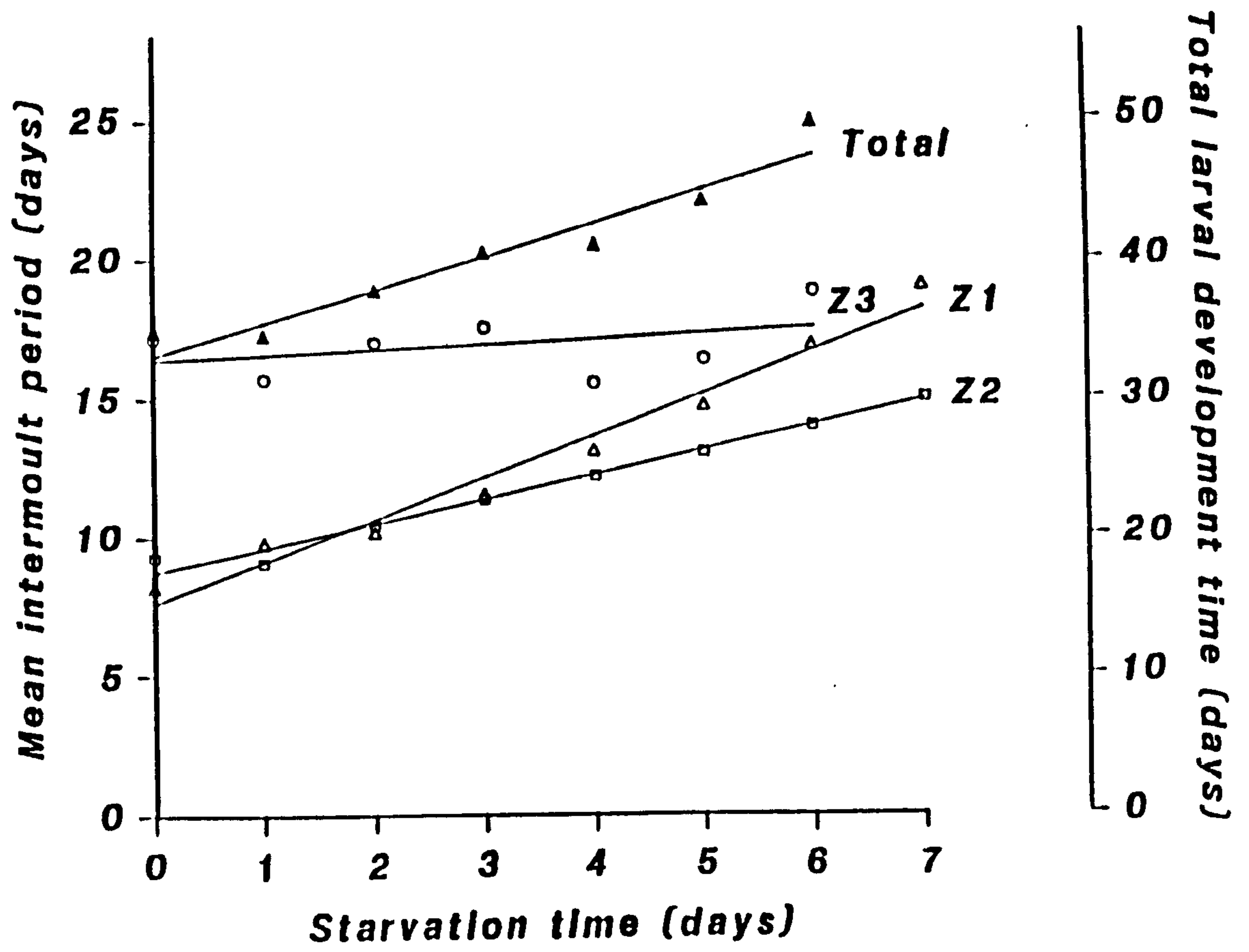


Table 3.7

Linear regression equations relating mean intermoult period (Y) to initial period of starvation (X). The correlation coefficients (r) are given.

Development stage	Equation of the line	r	P value
Zoea 1 - 2	$Y = 7.62 + 1.510 X$	0.99	< 0.001
Zoea 2 - 3	$Y = 8.75 + 0.874 X$	0.99	< 0.001
Zoea 3 - PL1	$Y = 16.4 + 0.184 X$	0.23	n.s.
Zoea 1 - PL1	$Y = 33.0 + 2.430 X$	0.96	< 0.001

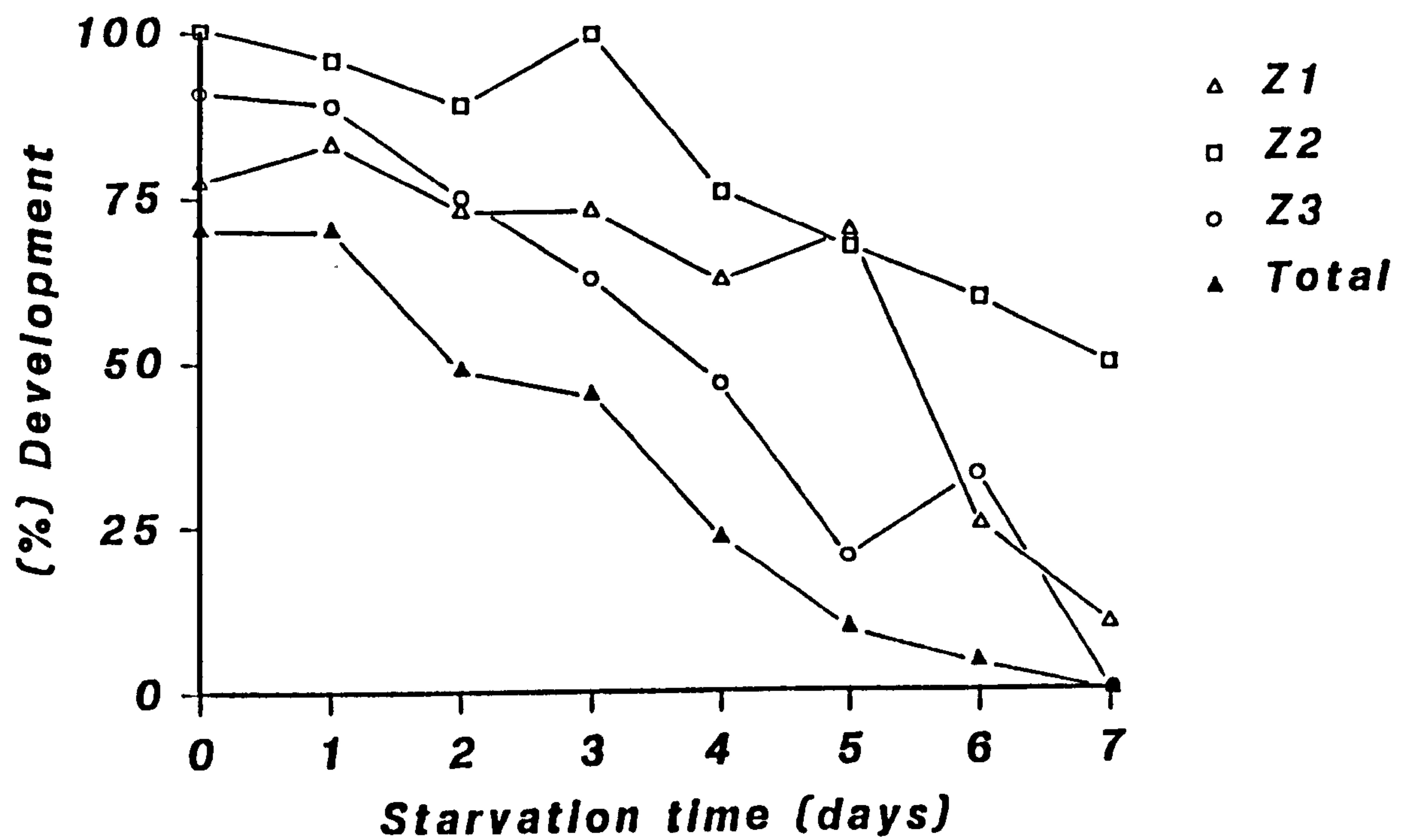
PL = Postlarva.

Figure 3.7 illustrates the survival throughout larval development for each period of initial starvation. It appears that one day of starvation has little influence upon future survival but periods in excess of this inflict increasing levels of mortality upon all 3 zoeal stages. Greatest mortality generally occurred after initial periods of 2 - 5 days of starvation. Survival of newly hatched larvae to the metamorphic moult decreased from 70% after no starvation or 1 day of starvation to 10% after 5 days of starvation. Survival appeared to be less adversely effected by initial periods of starvation during the second zoeal stage than for 1st and 3rd zoeae. The temperature conditions within the trial (13 - 15°C) may have been more compatible with the temperature tolerance of 2nd zoeae. The temperature conditions would therefore cause 1st and 3rd zoeae greater stress than those of the 2nd stage, compounding further the stress caused by the initial periods of starvation.

### Figure 3.7

The proportion of zoeae which successfully moult into the next stage after exposing newly hatched 1st stage zoeae to different initial periods of starvation.

Z1 = Zoea 1 - 2, Z2 = Zoea 2 - 3, Z3 = Zoea 3 - Postlarva 1, Total = Zoea 1 - Postlarva 1.





### 3.4. Discussion

Nephrops larvae can be reared under similar conditions to those used for the larvae of Homarus spp..

Figueiredo (1979) reported that ovigerous female Nephrops removed their eggs while maintained within brood stock aquaria. Consequently, in that study the eggs were stripped from the pleopods for incubation and hatching. Previous research involving H.americanus showed that eggs were maintained and hatched more effectively when retained naturally by the female than when they were removed to any artificial incubation system (Carlson, 1954). This was also found to be the case for Nephrops in this study, with very poor development recorded for artificially incubated eggs. The reason for this is probably the perpetual maintenance and removal of dead and diseased eggs by the female, as well as the dependence upon mechanical agitation for successful hatching (see Chapter 7).

Malachite green was first used as an aquaculture fungicide by Foster and Woodbury (1936) to treat fungal infection on bass, trout and trout eggs. It was then later used in conjunction with U.V. irradiation to successfully treat fungal and epibiotic infestations on the larval stages of H.americanus (Fisher *et al.*, 1976). The filamentous growth which afflicted the Nephrops larvae in this study was not specifically identified. Both filamentous fungi and filamentous bacteria have been reported to infest H.americanus (Fisher *et al.*, 1976). Anderson and Conroy (1968) reported infestations of Nephrops larvae by the ciliate Zoothamnion sp.. In the present study

the infestations entangled the larvae and appeared to mechanically inhibit locomotion, feeding, respiration and ecdysis (Plate 3.1). This effect is similar to that reported in previous larval studies on H.americanus (Nilson et al., 1975) and Nephrops (Anderson and Conroy, 1968). Treatment with malachite green appeared to be successful in controlling the infestation which had previously caused heavy larval mortalities.

Although considerable research has been conducted into the nutritional requirements and the development of artificial diets for H.americanus, live Artemia salina is still generally considered as the best available food source (Hughes et al., 1974 ; Van Olst et al., 1980). The first attempts to culture Nephrops larvae suggested that Artemia nauplii did not provide a sufficient food supply, live Crangon crangon eggs providing a satisfactory alternative (Figueiredo and Vilela, 1972). In a subsequent investigation Figueiredo (1979) fed Nephrops larvae A.salina nauplii, live C.crangon eggs, small pieces of Fucus spiralis and a pure culture of Nannochloris sp.. The results of the present investigation appear to contradict Figueiredo's findings in suggesting that Artemia can exclusively provide Nephrops larvae with a sufficient food supply, although cannibalism was also known to occur. Anger and Püschel (1986) also successfully reared Nephrops zoeae exclusively on a diet of A.salina. Nutritional studies by Van Olst et al. (1975) have shown that cannibalism promotes growth almost as well as brine shrimp in H.americanus.

The modified 'Hughes kriesels' (Hughes et al., 1974) were only used for a short period at the beginning of the rearing programme. However, the early results were promising, indicating that with the

provision of a readily available water supply these vessels would provide suitable culturing units for Nephrops larvae. Large scale rearing of larvae will depend upon the less labour intensive use of mass culture techniques (Jamieson et al., 1976). Further research is required to determine whether the qualities of these bins will permit mass culture of the older larval stages.

Under the competitive conditions of mass culture the stronger and faster developing larvae will have a better chance of survival. Therefore individual rearing will protect the more susceptible larvae from the pressures of competition and risks of cannibalism. Cannibalism in mass rearing systems may serve as a process of selection, removing the weaker and slower growing individuals (Van Olst et al., 1980). This could account for the observed differences between development times in mass culture and individual rearing ie. 25 to 29 days for development through the full larval phase under conditions of mass culture as compared to 28 to 43 days under conditions of individual rearing (Table 3.2b).

The results from the stocking density trials suggest that Nephrops larvae become more cannibalistic with development through the successive zoeal stages. Similar experiments comparing survival through the 3 zoeal stages under conditions of mass and individual culture were conducted by Figueiredo (1979). Survival was also reported to be greatly enhanced for all 3 zoeal stages under conditions of individual rearing, with comparatively better survival being recorded for individual rearing with each successive zoeal stage. The availability of larvae and the accepted level of mortality will determine the stocking density level which can be tolerated. With



development through the culture programme the investment in each individual increases and mortality will become less acceptable.

The effect of communal rearing upon growth and survival through the 3 zoeal and the early postlarval stages was also investigated in this study. Containers were stocked with either 1, 2 or 3 newly hatched larvae. For the communal trials the containers were larger than those used for individual rearing and in both rearing conditions, progressively larger containers were used for successive instars. In the present study communal rearing appeared to lead to the establishment of a faster growing dominant and a slower growing subordinate individual (or individuals) within most groups. As reported for H.americanus the dominant lobster moults first and controls access to both food and shelter (Cobb and Tamm, 1974).

In the present study it appeared that aggressive and cannibalistic behaviour became more evident with development. Under communal rearing conditions, after the metamorphic moult of the fastest developing 3rd zoeae, further development of the co-habitant(s) only occurred if they moulted within 2 days of the first, otherwise they were preyed upon. It was also found that if 2 or 3 first stage postlarvae were 'housed' together there was little evidence of agonistic behaviour. This behaviour changed, however, within 24h of the fastest developing postlarva completing ecdysis into the 2nd postlarval stage. This individual then became actively aggressive towards its co-habitant(s). This behaviour often resulted in cannibalism and invariably led to the death of the subordinate(s) before they were able to moult to the 2nd postlarval stage.

In H.americanus it has been established that aggression is dependent upon stage in the moult cycle (Tamm and Cobb, 1978). It has also been suggested (Cobb and Tamm, 1974) that the full repertoire of agonistic behaviour does not become fully established until the 7th stage (ie. 4th postlarval stage). If similar behavioural changes occur during development in Nephrops then this may explain the higher level of mortality encountered in the mass culture and communal rearing units for the older stages.

Individuals will be most vulnerable to cannibalism immediately after moulting when they are in the soft-shelled state. According to Tamm and Cobb (1978) H.americanus in this condition are much less aggressive. These authors reported that this often led to the dominant lobster swapping roles with the subordinate lobster immediately after moulting. This also appeared to be the case for Nephrops and occasionally led to the previously subordinate lobster eating the once dominant newly moulted co-habitant. The aggressive and solitary nature of nephropid lobsters is one of the major obstacles impeding the attainment of economically viable culture enterprises (Provenzano, 1985).

The optimum temperature for development will be a compromise between the requirement for fast development and for greatest survival. In Nephrops there appeared to be some variation between the 3 zoeal stages but the overall 'optimum' temperature was around 16°C. At this temperature development through the larval phase was completed in approximately 3 weeks. There was a slight increase in the rate of development at temperatures above 16°C but these higher temperatures appear to be associated with an increase in mortality, especially at



temperatures above 18°C. At 20°C only 1 hatched larva from an initial population of 80 survived to metamorphosis. Figueiredo (1979) reported that temperatures of around 20°C were lethal for the postlarval stages of Nephrops.

In previous investigations involving the culture of Nephrops the optimum temperature for the incubation of eggs and survival of larvae was reported as 11 – 14°C (Figueiredo and Vilela, 1972). In the same study the rate of development was approximately twice as fast at temperatures between 13 – 17°C, although, none of the larvae achieved metamorphosis. The rate of development from 2nd to 3rd stage zoeae achieved by Anger and Püschel (1986) at 12°C (mean 11.3 days) was considerably faster than that recorded at the same temperature in this present study (mean 15.3 days) or in all previous rearing experiments involving Nephrops (Phillips and Sastry, 1980). However, Anger and Püschel (1986) conducted their studies on larvae produced from a single ovigerous female and the growth characteristics demonstrated by larvae in their study may be atypical of the population. With improved husbandry the present study has shown that survival can be improved upon at these warmer temperatures (ie. 13 – 17°C). The levels of survival achieved for Nephrops zoeae by Anger and Püschel (1986) at 12°C supports this view. Results from this present study suggest that within the temperature range 8 to 20°C the intermoult period for each zoeal stage will be longer than the preceding stage. This supports evidence from previous investigations (Farmer, 1975).

The results from both the field and laboratory based studies (see Chapter 2 and 5 respectively) suggest that Nephrops larvae descend towards the sea bed during the 3rd zoeal stage. The larval phase,

however, is mainly completed in the upper warmer regions of the water column at temperatures of between 8 - 12°C. Throughout the larval season the temperature and salinity profiles were recorded at the same position (55°40.71'N, 05°00.00'W) and will therefore not take account of any regional variability. Under the culture conditions applied in this study 12°C was the lowest temperature for which complete larval development (to 1st stage postlarvae) was accomplished, taking 46 days. Limited development did, however, occur at 8°C and from previous field studies (Poulsen, 1946) it is known that Nephrops larvae can tolerate temperatures as low as 4°C. From laboratory observations involving a single individual Hillis (1974) reported that the full larval phase was completed in 39 days at 12°C, which agrees reasonably closely with the results obtained in this present work (ie. 46 days at 12°C).

The duration of the full larval phase will depend upon regional conditions which, over the full geographic range of the species will be very variable. Nephrops populations extend from the cool arctic waters off the Icelandic coast to the warm subtropical waters of the Mediterranean. From the temperature information provided by Poulsen (1946) for Danish waters it appears that his prediction of 2 to 3 weeks for completion of the larval phase may be underestimated. According to Hedgecock et al. (1982) there is a low incidence of inter-stock variability within conspecific decapod populations. It therefore seems unlikely that the L.F.C. and Danish Nephrops stocks have different rates of development determined by their genes. In the laboratory the larvae were exposed to artificial environmental conditions and an unnatural diet. In the sea natural conditions may promote faster development and, at temperatures of 10 - 12°C, the

pelagic larval phase may be considerably less than that recorded under laboratory conditions (minimum of 46 days). Using the same reasoning Hillis (1974) suggested that the full larval phase at normal sea temperatures in the Western Irish Sea (which during the larval season are similar to those found in the Lower Firth of Clyde, see Chapter 2) may not be much longer than 39 days, though the average sea temperature during the larval season is considerably colder than 12°C.

It has been suggested from the earlier investigations of Figueiredo and Vilela (1972) that the slow growth rate exhibited by juvenile Nephrops may affect the suitability of this species for aquaculture (Van Olst et al., 1980). Unfortunately, insufficient numbers of juveniles were obtained from the culture programme to ascertain the effect of temperature upon development for the postlarval stages. This would provide a useful subject for future research.

The starvation experiment gives an indication of the influence of food availability upon development in newly hatched larvae. Under laboratory conditions future development of Nephrops larvae was adversely affected by an initial period of starvation. Larval mortality and development time appeared to be directly related to the initial period of starvation. It has been well established from previous nutritional studies of crustacean larvae that, after a critical period of initial starvation, mortality will result even when food is subsequently made available (Sastry, 1983). Under the laboratory conditions of the present investigation starvation in excess of 1 - 2 days led to an increase in mortality of Nephrops larvae.



In the sea larvae may be required to expend more energy in swimming than when confined under the solitary culture conditions of this study. Swimming would probably be needed for maintenance of position in the water column, pursuit of prey and avoidance of potential predators. However, the colder temperatures in the sea (8 - 12°C compared to 13 - 15°C in culture) will induce a slower metabolic rate and a slower rate of development.

Although the effect of starvation upon swimming speeds was not investigated in this study it has been shown to adversely affect the ability of Paralithodes camtschatica (Alaskan king crab) larvae to catch prey (Paul and Paul, 1980). On this basis periods of starvation may have a more severe effect on Nephrops larvae in the sea than under culture conditions where food was provided. In the colder sea temperatures the longer development time may also give rise to a high risk of predation. The pelagic larval life is potentially more hazardous than the benthic postlarval existence (Thorson, 1964).

Final settlement of the early postlarval stages probably determines the eventual success of recruitment. According to Cushing's hypothesis (Cushing, 1982) successful recruitment may depend upon the synchronisation of larval production to that of their food supply (see Chapter 2 also). In temperate regions food availability follows a seasonal cycle which begins with the spring phytoplankton bloom (Russell and 'Yonge, 1975). This plant community is followed in the summer months by a rich fauna of zooplankton. This varied fauna includes Nephrops zoeae which derive their food supply from preying upon other members of the zooplankton. Peak larval abundance for Nephrops in the Lower Firth of Clyde occurred at the end of May and

beginning of June when the mean sea temperature to which the larvae were exposed was approximately 9°C. This was about 2°C below the corresponding value for the seasonal maximum of around 11°C at the beginning of August. In previous studies, a correlation is indicated between stock abundance and annual sea temperatures with a time lag of 3 - 6 years (Chapman, 1984). As yet no direct causal relationship has been demonstrated.

At the present time the commercial culture of Nephrops is technically possible but not economically feasible. Even with the continuing improvements in aquaculture technology and the anticipated eventual commercial viability of Homarus spp. culture (Provenzano, 1985) the continued strength of the traditional fisheries seems likely to deter development of Nephrops culture enterprises for the foreseeable future.

The fishery implications of the present study are discussed in Chapter 8.



## CHAPTER 4

### Growth and morphology of larval and early postlarval stages of Nephrops

#### 4.1. Introduction

This investigation involves both a qualitative and a quantitative study of the morphology of the early stages of Nephrops and the structural changes associated with metamorphosis.

General descriptions of the gross morphology of the zoeal and early postlarval stages of Nephrops are already available in the literature (Santucci, 1926, 1927 ; Karlovac, 1953 ; Andersen, 1962 ; Farmer, 1975). The purpose of this study was to supplement these existing descriptions through the use of light microscopy and Scanning Electron Microscopy. Changes occurring during the transition from the larval to the adult form were identified and examined in greater detail. Various biometric measurements have been used to assess absolute and relative growth during development through the zoeal and early postlarval stages.

Growth is a function of the intermoult period and the moult increment. A review of the four principal methods employed to determine age and growth in crustaceans is given by Hartnoll (1982). These are : 1, growth of captive individuals, 2, growth of tagged specimens, 3, size frequency analysis to discriminate successive instars and year classes and 4, synthesis from data on the intermoult

period and the moult increment. All four methods have been applied to determine growth in adult Nephrops (Thomas, 1965 ; Farmer, 1973 ; Charuau, 1977 ; Conan, 1978 ; Nicholson, 1979 ; Chapman and Bailey, 1987).

Due to the inability of fishing gear to sample the early postlarval stages (see Chapter 2) the present growth study was conducted using laboratory reared individuals. Caution must therefore be exercised in interpreting the results as it is known that in Crustacea both the intermoult period and moult increment vary with the environment (Hartnoll, 1982).

Crustaceans usually change shape as they grow, this is known as allometric growth. A study of allometric growth usually involves a comparison between the rate of growth of 2 structures, an independent or reference variable (which for most crustaceans is carapace length) and a dependent variable. Much of Huxley's pioneering work on allometric growth involved crustaceans (Huxley, 1924, 1932).

The physiological mechanism which controls allometric growth is not understood and any model used to describe this phenomenon can only be empirical. It is generally accepted, however, that growth occurs as a series of phases and that within each phase a pair of variables is related by the equation :

$$\log Y = \log a + b \log X$$

(Hartnoll, 1982). In this present study the relative growth demonstrated between carapace length and several different variables were examined for evidence of separate growth phases for the zoeal and postlarval stages. The allometric relationships were used in

conjunction with the morphological study to interpret the adaptational significance of the developmental changes in the different structures.

The morphology of the three 2nd stage zoeae which demonstrated accelerated development (two of which were reared during the stocking density trial in Chapter 3) has been investigated and discussed.

## 4.2. Materials and methods

All specimens used in this study were supplied from the culture facility.

### 4.2.1. Photographic examination

Photographs were taken from live specimens using a Nikon SMZ 10 stereomicroscope with Nikon HFX 2 Phototube and FX 35A camera. The S.E.M. material was prepared and scanned using the same methodology given in Chapter 5. As well as investigating the general morphology, regions of special interest, where structural change and adaptation appeared most evident, were examined in greater detail. These included the rostrum, eye (see also Chapter 5), mouthparts, pereopods, gills, pleopods and telson (Figure 4.1 and 4.2).

### 4.2.2. Biometry

All individuals from which biometric information was obtained were reared at ambient temperatures (12 - 15°C) under conditions of dim green laboratory lighting ( $1 - 5 \mu\text{Es}^{-1}\text{m}^{-2}$ ). First stage zoeae were reared under conditions of mass culture whereas individuals of all subsequent stages were reared in isolation. All measurements were taken with an eye-piece graticule fitted to a stereomicroscope (Nikon 102). Measurements were to the nearest 0.01 or 0.001mm depending upon the size of the structure. Due to the frailty of the larval and early postlarval stages morphological examination was not performed on live animals. All measurements were taken from specimens which had recently

died. For the postlarval stages, measurements were also taken from exuviae. Measurement were only made on undamaged structures.

The growth study involved an investigation of both absolute and relative growth. Carapace length, which is widely accepted as the standard measurement for Nephrops (Farmer, 1974e), was chosen as the basic index of growth.

Absolute growth, which is dependent upon intermoult period and moult increment (Hartnoll, 1982), was expressed as a function of premoult carapace length. The postlarvae were reared at  $13.5 \pm 1.5^{\circ}\text{C}$  and the intermoult periods for the zoeal stages were taken from the results of the  $14^{\circ}\text{C}$  temperature trial (see Chapter 3). Although there was a  $3^{\circ}\text{C}$  difference between the maximum and minimum temperature to which the postlarval stages were exposed it rarely fluctuated from  $14^{\circ}\text{C}$ .

In earlier studies growth was presented as a Hiatt diagram (postmoult length against premoult length) (Kurata, 1962) or as a 'growth factor' relationship (percentage increase in length against premoult length) (Mauchline, 1976, Hartnoll, 1982). Both these formulations incorporate premoult length into both variables. This will guarantee an association between them and mask any underlying biological relationship (Chapman, 1980). For this reason it was decided to plot the growth increment against carapace length as suggested by Chapman (1980).

For the study of relative growth, total length, eye stalk length, propodus length of 1st pereopods (ie. major chelipeds), protopod



length of 2nd pleopods, maximum width of 6th abdominal segment and maximum distance across splayed uropods, were compared on an individual basis with carapace length. For each determination carapace length was used as the independent variable. Before computer analysis the data were examined for the existence of separate growth phases. This revealed that for the Nephrops stages for which measurements were taken two growth phases existed, a larval and a postlarval phase. For each phase log-log linear regression lines were fitted to the data. The slopes of the lines were then examined to determine the relationship between the growth of the two variables and whether or not a significant difference existed between the slopes of the two growth phases. This analysis was performed by the Statistics Department of DAFS in Aberdeen.

Biometric data concerning the structure of the eye included the formulation of an 'eye index'. The greatest width and greatest length of the dark pigmented region was measured in both eyes. The values were combined and divided by 4 to give a mean which was used as the 'eye index'. Similar measurements were used to produce an 'eye index' for embryo development in Homarus americanus (Perkins, 1972).

### 4.3. Results

Simplified drawings of the general body plan of a Nephrops zoea (1st stage) and an early postlarva (2nd stage) are shown in Figure 4.1 and 4.2 respectively.

#### 4.3.1. Morphological description

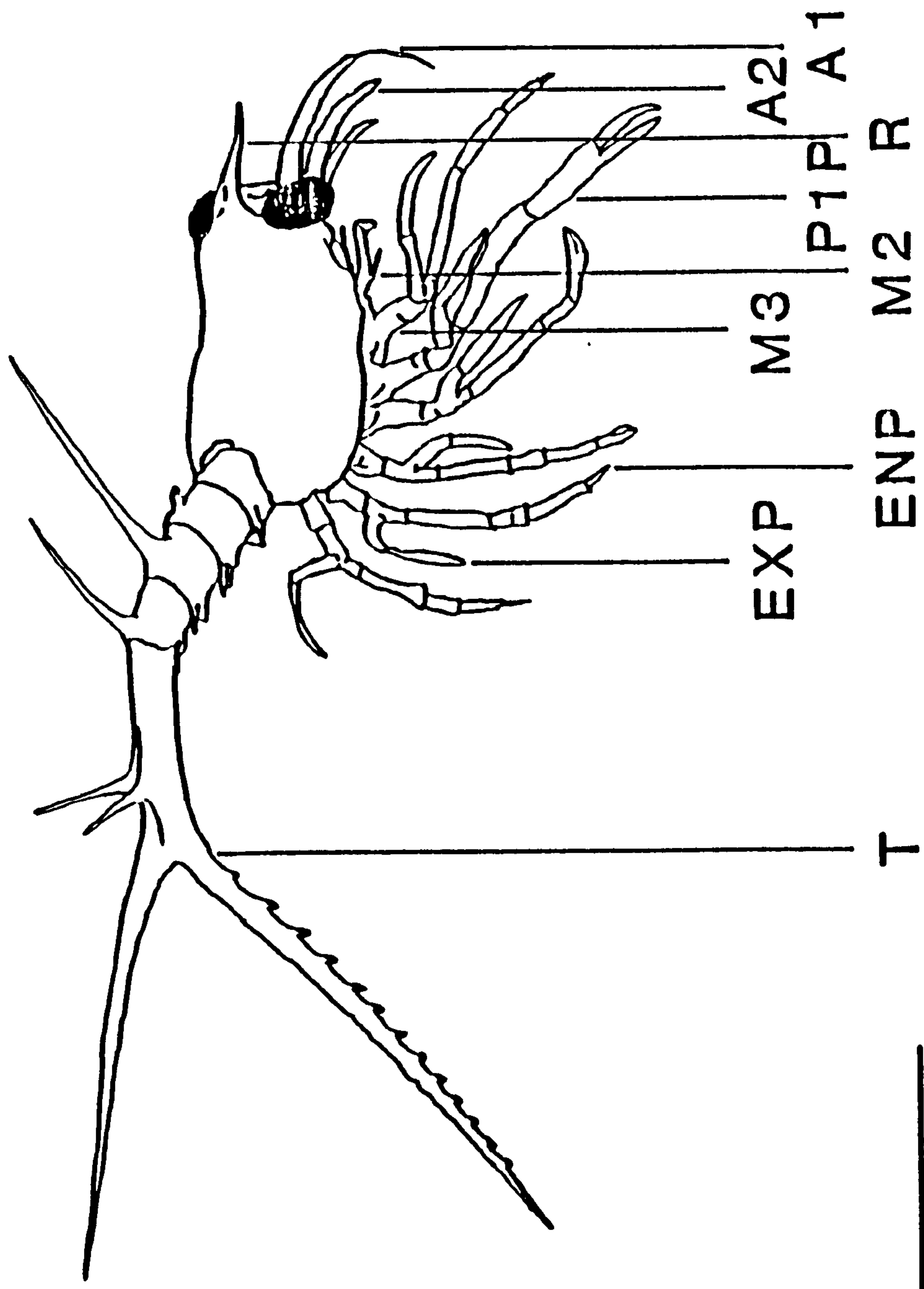
Colour photographs for Nephrops in the early stages of development (immediately prior to hatching and for each stage until the 4th postlarval stage) are shown in Plates 4.1 - 4.4. The embryo in Plate 4.1a has reached an advanced stage of development (stage 8 - 9). It is characterized by bright red chromatophores, 2 large black kidney shaped eyes and the remainder of the depleted yolk reserve (green). Dunthorn (1967) has provided a detailed description of embryonic development in Nephrops (see Chapter 7). The embryo initially hatches into a short-lived prezoea (Plate 4.1b). The illustrated individual was in the process of moulting into the 1st zoea and is less constricted by the prezoeal exoskeleton than when first hatched. After moulting (see Chapter 7) the larvae emerge as first stage zoeae (Plate 4.1c).

There is a progressive increase in size with development into the 2nd (Plate 4.1d) and 3rd (Plate 4.2a,b) zoeal stages. At this level of magnification there appears to be little change in their general shape, except for the development of uropods in 3rd stage zoeae. Zoeae of all 3 stages have chromatophore cells over most of their body surface. In the 1st stage zoeae the pigment was dispersed throughout

Figure 4.1

Simplified drawing of the general body plan of a Nephrops zoea (1st stage). Adapted from Andersen (1962).

Symbols : A1 = 1st antennae, A2 = 2nd antennae, END = endopod, EXP = exopod, M2 = 2nd maxilliped, M3 = 3rd maxilliped, P1P = propodus of 1st pereopods (ie. major chelipeds), R = rostrum, T = telson, UR = uropod.



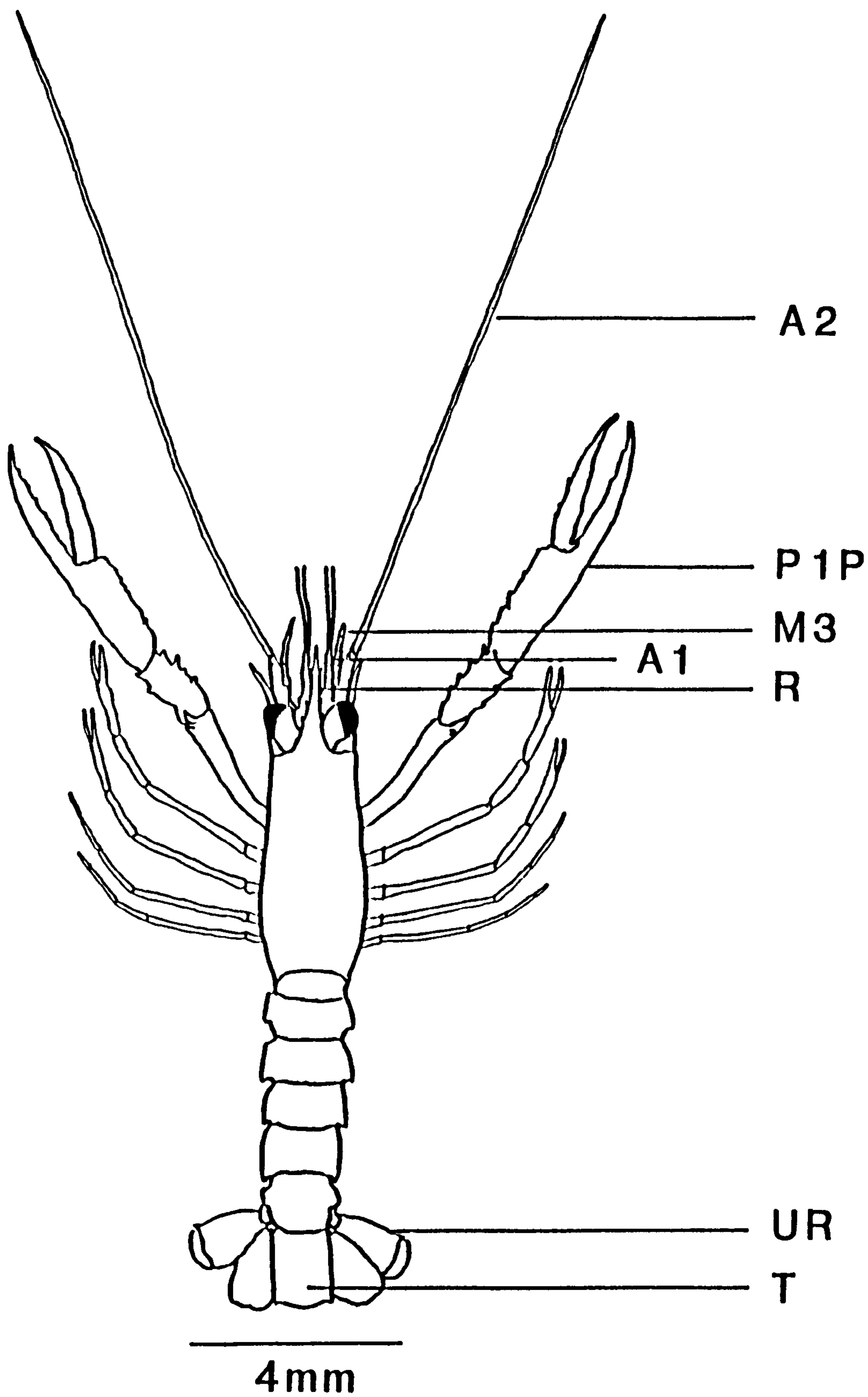
2mm

## Figure 4.2

Simplified drawing of the general body plan of an early Nephrops postlarva (2nd stage). Adapted from Andersen (1962).

Symbols as identified in Figure 4.1.





**Plate 4.1**

**(a) Embryo close to hatching (egg stage 8 - 9).**

**(b) Prezoea.**

**(c) First stage zoea.**

**(d) Second stage zoea.**

**Scale bar represents 0.5mm.**



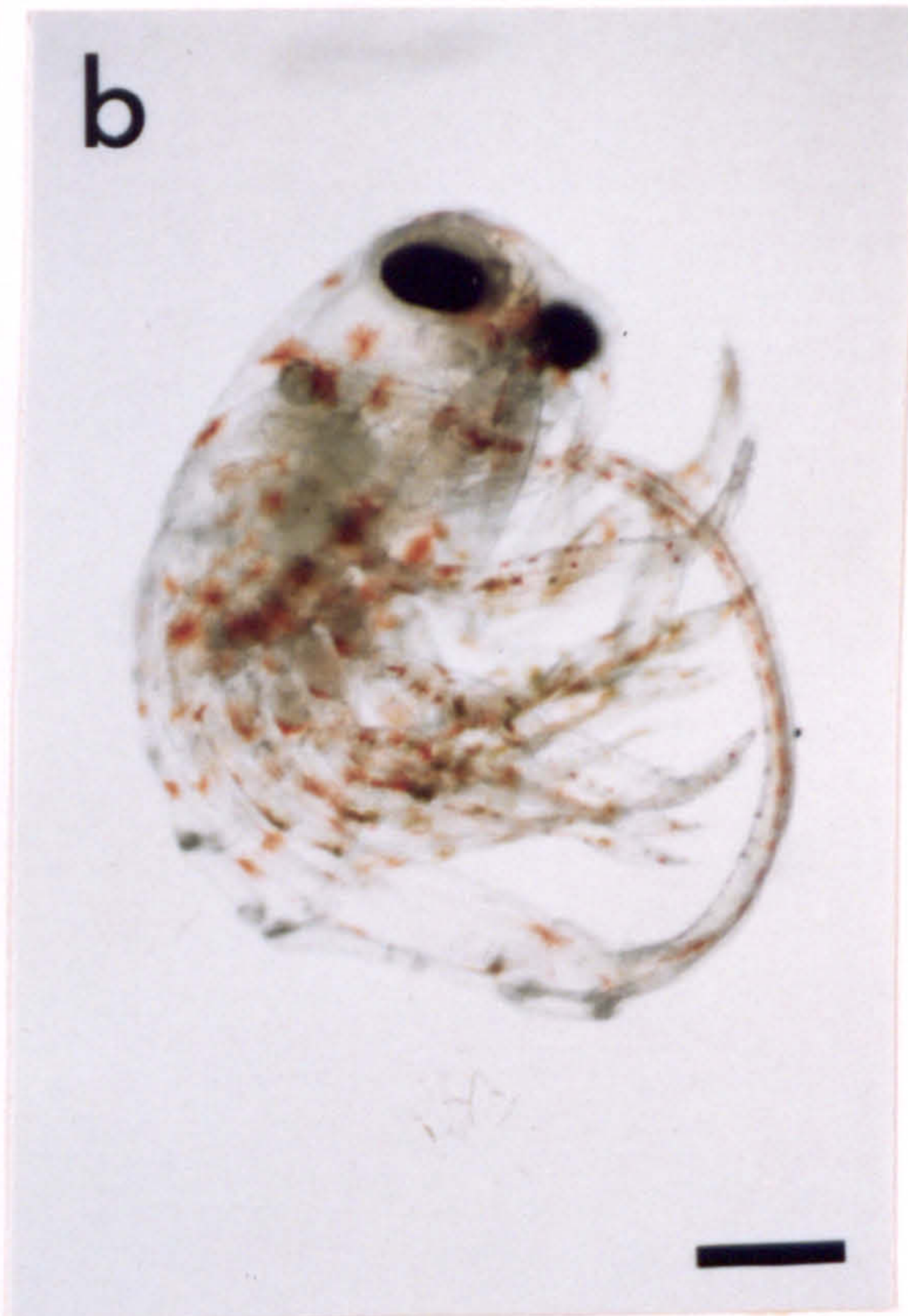
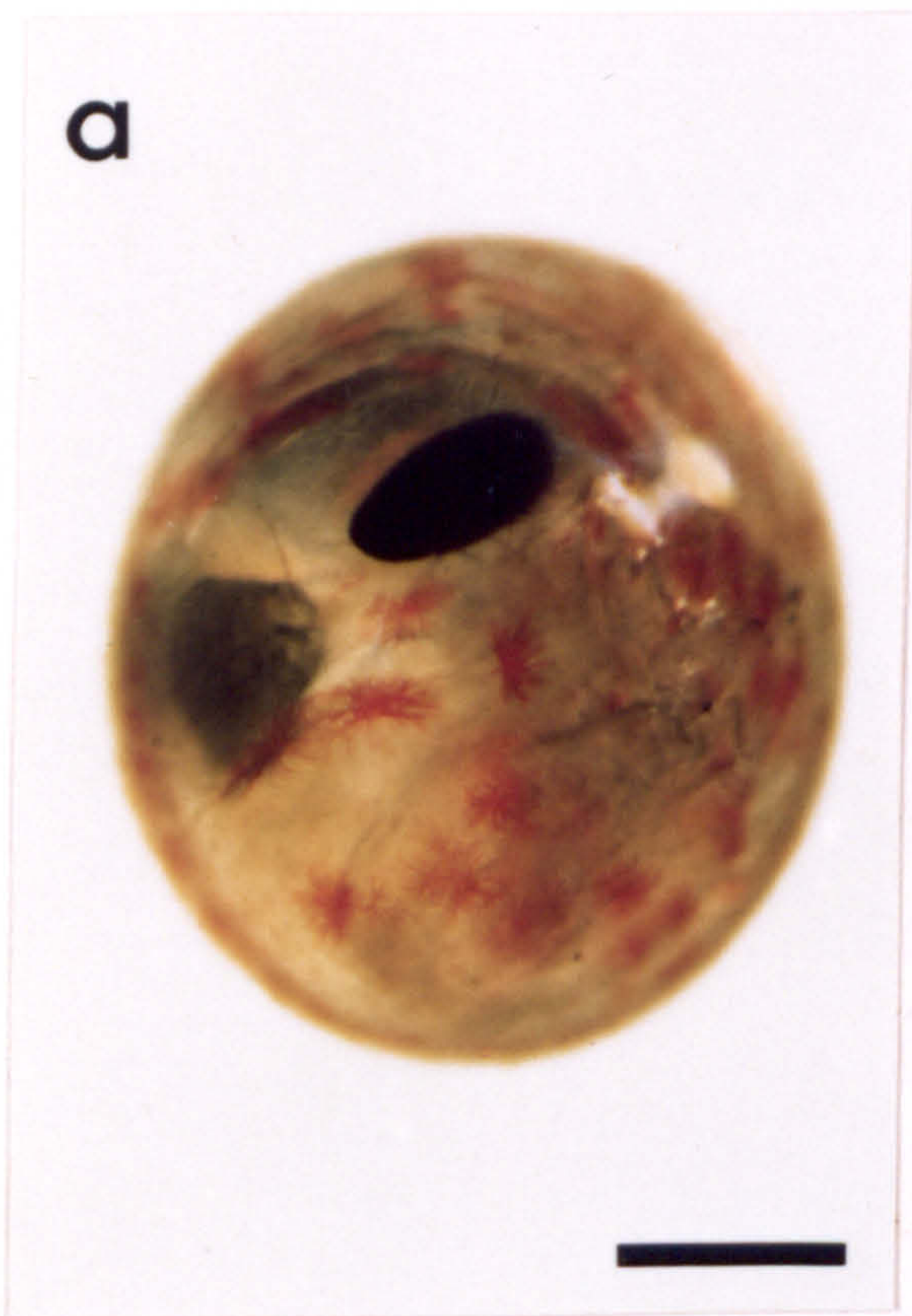




Plate 4.2

(a) Third stage zoea.

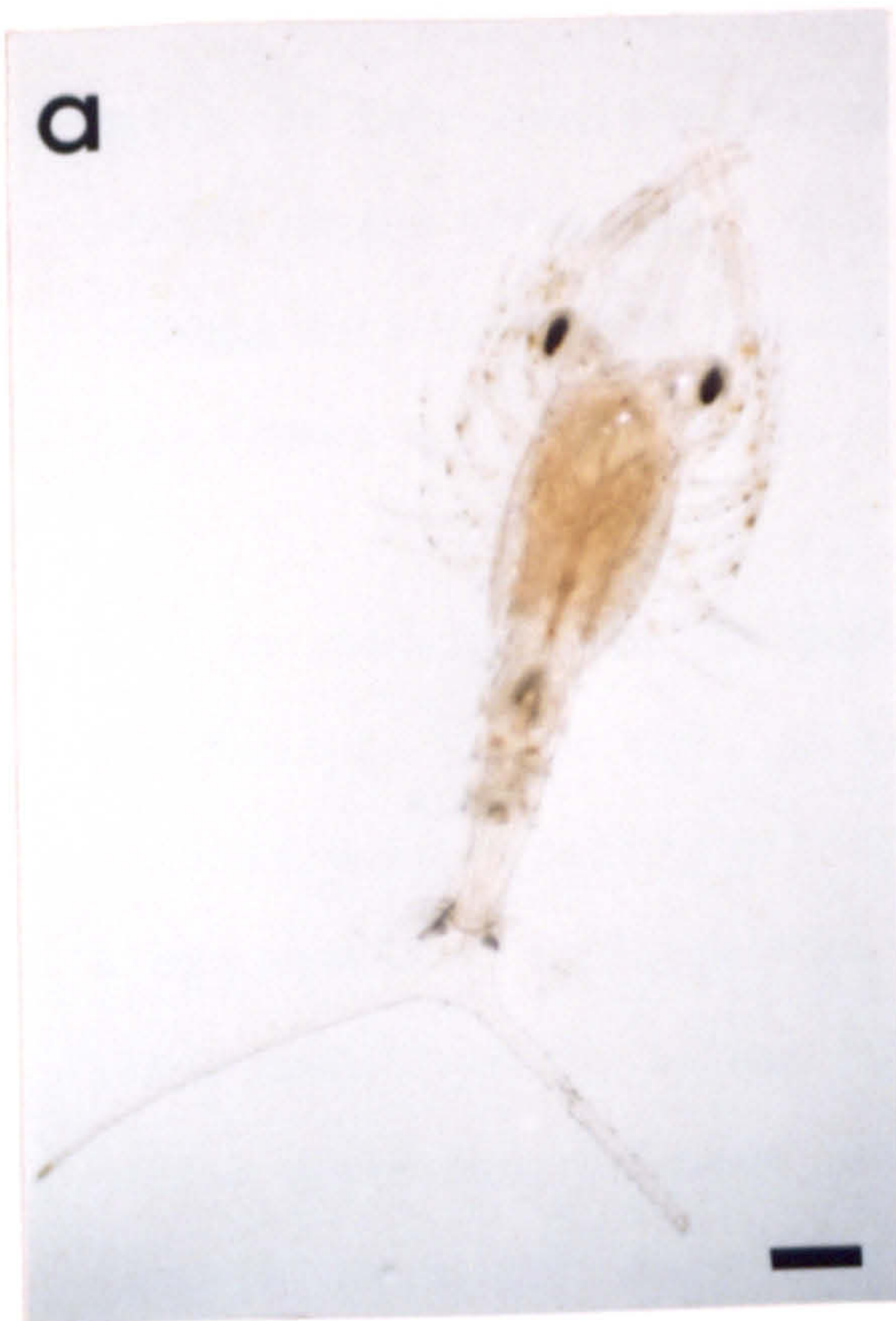
(b) Third stage zoea.

(c) Third stage zoea in the process of performing  
the metamorphic moult into the first postlarval stage.

(d) First stage postlarva.

Scale bar represents 1.0mm.







the ramifications of the chromatophore cells to give a brilliant red colouration to the larva. The pigment within the chromatophore cells of the 2nd and 3rd stage zoeae was concentrated at the centre of the cells. In this condition the red pigment was scarcely visible and both larvae were a translucent yellowish colour. The zoeae of all 3 stages possess well developed exopods on their 3rd maxillipeds and pereopods (see Figure 4.1 also), these are natatory in function (see Chapter 5).

An individual in the process of performing the metamorphic moult is shown in Plate 4.2c. At this level of magnification the most obvious morphological differences between 3rd stage zoeae and 1st stage postlarvae (Plate 4.2d and 4.3a) are the loss of the lateral projections from the telson (caudal fork) and the attainment of a pair of extremely long antennae (which are comparatively short in the zoeal stages). There also appears to be a slight difference in the shape of the carapace which is more rectangular in the postlarvae. The well developed thoracic exopods of the zoeae are greatly reduced in 1st stage postlarvae. The eye stalk is significantly shorter ( $P < 0.05$ ) in 1st stage postlarvae and the clear zone which previously existed between the outer surface (corneal lenses, see Chapter 5) and the dark staining pigment is no longer distinguishable.

1st stage postlarvae are less transparent than the zoeae and 2nd stage postlarvae (Plate 4.3b,c) are less transparent than 1st stage postlarvae. Following the moult into the 3rd postlarval stage (Plate 4.3d) the Nephrops resemble miniature adults. Postlarvae within the 3rd and 4th stages (Plate 4.4) show the same red and white markings as the adults and appear to possess fully calcified exoskeletons.

**Plate 4.3**

**(a) First stage postlarva.**

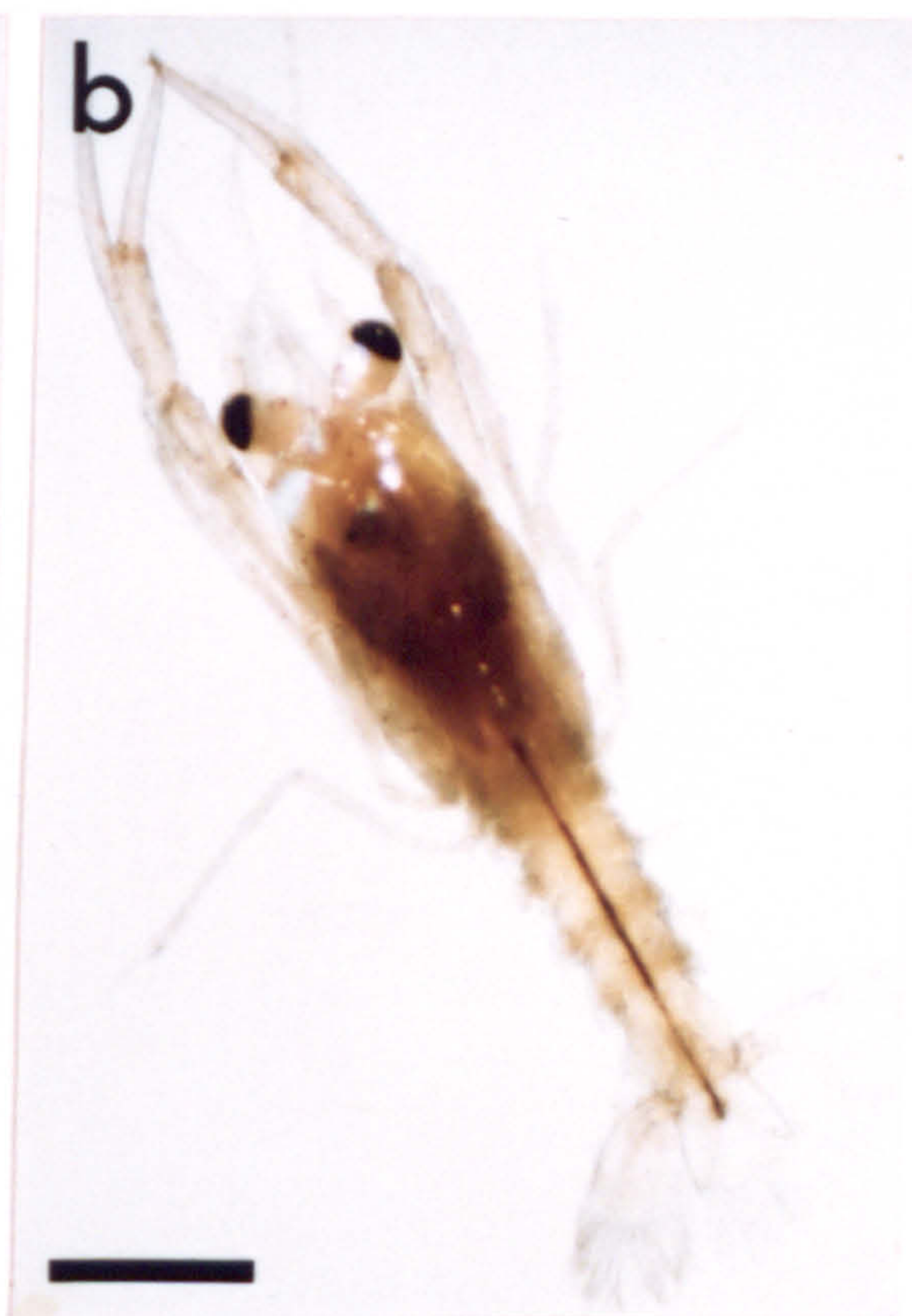
**(b) Second stage postlarva.**

**(c) Second stage postlarva.**

**(d) Third stage postlarva.**

**Scale bar represents 2.5mm.**







**Plate 4.4**

**(a) Third stage postlarva.**

**(b) Fourth stage postlarva.**

**(c) Fourth stage postlarva.**

**Scale bar represents 5.0mm.**







The S.E.M. photographs are shown in Plates 4.5 - 4.10. The general morphology of the 3 zoeal stages is shown in Plate 4.5. Although the specimens have suffered some distortion the general features are distinguishable. The zoeae of all 3 stages possess prominent rostrums and well developed, heavily setose thoracic exopods. The 3rd, 4th and 5th abdominal segments each bear a single median dorsal spine and on the 6th segment there are 2 divergent dorsal spines. The telson, which is divided into 2 lateral projections, is setose. Due to the distortion caused to the carapace, the gills (which normally lie underneath the carapace) are visible in the 1st and 2nd stage zoeae. Following development into the 2nd stage, the zoeae possess supraorbital spines. A supraorbital spine has been distorted from its normal 'live' position above the eye to below the eye in the 2nd stage zoea in Plate 4.5b. After moulting into the 3rd stage the zoeae possess uropods and well developed pleopods (Plate 4.5c and 4.3a,b).

Detailed descriptions of the mouthparts of the larval and early juvenile stages of Homarus americanus (a closely related species to Nephrops) have been provided by Factor (1978) and Hinton and Corey (1979). It was not the intention of this investigation to provide similar information for Nephrops though this would provide an interesting topic for a future study. Although Farmer (1974c) has described the mouthparts for adult Nephrops no similar study exists for the early stages.

The gross morphology of the mouthparts of the 3 zoeal and early postlarval stages is shown in Plate 4.6 and 4.7 respectively. There do not appear to be any major structural differences between the

Plate 4.5

S.E.M. of the general morphology.

(a) First stage zoea.

(b) Second stage zoea.

(c) Third stage zoea.

Scale bar represents 0.5mm for (b) and (c) and 1.0mm for (a).

Abbreviations are : g, gills ; pl, pleopod ; ss, supraorbital spine ;  
ur, uropod.



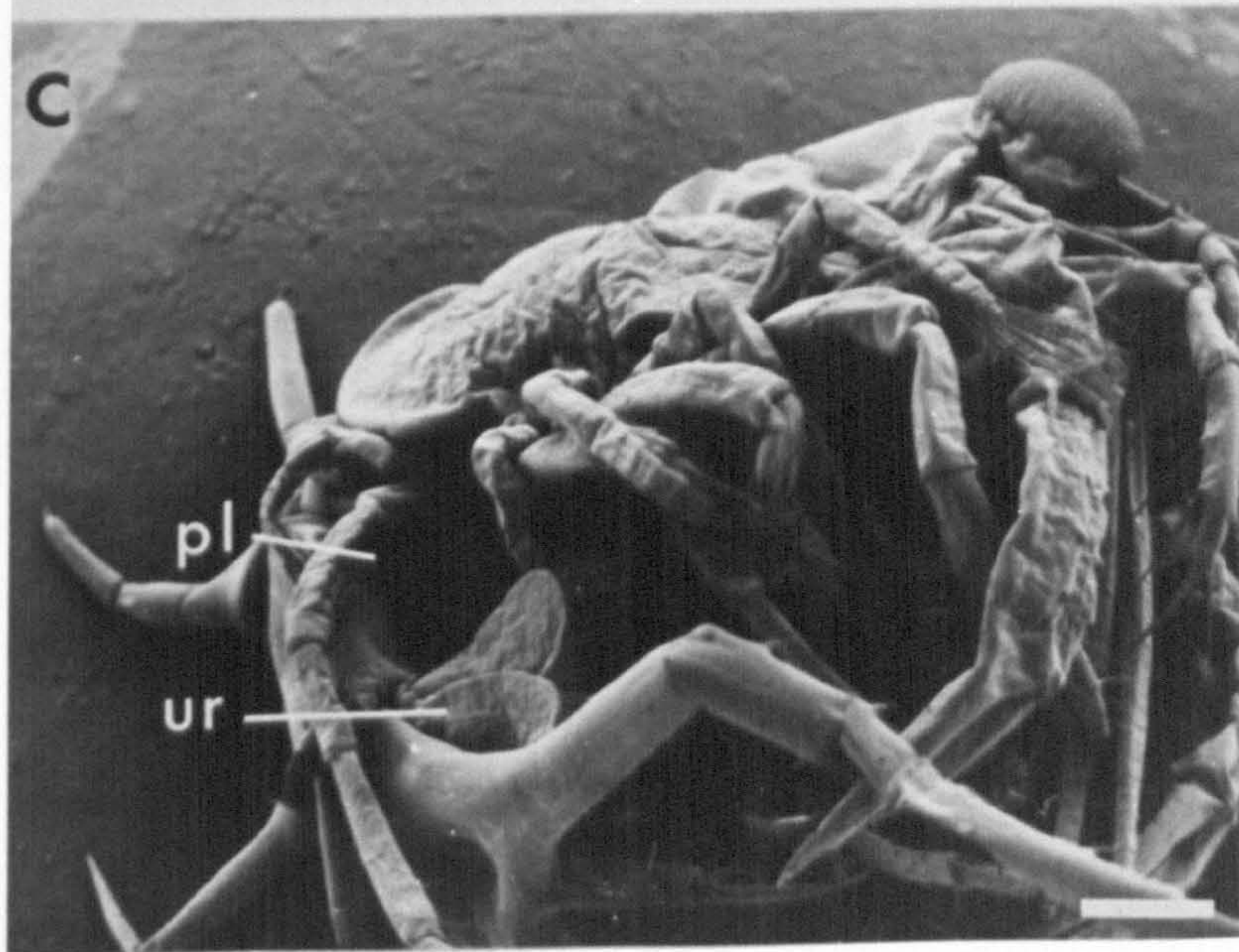
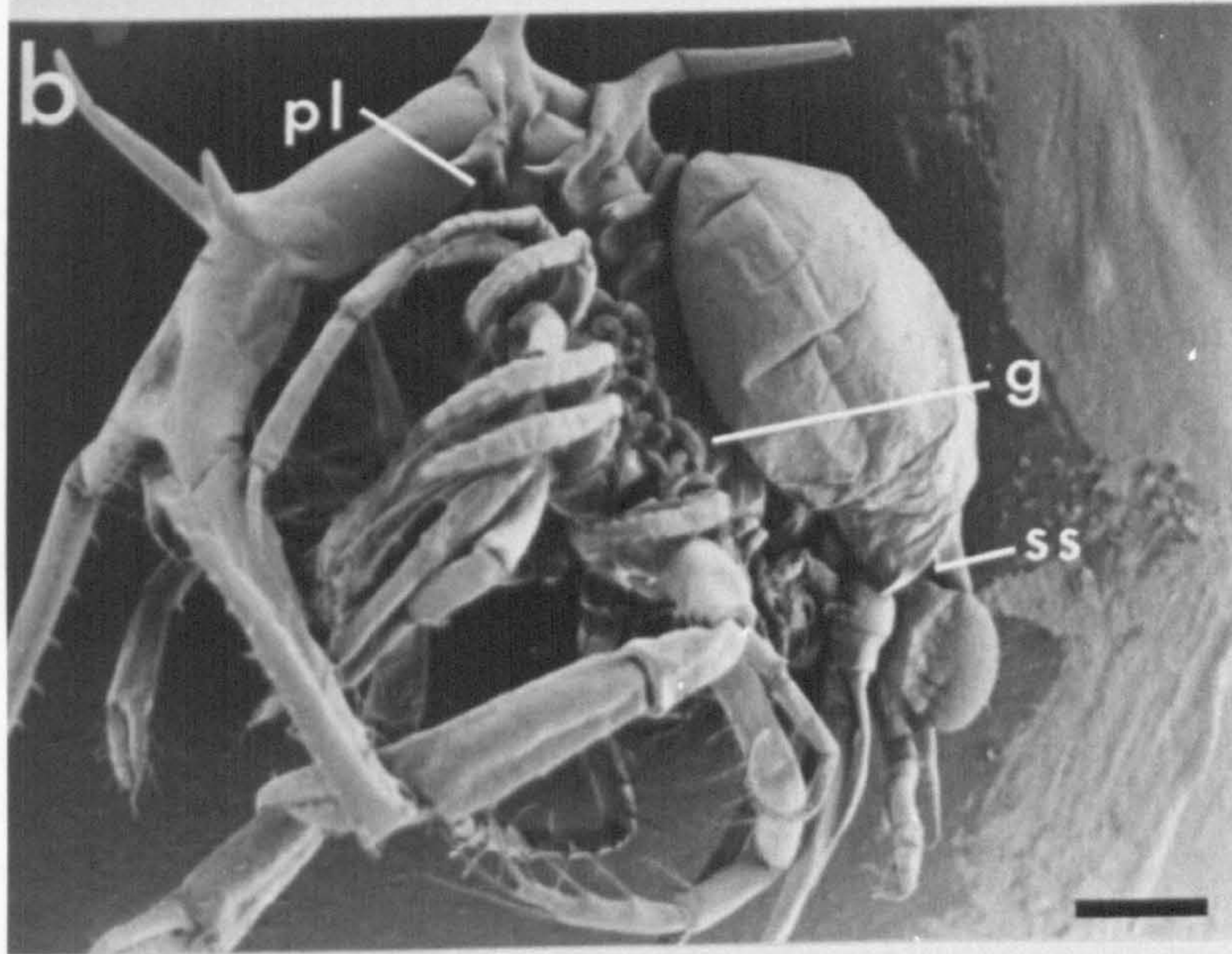
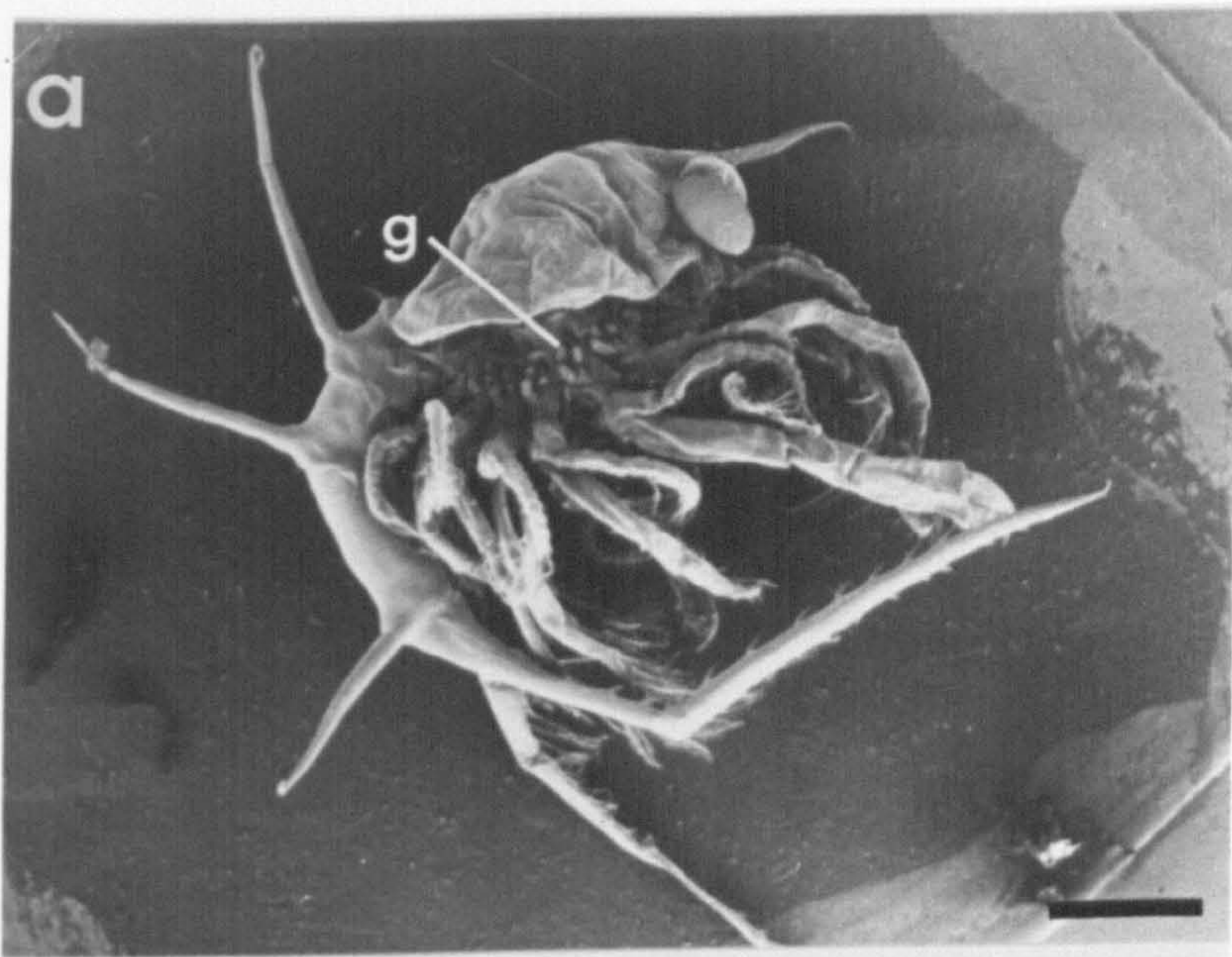




Plate 4.6

S.E.M. of the mouthparts.

(a), (b) First stage zoea.

(c), (d) Second stage zoea.

(e), (f) Third stage zoea.

Scale bar represents 0.05mm for (f), 0.1mm for (a), (b), (d) and (e) and 0.5mm for (c).

Abbreviations are : lb, labrum ; max 1, first maxilla ; max 2, second maxilla ; mb, mandible ; mbp, mandibular palp ; mxp 1, first maxilliped ; mxp 2, second maxilliped ; mxp 3, third maxilliped ; pg, paragnath.



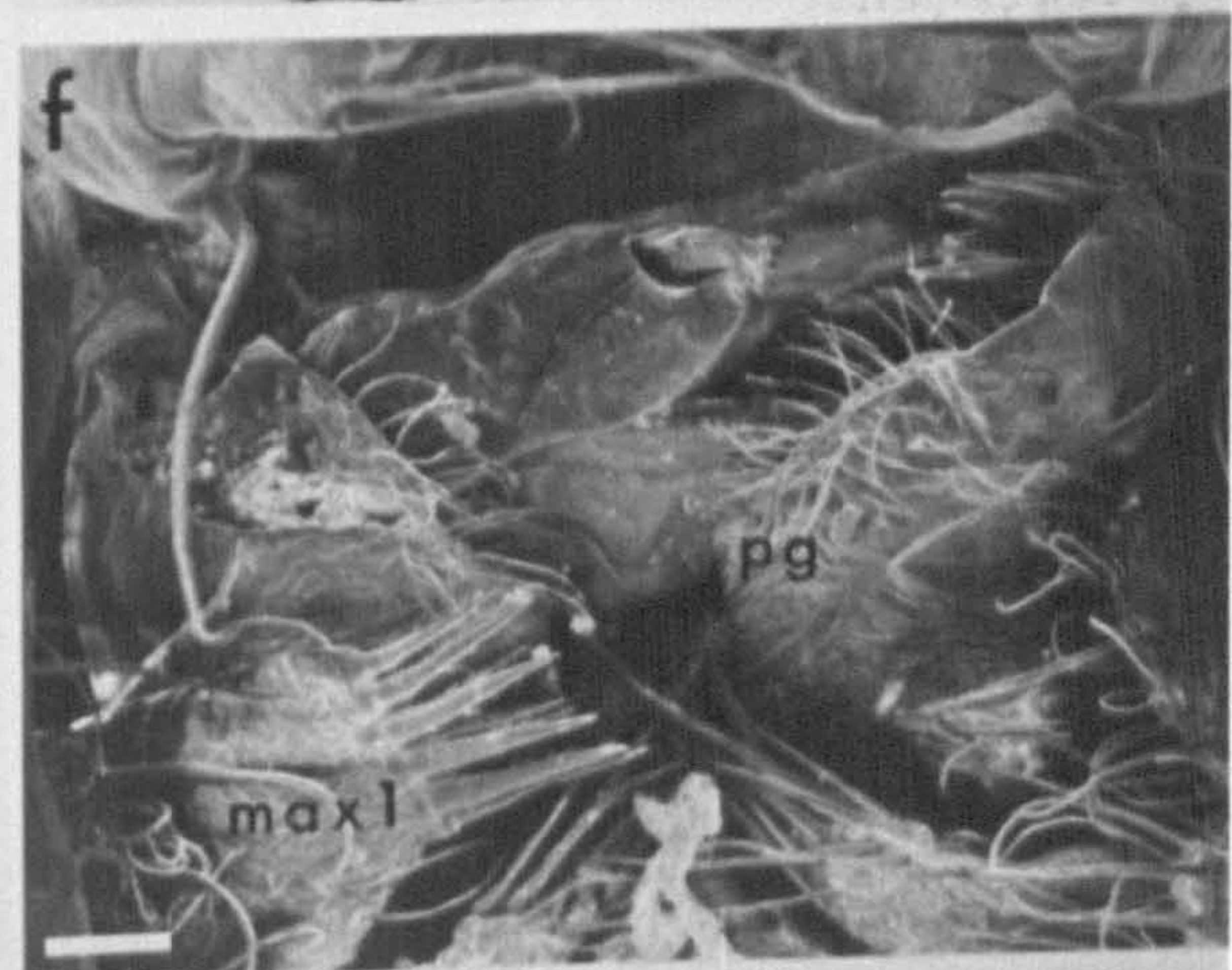
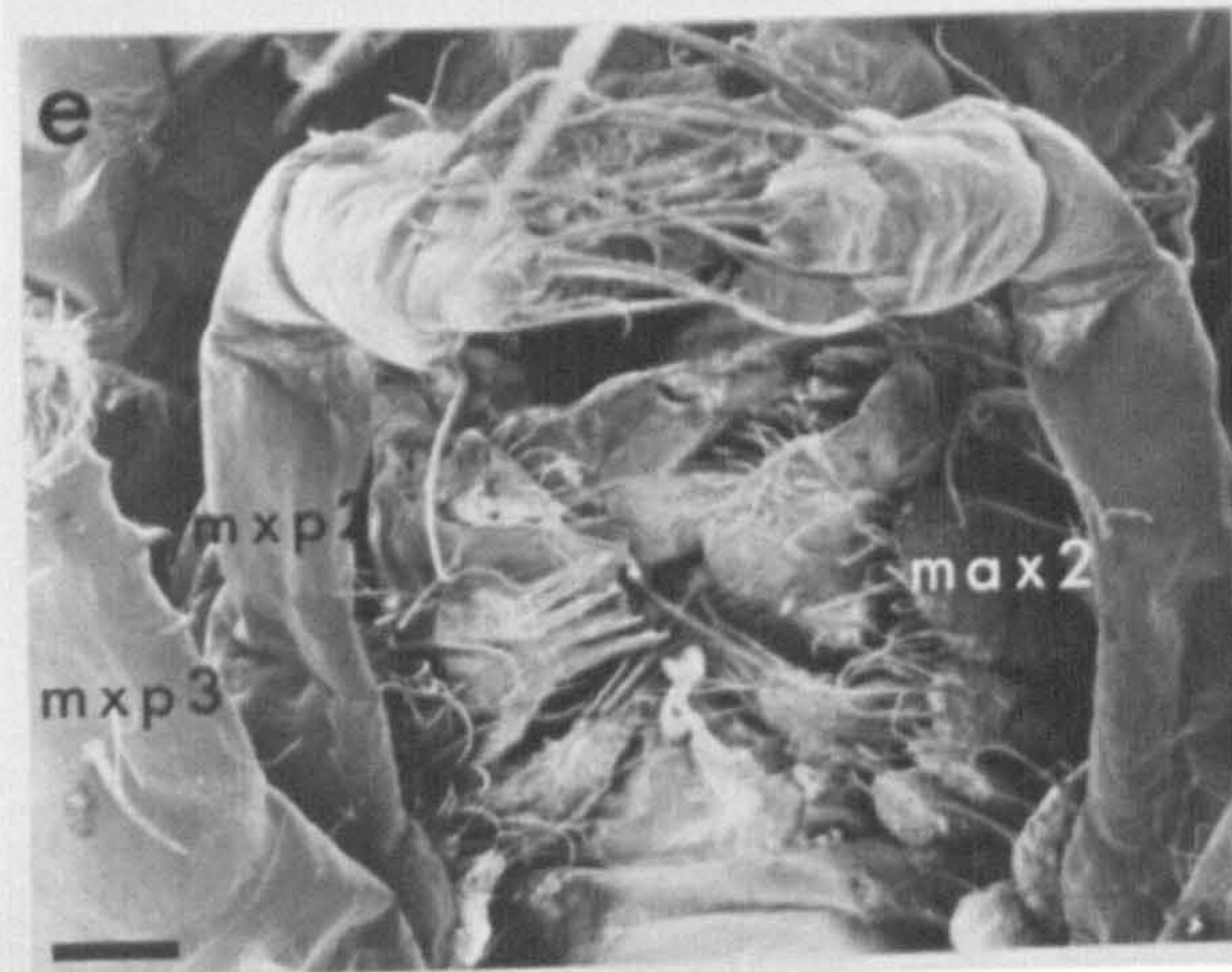
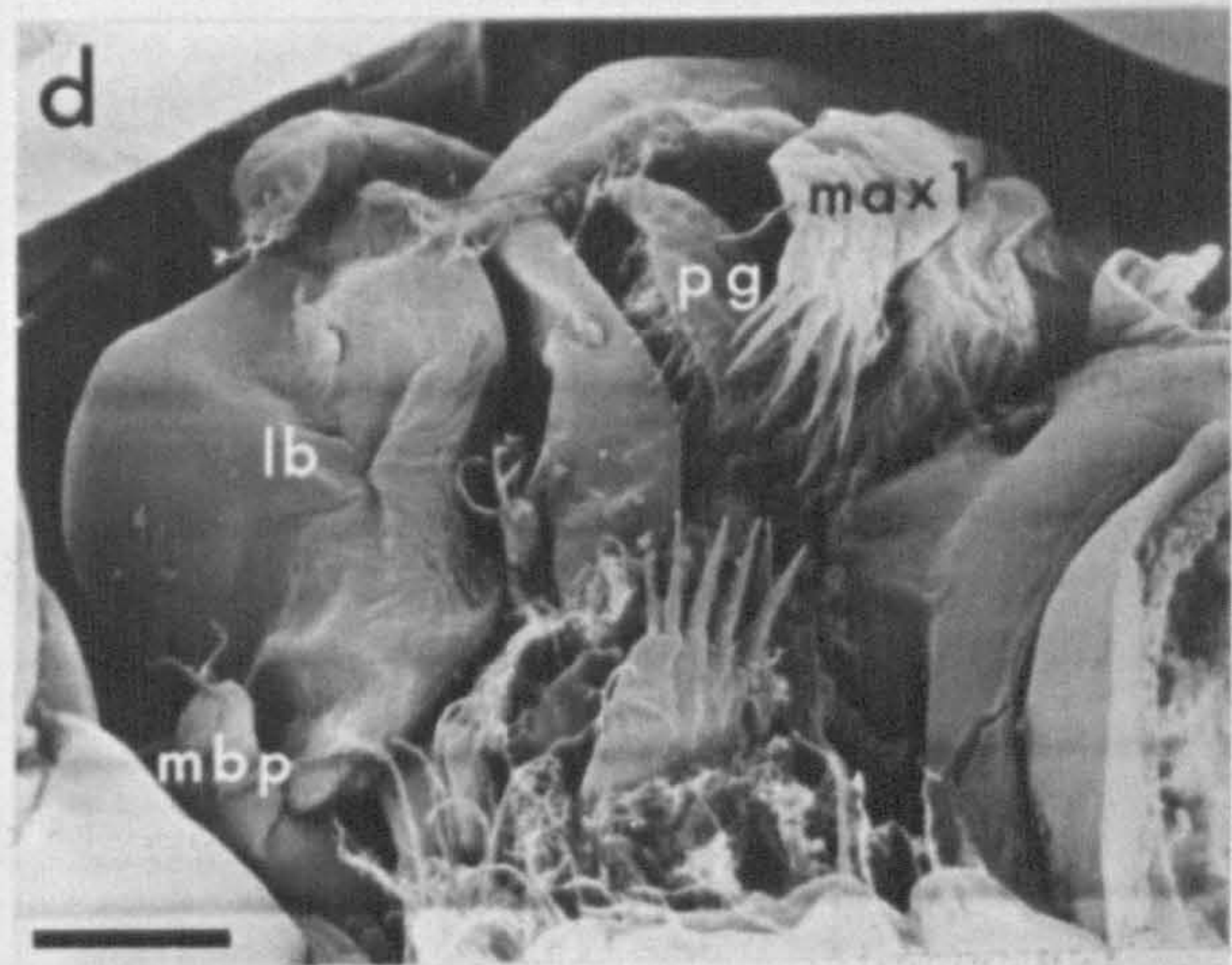
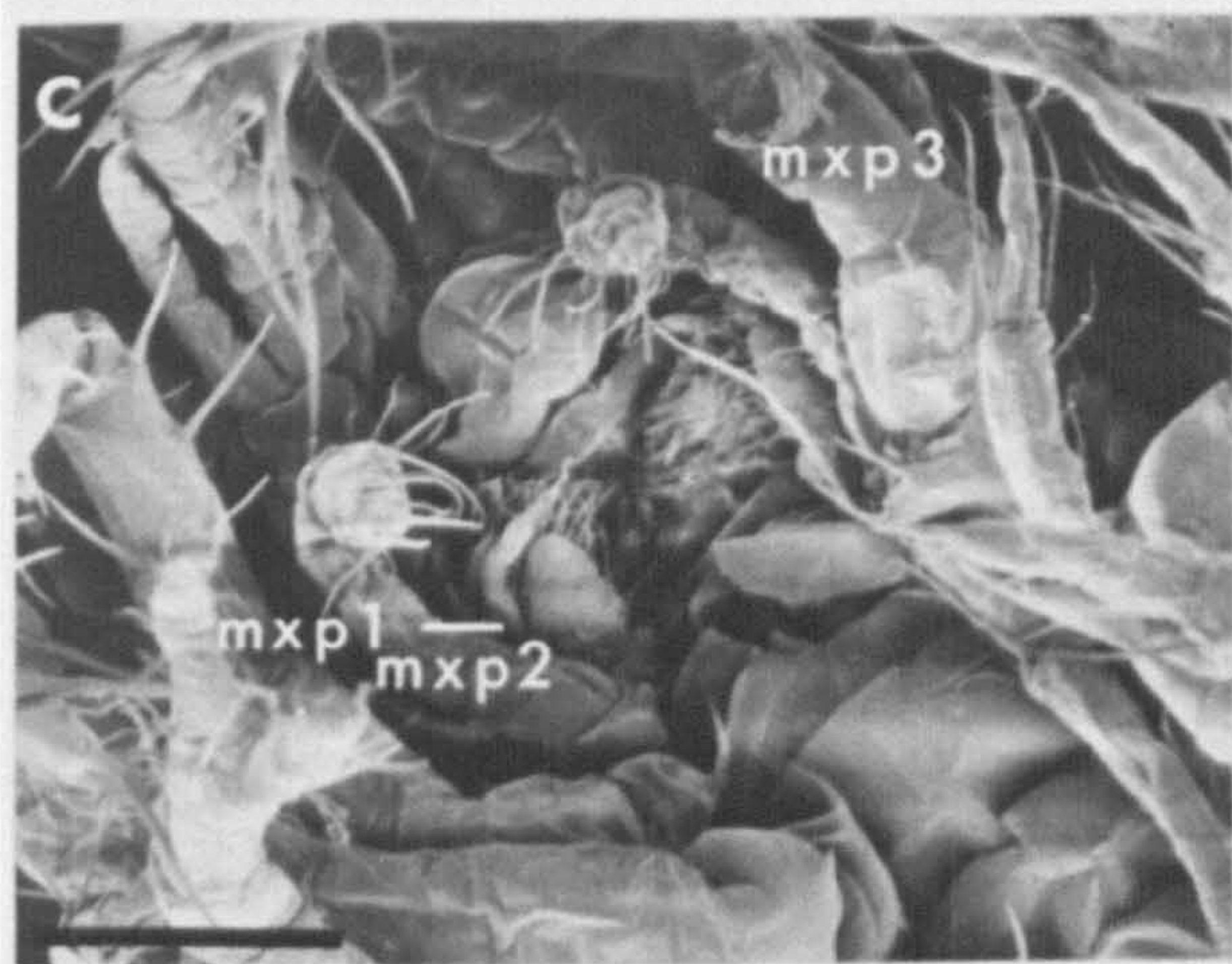
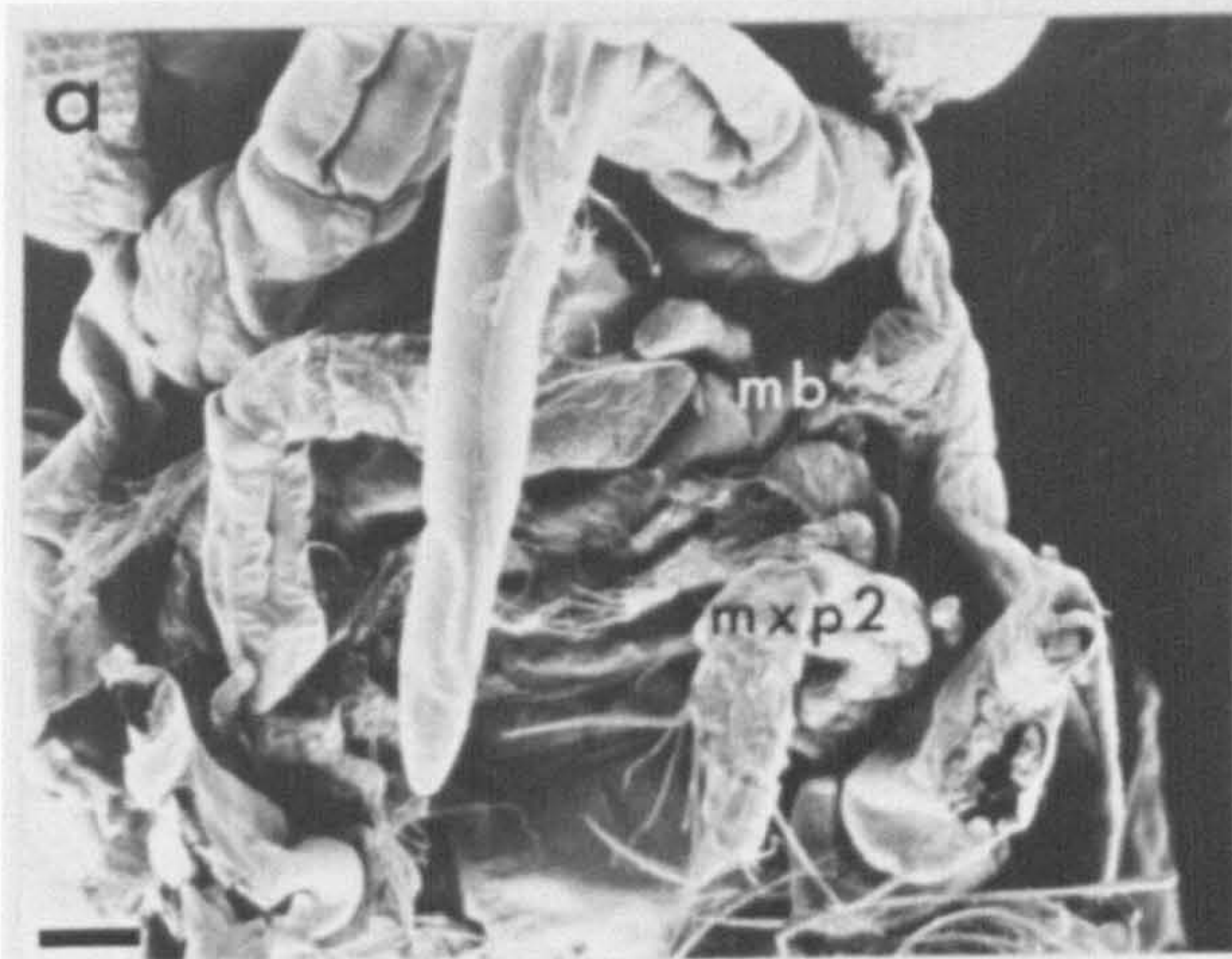




Plate 4.7

S.E.M. of the mouthparts.

(a), (b) First stage postlarva.

(c), (d) Second stage postlarva.

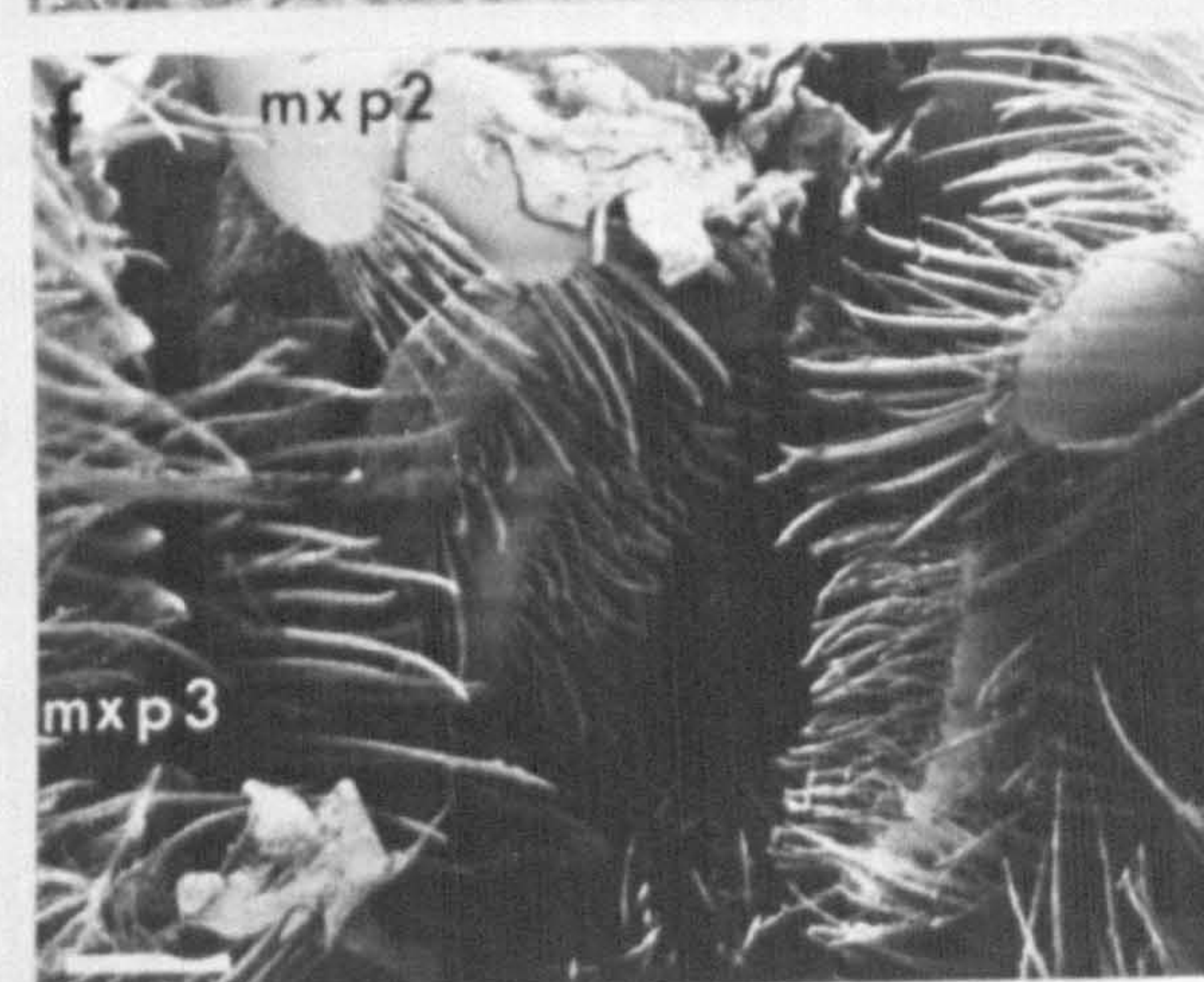
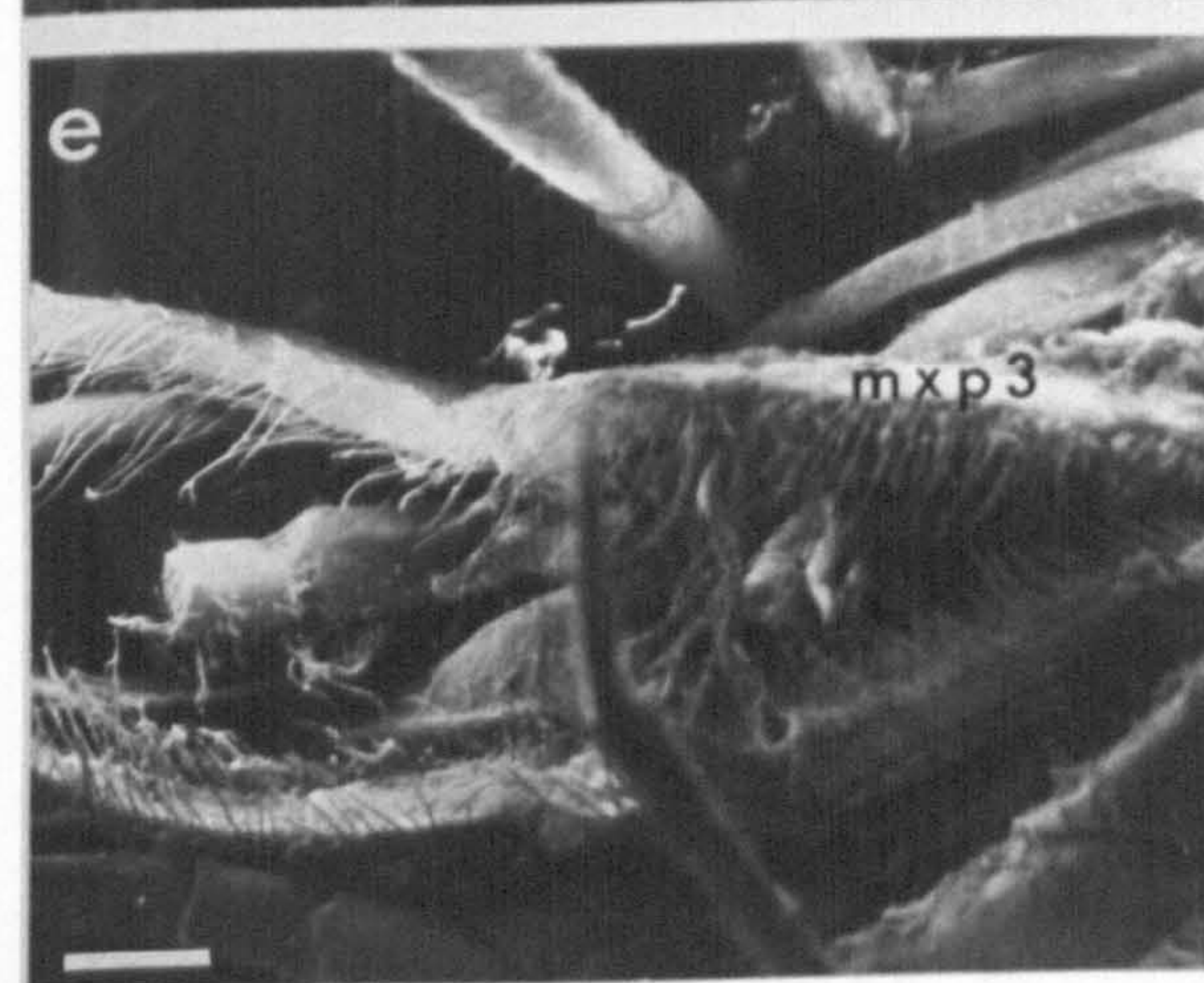
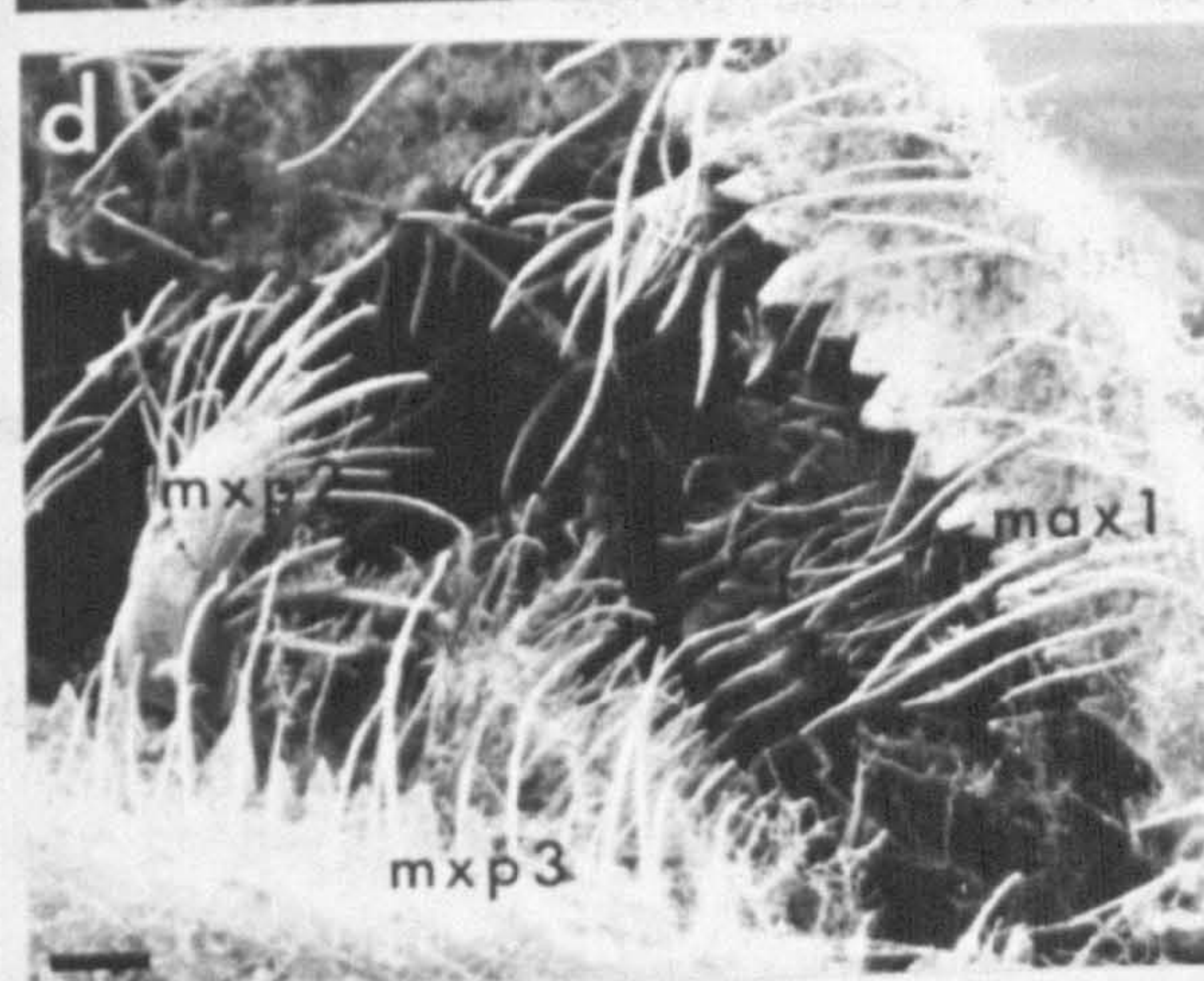
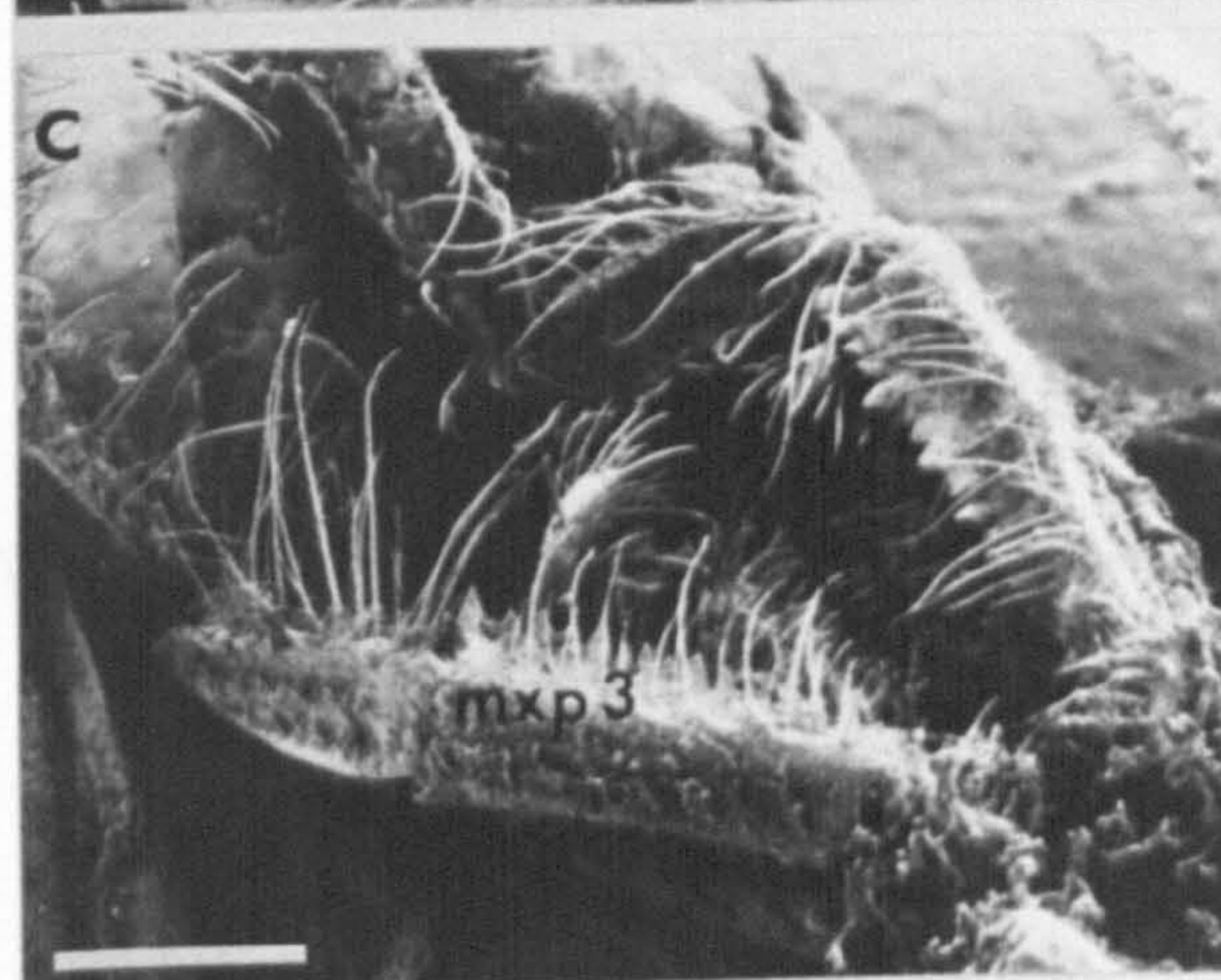
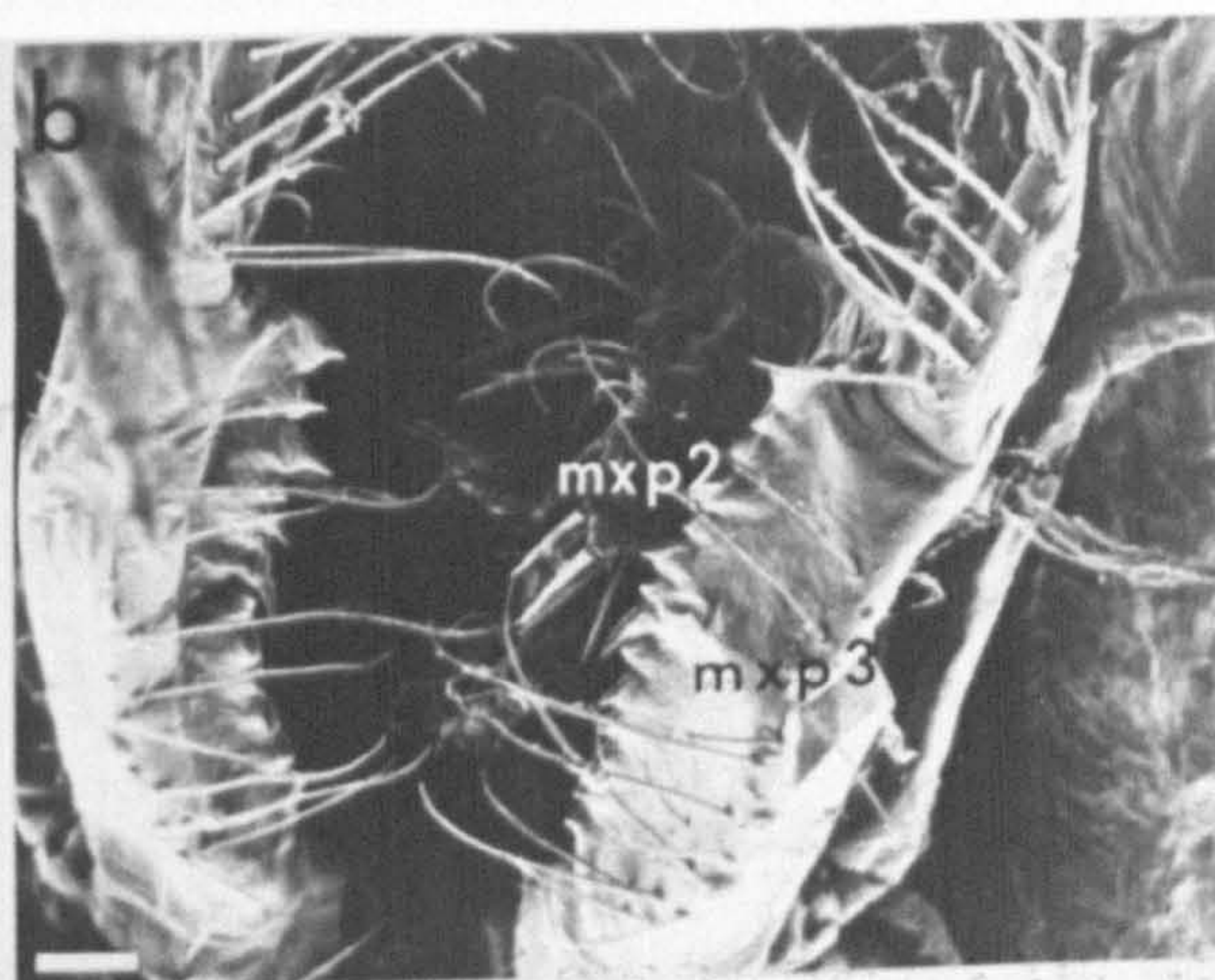
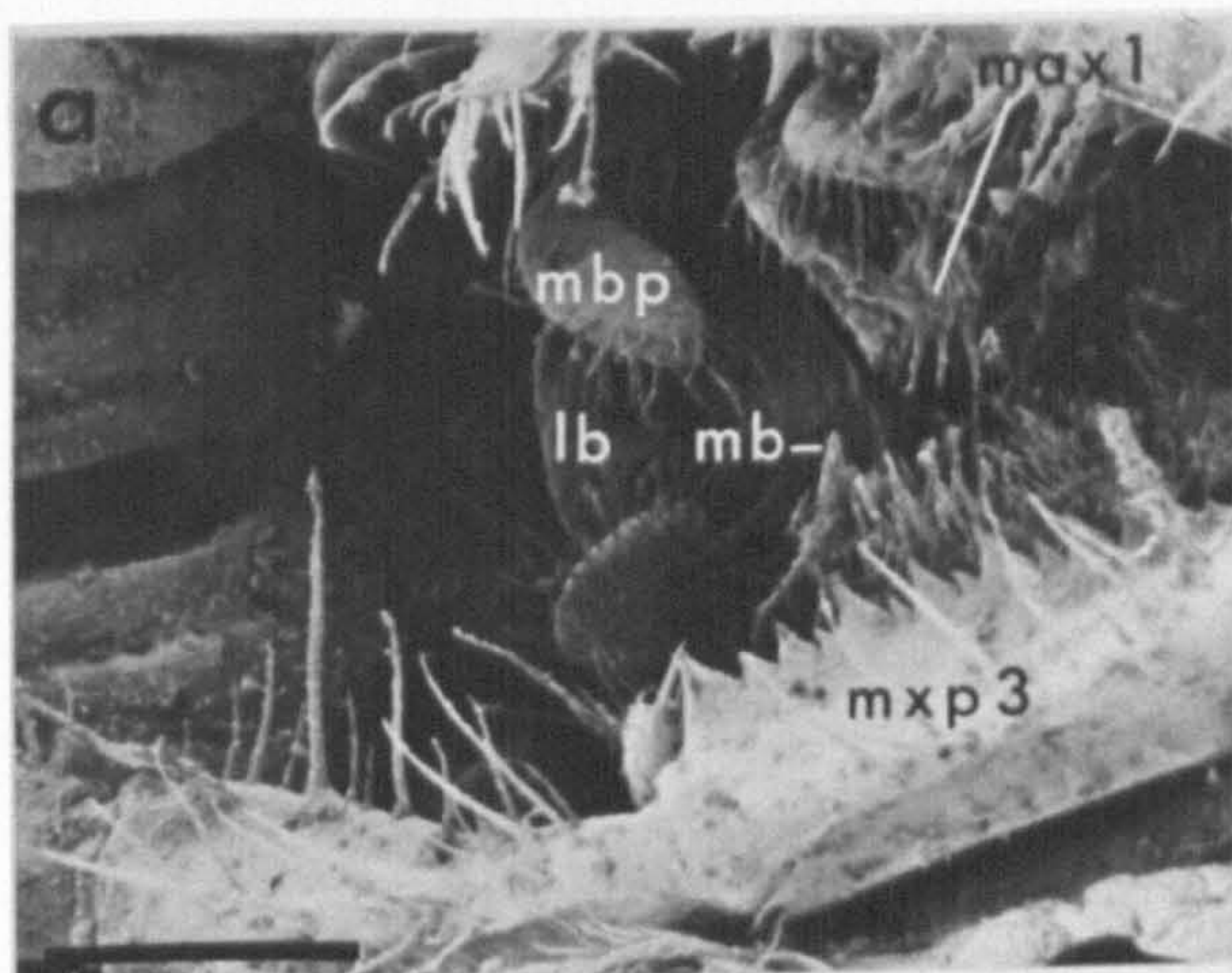
(e) Fourth stage postlarva.

(f) Adult.

Scale bar represents 0.1mm for (b) and (d), 0.5mm for (a), (c) and (f)  
and 1.0mm for (e).

Abbreviations as identified in Plate 4.6.







mouthparts of 1st, 2nd and 3rd stage zoeae. Apart from the obvious increase in the size of mouthparts as development proceeds, other changes also occur particularly following metamorphosis. The most obvious of these include an increase in the number of setae, greater development of the molar processes of the mandibles and the development of 'teeth' on the ischium of the 3rd maxillipeds.

The general morphology of the gills for the 3 zoeal and early postlarval stages are shown in Plate 4.8. The gills extend into the branchial cavity from the maxillipeds and pereopods. There are 3 types of gills in the nephropid lobsters, the differentiation being made by the site of implantation. Podobranchs extend from the coxae, arthrobranchs arise from the articulating membrane at the base of an appendage and pleurobranchs extend from the body wall (Plate 4.8b,c). The branchial formula for Nephrops is given by Farmer (1974c) and includes 6 podobranchs, 10 arthrobranchs and 4 pleurobranchs. The gills for all 3 zoeal stages appear to be simple lobes although by the 3rd stage there is evidence of surface dilations. After metamorphosis into the 1st stage postlarva although the gills are still rudimentary they have developed a major axis and pronounced secondary lobes have formed (Plate, 4.8d). This development continues over the next few moults and by the 4th postlarval stage (Plate 4.8f) the trichobranch gills are similar to those of the adult (and most other macrurans).

Although the major metamorphosis in Nephrops occurs between the 3rd zoeal and 1st postlarval stages there is also evidence of a slight secondary metamorphosis between the 1st and 2nd postlarval stages. Some of the most obvious differences between these 2 stages are shown in Plate 4.9. These include the possession of residual thoracic

Plate 4.8

S.E.M. of the gills.

(a) First stage zoea.

(b) Second stage zoea.

(c) Third stage zoea.

(d) First stage postlarva.

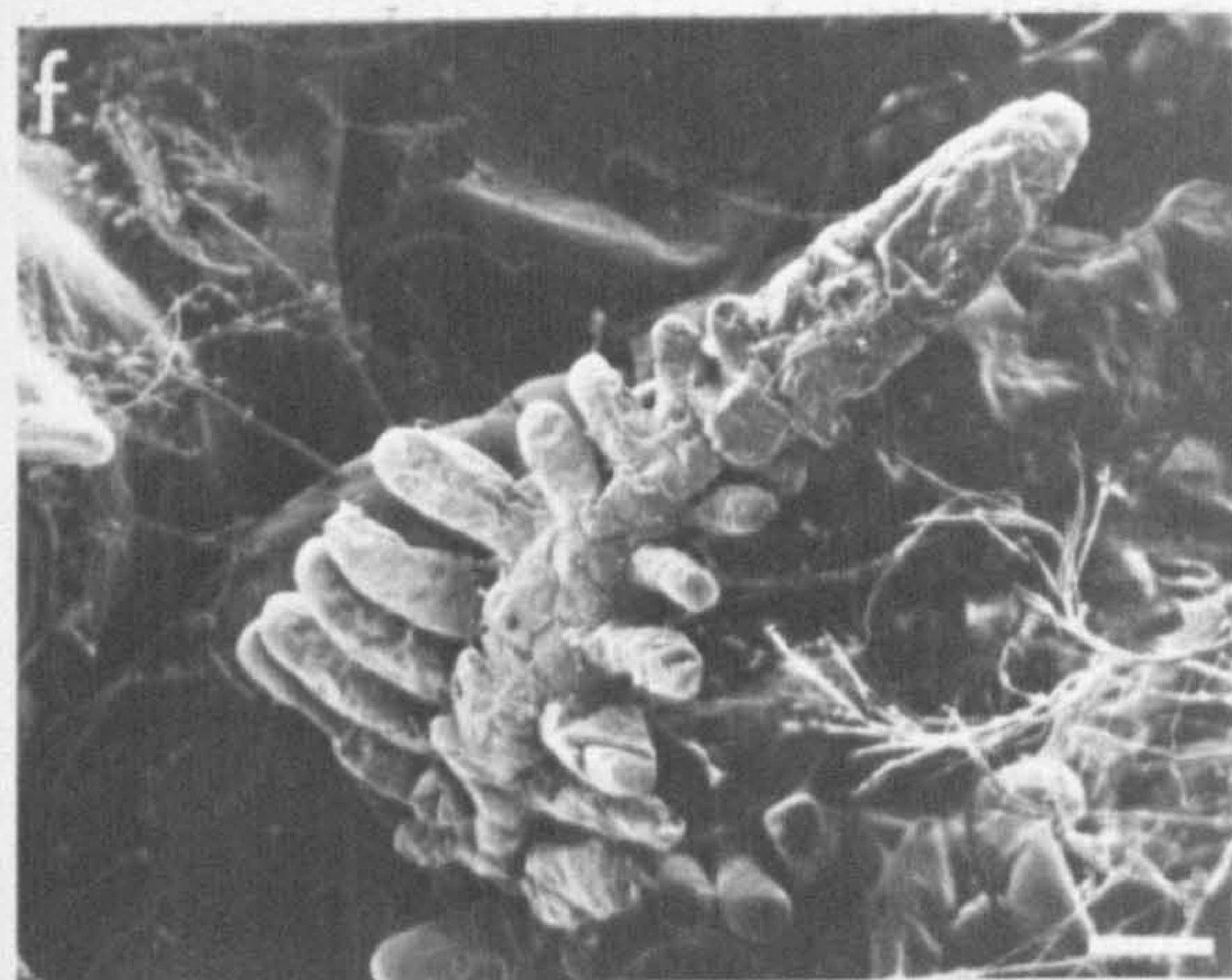
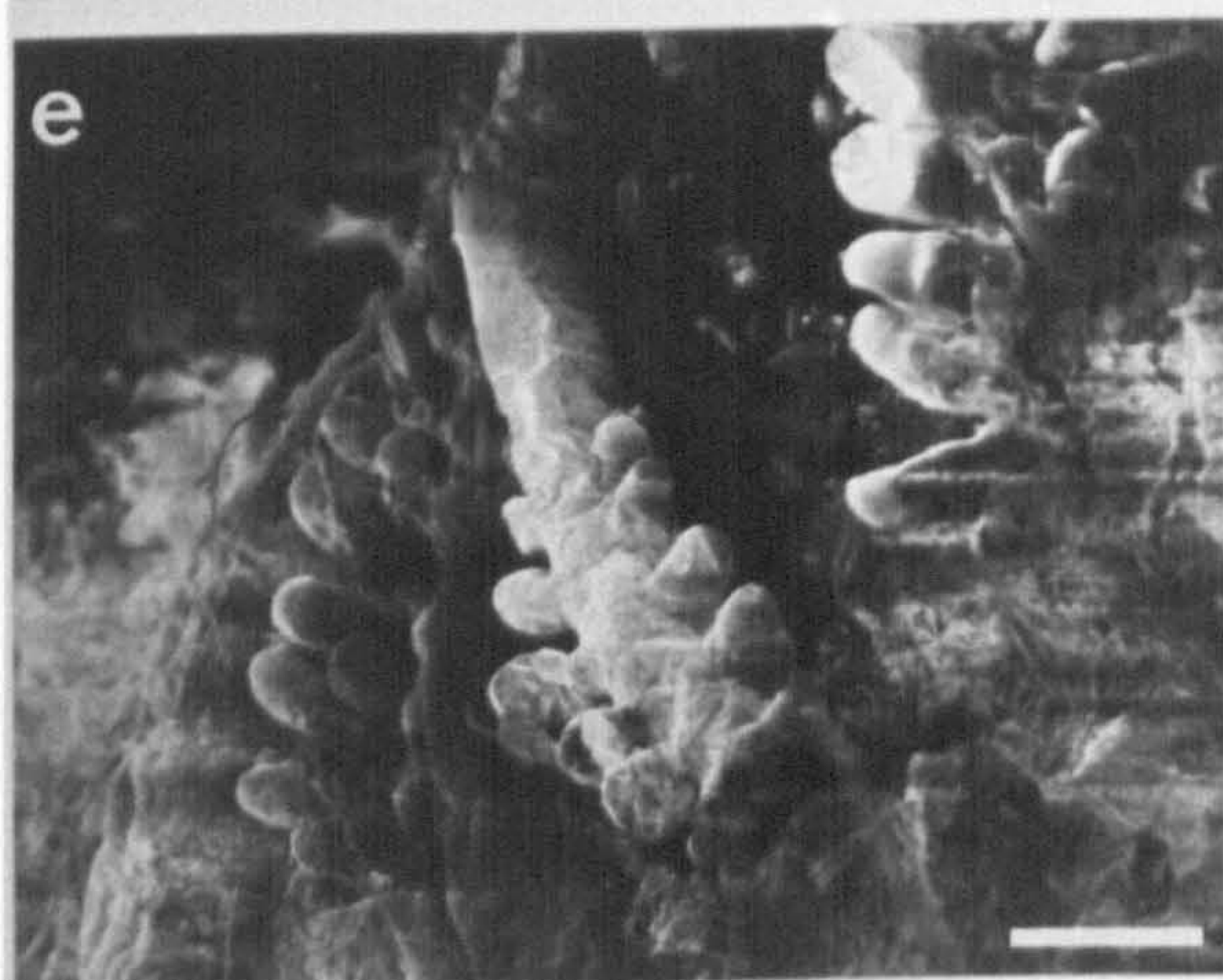
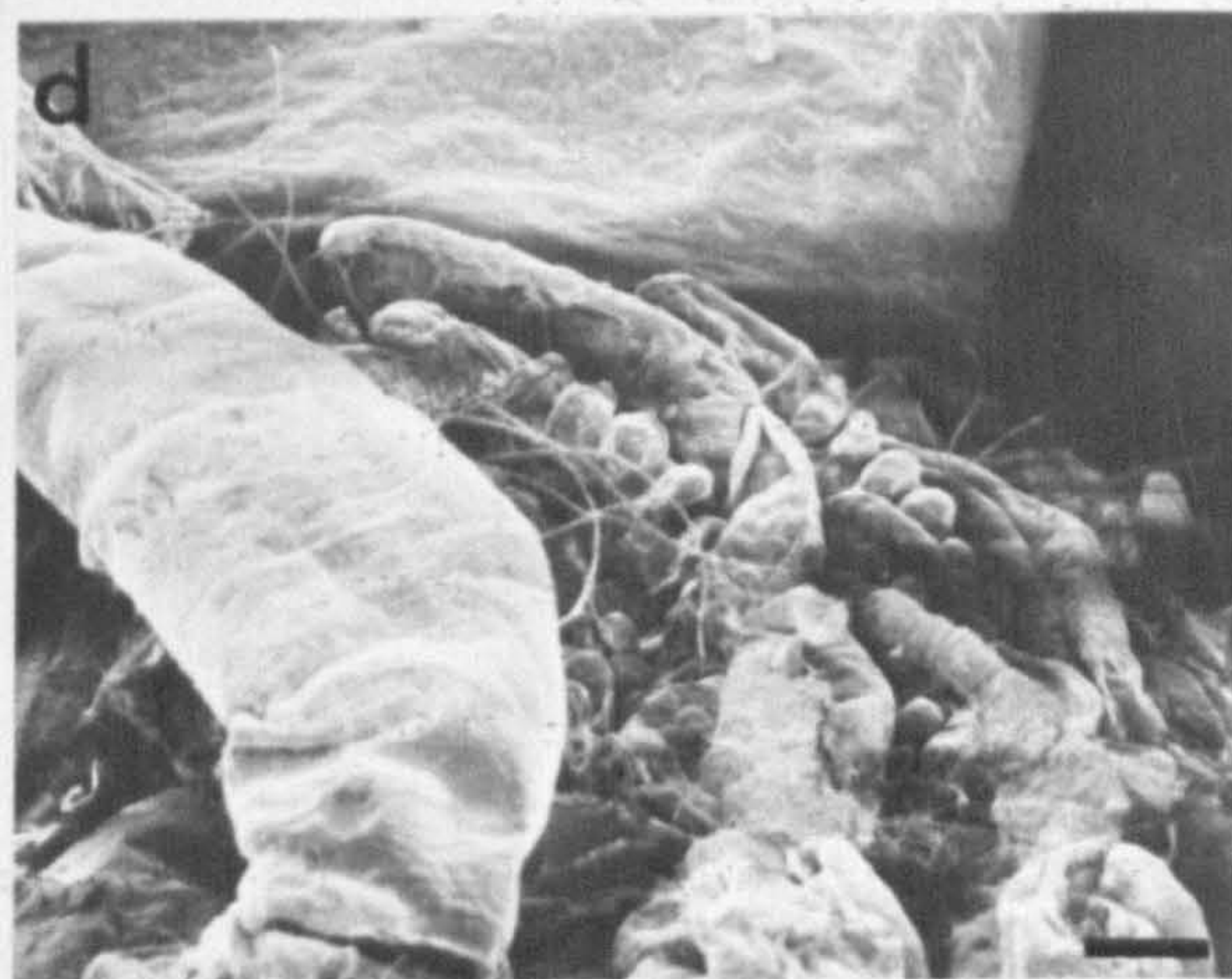
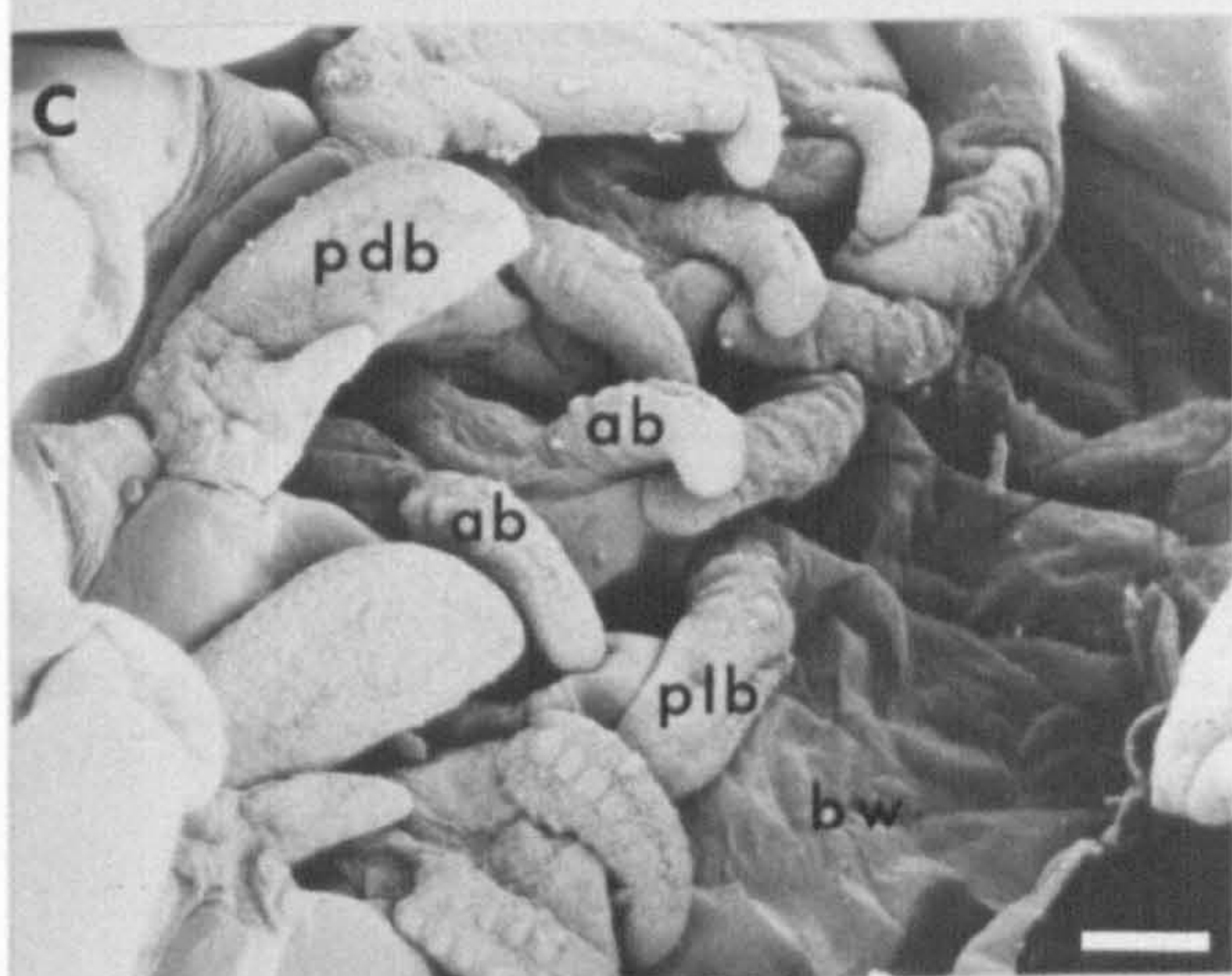
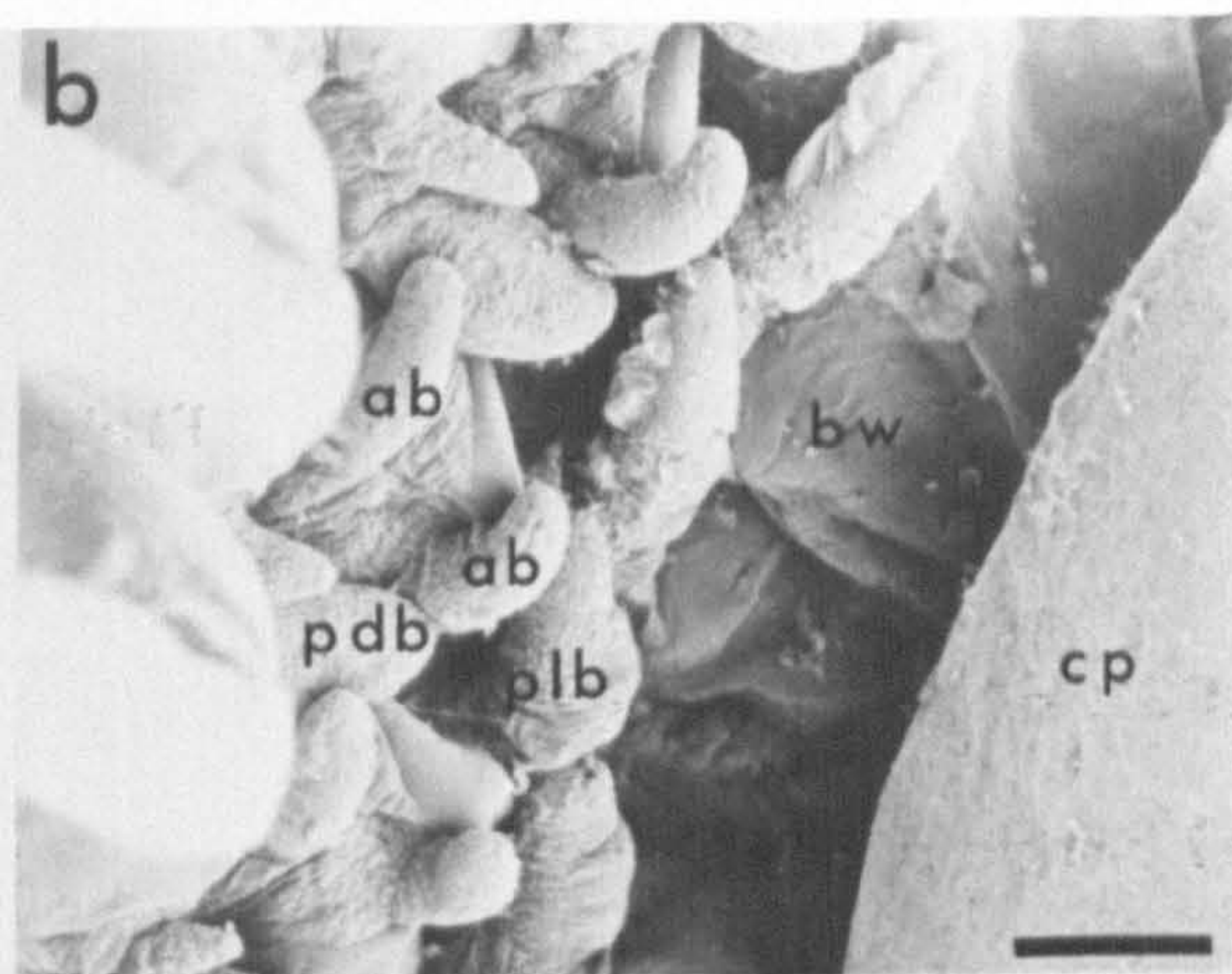
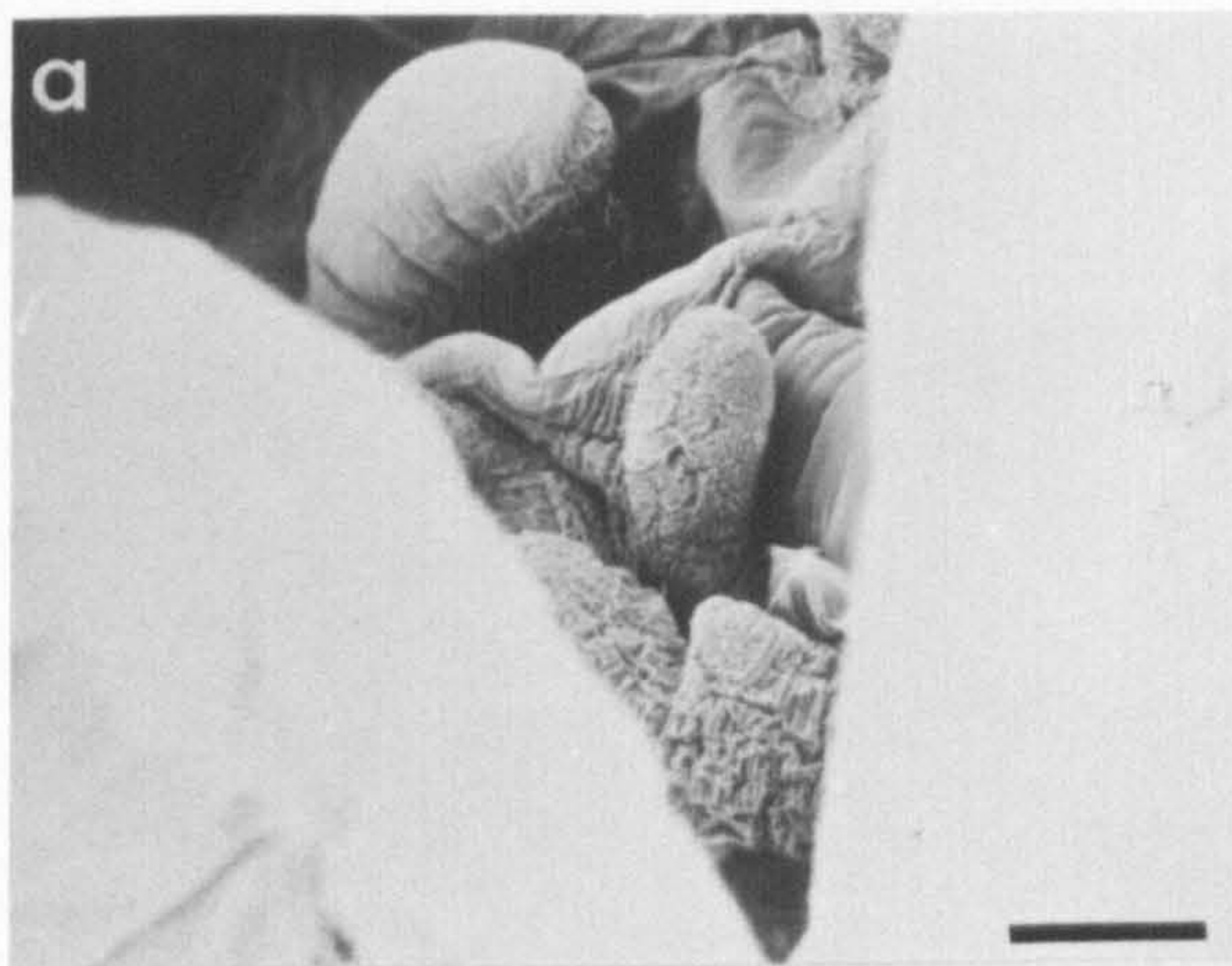
(e) Second stage postlarva.

(f) Fourth stage postlarva.

Scale bar represents 0.1mm, except for (a) where it represents 0.5mm.

Abbreviations are : ab, arthrobranch ; bw, body wall ; cp, carapace ;  
pdb, podobranch ; plb, pleurobranch.







# Plate 4.9

S.E.M. showing differences between the first and second postlarval stages.

(a) First stage postlarva. Ventral view of the residual thoracic exopods.

(b) Second stage postlarva. Ventral view of the thoracic appendages.

(c) First stage postlarva. Oblique view of the abdominal pleura.

(d) Second stage postlarva. Oblique view of the abdominal pleura.

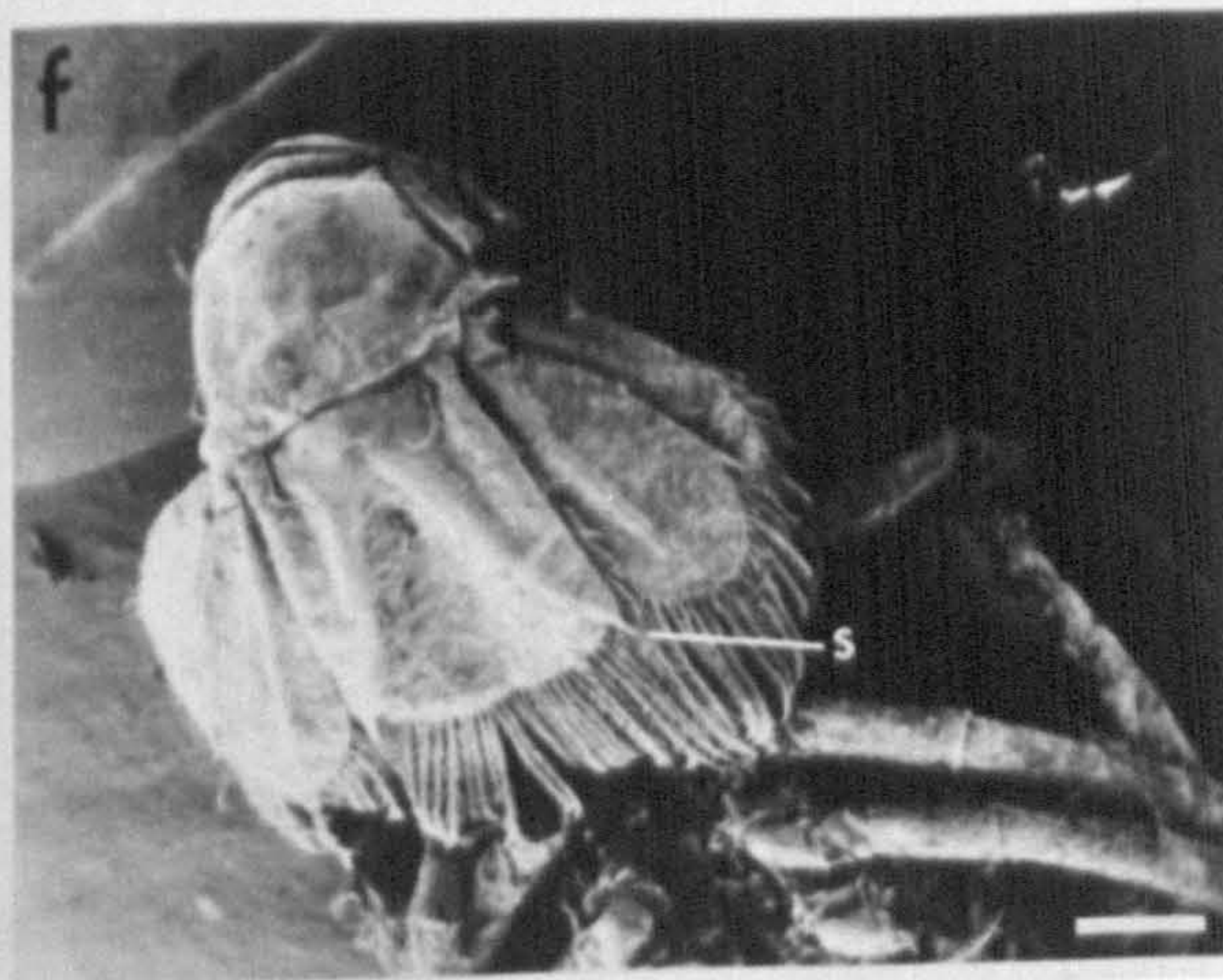
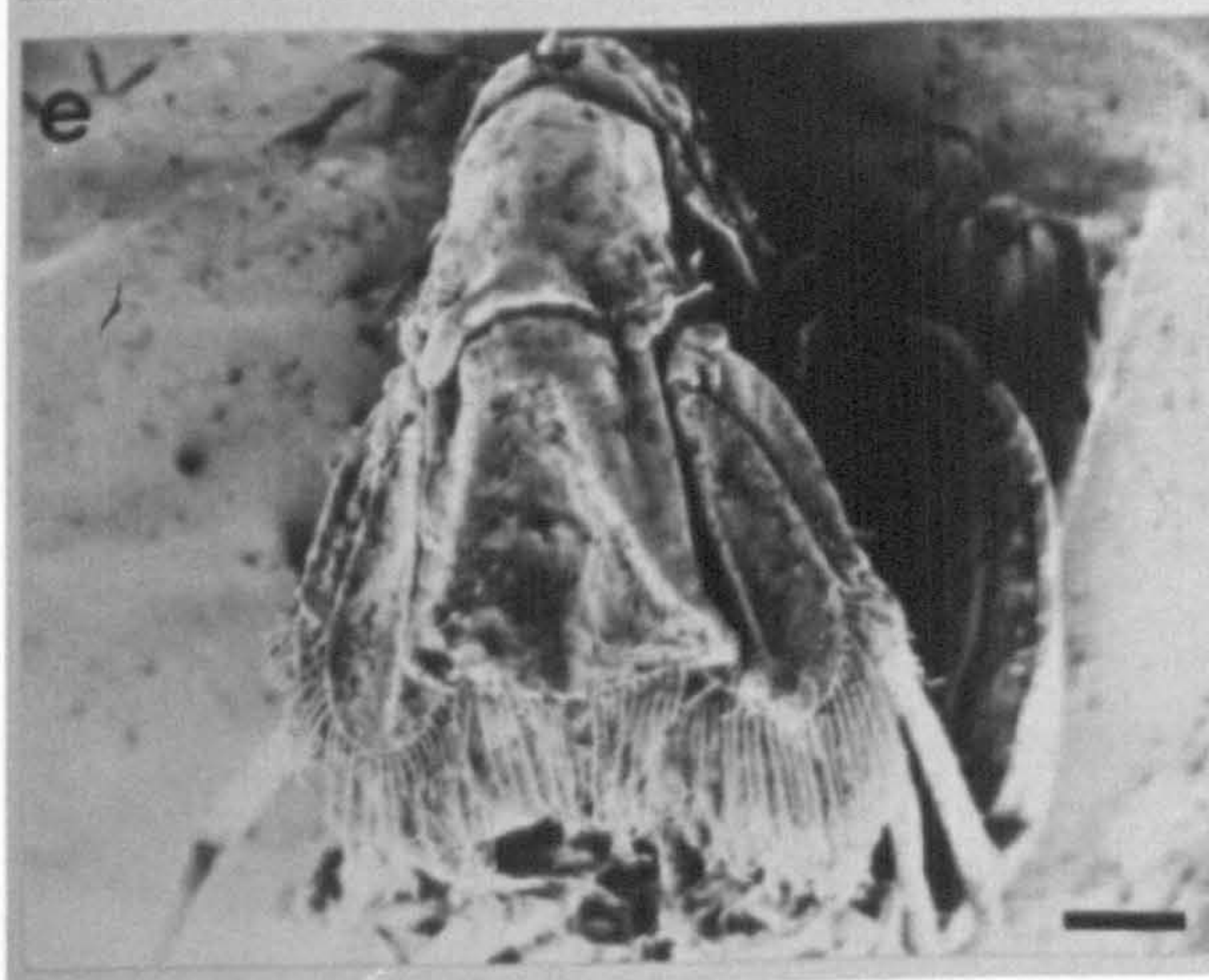
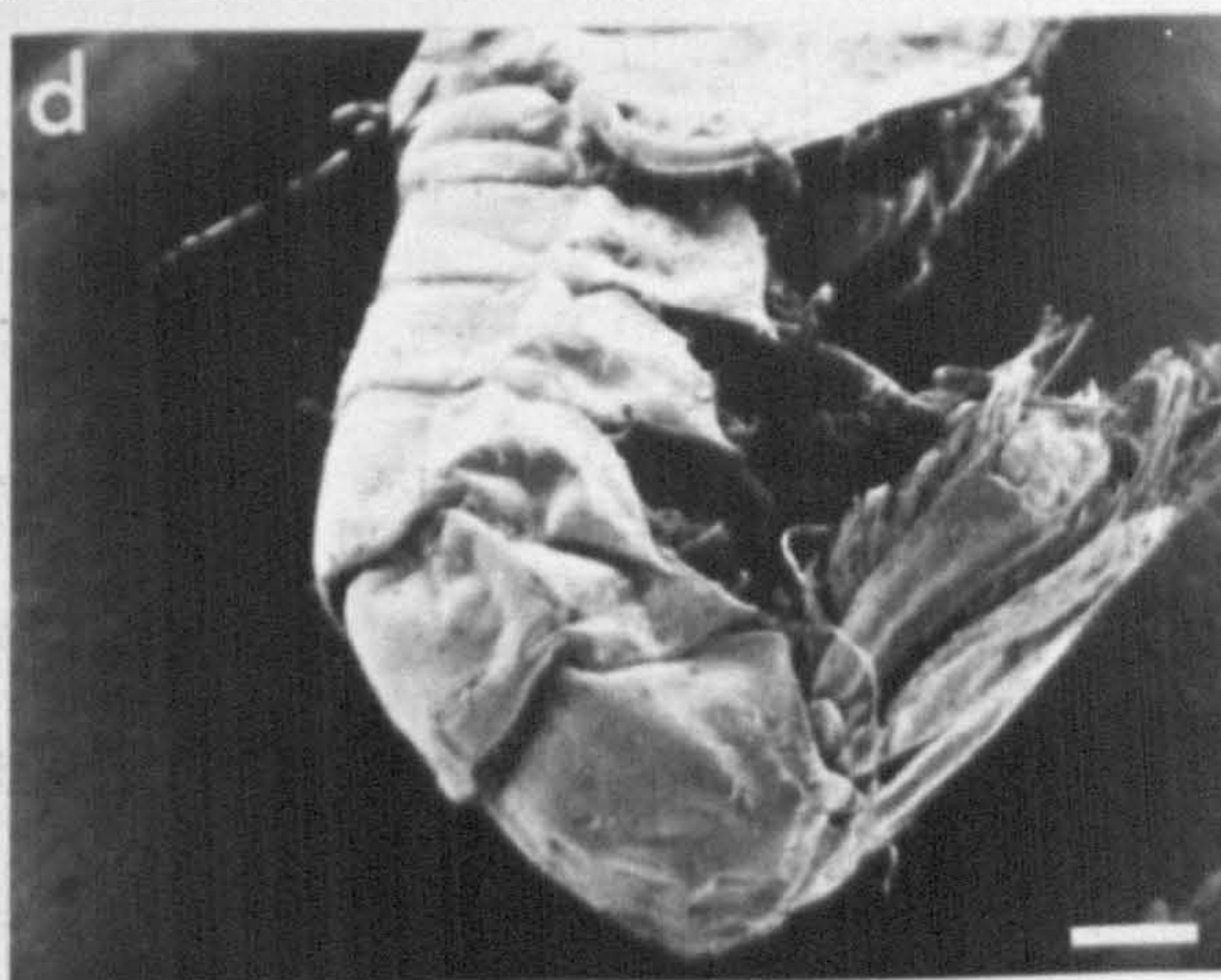
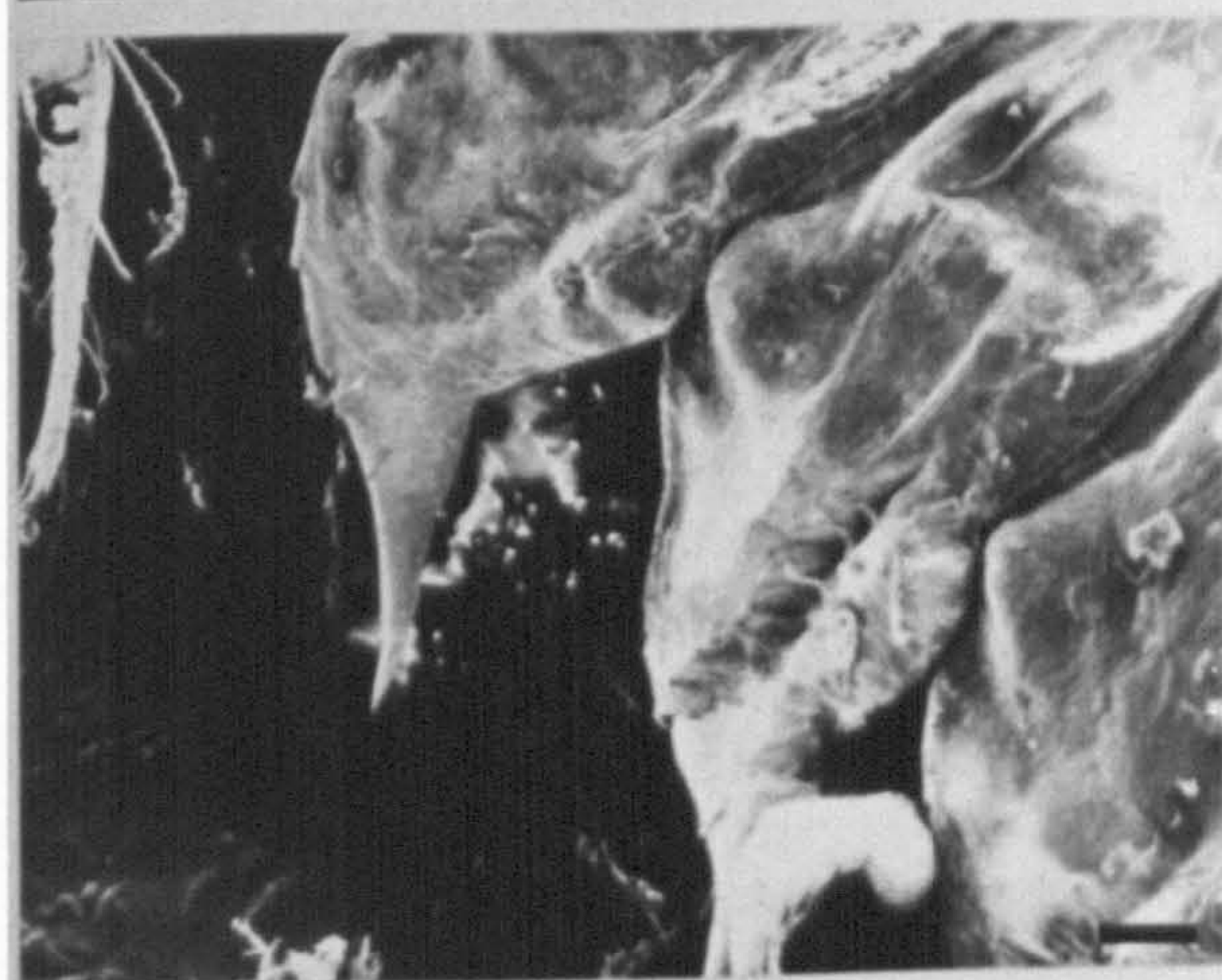
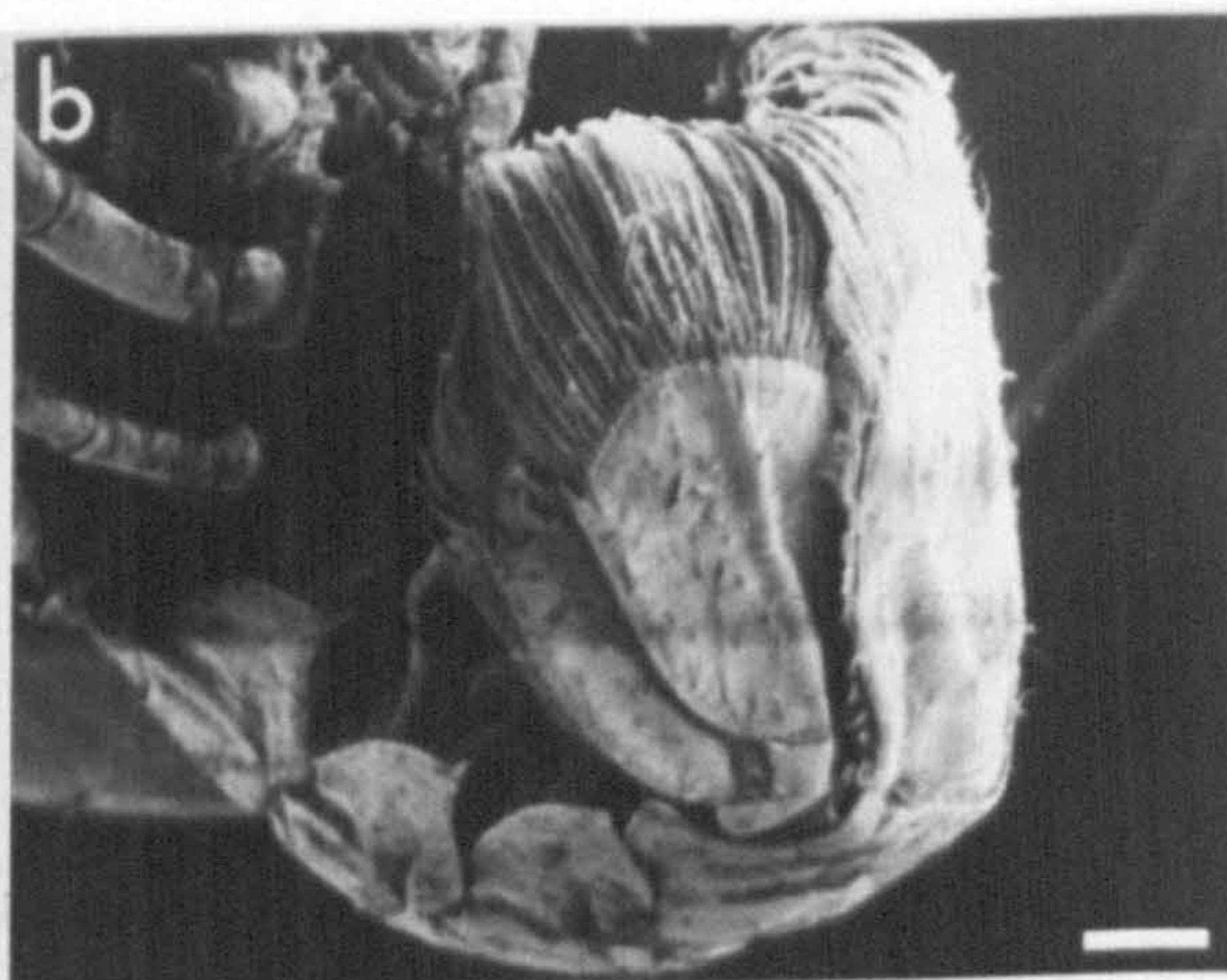
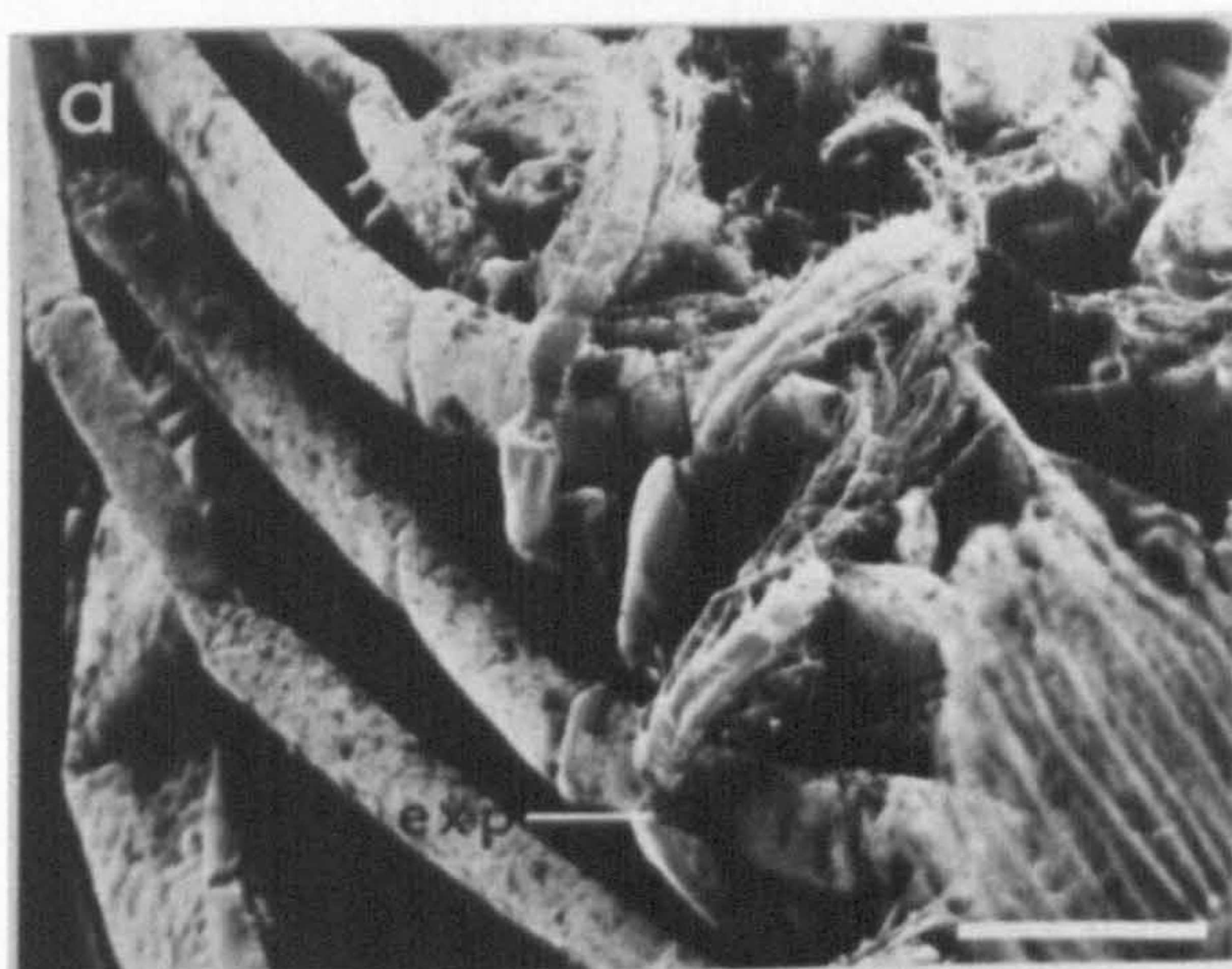
(e) First stage postlarva. Dorsal view of the telson.

(f) Second stage postlarva. Dorsal view of the telson.

Scale bar represents 0.05mm, except for (c) where it represents 0.1mm.

Abbreviations are : exp, exopod ; s, spine.







exopods in 1st stage postlarvae (the 2nd stage postlarvae have lost these structures), the extension of the abdominal pleura in 1st stage postlarvae into sharp posteriorly pointing spines (the corresponding feature in 2nd stage postlarvae is less pronounced) and the lack of spines at the posterior corners of the telson in 1st stage postlarvae (2nd stage postlarvae possess spines at the posterior corners of the telson).

#### 4.3.2. Growth

A summary of the biometric measurements taken for the 3 zoeal and early postlarval stages is shown in Table 4.1.

##### Absolute growth

Absolute growth is dependent upon the intermoult period and the moult increment. The mean intermoult periods (ie. stage durations) for the different stages is shown in Table 4.2. Hartnoll (1982) suggested that linear regressions of log intermoult period on length formed the best overall relationship between intermoult duration and size. The log mean intermoult period has therefore been plotted against mean carapace length (Figure 4.3).

Computer analysis revealed that the correlation coefficient was improved and the data better described if the zoeal and postlarval stages were treated separately. Regression lines were therefore fitted for the 2 phases. The equations of the lines are shown in Table 4.3. Both relationships were found to be significant and indicate that the intermoult period increased with size (and therefore with progressive

## Index for abbreviations and measurements

Z	=	Zoea
PL	=	Postlarva
Eye stalk +	=	Maximum length of the eye from the beginning of the stalk (centre of the median plate) to the outer perimeter of the cornea. The measurements were made along the inner axis of the removed eye, both eyes were measured and an average value was calculated.
Eye index	=	see text.
N	=	number of individuals contributing to the corresponding mean and standard deviation (S.D.).

In the zoeae the measurement for the maximum width of the 6th abdominal segment was taken in the region of the two divergent spines. The uropod width (maximum) was measured across the splayed uropods.





Table 4.1b  
Morphometric measurements (mm) for different development stages.  
(See Index for abbreviations.)

Development stage	Eye						Second Pleopod																
	N	Stalk length +		Index	N	Protopod length	Exopod					Endopod											
		Mean	S.D.				Mean	S.D.	Mean	S.D.	length	breadth	S.D.	Mean	S.D.	length	breadth	S.D.					
Z1	15	0.801	0.0252	0.317	0.0167	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z2	15	0.976	0.0457	0.362	0.0295	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z3	31	1.193	0.0478	0.396	0.0621	31	0.419	0.0401	0.512	0.0649	0.101	0.0135	0.542	0.0582	0.084	0.0119							
PL1	39	1.108	0.0522	0.372	0.0405	39	0.561	0.0401	0.772	0.0418	0.138	0.0127	0.811	0.0629	0.121	0.0125							
PL2	16	1.176	0.0910	0.512	0.0919	17	0.708	0.0715	0.897	0.0963	0.166	0.0202	0.933	0.0855	0.144	0.0153							
PL3	3	1.422	0.0575	0.660	0.0809	6	0.842	0.0723	1.118	0.0654	0.196	0.0129	1.156	0.0694	0.170	0.0123							
PL4	3	1.504	0.0603	0.691	0.0676	5	1.138	0.2950	1.357	0.1350	0.255	0.0258	1.392	0.1078	0.231	0.0467							
PL7	-	-	-	-	-	1	1.631	-	2.091	-	0.510	-	2.269	-	0.459	-							
PL8	1	1.900	-	1.651	-	1	1.658	-	2.346	-	0.510	-	2.499	-	0.485	-							
PL11	1	3.640	-	2.600	-	1	2.550	-	2.536	-	0.816	-	3.848	-	0.816	-							

**Table 4.2**

**The intermoult periods for development into the next stages  
(See Index for abbreviations).**

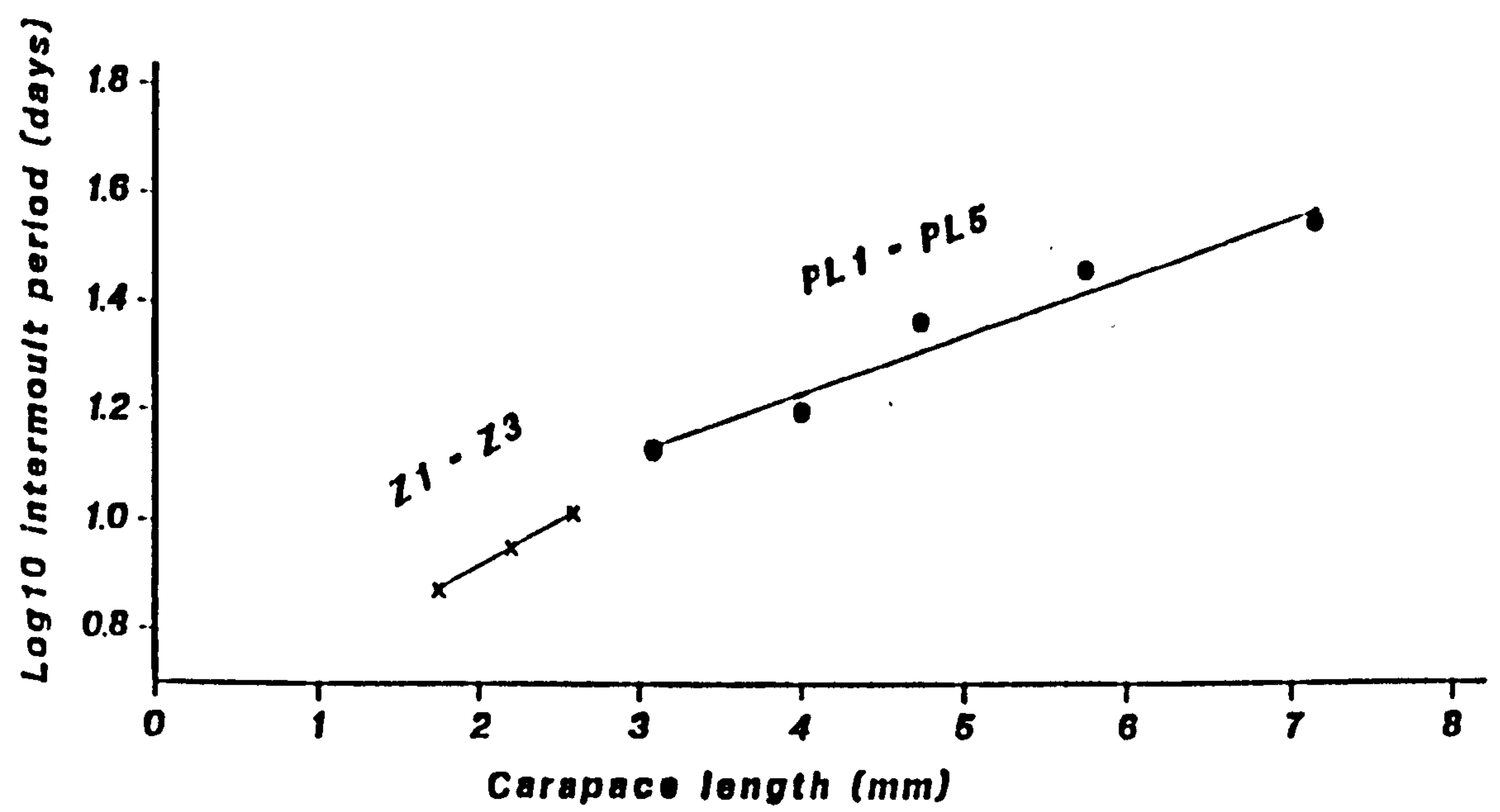
Development stage	Number of observations	Intermoult period (days)	
		Mean	S.D.
Z1	100	6.96	0.82
Z2	50	9.33	1.23
Z3	12	12.75	1.71
PL 1	10	13.70	0.82
PL 2	10	15.90	2.89
PL 3	5	23.20	3.27
PL 4	1	29.00	-
PL 5	1	35.00	-

Figure 4.3

The log linear relationship between the length of the mean intermoult period (Table 4.2) and the mean carapace length (Table 4.1a) during development through the zoeal growth phase (Z1 - Z3) and the first 5 stages of the postlarval growth phase (PL1 - PL5). Equations of the lines are shown in Table 4.3.

Z = Zoea (x), PL = Postlarva (●).





**Table 4.3**

Correlation coefficients and linear regressions between moult increment (Y) and premoult carapace length (X) for the 3 zoeal stages and the first 5 postlarval stages. Each stage was represented by an 'average' animal of mean carapace length and mean intermoult period (see Tab. 4.1a and Tab. 4.2 respectively). (See Index for abbreviations).

Development stages	Number of observations	Correlation coefficient	Equation	Significance of regression (P)
Z1 - Z3	3	0.996	$\text{Log } Y = 0.282 + 0.320 X$	< 0.05
PL1 - PL5	5	0.965	$\text{Log } Y = 0.817 + 0.106 X$	< 0.01

instars) for both the zoeal and postlarval phases. The relationships were derived from mean values and were therefore based on 3 and 5 data points (for the zoeal and postlarval relationships respectively). Although a difference in slope between larval and postlarval lines is indicated in Figure 4.3 because of the small data sets the difference was not statistically significant ( $P > 0.05$ ).

The moult increments were calculated for each stage by subtracting the mean carapace length for the given stage from the corresponding value for the next stage in Table 4.1. These values were plotted against the corresponding mean premoult carapace lengths for each stage (Figure 4.4). Computer analysis revealed that the correlation coefficient was improved and the data better described by combining the zoeal and postlarval stages and fitting a single regression line. The relationship was significant ( $P < 0.005$ ) and indicates that moult increment tended to increase with increasing premoult length over the size range and development stages examined.

#### Relative growth

Best fit log-log linear relationships were derived for each study of relative growth and are shown in Figure 4.5 and 4.6. The equations of the lines are shown in Table 4.4. All the relationships formed were highly significant ( $P < 0.001$ ). The slope of the line (b) defines the type of allometric growth (Hartnoll, 1982). If  $b = 1$  there is isometry, with both variables growing at the same rate. If  $b > 1$  then positive allometry exists with the dependent variable growing at a faster rate than the carapace length. If  $b < 1$  then negative allometry exists with the dependent variable growing at a slower rate than the

**Figure 4.4**

**The linear relationship between mean moult increment and mean premoult carapace length during development through the 3 zoeal and first 11 postlarval stages (Z1 - PL11).**

**Z = Zoea (x), PL = Postlarva (●).**



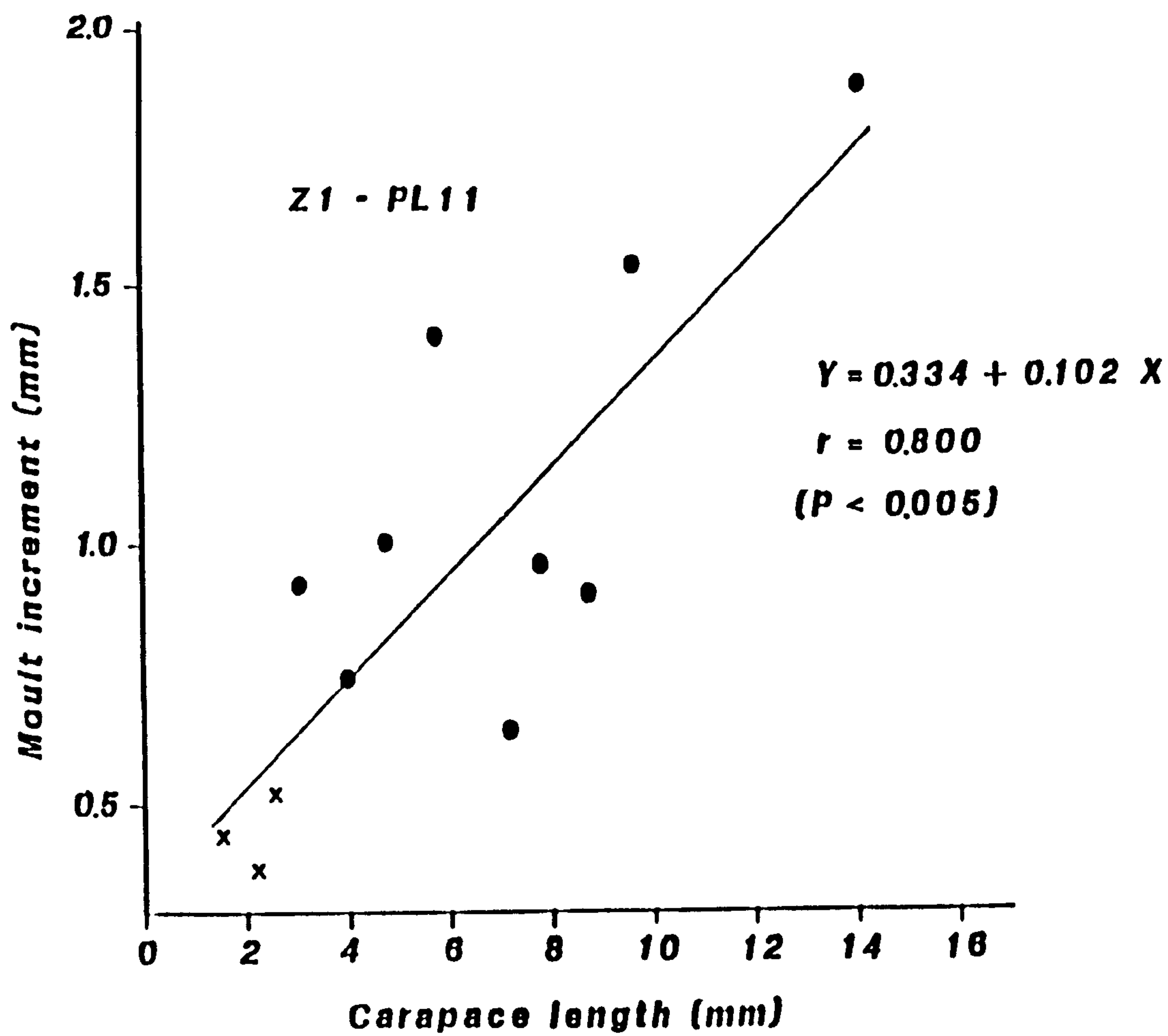


Table 4.4

Correlation coefficients and log-log linear regressions describing the relative growth between the dependent variable (Y) and carapace length (X, the independent variable) during zoeal (Z1- Z3) and postlarval (PL1- PL11) development. (See Index for abbreviations).

Dependent variable	Development stages	Number of observations	Correlation coefficient	Equation	Significance of regression (P)
Total length	Z1 - Z3	112	0.956	$\text{Log } Y = 0.542 + 0.953 \text{ Log } X$	< 0.001
	PL1 - PL11	134	0.985	$\text{Log } Y = 0.522 + 0.954 \text{ Log } X$	< 0.001
Abdomen width (6th seg.)	Z1 - Z3	112	0.959	$\text{Log } Y = -0.667 + 1.310 \text{ Log } X$	< 0.001
	PL1 - PL11	126	0.984	$\text{Log } Y = -0.544 + 1.210 \text{ Log } X$	< 0.001
Eye stalk length +	Z1 - Z3	63	0.908	$\text{Log } Y = -0.321 + 0.955 \text{ Log } X$	< 0.001
	PL1 - PL11	55	0.919	$\text{Log } Y = -0.251 + 0.609 \text{ Log } X$	< 0.001
Uropod width	PL1 - PL11	91	0.986	$\text{Log } Y = 0.002 + 1.080 \text{ Log } X$	< 0.001
Protopod length (2nd pleopod)	PL1 - PL11	70	0.960	$\text{Log } Y = -0.726 + 0.976 \text{ Log } X$	< 0.001
Propodus length (1st pereopods)	PL1 - PL11	92	0.983	$\text{Log } Y = -0.054 + 1.150 \text{ Log } X$	< 0.001

carapace length.

There was no significant difference ( $P > 0.05$ ) between the slopes of the relationships between carapace length and total length for the zoeal and postlarval stages (Figure 4.5a). Growth between these 2 variables during the zoeal stages was not significantly different ( $P > 0.05$ ) from isometric but at this level of significance there was evidence of slightly negative allometry for the postlarval stages (ie. the rate of growth for total length was slightly slower than that recorded for carapace length for the postlarval stages but was the same for the zoeal stages).

A significant difference ( $P < 0.05$ ) was recorded between the slopes of the relationships between carapace length and maximum width of the 6th abdominal segment for the zoeal and postlarval stages (Figure 4.5b). Positive allometric growth appears to have occurred for both growth phases ( $P < 0.05$ ) (ie. the rate of growth for abdominal width was faster than that recorded for carapace length) but was more exaggerated during zoeal development.

A significant difference ( $P < 0.05$ ) was detected between the slopes of the relationships between carapace length and eye stalk length + (see abbreviations) for the zoeal and postlarval stages (Figure 4.5c). Growth between these 2 variables during the zoeal stages was not significantly different ( $P > 0.05$ ) from isometric but there was evidence at this level of significance of negative allometry for the postlarval stages.

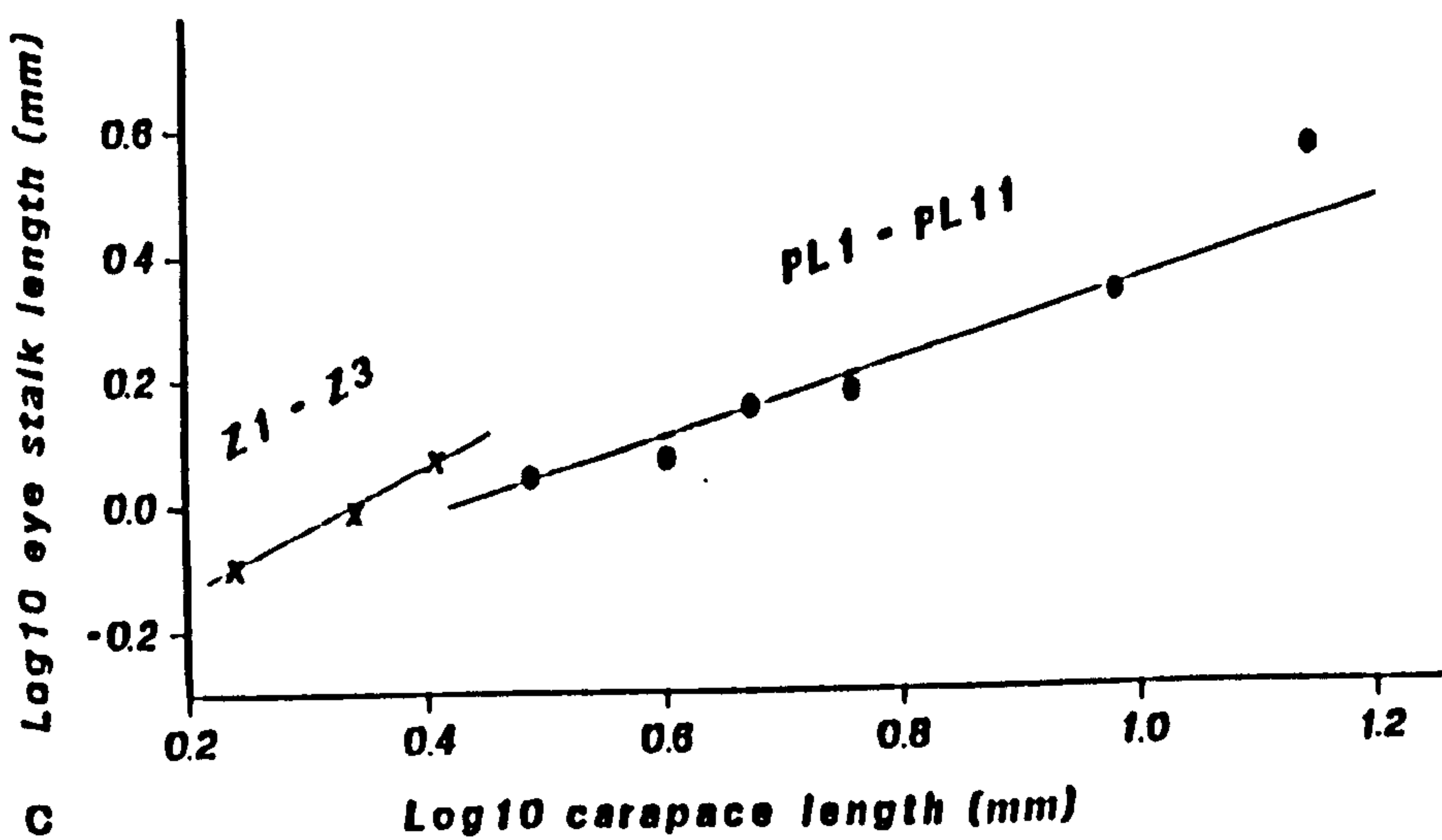
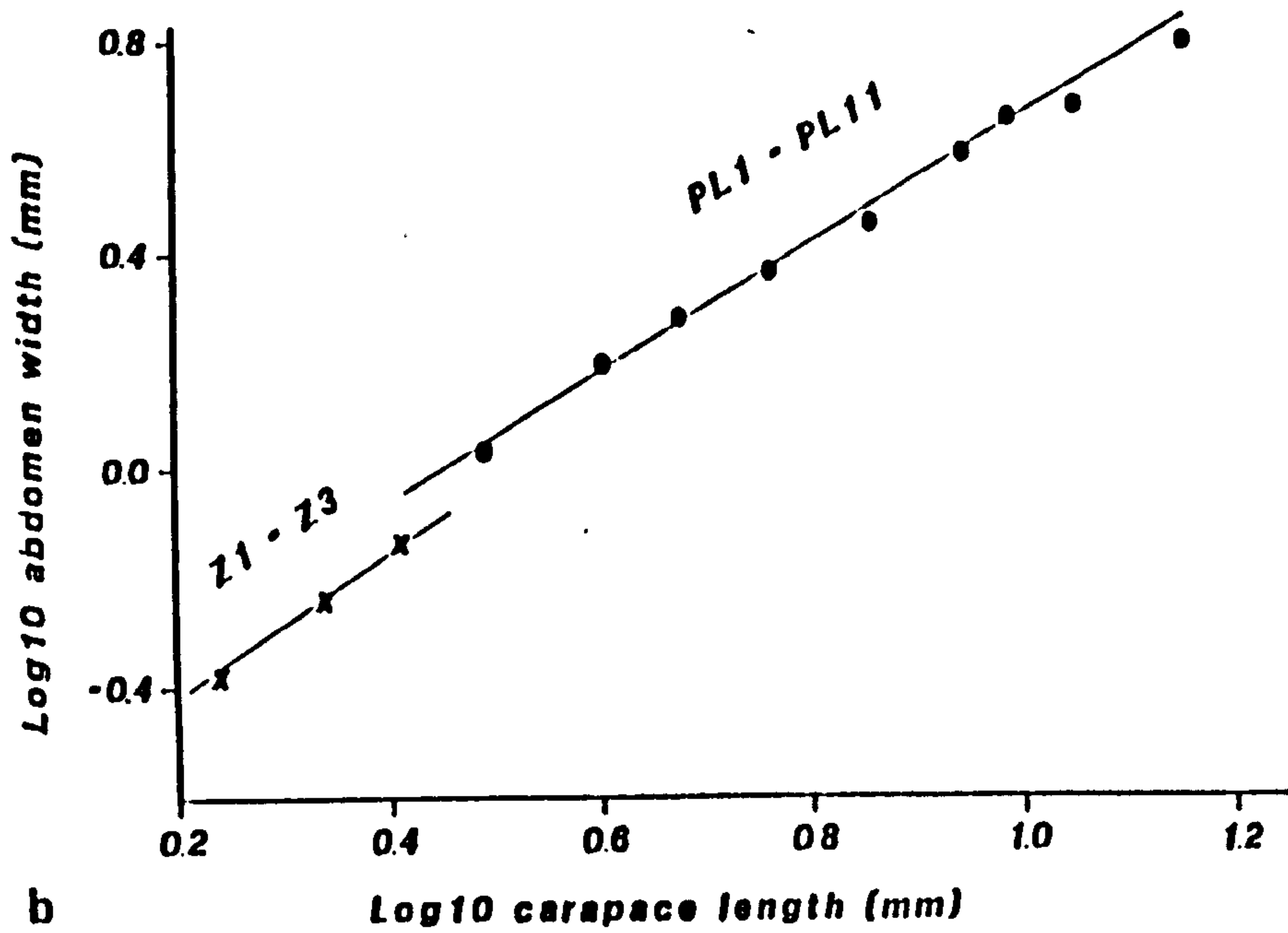
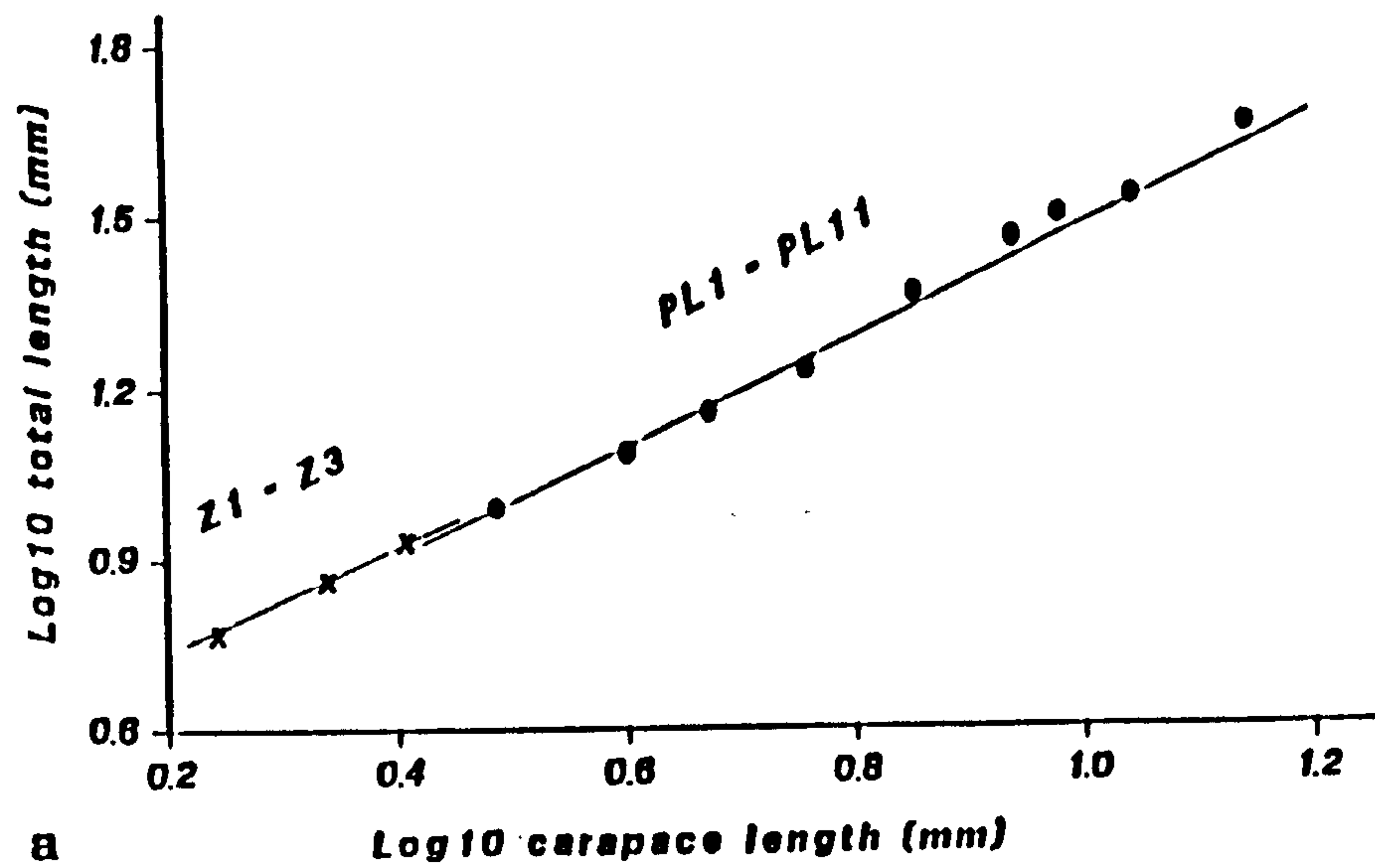
There was evidence of a marginally positive allometric

Figure 4.5

The log-log relationships describing relative growth between carapace length and (a) total length, (b) abdomen width and (c) eye stalk length during the zoeal (Z1 - Z3) and postlarval (PL1 - PL11) growth phases. The data points from which the relationships were derived have been excluded for purposes of clarity and only the mean sizes for each pair of variables is shown for each stage. For further information on the statistics associated with the data see Table 4.1.

Z = Zoea (x), PL = Postlarva (●).





relationship between the width of the splayed uropods and carapace length for the postlarval stages ( $P < 0.05$ ) (Figure 4.6a) (ie. the rate of growth for uropod width was slightly faster than that recorded for carapace length). Uropod width could only be measured in zoeae of the 3rd stage as zoeae of the earlier stages do not possess uropods. No significant difference ( $P < 0.05$ ) was recorded between the relative sizes of these 2 variables for 3rd stage zoeae from that predicted by the corresponding equation for the postlarval stages.

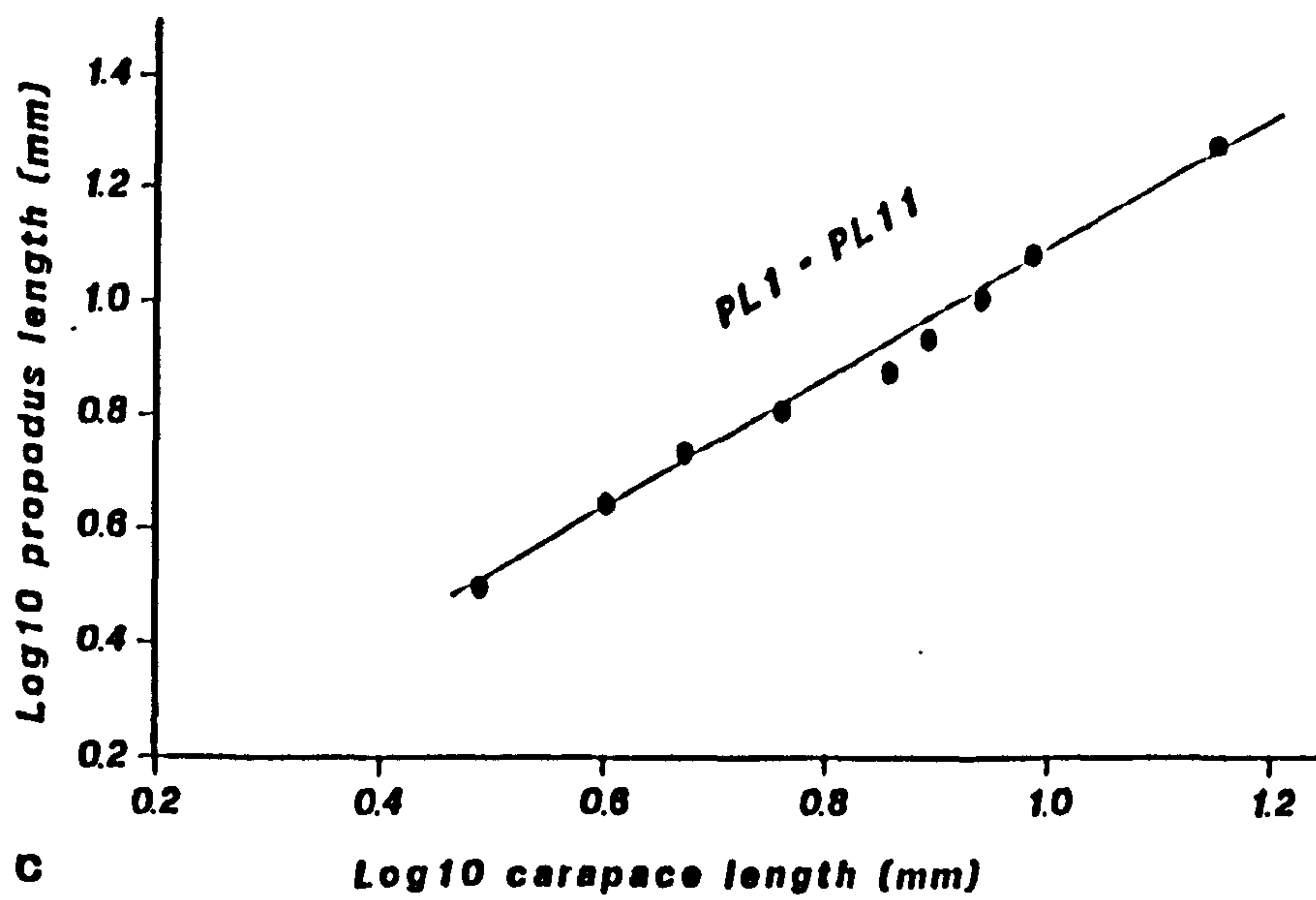
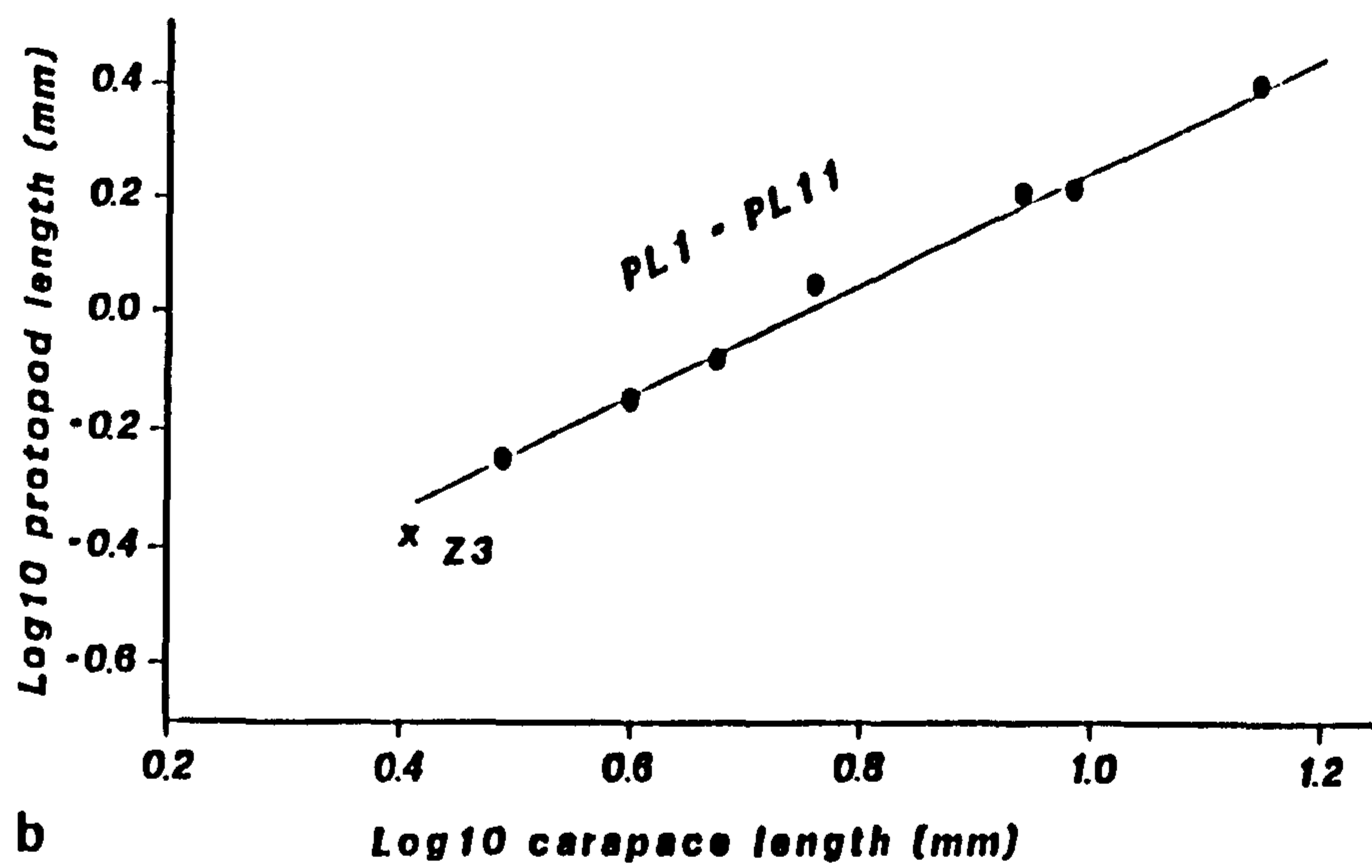
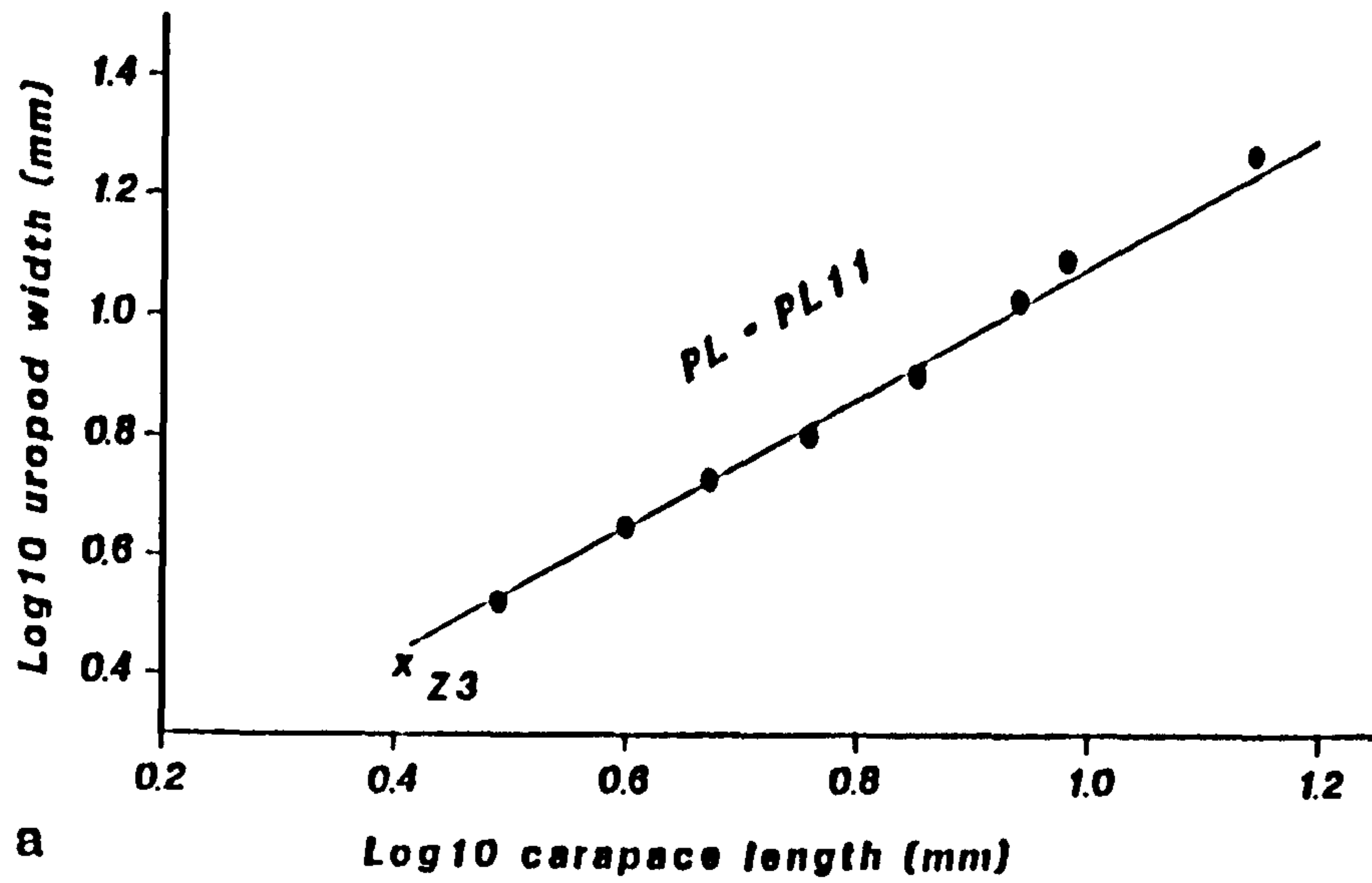
The relationship between protopod length of the 2nd pleopods and carapace length for the postlarval stages was not significantly different ( $P > 0.05$ ) from isometric (Figure 4.6b). Assuming that protopod growth gives a good indication of growth for the entire pleopod (the exopod and endopod measurements suggest that this is the case, see Table 4.1b) then the rate of growth for this structure was similar to that recorded for carapace length. Due to the small size of the pleopods in 1st and 2nd stage zoeae, protopod measurements were only made for 3rd stage zoeae. The relative size of the pleopod protopod of 3rd stage zoeae was significantly smaller ( $P < 0.05$ ) than in postlarvae.

There was evidence of a marginally positive allometric relationship between propodus length of the 1st pair of pereopods and carapace length for the postlarval stages ( $P < 0.05$ ) (Figure 4.6c) (ie. the rate of growth for the propodus was slightly faster than that recorded for the carapace length).

Figure 4.6

The log-log relationships describing relative growth between carapace length and (a) uropod width, (b) protopod length of the 2nd pleopods and (c) propodus length during the postlarval growth phase (PL1 - PL11). The data points from which the relationships were derived have been excluded for purposes of clarity and only the mean sizes for each pair of variables is shown for each stage. The corresponding mean value for 3rd stage zoeae (Z3) has been plotted for (a) and (b). For further information on the statistics associated with the data see Table 4.1.

Z = Zoea (x), PL = Postlarva (●).





#### 4.3.3. Accelerated development

During the stocking density experiment conducted in Chapter 3 two 2nd stage zoeae from the individual rearing trial and, a third individual in another experiment, appeared to moult directly into 1st stage postlarvae, missing out the intermediate 3rd zoeal stage (Plate 4.10).

The features which these individuals possess which most clearly establish them as 1st stage postlarvae as opposed to 3rd stage zoeae are shown in Plate 4.10. These include : (1) shape of the rostrum, lack of supraorbital spines, reduced eye stalk (Plate 5.2 and 5.3) ; (2) evidence of both circular and square corneal lenses (Plate 5.3) ; (3) mouthparts (Plate 4.7) ; (4) residual exopods on the pereopods (Plate 4.9) ; (5) secondary lobes on the gills (Plate 4.8) ; (6) shape of the telson, although residual lateral projections were retained (Plate 4.5).

A summary of the biometric measurements taken from these individuals is shown in Table 4.5a. The corresponding measurements for 3rd stage zoeae and 1st stage postlarvae are summarised in Table 4.5b. From the carapace length measurements all three individuals were significantly larger ( $P < 0.05$ ) than the 3rd stage zoeae but within the accepted size range, at this level of significance, for 1st stage postlarvae. The three individuals died shortly after completing the moult. They may not have achieved the full potential increase of the moult when the morphometric measurements were taken which may account for the generally smaller dimensions recorded for these postlarvae compared to the mean values recorded for the stage. It is evident

Plate 4.10

S.E.M. of individuals of the first postlarval stage which had demonstrated accelerated development, moulting directly into this form from the second zoeal stage.

(a) Thoracic region.

(b) Corneal lenses of the eye.

(c) Mouthparts.

(d) Residual thoracic exopod.

(e) Gills.

(f) Telson.

Scale bar represents 0.05mm for (b), 0.10mm for (c), (d) and (e) and 0.50mm for (a) and (f).

Abbreviations are : exp, exopod ; max 1, first maxilla ; mb, mandible ; mxp 3, third maxilliped ; rlp, residual lateral projection ; t, telson ; ur, uropod.



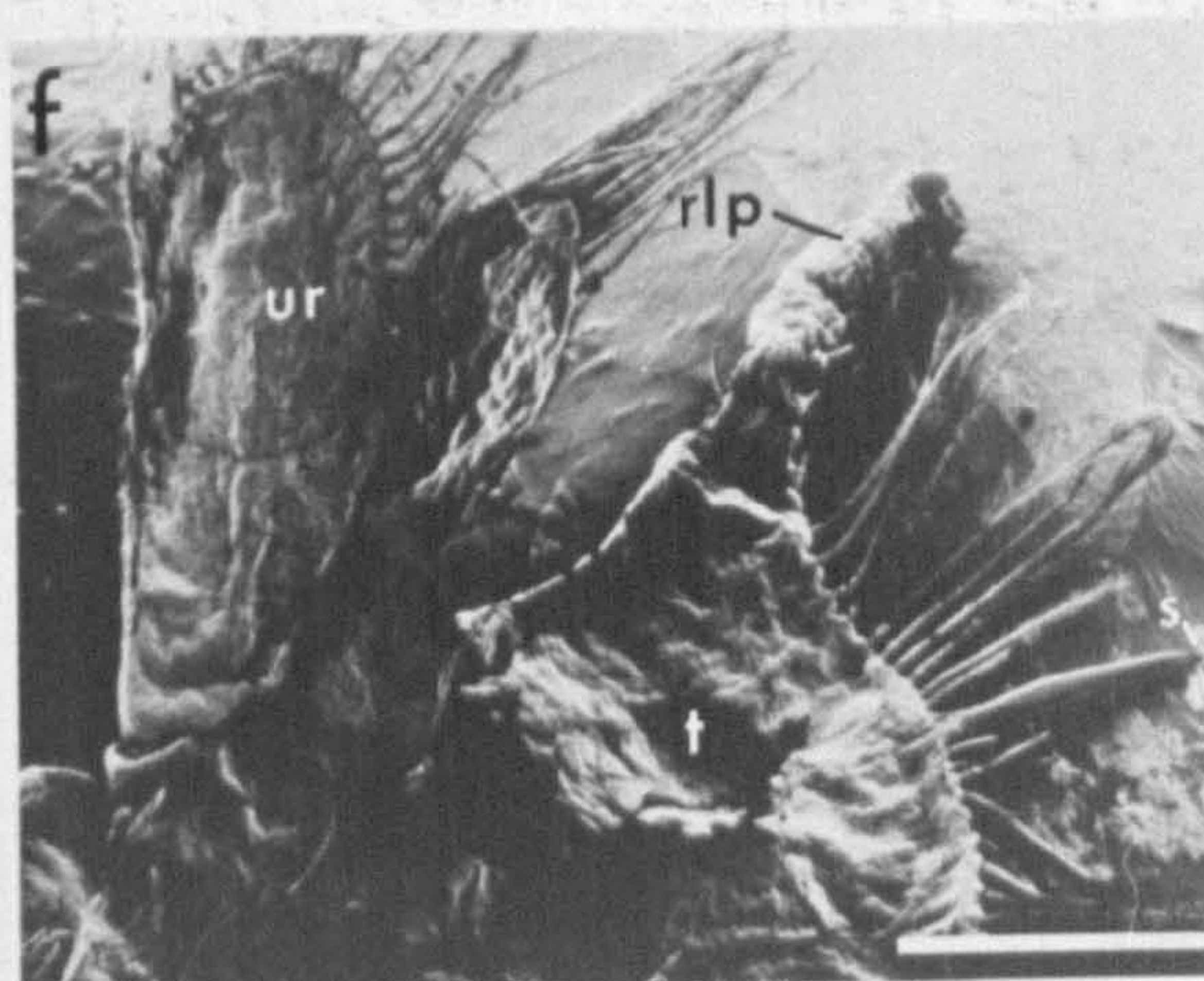
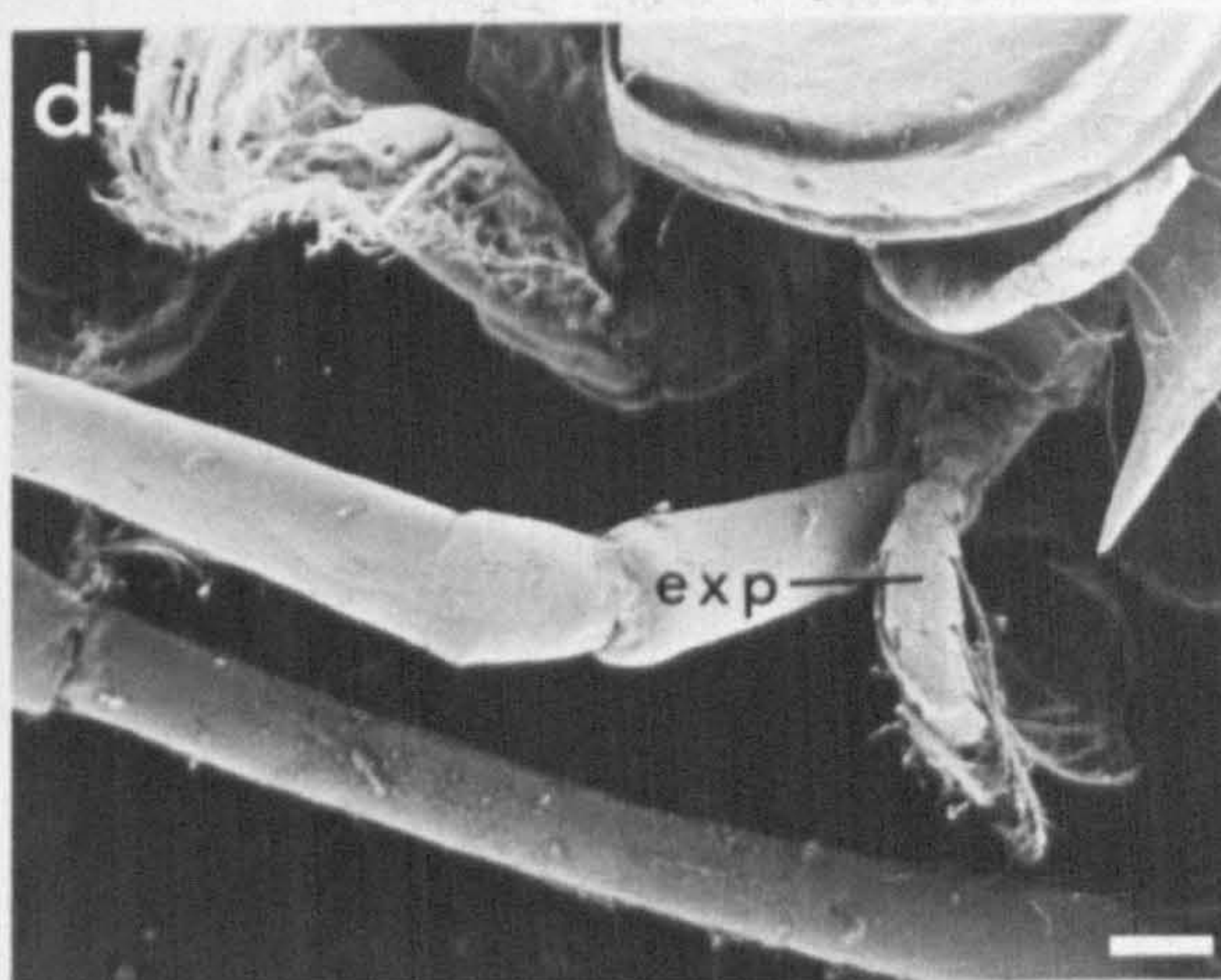
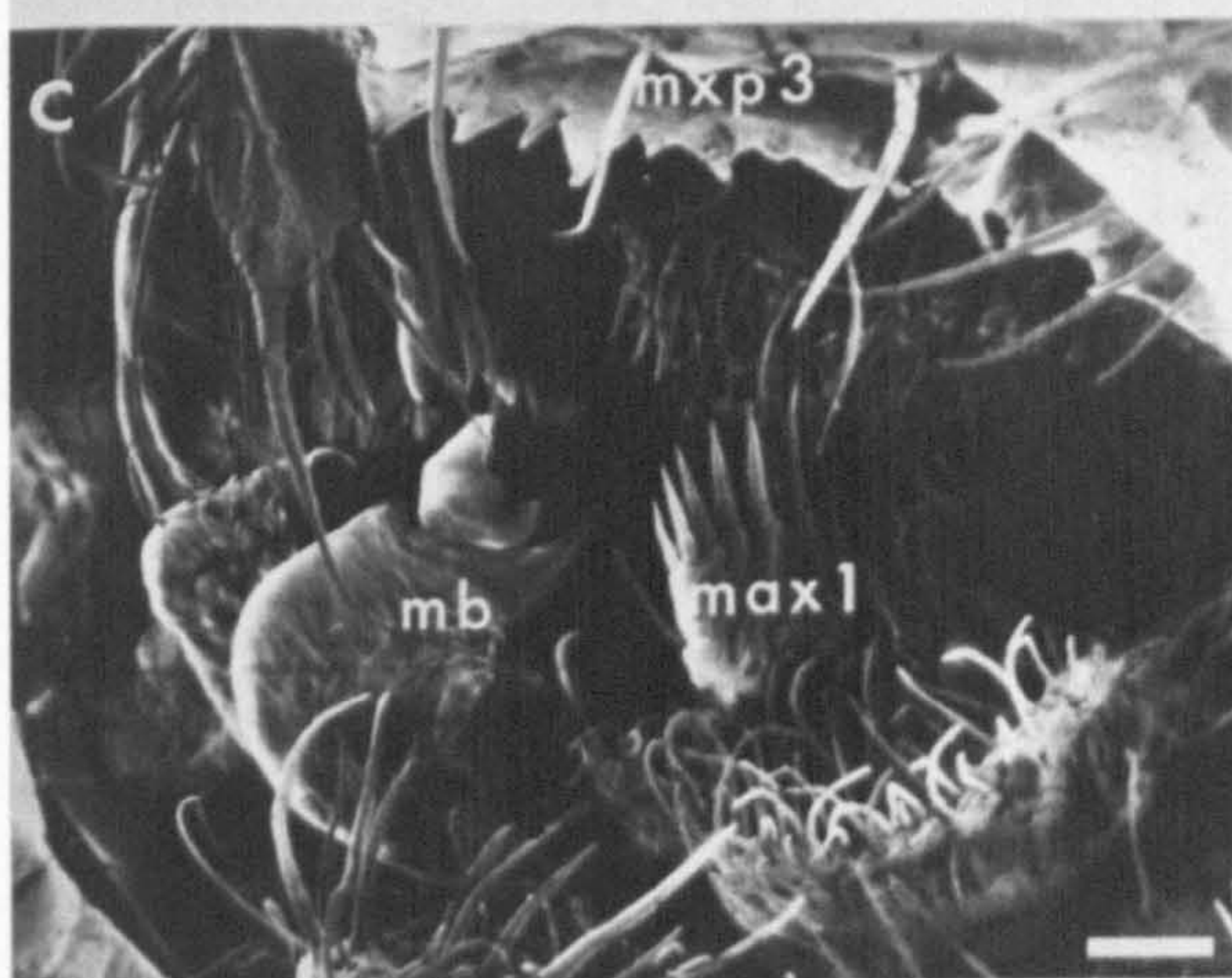
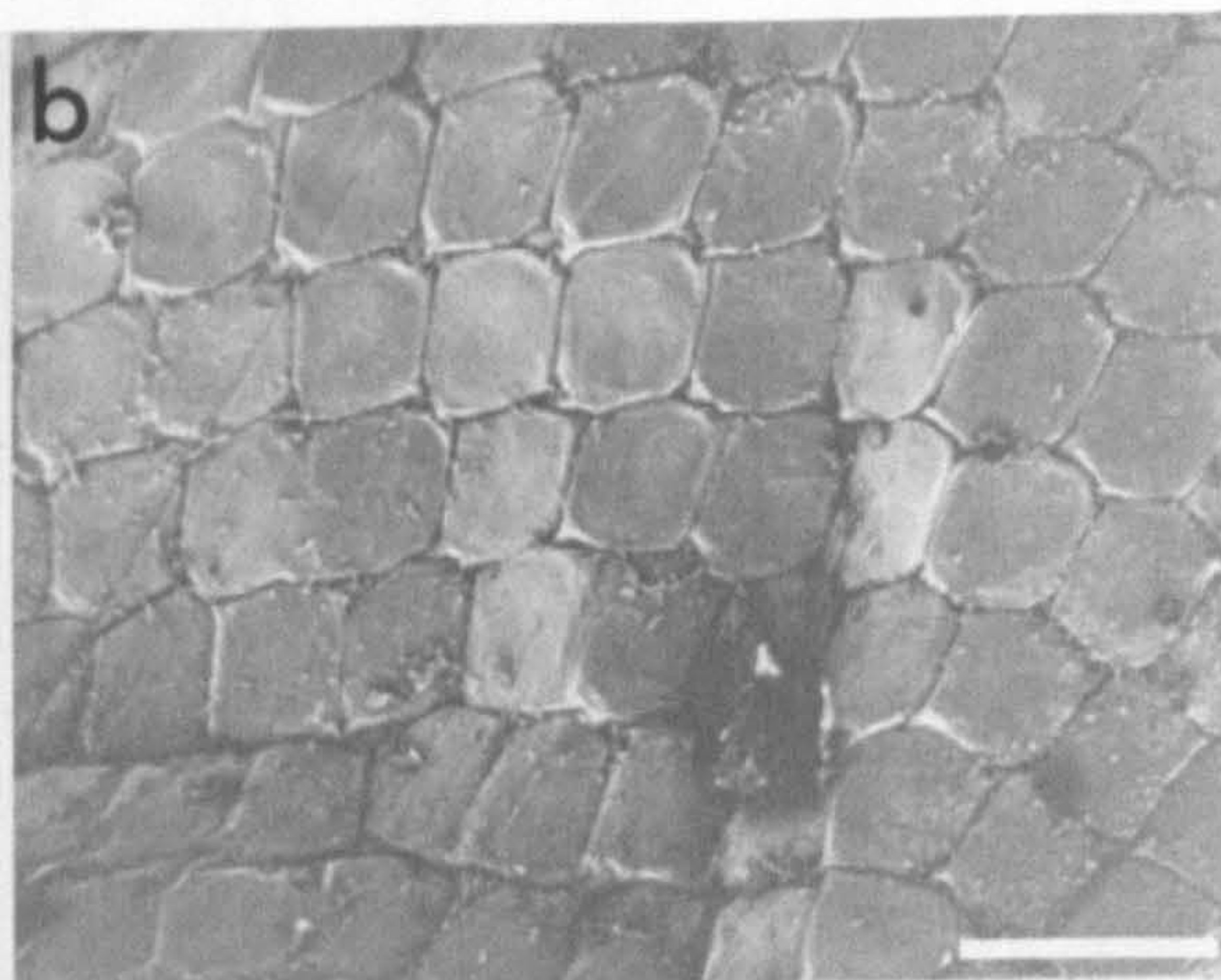
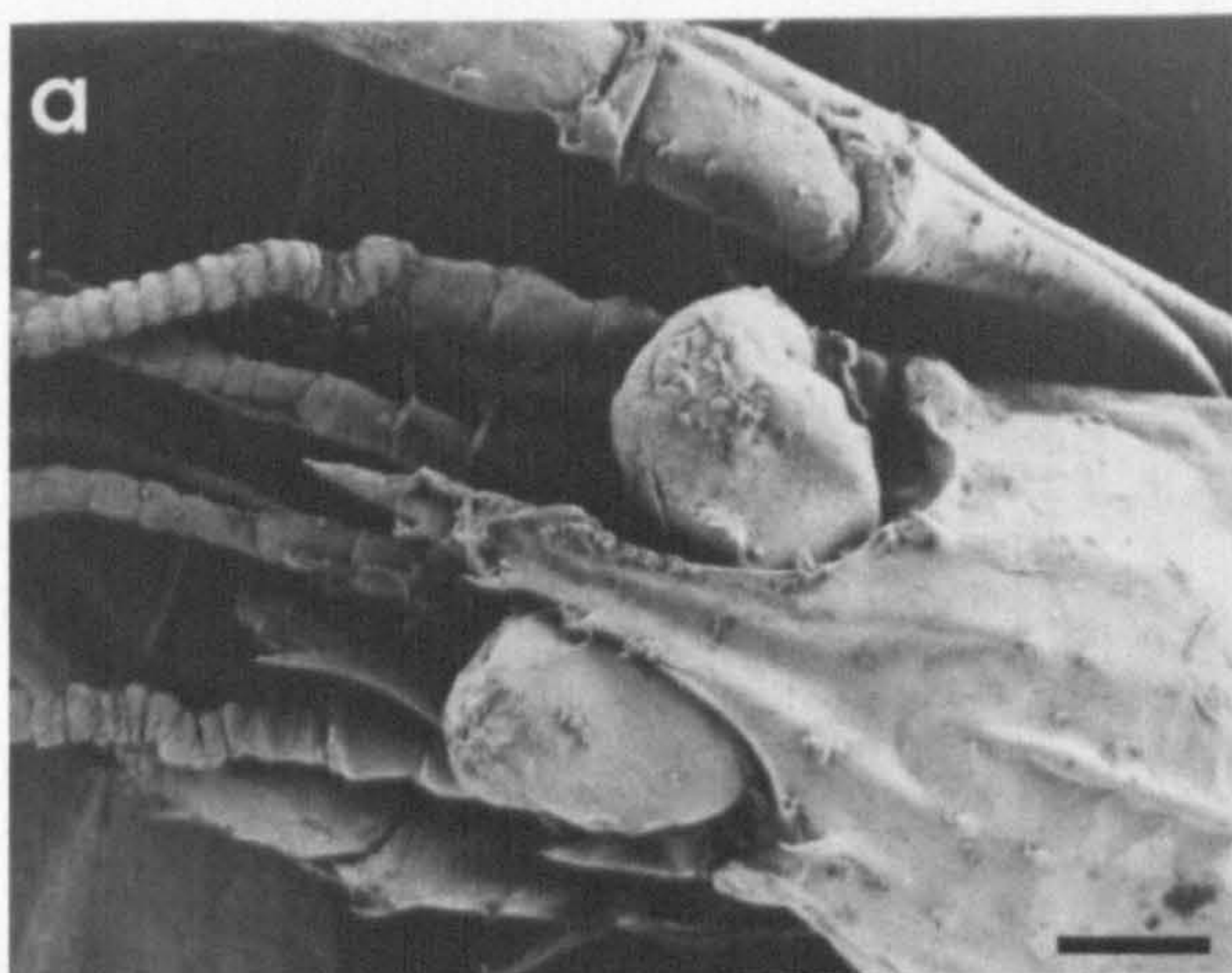




Table 4.5

A: Morphometric measurements (mm) for the 3 individuals (A, B, C) which demonstrated accelerated development from second stage zoeae (Z2) to 1st stage postlarvae (PL1).

B: The mean values and their associated 95% confidence limits ( $\pm$ ) for the corresponding measurements (mm) for 3rd stage zoeae (Z3) and for 1st stage postlarvae (PL1).

Individuals A and B were reared during the stocking density experiment (see Chapter 3, Table 3.2b) and both developed from newly hatched 1st stage zoea into 1st stage postlarvae in 21 days under conditions of individual rearing. This was significantly shorter ( $P < 0.05$ ) than the development rates recorded under conditions of individual or mass rearing. (See Index for abbreviations).

Individual	Total length	Carapace length	Abdomen width (6th)	Uropod width	Propodus length	Second Pleopod				Eye	
						Protopod length	Exopod length	Endopod breadth	Stalk+ Index		
Z2 Cast A		(2.08)									
PL 1	8.54	3.02	0.918	2.58	2.82	-	-	-	1.032	0.217	
Z2 Cast B		(2.13)									
PL 1	8.75	3.07	0.969	2.54	-	0.510	0.663	0.128	0.689	0.115	0.829 0.332
Z2 Cast C		(2.08)									
PL 1	8.95	2.86	1.020	2.95	2.69	-	-	-	0.867	0.332	
B											
Mean	8.59	2.56	0.730	2.63	2.35	0.419	0.512	0.101	0.542	0.085	1.193 0.396
Z3 95% Limits ( $\pm$ )	0.90	0.29	0.080	0.326	0.22	0.079	0.127	0.026	0.114	0.023	0.094 0.122
Mean	9.82	3.08	1.085	3.36	3.14	0.561	0.772	0.138	0.811	0.121	1.108 0.372
PL 1 95% Limits ( $\pm$ )	1.06	0.40	0.170	0.51	0.48	0.079	0.082	0.025	0.123	0.025	0.102 0.079



from both the S.E.M. photographs and the biometric data that these individuals have completed the metamorphic moult and are indeed 1st stage postlarvae.

#### 4.4. Discussion

##### 4.4.1. Morphological description

The colour photographs and S.E.M. plates provide a useful addition to the morphological descriptions and line drawings which have previously been provided for the early stages of Nephrops (Santucci, 1926 ; Kurian, 1956 ; Andersen, 1962). Although photographs describing these stages are already available in the literature (Figueiredo and Vilela, 1972) they were taken in black and white, reveal little detail and were not accompanied by a description.

In an early study of Homarus americanus, Herrick (1895) described the characteristic distribution of chromatophores in the larval stages and the ability of the larvae to change colour through the dispersion or contraction of the pigments within the chromatophore cells. It appears that Nephrops also possess a characteristic chromatophore distribution and the ability to change colour. The brilliant red colouration of the 1st stage zoea (Plate 4.1c) was caused by pigment migration throughout the ramifications of the chromatophore cells. The yellowish translucent qualities of the zoeae within the 2nd and 3rd stages were caused by the contraction of the pigments and concentration within a small region of the chromatophore cells. The extent of the dispersion or contraction of pigment in chromatophores is caused by hormones called chromatophorotropins (Warner, 1977). Earlier investigations involving fiddler crabs (Uca spp.) have shown that the most important environmental variables which control pigment migrations are light, heat and colour of the

background (Warner, 1977). It is uncertain which of these variables (if any) caused the variation between the condition of the chromatophore pigments for Nephrops zoeae in this present study. Short-term differences in lighting conditions may be the most likely cause as the culture temperature (14°C) and colour of the background (white) was similar for all zoeae. In the larval stages of H.americanus colour changes were stimulated by pressure and were normally completed within 10 - 15mins (Herrick, 1895). Light within the red region of the spectrum is strongly attenuated on transmission through water (Jerlov, 1968). In their natural environment the zoeae will therefore possess transparent qualities whether their chromatophores are in the contracted or expanded condition. This will enable them to remain inconspicuous to the attention of potential predators, transparency for predator avoidance is a common strategy amongst the zooplankters.

Nephrops zoeae are sufficiently distinct from other members of the zooplankton for chromatophore number and colouration to be unimportant as identification criteria. These features, however, have been of considerable importance in the identification of the larval stages of other decapods (Gurney, 1942). After metamorphosis Nephrops is still relatively transparent and the typical adult colouration does not become fully established until the 3rd postlarval stage. Similar developmental changes in colouration have been reported for H.americanus (Herrick, 1895).

The general shape of Nephrops zoeae is similar for all 3 stages. In common with the larvae of many other crustaceans (Williamson, 1982), Nephrops zoeae possess a heavily armed exoskeleton which

includes a well developed rostrum, abdominal spines and a furcate telson. The primary function of these spines and setae is probably one of armature for protection from potential predators (Williamson, 1982). However, they will also serve to increase the surface area and reduce the rate of passive sinking in the negatively bouyant Nephrops zoeae (see Chapter 5).

The possession of well developed and heavily setose exopods on the 3rd maxillipeds and the pereopods in Nephrops zoeae has also been reported for the equivalent stages of Homarus gammarus (Neil et al., 1976). Swimming is accomplished by the metachronous beating of these structures in H.gammarus zoeae (Neil et al., 1976). It appears that these appendages serve the same purpose in Nephrops zoeae (see Chapter 5). The precocious development of functional pleopods and uropods in 3rd stage zoeae provides the Nephrops with both thoracic and abdominal swimming appendages. The formation of a tail fan through the development of uropods in 3rd stage zoeae is a characteristic decapod feature (Williamson, 1982). Foxon (1934) suggested that the precocious development of this feature was due to the early need for a mechanism to enable a rapid reversal in the direction of migration. Such a mechanism has been described in 3rd stage zoeae of H.americanus (Bliss, 1982) and Neil et al. (1976) indicated that mobility was improved after development into the 3rd zoeal stage in H.gammarus.

After metamorphosis the thoracic exopodites are reduced to a residual form and there is a further increase in the size of the pleopods. Similar changes have been reported to follow development into this 'decapodid' stage (Felder et al., 1985) in H.gammarus (Neil et al., 1976) and the closely related New Zealand 'scampi'



Metanephrops challengeri (Wear, 1976). Locomotion in this and all subsequent stages is achieved either by swimming using the abdominal appendages or by walking on the substratum using the pereopods which, after development into the 2nd postlarval stage, become entirely uniramous. The change in the locomotory mechanism from the thoracic exopods to the abdominal appendages and walking legs has been widely used as a criterion for separating larval and postlarval phases in decapods (Gore, 1985).

The general developmental changes which occur in the mouthparts of early Nephrops closely resemble those which were described for the corresponding stages of H.americanus (Factor, 1978 ; Hinton and Corey, 1979). From observations of H.americanus, Factor (1978) concluded that the mandibles and the 3rd maxillipeds were the most important appendages for the mastication of food. It is not surprising therefore that the greatest developmental changes in the mouthparts were associated with these structures in both Nephrops (this study) and H.americanus (Factor, 1978). The development of these mouthpart components will enable Nephrops to cope better with the change in diet they will encounter after recruitment to the benthic environment. Recruitment occurs sometime after attainment of the 2nd postlarval stage (see Chapter 6). Factor (1981) also showed that the developmental changes in the mouthparts of the early stages of H.americanus were correlated with changes in the gastric mill. Further research may reveal that this is also true for Nephrops.

During the zoeal stages the Nephrops gills are simple lobes, similar to those previously described for the equivalent stages of H.americanus (Factor, 1978). In decapods, gills are features of adults

and they often do not appear until the end of larval life (Gurney, 1942). It appears that Nephrops zoeae possess the full complement of 20 gills which are present in the adults. In some other members of the Decapoda the series is not completed until late in the postlarval phase (Gurney, 1942).

Adult Nephrops, like most macrurans, possess trichobranchiate gills. The branches in this type of gill are filamentous but not sub-branched and there are several series along the axis (Barnes, 1974). The first evidence of development towards the trichobranch type of gill follows metamorphosis but at the 1st postlarval stage the filaments are only represented by small secondary lobes. In Nephrops the gills continue to develop during the early postlarval stages. By the 4th postlarval stage they resemble the 'bottle-brush' shape of the adult gills (light microscope observations). An increase in body size is accompanied by a decrease in the surface area to volume ratio of the organism. The evolution of well developed respiratory organs during the early postlarval stages will therefore help provide a greater surface for gas exchange and help compensate for the effect of increased size. The standard oxygen consumption per unit of body mass decreases with the increasing size of the animals (Dejours, 1981). This may explain why the gills do not appear to become further elaborated after the early postlarval stages.

It is known that adult Nephrops can tolerate and remain within the confines of their burrows under conditions of severe hypoxia (ie. less than 25% air saturation), an ability they share with many other burrowing decapods (Hagerman and Uglow, 1985). Under conditions of low oxygen concentrations these authors observed that Nephrops

increased the rate of ventilation by accelerating the scaphognathite beat rate and also raised their bodies on extended legs from the substratum surface to ventilate water of higher oxygen content. There is no equivalent information on the tolerance of the postlarval stages to hypoxic conditions. The lack of differentiation of the gills in 1st stage postlarvae may provide one possible explanation for the apparent restriction of these stages to the substratum surface (see Chapter 6). The underlying reasons which govern the time at which postlarvae construct burrows remain unknown and would provide an interesting topic for future research.

In relation to environmental adaptation, Rice (1981) considered the brachyuran megalopa to be morphologically intermediate and not well adapted for life as a plankter or a member of the benthos. Although the equivalent stage for Nephrops (1st postlarval stage) does not possess all the attributes of the adult it more closely resembles the adult form than the larval form. It therefore appears correct to consider the moult from the 3rd zoeal stage to the 'decapodid' stage as the true metamorphosis in Nephrops.

#### 4.4.2. Growth

Caution must be exercised in interpreting the results of growth studies conducted under laboratory conditions. This is especially so for this study as rigorous constant temperature conditions were not applied. The lack of such conditions is likely to have been most serious for the study of absolute growth as temperature variations may have affected not only the intermoult period but the moult increment as well.



## Absolute growth

A progressive increase was recorded in both the intermoult period and the moult increment during development in the early stages of Nephrops.

In most crustaceans the intermoult period lengthens with increased size but the relationship is not necessarily constant between the larval and postlarval phases (Hartnoll, 1982). There is some evidence from the results of this present study that a different relationship exists between the zoeal and postlarval phases for Nephrops but the difference was not statistically significant ( $P > 0.05$ ). The gradient of the slope was steeper for the zoeal phase. A steep slope to the regression line indicates that the moults become infrequent while the crustacean is still small. This will tend to limit the maximum size attainable and from the values presented by Hartnoll (1982) it is evident that steeper slopes are associated with the smaller crustaceans. Compared to the gradient of the slopes recorded for larger (0.013 for H.americanus, Templeman, 1948) and smaller (0.250 for Rithropanopeus harrisi, Hartnoll, 1978) crustaceans the slope recorded in this present study for the postlarval stages of Nephrops has an intermediate value (0.106) as might be expected.

Due to the inappropriate application of Hiatt diagrams and 'growth factors' by earlier researchers to describe moult increments (see section 4.2.2.) there are few studies which can be compared directly with the results of this investigation. Chapman (1980) has transformed the results obtained from earlier growth studies involving



captive adult Nephrops (Thomas, 1965) and presented them in the form of a plot of moult increment against premoult carapace length. It appears that the linear relationship so derived for adult male Nephrops was similar in form to that obtained in this study for the combined zoeal and postlarval stages of Nephrops.

### Relative growth

Crustaceans usually change shape as they grow and this change is described as allometric growth. Although the physiological mechanisms which control allometric growth are poorly understood it is generally accepted that within each growth phase a pair of variables are related according to the equation  $\log Y = \log a + b \log X$ , even though this is purely an empirical relationship. The instars which correspond to the same growth phase will therefore be represented by individuals in which the two variables are related by the same allometric equation. This criterion was adopted for differentiating between the zoeal and postlarval phases for Nephrops in this study. If the zoeal and postlarval stages had not been treated separately, the shape of the log-log plots would have assumed a sigmoid curve. This does not represent any actual growth phenomenon but is purely an artifact (Hartnoll, 1982).

The evidence from most studies involving crustacean growth indicates that after larval development there are generally two immature phases and one mature phase in each sex (Hartnoll, 1982). The two immature phases, which are separated by a pre-pubertal moult, were termed the 'undifferentiated' phase and the 'juvenile' phase by Hartnoll (1982). The moult between the two immature postlarval phases

is often not marked by obvious morphological changes (Hartnoll, 1982). In Nephrops, Farmer (1974d) found evidence for the initial development of external sexual characteristics at a carapace length of approximately 8mm. This therefore indicates the existence of a pre-puberty moult at this size, which corresponds to approximately the 6th postlarval stage. In this study few postlarvae beyond the 5th postlarval stage were measured so that an older 'juvenile' growth phase was not identified. All postlarvae appeared to be adequately described by a single growth phase. From the growth studies conducted by Farmer (1974e) for Nephrops in the Irish Sea, the end of postlarval growth (ie. the 'juvenile' phase) appeared to occur at a carapace length of approximately 16mm. This corresponds to the pubertal moult (as termed by Hartnoll, 1982) and separate growth phases were then described for male and female Nephrops.

Although Farmer (1974e) did not describe relative growth for Nephrops as a logarithmic regression his results indicate an almost isometric relationship between total length and carapace length for all sizes examined (4 - 55mm carapace length). A similar relationship was also obtained between these 2 variables in this study for both the zoeae and postlarvae (a marginally negative allometric relationship was identified for the postlarval stages). This provides further evidence for the existence of isometry between total length and carapace length in Nephrops of all sizes. For given values of carapace length the relationships derived for the postlarval stages from this study and Farmer (1974e) predict similar values for total length. The results of a growth study conducted by Rice (1968) indicate that carapace and abdominal growth (and hence total length) are nearly or completely isometric for the zoeal stages of many decapods.

Although the results of this study indicate that positive allometry existed between abdomen width and carapace length for both the zoeal and the postlarval phase, the relative increase in abdomen width was greater for zoeae. Farmer (1974e) reported a change in the relative growth of these 2 variables at a carapace length of about 16mm (pubertal moult) ; the relative growth of the abdomen being greater for both the male and female Nephrops. For given values of carapace length the relationships derived for the postlarval phase from both studies predict similar values for abdomen width.

It was apparent from the eye stalk measurements that not only was the relative growth of this structure faster during the zoeal phase but the absolute length was greater in 3rd zoeae than in first and second stage postlarvae. The possession of relatively long eye stalks in 2nd and 3rd stage zoeae (eyes are sessile in 1st stage zoeae) may be a structural adaptation to extend the total range of binocular vision in these pelagic individuals to longer distances (Meyer-Rochow, 1975).

It was evident from the measurements recorded for protopod length (as well as those for the exopod and endopod of the 2nd pleopods) that a significant increase ( $P < 0.05$ ) occurs in the size of the pleopods after metamorphosis. The development of the pleopods provides the postlarval and adult Nephrops with an abdominal locomotory mechanism. A significant increase was not recorded in the size of the tail fan (uropod width) after metamorphosis. This indicates that this structure is fully developed in 3rd stage zoeae and probably provides them with an effective mechanism for rapid reversal in the direction of migration. This probably functions as an escape response.



A positive allometric relationship was recorded between propodus length of the 1st pair of pereopods (ie. major chelipeds) and carapace length for the postlarval growth phase. Farmer (1974e) reported a similar relationship for Irish Sea Nephrops but recorded an increase in the growth rate in males at a carapace length of approximately 26mm. For given values of carapace length the relationship derived for the postlarval stages from both studies predict similar values for propodus length. This indicates that the estimates are probably reasonably accurate.

#### 4.4.3. Accelerated development

Regular development is defined as 'the numerically predominant type of ontogenetic growth and developmental strategy that consistently recurs in a particular decapod group' (Broad, 1957). In Nephrops early 'regular' development consists of a short prezoeal stage, 3 zoeal stages and a 'decapodid' stage (see introduction) which is the first postlarval stage. The type of accelerated development seen in this investigation in three 2nd zoeae corresponds to 'skipped staging', as termed by Gore (1985). According to Gore, 'skipped staging' is probably the most common type of accelerated development in decapod larvae but, appears most often in those groups with extended larval development. It is therefore surprising to find evidence of accelerated development in Nephrops as the larval phase in this species is relatively short. The 3 individuals which demonstrated accelerated development also appeared to possess some residual characteristics of the 3rd zoeal stage (ie. residual lateral projections on the telson). This type of development was termed 'combinatorial staging' by Gore (1985) and involves a combination of



characters from the preceding or succeeding instar. It is often seen in conjunction with 'skipped staging' (Gore, 1985).

Most investigations where 'irregular development' have been observed have been conducted under laboratory conditions. Le Roux (1970) demonstrated that Palaemonetes varians has a variable number of larval instars depending upon the rearing temperature. Under unfavourable conditions Templeman (1936b) reported that H.americanus can delay metamorphosis into the postlarval phase by producing a 4th larval instar which is intermediate between the preceding 3rd zoeal stage and the normal 'decapodid' stage (1st postlarval stage).

The identification of this phenomenon in Nephrops (this study) and H.americanus (Templeman, 1936b) depends upon individual rearing and so other workers may have overlooked it. Mass culture is the normal method employed for the culture of Nephrops (Figueiredo, 1971 ; Figueiredo and Vilela, 1972) and H.americanus (Sastry and Zeitlin-Hale, 1977 ; Aiken, 1981). Future investigations may reveal whether 'irregular development' in Nephrops is purely an artifact of artificial laboratory conditions or whether this phenomenon occurs in the field. Under laboratory conditions only a small proportion of the zoeae demonstrated accelerated development (approximately 1.5% of all individually reared Nephrops which completed metamorphosis). The temperatures to which these individuals were exposed (15 - 17°C, see Chapter 3) were considerably warmer than they would normally experience in the field (8 - 12°C, see Chapter 2). If increased temperatures promote 'skipped staging', as reported by Le Roux (1970) for P.varians, then it appears unlikely that a significant proportion

of the Nephrops zoeae will demonstrate this type of development in the field.

## CHAPTER 5

### Light and hydrostatic pressure sensitivity and a study of the developmental changes in the eye

#### 5.1. Introduction

##### 5.1.1. Behavioural responses to changes in light and hydrostatic pressure

Most marine invertebrates have a pelagic larval phase which permits dispersal of the species through utilization of the water currents. The importance and success of this strategy is evident from its convergent evolution within such a diverse group of animals. Despite the high level of mortality which is usually inflicted during this period (Thorson, 1964) the survival of the species which perpetuate their populations in this manner is testimony to its success.

After release, most larvae migrate upwards towards the sea surface under the influence of light and gravity (Sastry, 1983). Here they perceive their environment for orientation and movement. Larvae exhibit 2 types of responses, a tactic response which is an orientated response to an environmental stimulus (eg. light) and a kinetic response which is a non-directional scalar response to changes in the environmental conditions (Crisp, 1974).

Most malacostracan larvae possess well developed compound eyes

and poorly developed gravity receptors (Rice, 1964). They therefore orientate primarily to light (phototaxis) (Rice, 1964). This sensitivity to light can contribute to the occurrence of diel vertical migrations (Forward and Costlow, 1974). Such migrations have been inferred for Nephrops larvae (see Chapter 2).

The two most regular characteristics of the larva's environment from which it could achieve a 'sense of depth' are light and pressure. The purpose of this study was to investigate the effect of changes in light and hydrostatic pressure on the behavioural responses of the three pelagic larval stages and the first postlarval stage of Nephrops.

Attempts were made to determine the spectral sensitivity and light intensities (measured in radiometric units) which induced a response in the different stages. Forward and Costlow (1974) reported no change between the spectral sensitivity of the various larval stages of the crab Rithropanopeus harrisi. Conversely, Lang *et al.*, (1979) observed changes in the spectral sensitivity for the various larval stages of the barnacle Balanus improvisus. For Nephrops, the spectral sensitivity and light intensity results were compared to the lighting conditions which the Nephrops larvae are exposed to in the field (see Chapter 2). The spectral responses of the zoeal stages were compared to those recorded for the adult (Loew, 1976 ; Shelton *et al.*, in press) and the spectral transmittance qualities of the water in the Lower Firth of Clyde (see Chapter 2). The water in this region was found to most closely resemble Jerlov's coastal water type 3 (Jerlov, 1976). Light is best transmitted within the wavelength band 400 to 600nm for coastal water. Most crustaceans for which the spectral



sensitivity has been determined have a peak sensitivity near the middle of this range (White, 1924 ; Beeton, 1959 ; Goldsmith and Fernandez, 1968 ; Forward and Costlow, 1974). A peak sensitivity of approximately 500nm has been identified for adult Nephrops (Loew, 1976 ; P.M.J.Shelton, pers. comm.).

Pressure sensitivity was first shown to be a depth regulatory mechanism in decapod larvae by Hardy and Bainbridge (1951). Subsequently it was shown to be a depth regulatory mechanism in a variety of planktonic animals (Knight-Jones and Morgan, 1966 ; Knight-Jones and Qasim, 1967 ; Rice, 1964, 1967 ; Ennis, 1973a). Rice (1964) categorised the pressure response of marine animals into 3 major types depending upon their utilization of light and gravity for orientation. Type 1 describes movement in response to pressure changes which are orientated entirely with respect to gravity, pressure increase causing upward movement and pressure decrease causing downward movement. Type 2 describes movement in response to pressure changes which are orientated with respect to both gravity and light. The geotactic response prevails under conditions of vertical light from above or below but, in horizontal light there is some orientation with respect to the light direction. Increased pressure causes movement towards the light source and decreased pressure causes either decreased activity or active movement away from the light source. Type 3 describes movement in response to pressure changes which are orientated primarily to light. A pressure increase causes movement towards the light source. However, on the basis of the response to a decrease in pressure, Rice further divided this category into 3 subdivisions. Type 3a is where a pressure decrease causes decreased activity and consequent sinking with no active movement away from the light source.

Type 3b is where a pressure decrease causes active movement away from the light source. Type 3c is where a pressure decrease causes either active movement away from the light source, passive sinking or both (from Rice, 1964). His investigations for Nephrops were restricted to stage 1 zoeae, which demonstrated a type 3a response ie. increased pressure caused increased activity and movement towards the light source, decreased pressure caused decreased activity and consequent sinking. In the present study, pressure sensitivity (barokinesis) was investigated in all 3 zoeal stages and the first postlarval stage. Lincoln (1970, 1971) found that the pressure response in Daphnia magna was greater under conditions of darkness than of overhead light. He suggested that a 'pressure sense' operated when the light intensity was below a certain threshold level although, when in opposition, the light response overrode the response to hydrostatic pressure. Ennis (1973a) suggested that such a mechanism would prevent the water fleas from wandering outside their depth range during dark periods. Ennis (1973a) reported that light intensity and the absence of light had no influence on the responsiveness of Homarus gammarus larvae to pressure, except that a slower response was registered in darkness. In the present study attempts were made to determine the lowest pressure change to which the Nephrops larvae responded. Ennis (1973) reported that H.gammarus larvae responded to pressure changes which corresponded to a change in depth of approximately 0.7m.

Swimming speeds and sinking rates for both conscious and anaesthetised Nephrops larvae were investigated and compared to the migrations which appeared to occur in the field (see Chapter 2). Observations were made concerning the orientation and swimming motions. A brief description of the orientation of a Nephrops larva to

light was presented by Foxon (1934). Movement towards a light source was directed telson and abdomen first with the head and thorax in a plane at right angles to them. Neil et al., (1976) described the swimming motions of H. gammarus larvae which involved the beating of the thoracic exopodites in a metachronous wave.

#### 5.1.2. Structure of the eye

The compound eyes of insects and crustaceans were divided into 2 classes by Exner (1891). The apposition eye, in which the proximal ends of the cones are in direct contact with the distal ends of the rhabdoms and, the superposition eye, in which the cones extend into a 'clear zone' before connecting with the rhabdoms. In apposition optics the ommatidia are isolated by the high refractive index of the cones. Only the light rays entering through the corneal lens of each ommatidium are therefore available to the rhabdom. Apposition optics are therefore best suited to conditions of high illumination. They have been found in all decapod larvae (Fincham, 1980), which generally remain in the upper, well lit regions of the water column. Apposition optics are usually correlated with diurnal activity (Meyer-Rochow, 1975). From field observations it appears that most Nephrops zoeae remain in the upper regions of the water column (10 to 30m) and demonstrate limited vertical migrations (see Chapter 2).

Superposition optics have been developed for their improved light gathering capacity (Land, 1981). There are 2 types of superposition optics, one involves refraction in a lens cylinder gradient and the other involves reflection in an orthogonal system of mirrors (Nilsson, 1983). According to Nilsson (1983) the optical mechanism



involved in the apposition eyes of decapod larvae provides the animal with a pre-adapted functional basis for reflecting superposition optics. Adult Nephrops possess reflecting superposition eyes (Aréchiga and Atkinson, 1975).

The purpose of this investigation is to describe the structure of the apposition larval eye and the changes which occur in the eye during metamorphic development and transition to the superposition type. Most apposition eyes are characterized by hexagonal packing between the corneal lenses (Fincham, 1980). This gives the appearance of hexagonal facetting although closer inspection has revealed that in Palaemon serratus the lenses were in fact circular (Fincham, 1984). Reflecting superposition eyes are characterized by an orthogonal arrangement of square facets, a pre-requisite for the development of the mirror mechanism (Vogt, 1977, 1980). The 'squaring-off' of the facets in preparation for the transition from apposition to superposition optics has been described by Fincham (1984) for P.serratus. The development of superposition optics was gradual, occurring over a series of moults during the early juvenile stages. This was in contrast to the dramatic morphological changes which followed the metamorphic moult (Fincham, 1984).

It is known that 8 retinula cells contribute to each ommatidium in the eyes of the Decapoda (de Bruin and Crisp, 1957). The 8 proximal retinulae are arranged radially around the rhabdom and penetrate the basement membrane of the eye. In decapods one of the 8 retinula cells is placed eccentrically (8th cell). Recent investigations have been undertaken to determine the nature and function of this '8th' retinula cell (Meyer-Rochow, 1975 ; Cummins and Goldsmith, 1981 ; Shaw and



Stowe, 1982). The 8th retinula cell has recently been identified in the superposition eyes of adult Nephrops (P.M.J.Shelton, pers. comm.). In this study attempts are made to locate and describe the 8th retinula cell for the larval eye.

## 5.2. Materials and methods

All larvae and juveniles were supplied from the culture facility having been maintained under lighting conditions which were considered to be of a similar spectral composition and intensity to those experienced in the field (see Chapter 2).

### 5.2.1. Behavioural responses to light conditions and hydrostatic pressure changes

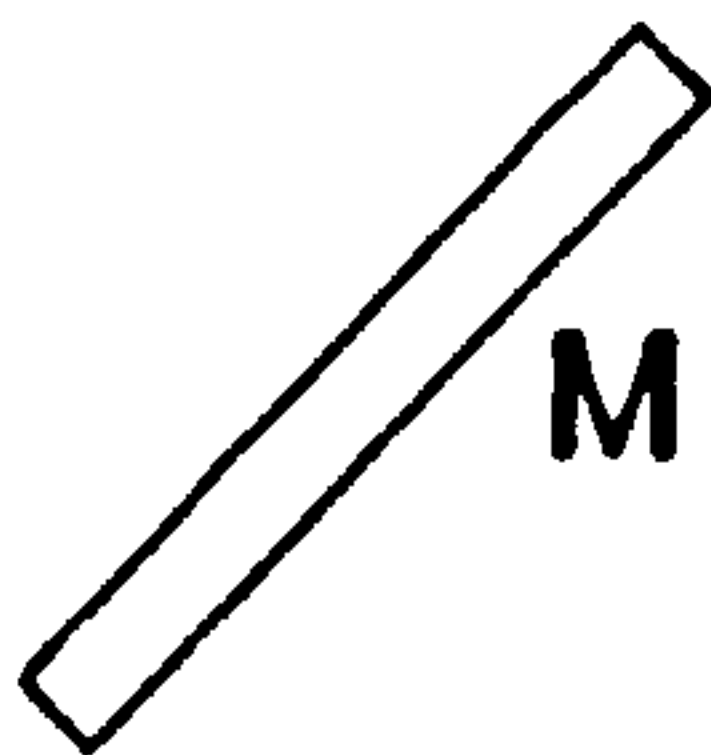
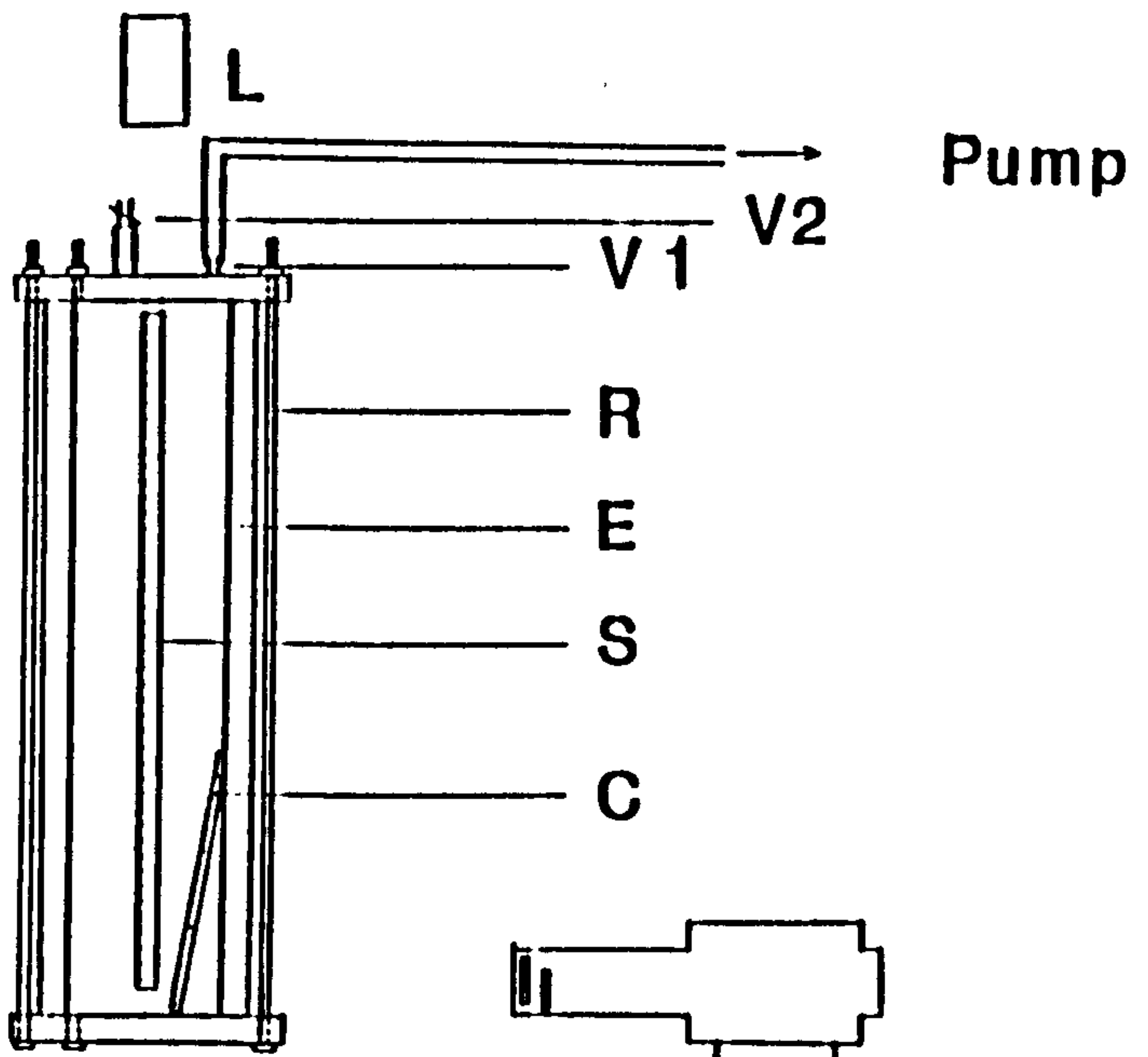
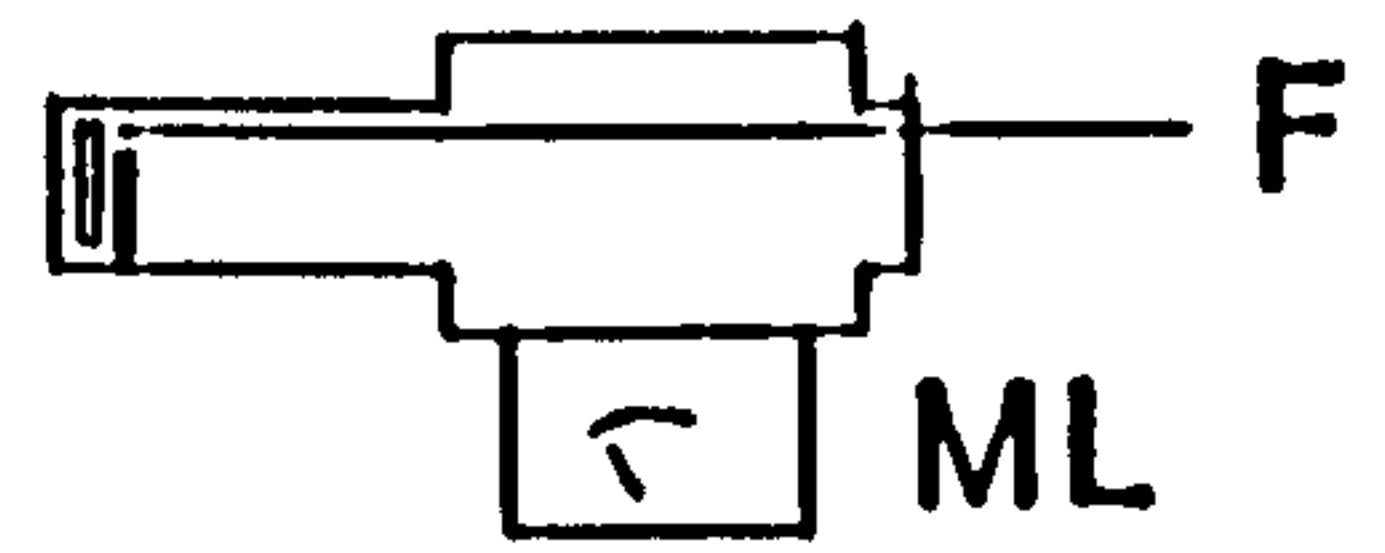
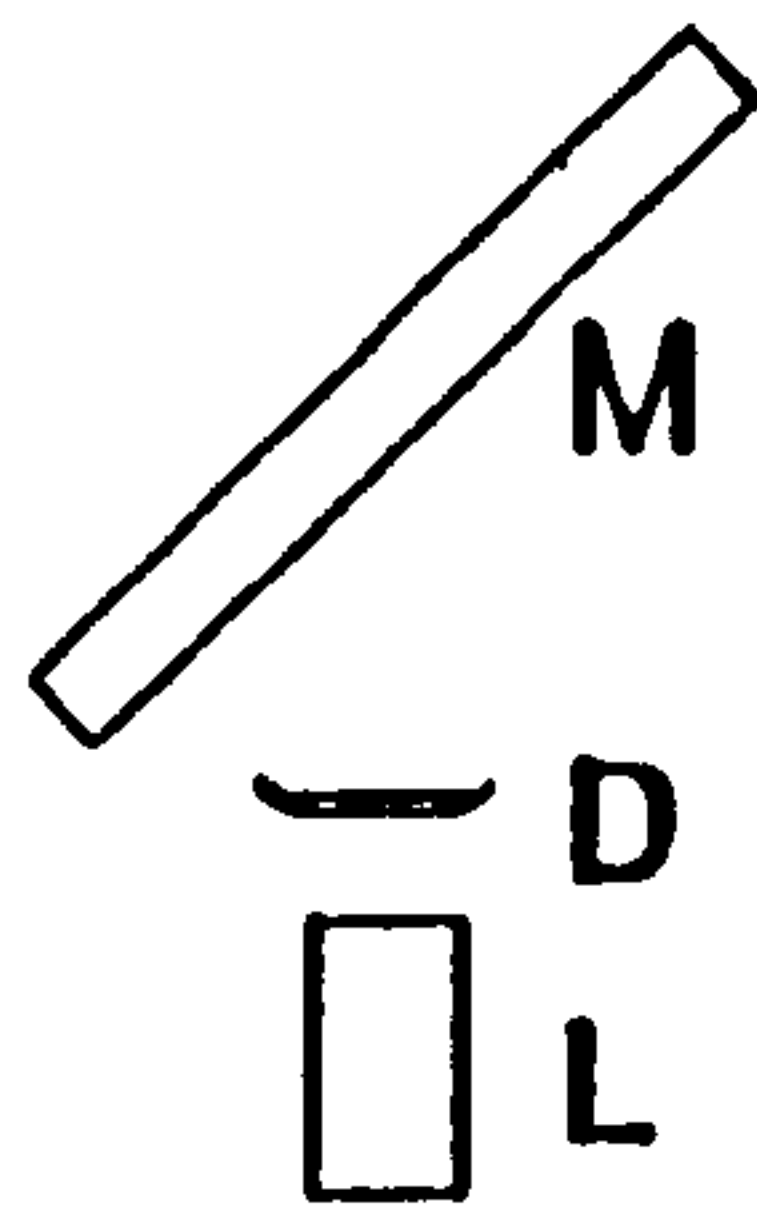
The experimental column (E) was a length of acrylic piping ; 330mm long, 78mm internal diameter and with walls 12mm thick (Figure 5.1). The base and cover of the column were constructed from a 12mm thick acrylic sheet. The base plate was permanently attached to the column and the cover was secured by three steel rods (R). The column was pressure sealed by an O-ring between the cover and cylinder surfaces. Two further holes were drilled through the cover. One was fitted with a one-way valve (V1) which was connected to a foot pump, the other was fitted with pressure tubing and screw clamps and served as a safety valve (V2) so that pressure could be applied and released instantaneously. Pressure changes in the column were monitored by means of a capillary tube (C), sealed at one end and previously calibrated by manometry in units of 0.1 Atmospheres (ats).

The column was graduated in cms (S) and filled to a depth of 290mm with filtered seawater at the same temperature (13 - 15°C) and salinity (32 - 34‰) as the culture facility where the test animals were reared and maintained.

**Figure 5.1**

**Apparatus in which light and pressure changes were induced.**

**Abbreviations explained in the text.**





Using a series of lenses (L) and mirrors (M), a parallel pencil beam of white light was produced from rheostat - controlled microscope lamps (ML) (Thorn B6F, 6V, 48W tungsten) and directed onto the column from above, below or horizontally from the side. The beam passed through a Wratten 64 filter (F) (Kodak) and its intensity was measured at each rheostat setting by a photomultiplier which had been previously calibrated in radiometric units with respect to this filter. This filter was known to approximate to the spectral response curve for adult Nephrops (Loew, 1976) (Figure 5.2) and was used previously in conjunction with the photomultiplier to measure light intensity at depth in the field (see Chapter 2). A small crystallizing dish filled with water was placed between the column and the light source to act as a heat filter when light was directed from above or below. The full complement of accessories used to produce the beam have only been shown for light directed from above (Figure 5.1).

Before each trial the test animal(s) was introduced to the experimental column under conditions of darkness for a 5min accommodation period. Its position was then recorded by short term exposure to light (4 to 5sec) which had been passed through a ruby filter (the characteristics of which are illustrated in Figure 5.2). Light in this region of the spectrum ( $> 620\text{nm}$ ) is known to be outside the range of perception of adult Nephrops (Loew, 1976) and is also considered to be outside the range of perception of decapod larvae (Thorson, 1964). During the accommodation period most zoeae remained at the bottom of the experimental column. When trials were conducted involving light exposure from below the photopositive individuals were initially attracted to the upper regions of the column using an overhead light source. This initial exposure was usually of 10 - 15s

duration.

Experiments were conducted on individual animals and small groups of six zoeae and four postlarvae. After each trial 1st and 2nd stage zoeae were discarded but 3rd stage zoeae and 1st stage postlarvae were reused from a pool of 36 and 24 animals respectively.

#### 5.2.1.1. Light responses

3 sets of experiments were conducted : the effects of the (a) direction, (b) intensity and (c) wavelength of light were investigated.

(a) The filtered green light at an intensity of  $4 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$  was directed from above, below and horizontally. This corresponds to the light intensity recorded in the field at a depth of approximately 35m under conditions of bright surface illumination ( $2 \times 10^3 \mu\text{Es}^{-1}\text{m}^{-2}$ ).

(b) The responses of the above stages to overhead green light of different intensities. The different intensities were achieved by varying the setting of the rheostat for the light source (microscope lamp). Because the light was filtered the variation in the colour temperature effects caused by the different rheostat settings were considered to be negligible. Stage 1 zoeae were also exposed to conditions of direct sunlight to determine whether positive phototaxis persisted at this higher level of light intensity.

(c) The spectral responses of the three zoeal stages were investigated by passing the light beam through Chance/Pilkington

filters for which the absolute transmission curves were known (Figure 5.2). Throughout this experiment the rheostat was set at the position which provided a light beam of intensity  $4 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$  when filtered through a Wratten 64 filter. After conducting a series of trials using this filter it was removed and replaced by different Chance/Pilkington colour filters. After each series of trials the light attenuation caused by each filter over the wavelength band 400 - 700nm was measured using a Licor quantum light meter (LI - 185B).

A photopositive response was considered to have been demonstrated when the test animal migrated towards the light source within the beam. Conversely, a photonegative response was considered to have been demonstrated when the test animal actively moved away from the light beam.

Throughout these light experiments the cover of the experimental column was removed and thus atmospheric pressure was ambient (1.0 ats).

All experiments with light of different intensities and spectral composition were carried out on two groups of animals. Those involving light from different directions were carried out on groups and individual animals.

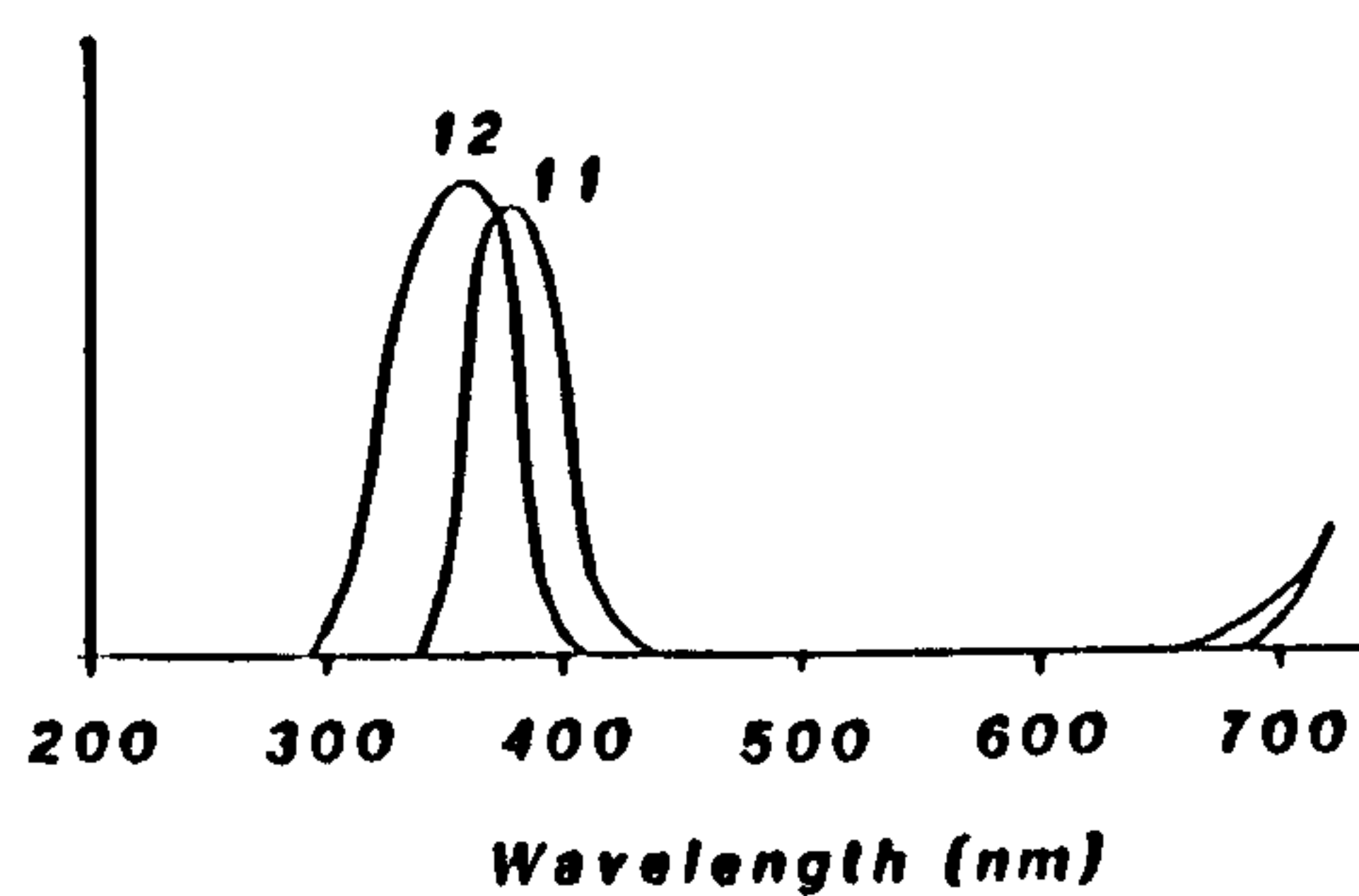
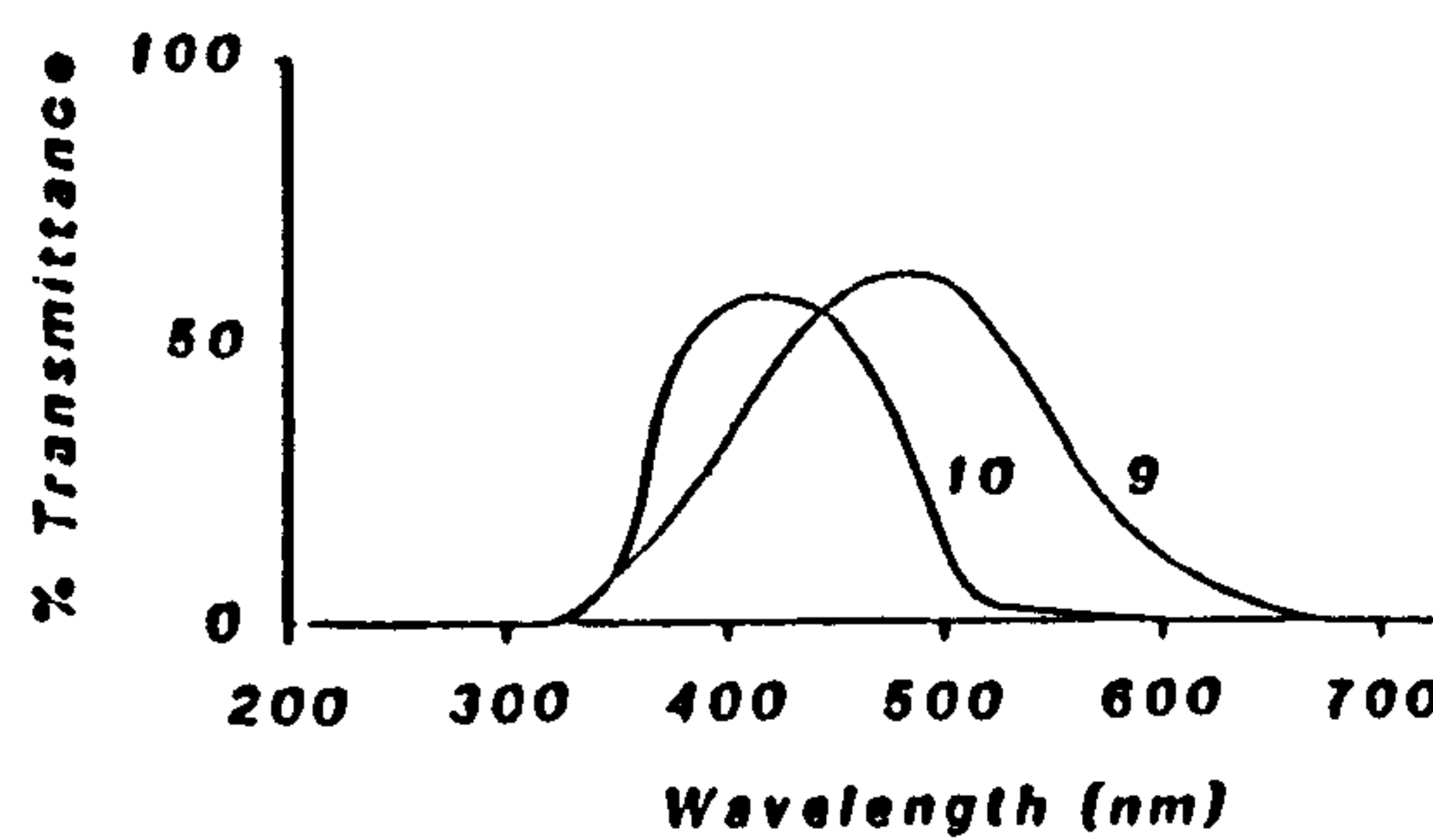
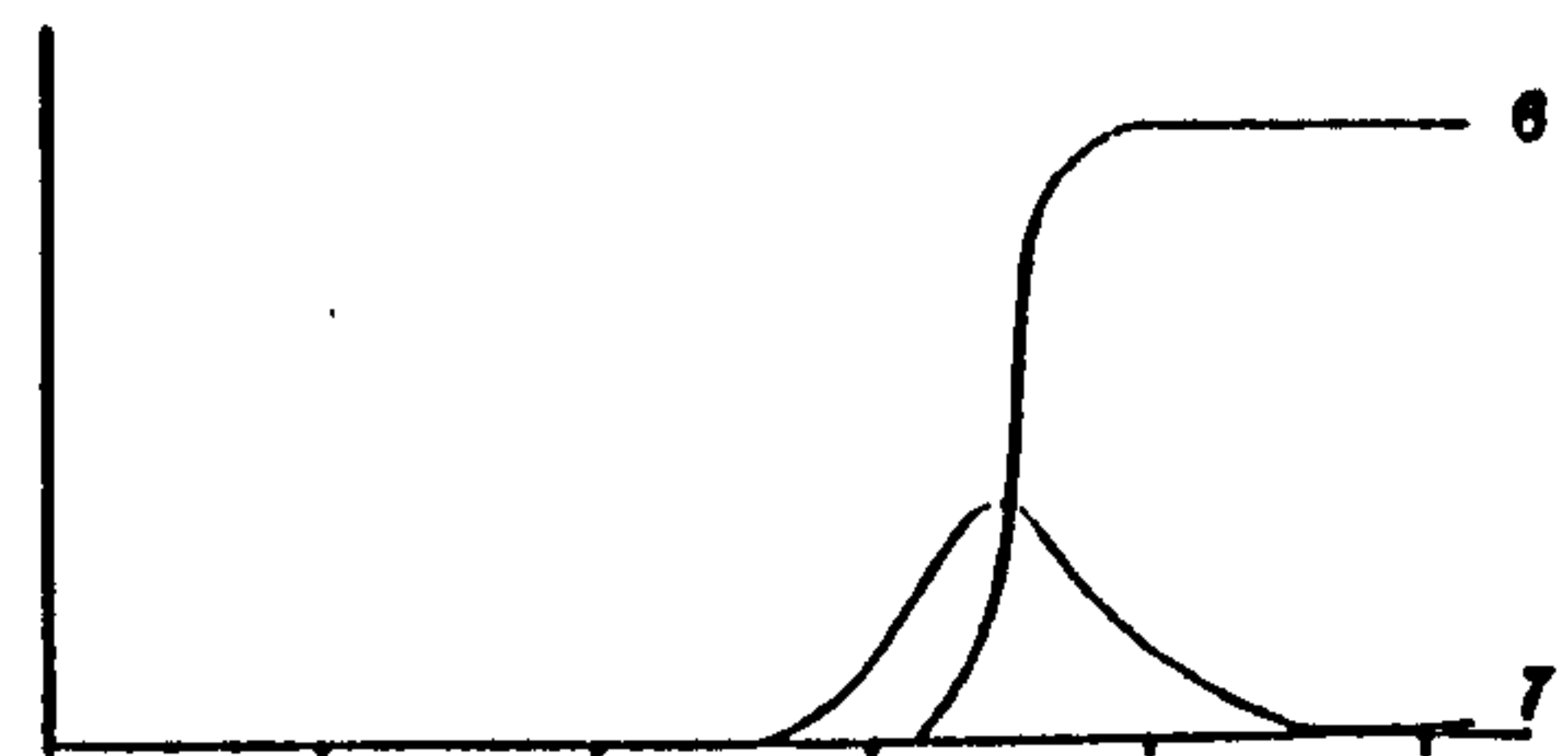
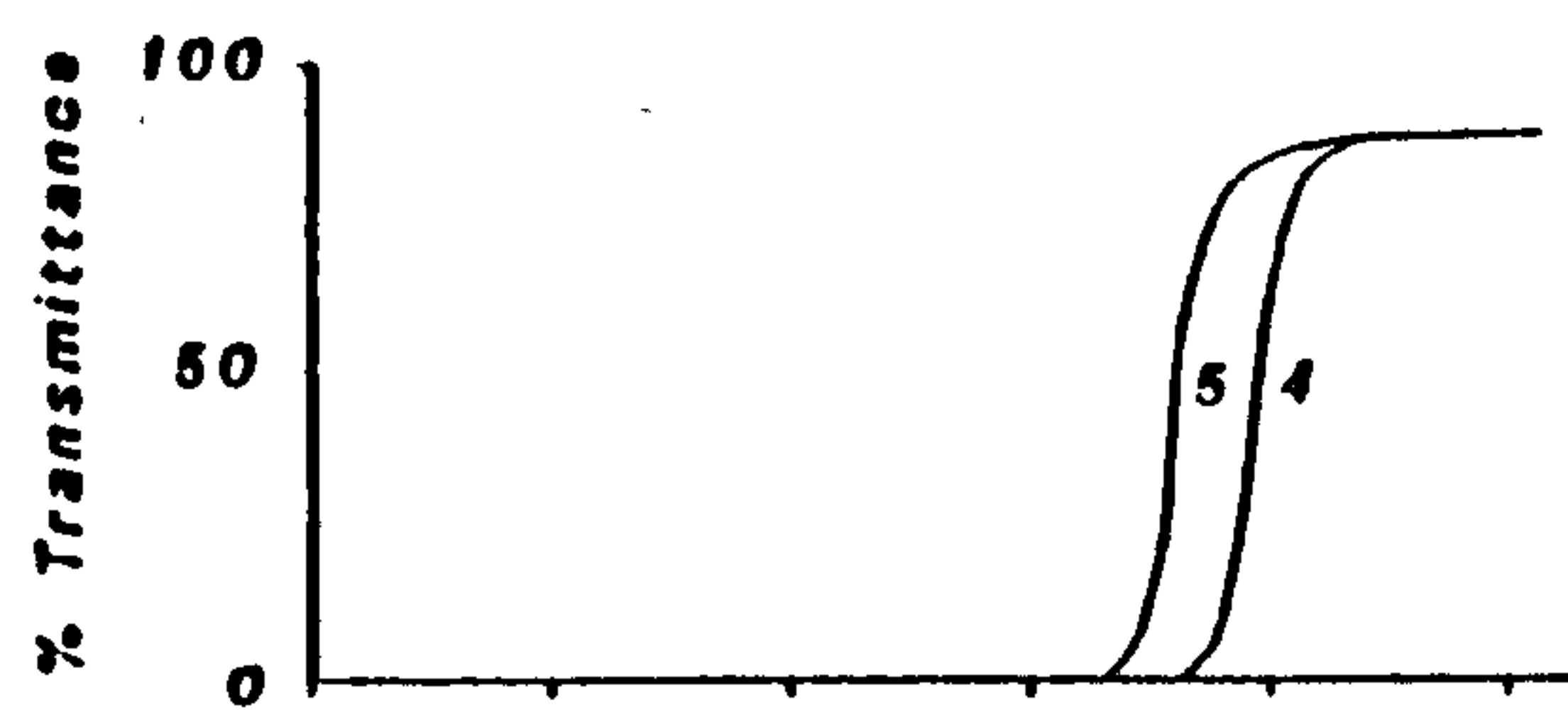
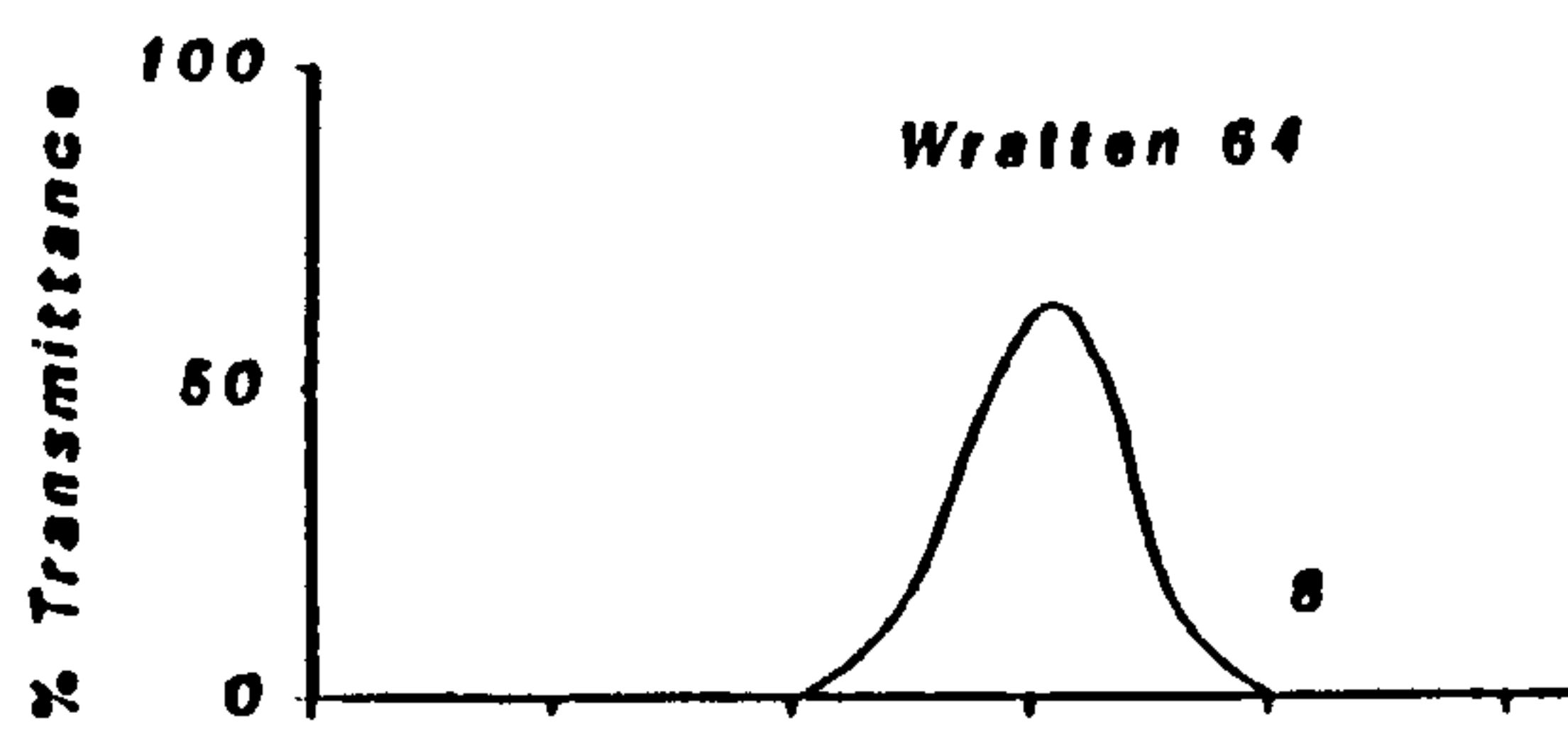
#### 5.2.1.2. Light and hydrostatic pressure

Having established the responses of the three zoeal stages and the first postlarval stage to light, pressure changes (approx. 2.0 ats) were then applied using individual animals. Trials were also

**Figure 5.2**

**Absolute transmission curves for the Wratten 64 and Chance/Pilkington filters. The numbers refer to the filters shown in Table 5.3.**





conducted to determine the minimum change in pressure which provoked a response. The pressure changes in both series of experiments involved an increase from or a decrease to ambient pressure (1 ats). All light supplied was filtered through a Wratten 64 filter and standardized at an intensity of  $4.0 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ . The test animals were preadapted to the conditions within the experimental column for 5 min before the pressure changes (approx. 2ats) were applied.

#### 5.2.1.3. Locomotion

During the response of the test animals to the various stimuli, their orientation and swimming motions were observed and recorded. The maximum swimming speeds attained by the different stages were also recorded as were the maximum rates of active descent. Individuals of each stage were anaesthetised using a 1% solution of propylene phenoxetol to determine the rate of passive descent for the three zoeal stages and the first postlarval stage. All speeds were recorded on a stopwatch with reference to a scale drawn down the outside of the experimental column.

#### 5.2.2. Structure of the eye

The methods employed to investigate the developmental changes which occurred in the structure of the eye over the 3 zoeal and early postlarval stages included high resolution photography, scanning electron microscopy (S.E.M.) and transmission electron microscopy (T.E.M.). All animals were provided from the culture facility.

##### 5.2.2.1. Light microscope photography

High resolution scientific photographs were taken of the general

morphology of the eye from live specimens using a Nikon SMZ 10 stereomicroscope.

#### 5.2.2.2. Electron microscopy

Lobsters were removed from the culture facility directly into a buffered aldehyde prefixative. The prefixative, which was made up within 5min of use, contained an addition of 0.05% osmium tetroxide. This extra procedure accelerates initial fixation (Eisenman and Alfert, 1982). Immediately after immersion within the prefixative the eyes were dissected from the animal and, in some cases, the eye stalks cut open, this promoted diffusion and penetration of the fixative into the tissue. After 5 - 10min the eyes were removed to the main buffered aldehyde fixative (Karnovsky, 1965), which had given good results in previous studies for the eyes of adult Nephrops (Shelton et al., 1985). After 2 - 3h the material was transferred to 1% osmium tetroxide in 0.2M sodium cacodylate buffer for 3h. It was then carefully rinsed in 0.1M sodium cacodylate buffer and, either used within 12h for further T.E.M. preparation or stored in the 0.1M sodium cacodylate buffer for future S.E.M. preparation at the Department of Zoology, Glasgow University.

#### S.E.M.

At Glasgow University, the material was rinsed in distilled buffer and then dehydrated through an ethanol series : 30%, 50%, 70%, 90%, 2 changes of absolute alcohol and 1 of dried absolute alcohol (15min in each). The material was then critical point dried, mounted on aluminium stubs using conductive silver paint, sputter coated with

gold (20 - 50nm) and scanned on a Philips 500 instrument.

#### T.E.M.

Material was transferred from the 0.1M cacodylate buffer into an aqueous 0.5% uranyl acetate solution for 1h, rinsed in distilled water and then dehydrated through a series of acetone : 30%, 50%, 70%, 90%, 3 changes in absolute acetone and a final change in dried absolute acetone (15min in each). Spurr's embedding resin (Spurr, 1969), which had been previously prepared from a 'one shot' kit (Polaron Equipment Ltd.), was subsequently added to the material while in the final acetone change making a 50% resin / acetone mix. The preparation was then transferred to a shaker (Luckham 805) for 8h at room temperature to promote initial embedding. The material was subsequently transferred to full resin and left overnight at room temperature to complete impregnation. The resin was then replaced with fresh resin which was poured with the material into shallow aluminium caps and removed to an oven for polymerisation at 70°C for 16h. The blocks were later trimmed and sections cut on an ultramicrotome. The sections, which were necessarily thick due to the nature of the material (1000 - 2000Å), were picked up on copper grids and viewed on an AEI 801 instrument at Glasgow University.



## 5.3. Results

### 5.3.1. Behavioural responses to light conditions and hydrostatic pressure changes

#### 5.3.1.1. Light responses

The responses of the three zoeal stages and the first postlarval stage to light from different directions are presented in Table 5.1. Zoeae of the first 2 stages were photopositive, migrating upwards, downwards and horizontally in response to light. 3rd stage zoeae demonstrated both photopositive and photonegative behaviour when provided with light from the 3 different directions. Individuals, however, were consistent in their responses. Intermoult information was available for those 3rd stage zoeae which were individually reared and it appeared that the photonegative response was more prevalent amongst the older larvae. The first postlarval stage juveniles were photonegative.

The individuals of the 3rd zoeal and 1st postlarval stages which demonstrated a photonegative response remained at the base of the experimental column moving along the bottom to avoid the pencil beam of light. The ability of these individuals to respond photonegatively appeared to be limited to the lower light intensities. Movement seemed to be inhibited at light intensities above  $2.0\mu\text{Es}^{-1}\text{m}^{-2}$  : animals remained immobile at the bottom of the column irrespective of beam direction with no attempt to avoid the beam. This may be indicative of stress.

Table 5.1

The proportion of lobsters within each stage which demonstrated positive phototaxis when the light source was provided from different directions and under conditions of darkness.

	Zoea 1		Zoea 2		Zoea 3		Postlarva 1	
Light direction	N	Propn.	N	Propn.	N	Propn.	N.	Propn.
Above	16	0.94	15	0.93	12	0.42	10	0.00
Horizontal	17	1.00	15	1.00	12	0.50	10	0.00
Below	16	1.00	13	1.00	12	0.58	10	0.00
Darkness	15	0.00	13	0.00	12	0.00	10	0.00

The results of the light intensity trials are shown in Table 5.2 and Figure 5.3. 1st and 2nd stage zoeae demonstrated a similar level of response at the different intensities and appeared to become photopositive at intensities above  $1.0 \times 10^{-2} \mu\text{Es}^{-1}\text{m}^{-2}$ . The level of response increased with increasing intensity (a 50% response was recorded at approximately  $1.0 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ ) up to an energy level of  $3.0 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ . This maximum level of response was maintained at greater energy levels, the maximum intensity to which the zoeae were exposed under artificial illumination overhead was  $2.0 \mu\text{Es}^{-1}\text{m}^{-2}$ . In natural light and ambient surface pressure (1.0 ats) stage 1 zoeae responded photopositively to sunlight from a horizontal direction and intensity of approximately  $1.0 \times 10^3 \mu\text{Es}^{-1}\text{m}^{-2}$ . No further trials were conducted using natural illumination. As expected from the results of the light direction experiment, the level of response demonstrated by 3rd stage zoeae was much less than that recorded for zoeae of the two preceding stages. When photopositive behaviour was demonstrated, however, all three zoeal stages responded similarly to the different levels of intensity employed.

Throughout the range of light intensities the highest proportion of 3rd stage zoeae which responded photopositively was less than 46% (Figure 5.3). The remaining zoeae of this stage and all juveniles of the first postlarval stage remained at the bottom of the column. Any of these individuals which became exposed to the pencil beam moved along the bottom to avoid the beam, suggesting negative phototaxis. The ability of these stages to respond appeared to be limited to intensities below  $2.0 \mu\text{Es}^{-1}\text{m}^{-2}$ , which is consistent with the results obtained during the light direction trials.

Table 5.2

The phototactic responses demonstrated by 1st, 2nd and 3rd stage zoeae when exposed to light of different intensities after a 5 min accommodation period in darkness. All post-larvae demonstrated negative photoaxis.

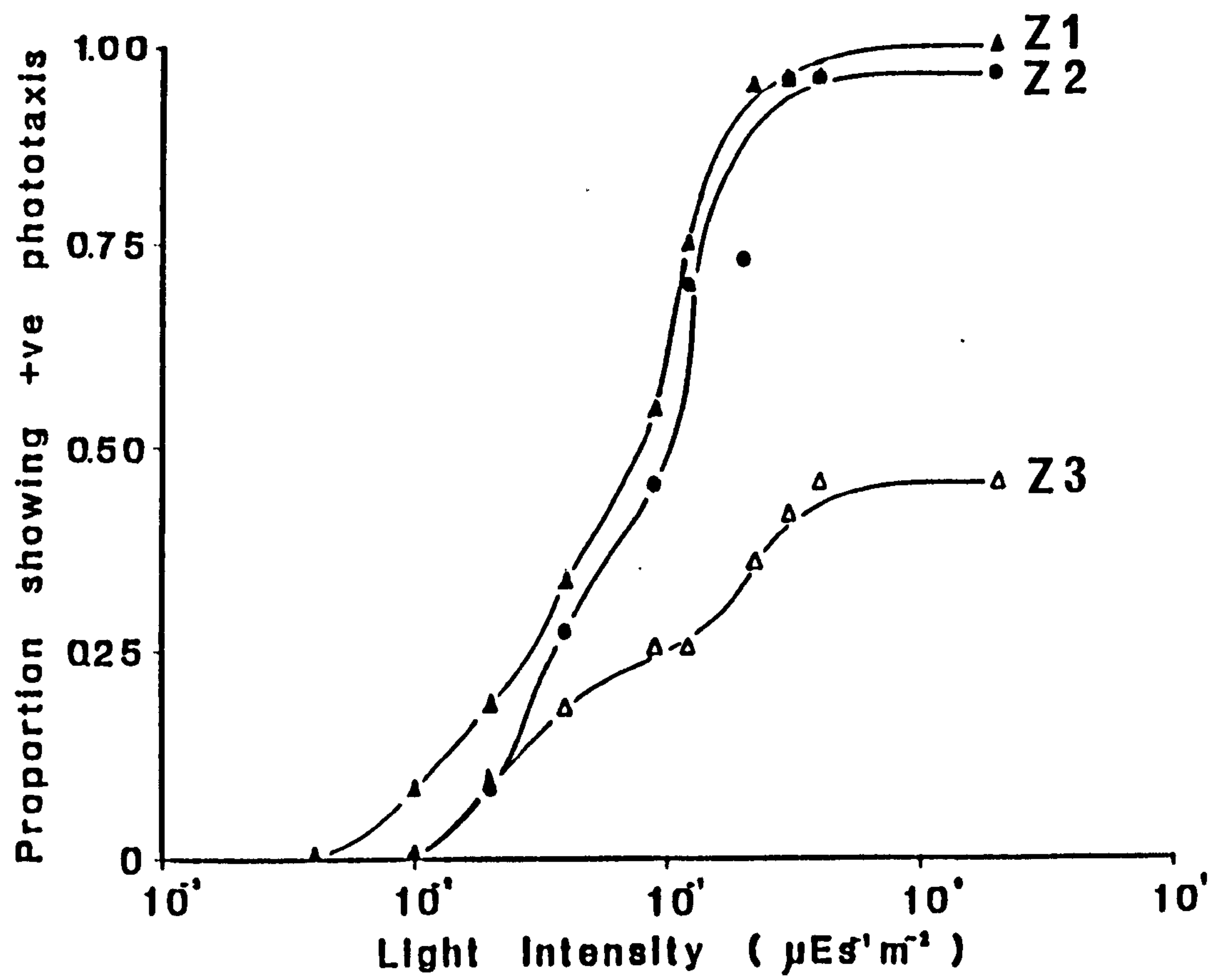
Zoea 1			Zoea 2		Zoea 3	
Light intensity ( $\mu\text{Es}^{-1}\text{m}^{-2}$ )	No. at bottom in darkness	No. migrate upwards on light exp.	No. at bottom in darkness	No. migrate upwards on light exp.	No. at bottom in darkness	No. migrate upwards on light exp.
$4.0 \times 10^{-3}$	10	0	11	0	12	0
$1.0 \times 10^{-2}$	12	1	11	0	11	0
$2.0 \times 10^{-2}$	11	2	12	1	11	1
$4.0 \times 10^{-2}$	12	4	11	3	11	2
$9.0 \times 10^{-2}$	11	6	11	5	12	3
$1.2 \times 10^{-1}$	12	9	10	7	12	3
$2.2 \times 10^{-1}$	10	9	11	8	11	4
$3.0 \times 10^{-1}$	11	10	11	10	12	5
$4.0 \times 10^{-1}$	12	11	12	11	11	5
$2.0 \times 10^0$	10	10	12	11	11	5



Figure 5.3

The proportion of zoeae in each stage which demonstrated a photopositive response when exposed to light of different intensities after a 5min accommodation period in darkness. The curves were constructed from the results shown in Table 5.2.

Z = Zoea.



The responses of the three zoeal stages to coloured light are presented in Table 5.3 and Figure 5.4. As expected from the results of the light direction and intensity trials the level of response demonstrated by the 3rd stage zoeae was less than in the two preceding stages. The spectral sensitivity appeared to be similar for all zoeae which demonstrated a photopositive response. The larvae appeared to be most sensitive to light from the blue and green regions of the spectrum (350 - 570nm). There also appeared to be a reduced response to light of shorter (violet and ultraviolet) and longer (orange and red) wavelengths. Ruby light (> 620nm) was the only colour which these stages appeared incapable of perceiving.

#### 5.3.1.2. Hydrostatic pressure and light

The responses of 1st, 2nd and 3rd stage zoeae and 1st stage postlarvae to changes in hydrostatic pressure (approx. 2 ats) under different light conditions (intensity  $4 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ ) are shown in Table 5.4. 3rd stage zoeae demonstrated two different behavioural responses under each treatment condition, which is consistent with the behaviour demonstrated by 3rd zoeae in the earlier study. One was a photopositive response and was similar to that demonstrated by 1st and 2nd stage zoeae and the other was a photonegative response and similar to that demonstrated by 1st stage postlarvae.

##### Photopositive individuals

An increase in hydrostatic pressure caused increased activity, enhancing migration in the direction of the light stimulus. Under conditions of darkness increased activity seemed to result in random

Table 5.3

The phototactic reponse of 1st, 2nd and 3rd stage zoeae to light from different regions of the spectrum. N is the number of individuals at the bottom of the column at the end of the 5 min accommodation period (darkness) immediately before light exposure. Individuals of the first postlarval stage were previously shown to be photonegative and were therefore not used.

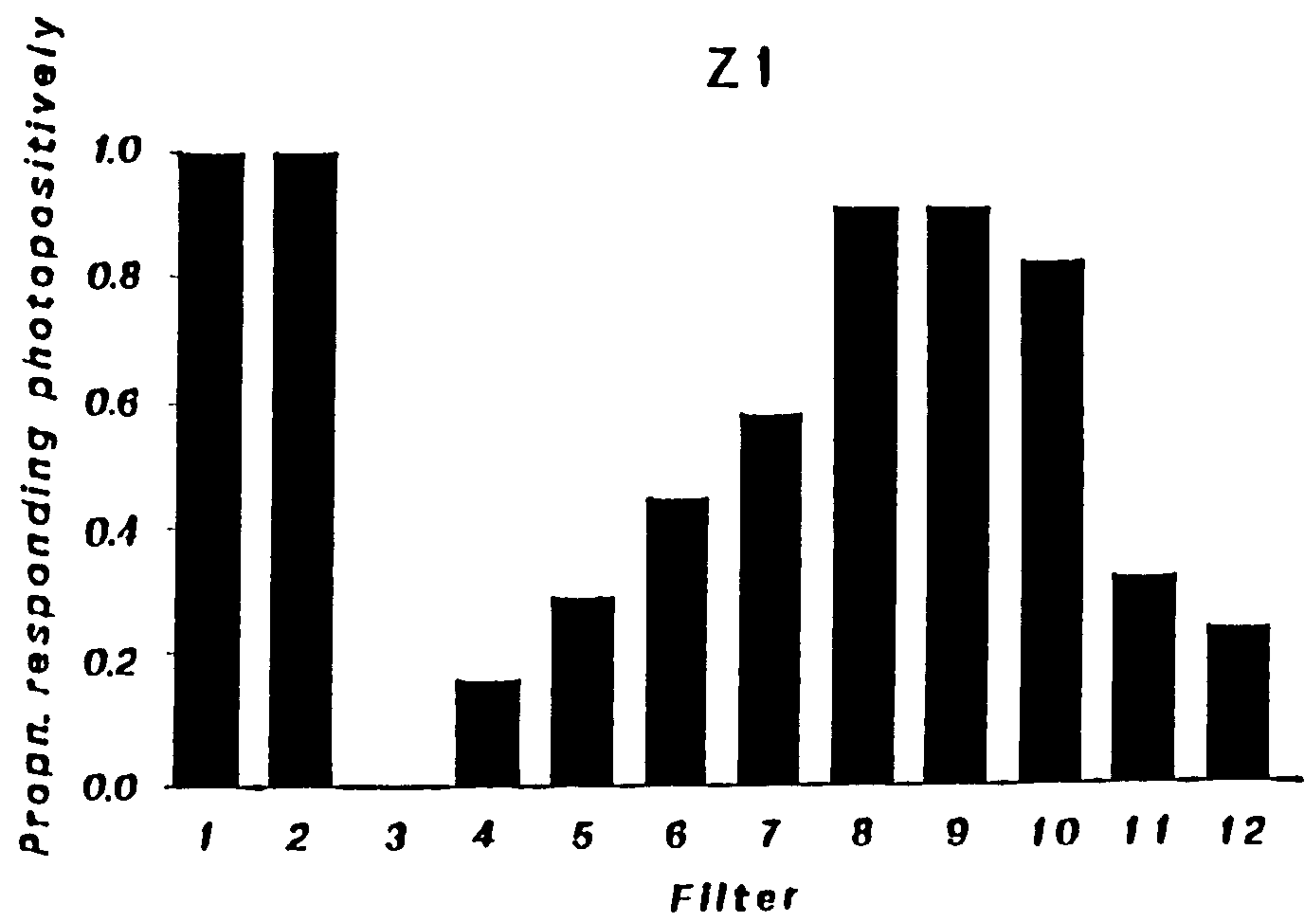
		Zoea 1			Zoea 2		Zoea 3	
Filter	Code	% Attenuation of filter	N	Propn. photo +ve	N	Propn. photo +ve	N	Propn. photo +ve
No filter	1	0	12	1.00	11	1.00	12	0.42
Day light	2	92.5	11	1.00	12	0.92	12	0.33
Ruby	3	66.0	11	0.00	11	0.00	12	0.00
Red	4	33.5	12	0.17	12	0.33	11	0.09
Deep orange	5	25.4	10	0.30	11	0.36	12	0.17
Light orange	6	23.3	11	0.45	12	0.42	12	0.17
Green	7	93.1	12	0.58	11	0.73	11	0.27
Blue- green (Wratten 64)	8	94.8	11	0.91	11	1.00	12	0.42
Blue- green	9	95.5	12	0.91	12	0.83	12	0.42
Blue	10	98.6	11	0.82	12	0.75	11	0.36
Purple	11	98.5	12	0.33	11	0.36	11	0.18
Ultra- violet	12	90.6	12	0.25	11	0.18	12	0.008



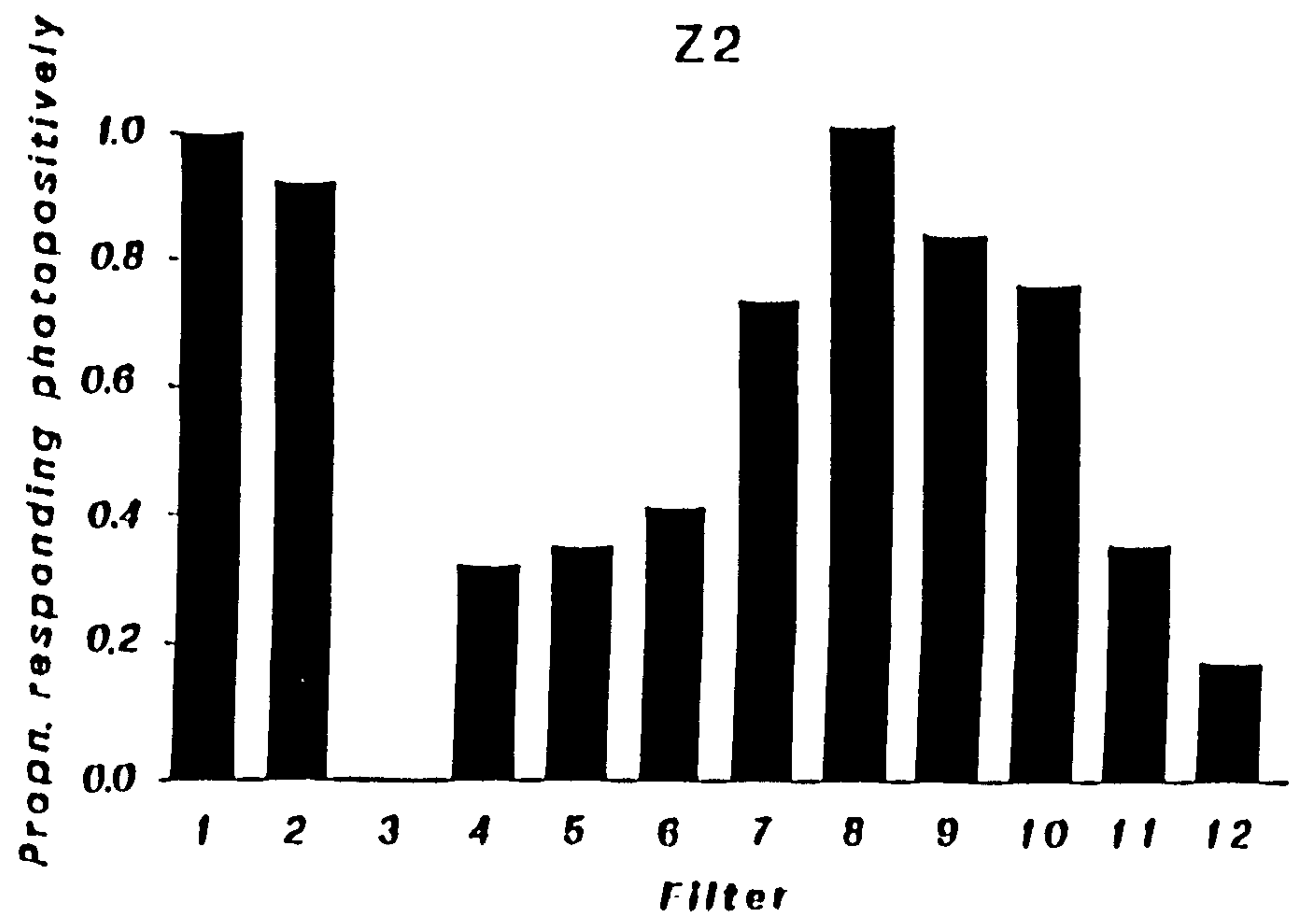
#### Figure 5.4

The proportion of zoeae in each stage which demonstrated a photopositive response when exposed to light which had been passed through a range of different coloured filters, thus altering the spectral composition of the light beam. The numbers refer to the filters shown in Table 5.3 and the transmission curves for each filter are illustrated in Figure 5.2.

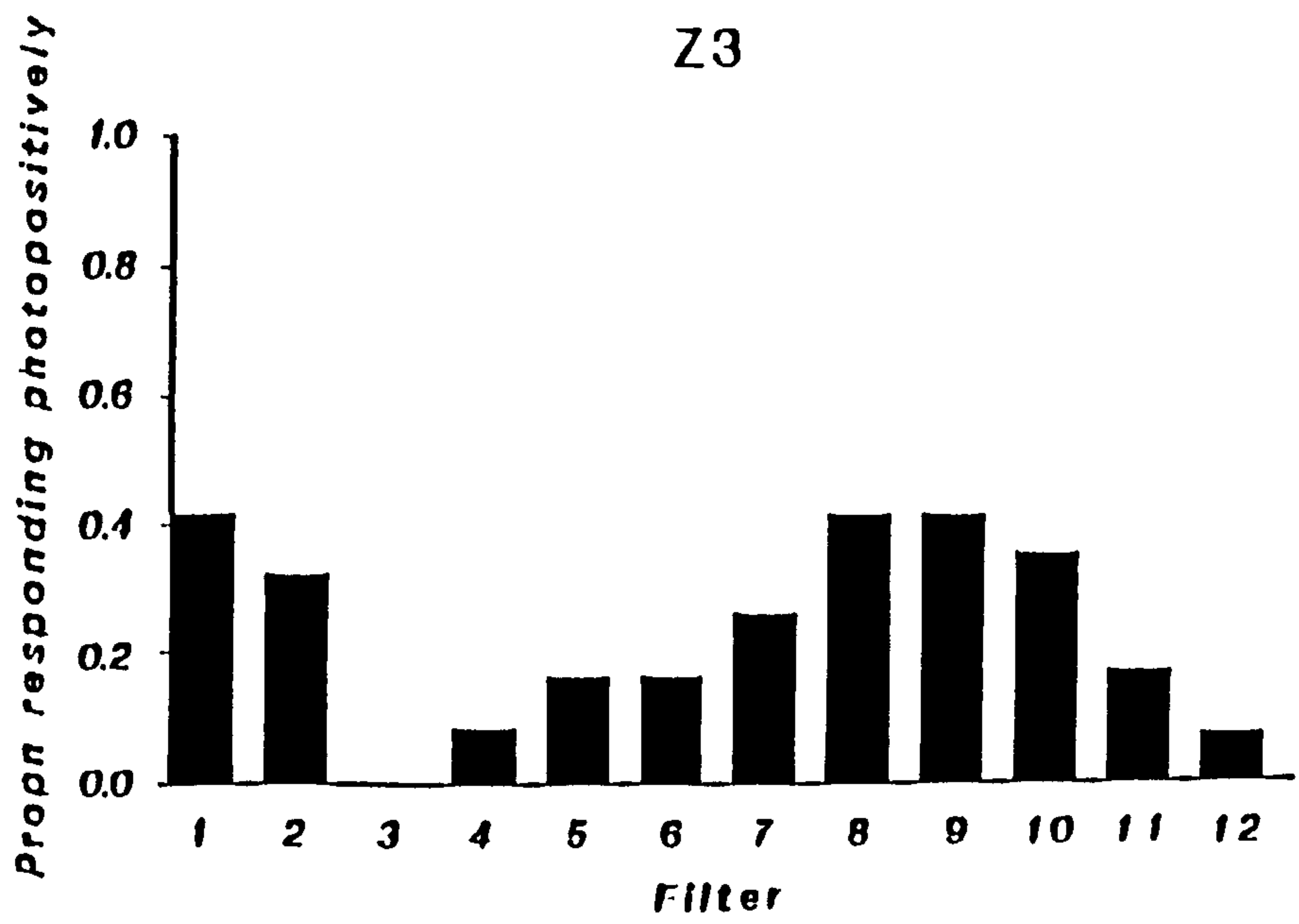
Z = Zoea.



**a**



**b**



**c**

Table 5.4

The responses of 1st, 2nd and 3rd stage zoeae and 1st stage postlarvae to changes in hydrostatic pressure (approx. 2 ats) under different light conditions (intensity  $4.0 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ ). The proportion of larvae reacting to an experimental change is expressed as a ratio. Arrows indicate the direction of movement.

		Zoea 1		Zoea 2		Zoea 3		Postlarva 1	
Light direction	Change in hydrostatic pressure	N	Propn.	N	Propn.	N	Propn.	N	Propn.
ABOVE:									
	Increase	10	↑ 1.00	10	↑ 1.00	8	↑ 0.50	8	0.00
	Decrease	10	↓ 0.80	10	↓ 0.70	9	↓ 0.22	8	0.00
HORIZONTAL:									
	Increase	8	↔ 1.00	11	↔ 1.00	9	↔ 0.44	8	0.00
	Decrease	10	↔ 0.30	9	↔ 0.33	9	↔ 0.22	8	0.00
BELOW:									
	Increase	12	↓ 1.00	8	↓ 1.00	10	↓ 0.40	9	0.00
	Decrease	10	↓ 1.00	8	↓ 1.00	9	↓ 0.44	8	0.00
NONE: (Darkness)									
	Increase	8	↑ 0.38	9	↑ 0.33	8	↑ 0.25	8	0.00
	Decrease	9	↓ 0.44	9	↓ 0.33	8	↓ 0.25	8	0.00

movement and although some vertical migration did occur this became much more evident when an overhead light source was introduced.

A decrease in pressure resulted in decreased activity causing sinking. Under conditions of overhead light combined with decreased pressure the zoeae moved out from the light beam and descended before moving back into the light beam and ascending photopositively. The period of descent was variable and depended upon movement back into the light source. This either occurred during the descent or once the zoeae had reached the bottom of the column. When light was directed from below the decreased activity which resulted from the decrease in pressure enhanced the photopositive response, promoting downward migration in the direction of the light source. Under conditions of darkness a decrease in pressure caused a decrease in activity and passive sinking in those individuals which were not already at the bottom of the column. The minimum change in pressure to which these photopositive zoeae responded was between 0.3 - 0.5 ats which corresponds to a change in depth of 3 - 5m.

#### Photonegative individuals

Pressure changes appeared to have little influence on the light responses of these individuals. They remained at the base of the column throughout all the experimental conditions making short migrations along the bottom to avoid the light beam.

#### 5.3.1.3. Locomotion

When responding to an overhead or horizontal light source the



photopositive zoeae orientated with their telson and abdomen directed towards the light source, with the head and thorax in a plane at right angles to them (Figure 5.5a). In this orientation swimming appeared to be achieved by the downward beating of the heavily setose exopods of the pereopods (see Plate 4.5). Zoeae responded to a change in the direction of the light stimulus within 2 - 5 sec, changing their direction of migration from upwards to downwards as the direction of the source was changed from above to below. The initial descent often involved a period of tumbling, this developed into a spiralling motion which persisted throughout the remainder of the descent. During the spiralling descent the zoeae retained the same <sup>orientation</sup>  $S/V_{\text{F}}$  as demonstrated during the ascent. Orientation was different, however, zoeae descended head first with their eyes and anterior thoracic region directed towards the light source, the plane of the thorax making an angle of approximately  $45^\circ$  with the horizontal (Figure 5.5b).

The maximum speeds of ascent and descent achieved by the 3 zoeal stages are shown in Table 5.5. These results suggest that there is a slight increase in the swimming speeds and sinking rates with larval development, average speeds increasing between the 1st and 3rd zoeal stages from  $21.2$  to  $30.0\text{mms}^{-1}$  ( $n = 21$ ) and  $22.0$  to  $30.4\text{mms}^{-1}$  ( $n = 21$ ) for the upward and downward migrations respectively. All individuals of the first postlarval stage remained at the base of the column during these trials, although vertical migrations were observed during substratum selection studies (see Chapter 6).

The mean rates of passive descent for anaesthetised individuals of the 3 zoeal stages and the 1st postlarval stage are presented in Table 5.5. There appeared to be a slight increase in the rate of

### Figure 5.5

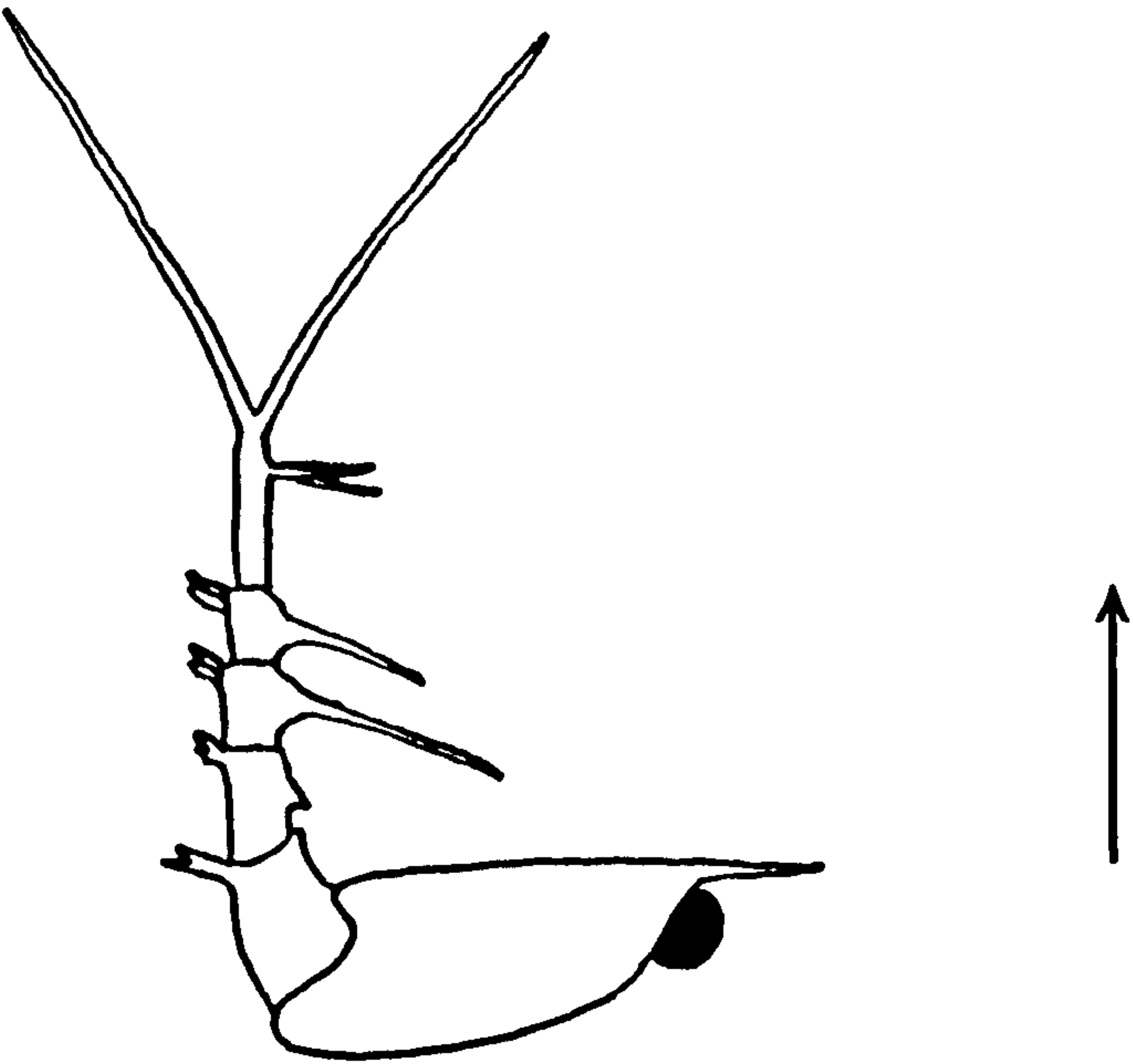
(a) Diagram to show the orientation of a zoea when swimming upwards.

Adapted from Foxon (1934).

(b) Diagram to show the orientation of a zoea when swimming downwards.

The arrowed hoop above the zoea indicates a spiralling descent.

a



b

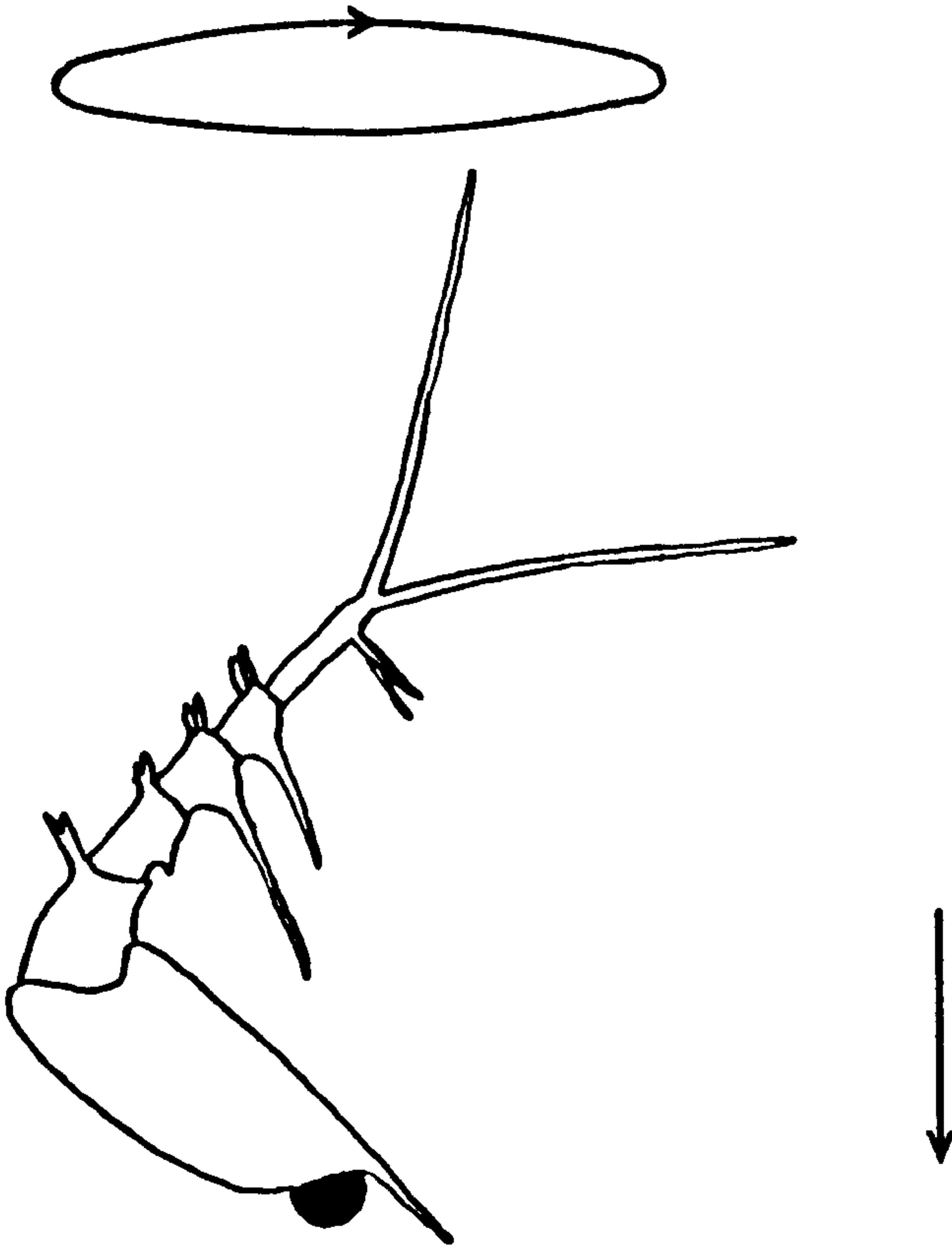


Table 5.5

The average maximum speeds of ascent and descent recorded for individuals of the 3 zoeal stages and the rate of passive descent for individuals of the 3 zoeal and 1st postlarval stages. The figures are derived from at least 5 measurements. All conscious 1st stage postlarvae remained at the bottom of the column and therefore did not demonstrate either active upward or downward movements.

	Zoea 1		Zoea 2		Zoea 3		Postlarva 1	
Movement	Mean speed (mm.s <sup>-1</sup> )	S.D.	Mean speed (mm.s <sup>-1</sup> )	S.D.	Mean speed (mm.s <sup>-1</sup> )	S.D.	Mean speed (mm.s <sup>-1</sup> )	S.D.
Passive descent (anaesthetised)	8.6	1.1	9.1	1.3	9.5	1.7	10.8	3.0
Active descent	22.0	3.2	25.0	4.6	30.4	1.1	-	-
Active ascent (swimming)	21.2	3.6	28.0	3.2	30.0	2.6	-	-



descent with development, average speeds increased from 8.6 to 10.8mms<sup>-1</sup> (n = 24) between the 1st zoeal and 1st postlarval stages. The variation within stages was high, however, and the rate of descent was influenced by the orientation adopted by each individual.

### 5.3.2. Structure of the eye

#### 5.3.2.1. Light microscope photography

The general form of the eye in live specimens of the 3rd zoeal and first four postlarval stages is shown in Plate 5.1. The greatest structural change appears to occur between the 3rd zoeal and 1st postlarval stages. It is known that the eye stalk is significantly longer ( $P < 0.05$ ) in 3rd stage zoeae than in 1st stage postlarvae (see Chapter 4). There also seems to be a wider transparent margin around the perimeter of the eye between the outer cornea and the black pigment in 3rd stage zoeae.

#### 5.3.2.2. S.E.M.

##### Larval stages

The packing between the corneal lenses in the 1st and 2nd zoeae is hexagonal and at low magnification (Plate 5.2a,b) gives the superficial impression of hexagonal facetting. Higher magnification reveals that the corneal lenses are in fact circular (Plate 5.2b,d). At low magnification, the facetting of the eye of 3rd stage zoeae also appears to be hexagonal (Plate 5.2e). Higher magnification again reveals the circular shape of the lenses and their hexagonal packing

## Plate 5.1

The general form of the eye and rostrum in live specimens.

(a) Third stage zoea.

(b) First stage postlarva.

(c) Second stage postlarva.

(d) Third stage postlarva.

(e) Fourth stage postlarva.

Scale bar represents 0.50mm.



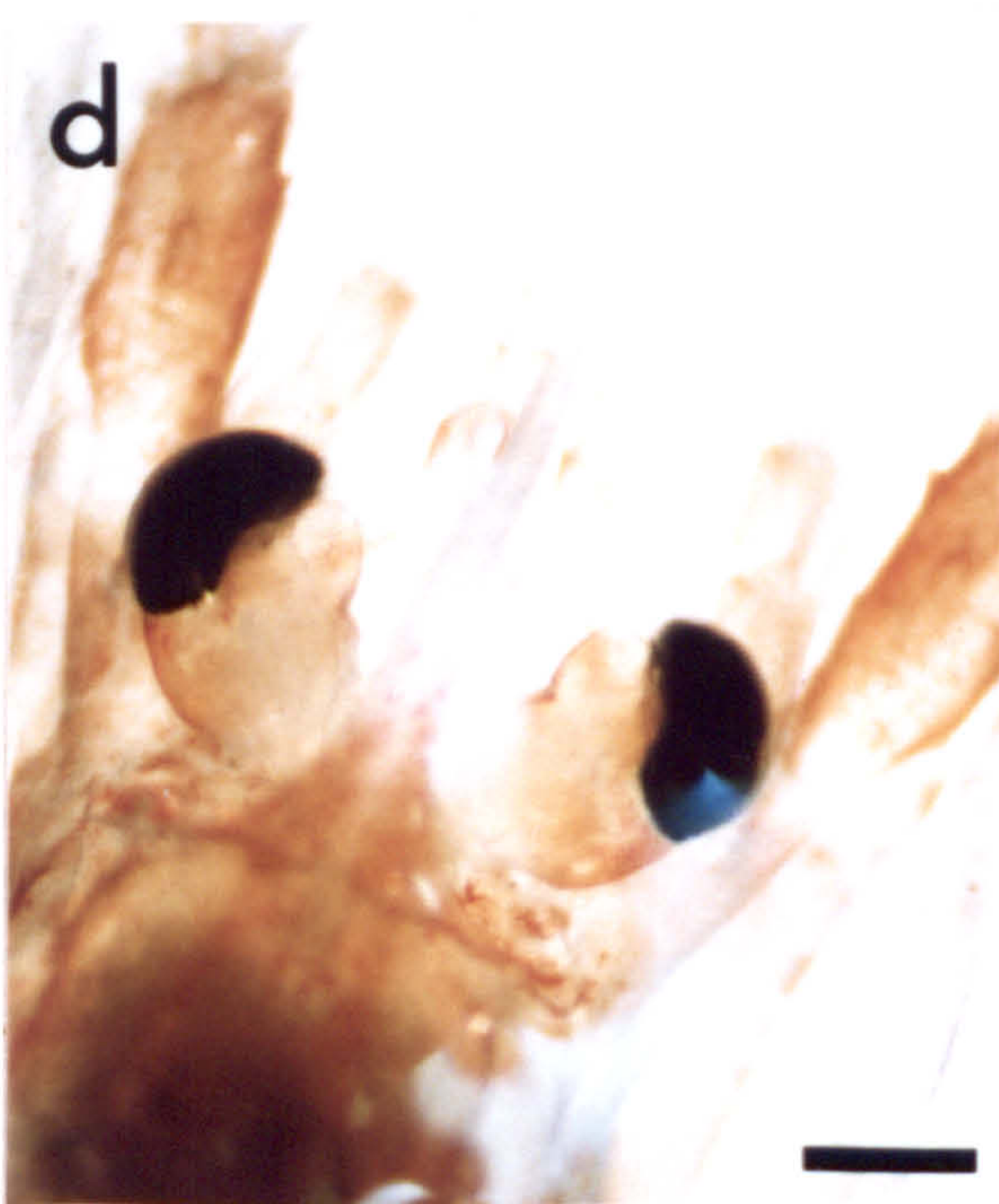
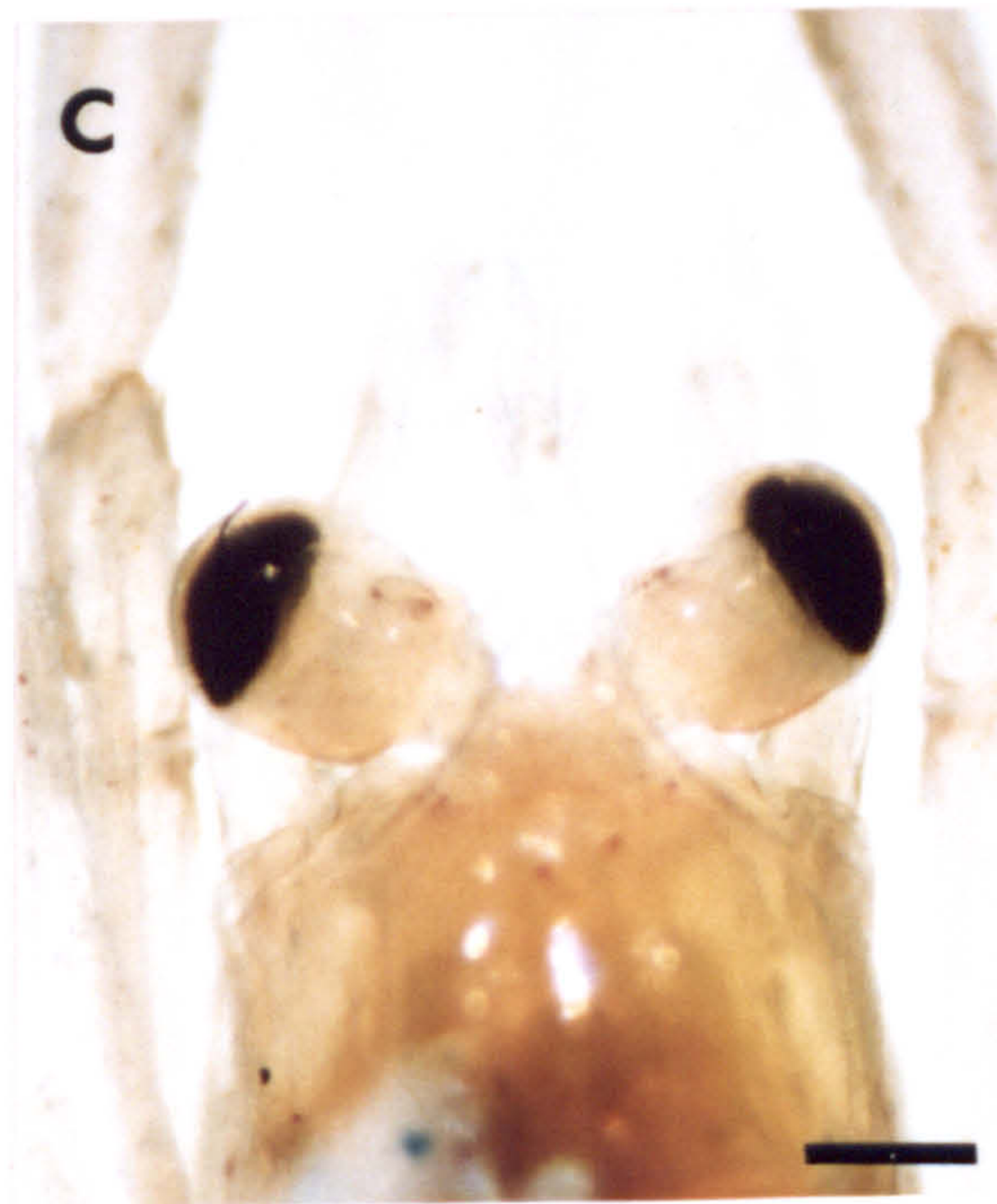
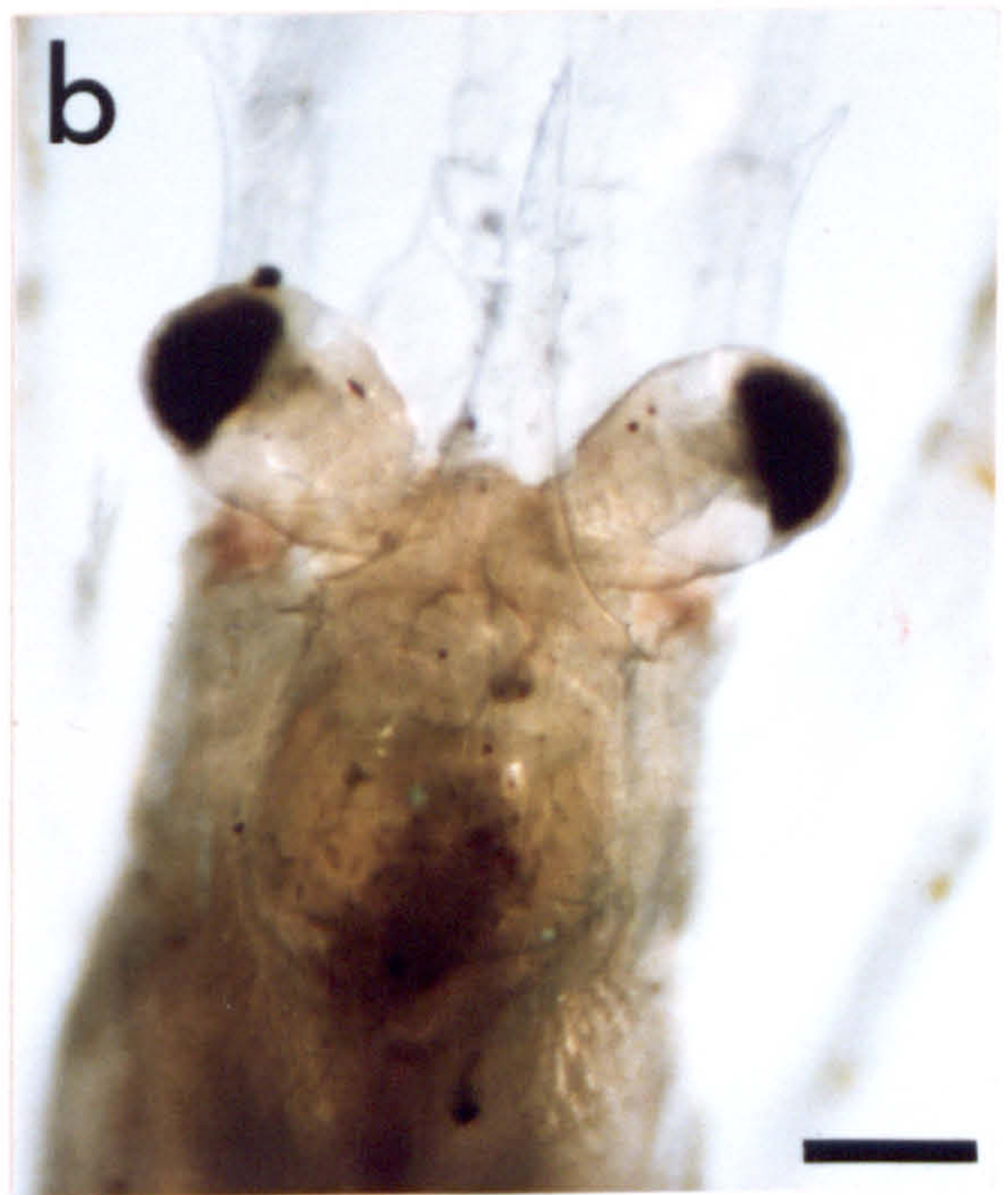




Plate 5.2

S.E.M. of the eye showing the patterning of the corneal lenses.

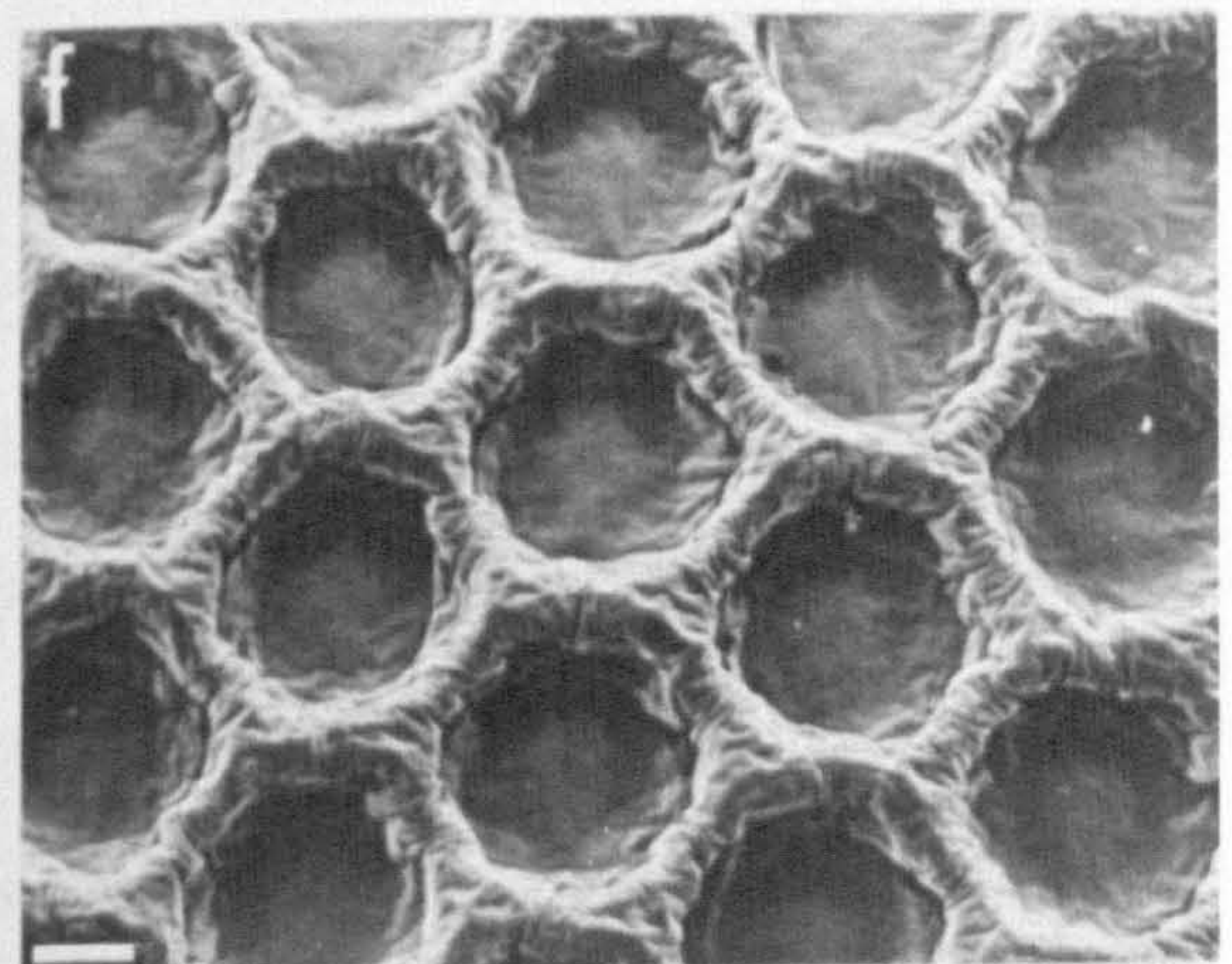
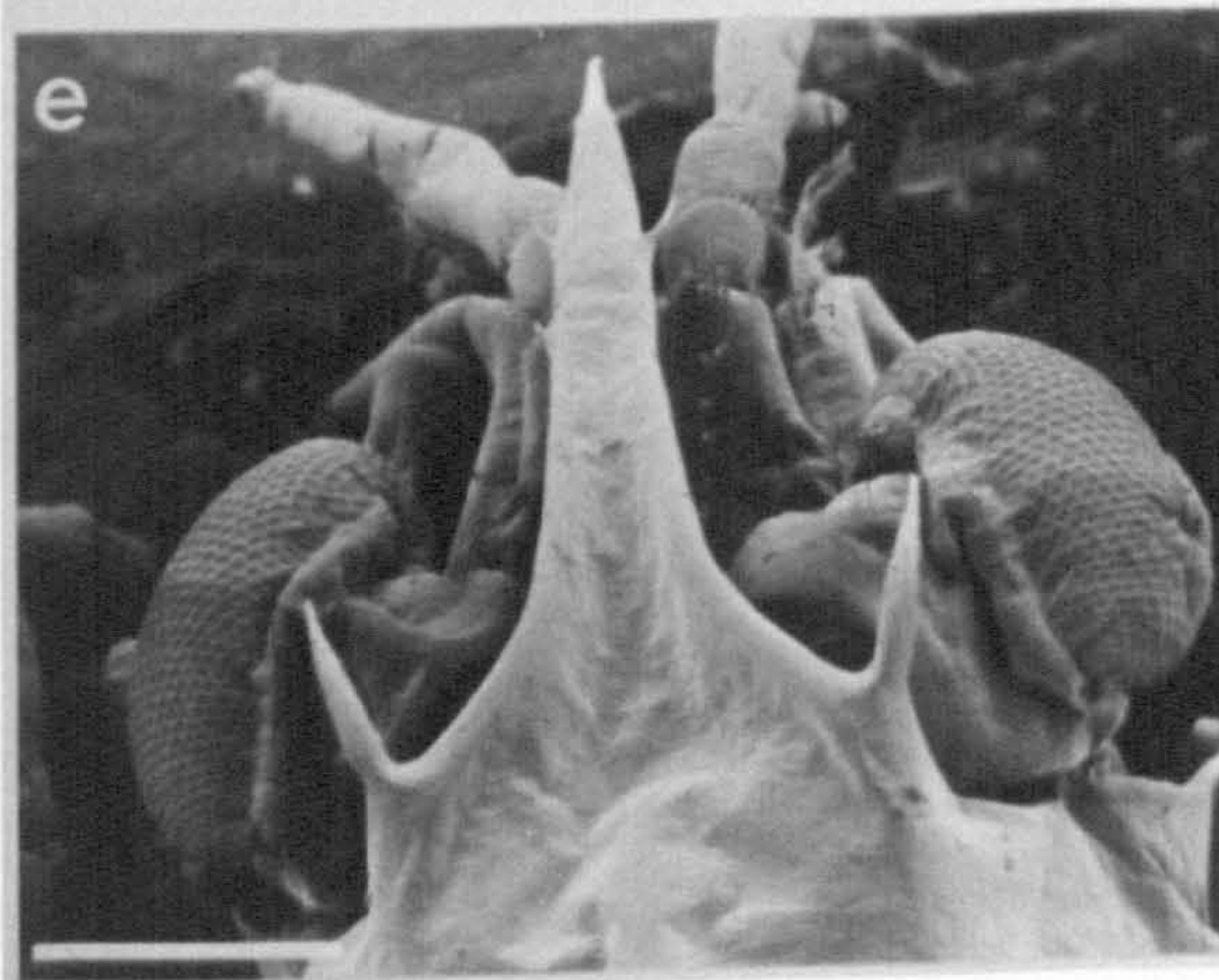
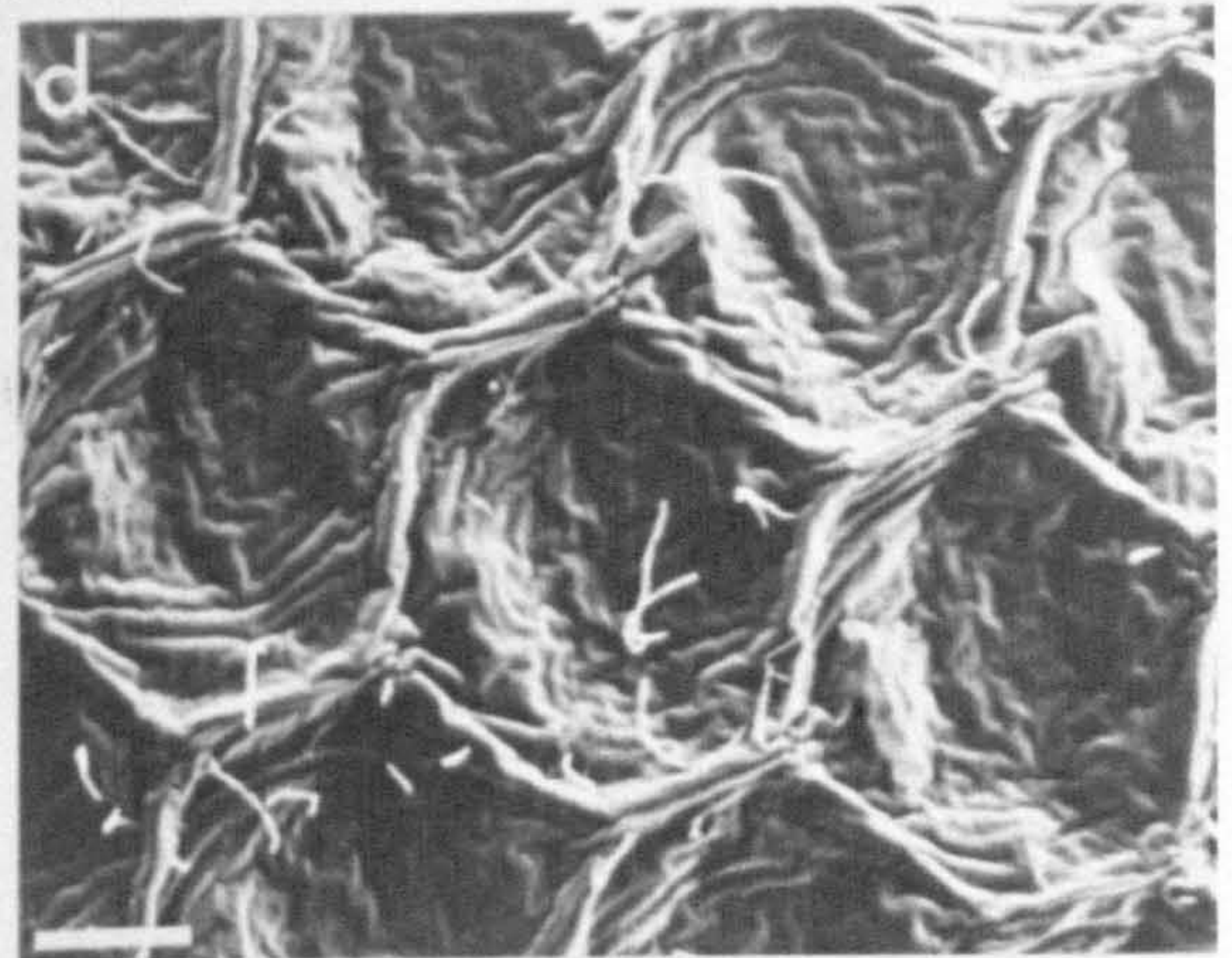
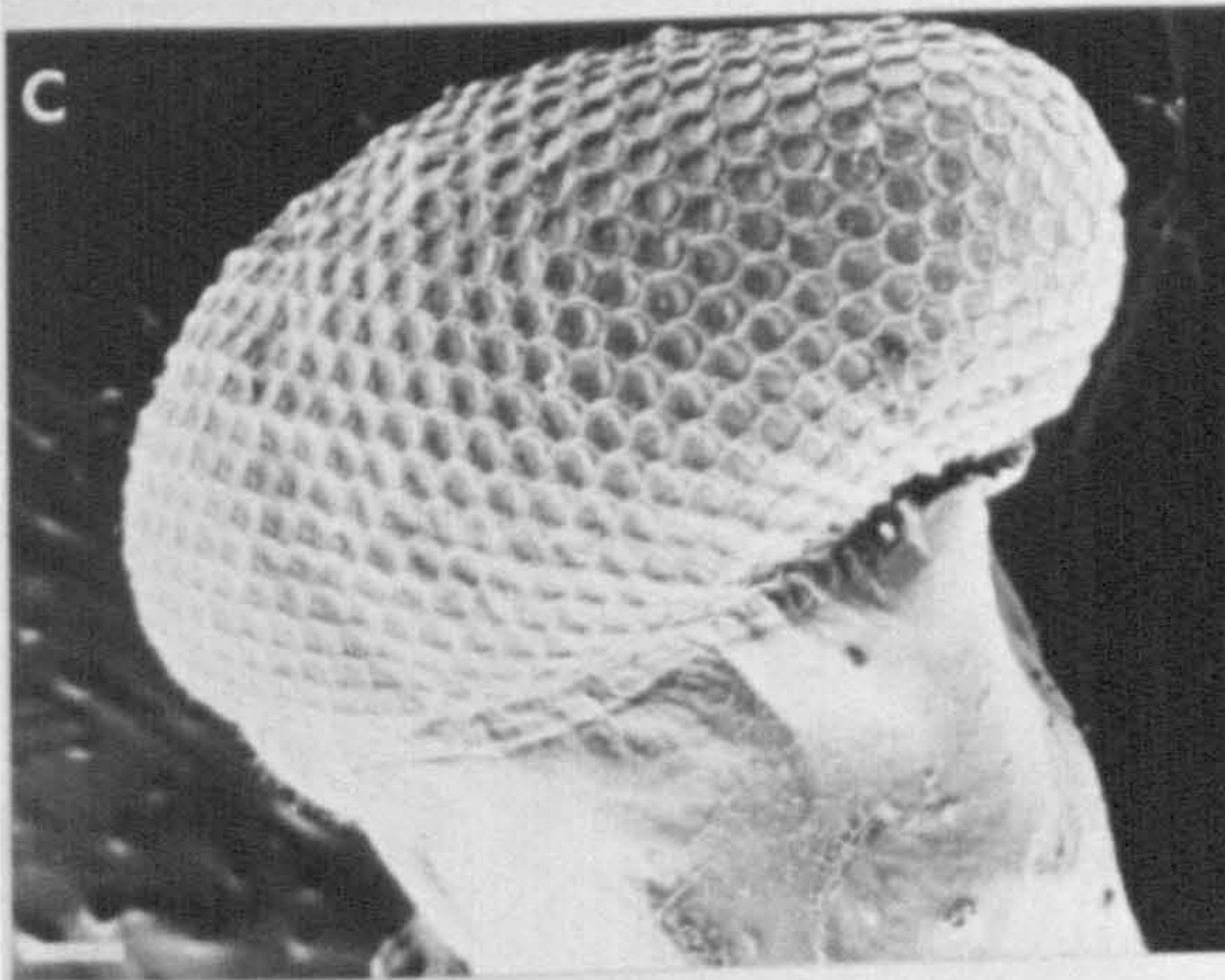
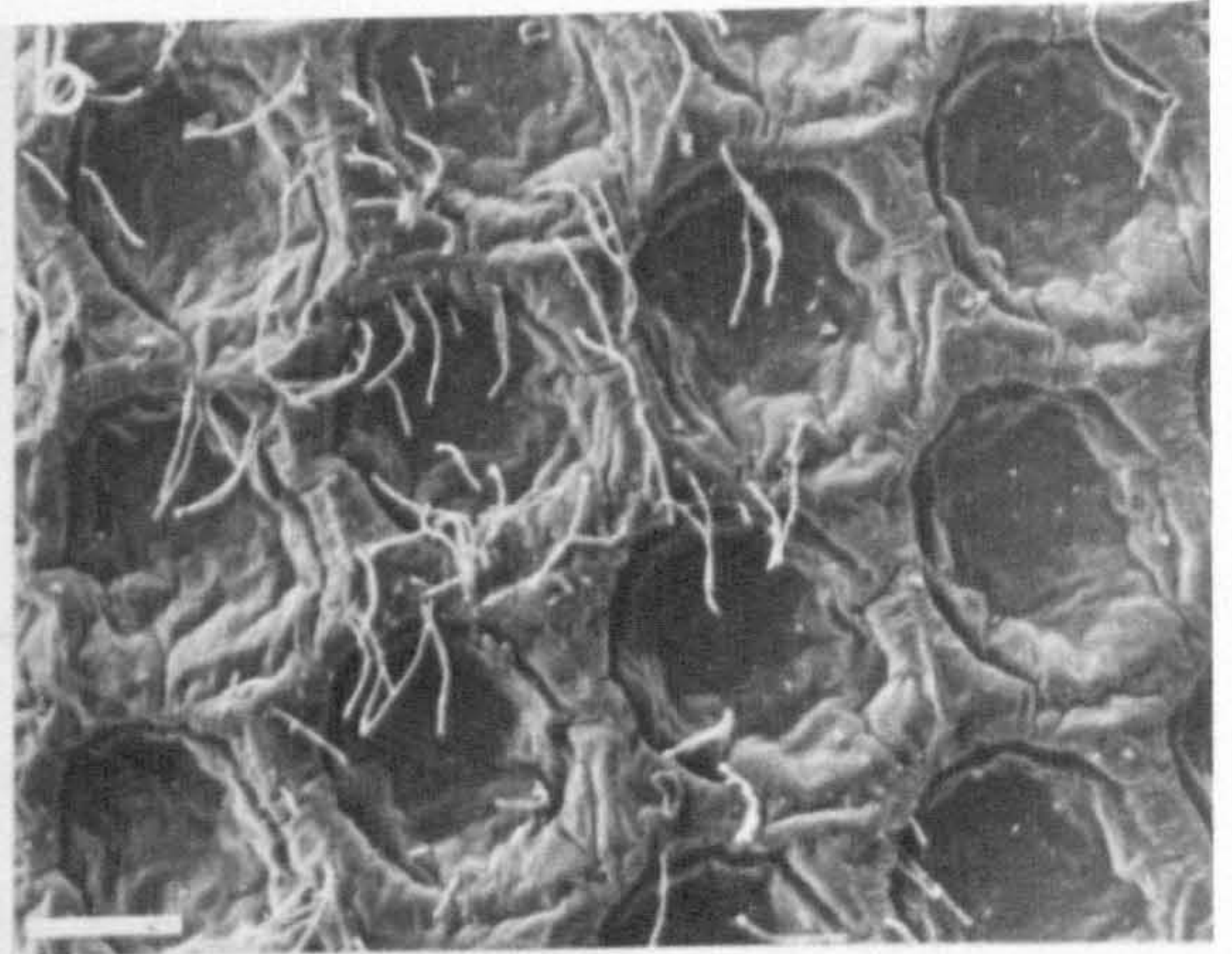
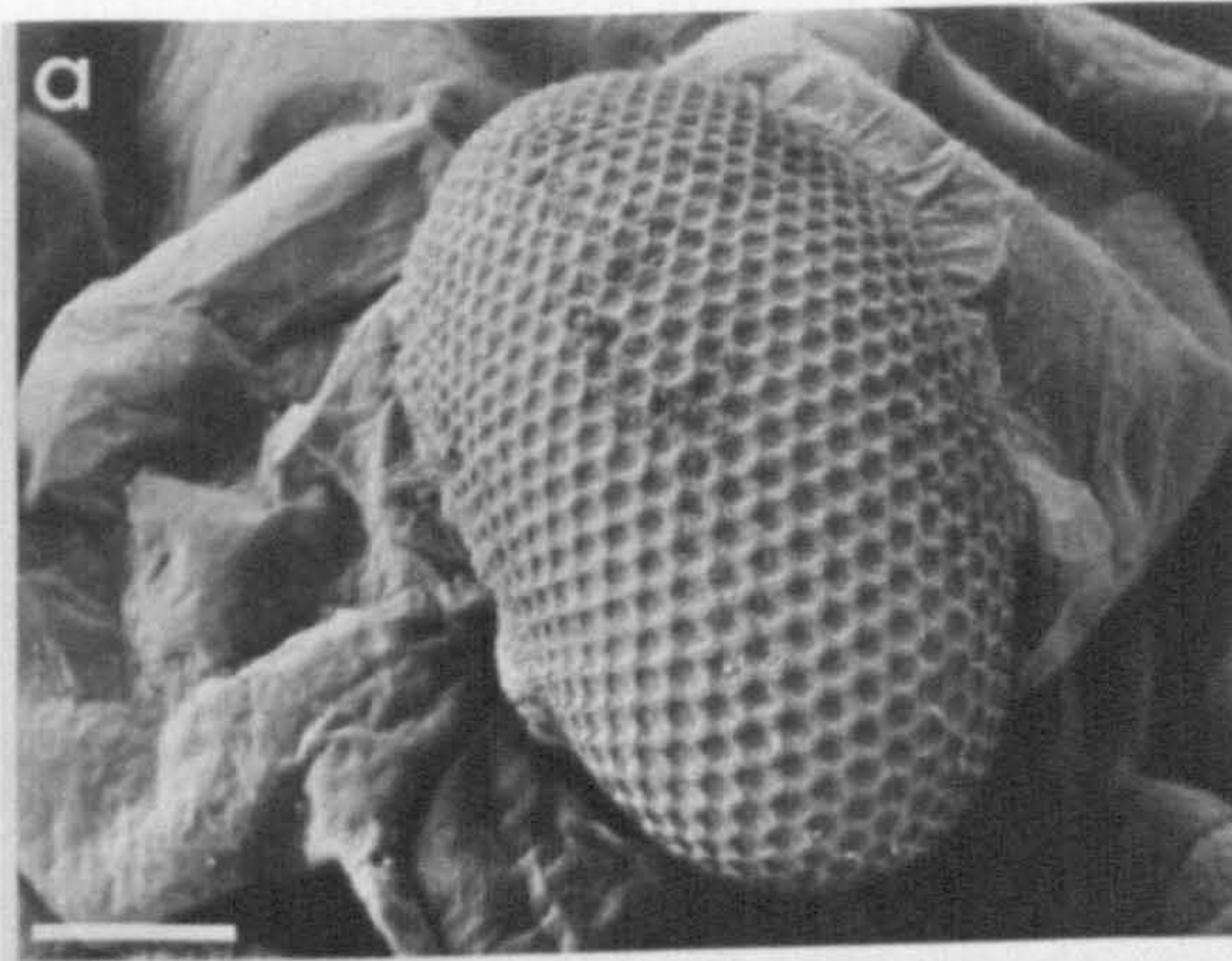
(a), (b) First stage zoea.

(c), (d) Second stage zoea.

(e), (f) Third stage zoea.

Scale bar represents 0.01mm for (b), (d) and (f), 0.10mm for (a) and (c) and 0.50mm for (e).







(Plate 5.2f). Within this stage there is evidence of the lenses and surrounding hexagonal patterning beginning to 'square - off' at the margins (Plate 5.3a,b). These facets are in the region of the growth zone (Shelton et al., 1981) and will probably represent new ommatidia which have been added at the preceding moult.

#### Juvenile and adult stages

At low magnification the surface of the eye in 1st stage postlarvae demonstrates both the hexagonal patterning of the larval stages and the square patterning of the later postlarval and adult stages (Plate 5.3c). The square facets occur in bands of rows and are located towards the margin of the eye, forming the orthogonal arrangement of adult eyes in this region (Plate 5.4e). They were probably added at metamorphosis. Higher magnification reveals the localised orthogonal arrangement between the square lenses and the occurrence of proximal subcircular lenses (Plate 5.3d).

The lenses in the eye of 2nd stage postlarvae are predominantly arranged orthogonally (Plate 5.3e,f). There is some variation, however, with several lenses still possessing the subcircular shape. The arrangement of facets still appears to be relatively disorganised during this stage. In third and fourth stage postlarvae there appears to be no further evidence of hexagonal patterning and circular lenses (Plate 5.4a,b,c). Here the orthogonal arrangement of ommatidia is more organised, resembling that of the adult eye (Plate 5.4d,e). The surface of the eye in these older postlarvae and in adult Nephrops comprises an almost spherical array of square-faceted ommatidia and is similar to that described for adult Homarus gammarus

Plate 5.3

S.E.M. of the eye showing the patterning of the corneal lenses.

(a), (b) Third stage zoea (at the margins of the eye).

(c), (d) First stage postlarva.

(e), (f) Second stage postlarva.

Scale bar represents 0.01mm for (b), (d) and (f), 0.05mm for (a) and (c) and 0.10mm for (e).



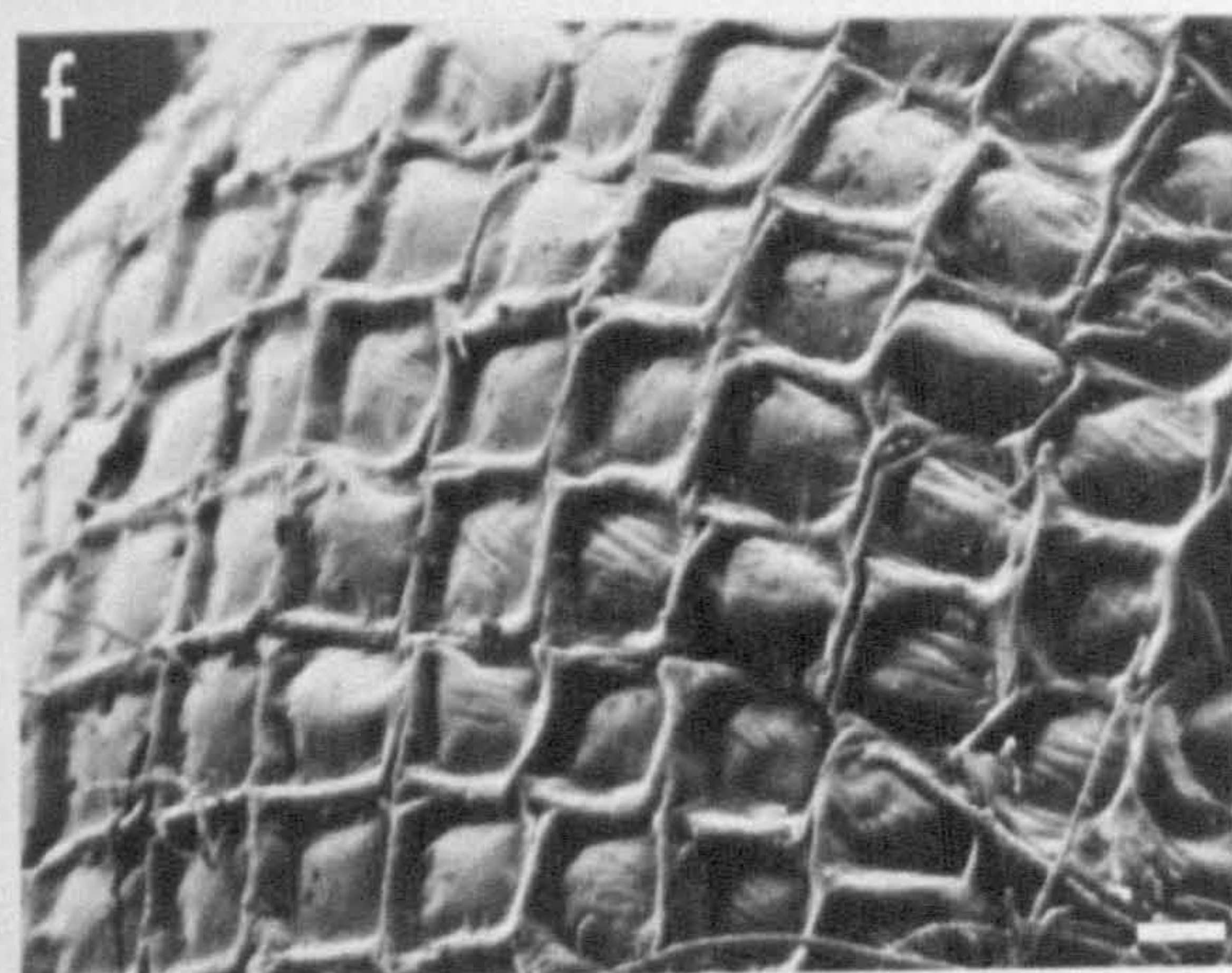
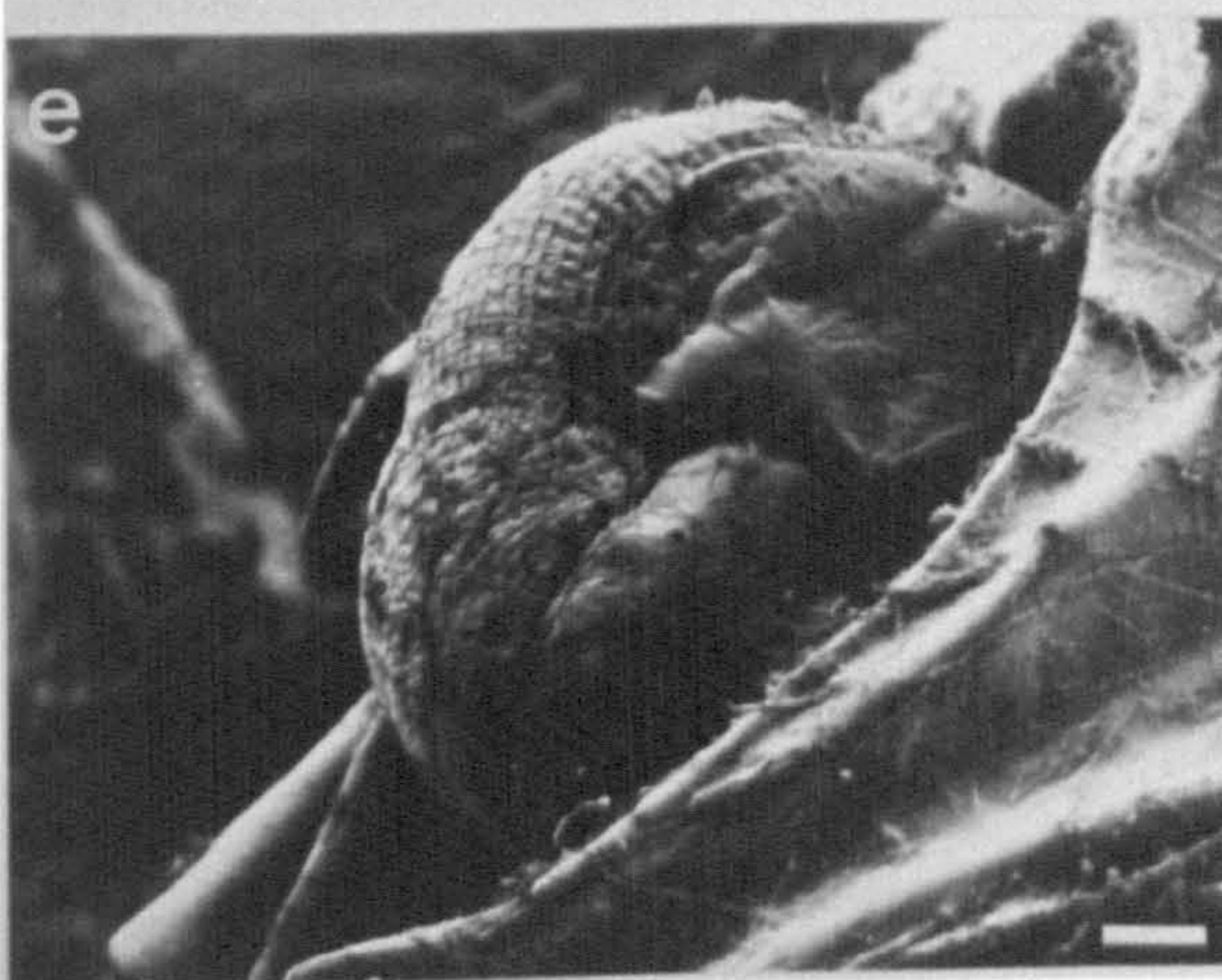
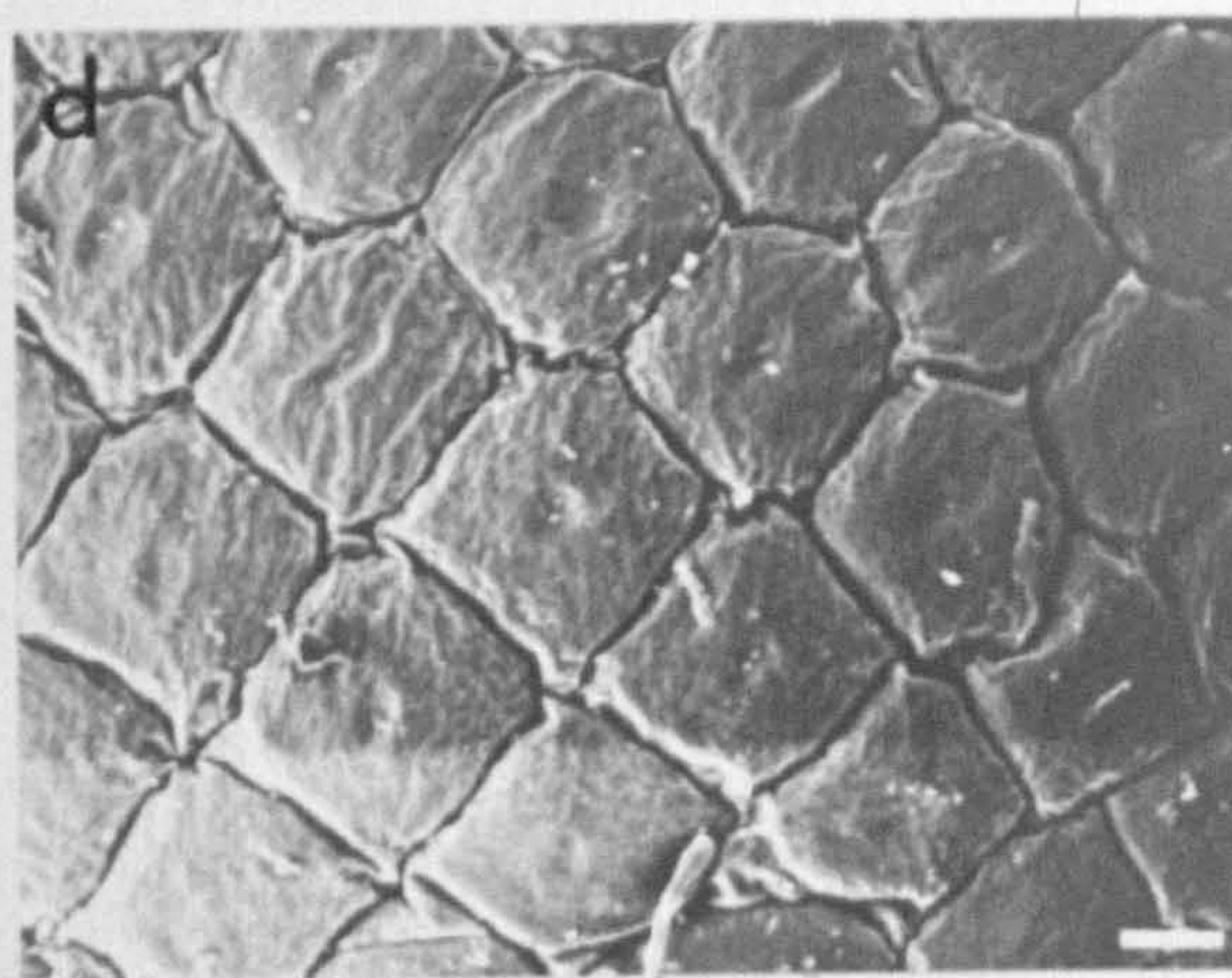
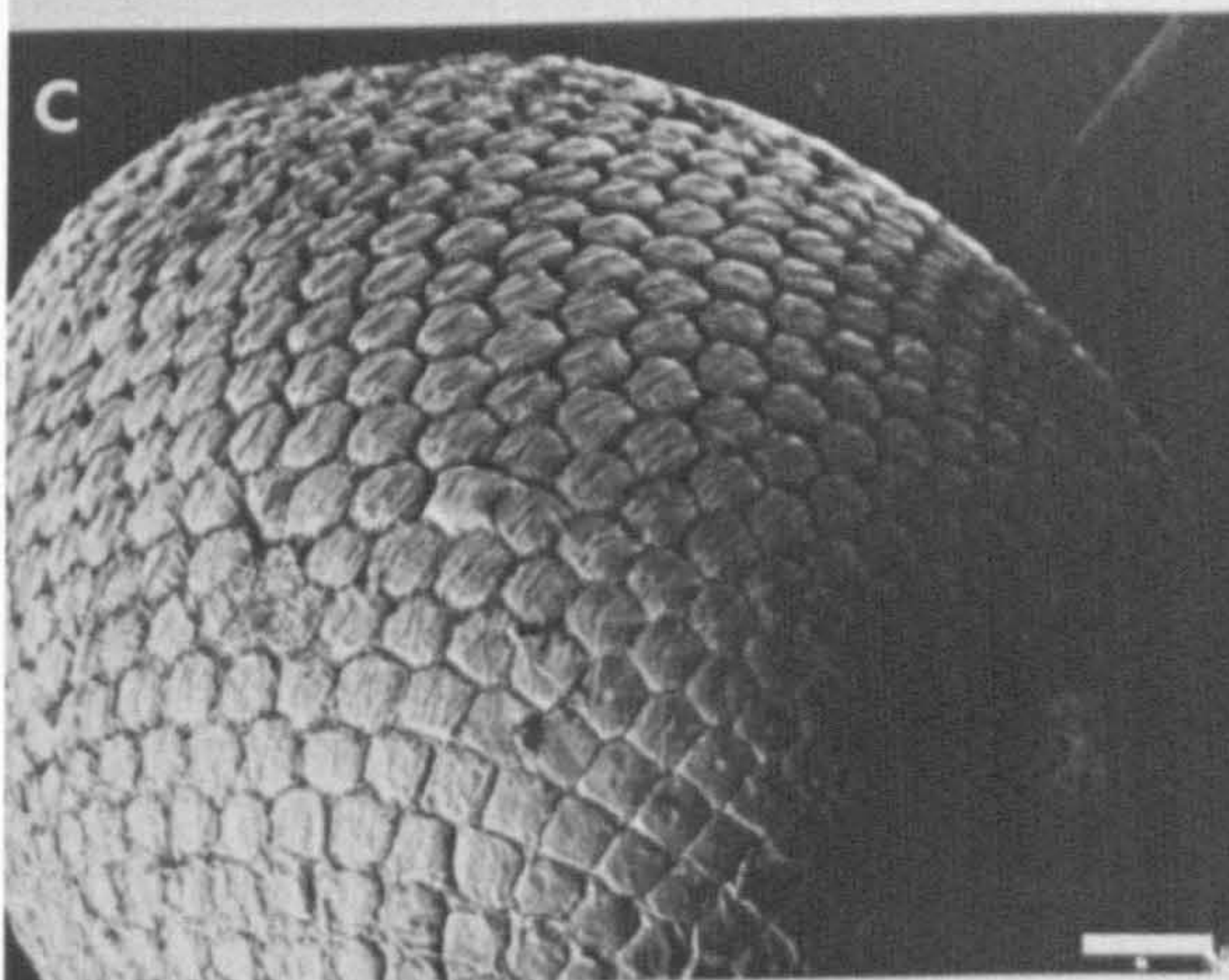
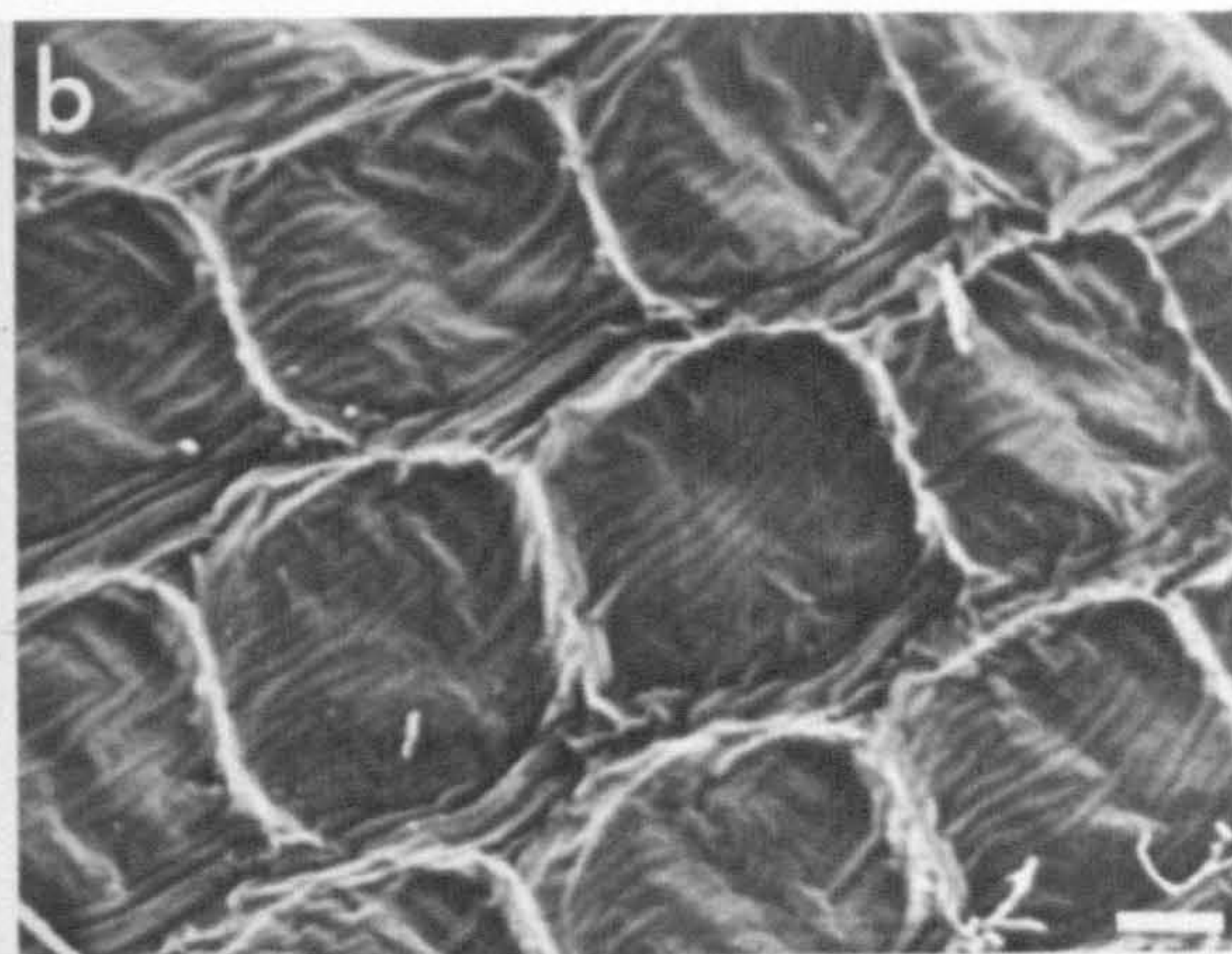
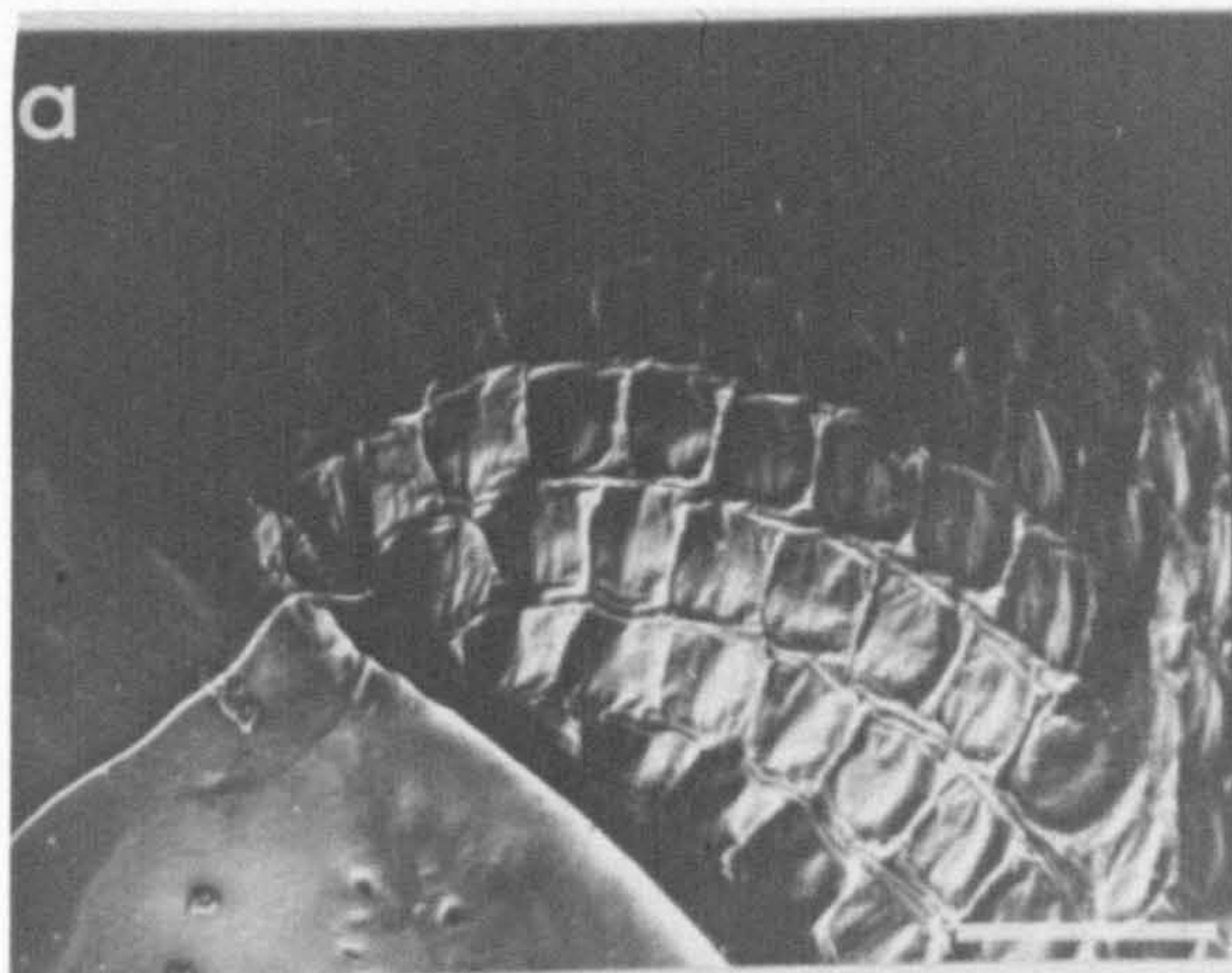




Plate 5.4

S.E.M. of the eye showing the patterning of the corneal lenses.

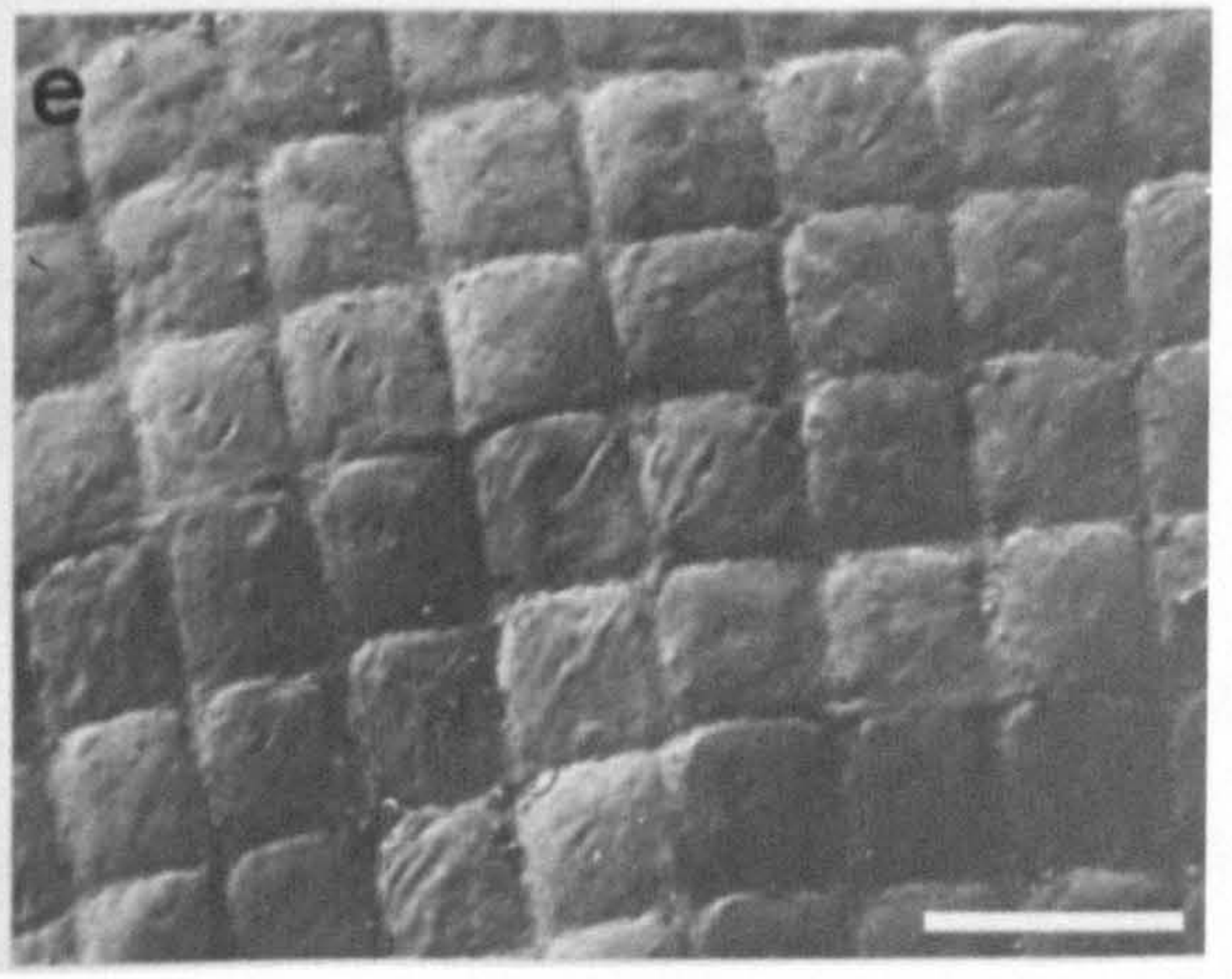
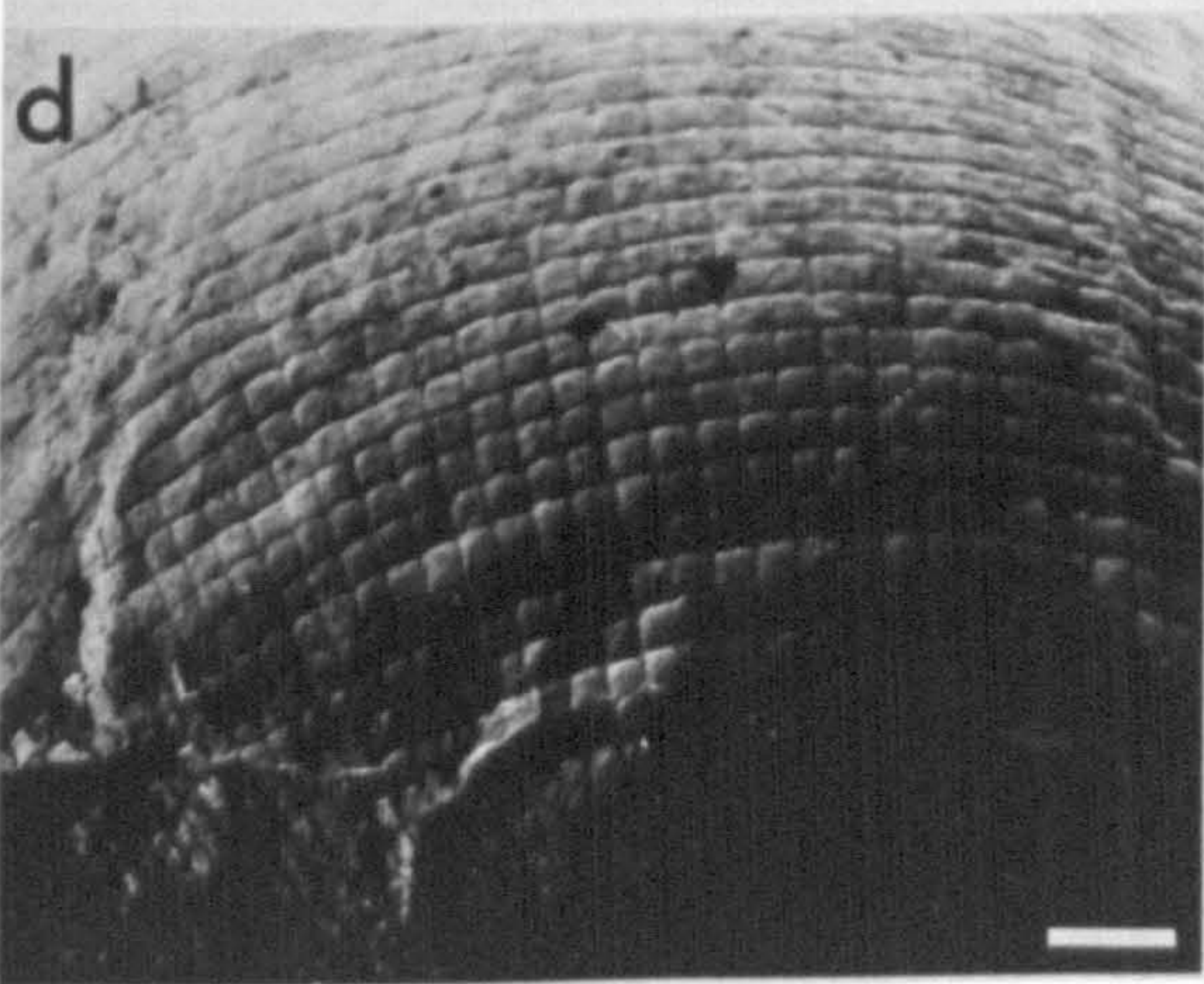
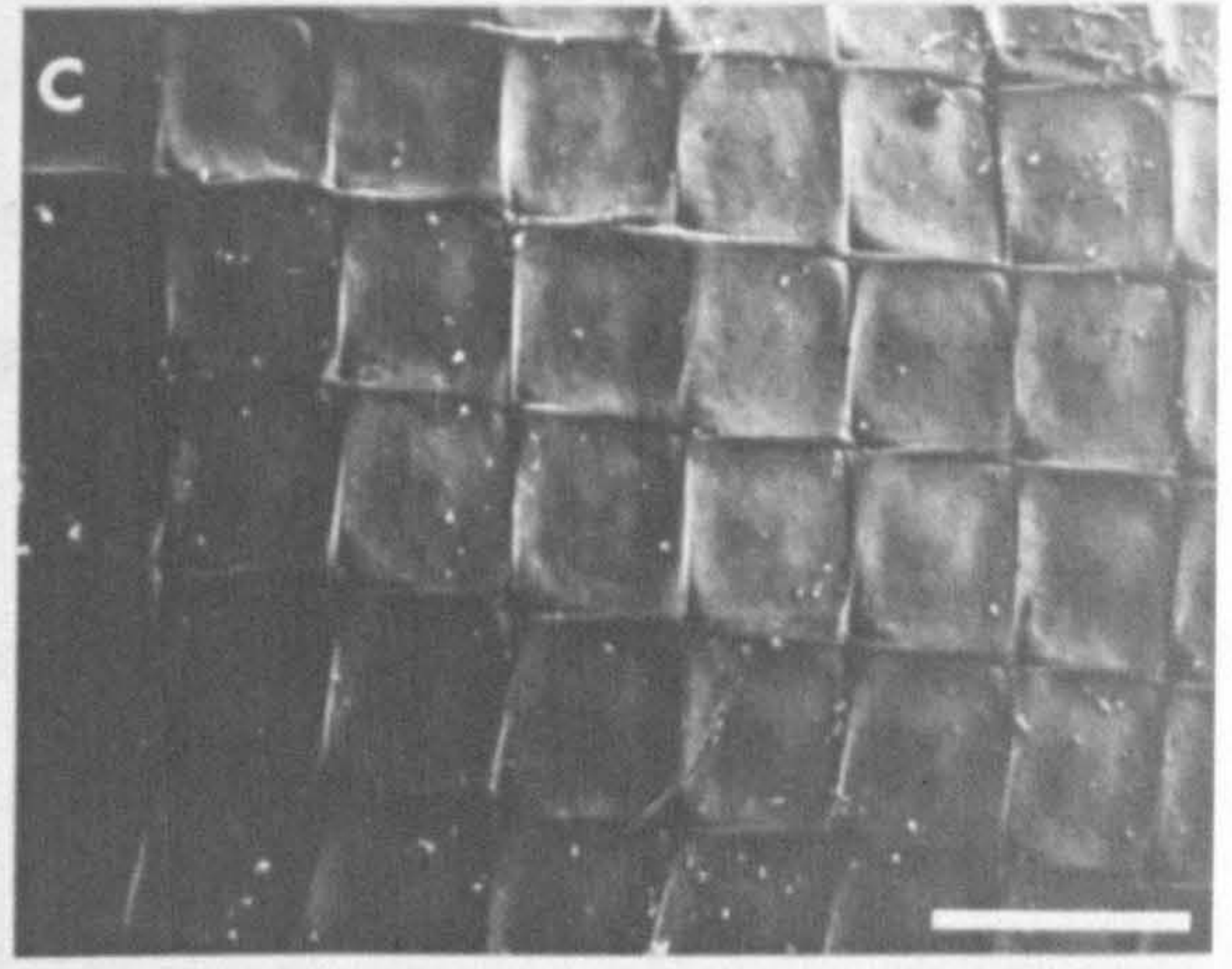
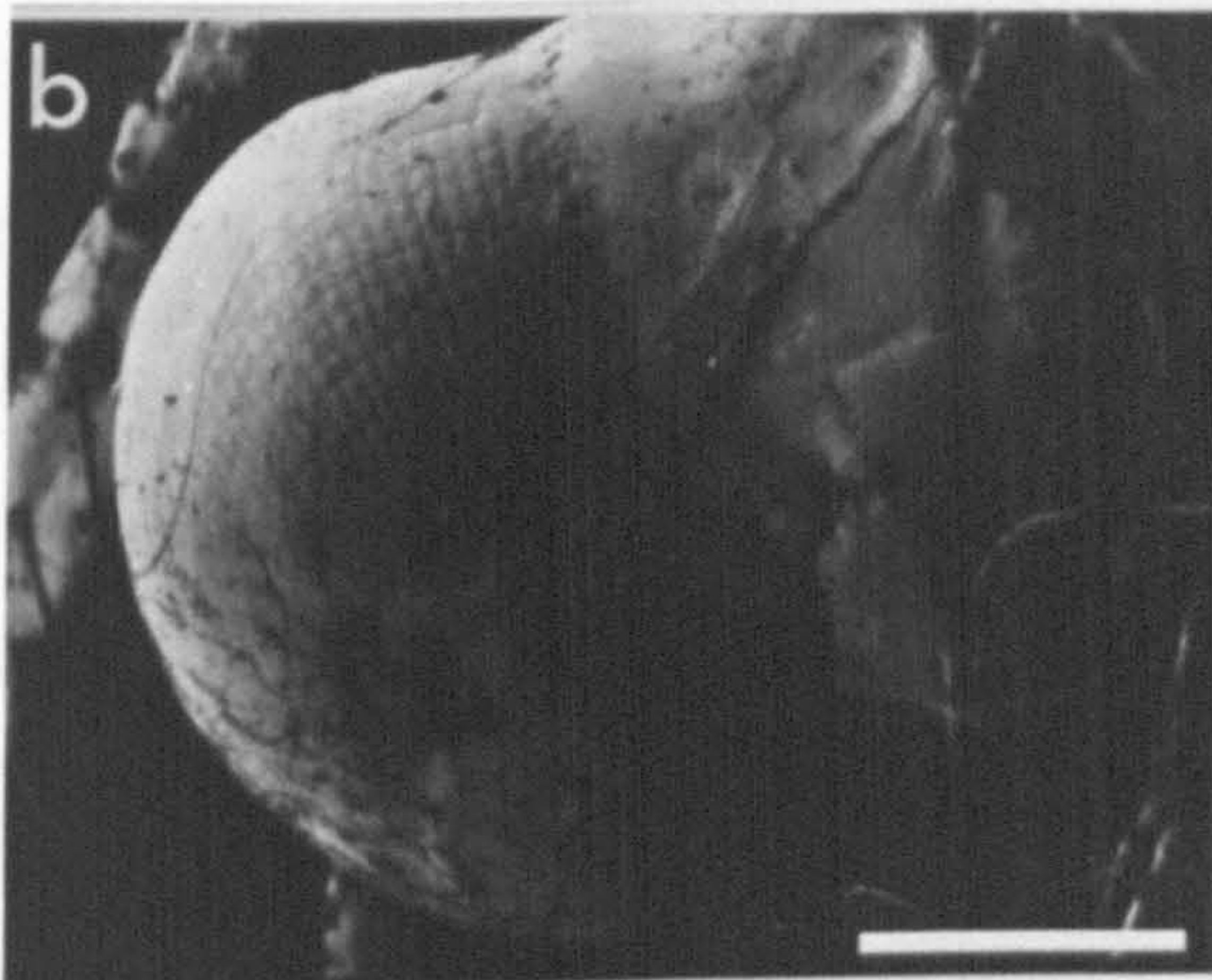
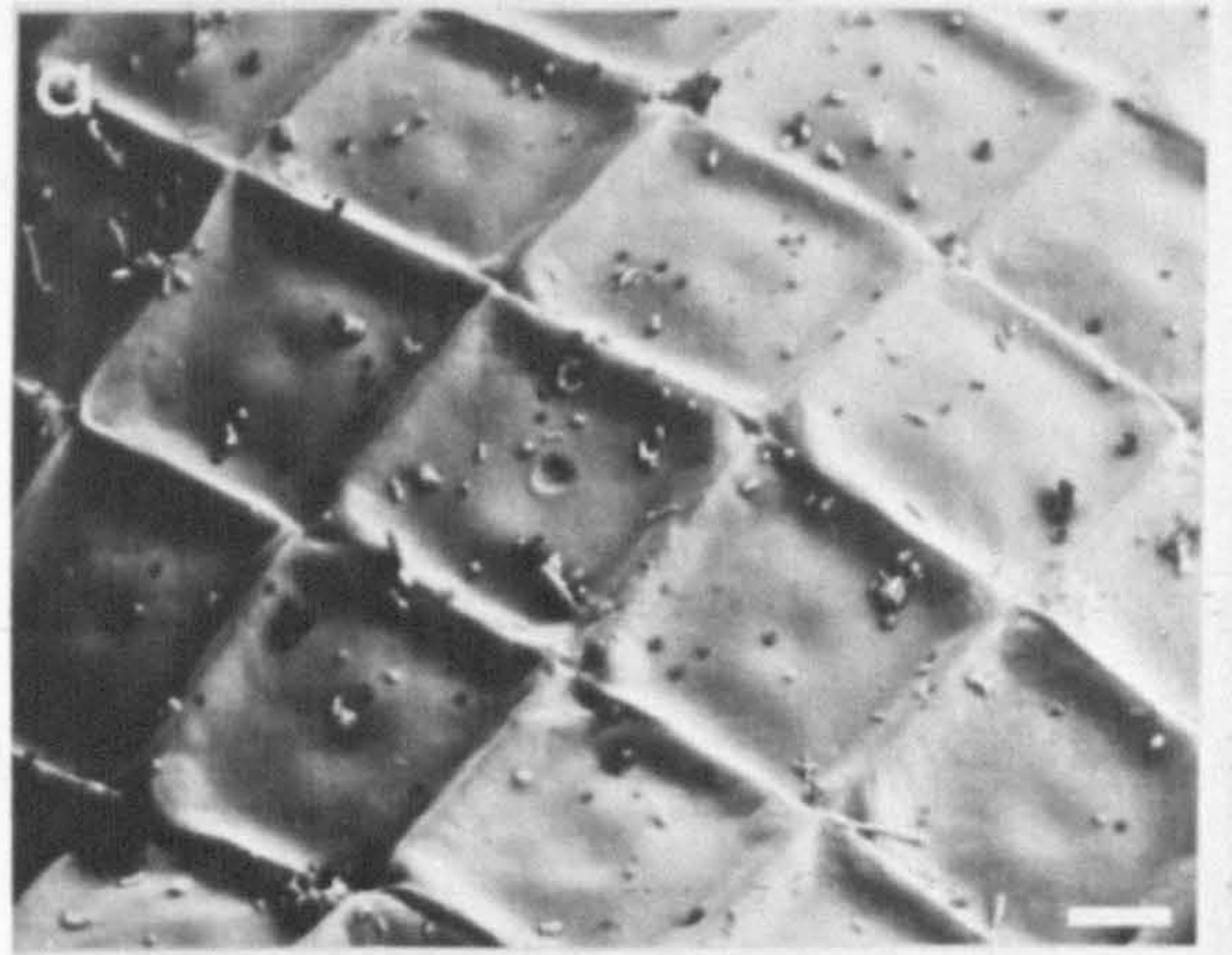
(a) Third stage postlarva.

(b), (c) Fourth stage postlarva.

(d), (e) Adult.

Scale bar represents 0.01mm for (a), 0.05mm for (c) and (e),  
0.10mm for (d) and 0.50mm for (b).







(Shelton et al., 1981).

The heavy packing evident between the lenses in the larval stages is reduced after metamorphosis. This reduction continues over the next three moults and by the fourth postlarval stage such packing is minimal and similar to that observed for the adult eye.

#### 5.3.2.3. T.E.M.

##### Second stage zoea

The T.E.M. photographs of the eye of the 2nd stage zoea are presented in Plate 5.5. The cornea of each ommatidium is a cube of cuticle and forms a continuous plate (Plate 5.5a,b) of approximately 4µm thickness over the surface of the compound eye with little curvature of the individual facets. The cornea is differentiated into two regions, an extremely thin (< 0.1µm) darker - staining outer ectocuticle and an inner lighter - staining multi-layered endocuticle (Plate 5.5a). The underlying corneagen cells which secrete the cornea do not appear to be differentiated fully. The cone, which is packed with mitochondria, lies immediately distal to the corneagen cells and has not yet become differentiated fully (Plate 5.5b). The cone is a quadripartite structure involving the association of four cone cells (Plate 5.5d). It extends from the cornea to the distal ends of the rhabdoms. There is no evidence of a 'clear zone' which characterizes the superposition optics of the adults (Loew, 1976) and, in common with other decapod larvae (Fincham, 1984), the Nephrops larvae seem to possess apposition type vision.

Plate 5.5

T.E.M. of the eye of a second stage zoea.

(a) Longitudinal section showing the structure of the cornea.

(b) Longitudinal section showing the cornea and underlying cone.

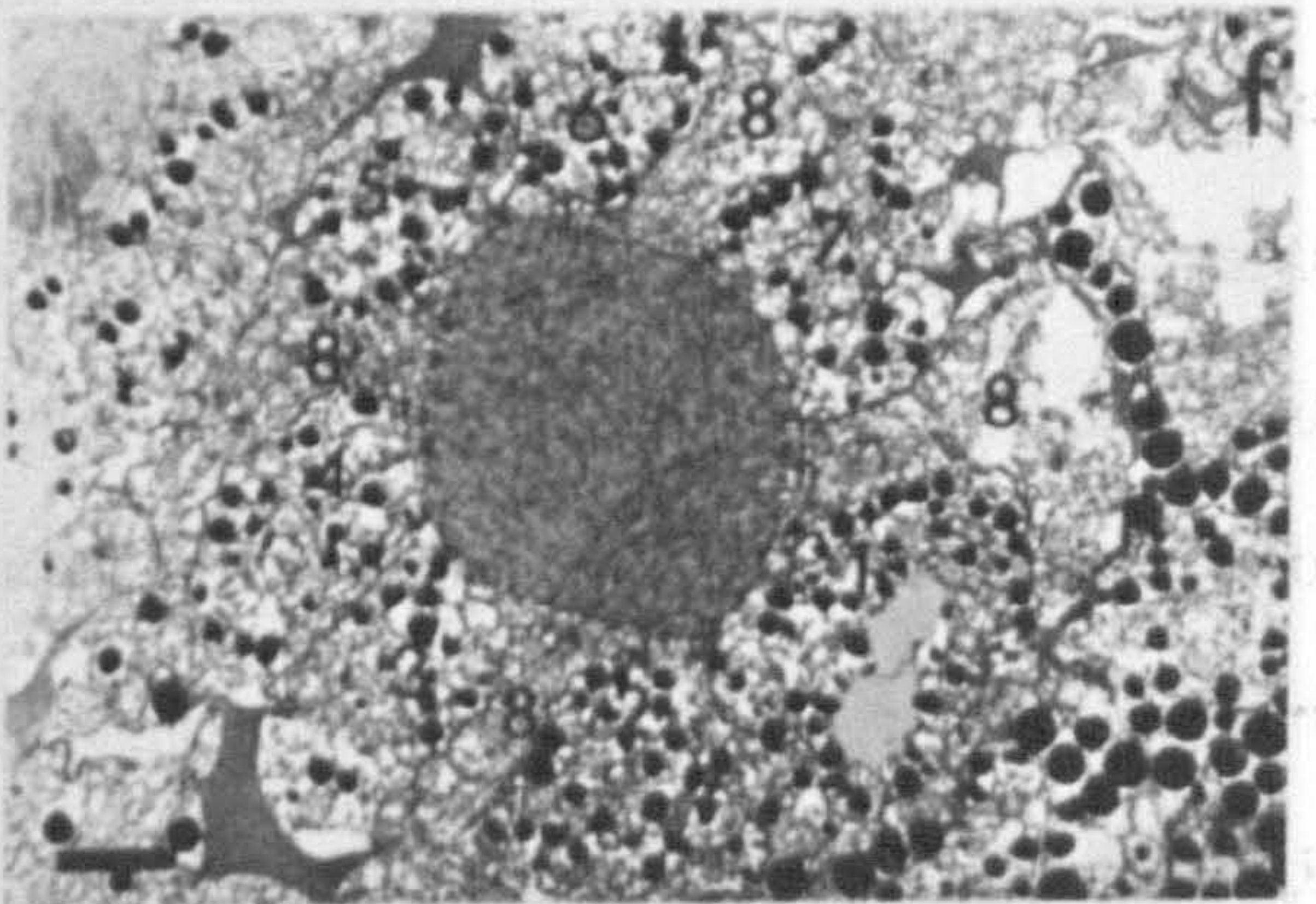
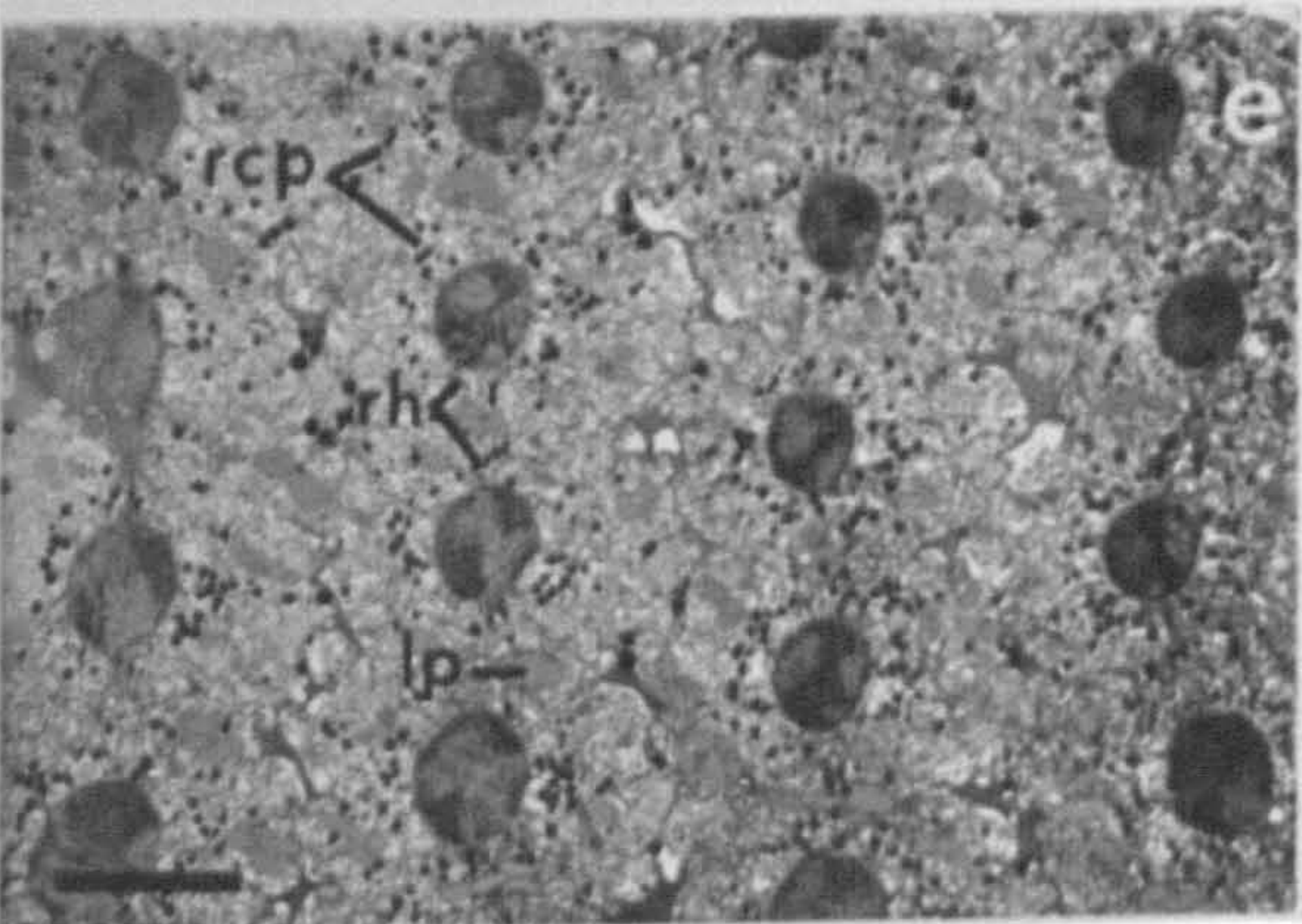
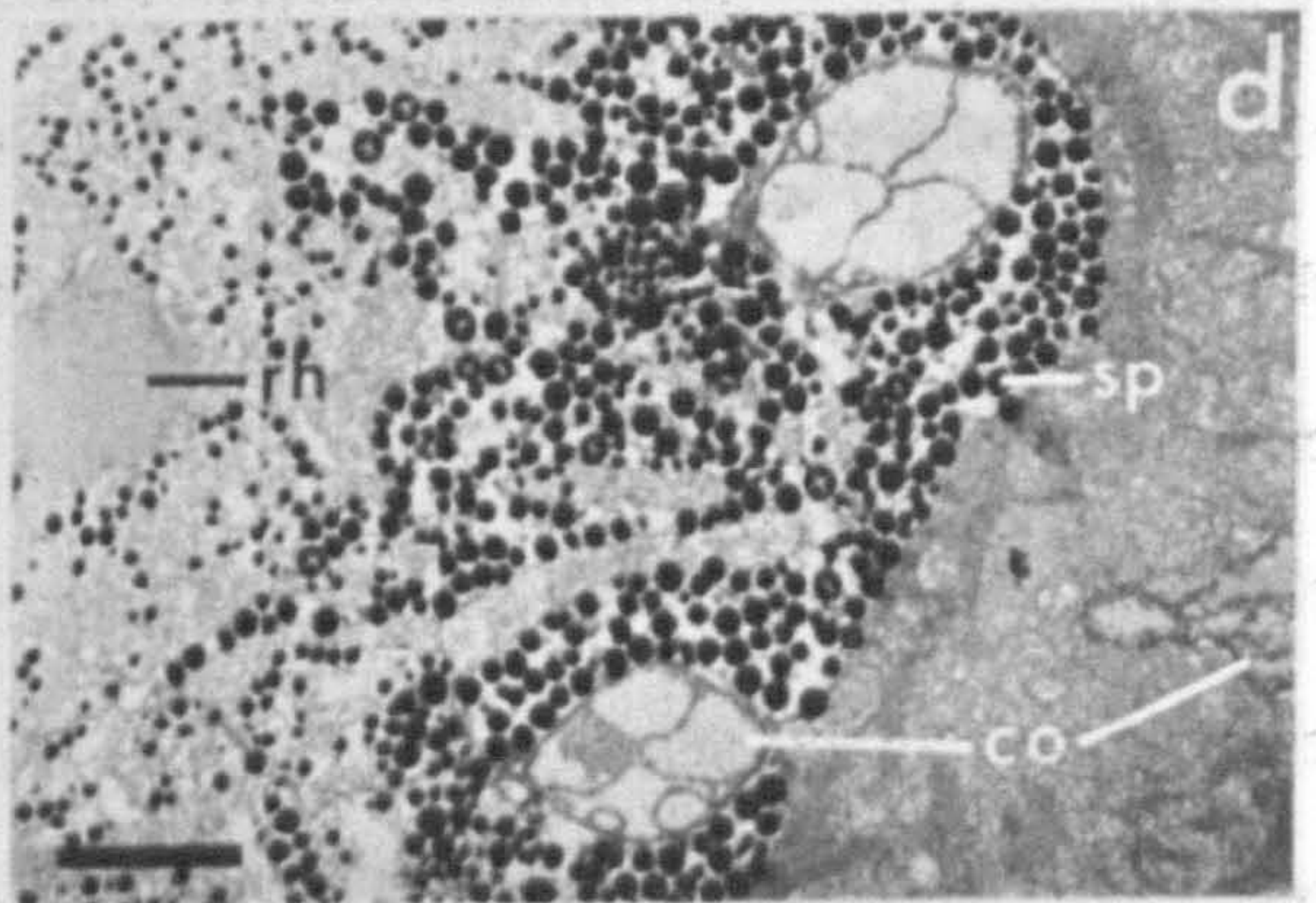
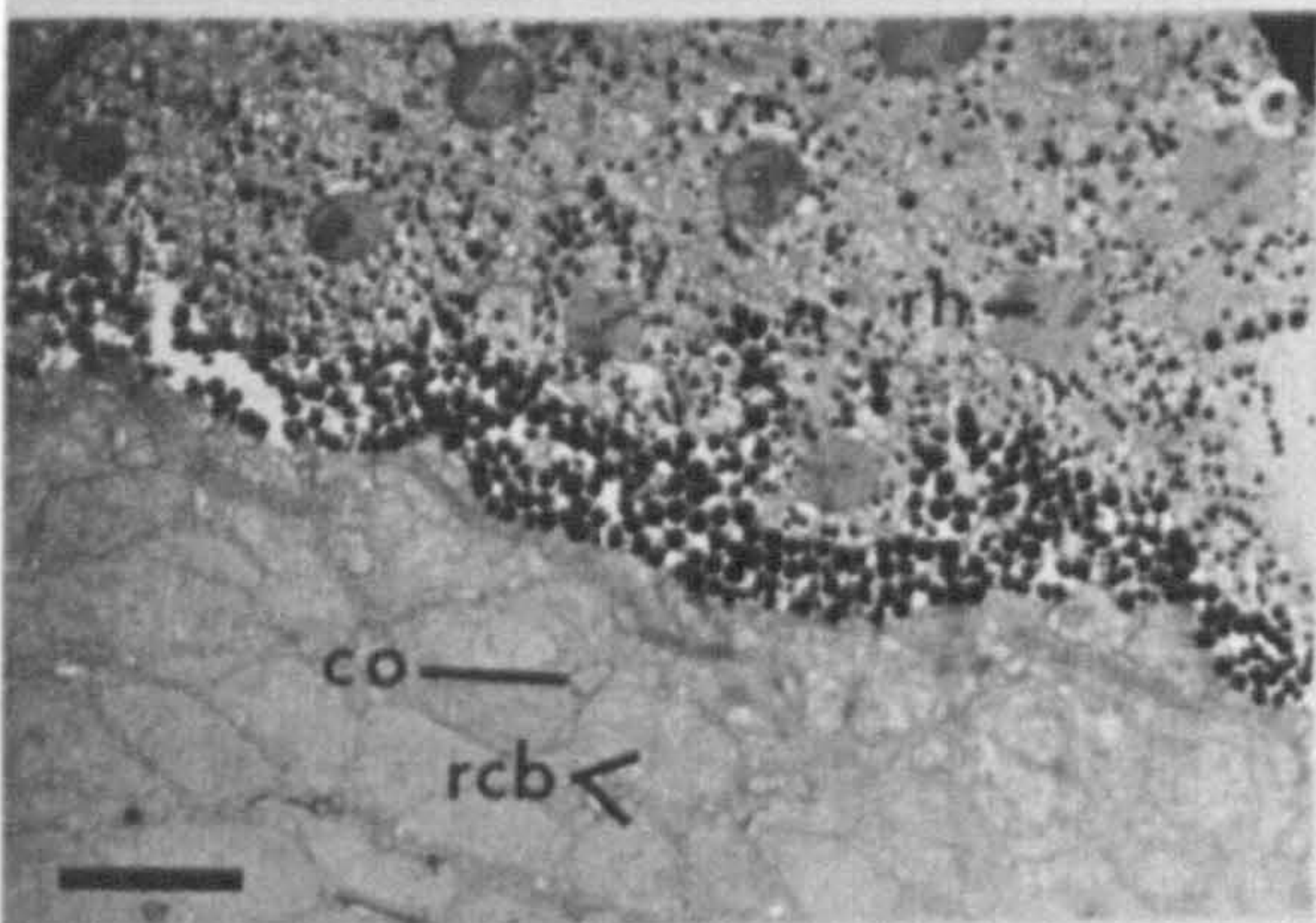
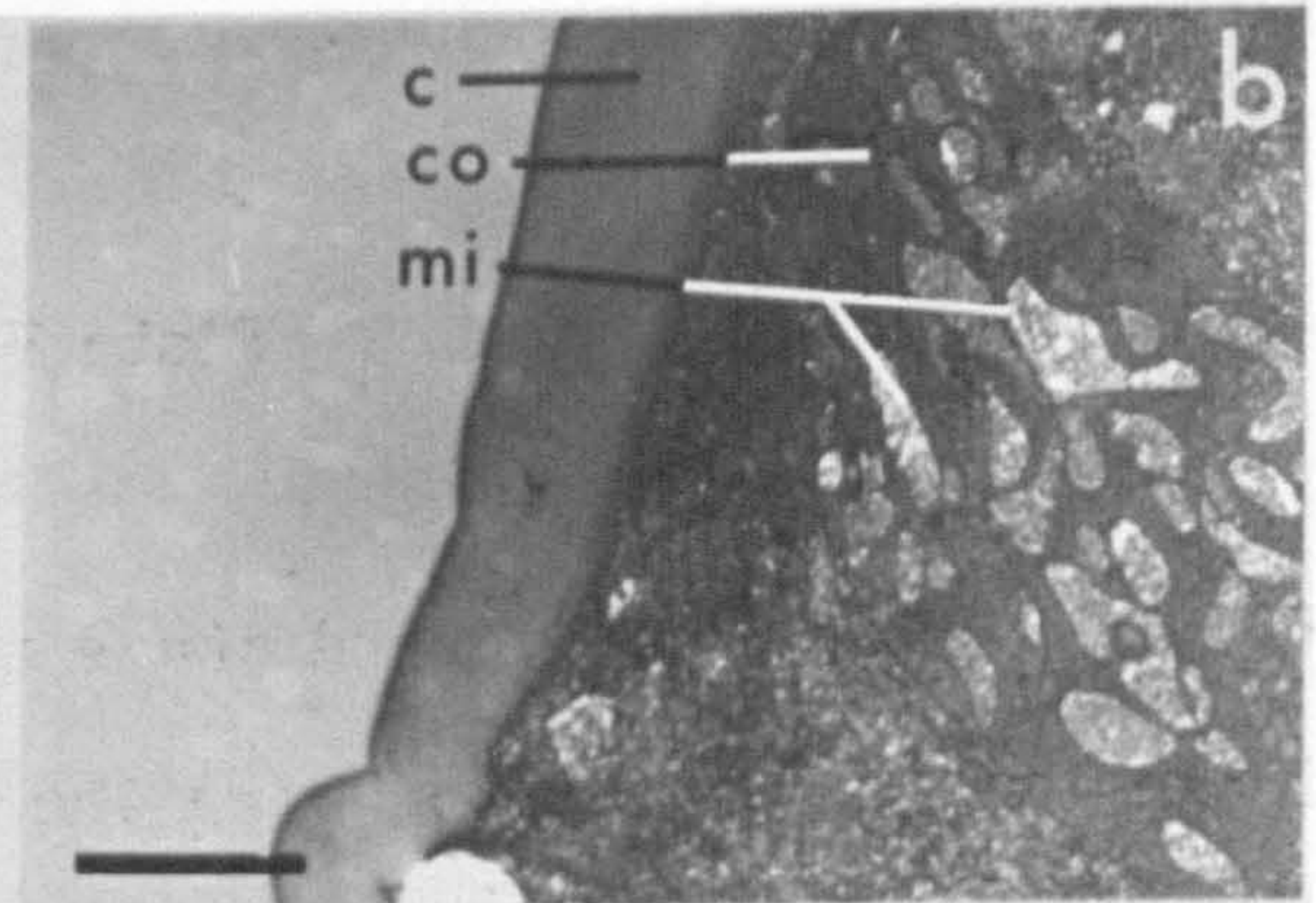
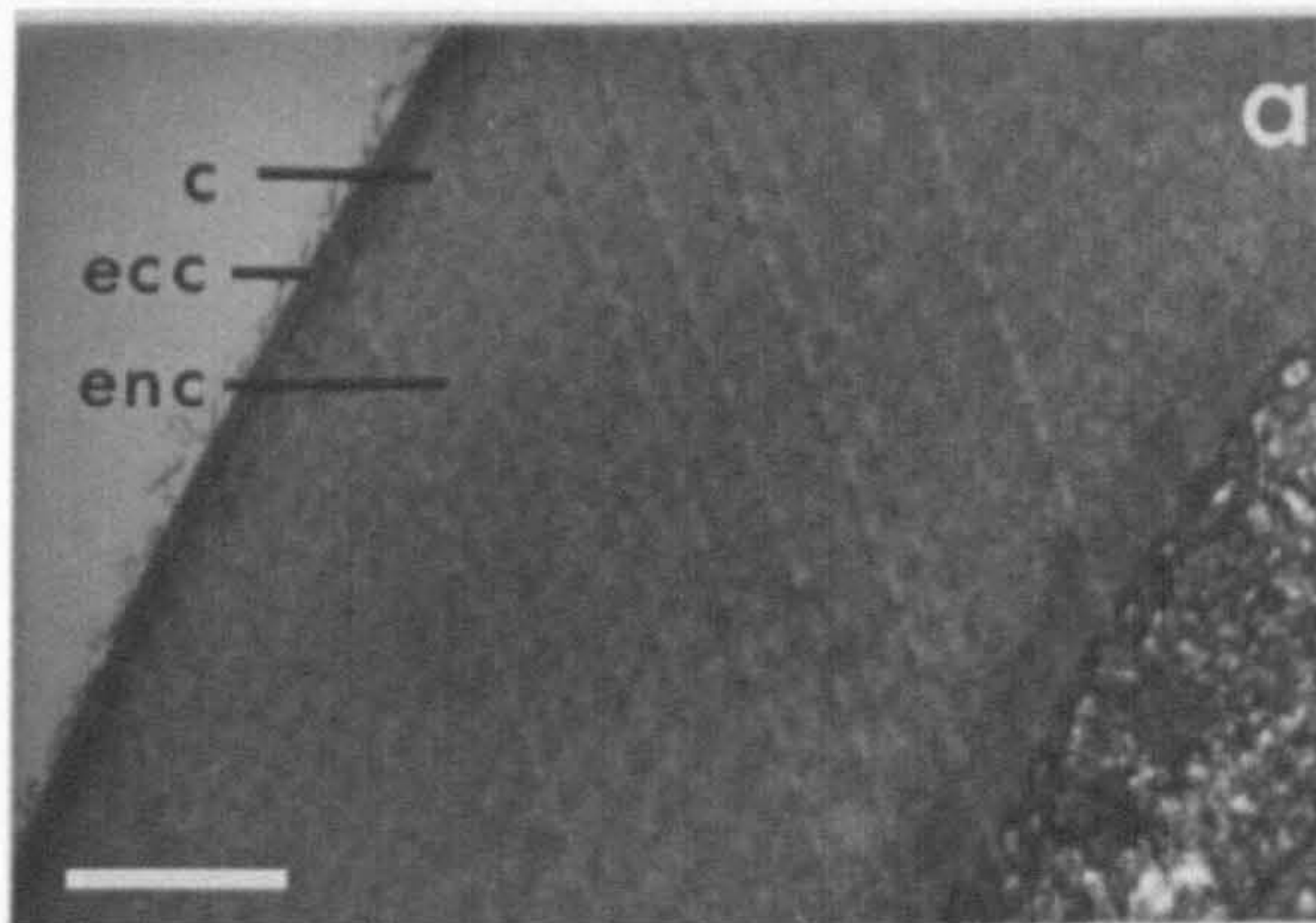
(c), (d) Oblique transverse section showing the retinula cell bodies (which contain the proximal ends of the cones) and the distal rhabdom region.

(e), (f) Transverse section of the proximal rhabdom region showing the regular arrangement of the 8 retinula cells (7 ordinary cells and the four proximal processes of an 8th cell).

Scale bar represents 1 $\mu$ m for (a), 2 $\mu$ m for (f), 5 $\mu$ m for (b) and (d) and 10 $\mu$ m for (c) and (e).

Abbreviations are : c, cornea ; cc, corneagen cell ; co, cone ; coc, cone cell ; cocn, cone cell nucleus ; ecc, ectocuticle ; enc, endocuticle ; l, lamina ; ln, lamina neuron ; lp, lipid deposit ; mi, mitochondria ; r, retina ; ra, retinula axon ; rcb, retinula cell body ; rcp, retinula cell pigment ; rh, rhabdom ; rpc, reflecting pigment cell ; sp, screening pigment.







Screening pigment appears to be restricted to the photoreceptive regions of the ommatidia (retinula cells and rhabdoms) with no evidence for migration of pigments along the perimeter of the cones. The dense pigment layer which occurs between the retinula cell bodies (which contain the proximal ends of the cones) and the distal ends of the rhabdoms (Plate 5.5c,d) may be associated with distal pigment cells which have not yet moved to their adult position between the cones. The smaller pigment granules surrounding the rhabdoms are retinula cell pigments (Plate 5.5e). In transverse section the rhabdoms are shown to be arranged into rows and columns, reflecting the regular arrangement of the corneal facets (Plate 5.2c). The individual rhabdoms demonstrate the same quarter plate organisation as described by Rutherford and Horridge (1965) for Homarus gammarus and are composed of microvilli which extend from the walls of the surrounding retinula cells. They appear subcircular in transverse section and have a diameter of approximately 7.5µm. This size will obviously vary with the direction and position of the cut in relation to the axis of the rhabdom. Each rhabdom is associated with 8 retinula cells (Plate 5.5f). 7 contribute microvilli, 2 cells contributing to each of 3 quarter plates with the 4th plate formed from a single retinula cell. The 8th retinula cell has 4 proximal processes which extend between the other 7 ordinary retinula cells. Lipid deposits are found within the retinula cells.

#### First stage postlarva

The T.E.M. photographs of the eye of the 1st stage postlarva are presented in Plate 5.6. The cornea forms a continuous plate of 4 to 5µm thickness over the surface of the compound eye. It is

differentiated into an outer ectocuticle which is approximately  $0.25\mu\text{m}$  thick and an inner multi-layered endocuticle (Plate 5.6a,b). Immediately below the cornea two triangular shaped corneagen cells are visible (Plate 5.6b). Aréchiga and Atkinson (1975) reported that four corneagen cells are responsible for the secretion of each corneal lens. They are immediately above the cone which appears to achieve direct contact with the cornea between the corneagen cells. The cone is a quadripartite structure and each cone cell contains a Semper nucleus. Two of the four cells are visible in longitudinal section (Plate 5.6a,b). The size of the cones will vary depending upon their position in the eye (Aréchiga and Atkinson, 1975 ; Meyer-Rochow, 1975) but in these sections appeared to be short and robust ( $45$  to  $55\mu\text{m}$  long and  $14$  to  $16\mu\text{m}$  in diameter, Plate 5.6a). The cone extends to the distal end of the rhabdoms. The 'clear zone' is not evident in these sections. 1st stage postlarvae therefore appear to be restricted to apposition type vision.

Screening pigment granules appear to be generally restricted to the photoreceptive regions. There is some evidence of their occurrence at the proximal ends of the cones but this may be an artifact of distortion in the sections. The rhabdoms are fusiform in shape ( $15$  to  $17\mu\text{m}$  long and maximum diameter of approximately  $6\mu\text{m}$ ) and banded throughout with microvilli of alternate layers lying at right angles to one another (Plate 5.6c,d). Each rhabdom is associated with 8 retinula cells (Plate 5.6e), although at the proximal level at which this section was taken the 8th cell is not evident and will be represented by a single small process to one side of the other retinula cells (Meyer-Rochow, 1975). The rhabdoms appear subcircular in transverse section and are surrounded by retinula cell pigments.



## Plate 5.6

T.E.M. of the eye of a first stage postlarva.

(a), (b) Oblique section showing the structure of the cornea, the proximal corneagen cells and the cone.

(c), (d) Oblique longitudinal section showing the elliptical shape of the distal rhabdom and the regular arrangement of the microvilli.

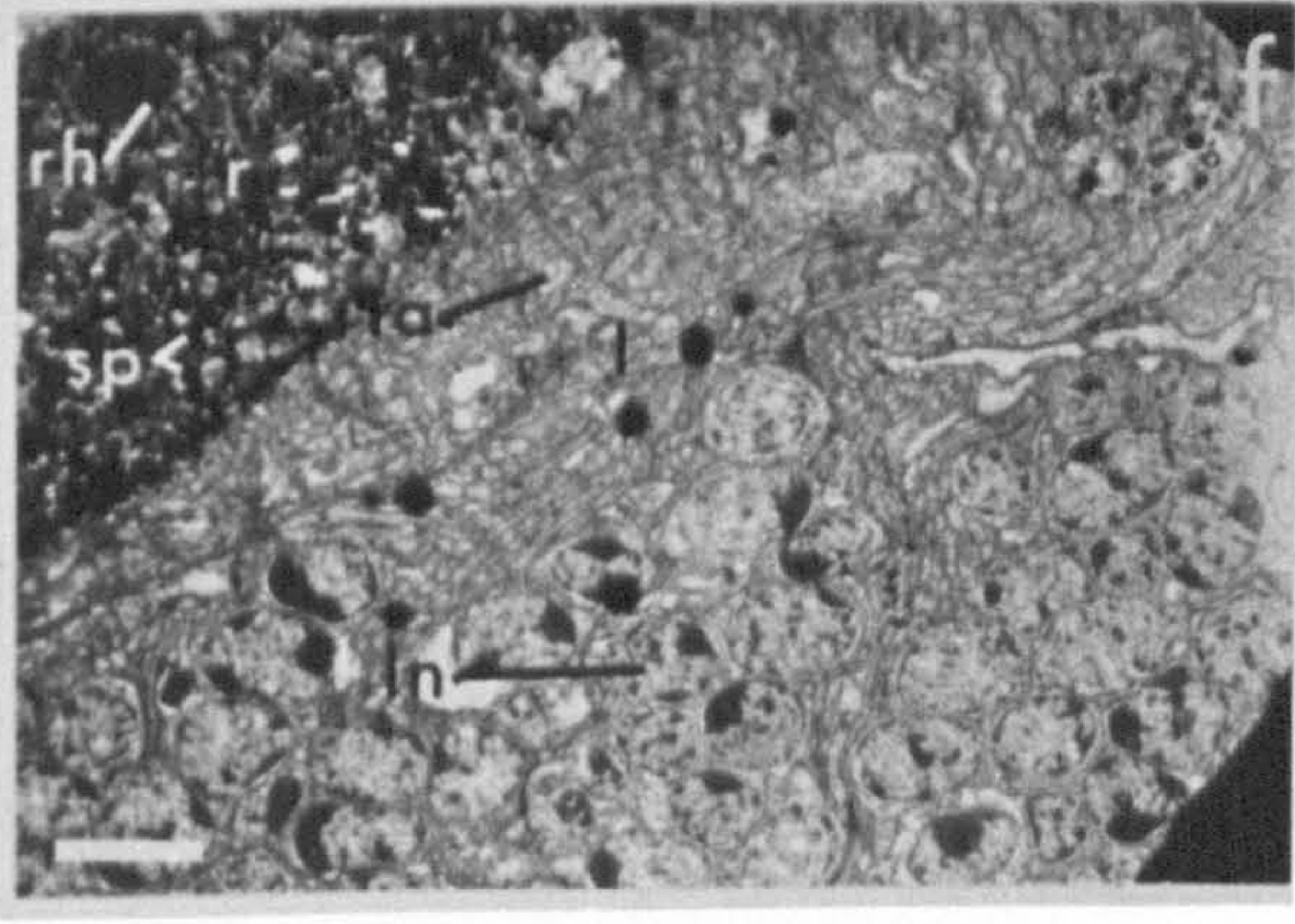
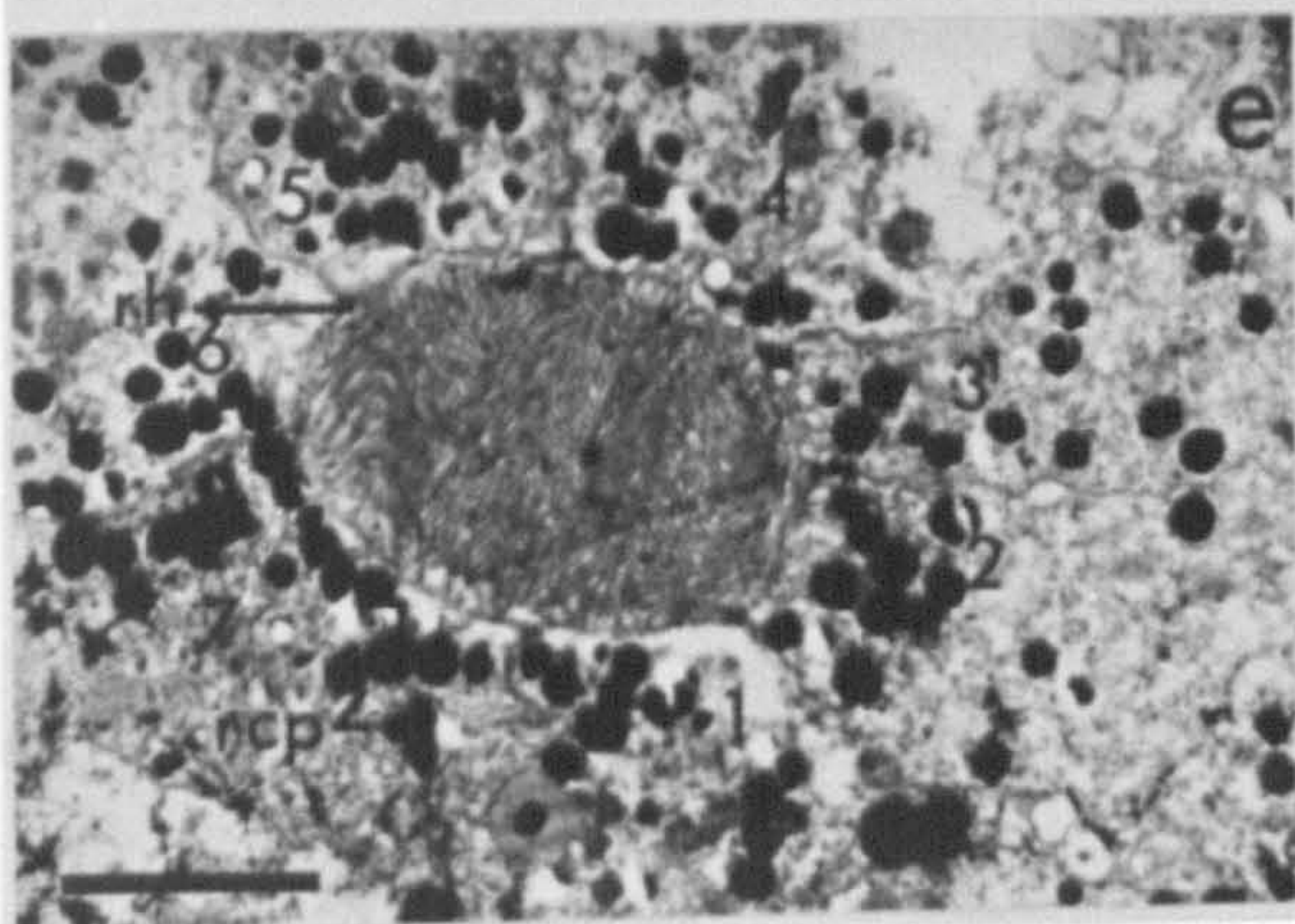
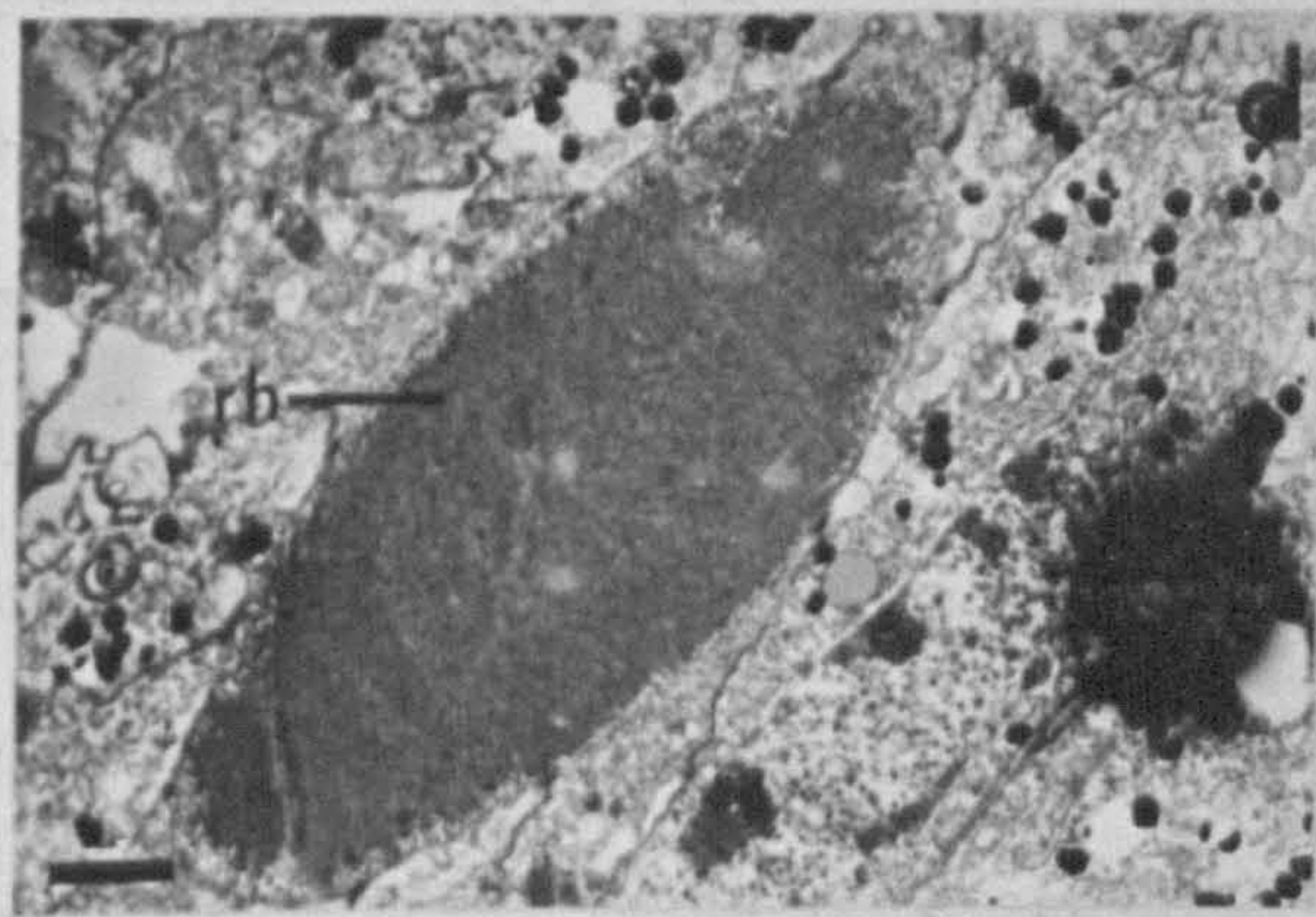
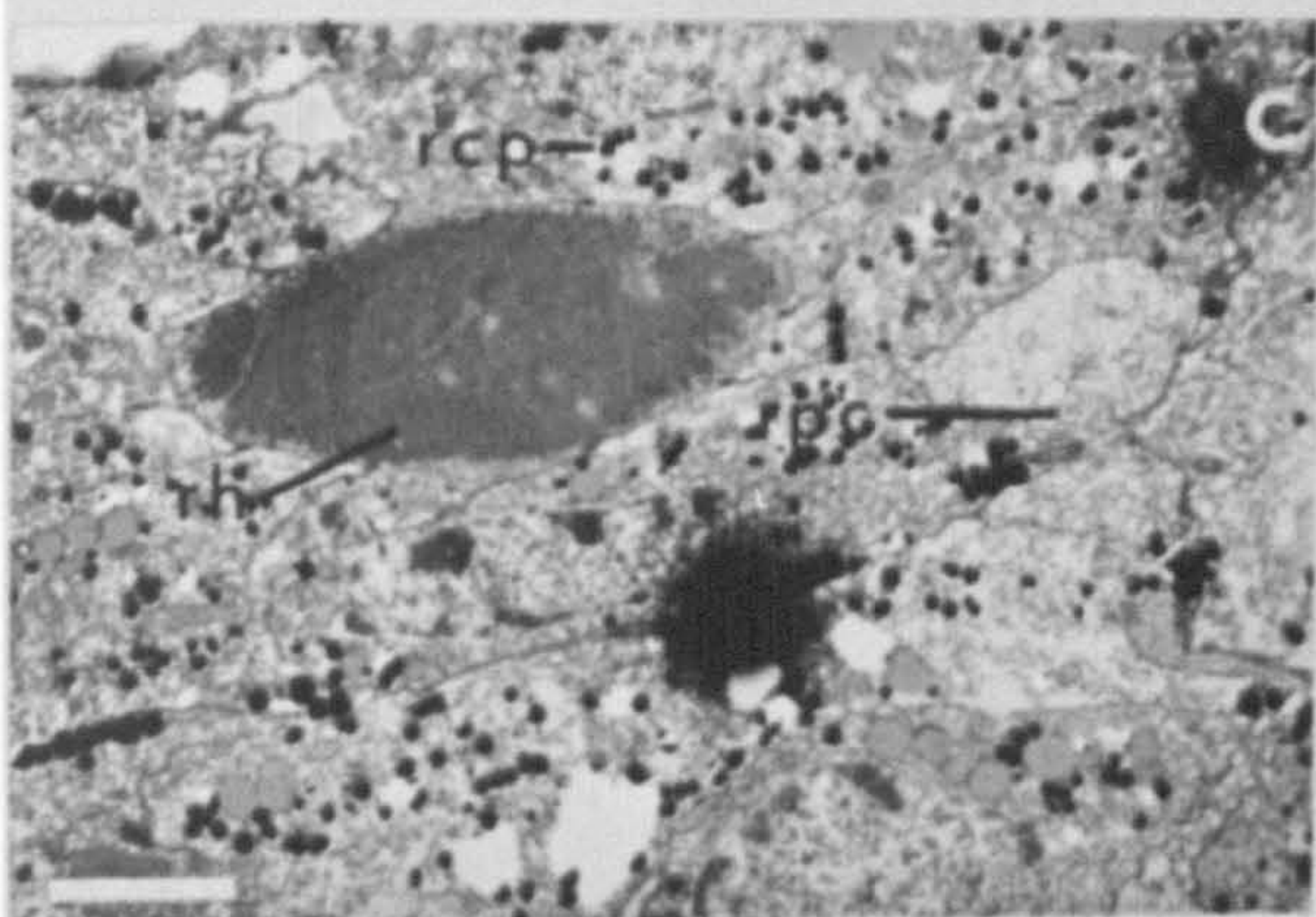
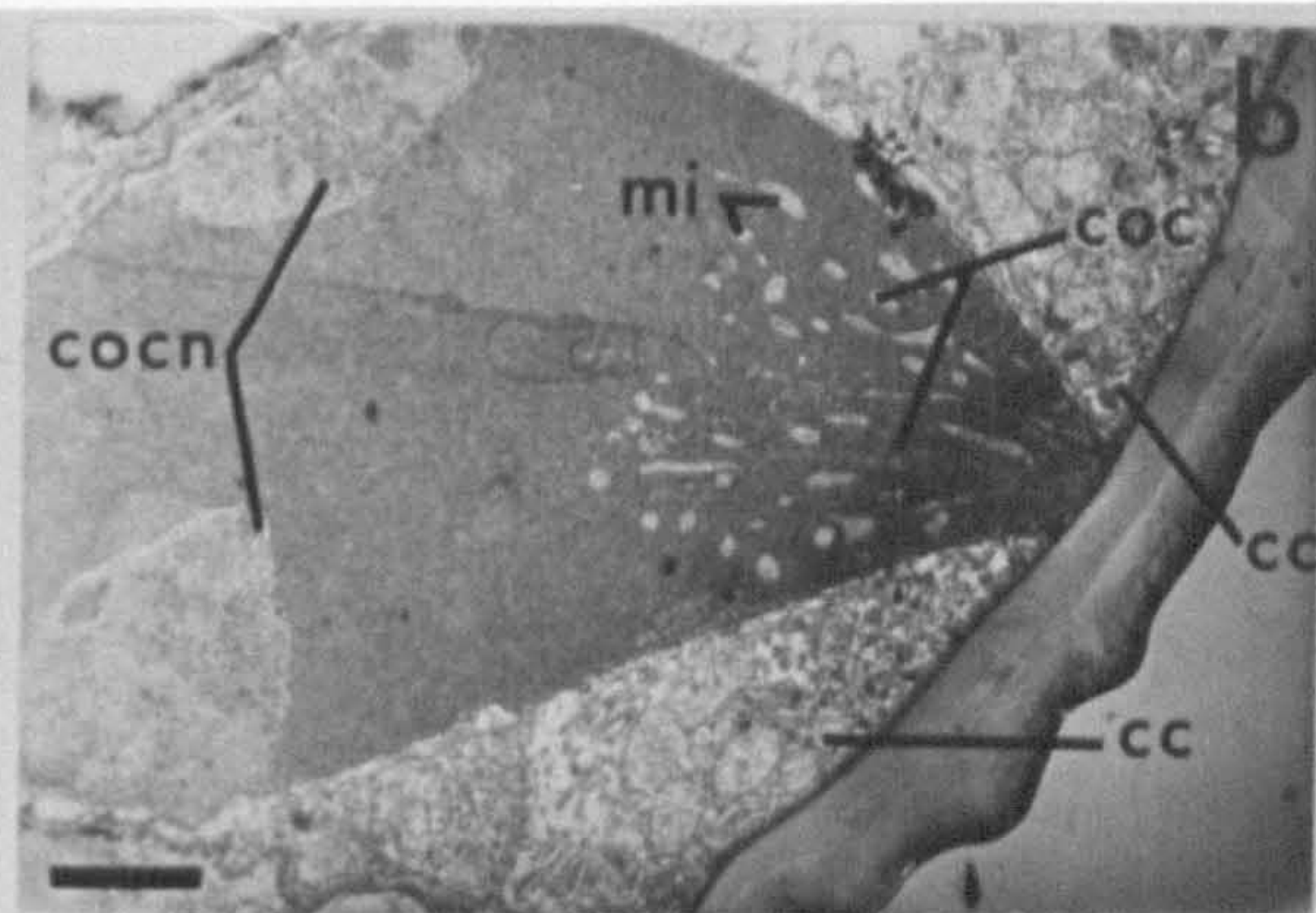
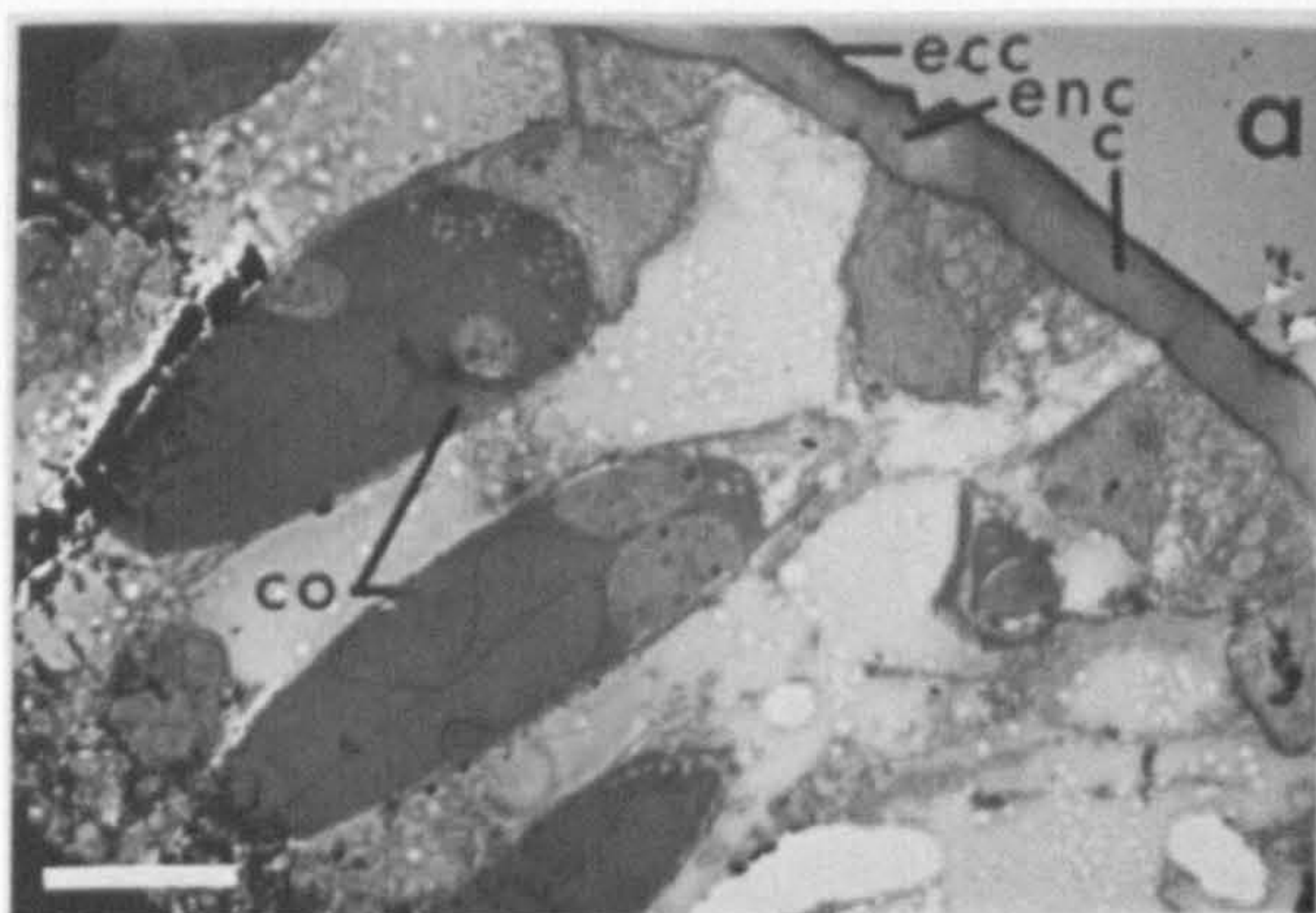
(e) Oblique transverse section of the rhabdom showing the regular arrangement of the 7 ordinary retinula cells. At the proximal level at which this section was taken the 8th retinula cell is not evident.

(f) Oblique transverse section showing the proximal edge of the retina and the underlying lamina.

Scale bar represents 2 $\mu$ m for (d), 5 $\mu$ m for (a) and (c) and 10 $\mu$ m for (b), (e) and (f).

The abbreviations are identified in Plate 5.5.







There is no evidence for the development of a tapetum but there are a few reflecting pigment cells within the retina (Plate 5.6c). A dense layer of screening pigment granules appear to occur towards the proximal edge of the retina (Plate 5.6f). Immediately below this layer lies the lamina. The basement membrane is not visible and is probably masked by the pigment layer. Retinula axons penetrate the lamina where they synapse with secondary neurons whose cell bodies lie in the most distal layer of the ganglion (Plate 5.6f). The lamina is the first of four optic ganglia in stalk-eyed malacostracans. The others are the medulla, lobula complex and lateral protocerebrum (Fincham, 1984).

## 5.4. Discussion

### 5.4.1. Behavioural responses to light conditions and hydrostatic pressure changes

Laboratory based behavioural studies at best give only an indication of how an organism may respond to a particular stimulus in the field. Caution must therefore be used in interpreting the results of this study in terms of the behaviour of the larvae and juveniles in nature.

The behavioural responses of the larvae appeared to change during the final larval stage with a change from positive to negative phototaxis. It has been shown that statocysts are absent in the larval stages of another nephropid lobster, H. gammarus (Neil et al., 1976), first appearing in the 1st postlarval stage. This may indicate that the change in behaviour demonstrated during the 3rd zoeal stage in Nephrops was entirely caused by a change in the light response. In the field, this reversal in phototaxis will cause the older larvae to sink to the bottom where, they may test the substratum at intervals. From the substratum selection experiments (see Chapter 6) it appears that the larvae are able to postpone metamorphosis in the absence of a suitable substratum. Thorson (1964) postulated that a change in the phototactic response from positive to negative probably occurred in the later larval stages for most deep water sublittoral animals. In several species of brachyuran the migration of the later larval stages into the lower regions of the water column has been attributed to a decreasing phototactic response and a shift from geonegative to

geopositive behaviour (Sulkin, 1973 ; Forward and Costlow, 1974 ; Castro, 1978 ; Bigford, 1979 ; Sulkin et al., 1980). The Nephrops zoeae which were photopositive generally remained at the base of the experimental column in darkness. Under those conditions the photopositive individuals behaved similarly to the photonegative individuals of the 3rd zoeal and 1st postlarval stages which remained at the bottom of the column under all lighting conditions. This may indicate the existence of a positive geotactic response but without further investigation this is only conjecture. A more likely explanation may be that swimming activity ceased in photopositive individuals on removal of a light stimulus resulting in passive sinking.

A change in pressure of between 0.3 to 0.5 ats appeared to induce a response. Therefore descent under the influence of gravity should be reversed after sinking 3 to 5m. The pressure changes were presented with reference to an ambient pressure of 1.0 ats. Whether the zoeae would have shown the same responses when adapted to the higher ambient pressures they normally experience in the field (2.0 - 4.0 ats) was not determined. If the pressure response for Nephrops zoeae is similar to that reported for Calanus finmarchicus (Lincoln, 1971) pressure sensitivity is not likely to be greatly affected by the ambient pressure. Homarus gammarus larvae respond to changes in pressure equivalent to a 0.7m change in depth (Ennis, 1973a).

Photopositive 1st, 2nd and early 3rd stage zoeae demonstrated the type 3a response (as categorised by Rice, 1964) to changes in hydrostatic pressure. Thus pressure increase enhanced movement towards the light source when light was directed from above, below or



horizontally. Pressure decrease caused reduced activity and consequent sinking. These responses to changes in pressure were previously reported by Hardy and Bainbridge (1951) for decapod crustacean larvae and pressure sensitivity is known to be a common depth-regulatory mechanism in planktonic animals (Knight-Jones and Morgan, 1966 ; Knight-Jones and Qasim, 1967 ; Rice, 1964, 1967 ; Ennis, 1973a). When a change in pressure evoked a response in Nephrops zoeae which opposed their phototactic response ie. pressure decrease with an overhead light source, the influence of the change in pressure was very short-lived. In this case, the larvae descended over a short distance before moving back into the light beam and reversed the direction of migration, swimming upwards. It therefore appears that the response to light in photopositive Nephrops zoeae over-rides the response to hydrostatic pressure. Similar behaviour was reported for the planktonic freshwater cladoceran Daphnia magna (Lincoln, 1970).

In the light intensity experiments zoeae appeared to become photopositive to an overhead light source at intensities which corresponded to a depth of approximately 65m ( $1 \times 10^{-2} \mu\text{Es}^{-1}\text{m}^{-2}$ ) on a bright day (surface light level,  $1 - 2 \times 10^3 \mu\text{Es}^{-1}\text{m}^{-2}$ , see Chapter 2). The level of the response seemed to increase with increasing light intensity ( $3 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ ) equivalent to about 40m depth. This level of response was maintained at the highest intensity to which they were exposed ( $2.0 \mu\text{Es}^{-1}\text{m}^{-2}$ ), corresponding to a depth of approximately 25m. The diffuse lighting conditions which occur in the field are very different from those which the zoeae experienced in this laboratory study. Caution must therefore be exercised when comparing field and laboratory data. Nevertheless light still provides a good directional stimulus for organisms living at depth in the sea (Blaxter, 1970).

First stage zoeae seemed to respond positively to horizontal light comparable to sea surface intensity ( $1 \times 10^3 \mu\text{Es}^{-1}\text{m}^{-2}$ ) when maintained in glass beakers. Older zoeae were not used in this experiment. However, from their responses to lower light intensities photopositive zoeae from all three stages appeared to demonstrate a similar sensitivity to light. The level of the positive phototactic response demonstrated by newly hatched H.gammarus larvae was reported to be directly related to light intensity over a wide range of values (Ennis, 1973a) ie. more larvae respond at stronger light intensities.

It is known from field observations that Nephrops zoeae are seldom caught in the surface waters. The majority remain at a depth of between 10 - 30m, with limited vertical migration occurring between sunset and sunrise (see Chapter 2). Some means of depth control must therefore exist. The field evidence obtained in Chapter 2 suggests that light and pressure are the two principal variables used by Nephrops zoeae for depth regulation. The laboratory study indicated that Nephrops zoeae are unable to perceive light intensities below  $4 \times 10^{-3} \mu\text{Es}^{-1}\text{m}^{-2}$ . In the field there was weak evidence for an initial midnight dispersion, which preceded the night-time ascent. This seemed to correspond to a reduction in light intensities below the predicted 'threshold' level. Pressure sensitivity will prevent excessive nocturnal sinking and may have been implicated in the nocturnal ascent of larvae to the surface waters. At around dawn the 'threshold' intensity is exceeded and the larvae descend to their normal day-time depth. Depth regulation during the day therefore seems to involve both light and pressure sensitivity and the diel rhythm, which appears to be associated with the vertical migrations, seems to occur as a consequence of diel changes in light intensity (see Chapter 2).

Most zooplankton demonstrate a diel vertical migration pattern. This typically consists of a series of consecutive steps, a dusk ascent from the day-time depth, midnight dispersion, regathering at the surface around dawn and a final retreat to the original day-time depth with increasing light intensity (Cushing, 1951). Naylor and Atkinson (1972) pointed out that endogenous activity rhythms synchronised by dark/light cycles were important in diel vertical migrations for a variety of planktonic crustaceans. The environmental factors which have been implicated in association with biological rhythms for the control of these migrations include light intensity, light quality, gravity and hydrostatic pressure (Rebach, 1983). For H.gammarus zoeae it has been suggested that endogenous biological rhythms, which may be important in vertical migration or maintaining vertical position, are synchronised or reinforced by pressure as well as light/dark cycles (Ennis, 1973a). From Lincoln's observations concerning Daphnia magna (Lincoln, 1970) Ennis (1973a) suggested that the replacement of light by pressure as the dominant sense when light intensity is below a certain minimum level would prevent D.magna from being displaced outside their depth range during hours of darkness. This depth regulatory mechanism was not found to operate for H.gammarus larvae (Ennis, 1973a). Further research is required to determine the mechanisms employed which prevent the ascent of Nephrops larvae into the surface waters during the hours of daylight.

It is known that the diel vertical migration pattern demonstrated by zooplankton involves a descent to deeper cooler waters at around dawn, where they remain during daylight hours. This may therefore remove the organisms from the region of greatest food availability, the euphotic zone, where phytoplankton provides the lowest trophic



level, on which all subsequent food webs are based (Russell-Hunter, 1970). It is generally accepted that diel vertical migrations are involved with feeding (Rebach, 1983) and other hypotheses have been proposed to explain the adaptive significance of migrations which may result in reduced access to food. The two most plausible theories are predator avoidance and improved food assimilation. The former explanation was based on the assumption that during daylight hours visual feeding predators make surface waters unsafe. Enright (1977), while accepting the importance of predator avoidance suggested that this was of secondary importance. He proposed a metabolic model based on the idea that nocturnal feeding might provide a greater net energy gain for growth and reproduction than continuous feeding.

The positive phototactic response demonstrated by 1st stage zoeae to horizontal light under conditions of bright surface illumination may be an artifact of the unnatural laboratory conditions. In the field migration will be less restricted and other 'factors' may prevent migration of larvae into the surface waters (Chapter 2). In addition to pressure these 'factors' may include temperature, salinity and/or biological considerations such as the nutritional state of the zoeae and feeding conditions. The phototactic response of Balanus spp. larvae have been shown to change from positive to negative with an increase in temperature (Ewald, 1912). Exposure to low salinity is known to generally induce a negative phototaxis in normally photopositive larvae (Thorson, 1964).

Although the effects of temperature and salinity changes were not investigated in this laboratory study, during the Nephrops larval season (April to August) the water column is known to become



stratified with the formation of a thermocline and a halocline (see Chapter 2). At the period of peak larval abundance (end of May and beginning of June) the thermocline and halocline are located between 5 - 20m depth. If the phototactic response is reversed under conditions of increased temperature and decreased salinity then the pycnocline boundary may prevent further ascent. During the field study the discontinuity layer was not fully defined and did not prevent a large proportion of the zoeae ascending into the upper regions of the water column at night though few zoeae ascended past it during the day. However, the pycnocline may form a more formidable barrier to the ascent of the Nephrops zoeae later in the season (see Chapter 2).

The attraction of the zoeae to strong light intensities under laboratory conditions may be in itself an artifact of the artificial conditions. In the sea high light intensities may repel the larvae and prevent them ascending to the surface,  $1.6\mu\text{Es}^{-1}\text{m}^{-2}$  was the highest light intensity at which the mean larval depth was recorded for both the spring and neap tidal surveys combined (see Chapter 2). The avoidance of high light intensities may therefore provide a possible explanation for the early morning descent. During daylight hours the light response may therefore reinforce the effect of the discontinuity layer. If it is the combined effect of these 2 factors which prevents the zoeae from ascending into the surface waters during the day, at night field evidence suggests that the pycnocline alone does not prevent an upward migration. The nocturnal ascent may have been a response to decreasing light or may have been a barokinetic over-compensation for night-time sinking (see Chapter 2).

Phototactic responses of larvae may be altered by feeding conditions (Sastry, 1983). Barnacle nauplii were found to demonstrate a more positive phototactic response when starved (Singarajah et al., 1967) as were the first zoeae of the sand crab Emerita onaloga (Burton, 1979). The effect of starvation was not investigated in this study but in the field the majority of the larvae were caught immediately below the pycnocline, where the abundance of food organisms (zooplankton) may be greatest (Russell and Yonge, 1975).

The spectral sensitivity of the Nephrops zoeae which demonstrated a photopositive response did not appear to change during development. Forward and Costlow (1974) also reported no change in the spectral sensitivity between larval stages of the crab Rithropanopeus harrisi. Conversely, Lang et al., (1979) observed changes in the spectral sensitivity for the various larval stages of the barnacle Balanus improvisus.

The Nephrops zoeae appeared to be most sensitive to light within the blue and green regions of the spectrum (400 - 600nm). Although electro-retinogram (ERG) spectral sensitivity curves are not available for the larval and juvenile stages they have been determined for the adult Nephrops (Shelton et al., in press). As expected, the adult ERG determined spectral sensitivity curves reflected the absorbance spectra recorded by Loew (1976) for isolated rhabdoms from adult eyes. The adults showed a maximum sensitivity to flashes of light at a wavelength of about 500nm at an intensity of  $3 \times 10^{-2} \mu\text{Es}^{-1}\text{m}^{-2}$  (P.M.J. Shelton, pers. comm.). This intensity is an order of magnitude below that which brought about a maximum response in the zoeal stages ( $3 \times 10^{-1} \mu\text{Es}^{-1}\text{m}^{-2}$ ). The zoeae do perceive light at lower intensities

but with a reduced response. It seems however that Nephrops zoeae are most sensitive to light within the same wavelength range as the adults.

The depth profile for light attenuation in the Lower Firth of Clyde was measured with a Licor quantum light meter (see Chapter 2). The light curve constructed to describe these readings most closely resembled Jerlov's coastal water type 3 (Jerlov, 1976). Light is best transmitted within the wavelength band 400 – 600nm for coastal water. The results from the present study suggest that Nephrops zoeae show a reduced response to light of shorter (purple and ultraviolet) and longer wavelengths (orange and red). In the sea less than 5.0% of light at the ends of the spectrum will be transmitted below 10m (Jerlov, 1976).

Based on phototactic behaviour the action spectra for the zoeal stages of the crab R.harrissii (Forward and Costlow, 1974) and shrimp Palaemonetes vulgaris (White, 1924) show a maximum at approximately 500nm. Forward and Costlow (1974) reported 2 secondary peaks occurring at 400 and 280nm for R.harrissii. Violet peaks have also been recorded in the action spectra for adult shrimps Palaemonetes sp. (Goldsmith and Fernandez, 1968 ; Seldin, 1968) and the mysid Mysis relicta (Beeton, 1959). There appears to be a secondary peak in the action spectra for adult Nephrops at approximately 400nm and this may correspond to the response of the 8th retinula cell (P.M.J.Shelton, pers. comm.). Further investigation is required to determine the spectral sensitivity curve for the zoeal stages and to determine the function of the 8th retinula cell.



The orientation adopted by the Nephrops zoeae during swimming was originally reported by Foxon (1934), the telson and abdomen are directed towards the light source and the head and thorax lie in a plane at right angles to them. Although this is a different posture from that reported for H.gammarus (Foxon, 1934 ; Neil et al., 1976), which swims with its abdomen downcurved, the method of propulsion appears similar. Each species has invested in a specialized swimming system for the larval stages involving the development of the thoracic exopodites. A qualitative description of limb co-ordination and the development of a metachronous wave motion created by the exopodites during active swimming in the larval stages of H.gammarus has been presented by Neil et al. (1976). Although this investigation did not involve a detailed study of the swimming motions in Nephrops zoeae they also appeared to utilize a metachronous wave motion with forward propulsion provided by the downward 'powerstroke' of the exopodites.

The method of reversal (ie. change in direction of movement) demonstrated by the Nephrops zoeae in response to strong illumination from below often involved initial tumbling before descending in a spiral swimming path. Similar behaviour has been reported for H.gammarus larvae (Neil et al., 1976). This disorientated behaviour suggests the lack of a geotactic response and indicates that Nephrops zoeae, like the equivalent stages of H.gammarus (Neil et al., 1976), lack statocysts. This is not surprising as these animals appear to orientate primarily to light and possess well developed compound eyes.

The swimming speeds and sinking rates recorded for the Nephrops zoeae increased from 21.2 to 30.3 mms<sup>-1</sup> and 22.0 to 30.4 mms<sup>-1</sup>,



respectively, between the 1st and 3rd zoeal stages. These speeds were recorded within the experimental column and were therefore conducted over short distances (approximately 0.3m). Even if these swimming speeds and sinking rates (which equate to approximately 76 to 108  $\text{mh}^{-1}$  and 79 to 109  $\text{mh}^{-1}$  respectively) are only short lived, the zoeae are clearly capable of adjusting their position in the water column. These swimming speeds may also be important in providing the zoeae with the ability to avoid potential predators. From field observations concerning H.gammarus, Ennis (1973a) reported an increase in the maximum rate of both upward and downward movement between zoeae representing the 1st and 3rd stages. Between these stages the maximum rate of upward movement increased from 144 to 180  $\text{mh}^{-1}$  and the maximum rate of downward movement from 90 to 120  $\text{mh}^{-1}$ . Rimmer and Phillips (1979) also recorded an increase in the swimming speeds and sinking rates between the early and later larval stages of the western rock lobster Panulirus cygnus.

The rate of passive descent appeared to increase slightly between 1st stage zoeae and 1st stage postlarvae (8.6 to 10.8  $\text{mmh}^{-1}$ , equivalent to 31 to 39  $\text{mh}^{-1}$ ). If the larvae were entirely dependent upon a phototactic response for maintaining their position in the water column, one might expect that during darkness the negative buoyancy of the larval stages would cause them to sink to much greater depths than was evident from the field observations (see Chapter 2). However, the laboratory studies were conducted under static conditions which are very different from those which the larvae would experience in the field.

Although pressure sensitivity in decapod crustacean larvae was discovered in the early 1950's by Hardy and Bainbridge (1951) the mechanisms involved in its perception are not fully understood. It is known that these pelagic stages can detect very small changes in pressure and it has been suggested that this sensitivity may involve electrode action (Digby, 1965). In a later investigation Digby (1972) suggests that pressure sensitivity is achieved through the compression of a thin layer of hydrogen gas. According to Digby (1972) this would create a current flow through the semi-conducting tanned cuticle and hence provide a mechanism for detecting small changes in pressure. He concludes that deposition of calcium salts occurs in a similar way and that systems involving calcification may be associated with sensitivity to hydrostatic pressure.

#### 5.4.2. Structure of the eye

Metamorphosis in Nephrops corresponds to the moult from the 3rd zoea to the 1st postlarva, during which they undergo dramatic morphological changes (see Chapter 4). These include the development of fully setose abdominal pleopods which replace the now residual exopodites of the thoracic appendages for swimming. This causes a change in posture and direction of swimming, with the postlarva moving head first instead of the telson first swimming posture of the zoeal stages. Similar changes accompany metamorphosis in the common prawn Palaemon serratus (Fincham, 1984).

As in the larvae of other decapod crustaceans (Meyer-Rochow, 1975 ; Fincham, 1980), Nephrops larvae possess apposition type compound eyes. At metamorphosis there does not appear to be an abrupt change to

the reflecting superposition type of eye characteristics of the adult Nephrops (Aréchiga and Atkinson, 1975).

Apposition optics are usually correlated with diel activity involving migration into regions of high illumination (Meyer-Rochow, 1975) and characterized by hexagonal corneal facetting (Fincham, 1980). Hexagonal patterning is a feature of the Nephrops zoeal eye and forms an economic shape for packing the circular corneal lenses of each ommatidium. The zoeae also undergo limited vertical migrations (see Chapter 2).

In apposition type eyes, because of the high refractive index of the cones, each ommatidial unit is isolated and only rays parallel to the ommatidial axis fall on the retina (de Bruin and Crisp, 1957 ; Nilsson, 1983 ; Fincham, 1984). The light-receiving ommatidia cooperate to form an inverted mosaic image (Horridge, 1980 ; Fincham, 1984). The dependence upon a high refractive index for optical isolation of the ommatidia appears to be a feature of the eye of Nephrops zoeae. The dioptric region of the ommatidia (cornea and cones) appears to be completely transparent in live animals with all pigment restricted to the underlying photoreceptive region (retinula cells and rhabdoms) (Plate 5.1). According to Nilsson (1983) the planktonic larvae of most euphausiids and decapods possess a transparent type of apposition eye which is pre-adapted for superposition optics. This will form the basis of the reflecting superposition optics of the adult Nephrops (Fincham, 1980).

The development of a clear zone distal to the retina cell layer and the transformation of the robust larval cones into rectangular



boxes are pre-requisites for conversion from apposition to superposition type optics (Kunze, 1979 ; Land, 1980 ; Fincham, 1984). These features have been described for the reflecting superposition eyes of the adult Nephrops (Aréchiga and Atkinson, 1975 ; Loew, 1976). There is no evidence, however, for their development within the optical apparatus of the first postlarval stage which still appears to possess short elliptical cones. Also, a tapetum has not been formed by this stage and only isolated reflecting pigments occur within the retina. The transformation to the superposition eye appears to be gradual beginning with a gradual 'squaring - off' of the corneal facets during the 3rd zoeal stage. The development of square facets is essential to the mirror mechanism of superposition optics (Vogt, 1977, 1980) and this appears to have been completed by the 3rd postlarval stage. The facets are then perfectly square and arranged in an orthogonal matrix. Similar changes for the development of superposition optics were observed for palaemonid shrimps (Fincham, 1980, 1984).

Superposition optics have been developed for their improved light-gathering capacity under low ambient light conditions (Land, 1981). Not surprisingly therefore, superposition eyes are only generally found in decapods living in low light levels. From recent studies it appears that eye structure may provide a useful feature for future taxonomic schemes for malacostracan crustaceans (Fincham, 1980).

In adult Nephrops the movement of the proximal shielding pigment facilitates light and dark adaptation. (Shelton et al., 1986). Previous reports of distal pigment migration are considered by these authors to have been an artifact of ommatidial damage. In the dark



adapted state the proximal pigment migrates to a position level with the bottom of the rhabdoms (Shelton et al., 1986). This, therefore, exposes the photoreceptive pigments within the microvilli of the rhabdom to the full extent of the superposition reflected rays. In this position the tapetum is also unshielded and the rays are reflected through the visual pigments of the rhabdom for a second time, further enhancing the use of available light. During light adaptation the proximal pigment moves distally surrounding the rhabdoms and shielding the tapetum (Shelton et al., 1985).

If the distal pigments are stationary (as suggested by Shelton et al., 1986) then during light adaptation screening pigment migration accompanied by certain morphological changes in the retinula cells (Rao, 1985) will contribute the principal photomechanical adaptations to reduce eye sensitivity. The redistribution of the proximal screening pigment will help to control the effective aperture and field of view of the ommatidia (Rao, 1985). Depending upon the extent of the migration, the angle of acceptance may effectively isolate each ommatidium. This, would, therefore temporarily restore the apposition type optics of the larval stages.

In Nephrops an 8th retinula cell has now been located in both the apposition eyes of the larval stages (this study) and the reflecting superposition eyes of the adults (P.M.J.Shelton, pers. comm.). In common with several other decapods, the 8th retinula cell does not appear to contribute to the main rhabdom but probably forms a smaller rhabdomere overlying the main structure (Shaw and Stowe, 1982).

In previous studies attempts have been made to determine the function of the 8th retinula cell, which probably filters all light which reaches the main rhabdom (Shaw and Stowe, 1982). Cummins and Goldsmith (1981) have shown that the 8th retinula cell is the only violet receptor in the ommatidia of crayfish. By analogy, the 8th retinula cell may be responsible for the ERG responses to violet light in adult Nephrops (P.M.J.Shelton, pers. comm.). The ability of adult Nephrops to perceive light within this region of the spectrum may have no functional value, however, as less than 0.6% penetrates below 10m (Jerlov, 1976).

At the distal end of the 8th retinula cell in the western rock lobster, Panulirus longipes, Meyer-Rochow (1975) reported the existence of a 'crystal-like' lens structure. He suggested that this structure may function as a lens to increase acuity in the light adapted state by focusing incident light on to the thin column of the distal rhabdom. Further research to determine the anatomical nature and spectral sensitivity of the 8th retinula cell in Nephrops is currently being undertaken (P.M.J.Shelton, pers. comm.). This should help promote a better understanding of its function.

## CHAPTER 6

### Substratum selection and burrowing behaviour in juvenile Nephrops

#### 6.1 Introduction

Despite the economic importance of Nephrops the ecology and behaviour of the early postlarval stages are poorly understood. This may be partly attributed to the use of inappropriate sampling gear (Farmer, 1975) and also to the fact that juveniles appear to remain within their burrows and so are not available to towed gear (Chapman, 1980). All stage 3 zoeae and juveniles used in this laboratory study have therefore been supplied from the culturing facility.

It is generally considered that moulting from the third zoeal stage to the first postlarval stage represents the metamorphosis between larval and juvenile phases in Nephrops (Farmer, 1975) and in this investigation the first postlarval stage is synonymous with the first juvenile stage. In this study inconsistent phototactic responses were observed for third stage zoeae (Chapter 5). Therefore, there was reason to believe that during this final larval stage Nephrops may descend through the water column with metamorphosis into the first postlarval stage occurring on or within the vicinity of the bottom. If this is the case then substratum type may influence the duration of the intermoult period in this stage. Similar studies involving Homarus americanus (Cobb, 1968 ; Botero and Atema, 1982) have established that the larval stage immediately prior to metamorphosis was able to extend the intermoult period when provided

with an unsuitable substratum for settlement. It is known that adult populations inhabit cohesive mud substrata (Farmer, 1975) consisting of various proportions of silt and clay (Chapman, 1980 ; Bailey et al., 1986) and an investigation was carried out to determine the selectivity of the early postlarval stages when provided with different substrata. Substratum selection trials with H.gammarus (Howard and Bennett, 1979 ; Howard, 1980, 1983) and H.americanus (Pottle and Elner, 1982 ; Botero and Atema, 1982) have demonstrated active selection of certain substratum types by the juvenile stages of these lobsters. These substrata tended to be different from those inhabited by the adults.

It is reported that the mechanisms involved in substratum selection by the juvenile stages of H.americanus include negative phototaxis and positive thigmotaxis (Hadley, 1908 ; Cobb, 1971 ; Botero and Atema, 1982). In the present work attempts were made to determine some of the controlling influences involved in substratum selection by juvenile Nephrops.

Juveniles beyond the first postlarval stage have been recorded in the plankton (see Chapman, 1980). In the present work a comparison of the settling and burrowing activities of different early postlarval stages has provided a basis for the interpretation of these pelagic records.

The burrows constructed by juveniles are described in detail. There are few previous records. Crnković (1968) looked at the burrows and burrowing behaviour of a 5th and a 7th stage postlarval Nephrops in laboratory aquaria. The continual excavation of new shafts led



Crnković to conclude that they burrowed to feed. In the field it is known that juvenile burrows may be associated with adult burrows (Rice and Chapman, 1971, 1974 ; Chapman, 1980 ; Nash, 1980 ; Atkinson, 1986). The present study investigates burrowing in juveniles, examines juvenile / adult burrowing interactions for the first time and discusses the implications and benefits derived from the behavioural patterns demonstrated in the laboratory by the various postlarval stages.

## 6.2. Materials and methods

Mud was collected for this study using a mud bucket from a position 2km south of Little Cumbrae (depth 80m). This area was within the extensive silt-clay facies recorded and described by several authors (Gair, 1967 ; Deegan *et al.*, 1973 ; Pye, 1980 ; B.G.S., 1985). Although some variation has been found to occur within this area (Pye, 1980 ; B.G.S., 1985), the bulk of the sediment consists of silt and clay particles with sand accounting for less than 10%. Such a sediment is classified as mud (B.G.S., 1985) according to the system devised by Folk (1974) and supports an extensive *Nephrops* fishery (Bailey *et al.*, 1986). Before depositing the sediment in the various containers it was carefully examined and the carnivorous macrofauna was removed.

All units containing mud were made up one month prior to use, allowing the mud based substrata time to consolidate. During this period and when the units were not in use, they were kept immersed under flowing water conditions to maintain aerobic conditions within the substrata. Immediately before the trials were begun the water supply was disconnected. The overlying water was carefully siphoned off and, using the same procedure, replaced with water from the pretreatment tank (Chapter 3) which had been adjusted to the temperature of the water it was replacing (14 - 16°C).

Sand, obtained from Kames Bay (Isle of Cumbrae), had a median grain size of approximately 0.25mm, which is classified as fine-medium sand according to the Wentworth scale (Folk, 1974). Gravel, of unknown source, had an average particle diameter of between 5 - 10mm, which is

within the size range of commercial gravel (Deegan et al., 1973) and corresponds to the small to medium pebble category as devised by Folk (1974).

All larvae and juveniles used in these studies were provided from the culture facility (Chapter 3) having been maintained under conditions of controlled dim laboratory lighting at levels of intensity below those considered to cause rhabdomic degeneration (Shelton et al., 1985).

#### 6.2.1. Effect of substratum type on intermoult period prior to metamorphosis

Newly moulted third stage zoeae were removed from the culturing facility and maintained individually in polypropylene bowls (0.3l volume, 110mm diameter, 50mm height) which contained either mud, sand, gravel or no sediment (control). The substratum was added to a depth of 10mm in each bowl and water added to a depth of 30mm above the substratum surface. Partial water change was performed daily. Conditions of dimmed (curtains) natural illumination were provided and food was administered as in the culturing programme (Chapter 3), with uneaten remains removed before further additions. Behavioural observations were made throughout the investigation and the intermoult period for each individual was recorded as was percentage survival to metamorphosis during each treatment.

#### 6.2.2. Substratum selection

Throughout these trials substratum selection refers to the active

selection and migration on to a particular substratum. Settlement denotes further development of this initial response and involves the demonstration of burrowing behaviour and evidence for final settlement to the substrata.

In sections 6.2.2. - 6.2.3. all trials were conducted under static water conditions and after the completion of each trial the test animal was removed and the water was changed. In sections 6.2.2.- 6.2.4. the experiments were conducted under conditions of green illumination on the assumption that the spectral range of the postlarval stages is likely to be similar to that of adults (Loew, 1976). Green fluorescent tubes (Thorn : 95% transmission between 500 and 600nm, wavelength maximum 550nm) provided the light source. Light intensities were adjusted to levels found in the field (Chapter 2) during summer at midday at 25 - 30m depth ( $1.0 - 1.4 \mu E.s^{-1}m^{-2}$ ). This intensity range is below that which causes damage in adults (Shelton *et al.*, 1985). Behavioural observations were made throughout these trials.

The investigation in this section was divided into two parts, the first involved a choice of different substratum types and the second a choice of different mud / sand percentage mixtures. All trials were conducted with individual early postlarvae of the first 4 stages.

In the first experiments the choice chambers consisted of square polypropylene tanks (200 X 200 X 90mm) which had been subdivided into 4 equal sized areas by the insertion of 4 square petri dishes (100 X 100 X 20mm). Each dish was then filled with the appropriate substratum and, after the period of consolidation, water was supplied to a depth



of 60mm above the substratum surface.

The substratum types chosen were mud, sand, a mixture of the two previous substrata in equal proportions and gravel. The mixed substratum will be composed of between 50 to 56% sand depending upon the initial sand content of the mud substratum. The mixture therefore conformed to the muddy-sand category as defined by Folk (1974). 5 of the 6 units constructed provided a combination of choices of 2 of the substratum types, with the same substratum represented in 2 of the 4 regions at diagonally opposite positions (Figure 6.1a). The 6th unit provided a choice of all 4 substrata (see Table 6.2).

During placement the test animal was confined within a 25mm diameter plastic pipe placed vertically on a square glass slide (30 X 30mm) over the central region of the choice chamber. After 5min acclimation the piping was slowly lifted to release the lobster. The glass slide was removed from the chamber once the juvenile had moved off it. The position of the animal was monitored at 30s intervals for a period of 15min and then at 15 min intervals for a further 2h. If settlement appeared to occur within the 2h period the individual was removed to another substratum type within the choice chamber and observed for evidence of migration back to the substratum it had been displaced from.

In the second series of experiments the choice chamber consisted of a single rectangular polypropylene tank (700 X 460 X 300mm) which had been partitioned into 12 equal sized areas (170 X 145 X 165mm). Each area was completely filled with the appropriate substratum and, after the period of consolidation, water was supplied to a depth of

**Figure 6.1**

**(a) Diagram of the chamber used to provide a choice of two (or in one case four) sediment types. In this example the choice was mud (M) or sand (S).**

**Scale bar represents 0.05m.**

**(b) Diagram of the chamber used to provide a choice of different proportions of mud and sand. The figures refer to the percentage of mud that contributed to the substrata in each area.**

**Scale bar represents 0.05m.**

**a**

<b>M</b>	<b>S</b>
<b>S</b>	<b>M</b>

---

**b**

<b>80</b>	<b>60</b>	<b>0</b>
<b>100</b>	<b>40</b>	<b>20</b>
<b>60</b>	<b>0</b>	<b>40</b>
<b>20</b>	<b>100</b>	<b>80</b>

---

120mm above the substratum surface. Any substratum which had been displaced into neighbouring areas was siphoned off. The mud and sand substrata were mixed in various proportions to provide substrata of 100, 80, 60, 40, 20, and 0% mud (Figure 6.1b). This choice therefore included the Folk (1974) sediment categories ; mud, sandy-mud, muddy-sand and sand. Each of the 6 mixtures were represented in 2 of the 12 areas within the choice chamber and arranged so that like sediments were distant from each other.

The method of placement was the same as used in the first series of experiments and the animal positions were monitored continually for the first 30min and then at 5min intervals for the remainder of the trial. The period of observation for each trial was 4h with locomotor activity plotted on a diagram of the chamber to provide an approximate record of the time spent on each mud / sand mixture. If settlement had appeared to occur within the 4h period the individual was removed to a different substratum type and observed for confirmation of this 'choice' by migration back to the original substratum or to one of a similar type. Qualitative data on initial burrowing and exploratory behaviour were also recorded during these trials. Before starting a new trial or relocating an individual for confirmation of its choice, all previous excavation was carefully filled in so as not to bias further selection.

Additional observations were made of the burrowing and exploratory behaviour demonstrated by older postlarvae (stages 4 and 5) for which field evidence suggests a benthic habit (Chapman, 1980). These were introduced to acrylic tanks (330 X 220 X 200mm) without sediment or containing sand or gravel (unsuitable for normal



burrow excavation). This allowed observations to be made on their ability to leave the 'unsuitable' substrata and make vertical migrations into the overlying water.

#### 6.2.3. Settlement behaviour in relation to existing burrow openings

Stages 1 to 4 postlarvae were individually introduced into acrylic bowls (4l volume, 200mm diameter, 160mm height) which contained consolidated mud deposits of approximately 70mm depth. These deposits were modified in various ways to imitate burrow openings. This included the hand excavation of 'burrows' or depressions (or the use of burrows excavated by <sup>other</sup> juveniles) and the introduction of lengths of plastic piping and circles of black card, with various combinations of each being presented to the test animals.

On occasions when juveniles formed a burrow, either in association with, or completely independently of the artificial structures presented to it, further trials were conducted to determine the response of other juveniles towards the vacated burrow. These juveniles were introduced individually once the original resident had been removed from the bowl.

#### 6.2.4. The burrows and burrowing behaviour of the juvenile stages

Members of all the postlarval stages (stages 1 to 11) that were reared during the course of the ongoing culture programme (Chapter 3) were individually provided with a consolidated mud substratum. This was contained in acrylic bowls (4l volume, 200mm diameter, 160mm height). The mud depth was approximately 70mm for all stage 1 to 5

postlarvae. Larger acrylic tanks (260 X 100 X 360mm) containing 200mm depth of mud were used for the older juveniles. The bowls were maintained under static water conditions and the tanks within a continuous water flow. Air-stones suspended just below the water surface providing aeration in both cases. Food was provided daily in the form of live Artemia and frozen mysids and qualitative data on burrowing, exploratory and feeding behaviour were recorded for each individual.

Adult Nephrops were collected from creels (see Chapter 1) and brought back to the laboratory for introduction to holding units containing previously prepared mud substrata. Eight rectangular polypropylene baths (270 X 190 X 230mm), six acrylic rectangular tanks (450 X 100 X 380mm) and one glass aquarium (1850 X 500 X 500mm) were used. The mud depths in the baths and tanks were approximately 120 and 240mm respectively and each was stocked with one adult Nephrops and supplied with water in a flow through system. The aquarium was filled to a depth of approximately 200mm and stocked with four adults and partial water change was performed twice a week. The water temperatures fluctuated in the flow-through systems from 10 - 14°C and between 13 - 16°C within the aquarium. Air-stones were suspended to within 50 - 100mm of the substratum and conditions of dim green laboratory lighting were maintained throughout.

The adult Nephrops were fed quantities of frozen brown shrimp (Crangon crangon) daily. Once burrow systems had been excavated, a single juvenile Nephrops was added to each of the baths and tanks, and two juveniles were introduced to the aquarium. The behaviour of the juveniles was continuously monitored until settlement was considered

to have occurred, either through prolonged disappearance into an adult burrow or independent excavation of a separate burrow.

Some of the burrows constructed by individual juveniles and all adult burrows to which juveniles had been added, were cast using polyester resin (AP 101 PA with liquid catalyst, marketed by Trylon Ltd.). The method was similar to that used by Atkinson (1974), Farrow (1975) and Nash (1980) and detailed in Atkinson and Chapman (1984). Immediately before the resin was introduced the juveniles were successfully removed from their individual burrows by injecting a hypersaline solution down one of the surface openings. This was also successful in removing some of the lobsters from the adult / juvenile systems but others were only removed during resin casting when they appeared at the surface. Only in one case did an individual remain trapped within a cast (Plate 6.8).

After a period of 1 - 2 days when the resin was fully set, the casts were carefully removed from the sediment to be individually described (Table 6.5) and photographed (Plates 6.2 - 6.10).

### 6.3. Results

#### 6.3.1. Effect of substratum type on intermoult period prior to metamorphosis

Immediately following introduction on to the substratum most of the stage 3 zoeae spent a considerable time swimming over the substratum surface making frequent migrations up into the surface waters. This activity appeared to decrease with time and within a few days most zoeae remained generally stationary on the substratum surface. During the course of these trials some of the stage 3 zoeae which died before moulting into the first postlarval stage remained within this zoeal stage for periods well in excess of the average intermoult period (9.45, 9.89, 12.73 and 13.25 days for sand, mud, gravel and control respectively) for the given substratum, with some surviving for as long as 30 days. Some of the larvae which had been provided with a gravel substratum became hidden within the crevices. Table 6.1 suggests that sediment type appears to exert little influence over the proportion of zoeae metamorphosing successfully into the first postlarval stage. The values were similar under all 4 conditions with an average of approximately 44%. On the other hand there were differences in the intermoult period for zoeae maintained on the 4 substratum types (Table 6.1). The intermoult period was significantly shorter ( $P < 0.05$ ) on mud and sand substrata than on gravel or no sediment (ie. control). The small difference in intermoult period of zoeae maintained on the mud and sand substrata was not significant. The small difference in intermoult period of zoeae on gravel and no sediment (ie. control) was not significant.



Table 6.1

Effect of substratum type on survival to metamorphosis and on intermoult period prior to metamorphosis.

3rd stage zoeae			1st stage postlarvae		
			Intermoult period (days)		
Substratum	N	Percentage survival to metamorphoses	Mean durn.	S.D.	Range
Control	9	44.44	13.25	2.22	11-16
Mud	21	42.86	9.89	1.90	8-14
Sand	23	47.83	9.45	0.93	8-11
Gravel	27	40.74	12.73	2.24	10-17

### 6.3.2. Substratum selection

The substratum choices provided for each test in the first part of the substratum selection experiment are shown in Table 6.2. There appeared to be no difference in the sediment choices of 2nd, 3rd and 4th stage postlarvae so that the results were combined. The results of the substratum selection tests for 1st stage postlarvae and the older juveniles of the 2nd, 3rd and 4th postlarval stages are shown in Figure 6.2. These data were then used to test the hypothesis that choice of substratum is independent of the substrata available. If this was the case the proportion (P) of animals found in each substratum, for the tests where two substrata were involved, would be 0.5. The observed proportions are given in Table 6.3 along with the probability of obtaining, for each binomial test, those proportions, or more extreme ones, when 'P' equals 0.5.

At the 5% level of significance the hypothesis is accepted for tests 1 to 5 for 1st stage postlarvae. Given a choice of four substrata test 6 suggests that 1st stage postlarvae chose the finer sediment of those provided. Overall it appears that 1st stage postlarvae have no marked substratum preference; as shown in Figure 6.2 the majority made no choice at all.

For tests 1 to 4 using 2nd to 4th stage postlarvae the hypothesis is rejected since the probability of obtaining the observed proportions, or more extreme ones, is very small (less than 0.05). Where mud was available the animals showed a preference for it and preferred muddy-sand to sand. Where the choice was between sand and gravel no significant preference was demonstrated but more animals

Table 6.2

The substratum choice provided for each test and the corresponding number of animals involved. The number of times each substratum type was used over the 6 tests has also been shown.

Test	Substratum type				Number of animals	
					PL1	PL2-4
	M	MS	S	G		
1	X	X			18	16
2	X		X		20	14
3	X			X	19	14
4		X	X		18	14
5			X	X	20	14
6	X	X	X	X	18	15
Number of times substratum used	4	3	4	3		

#### Abbreviations

M = Mud, MS = Muddy-sand, S = Sand, G = Gravel, PL = Postlarva.

### Figure 6.2

(a) The results of the substratum selection tests, showing the choice made by 1st stage postlarvae (PL1) and individuals of the 2nd, 3rd and 4th postlarval stages combined (PL2 - PL4). The substratum choice provided for each test is shown in Table 6.2.

(b) The proportion of individuals which selected each substratum type or made no discernible choice during the 6 test conditions for the 1st postlarval stage and 2nd, 3rd and 4th postlarval stages combined.

Abbreviations are : M, Mud ; MS, Muddy-sand ; S, Sand ; G, Gravel ; 0, no discernible choice ; PL, Postlarva.



**a**

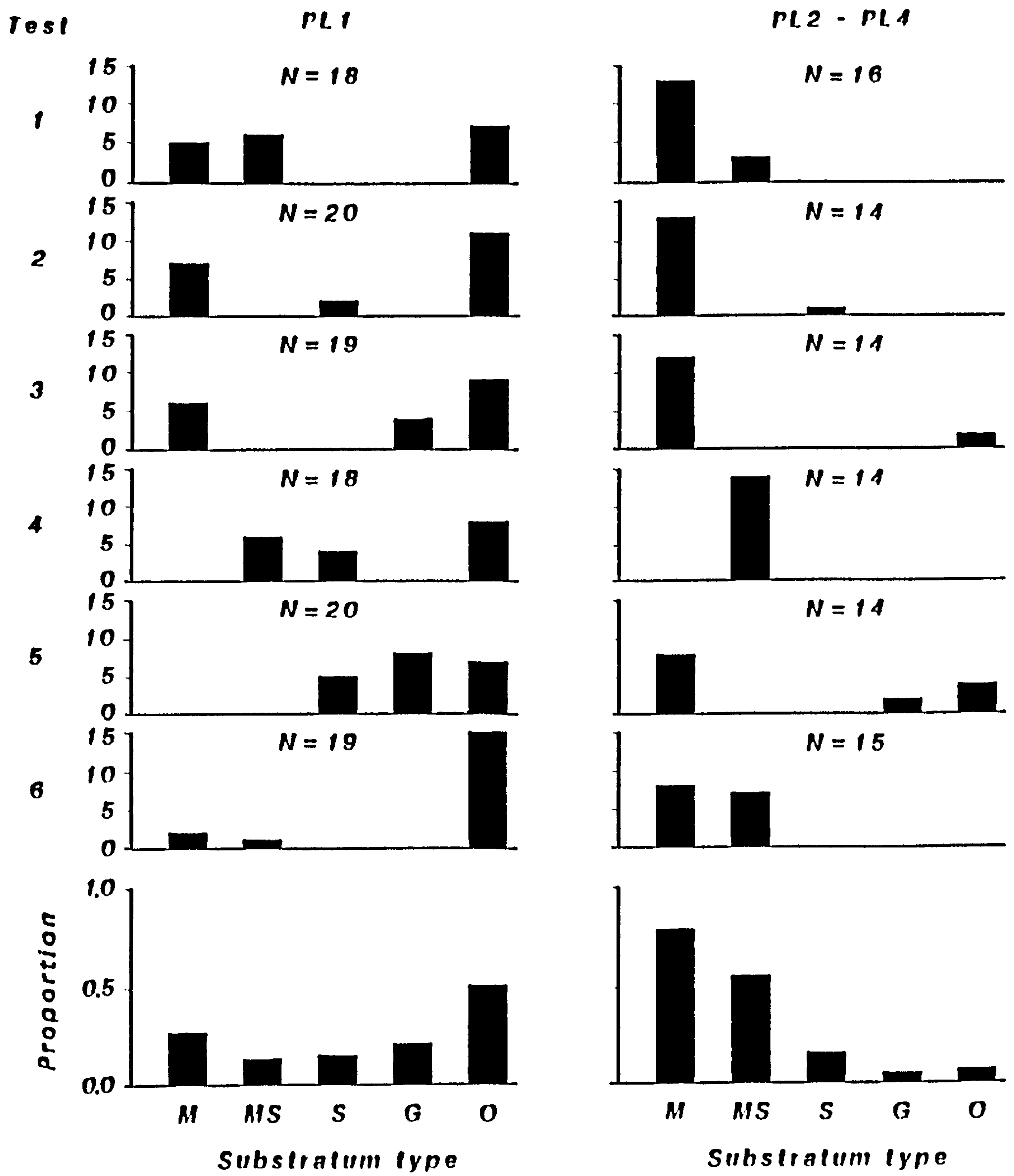


Table 6.3

Observed proportions and calculated probabilities of obtaining these, or more extreme, proportions. The abbreviations are presented with Table 6.2.

Stage	Test	Substratum type				Probability
		M	MS	S	G	
PL 1	1	0.45	0.55	-	-	0.5000
	2	0.78	-	0.22	-	0.0900
	3	0.60	-	-	0.40	0.3770
	4	-	0.60	0.40	-	0.3770
	5	-	-	0.38	0.62	0.2910
	6	0.67	0.33	0.00	0.00	-
PL 2-4	1	0.81	0.19	-	-	0.0110
	2	0.93	-	0.07	-	0.0009
	3	1.00	-	-	0.00	0.0002
	4	-	1.00	0.00	-	0.0001
	5	-	-	0.80	0.20	0.0550
	6	0.53	0.47	0.00	0.00	-

settled on the sand. In test 6 a preference was shown for mud and muddy-sand rather than sand and gravel. The mud and muddy-sand were similarly acceptable to the animals under test which is surprising in view of the results of test 1 where a significant preference for mud was found. These substratum selection trials therefore indicate that for postlarvae of the 2nd, 3rd and 4th stages the overall order of preference was mud, muddy-sand, sand and lastly gravel.

The second set of substratum selection trials offered a choice of 6 different combinations of mud and sand. The results are summarised in Table 6.4.

The indifference to substratum type demonstrated by 1st stage postlarvae in the first part of this investigation was verified in these trials. Individuals within this stage generally spent much of their time in exploratory behaviour but showed no inclination to burrow. This was also true for the 4 individuals which appeared to 'select' a substratum (Table 6.4). The 1st stage postlarvae made frequent vertical migrations up into the overlying water although the periods of continued swimming activity rarely exceeded 30 seconds.

Examples of the substratum selection behaviour demonstrated by individuals of the 2nd, 3rd and 4th postlarval stages are presented in Figures 6.3 - 6.5. On introduction to the tank Nephrops within these stages usually began walking around the tank perimeter maintaining contact with the tank sides. During this period little swimming activity was observed, and when it did occur, it was generally up the walls of the tank. After this initial behaviour the lobsters began to show increased attachment to a particular area of substratum which

Table 6.4

The number of individuals that selected each substratum type.  
The abbreviations are presented with Table 6.2.

		Substratum type (% Mud)						No choice
	N	100	80	60	40	20	0	
PL 1	10	0	1	2	1	0	0	6
PL 2-4	28	10	15	2	0	0	0	1



Figure 6.3

An example of the ambulatory activity and substratum selection / settlement behaviour demonstrated by a 2nd stage postlarva when provided with a choice of different proportions of mud and sand (the percentage of mud in each area of the tank is shown). The activity is shown over different time intervals after introduction to the choice chamber at 0min.

Symbols are : x = Introduction to choice chamber.

▲ = Moves away from perimeter of tank.

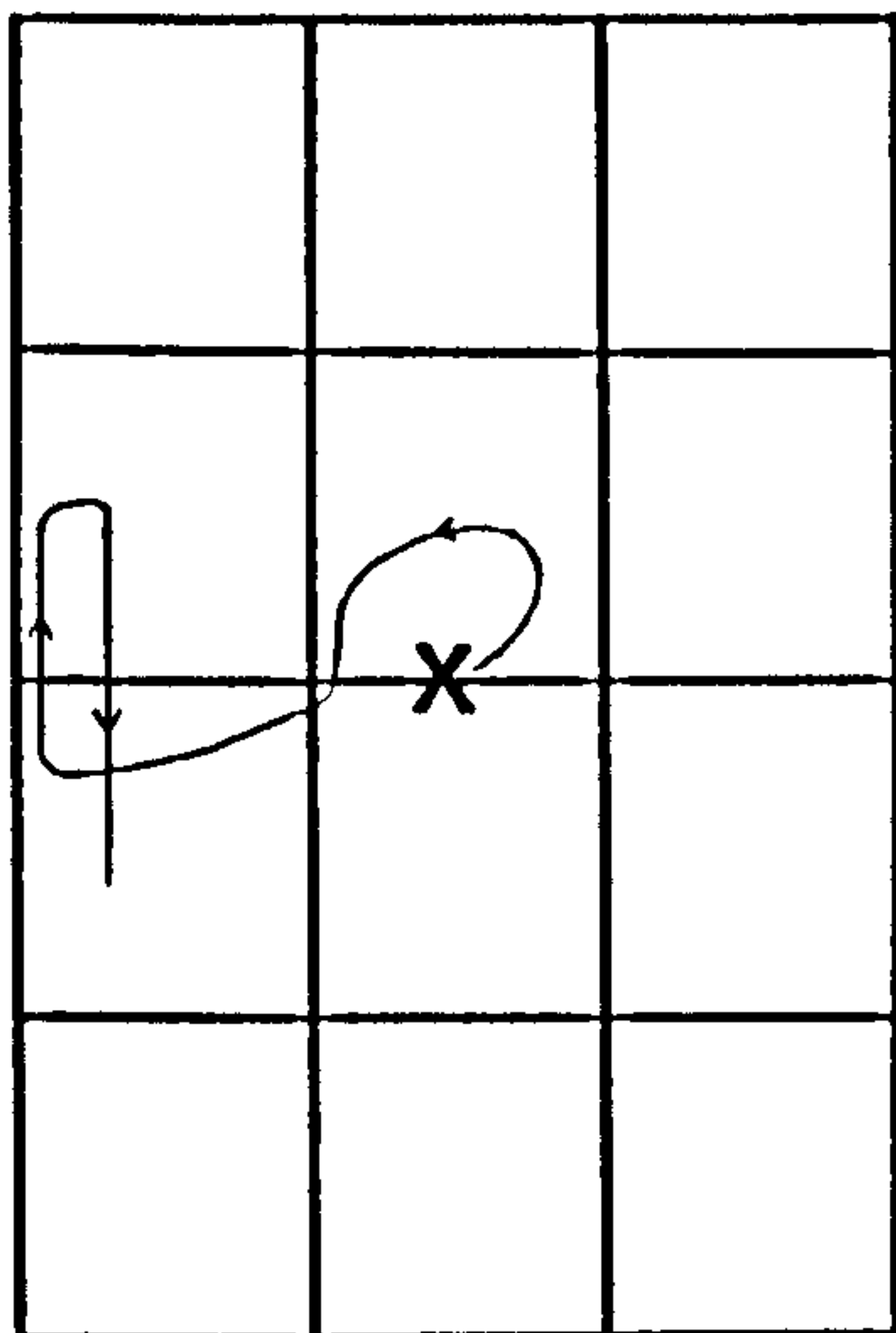
▽ = Remains stationary for a prolonged period.

+ = Some limited burrowing behaviour.

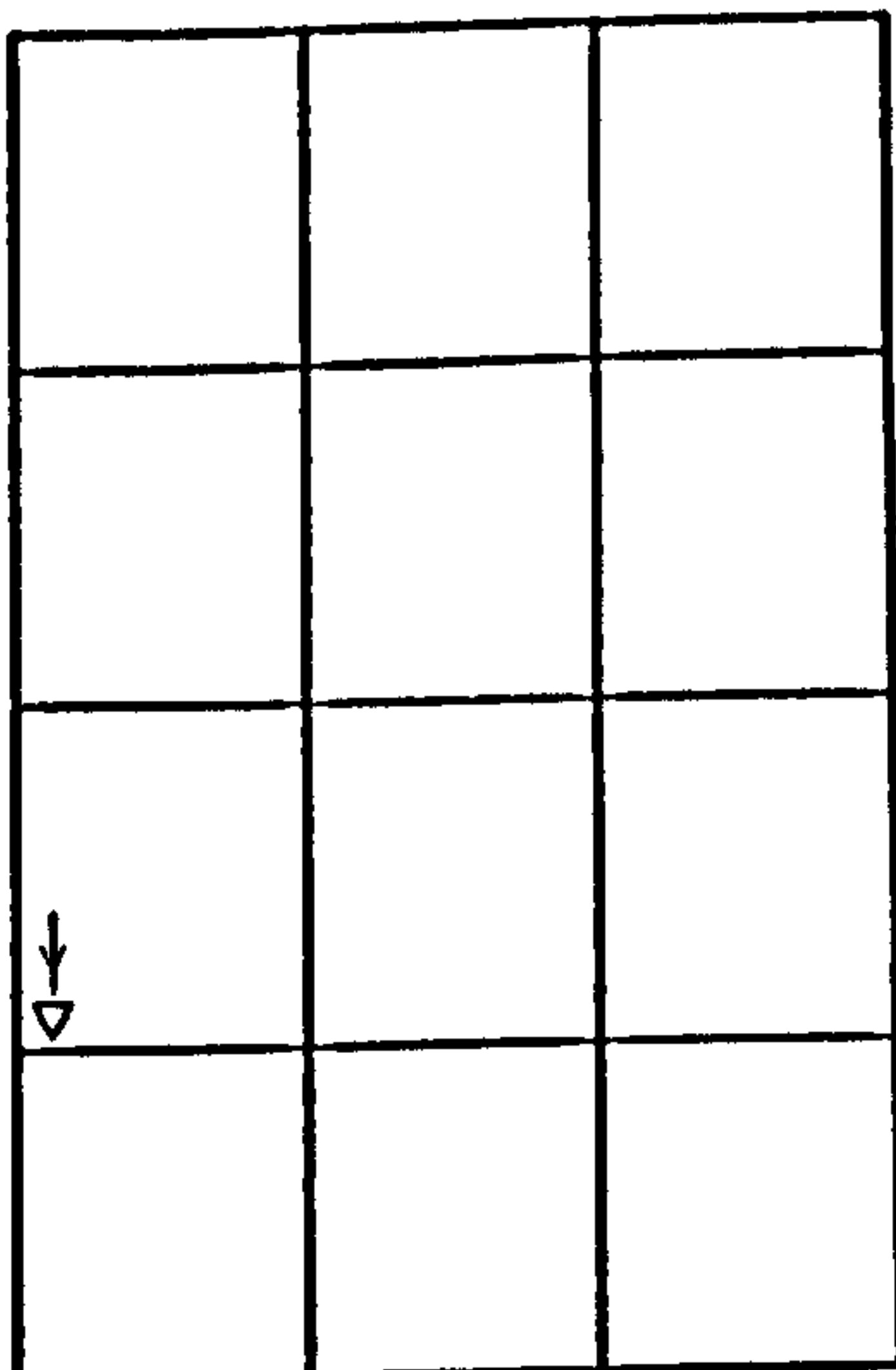
● = Final selection and burrow construction.

All movement was along perimeter of tank except when indicated by ▲.

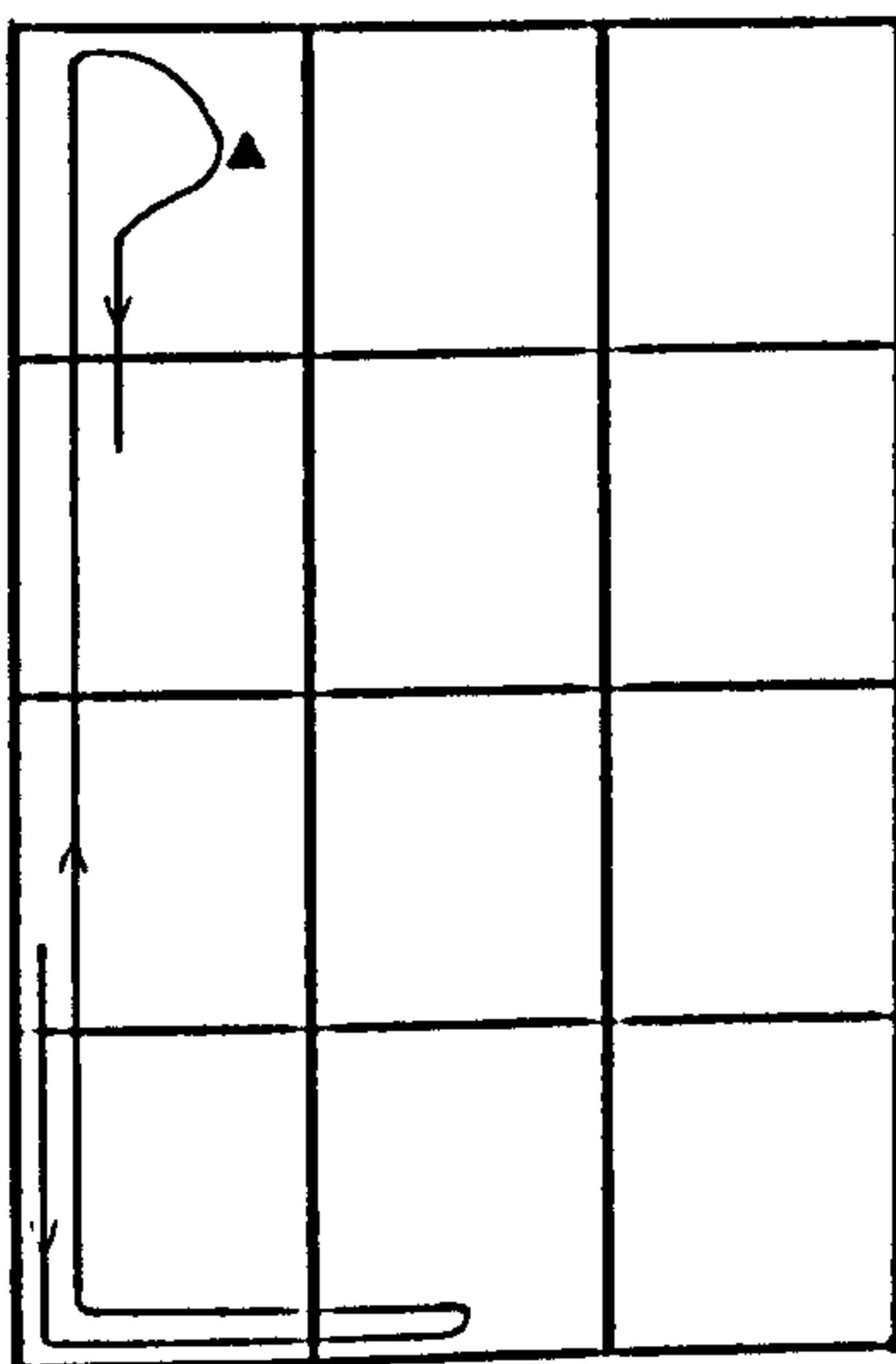
0 - 5min.



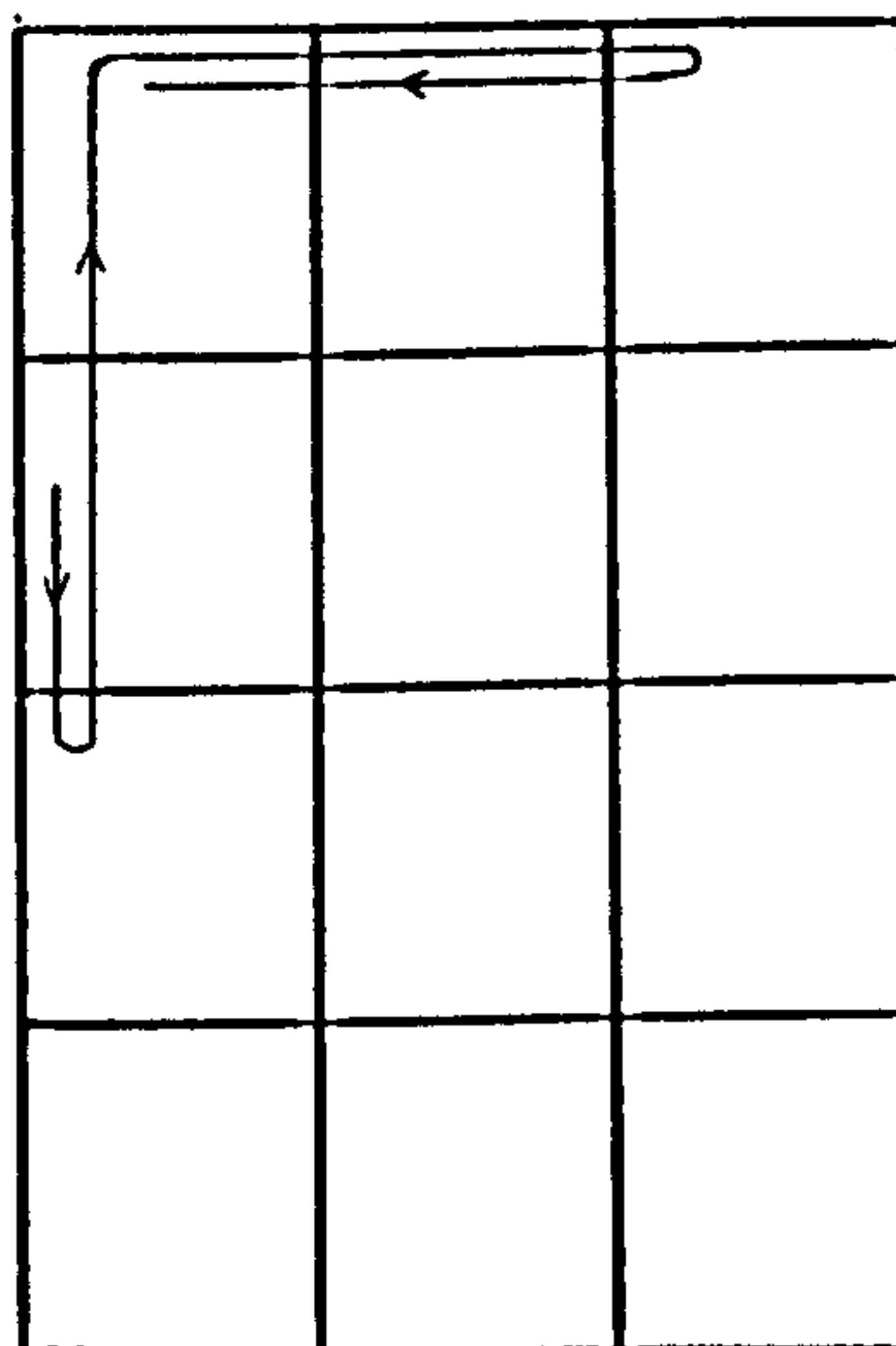
5 - 15min.



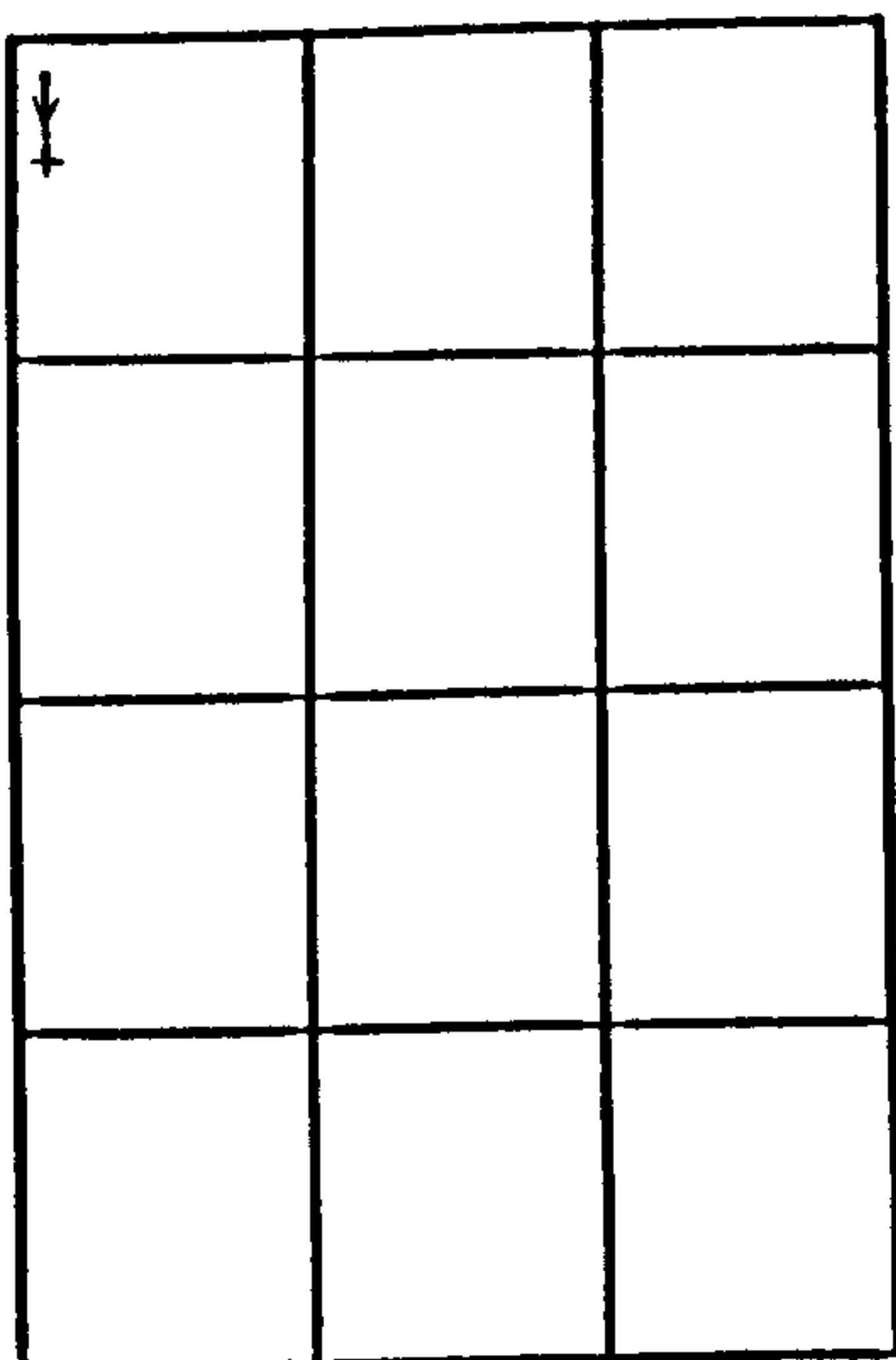
15 - 30min.



30 - 45min.



45 - 60min.



60 - 90min.

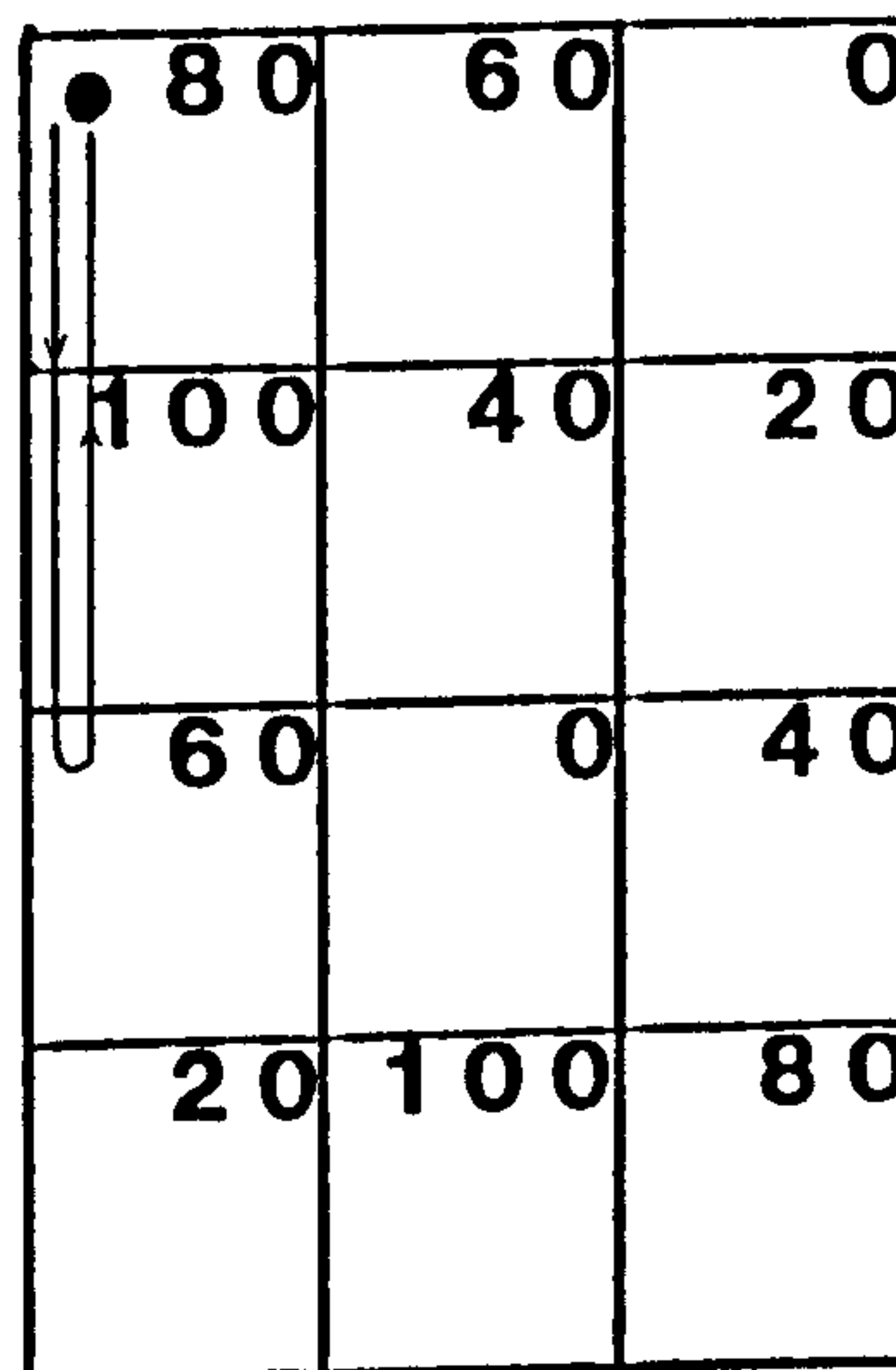
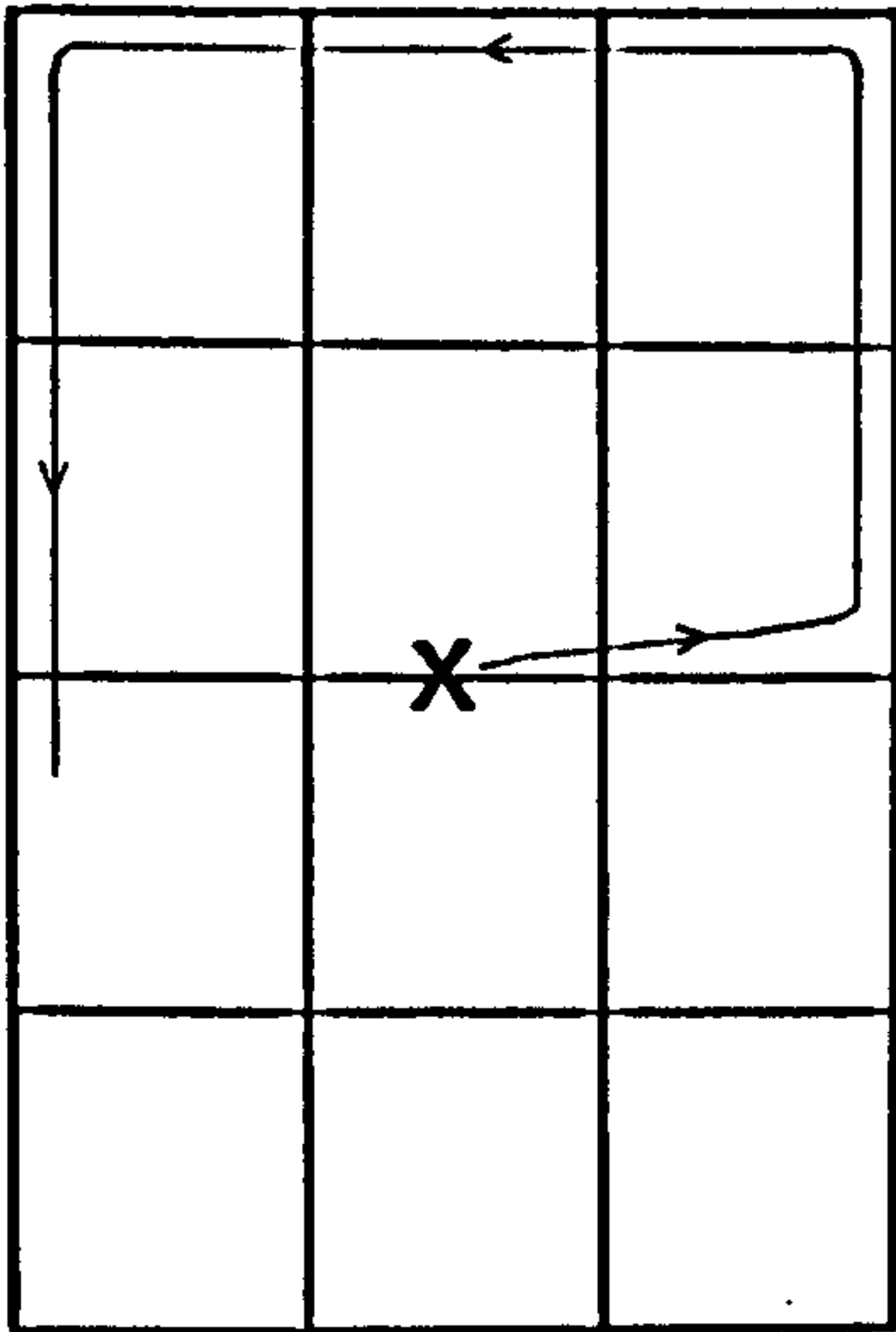


Figure 6.4

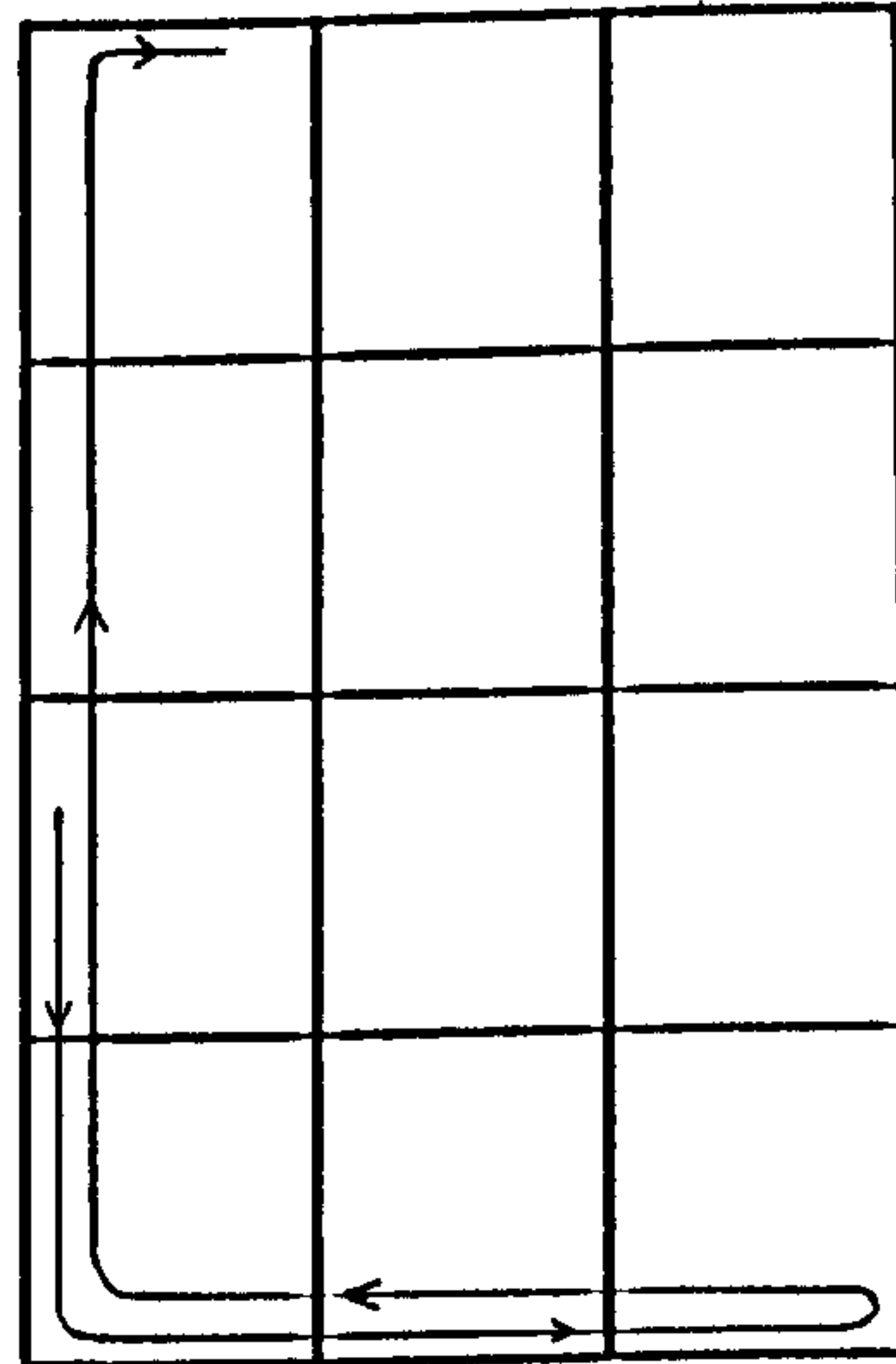
An example of the ambulatory activity and substratum selection / settlement behaviour demonstrated by a 3rd stage postlarva when provided with a choice of different proportions of mud and sand (the proportion of mud in each area of the tank is shown). The activity is shown over different time intervals after introduction to the choice chamber at 0min.

Symbols are identified in Figure 6.3.

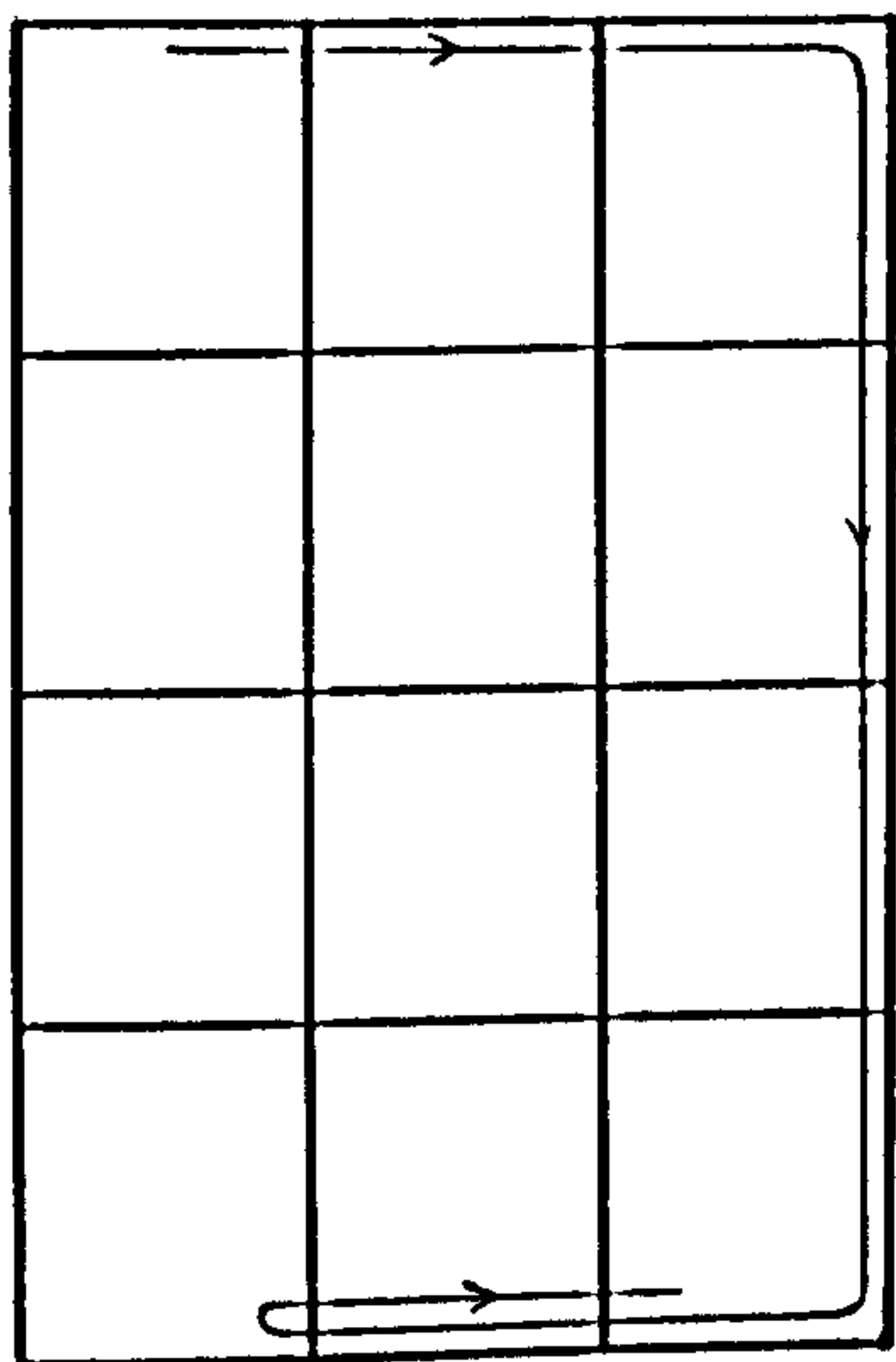
0 - 5min.



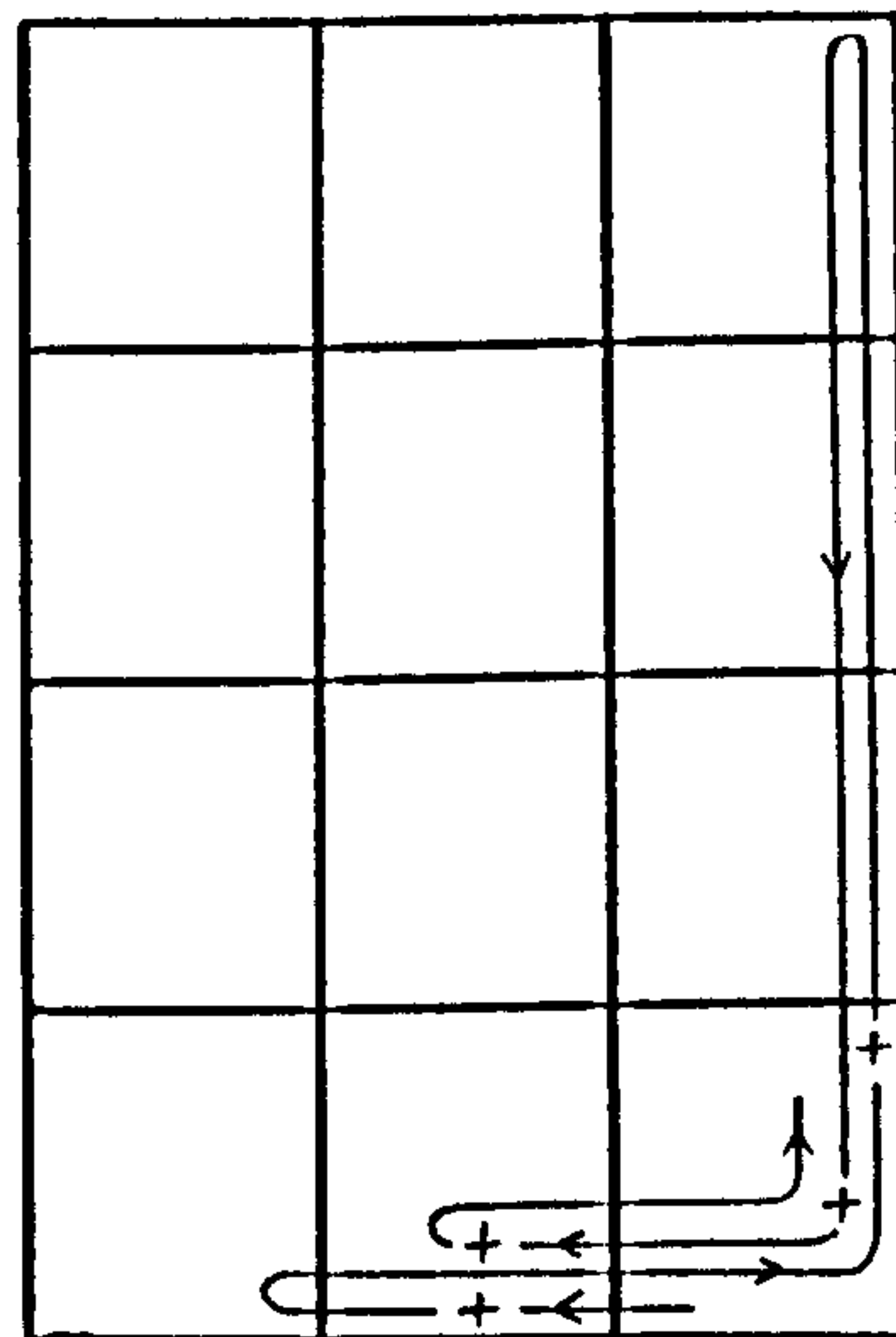
5 - 10min.



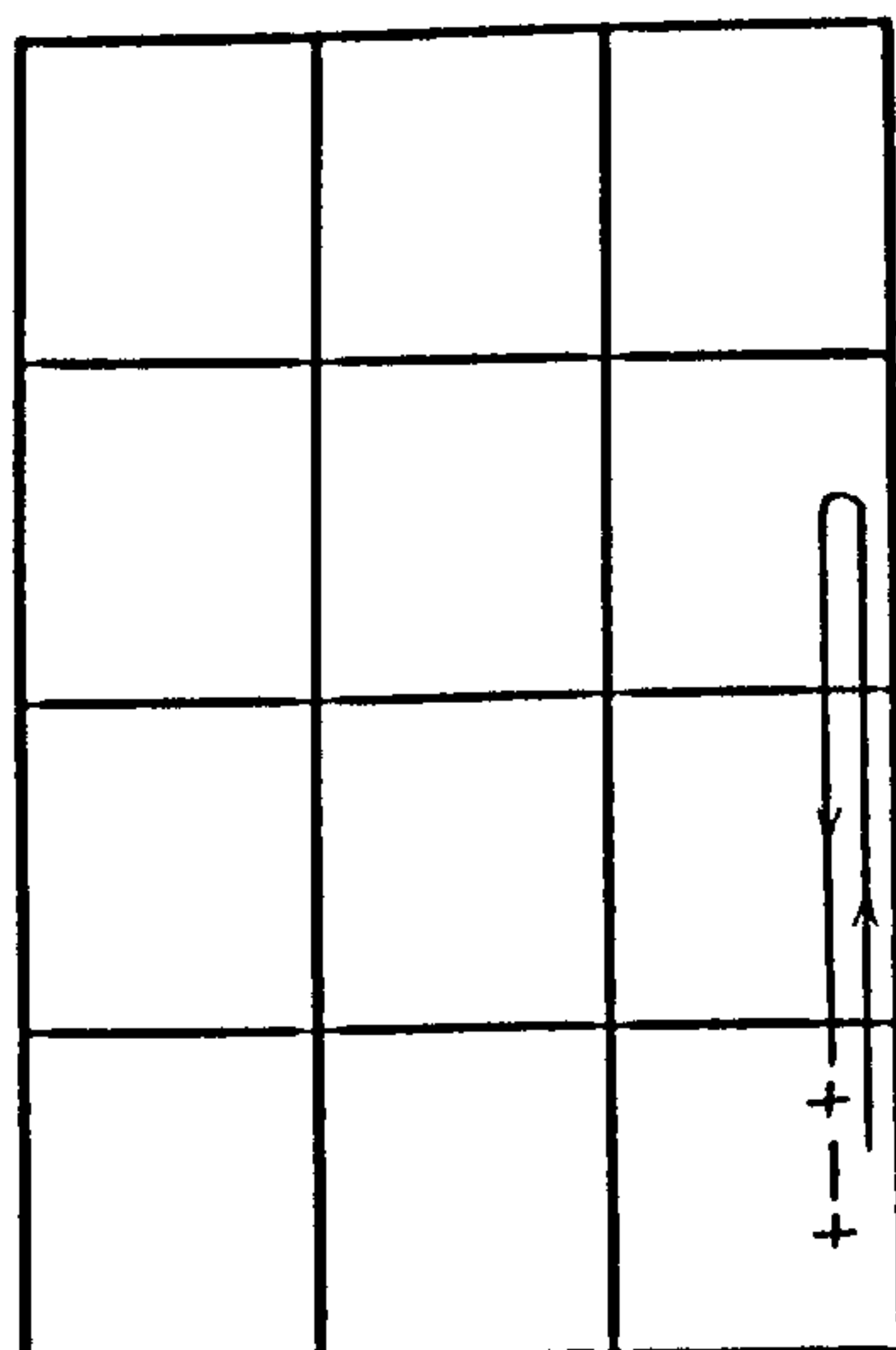
10 - 20min.



20 - 40min.



40 - 60min.



60 - 90min.

80	60	0
100	40	20
60	0	40
20	100	80



### Figure 6.5

An example of the ambulatory activity and substratum selection / settlement behaviour demonstrated by a 4th stage postlarva when provided with a choice of different proportions of mud and sand (the proportion of mud in each area of the tank is shown). The activity is shown over different time intervals after introduction to the choice chamber at 0min.

Symbols are identified in Figure 6.3.

A 3x3 grid with a central 'X'. Arrows indicate a path starting from the top-right corner, moving left, then down, then left again, ending at the bottom-left corner.

A 3x3 grid with arrows indicating a path starting from the top-left cell, moving right, then down, then right again, and finally down to the bottom-right cell.

A 4x4 grid with a path starting at (1,1), going down to (4,1), then right to (4,4), then up to (2,4), then left to (2,1), and finally right to (3,1). A small triangle is at (2,2).

A 4x3 grid of numbers. The numbers are arranged as follows:

80	60	0
100	40	20
60	0	40
20	100	80

A path is indicated by a solid line starting from a black dot in the second row, first column, moving vertically down to the bottom of the grid, then horizontally to the right, ending with an arrow pointing right.

became a focal point for all future activity. Explorations, which occasionally included migrations into the central regions of the tank, became progressively confined to smaller areas as 'unsuitable' substrata were avoided. The 'unsuitable' substrata tended to be the coarser, sand ones, with active avoidance of these areas becoming increasingly apparent as the animal spent progressively less time at the boundaries of these regions before retreating back to its 'selected' area. The 'selected' areas tended to be the finer mud ones and, except on two occasions when burrows were constructed in a mixture of 60% mud / 40% sand, all other individuals eventually settled and formed burrows or depressions on sediments comprising 100 or 80% mud. Most excavation was performed along the sides of the tank, with the tank wall providing support for the burrow. Final selection and burrow construction usually occurred within 1.5h of introduction, though in some cases only 40 minutes was required. Thereafter, very little further exploration took place and the individual tended to remain within the confines of its burrow or depression.

Stage 4 and 5 postlarvae, on introduction to tanks containing only one substratum type (either sand, gravel or acrylic sheet), immediately became involved in exploratory behaviour and moved continually around the circumference of the tanks, except during frequent vertical migrations up into the surface waters. In addition to these vertical excursions, some swimming also occurred close to the sediment surface, alternating with periods of walking. Intermittent swimming activity continued throughout the trials, but was only sustained for short periods. Most swims were of about 4 - 5s duration, with 25s being the longest period of continual swimming recorded. The vertical component of this swimming behaviour tended to decrease with

time. Each individual presented with a sand substratum attempted to construct a burrow. The site of excavation was usually at the corner of the tank. Excavation activity persisted for prolonged periods (2 to 3h) but was eventually abandoned without the formation of a burrow. The animal then remained stationary within the slight depression it had created until the end of the trial. The juveniles provided with gravel explored the interfaces between particles but none provided crevices large enough for concealment. Eventually they moved to the corner of the tanks where they remained for the remainder of each trial. Under conditions where no substratum was added and the juveniles were presented with an acrylic surface, exploratory behaviour tended to be restricted to the perimeter of the tank, with animals eventually remaining inactively at one of the corners.

### 6.3.3. Burrow selection behaviour

As expected from the substratum selection trials, most of the first stage postlarvae failed to show any clear responses to any modification of the mud substratum intended to imitate burrow openings. A few of the older stage 1 individuals, however, entered the lengths of plastic piping or occupied the vacated depressions and tunnels which had been previously excavated by older juveniles.

Individuals of the older postlarval stages (ie. stage 2 onwards) appeared to show varying degrees of interest in all of the modifications and additions made to the substratum. Some of the juveniles provided with circles of black card appeared to show initial interest in them, walking over them several times but after this early investigative behaviour no further interest was demonstrated. When



lengths of plastic piping were provided the juveniles readily entered them and later often utilized them as a starting point for burrow construction. Depressions and holes in the substratum which had been created manually were usually investigated by these older postlarval stages but interest in them was always brief and no attempt was ever made to develop them into burrows. When presented with a vacated burrow which had been constructed by another juvenile, immediate interest was shown. The juvenile took up residence within 5 minutes of having been introduced to the substratum.

In one experiment, when a first stage postlarva entered a burrow occupied by an older juvenile, the former was eaten. Since the number of first stage postlarvae was limited no attempt was made to repeat this experiment.

On one occasion a third stage postlarva investigated, modified and then inhabited a vacated burrow of either a polychaete or a bivalve. Whether the original inhabitant had been eaten by the juvenile was not discovered.

#### 6.3.4. Burrowing behaviour of juvenile stages

On introduction to consolidated mud surfaces first stage postlarvae did not burrow and it was only after the next moult that the burrowing habit became apparent. In comparison with the older postlarvae, however, stage 2 individuals appeared to remain on the substratum for longer periods before initiating burrow excavation.

The method of burrow construction demonstrated by juvenile

Nephrops was similar to that described for adult Nephrops (Dybern, 1965 ; Rice and Chapman, 1971 ; Farmer, 1974a) and juvenile H.americanus (Berrill and Stewart, 1973). Movement of the sediment was facilitated by its thixotropic properties, becoming more fluid under the continual probing and thrusting of the lobsters' third maxillipeds. This initial probing and thrusting behaviour produced a small depression in the mud. The loose mud was then gathered together by the third maxillipeds and the second and third pereopods. These appendages formed a 'basket' (Dybern, 1965) in which to transport sediment and initiate excavation of the burrow.

When food was provided (frozen mysids) the postlarvae emerged rapidly from their burrow openings, grasped the food in one or both major chelipeds and, without turning, retreated immediately into their burrows. The entire exercise was completed within several seconds. Having removed the food to its burrow it was either eaten immediately or buried. Some individuals were observed re-excavating and eating the food on a later occasion. Postlarvae of stages 2, 3 and 4 are shown within their burrows in Plate 6.1. In Plate 6.1a the remains of a polychaete can be seen. This was recovered by the postlarva from the sediment and brought back into its burrow.

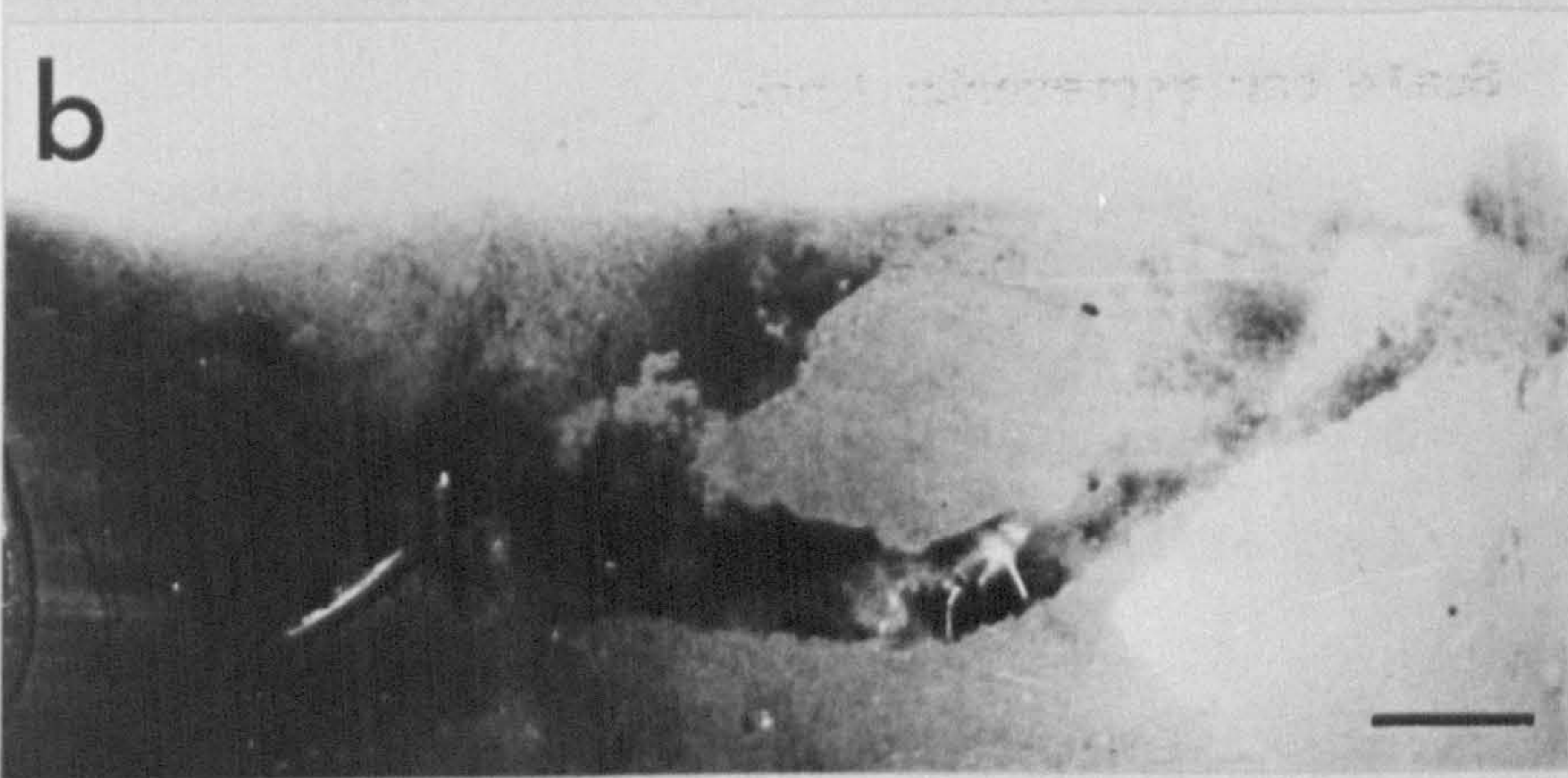
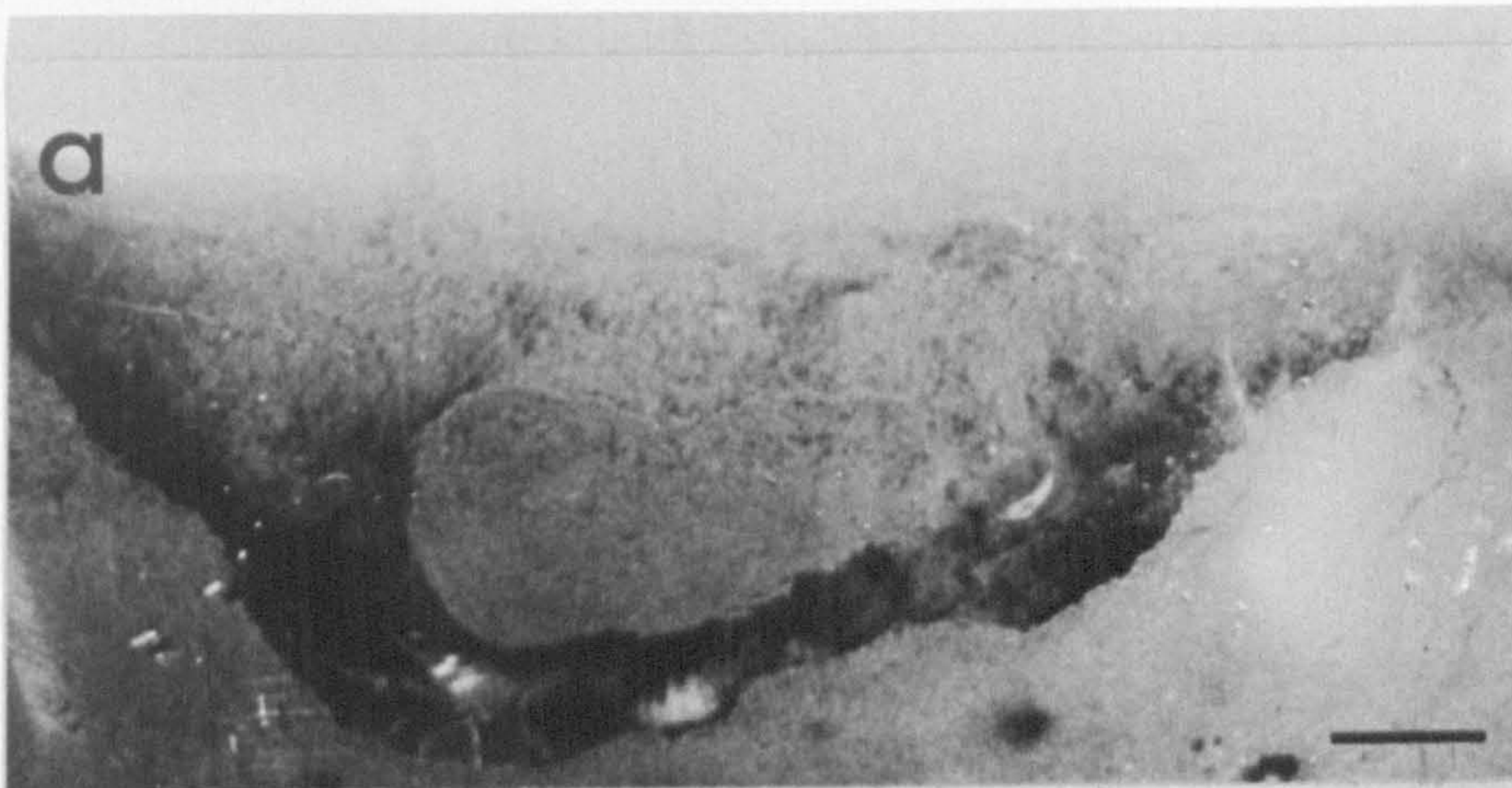
Postlarvae introduced on to mud in which adult Nephrops had already constructed a burrow attempted to enter the adult burrows through one of the surface openings. In the small baths and tanks this resulted in the juvenile being chased out by the inhabitant. Having evicted the juvenile from the burrow, the adult did not give further pursuit and remained within its burrow. The burrows which were formed by individual adults within the relatively small rectangular baths

**Plate 6.1**

**Postlarva of the 2nd, 3rd and 4th stages shown within their burrows.**

**Scale bar represents 10mm.**







(270 X 190 X 230mm) and tanks (450 X 100 X 380mm) usually had only two or three surface openings (Plate 6.8), tunnel lengths were restricted by container size. The adults were therefore able to successfully patrol their burrows against intrusion by the juvenile. After repeated attempts at entry the juvenile ceased to enter the adult burrow and excavated its own burrow.

In the aquarium (1850 X 500 X 500mm) two adults formed a burrow system which was much more extensive than the burrows formed in the smaller containers by single adults. The aquarium provided two juveniles with more space and many more surface openings. After being chased out from several different surface openings both juveniles eventually succeeded in entering the system shared by the two adults and in constructing their own burrows from within it (Plate 6.10).

#### 6.3.5. Description of the burrows of juveniles and adults

Details of 20 Nephrops burrows cast in the laboratory with polyester resin are given in Table 6.5 and illustrated in Plates 6.2 - 6.10.

The simplest burrows formed by both juvenile and adult Nephrops were U - shaped tunnels (Plate 6.2). Although there was considerable variation between burrows and between the two shafts forming the U - shaped tunnel, the angle of descent from the mud surface over the first section of the shaft was generally greater in the juvenile (Plates 6.2 - 6.6) than the adult (Plates 6.7 - 6.9) burrows. After this first section, where the shaft descended almost vertically, the angle changed to approximately 20 - 30°, before continuing almost

horizontally, finally turning upwards again to form the second shaft (Plate 6.5 and 6.7). From the mid-point both shafts ascended in a similar manner in the juvenile burrows (Plate 6.2), giving a symmetrical U - shaped appearance. In the adult burrows there was an obvious difference in the gradient of the two components opening to the surface (Plate 6.7), with one constructed at a much shallower angle than the other. The shallower tunnel descended from the horizontal at an angle of 25 to 30° which on returning to the surface formed an almost vertical shaft. This type of burrow has been previously described for adult Nephrops in the field (Rice and Chapman, 1971) and was considered by Chapman (1980) as typical of initial burrow excavation.

The shafts tended to become more flattened in cross-section on progression from the vertical to the horizontal plane and in corresponding regions were more circular in the burrows of juveniles than in those of adults (Table 6.5).

The angles at the first burrow junction normally formed a Y shape in the early postlarval burrows (Plates 6.3 - 6.5), becoming progressively more T - shaped in the older postlarval (Plate 6.6) and adult burrows (Plates 6.7 - 6.9). The tendency for the younger Nephrops to form Y - shaped and the older to form T - shaped junctions was a fairly consistent feature.

Based on the information of the resin casts, the depth to which the initial U - shaped tunnel descended appeared to increase with age over the postlarval stages. For postlarval stages 3 and 4 the depth of construction was found to be between 30 - 35mm (Plates 6.2 - 6.5)

increasing to 50 - 60mm for postlarval stage 7 and 90mm for postlarval stage 11 (Plate 6.6). The two adult Nephrops (carapace lengths 24 and 32mm) for which the depth of the substratum did not appear to limit the depth of their burrows, developed their initial U - shaped burrows to depths of 150 and 190mm respectively (Plate 6.7).

From approximately the deepest position along the U - shaped burrow a side branch developed forming the first junction in both the juvenile (Plates 6.3 - 6.6) and adult (Plate 6.7) burrows. Usually the burrow was progressively elaborated with lateral tunnels and further openings to the surface. Juvenile burrows were often more elaborate than those of adults. In one postlarval burrow there was evidence of there having been as many as 11 surface openings (Plate 6.5). Normally the first side branch from the initial U - shaped burrow of juveniles sloped downwards at approximately  $30 - 40^\circ$  to the horizontal before levelling off and then ascending (Plate 6.5 and 6.6). This ascent was initially at approximately the same angle as the descent ( $30 - 40^\circ$ ) but then steepened so that the terminal section formed an almost vertical shaft to the surface opening. The vertical section of the burrow was circular in cross-section and tended to be narrower in diameter than the descending and ascending shafts of the initial U - shaped burrow (Plate 6.5 and 6.6). The adults observed did not construct this type of shaft. Their burrows generally made shallower angles with the sediment surface and subsurface horizontal sections were relatively longer than those of juveniles (Plates 6.7 - 6.9). Their burrowing habits were more restricted by the holding facilities, however, and field evidence suggests considerable variation in burrow form (see discussion). The length of the burrows of juveniles, measured from the mud surface to the first junction,



increased with the size of the individual from an average value of approximately 60mm for stage 3 postlarvae (Plate 6.3) to 300mm for stage 7 postlarvae (Plate 6.6).

There appeared to be a tendency for the postlarvae to continually excavate new shafts (Plate 6.5), allowing older ones to collapse. This behaviour was less apparent in the adults where more effort appeared to be directed towards maintenance of the existing burrows which usually had one side branch and three openings to the surface (Plate 6.7).

Burrow volume appeared to increase with increasing size of the inhabitant, especially over the postlarval stages, tank size possibly limiting further excavation and elaboration of the adult burrows. There was no evidence to suggest that the complexity of the burrows of juveniles increased with age of inhabitant. The most elaborate burrow observed was developed by a 4th stage postlarva (Plate 6.5). The burrows of juveniles increased in length with increasing size of the inhabitant and also increased in circumference as would be expected (Table 6.5). The average circumference of the burrows increased from 35 - 40mm in stage 3 postlarvae (Plate 6.3) to 60mm in stage 7 postlarvae (Plate 6.6), with an adult of carapace length 47mm occupying tunnels of 300mm average circumference (Plate 6.10).

#### 6.3.6. Burrow systems involving associations between individuals

In the aquarium, two burrow systems were constructed which involved associations between individual Nephrops (Table 6.5c).



Plate 6.9 shows a burrow system incorporating the burrows constructed by two Nephrops (a and b) of carapace lengths 46 and 23mm respectively. Burrow 'a' was constructed by the larger animal and consisted of a single tunnel 1100mm long. Towards the surface the cross-sectional shape of the tunnel changed from a flattened ellipse to an ellipse. Burrow 'b' was connected at right angles to burrow 'a' at a depth of 100mm and was 220mm long. Midway along the shaft there was evidence of two previous lateral tunnels which had subsequently collapsed. They branched from the existing shaft almost at right angles and at opposite positions as two T junctions. The cross-sectional shape of the shaft was elliptical.

The burrow system shown in Plate 6.10 was attributable to four Nephrops, two adults of carapace lengths 47 and 29mm (a and b) and two juveniles of carapace lengths 16 and 14mm (c and d) which were obtained from the culturing facility. Burrow component 'a' comprised two tunnels (ax and ay) 920 and 410mm long respectively. The angle of the tunnel intersection was about 80°. Towards the surface the cross-sectional shape of the tunnel changed from a flattened ellipse to an ellipse. Tunnel 'b' was a simple T - shaped burrow. The major branch was 180mm long and it was connected to tunnel 'ax' just below its surface opening. In cross-section the shafts were an elliptical shape. Component 'c' was connected to both tunnels 'ax' and 'ay' in 3 places. Component 'c' consisted of 3 tunnels (c1, c2 and c3) with evidence of a further, now collapsed connection. Tunnel 'c1' (which joined both ax and ay) was constructed almost parallel to tunnel 'c2' and joined to it by tunnel 'c3'. Tunnel 'c2' was connected to component 'a' just below the surface opening where tunnels 'ax' and 'ay' joined. After the Y - shaped junction where it connected with tunnel 'c3', 'c2'

turned abruptly upwards forming an almost vertical shaft. This provided the juvenile with independent access to the surface. The shafts were subtriangular in cross-section. Component 'd' consisted of a single tunnel. This was constructed between tunnels 'c1' and 'ay' so that this juvenile was without independent access to the mud surface. This solitary tunnel was connected at right angles to tunnel 'c1' near its junction with tunnel 'ax' and to tunnel 'ay' just below the surface opening between tunnels 'ax' and 'ay'. The tunnel 'd' was 110mm in length rising from a depth of 120mm at the junction with burrow 'c1' at a constant gradient of approximately 20° to the horizontal. Towards the other junction the angle increased forming a vertical shaft on joining tunnel 'ay' at a depth of 40mm. The shaft was circular in cross-section.

Table 6.5  
Details of Nephtopsis casts

(a) Juvenile casts involving one individual.

Ident.	Postlarval stage	Number of openings to surface	Evidence of collapsed or almost collapsed openings	Volume of burrow (ml)	Max depth in sediment (mm)	Cross-section of burrow		Description
						Shape	Av. circum. + (mm)	
1	3	2	-	19	42	S.C.	40	Simple U-shaped tunnel (Plate 6.2a,b).
2	3	3	-	14	40	S.C.	35	Simple U with side tunnel to surface forming narrow vertical shaft (Plate 6.3a,b).
3	3	3	-	13	45	C	40	Simple U with side tunnel to surface (Plate 6.3c,d).
4	3	3	1	15	41	S.C.	40	Simple U with side tunnel to surface, beginnings of 4th branch (Plate 6.4a,b).
5	4	2	1	10	35	-	-	Simple U with beginnings of side branch. Poorly developed with both branches constructed along the wall of the bowl, preventing cross-sectional measurements from being taken (Plate 6.2c,d).
6	4	4	1	25	70	C	40	Relatively simple with 2 branches from main tunnel and the beginnings of a 3rd (Plate 6.5a,b).
7	4	5	6	35	45	C	45	Relatively simple with 2 branches from main tunnel, one of which divides forming 2 surface openings (Plate 6.5c,d).
8	5	3	1	31	60	S.T.	55	Relatively simple with 2 branches from main tunnel which interconnect to form a circuit. One of the branches forms an incomplete side branch (Plate 6.4c,d).
9	7	4	2	81	60	S.T.	60	Relatively simple with 4 branches from the main tunnel, 2 of which interconnect forming a circuit.
10	7	3	-	136	200	S.T.	60	Main tunnel forms 2 U-shaped junctions, one of which is at a much greater depth and leads to a narrow vertical shaft which provides a 3rd surface opening (Plate 6.6a,b).
11	11	2	3	136	150	S.T.	70	The main tunnel is U-shaped with a single side branch which divides forming a 2nd U-shape of 2 incomplete branches. Beginnings of a 3rd side branch between the 2 U-shaped sections (Plate 6.6c,d).

(b) Adult casts involving one individual

Ident.	Size of inhabitant (carapace length mm)	Number of openings to surface	Evidence of collapsed or almost collapsed openings	Vol. of burrow (ml)	Maximum depth in sediment (mm)	Average cross-section of burrow		Description
						Shape	Circumference (mm)	
12	19	4	2	390	110	S.T.	75	Main tunnel formed a circuit with 4 branches forming vertical shafts to the surface. Evidence of a collapsed lateral branch which would have crossed the circuit. Burrow restricted by depth of mud and influenced by walls of tank. Inhabitant within cast (Plate 6.8a,b).
13	24	3	1	310	150	S.T.	65	Simple burrow, T-shaped in plan. Main shaft influenced by walls of tank (Plate 6.7a,b).
14	26	3	2	305	85	F.E.	75	Burrow branches several times forming one complete circuit, with evidence of collapsed regions which have formed blind tunnels. Burrow restricted by depth of mud and influenced by walls of tank.
15	27	2	1	152	100	-	-	Burrow would probably have formed a T-shaped plan had the other branch of the main tunnel not collapsed. The perpendicular side branch of the T-shape forms a vertical shaft which is much narrower than the other branch. Burrow restricted by depth of mud, both branches were constructed along the walls of the tank therefore preventing cross-sectional measurements from being taken.
16	29	4	1	257	70	F.E.	80	Burrow branches 3 times in a series of T-shaped junctions. Evidence of a collapsed vertical shaft which may have previously extended to the surface to join an existing shaft and therefore have formed a circuit. Burrow restricted by depth of mud and influenced by walls of tank (Plate 6.8c,d).
17	32	3	1	238	190	-	-	Burrow tending towards a T-shape (plan). Branches influenced by walls of the tank preventing the attainment of cross-sectional measurements (Plate 6.7c,d).
18	40	3	-	310	170	S.T.	90	Burrow a simple T-shape in plan. Burrow restricted by depth of mud and side branch influenced by walls of tank.



(c) Casts involving more than one individual.

Ident.	Size of inhabitant (carapace length mm)	Number of openings to surface	Evidence of collapsed or almost collapsed openings	Vol. of entire burrow system (ml)	Maximum depth in sediment (mm)	Average cross-section of burrow		Description
						Shape	Circumference + (mm)	
19	46	2	2	2762	160	E	220	Burrow system consisting of 2 interconnected adult burrows. Burrow system restricted by depth of mud and influenced by walls of aquarium. See text for further description (Plate 6.9).
	23	1	2			E	65	
20	47	3	1	5071	190	E	300	Burrow system consisting of 4 interconnected burrows, 2 of which were constructed by juveniles obtained from the culturing facility and 2 by adults. Burrow system restricted by depth of mud and influenced by walls of aquarium. See text for further description (Plate 6.10).
	29	2	-			E	80	
	16 (PL13)	1	1			S.T.	70	
	14 (PL11)	0	0			C	60	

Abbreviations:-

C = circular, S.C. = subcircular, S.T. = subtriangular, E = elliptical, F.E. = flattened ellipse, PL = Postlarva.

+ = all cross section details are taken from regions below the surface openings before the tunnels develop into craters.

Plate 6.2

Polyester resin casts of burrows of juveniles.

(a) Elevation and (b) plan of burrow 1 in Table 6.5a.

(c) Elevation and (d) plan of burrow 5 in Table 6.5a.

Each gradation on the scale bar represents 10mm.



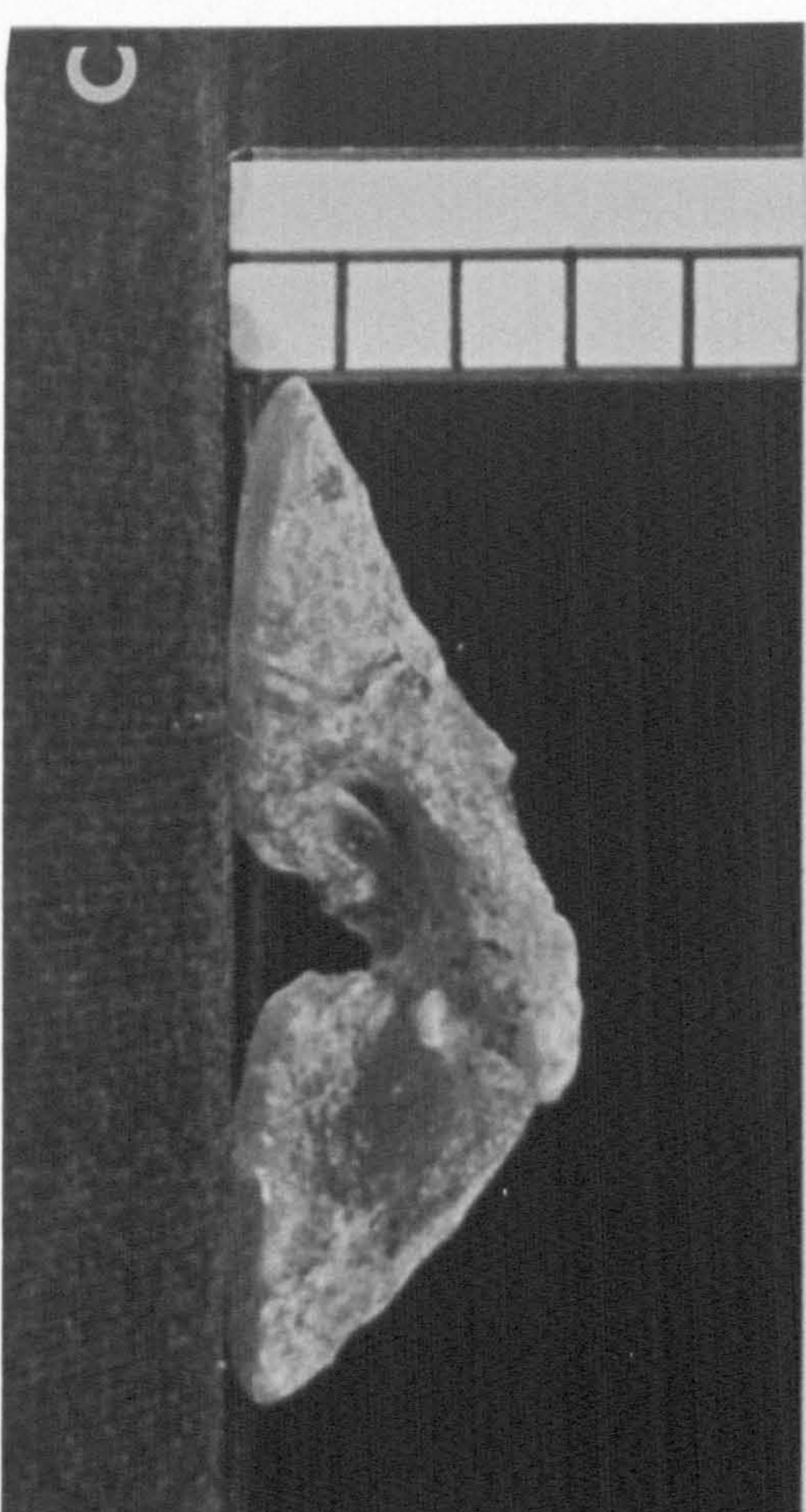
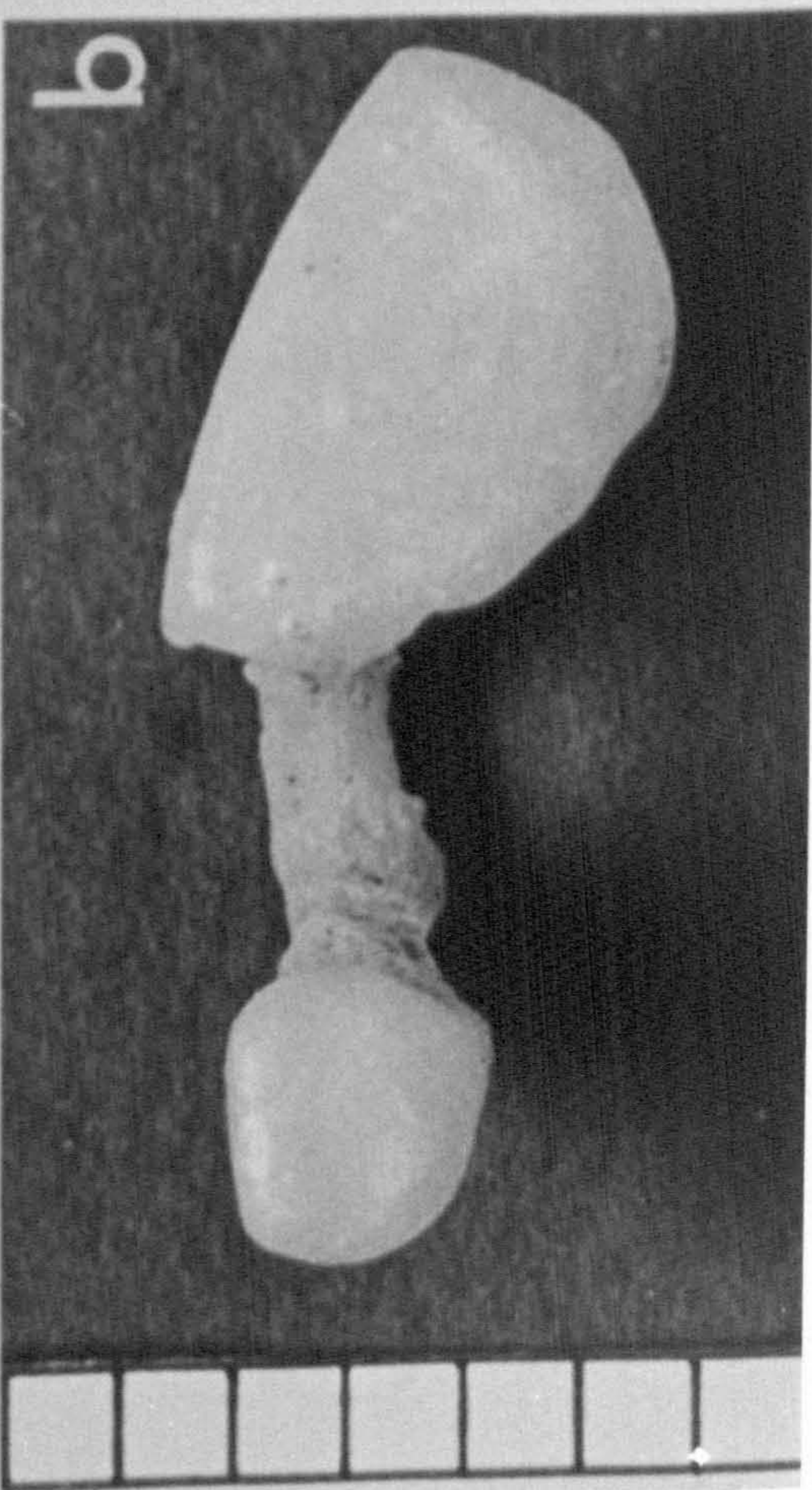
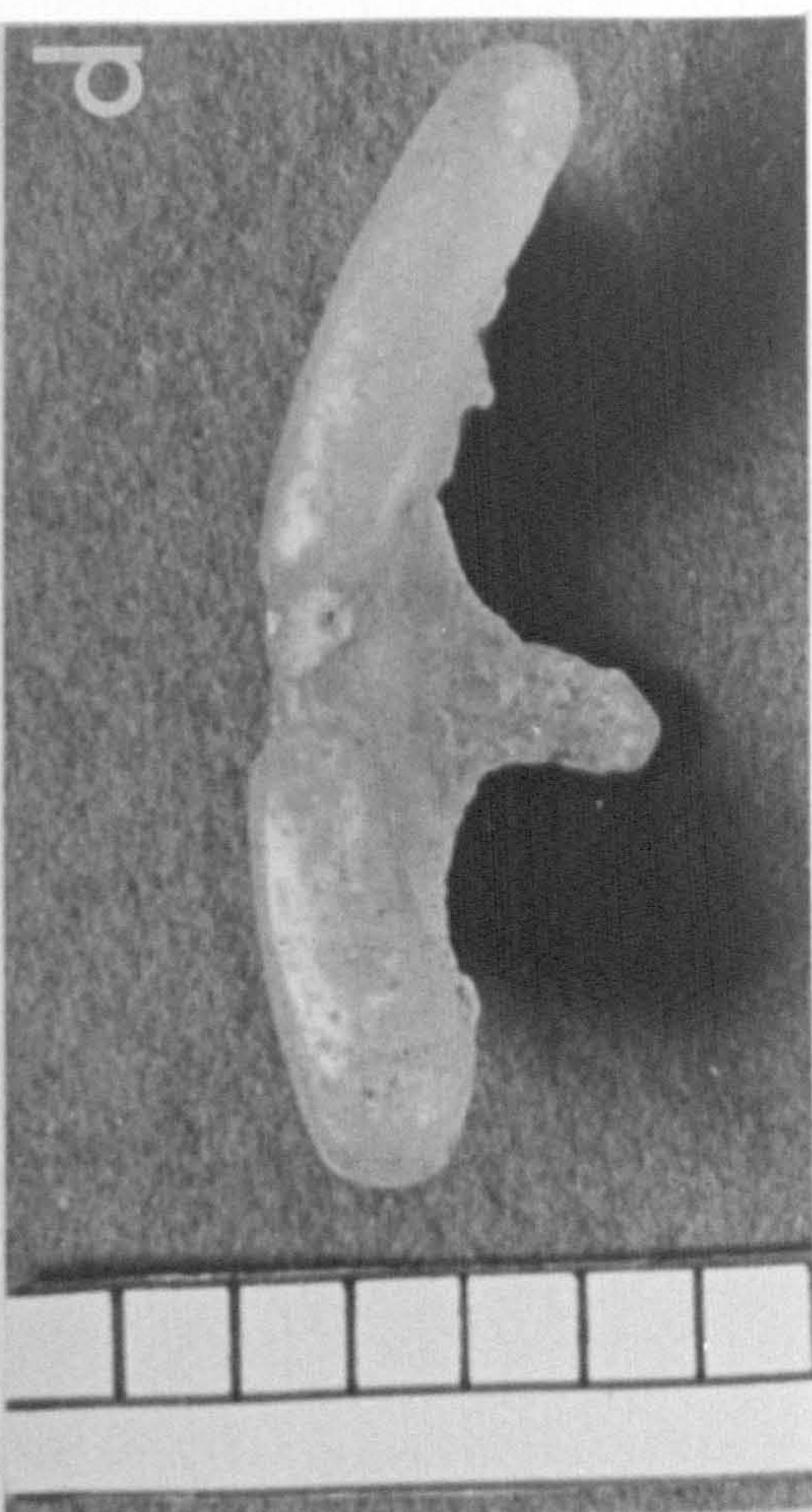




Plate 6.3

Polyester resin casts of burrows of juveniles.

(a) Elevation and (b) plan of burrow 2 in Table 6.5a.

(c) Elevation and (d) plan of burrow 3 in Table 6.5a.

Each gradation on the scale bar represents 10mm.



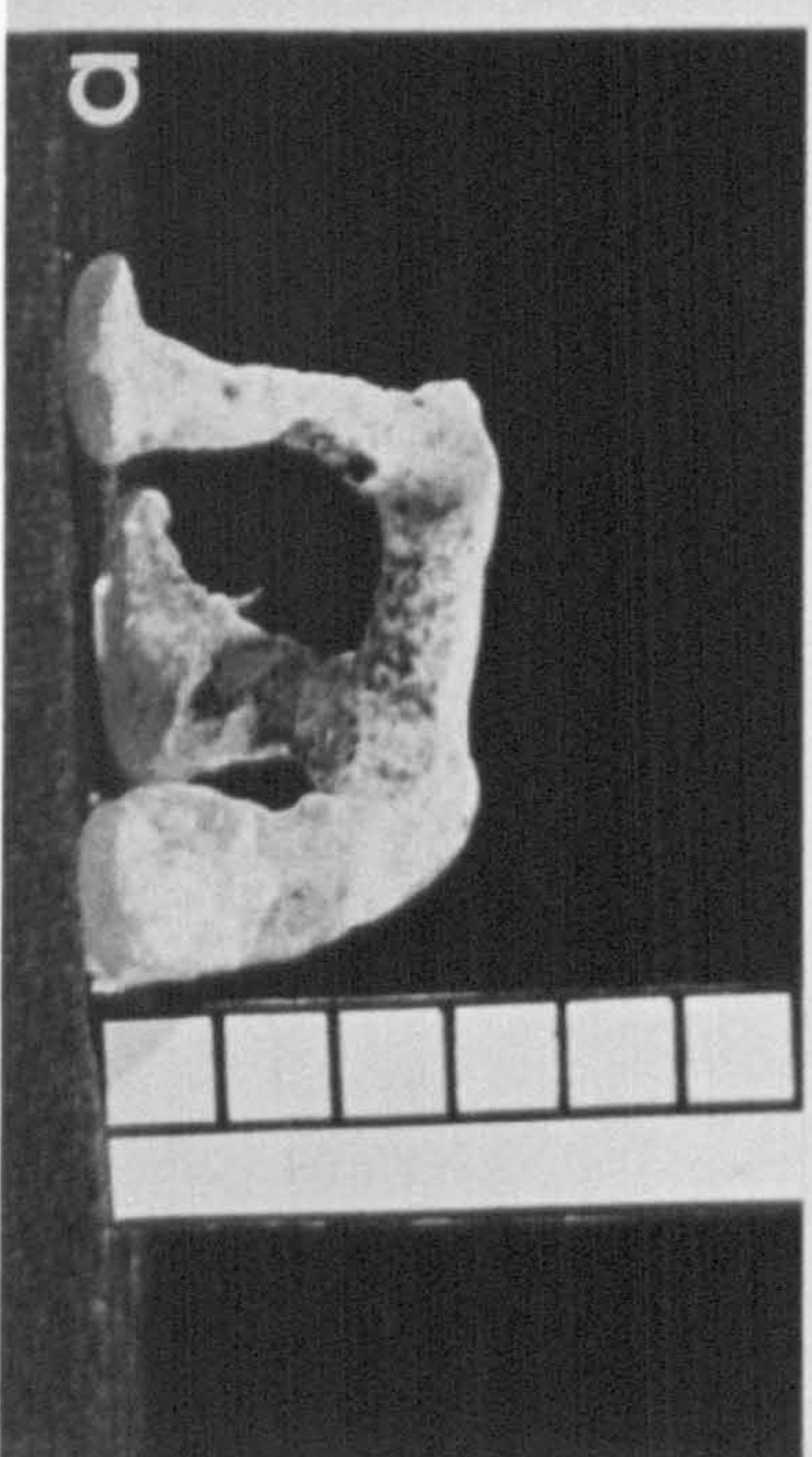
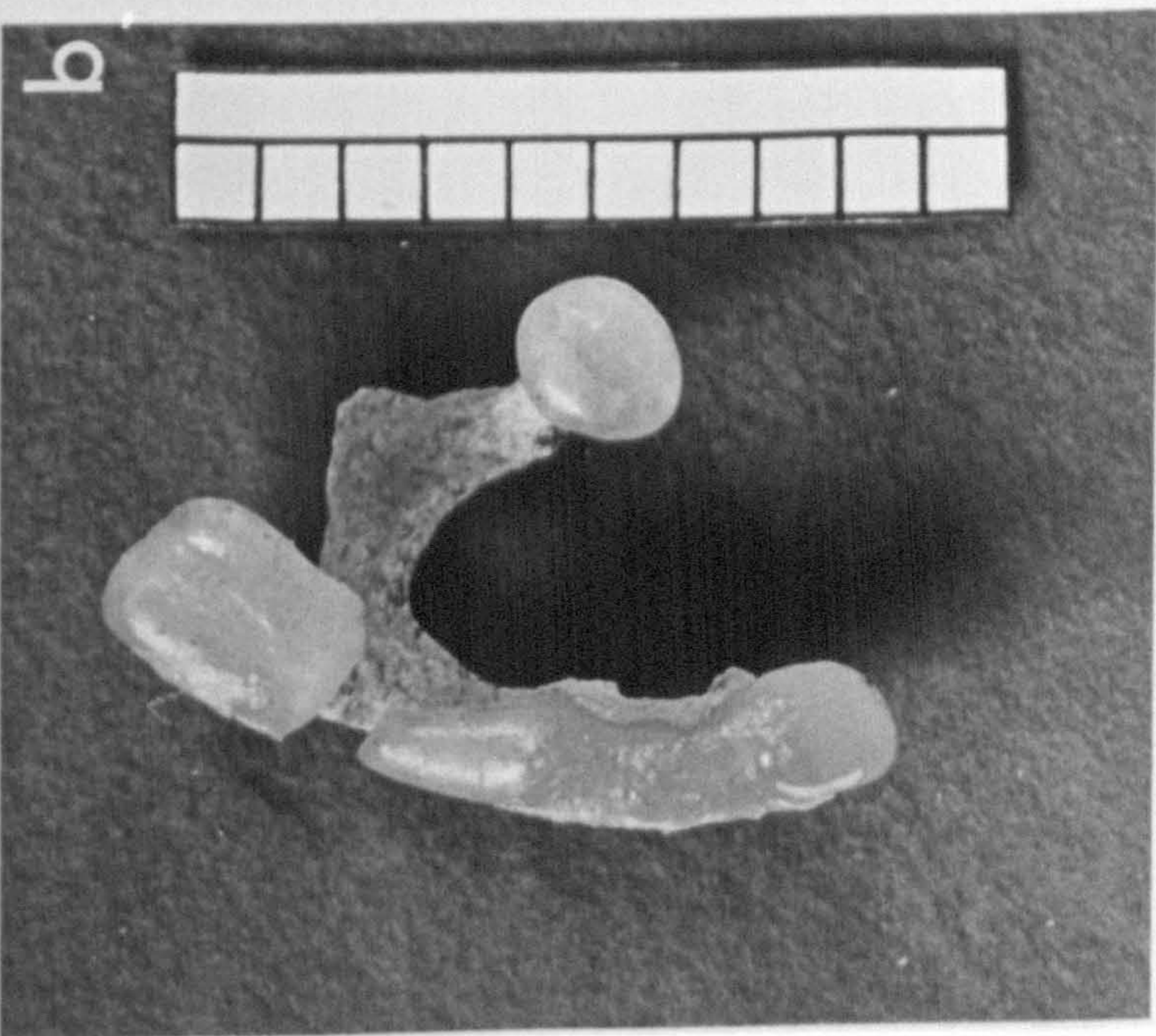
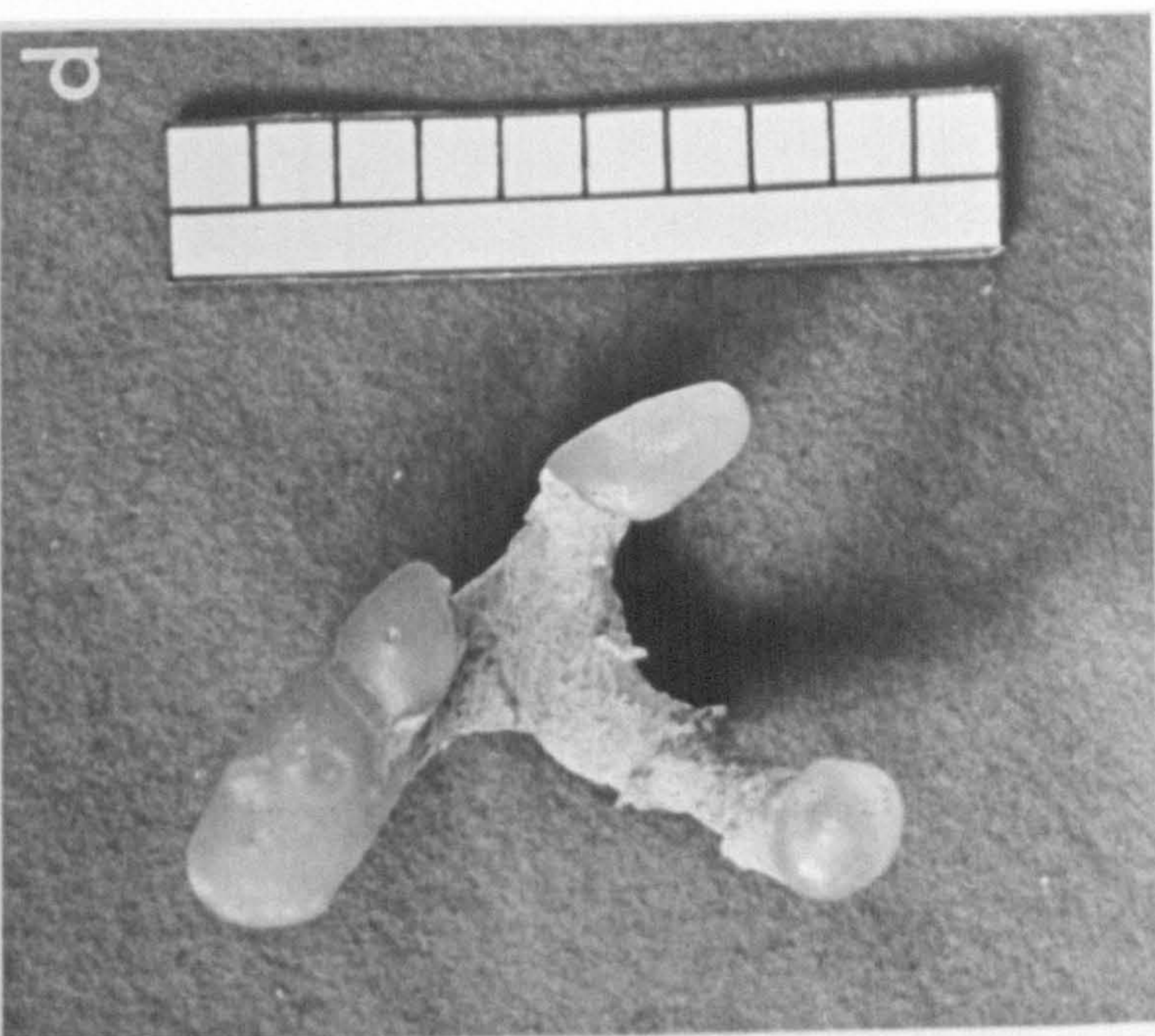




Plate 6.4

Polyester resin casts of burrows of juveniles.

(a) Elevation and (b) plan of burrow 4 in Table 6.5a.

(c) Elevation and (d) plan of burrow 8 in Table 6.5a.

Each gradation on the scale bar represents 10mm.



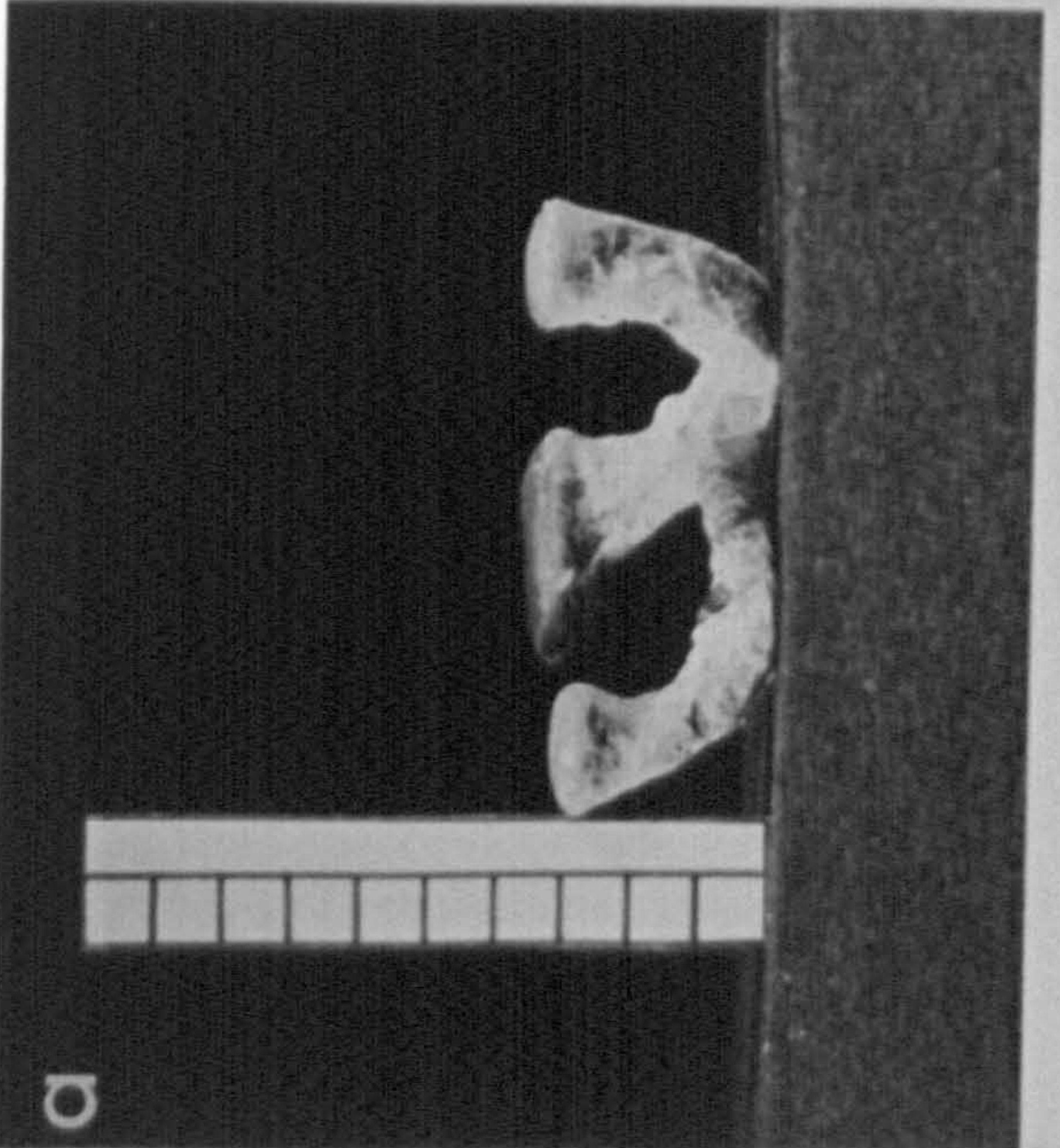
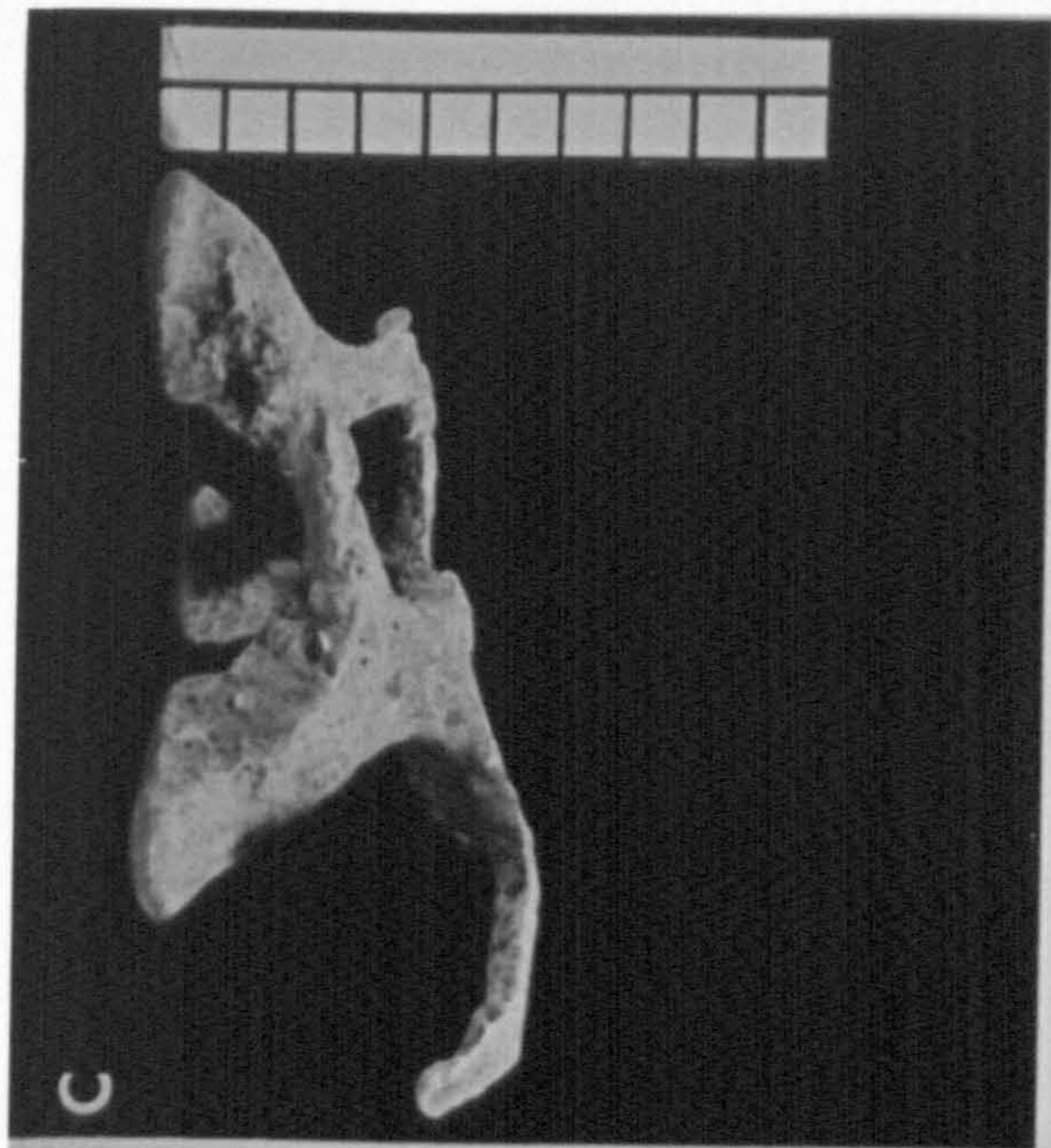
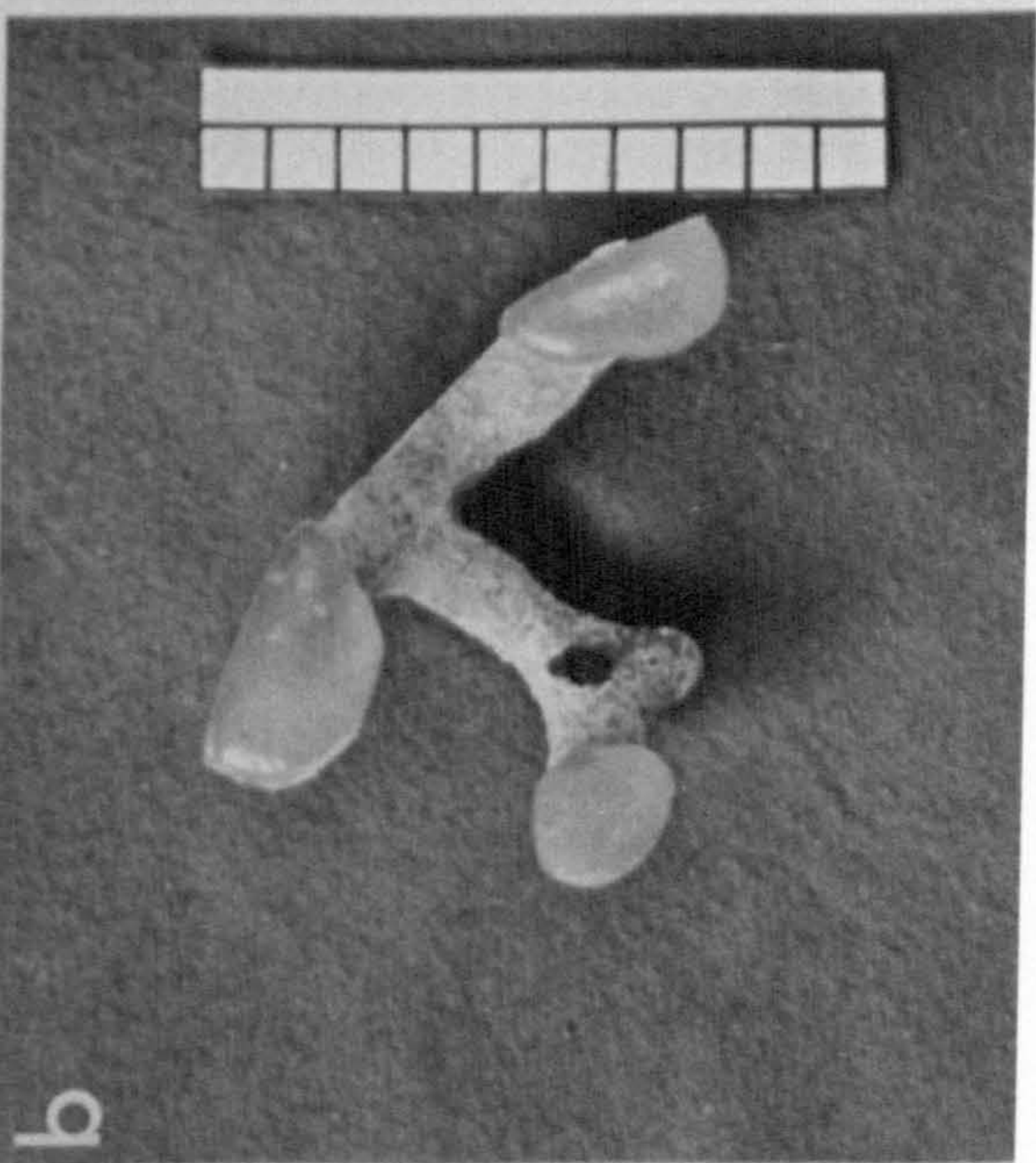
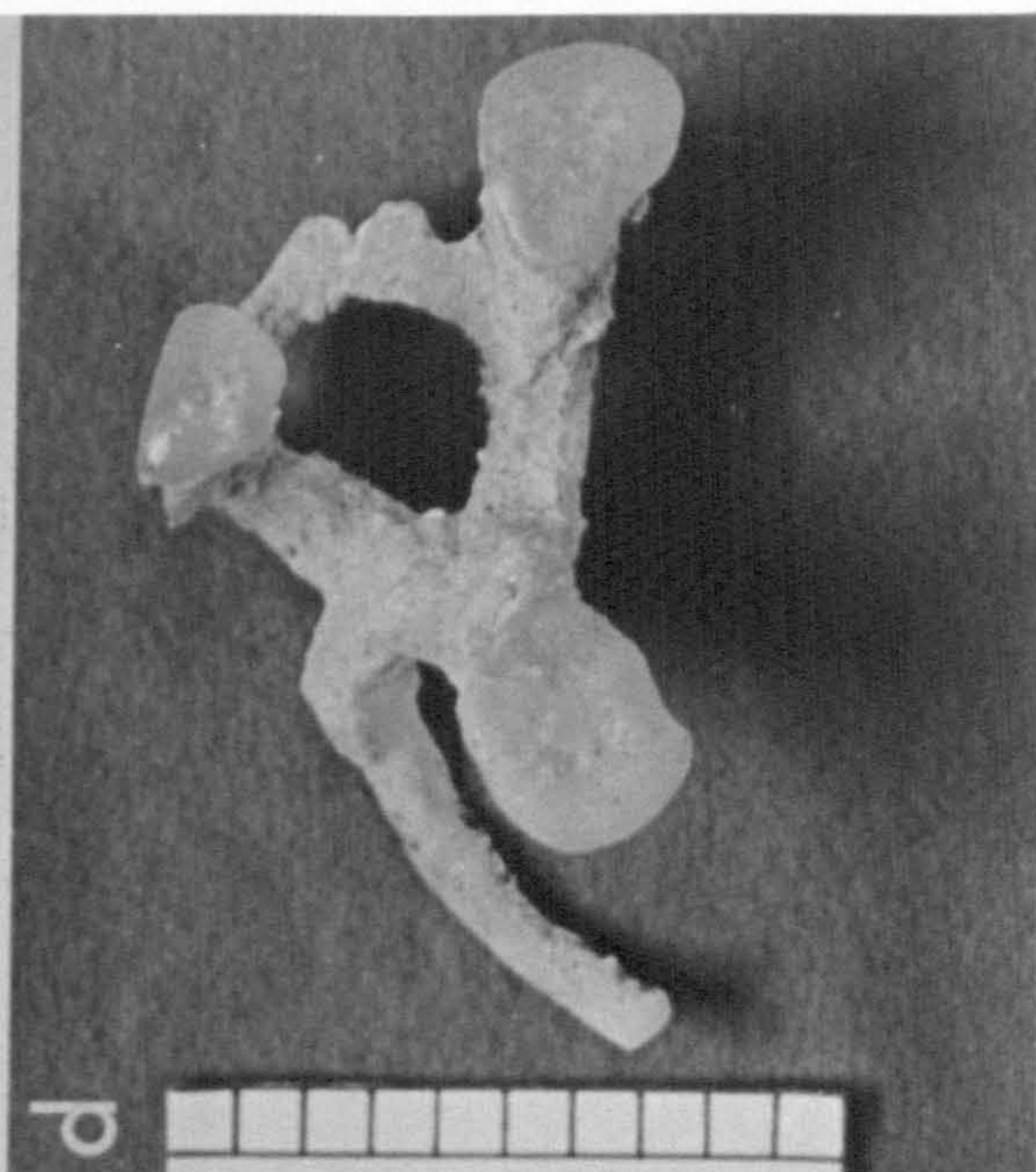




Plate 6.5

Polyester resin cast of burrows of juveniles.

(a) Elevation and (b) plan of burrow 6 in Table 6.5a.

(c) Elevation and (d) plan of burrow 7 in Table 6.5a.

Each gradation on the scale bar represents 10mm.



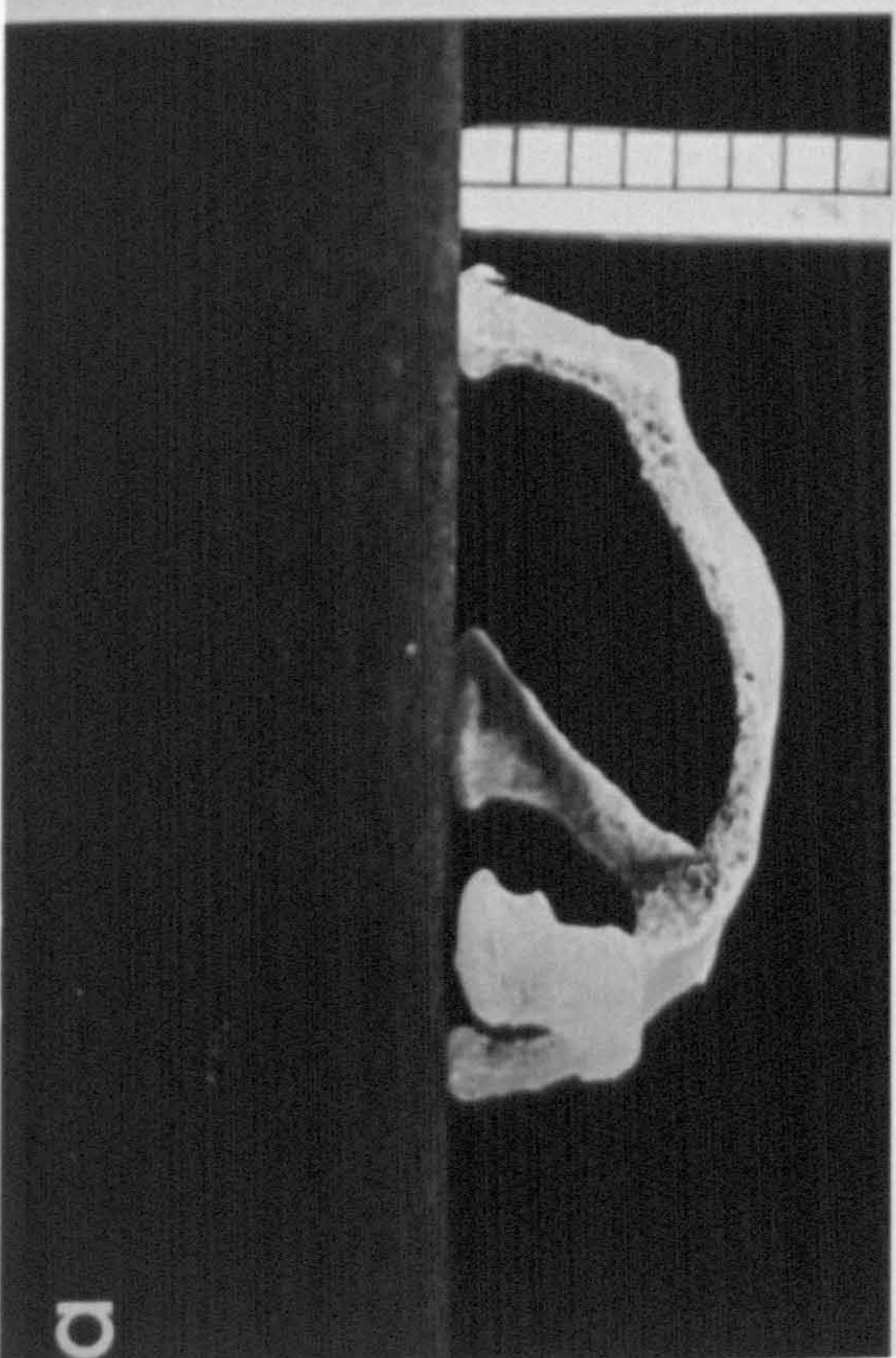
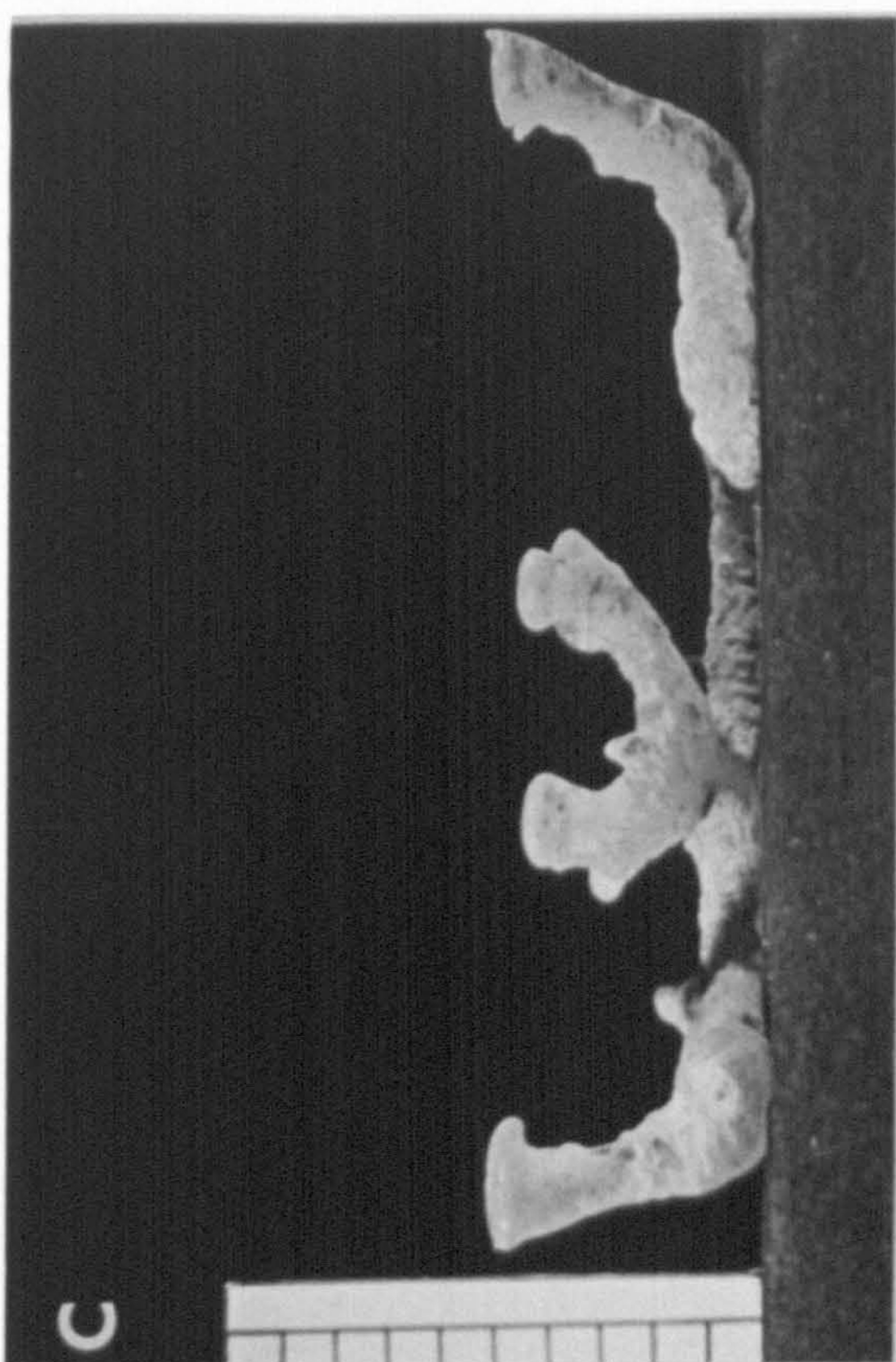
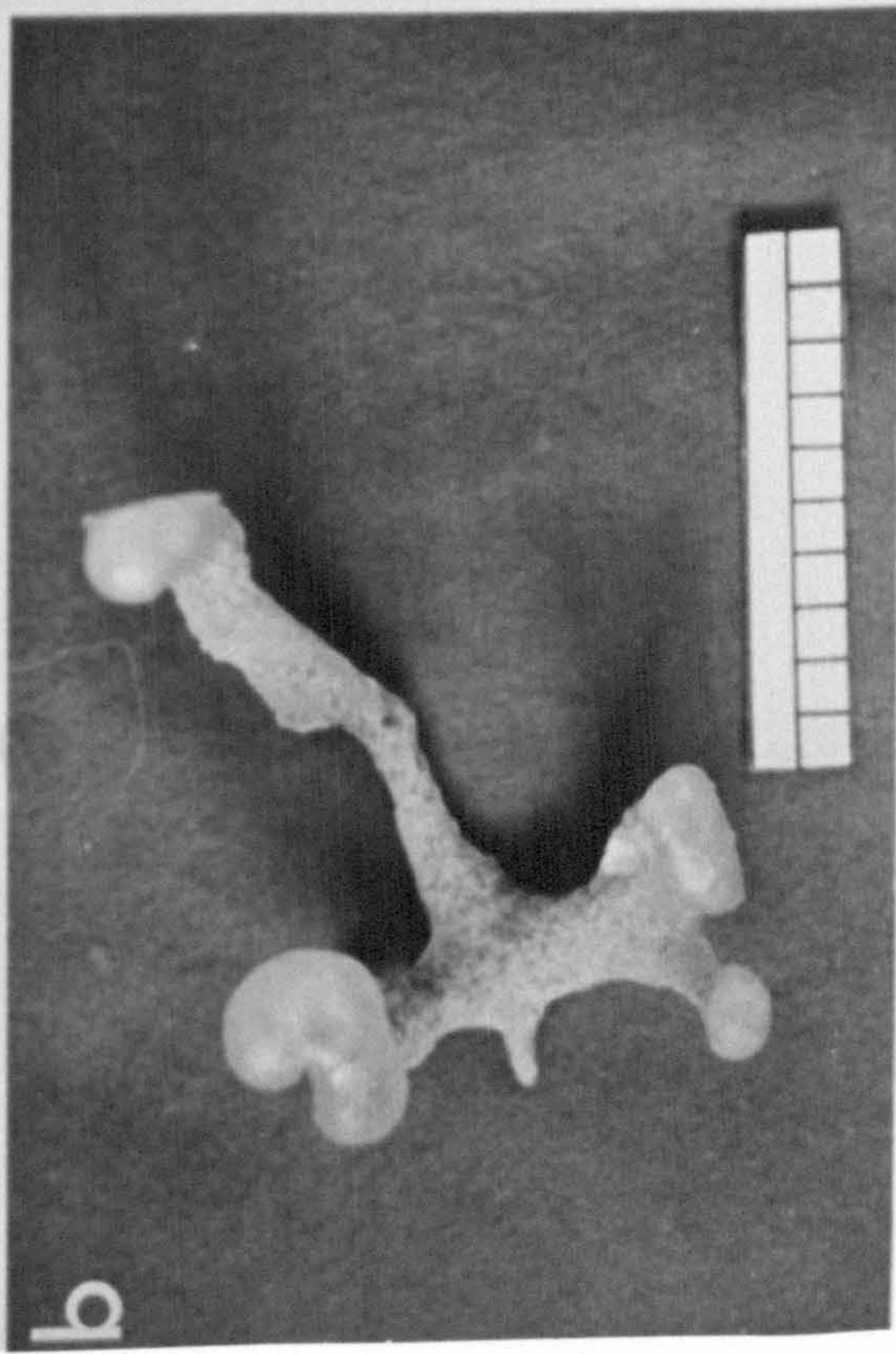
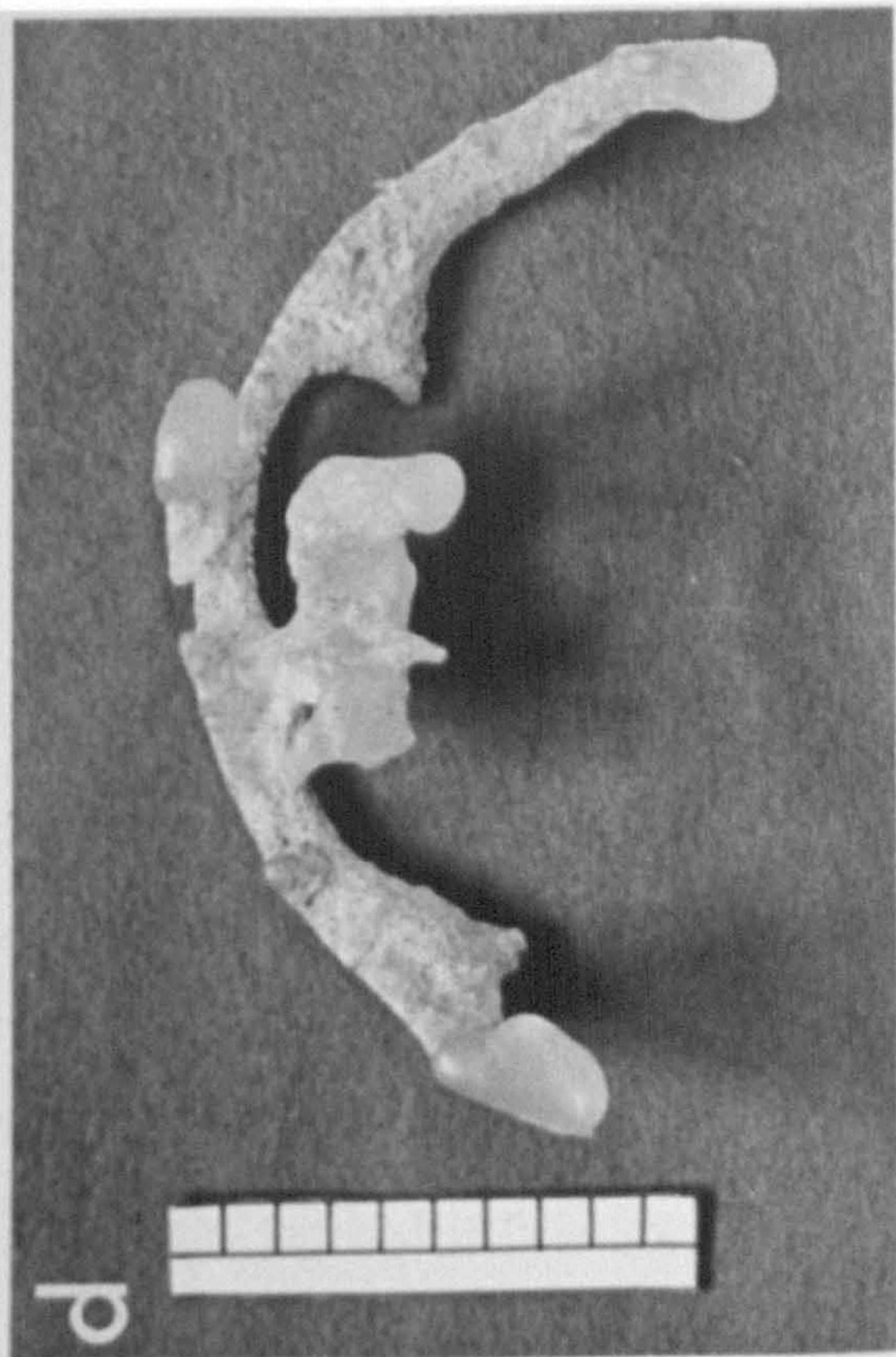




Plate 6.6

Polyester resin cast of burrows of juveniles.

(a) Elevation and (b) plan of burrow 10 in Table 6.5a.

(c) Elevation and (d) plan of burrow 11 in Table 6.5a.

Each gradation on the scale bar represents 10mm.



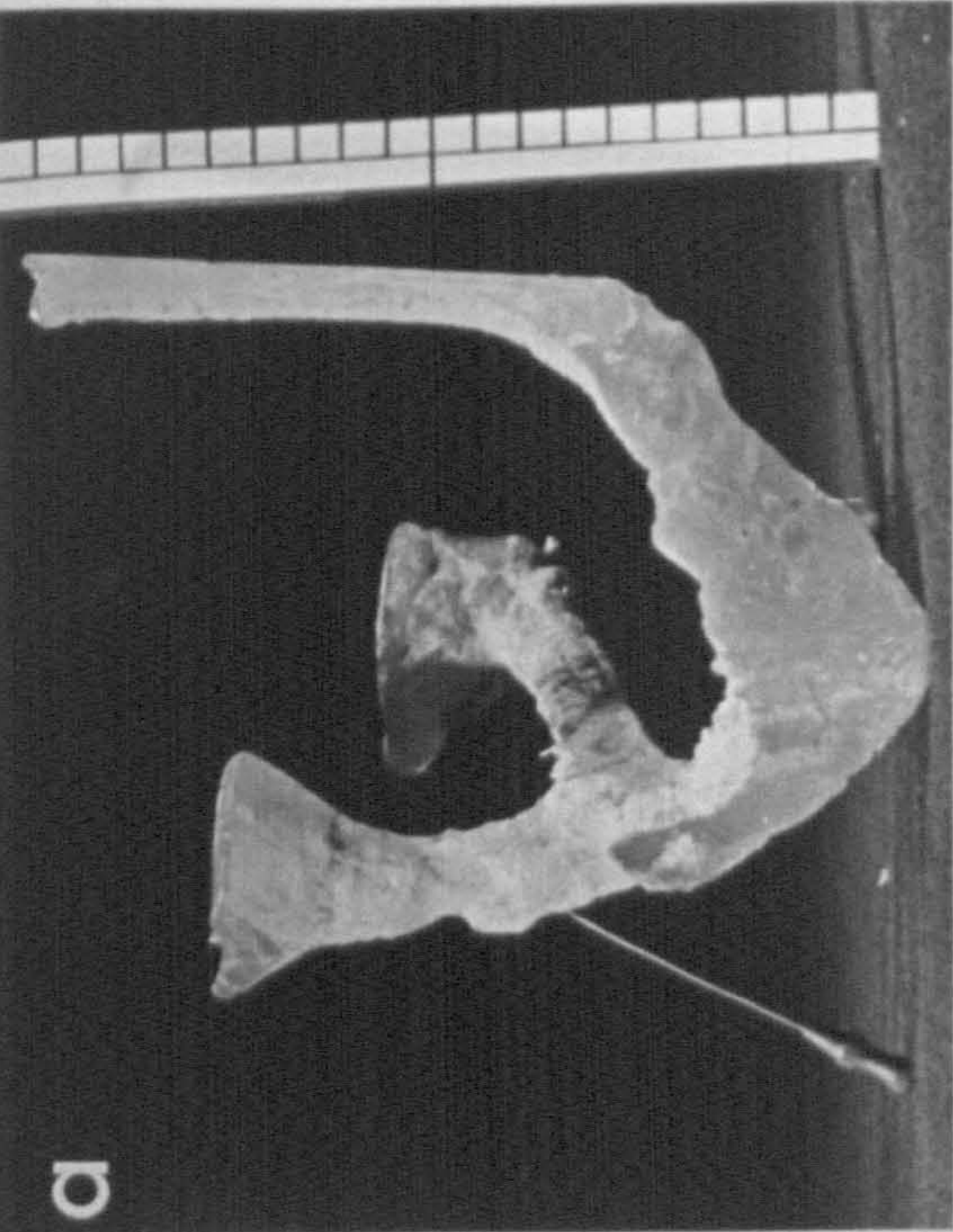
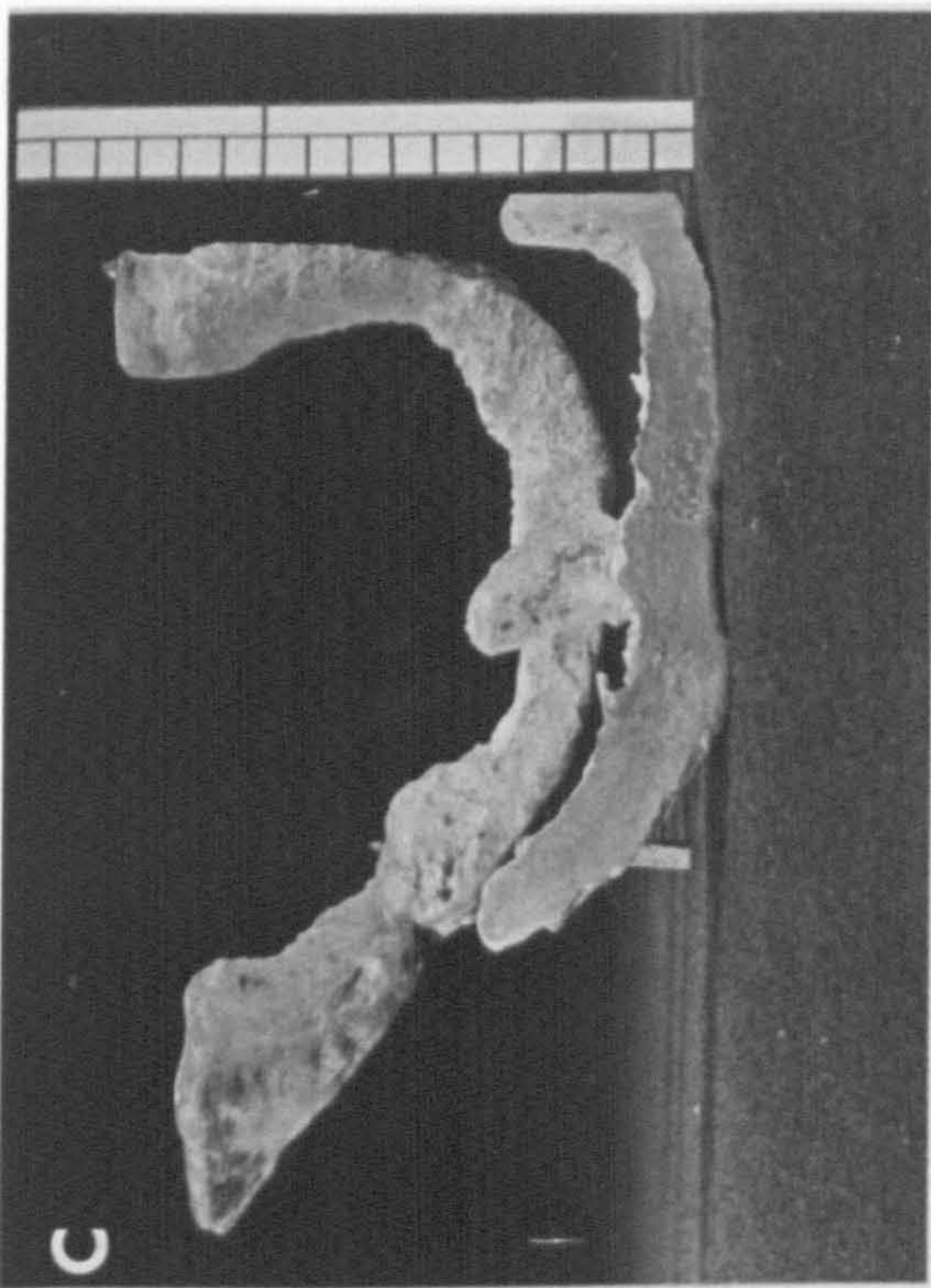
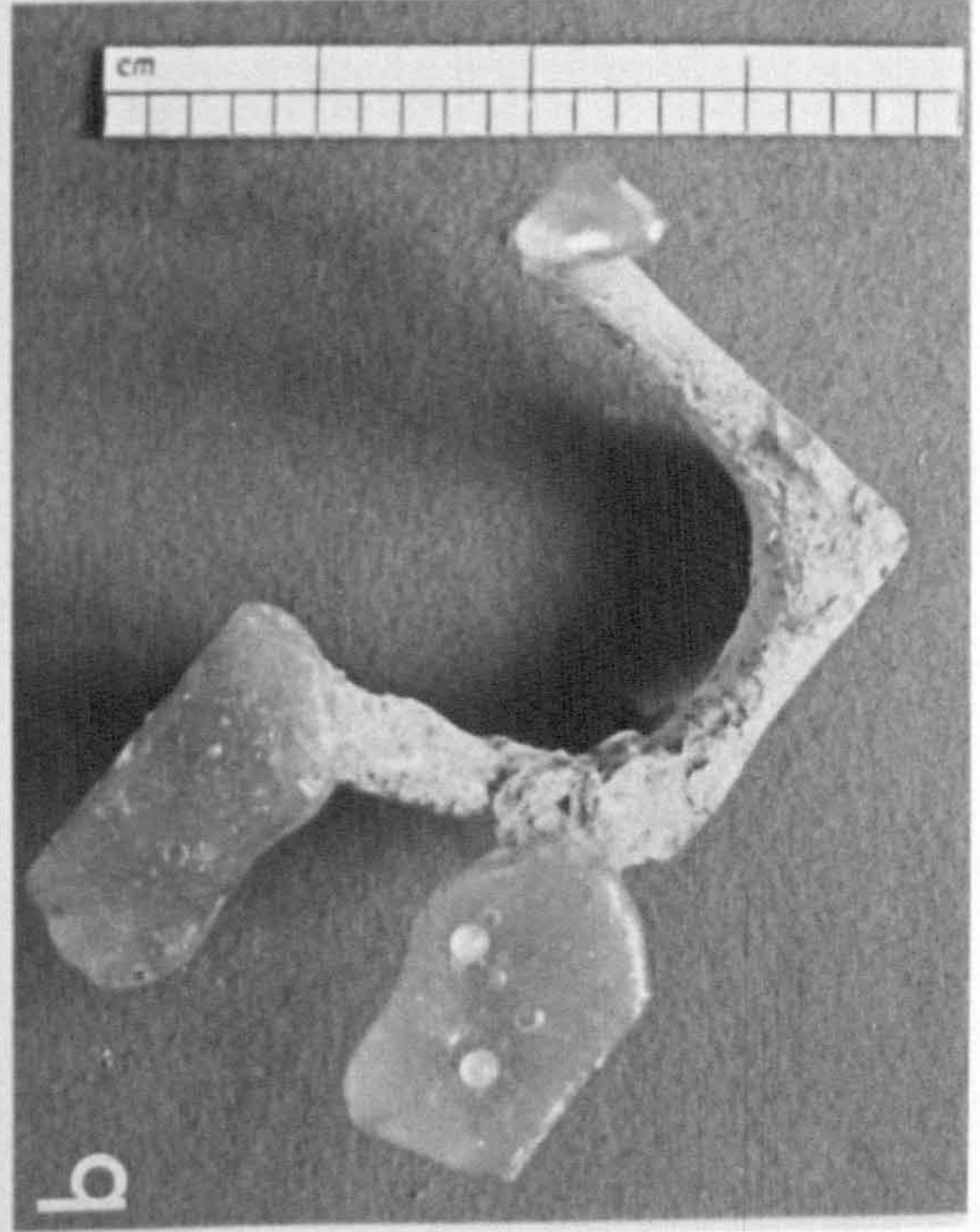
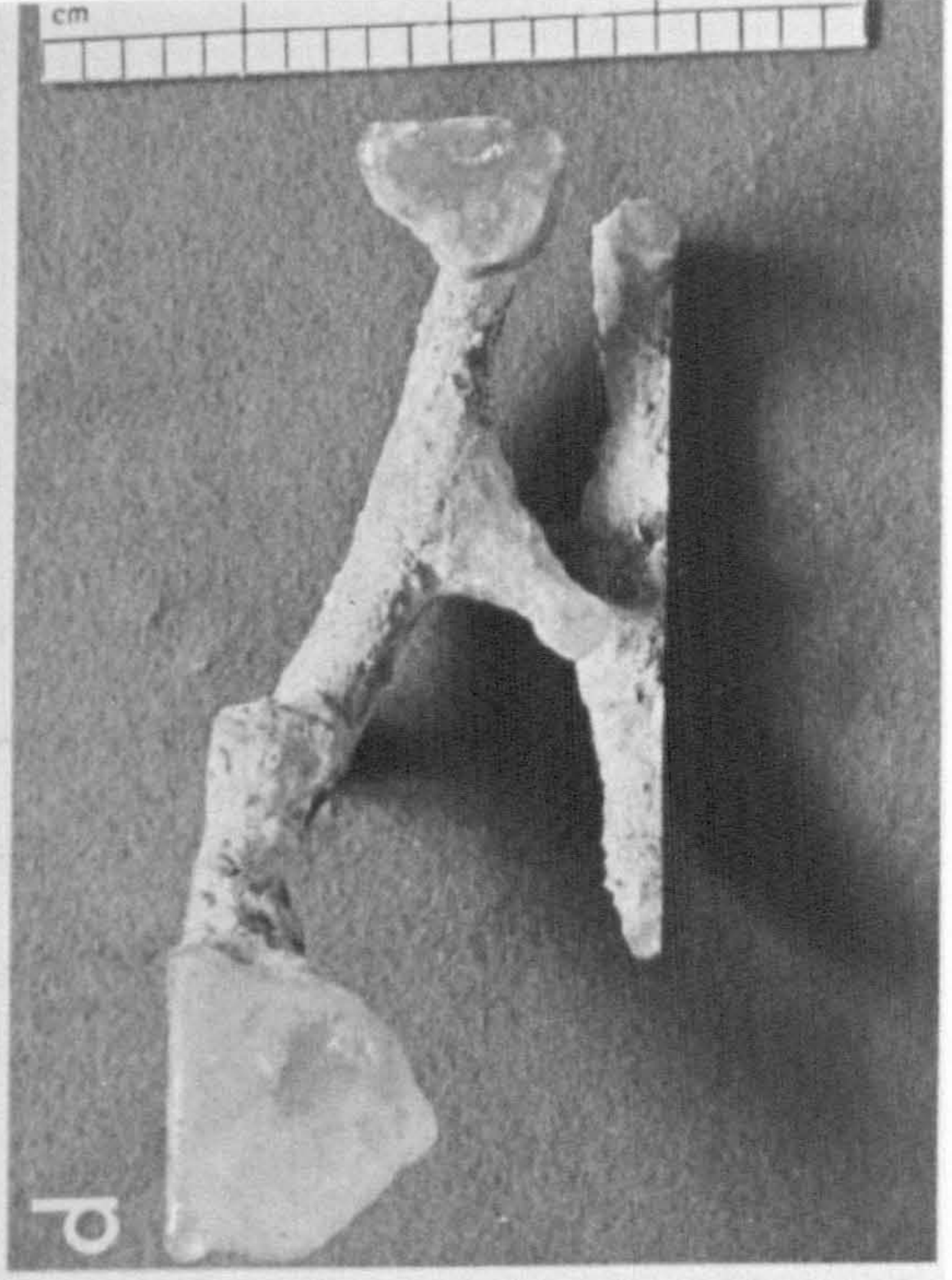




Plate 6.7

Polyester resin casts of burrows of adults.

(a) Elevation and (b) plan of burrow 13 in Table 6.5b.

(c) Elevation and (d) plan of burrow 17 in Table 6.5b.

Each gradation on the scale bar represents 10mm.



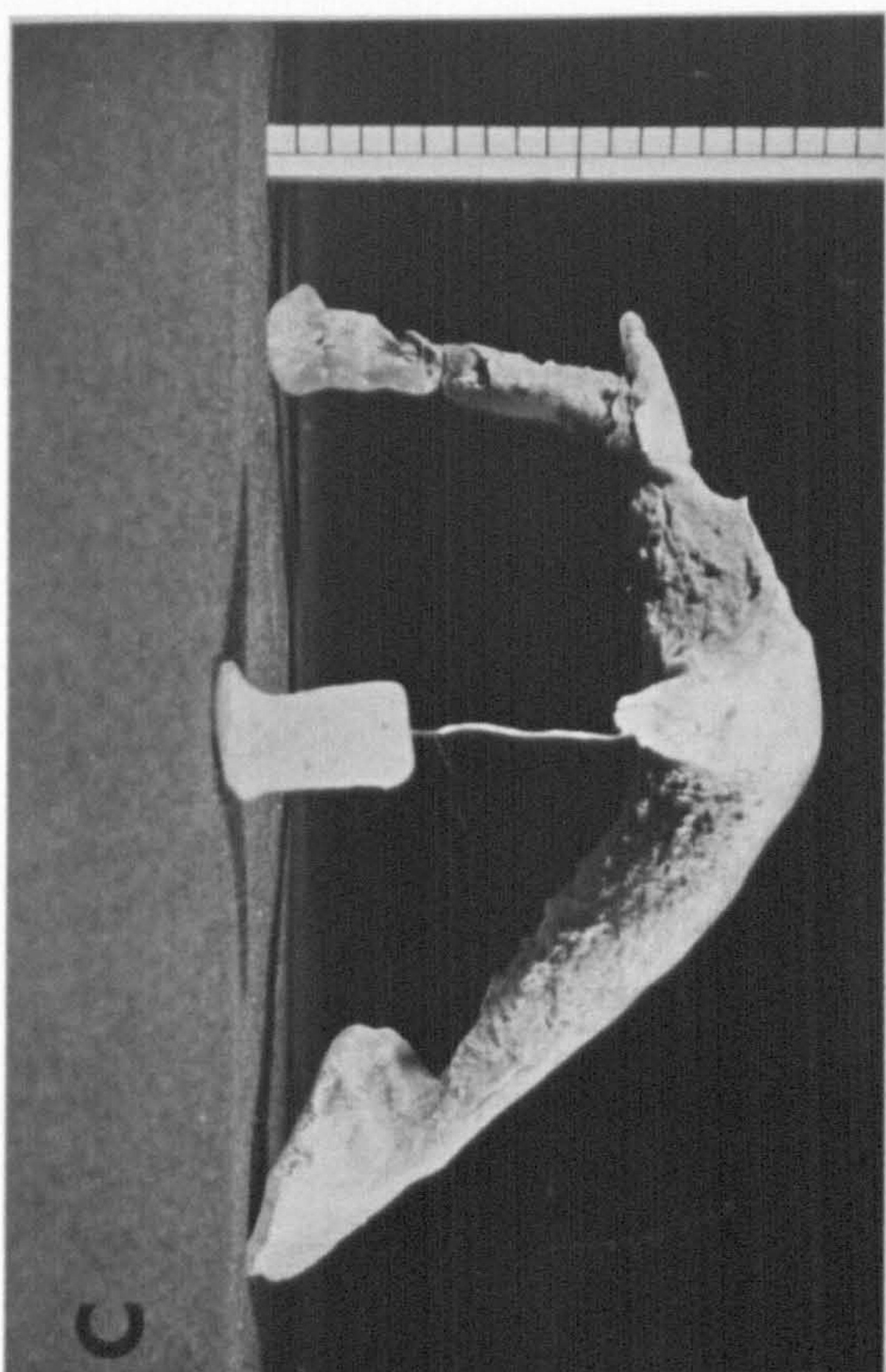
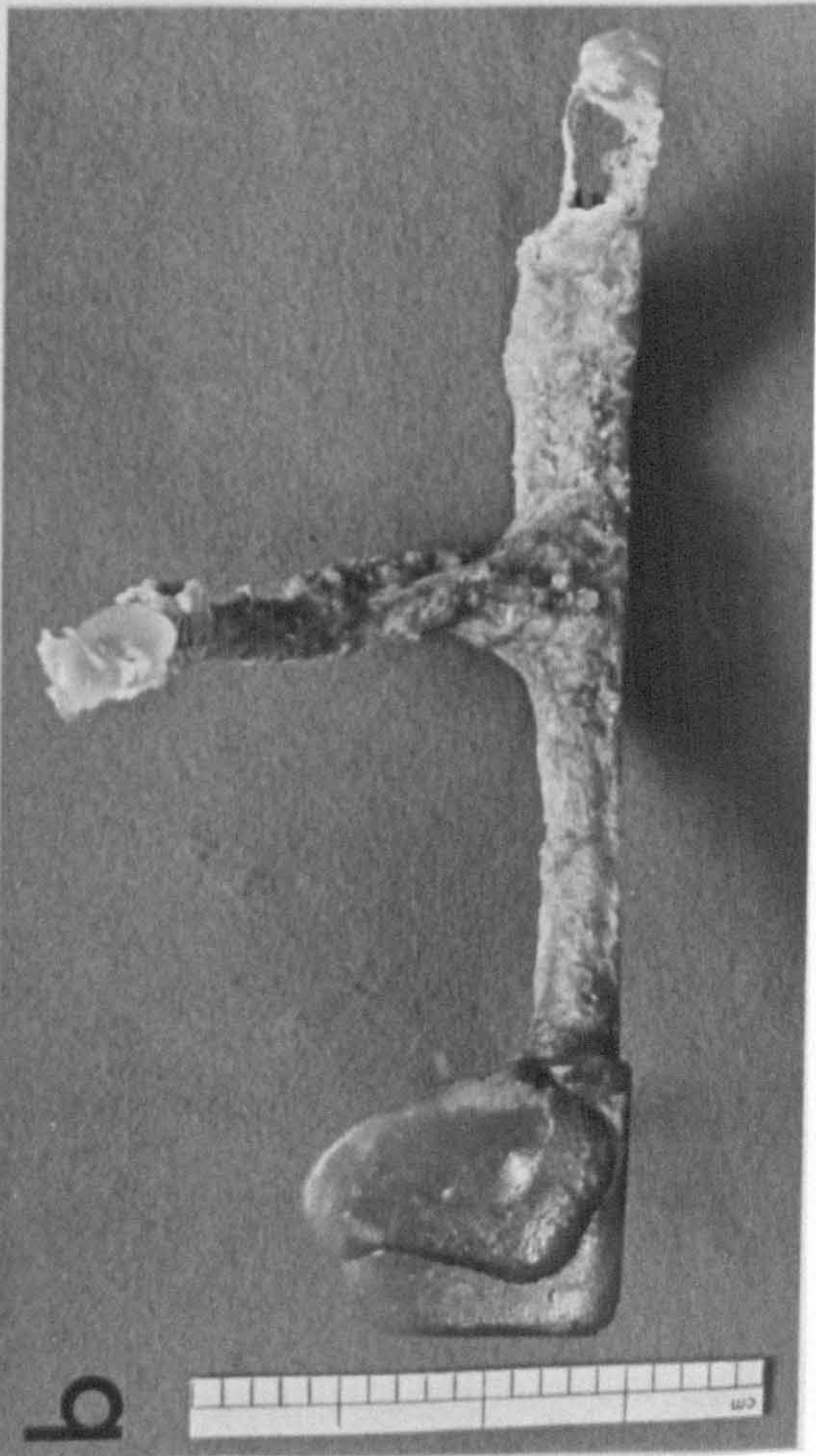
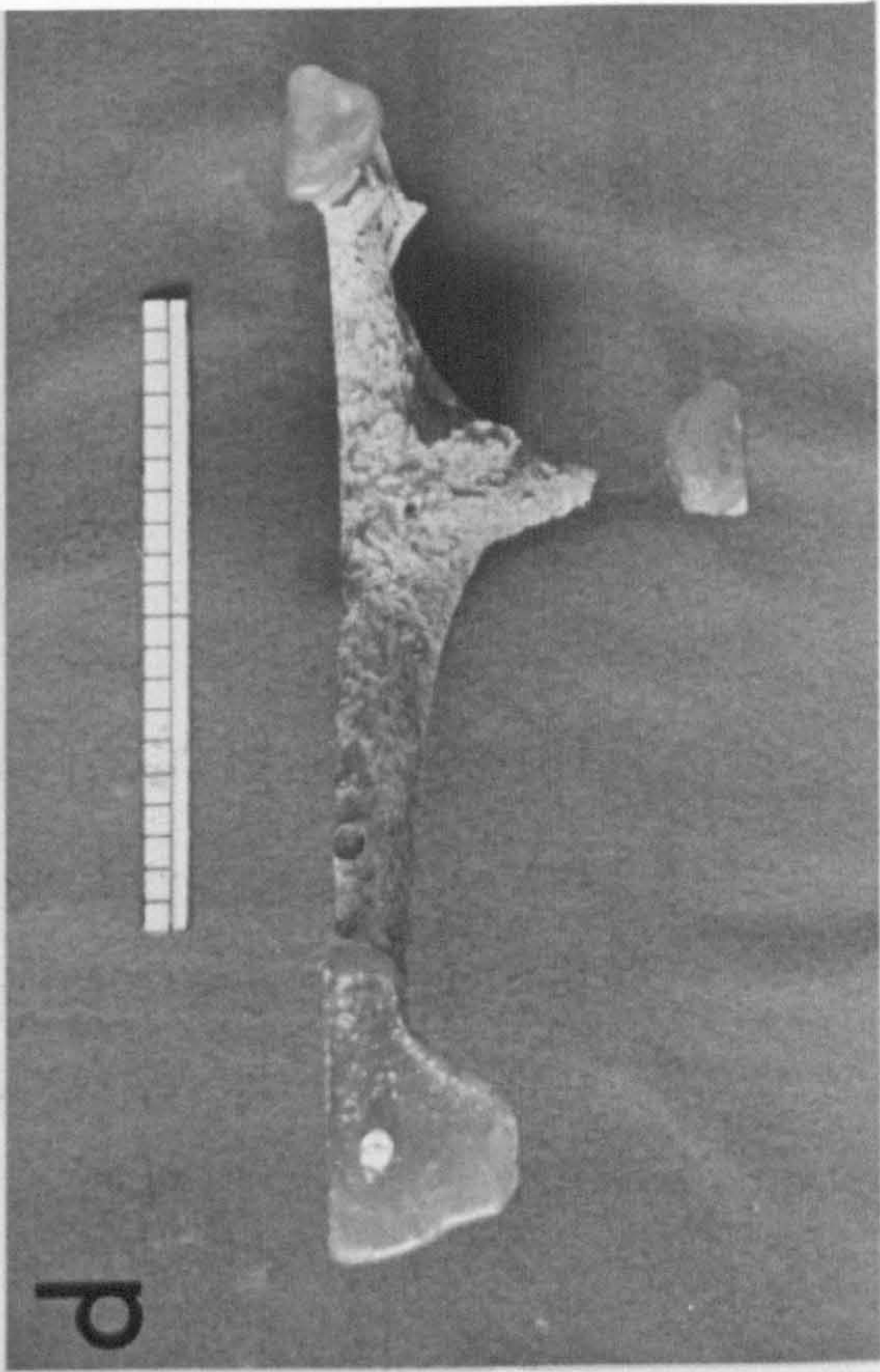




Plate 6.8

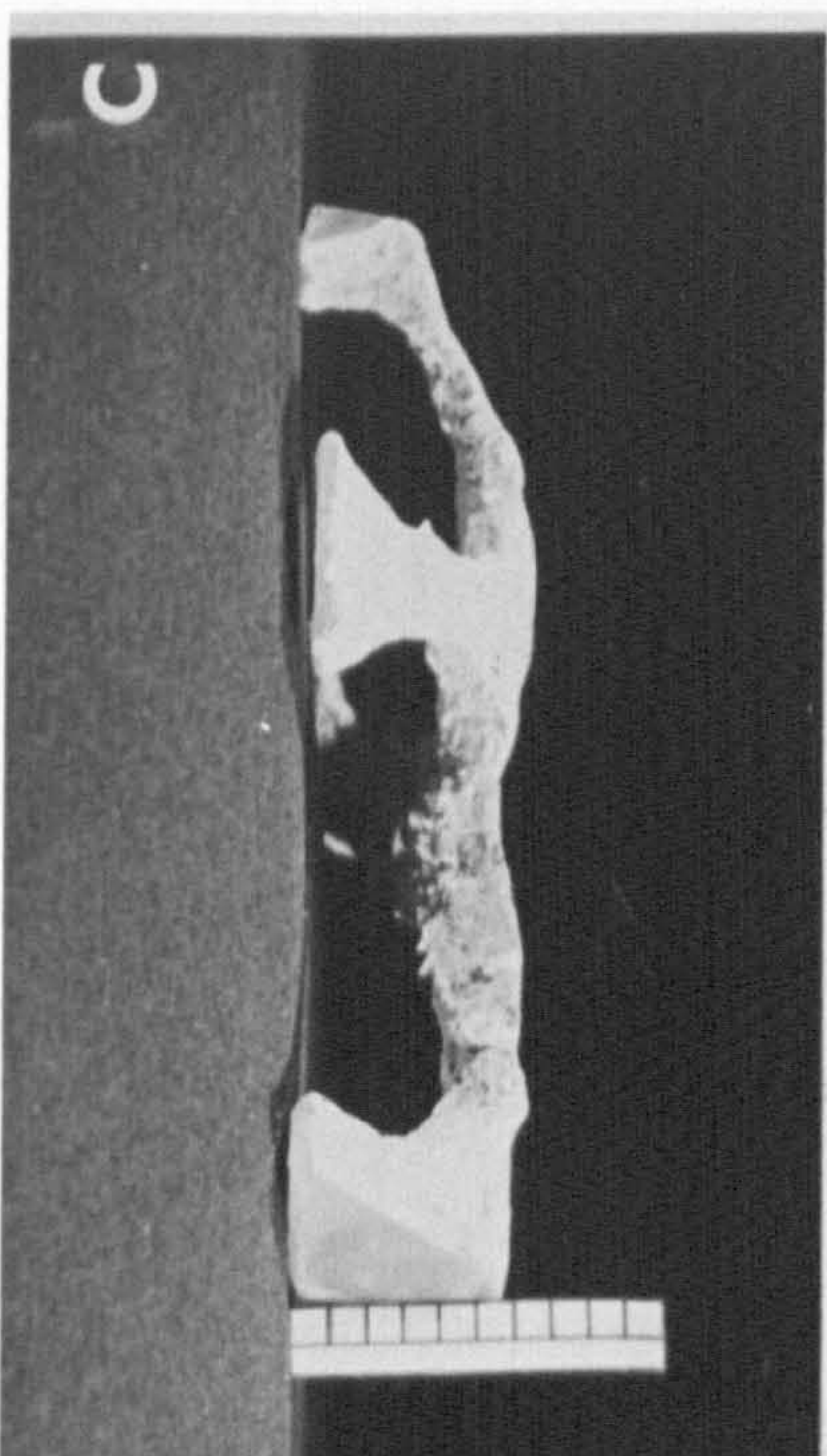
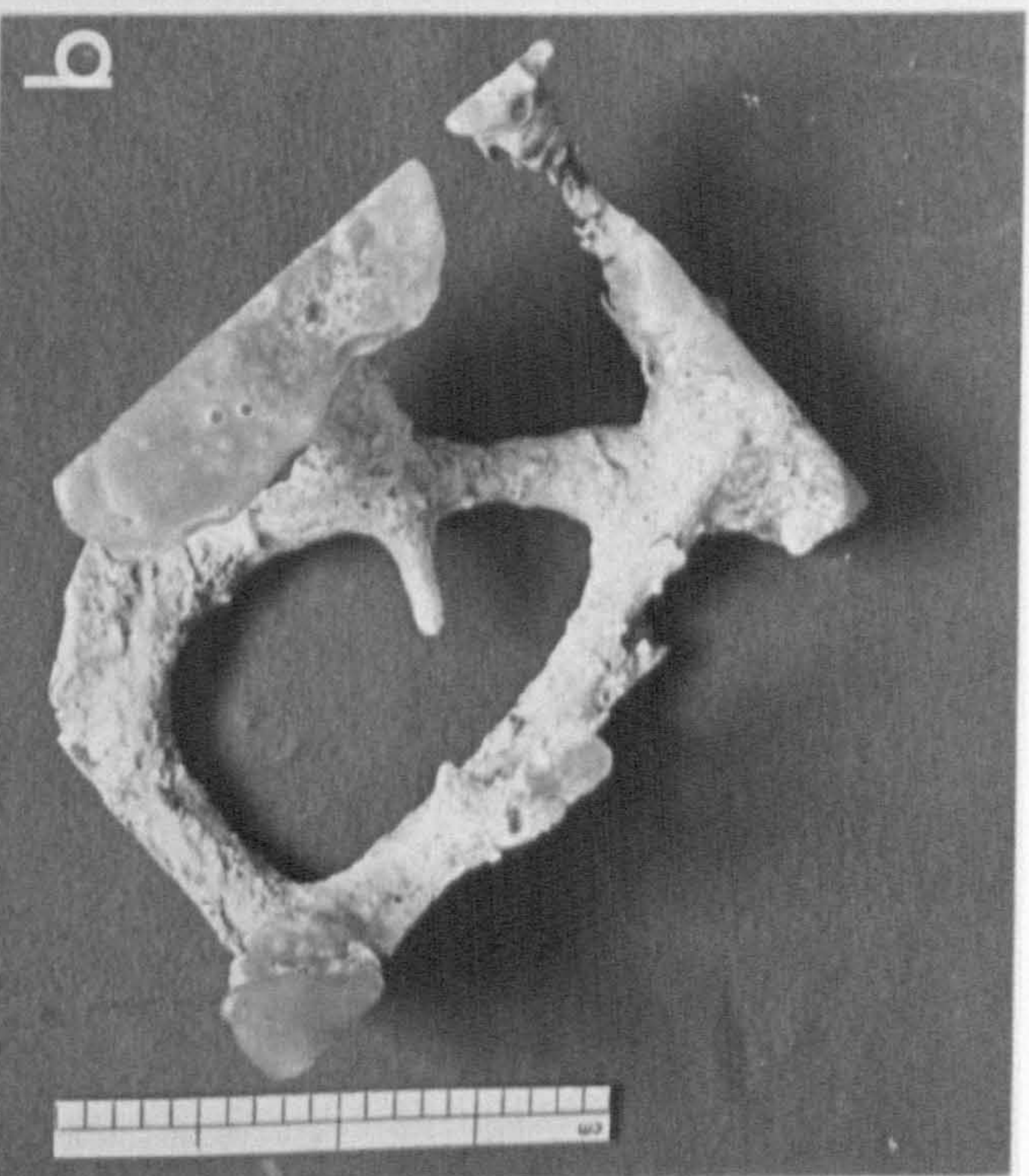
Polyester resin casts of burrows of adults.

(a) Elevation and (b) plan of burrow 12 in Table 6.5b.

(c) Elevation and (d) plan of burrow 16 in Table 6.5b.

Each graduation on the scale bar represents 10mm.







### Plate 6.9

Polyester resin cast of a burrow system involving the burrows of two adults of differing sizes (a,b).

(a) Elevation and (b) plan of burrow 19 in Table 6.5c.

Each gradation on the scale bar represents 10mm, the total length of the scale bar represents 0.2m.



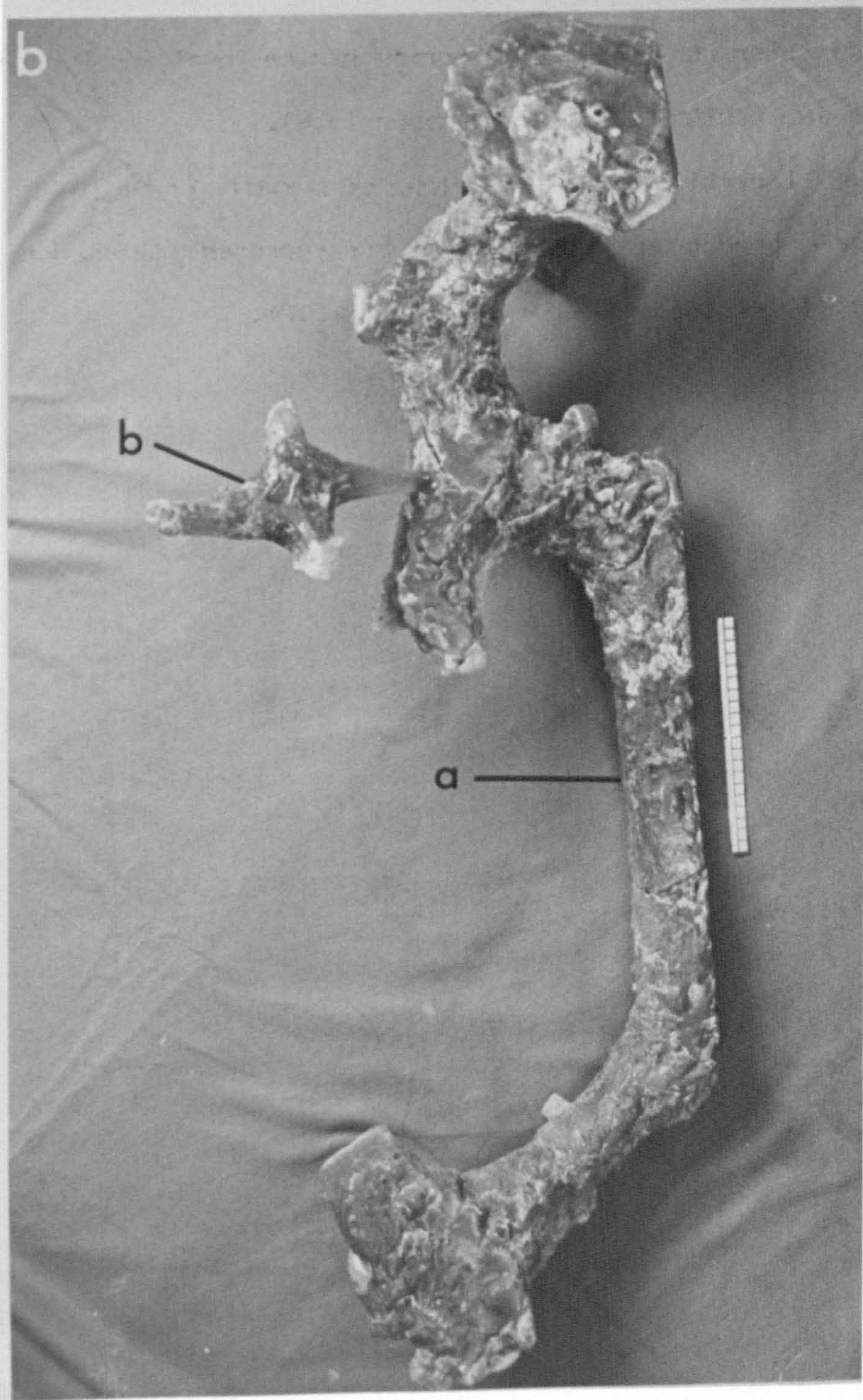
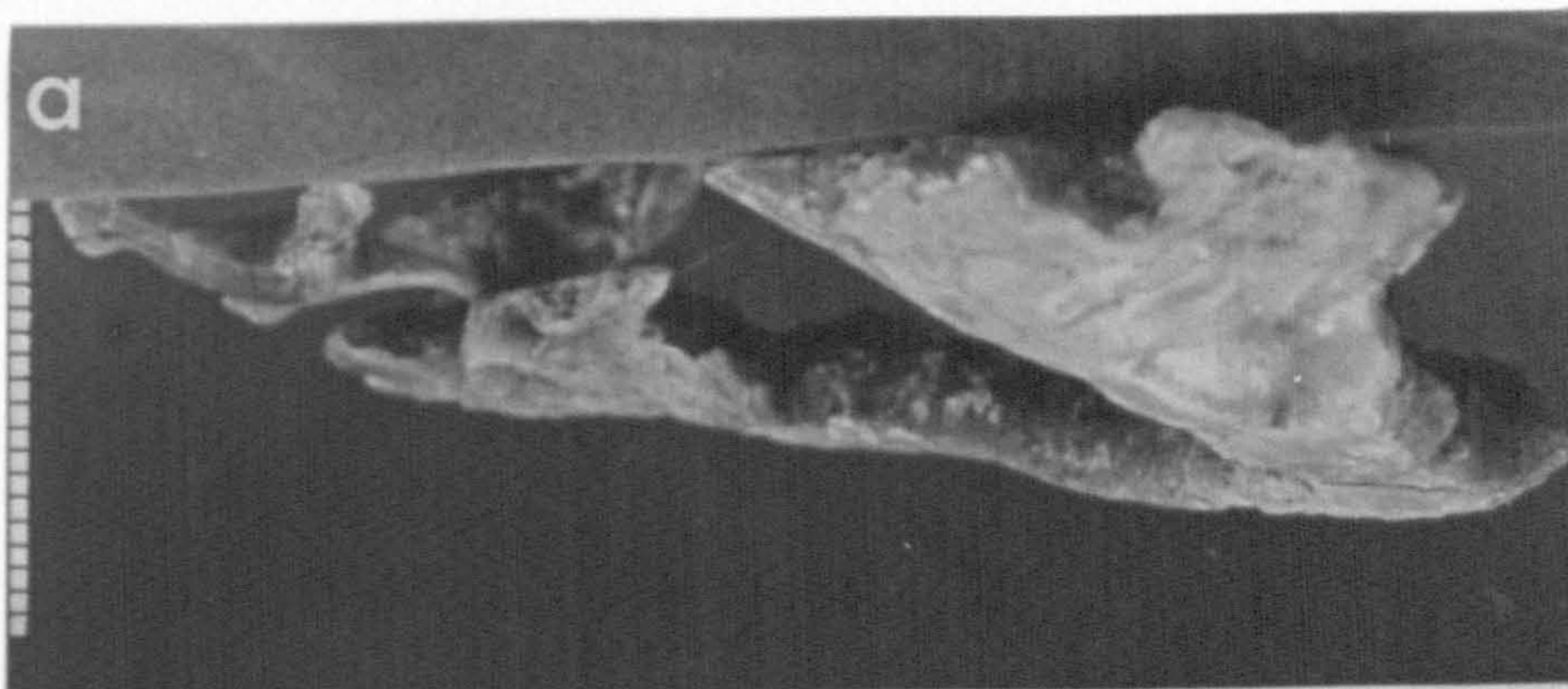




Plate 6.10

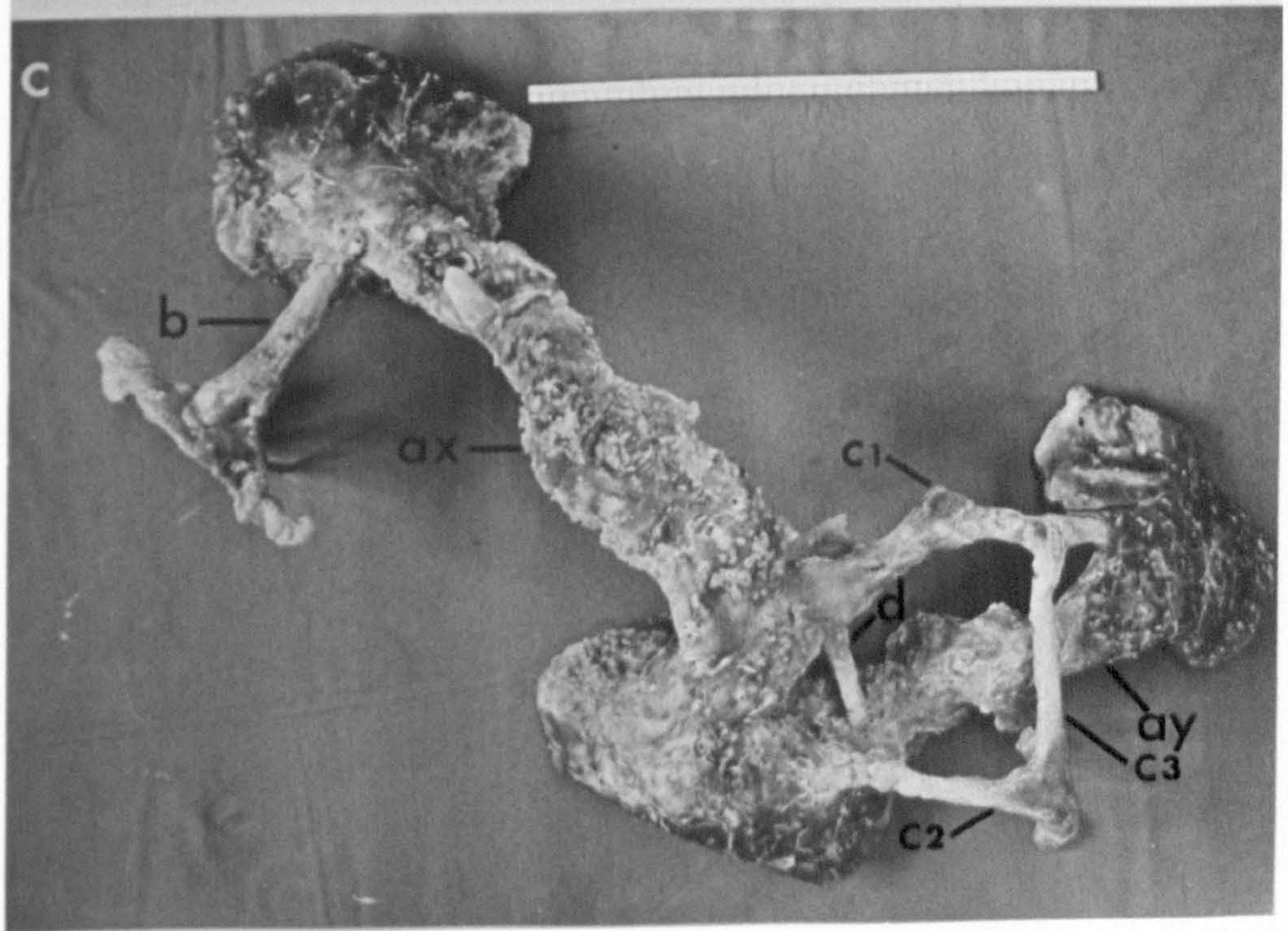
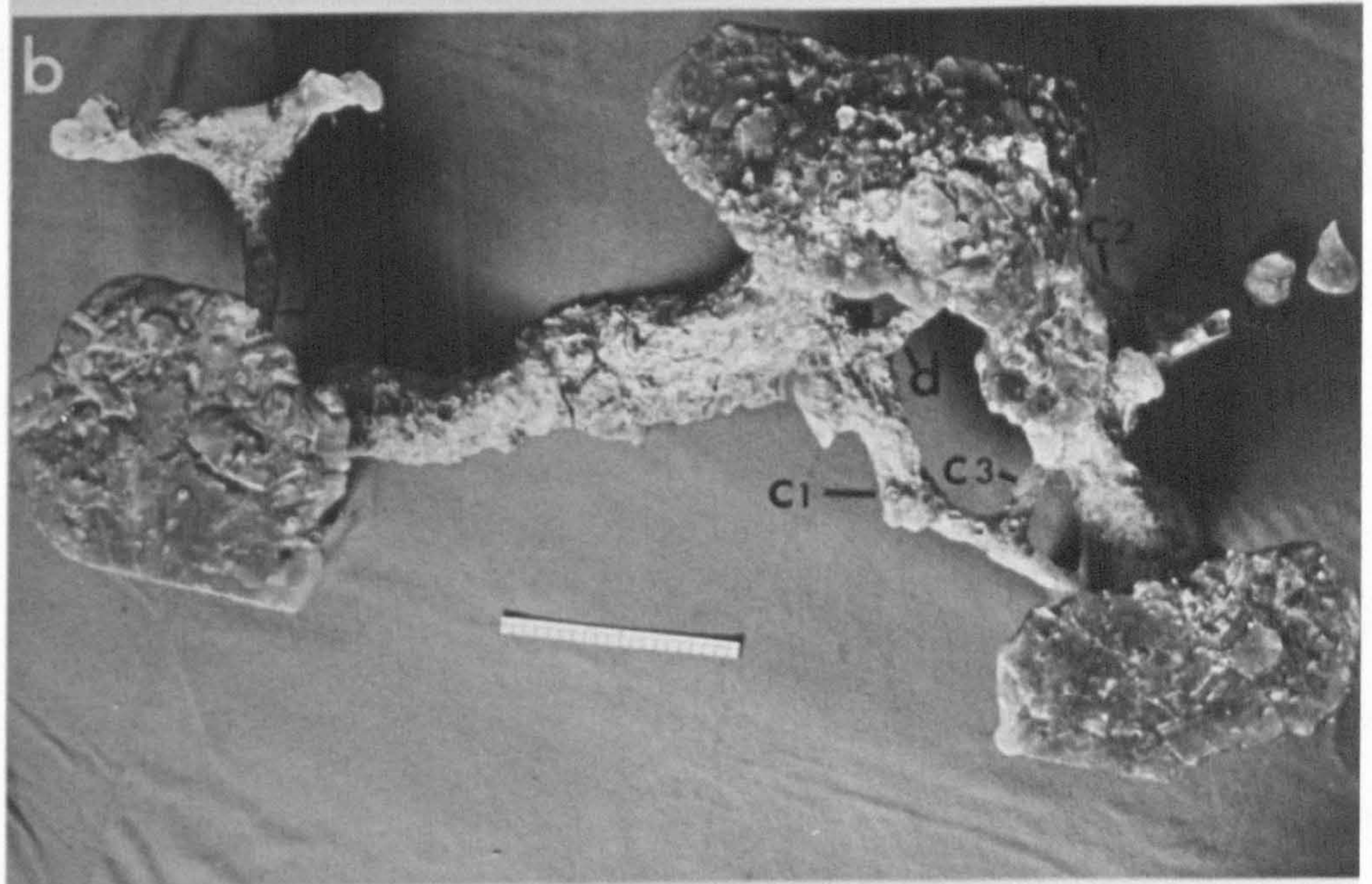
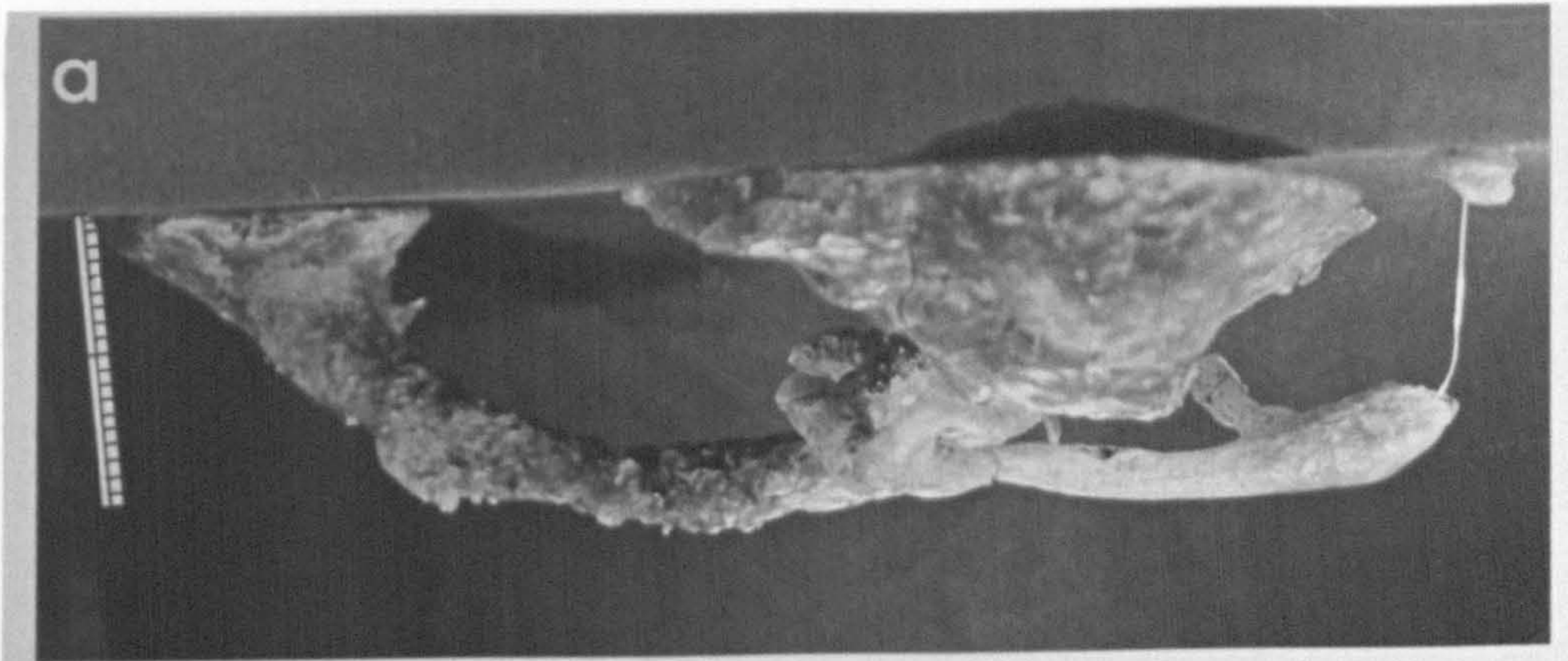
Polyester resin cast of a burrow system involving two juveniles and two adults.

(a) Elevation and (b), (c) plan of burrow 20 in Table 6.5c.

Each gradation on the scale bar represents 10mm, the total length of the scale bar represents 0.2m in (a) and (b) and 0.5m in (c).

For labelling see text.







## 6.4. Discussion

### 6.4.1. Effect of substratum type on intermoult period prior to metamorphosis

The results from this present study appeared to indicate that stage 3 zoeae can delay metamorphosis into the juvenile phase in the absence of a suitable substratum. Moulting into the first postlarval stage occurred, on average, after 9 - 10 days with a mud or sand substratum but metamorphosis was delayed about three days and in some cases up to one week when the substratum was gravel or an acrylic sheet. This suggests that the larvae may be searching for a suitable substratum. Under natural conditions, this delay would enable the larva to exploit the bottom currents and thus investigate a larger area. Although the strategy of delayed metamorphosis carries a greater risk of predation, the location of a suitable substratum is a pre-requisite for successful settlement and adoption of a burrow (Farmer, 1975 ; Chapman, 1980). Delayed metamorphosis has also been recorded in H.americanus (Cobb, 1968 ; Botero and Atema, 1982) in which the transition from the larval to the juvenile phase was considered to occur between stages 4 and 5 ie. between postlarval stages 1 and 2 according to the classification adopted in this present study (see introduction). In view of this, it would have been interesting to have continued this investigation using the first postlarval stage but, unfortunately, there were insufficient numbers of these available.



#### 6.4.2. Substratum selection

The first postlarval stage appeared to be a transitional stage between the planktonic and benthic environments. Substrata were apparently explored during this stage but final settlement did not occur until towards the end of this stage or until the second postlarval stage. It therefore appears that the function of the 1st postlarval stage in Nephrops is primarily one of exploration for eventual recruitment after the subsequent moult, which is slightly metamorphic (see Chapter 4). The changes which occur after this moult (notably in the gills) may be pre-requisites for the adoption of the benthic habit. Early extensive reconnaissance behaviour has been well documented for the settling stages of other marine organisms (Wisley, 1960 ; Cranfield, 1973 ; Crisp, 1976) reflecting the previously established ability of these stages to discriminate between favourable and unfavourable habitats (see Crisp, 1976). The older postlarvae (ie. beyond stage 1) showed a strong preference for mud, followed by muddy-sand, sand and gravel. From sediments with various mixtures of mud and sand most postlarvae selected those with the highest proportion of mud. As the sand content of the mud was less than 10% and the sand contained a negligible amount of silt and clay particles the coarsest substratum on which a postlarva successfully excavated a burrow would have comprised between 40 and 46% by weight of sand.

Field studies have shown that adult Nephrops can inhabit a wide range of sediments in which the silt and clay component varies from 30% to almost 100% (Chapman and Bailey, 1986), sand comprising the remaining fraction. These authors suggest that within this range the highest densities of Nephrops tend to occur on the coarsest 'muds'.

The results from this present study indicate that these higher densities may not occur as a direct consequence of the intrinsic qualities of the sediment. However, although the mud based substrata used in the choice cell experiments were given a month to consolidate before the trials were begun they would not have consolidated to the same degree as in the field. Caution must therefore be exercised in the interpretation of the results obtained from this laboratory based study.

After attainment of the third postlarval stage most juveniles selected a suitable substratum and commenced burrowing within 40 minutes of introduction. This was faster than in younger juveniles of the second postlarval stages which sometimes took several hours before initiating burrowing behaviour. When provided with an unsuitable substratum, however, the older stages (postlarval stages 4 and 5) demonstrated limited swimming ability by making short vertical excursions from the bottom. Swimming activity appeared to decrease with increasing size, although juveniles with carapace lengths of up to 5 - 7mm (corresponding to postlarval stages 3 - 5 respectively) have been caught in small mesh pelagic trawls (Chapman, 1980).

The present work supports the generally held view that juvenile Nephrops actively select substrata of a similar type to that which supports adult populations (Chapman, 1980). This was not found to be the case for juvenile H.americanus (Botero and Atema, 1982) and H.gammarus (Howard, 1980) which inhabited different types of substrata from those inhabited by the adult lobsters.

#### 6.4.3. Burrow selection behaviour

The change from positive phototaxis to negative phototaxis during the third zoeal stage (Chapter 5) would probably cause Nephrops to come into contact with the sea bed for the first time towards the end of this final larval stage. A negative phototactic response was probably directly responsible for the attraction of the juveniles to circles of black card. As settlement did not finally occur before the end of the first postlarval stage it obviously involved the interaction of several responses. The attraction of postlarvae to crevices and artificial shelters could involve a negative phototaxis and/or positive thigmotaxis. Thigmotactic behaviour was more clearly demonstrated, however, by the tendency of the juveniles to remain in contact with the sides of the experimental containers while conducting exploration and in their preference for tank corners if no suitable substratum was provided. Similar results have been obtained for the juvenile stages of H.americanus (Hadley, 1908 ; Cobb, 1971 ; Botero and Atema, 1982) where both negative phototaxis and positive thigmotaxis have been proposed as important mechanisms involved in the process of substratum selection. A possible chemotactic and/or rheotactic response may also have been involved in the settlement behaviour demonstrated by postlarval Nephrops as these stages only showed a brief interest in the imitation burrow openings.

#### 6.4.4. Burrows and burrowing behaviour

Although it has been suggested that negative phototaxis and positive thigmotaxis (as well as a possible chemotactic and/or rheotactic response) are probably involved in substratum selection the



stimuli that are required to initiate the burrowing behaviour and final settlement within the substratum remain unknown.

The current investigations have shown that juveniles older than the first postlarval stages are capable of constructing their own burrows. Crnković (1968) also observed independent burrow excavation for juvenile Nephrops but the 2 specimens he studied corresponded to much later postlarval stages (approximately 5 and 7 based on sizes given). Following introduction to a suitable substratum a period of exploration always appeared to precede excavation. The duration of this exploratory behaviour tended to decrease with older postlarvae.

The initial burrow constructed by the juveniles was always a simple shallow U shape and excavation was usually completed within 20 - 60 minutes of initiating burrowing behaviour. This relative rapid initial excavation was probably a behavioural adaptation directed towards achieving cover as quickly as possible and thus reducing the risks of predation (Chapman, 1980). Further burrow elaboration involved the formation of a side tunnel from a Y - shaped junction. This descended deeper than the initial U - shaped tunnel before ascending as a vertical shaft which opened to the mud surface. Chapman (1980) suggested that narrow vertical shafts may be primarily ventilatory in function. The field evidence is that early juveniles do not normally emerge from their burrows (Chapman, 1980).

There were insufficient juvenile burrows constructed during the course of this investigation to allow a statistical analysis to determine whether a direct relationship existed between the length and depth of tunnels below the mud surface and size of the occupant. Such

relationships have been reported for adult Nephrops (Dybern and Høisaeter, 1965).

The burrow volume indicates the amount of sediment removed to the sediment surface. Bioturbation effects include oxygenation of the sediment, nutrient recycling and the provision of reactive interfaces. In the field the provision of an extended aerobic layer within the sediment around the vicinity of the burrow will promote both microbial and faunal colonisation, therefore increasing productivity.

When a vacated juvenile burrow (or on one occasion a polychaete or bivalve burrow) was presented, the juvenile Nephrops always chose to inhabit it rather than construct its own burrow. This preference for existing burrow systems was also demonstrated when the juveniles were introduced to substrata in which adult burrows were present. Under natural conditions adult burrows are much more extensive than the ones formed under the restrictive laboratory conditions of this study (Chapman, 1980 ; Atkinson, 1986) and juvenile recruitment to them would probably be more easily achieved than observed in the laboratory. It has been established that juvenile Nephrops do become associated with adult burrows in the field (Rice and Chapman, 1974 ; Chapman, 1980 ; Atkinson, 1986) and as yet independent burrows have not been found. Rice and Chapman (1974) postulated that this association may occur through direct entry of the juveniles at the time of settlement. These authors also suggested that under conditions of high density such associations could occur by chance, but from the evidence of the present studies this explanation seems unlikely. Frey and Howard (1975) found adult / juvenile burrow associations in the burrowing thalassinid mud shrimp Upogebia affinis and these authors

suggested that they arose by direct entry of the postlarva through the surface openings of the adult burrows at the time of settlement.

Reconnaissance patterns are the rule for the settling stages of marine invertebrates (Mathews, 1917 ; Cole, 1938 ; Wilson, 1952) and form the second of the 3 steps (migration onto the substratum, exploration of the substratum, settlement onto or within the substratum) which are involved in the settling response (Crisp, 1976). Present studies have shown that the postlarval stages of Nephrops (beyond the first stage) can conceal themselves within 20min and form complete U shaped burrows within 60min of initiating burrowing activity. The time that these individuals were engaged in preliminary exploratory behaviour often exceeded that required for concealment. The ability of these stages to rapidly construct their own burrows suggests that recruitment to adult burrows, following reconnaissance, may provide them with advantages other than removal from the substratum surface and the associated risks of surface predation.

In the case of successful entry to an adult burrow, the juvenile immediately excavates a tunnel in one of the walls of the adults burrow (Plate 6.10). This provides the juvenile with its own separate burrow and gives protection from the likely cannibalistic tendencies of the adults. Advantages of this association might include protection from other predators, improved ventilation, access to a comparatively extensive and deep gallery system (Dybern and Höisaeter, 1965) and, possibly most importantly, the provision of areas of organically enriched sediment (McIntyre, 1973). It has been observed in the present investigation and in previous studies (Crnković, 1968) that juveniles seem to continually excavate new tunnels. If this is a



feeding behaviour, as concluded by Crnković (1968), then the Nephrops will benefit from an organically enriched substratum. When comparing two Nephrops populations, Bailey and Chapman (1983) reported a faster growth for the population supported within the more productive and organically richer sediment. These authors suggested that food availability may set a limit to growth. If food burial at the entrance to the burrow is a feature of adult Nephrops behaviour (Chapman, 1980) it may explain the organic enrichment of the sediment which has been reported to occur in the vicinity of these burrows (McIntyre, 1973). Whether the source of nourishment for the postlarval stages of Nephrops was the buried food itself, or the fauna and microflora that becomes associated with it was not determined in this present study. The strategy of feeding on an enriched fauna and flora, termed 'gardening' by Hylleberg (1975), has been suggested for several benthic crustaceans (Buchanan, 1963 ; Farrow, 1971 ; Frey and Howard, 1975 ; Ott et al., 1976 ; Pye, 1980). The provision of a readily available food supply buried within close proximity of the adult burrow may therefore benefit the juvenile directly or indirectly by attracting more food organisms to the region of its own burrow. This would avoid the necessity of having to make hazardous foraging trips out on to the sediment surface and explain why few juveniles are caught on the sea bed (Chapman, 1980).

It has been suggested that the density, growth and size composition of Nephrops populations are related to the nature of the substratum (Chapman and Bailey, 1987). Compared with fine mud sediments it has been demonstrated that coarser sandy-mud sediments generally support higher densities of Nephrops with slower growth rates and smaller individuals (Bailey et al., 1986). Therefore, on

these latter grounds, there will be greater numbers of adult burrows for the recruiting juveniles to associate with. Thus it is possible that a high density population may be sustained by high levels of recruitment.

#### 6.4.5. Résumé

The roles of the early stages in the life cycle of Nephrops and the associated responses demonstrated by these stages are summarised in Figure 6.6 and Table 6.6. The zoeal stages, which demonstrate positive phototaxis and high barokinesis, exploit the hydrographic conditions to effect dispersion of the species. Towards the end of the 3rd zoeal stage the light response appeared to change to negative phototaxis and the lobster seemed to become indifferent to pressure (see Chapter 5). This may cause the zoeae to descend through the water column and, while still within this larval form, Nephrops probably first comes into contact with the sea bed. After a possible brief exploratory period metamorphosis occurs with development into the first postlarval stage. The main purpose of this transitional stage appears to be exploration of the seabed. The continual ambulatory activity demonstrated by most individuals within this stage and the ensuing lack of commitment to a particular choice cell may explain why, under the confined conditions of the laboratory, most appeared to demonstrate no substratum preference. Only one individual within the 1st postlarval stage showed any evidence of burrowing behaviour.

Burrowing behaviour first became evident after development into the 2nd postlarval stage. In the field, recruitment to the benthic habit may be expected to occur sometime after this moult. Although

**Figure 6.6**

**Simplified life cycle for Nephrops.**

**Abbreviations are : Pz, Prezoea ; Z, Zoea ; PL, Postlarva.**



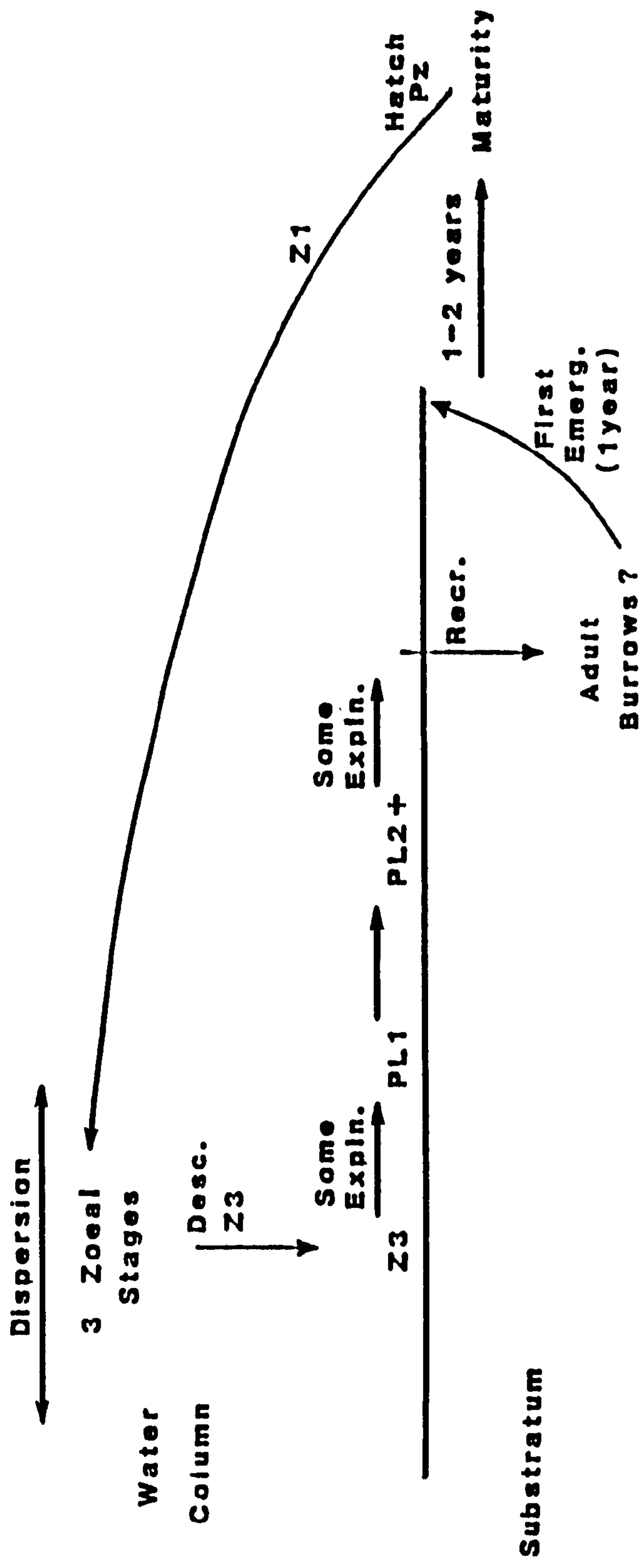


Table 6.6

A summary of the behaviour, distribution and involvement of the different early stages in the life cycle of Nephrops.

Stage	Habitat	Depth distn.(m)	Phototaxis	Pressure response (Barokinesis)	Substrate selection	Settlement
Z1	pelagic	10-30	+	high	0	0
Z2	pelagic	10-30	+	high	0	0
Z3	pelagic	10-S.S.	+ -	variable	0 +?	0
PL 1	pelagic/ benthic	S.S.	-	indifferent	+?	0
PL 2	benthic	S.S.-W.S.	-	-	+	+
PL 3	benthic	S.S.-W.S.	-	-	+	+
PL 4	benthic	S.S.-W.S.	-	-	+	+

#### Abbreviations

Z = Zoea, PL = Postlarva, S.S. = Substratum surface, W.S. = Within substratum.

In the last two columns 0 = no evidence of substrate selection or settlement,  
+ = evidence of substrate selection and settlement behaviour.

these stages are capable of forming their own independent burrow systems in the field, most postlarvae may gain initial entrance to the substratum directly through the burrow openings of adult Nephrops (Figure 6.6). It has been suggested that following settlement the juveniles generally remain within their burrows during their first year, until they have reached a size of 10 - 15mm CL (Chapman, 1980). Sexual maturity is attained after a further 1 - 2 years (see Figure 6.6).



## CHAPTER 7

### Biochemical changes and behaviour associated with egg development and hatching

#### 7.1. Introduction

Female Nephrops may carry several thousand fertilised, non-cleidoic eggs (ie. not protected by a shell or hard protective membrane) attached by ovigerous setae to the pleopods (Farmer, 1974b). Egg number varies with female size (see Chapter 1). During the 6 - 10 month period of embryo development (Farmer, 1975), changes occur in the biochemical composition of the eggs in preparation for hatching. These changes in Nephrops eggs are dealt with in this Chapter and the results are compared with earlier work on other marine Crustacea which also possess non-cleidoic eggs namely, Crangon crangon, Homarus gammarus, H.americanus, Ligia oceanica (Pandian; 1967, 1970a,b), and Pagurus bernhardus (Pandian and Schumann, 1967).

Determination of egg membrane composition proved difficult for Nephrops and Pandian's earlier studies of the structure in H.gammarus (Pandian, 1970a) are used to permit interpretation of water and salt metabolism and changing energy content.

In common with other lobsters (Ennis, 1973b, 1975), the ovigerous female Nephrops hatches its larvae in batches over a period of several days (Farmer, 1974b ; Moller and Branford, 1979) This staggered hatching behaviour was also documented in the present study. It has

also been reported that much energy is expended by the embryo just before hatching, for example, in H.americanus (Pandian, 1970a) and Semibalanus balanoides (= Balanus balanoides) (Barnes, 1965) and that larvae released in later batches contain less energy than those hatched in the first batch in H.americanus (Pandian, 1970b) and Macrobrachium idae (Pandian and Katre, 1972). The behavioural events occurring at eclosion in H.americanus have been investigated by Davis (1964). These various aspects are investigated in this present study of Nephrops.

## 7.2. Materials and methods

Ovigerous Nephrops were collected using creels (from same site as animals used for the fecundity estimation, see Chapter 1), and brought back to the laboratory for examination. Using forceps, eggs were stripped from the pleopods of the female and under stereomicroscopic examination classified according to the scale devised by Figueiredo and Barraca (1963) and later adapted by Dunthorn (1967). In this scale the embryonic development of the lobsters has been subdivided into 9 readily distinguishable stages (Table 7.1). Some of the ovigerous females incubating eggs that were near the hatching condition (stage 9), were maintained in the brood stock facility (Chapter 3), which subsequently allowed individual assessments to be made of fecundity and times and numbers of larvae hatched.

200 to 400 eggs of each of the 9 egg stages and as many hatched larvae as were available, were subjected to three 30sec washes in distilled water to remove seawater. After blotting, the test material was weighed on a Mettler digital balance (Type AE 163 : sensitivity 10 $\mu$ g). The material was then divided into two portions, the first was used for the determination of water and ash content and the second was freeze dried for future biochemical analysis and determination of energy content.

Water content and dry weight were determined by recording the numbers of eggs or larvae within each given sample and comparing the weight of the sample before and after drying at 60°C for 12h. Mean egg



Table 7.1a

Egg development (taken from Dunthorn, unpublished manuscript).





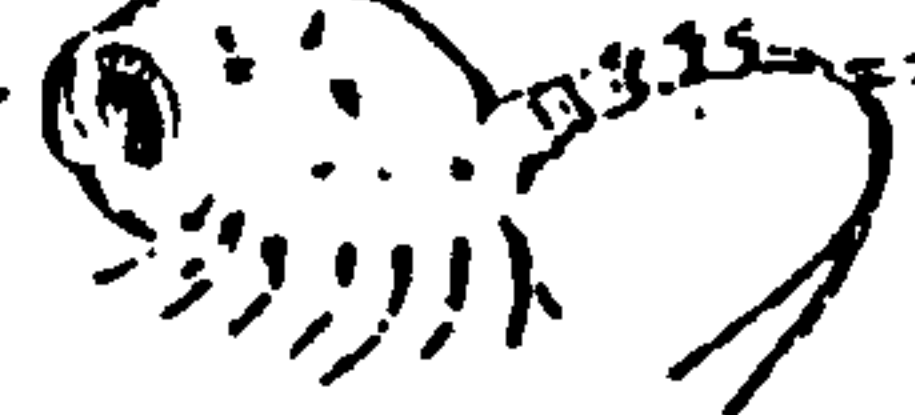
EGG DEVELOPMENT RATES		DAYS		
		8°c	12°c	15°c
1—		42	35	26
2—		70	53	40
3—		97	71	54
4—		122	88	67
5—		155	115	88
6—		190	140	109
7—		230	170	130
8—		265	195	150
9—		300	220	170
LARVA—				
				

Table 7.1b

Egg development (taken from Dunthorn (1967)).

Stage	Colour	Description
1	Dark green	Yolk segmented. No embryo.
2	Dark green	First appearance of embryo.
3	Dark green	Optic lobes and appendages present.
4	Dark green	Eye pigments present as thin crescentic shaped areas. Triangular shaped ocellus.
5	Dark green/ greyish green	Well formed embryo occupying about 1/2 egg capsule. Eye pigment comma-shaped. Ocellus sometimes present.
6	Greyish green/ to olive	Chromatophores present on limbs.
7	Olive	Embryo occupying about 2/3 capsule. Chromatophores on limbs and telson.
8	Light orange/ brownish orange	Yolk almost all absorbed. Eye pigment oval. Chromatophores on carapace.
9	Pale orange/ brownish orange	Formation of complete larva within capsule prior to hatching.

and larval values were then computed. Ash content was then determined by incinerating the dried material at 560°C for 5h and weighing the residue, as recommended by Paine (1964).

Biochemical analysis was carried out to determine the relative composition of protein, carbohydrate and fat as well as the energy content of the various developmental stages. Protein determination was carried out using the standard procedure of Lowry *et al.* (1951), as cited by Taylor and Venn (1979). The optical density at 750 nm was determined using a Cecil CE 273 spectrophotometer. A calibration curve using casein as a standard was constructed. Carbohydrate was determined using the method of Hewitt (1958) as cited by Taylor and Venn (1979). Lipid (fat) determination was carried out using the sulphophosphovanillin method (Barnes and Blackstock, 1973 ). Lipid was extracted using the procedure recommended by Folch, Lees and Sloane Stanley (1957) as cited by Taylor and Venn (1979).

Energy content was determined with a Phillipson A.H.9 microbomb calorimeter (Phillipson, 1964). <sup>Seven</sup> to 20mg dry weights test material was used for each determination. A calibration curve was determined using benzoic acid as a standard.

### 7.3. Results

#### 7.3.1. Changes in chemical composition

In this section the changes in composition are expressed in percentage form. The proportion of any one component within the egg or larva is therefore dependent upon changes in all other components.

The water content of freshly spawned eggs (stage 1) averaged 54.1% and increased to 88.4% in newly hatched first zoeae (Table 7.2). There appeared to be a steady increase in water content throughout egg development and roughly a 12% increase in water content between stage 9 eggs and newly hatched larvae. Deviation from mean water content for all developmental stages was less than 5%, indicating little variability within stages from different ovigerous females. Because different numbers of estimates and eggs were analysed for each development stage, direct comparisons between the level of variation recorded for each stage cannot be made. There was very little variation in water content of different groups of newly hatched larvae (coefficient of variation 0.6%) (see also Table 7.8).

Changes in the chemical composition of the eggs are presented in Table 7.3. The ash content increased from 3.44% in freshly spawned stage 1 eggs to 18.72% in newly hatched larvae. The increase in ash content was most evident over the final phase of development when it more than doubled. For the same reasons as applied for water content, direct comparisons between the level of variation recorded for each development stage cannot be made. There was, however, much variation



**Table 7.2**

**Water content for each egg stage (1-9) and for newly hatched first stage zoeae (Z1). Each analysis was performed on groups of 200-400 eggs or 50-100 zoeae obtained from different females.**

Developmental stage	No. of estimations (females)	Mean water content (%)	Standard deviation	Coefficient of variation (%)
1	6	54.1	2.0	3.7
2	6	58.5	1.8	3.1
3	10	60.4	1.4	2.3
4	8	64.3	2.1	3.3
5	9	65.5	2.8	4.3
6	10	69.7	2.2	3.2
7	14	70.3	2.9	4.1
8	10	73.7	2.9	3.9
9	9	78.7	3.1	3.9
Z1	19	88.4	0.5	0.6

in ash content of different groups of newly hatched larvae (coefficient of variation 11.11%). An investigation into the cause of this variation led to a separate analysis of larvae hatched on successive days from the same ovigerous female (see section 7.3.4). The ash residue appeared to change colour from greyish black over the first 5 egg stages to a progressively more milky white with subsequent egg development, leading to an almost pure white ash from incinerated larvae.

Fat content in freshly spawned eggs (stage 1), was relatively high (41.17%) but was reduced to less than half this value (19.03%) immediately prior to hatching. The reduction in fat content was greatest between stages 1 and 2. After which it remained fairly constant until stage 7 and declined further between stages 7 - 9 from 30.38 to 19.03%.

Carbohydrate concentrations were very low throughout egg development and decreased from 1.14% in freshly spawned eggs to 0.70% in stage 9 eggs. It seems that carbohydrates are of secondary importance to lipids as an energy reserve.

The results from the protein content determination appear spurious. Values have therefore been derived for protein based on the combined percentage composition of ash, carbohydrate and fat. Fat content showed greatest change over the egg development stages and therefore the derived values for percentage protein are most influenced by fat metabolism. Consequently, the periods of greatest fat metabolism (between stages 1 and 2 and stages 7 and 9) have associated with them the greatest increases in percentage protein.

TABLE 7.3

The chemical composition of eggs at each development stage (1-9) and for newly hatched first-stage zoeae (Z1). The percentage values have been derived from dry weight measurements. The protein estimates have been derived for each stage from the cumulative percentage values for the other 3 components.

Developmental stage	Ash (%)			Fat (%)			Carbohydrate (%)			Protein (%)			Protein % (estimate) (incl. non-protein Nitrogen)				
	n	Mean	S.D.	V(%)	n	Mean	S.D.	V(%)	n	Mean	S.D.	V(%)					
1	4	3.44	0.11	3.20	3	41.17	6.85	16.64	3	1.14	0.25	21.93	3	79.53	1.80	2.26	54.25
2	7	3.86	0.46	11.92	4	34.73	6.26	18.02	4	1.10	0.22	20.00	4	83.88	4.81	5.74	60.31
3	7	4.06	0.39	9.61	4	33.65	7.52	22.35	4	1.00	0.14	14.00	4	79.65	6.88	8.64	61.29
4	6	4.62	0.50	10.82	3	33.20	8.80	26.51	3	0.77	0.25	32.47	3	61.67	6.36	10.31	61.41
5	8	4.88	0.40	8.20	4	35.00	4.03	11.51	4	0.78	0.10	12.82	4	74.38	21.51	28.92	59.34
6	6	5.76	0.58	10.07	4	29.45	4.52	15.35	4	0.78	0.15	19.23	4	74.93	6.30	8.41	64.01
7	15	6.03	0.86	14.26	4	30.38	5.00	16.46	4	0.73	0.26	35.62	4	71.80	13.38	18.64	62.86
8	11	6.41	0.60	9.36	6	24.12	6.92	28.69	6	0.93	0.18	19.35	6	69.00	11.19	16.22	68.57
9	6	8.22	0.98	11.92	3	19.03	0.38	2.00	3	0.70	0.10	14.29	3	71.57	11.34	15.84	71.05
Z1	17	18.72	2.08	11.11													

The abbreviations are: - n = number of observations, S.D. = standard deviation, (V%) = percentage coefficient of variation.



From these estimated values, which will include non-protein nitrogen, protein appears to be the major component present in the eggs of Nephrops throughout all stages of development. During egg development protein content seemed to increase from approximately 54 to 71%.

Table 7.4 shows the change in energy content over the developmental stages. The energy content decreased from 24,805 joules  $\text{g}^{-1}$  dry weight in freshly spawned stage 1 eggs to 14,750 joules  $\text{g}^{-1}$  dry weight in newly hatched larvae. Apart from an initial decrease of 655 joules  $\text{g}^{-1}$  dry weight between stages 1 - 2, which may reflect the reduction in fat content recorded over this period and signify early embryonic metabolism, there is a fairly constant slow depletion of energy content over the next 6 stages. From stage 7 onwards the energy content (per gram egg material) dropped more dramatically. The egg membrane is sloughed off at hatching. No attempt was made to separately analyse this portion of the egg as dissection from the egg proved to be difficult due to its delicate nature and the small size of the eggs. If the Nephrops egg membrane is similar to that in H.gammarus (Pandian, 1970a), by stage 8 it will contribute approximately 4% towards the total energy content of the egg.

In general, the trend in energy content resembles that of fat changes. Unfortunately no analysis of fat content was carried out for the newly hatched larvae. If, however, this trend continues over the final developmental stage, then the fat content would be much reduced from the 19.03% recorded for stage 9 eggs. There was much variation in the energy content of different groups of newly hatched larvae (coefficient of variation 9.34%), as in the case of ash content. The effects of hatching time upon larval composition was investigated in

**Table 7.4**

**The energy content of eggs at each stage of development (1-9) and for newly hatched first stage zoeae (Z1).**

Development stage	Number of estimates	Mean energy content (joules g <sup>-1</sup> dry wt)	Standard deviation	Coefficient of variation (%)	Mean energy cont. (joules g <sup>-1</sup> dry organic substance)
1	2	24805	332	1.34	25020
2	2	24150	679	2.81	25265
3	2	24220	1075	4.44	25060
4	2	23485	714	3.04	24530
5	2	23290	788	3.38	24380
6	2	22860	721	3.15	23965
7	2	22395	969	4.33	23702
8	2	21610	495	2.29	23295
9	2	19255	1280	6.65	22155
Z1	8	14750	1377	9.34	17978

#### section 7.3.4.

##### 7.3.2. Biochemical changes in relation to a single egg

In order to relate the developmental changes in chemical composition per unit weight to actual changes in the embryo, the data have to be fitted to the mean dry weight of a single egg for each developmental stage. This information is presented in Table 7.5 and shows that the mean dry weight of a freshly spawned egg was 513 $\mu$ g, which progressively decreased to 446 $\mu$ g in stage 9 eggs. From stage 9 eggs to the newly hatched larvae there was a more dramatic reduction of 44 $\mu$ g to 402 $\mu$ g. Much of this decrease, however, will be due to the liberation of the embryo from the membrane. This will contribute approximately 22 $\mu$ g towards the reduction if, as in H.gammarus, it makes up approximately 5% of the total dry weight in stage 9 eggs. The number of estimates made to determine the mean dry weight for each developmental stage and their respective variation ranges are also given in Table 7.5. From these estimates it can be seen that variability within each developmental stage was high. It is evident, however, that egg dry weight and size of newly hatched larvae are not directly related to the size of the spawning female (Figures 7.1a and 7.1b).

From the values presented in Tables 7.2 to 7.5 average changes in chemical composition and energy content of a single egg from stage 1 to newly hatched larva have been calculated ; the values obtained are presented in Table 7.6. Over the total period of development there was a continual and progressive increase in water content (605 - 3,466 $\mu$ g) and ash content (18 - 75 $\mu$ g) and a steady and progressive decrease in



**Table 7.5**

**Dry weight estimates for eggs at each stage of development (1-9) and for newly hatched first stage zoeae (Z1). Each analysis was performed on groups of 200-400 eggs or 50-100 zoeae obtained from different females.**

Developmental stage	No. of estimates (females)	Mean dry wt. of 1 egg or zoea ( $\mu$ g)	Standard deviation	Coefficient of variation (%)
1	6	513.0	25.6	5.0
2	9	492.4	35.2	7.2
3	9	480.7	26.1	5.4
4	8	474.1	47.0	9.9
5	6	476.5	52.2	11.0
6	9	475.4	52.4	11.0
7	8	481.3	25.7	5.3
8	14	460.0	54.8	11.9
9	10	445.8	53.5	12.0
Z1	17	401.5	30.0	7.47

**Figure 7.1**

**(a) Relationship between the mean dry weight of a single stage 2 egg (Y) and the carapace length of the female (X). The best fit log-log relationship was :**

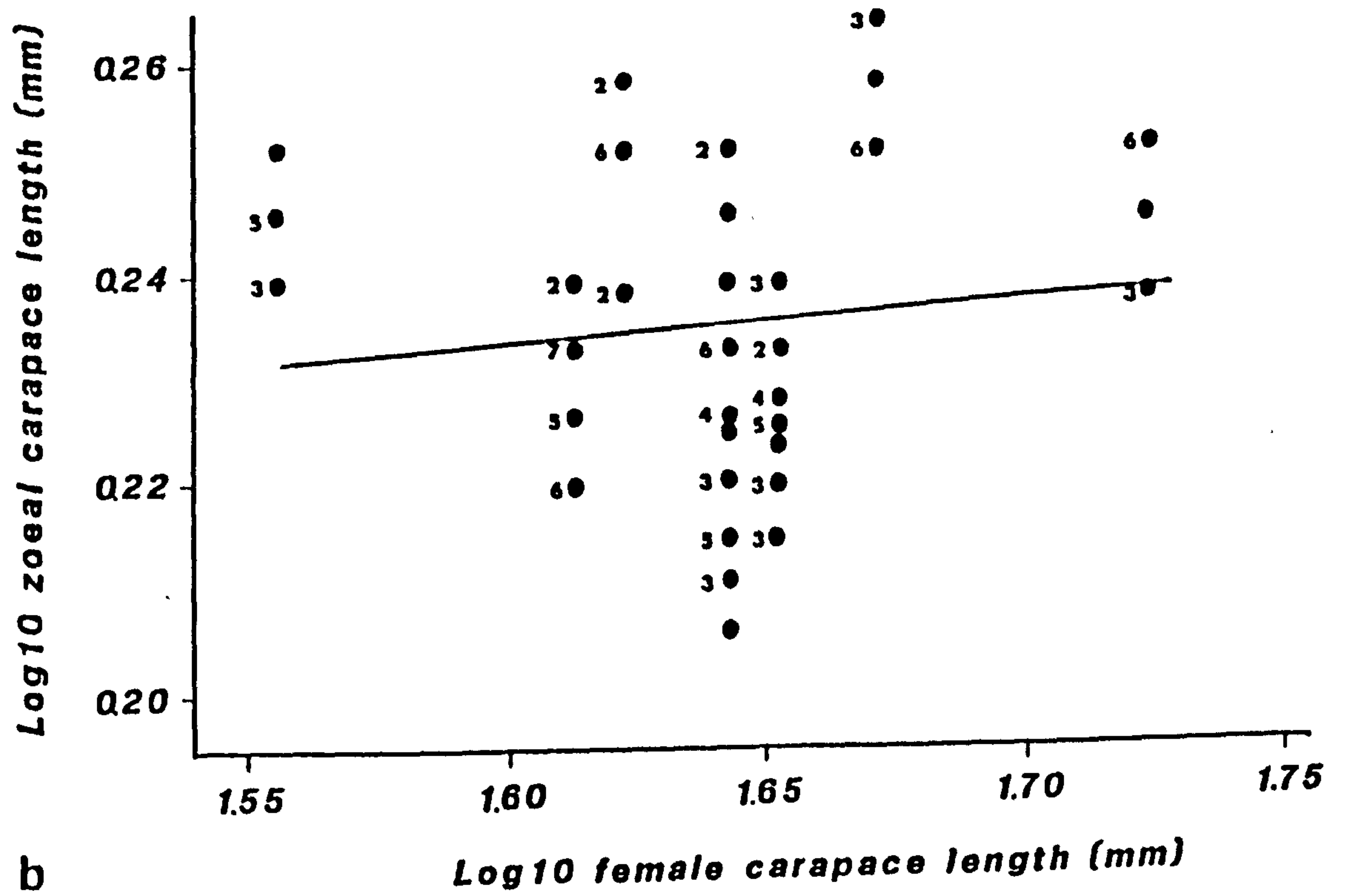
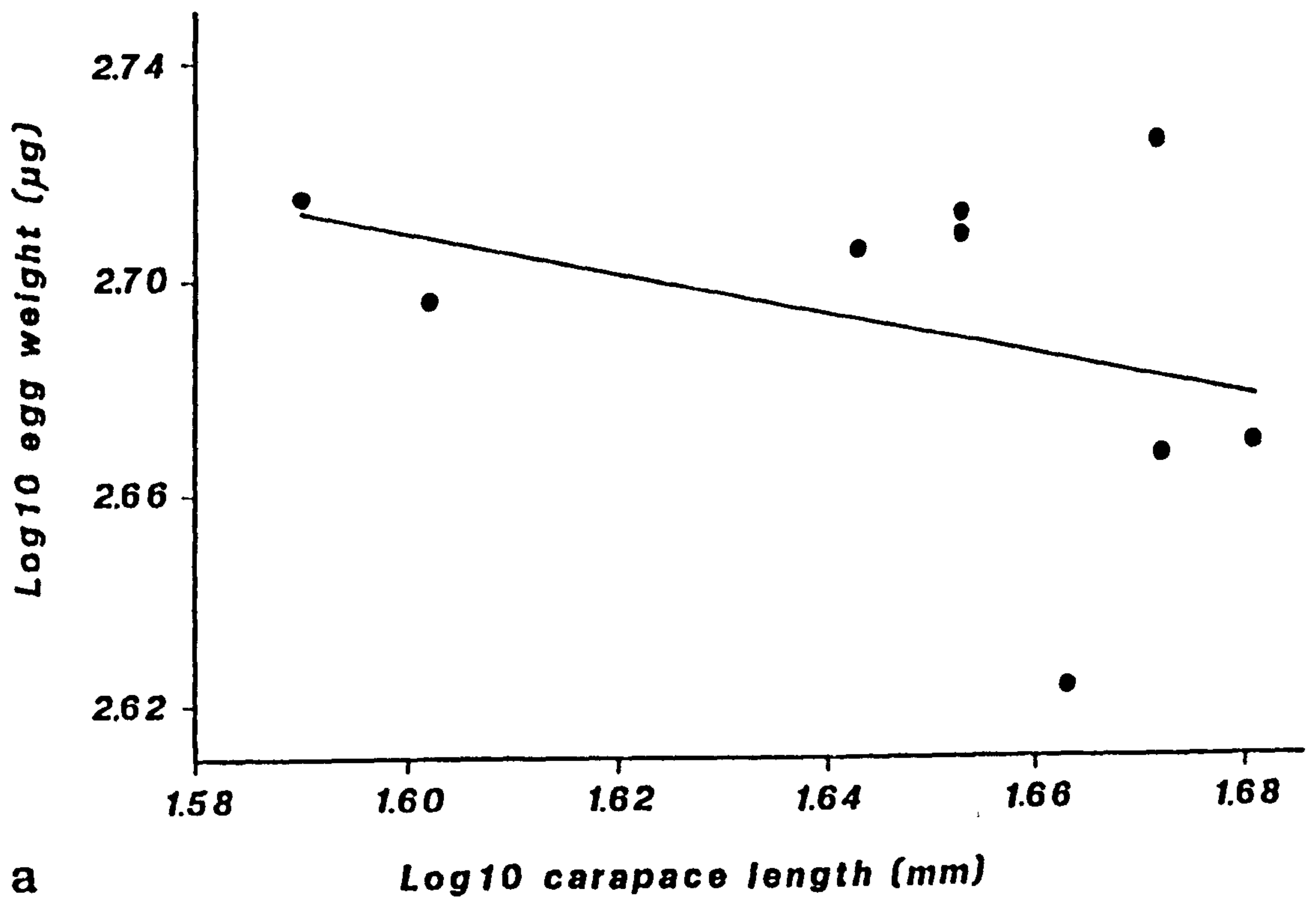
$$\text{Log Y} = 3.310 - 0.376 \text{ Log X}$$

**correlation coefficient = 0.095, which was not statistically significant ( $P > 0.1$ ).**

**(b) Relationship between the carapace length of newly hatched first stage zoeae (Y) and the carapace length of the female (X). The best fit log-log relationship was :**

$$\text{Log Y} = 0.165 + 0.043 \text{ Log X}$$

**correlation coefficient = 0.071, which was not statistically significant ( $P > 0.1$ ). Figures on graph indicate the number of larvae contributing to each data point.**





organic substance (495 - 327 $\mu$ g) and energy content (12.72 - 6.03 joules). Although fat, carbohydrate and protein assays were not carried out for the newly hatched larvae, over the 9 egg stages fat shows a dramatic decline (211 - 85 $\mu$ g). Carbohydrate appeared to be metabolised only during the first three developmental stages and contributed little to the overall energy budget of the developing embryo (7 - 3 $\mu$ g). Protein levels seemed to increase slightly during development (277 - 321 $\mu$ g).

The cumulative yolk utilization efficiency values (CYUE) (ratio 'body formed / body formed + yolk used for metabolism') have been calculated (Pandian, 1970a). For complete development from freshly spawned eggs to newly hatched larvae CYUE was 78.4 %, 66.1 % and 47.4% for dry weight, organic substance and energy content, respectively. The corresponding values for development from stage 1 - 9 for fat and carbohydrate were 40.3 and 42.9% respectively, with no net utilization recorded for protein but an increase of 15.9%. The actual amount of yolk material oxidised during this period of development was 130 $\mu$ g, fat contributing 126 $\mu$ g and carbohydrate 4 $\mu$ g. The net reduction in organic substance was 86 $\mu$ g with 44 $\mu$ g of protein being synthesised. The oxidation of 1g of fat, carbohydrate and protein produces 39,348, 17,372 and 23,651 joules, respectively. Therefore, during embryonic development from stage 1 - 9, the contribution towards metabolic processes from the oxidation of the three major chemical components were : fat (126 $\mu$ g x 39,348 joules g<sup>-1</sup> dry weight = 4.96 joules), carbohydrate (4 $\mu$ g x 17,372 joules g<sup>-1</sup> dry weight = 0.07 joules) and protein (nil). The combined products of oxidation over developmental stages 1 - 9 were therefore 5.03 joules. The energy gain from the synthesis of 44 $\mu$ g of protein during egg development was 1.04 joules

( $44\mu\text{g} \times 23,651$ ). Therefore the energy expended on metabolic processes during egg development was  $5.03 - 1.04 = 3.99$  joules. The estimated change in energy content over this period from the calorimetric study was  $12.72 - 8.59 = 4.13$  joules which compares well with the figure derived from the biochemical data.

During development from stage 9 to the newly hatched larvae 2.56 joules were expended. If the energy content per gram dry weight of the discarded membrane was similar to that of H.gammarus, as recorded by Pandian (1970a), then the  $22\mu\text{g}$  membrane would account for  $22 \times 16,949$  joules  $\text{g}^{-1}$  dry weight = 0.37 joules. The remaining 2.19 joules were expended in metabolic processes over this period of development.

### 7.3.3. Water and salt metabolism

During development of the freshly spawned egg ( $1,118\mu\text{g}$  wet weight),  $2,459\mu\text{g}$  of water was required for the completion of development (Table 7.6). Water uptake (Figure 7.2) appeared to be relatively slow and erratic over the first 8 egg stages, but was much greater between stages 8 and 9 and between egg stage 9 and the newly hatched larva.

Salt (= ash) was taken up more or less continuously throughout embryonic development. Salt uptake appeared to increase between egg stages 8 - 9 and again between stage 9 and the newly hatched larva (Figure 7.2). The mean energy content of the eggs declined gradually over the first 7 development stages and more rapidly over the final 3 stages, with newly hatched larvae containing less than 50% of the initial energy content of freshly spawned eggs (Table 7.6).

**Table 7.6**

**The average composition and energy content of a single egg at each development stage (1-9) and for a single newly hatched first stage zoeae. All weights given in  $\mu$ g. (Derived from Tables 7.2-7.5).**

Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Zoea 1
Live wet weight	1118	1186	1215	1328	1383	1568	1620	1749	2094	3466
Water	605	694	734	854	906	1093	1139	1289	1648	3064
Dry wt.	513	492	481	474	477	475	481	460	446	402
Ash	18	19	20	22	23	27	29	29	37	75
Org.subst.	495	473	461	452	454	448	452	431	409	327
Fat	211	171	162	157	167	140	146	111	85	-
Carbohyd.	7	5	5	4	4	4	4	4	3	-
+Protein	277	297	294	291	283	304	302	316	321	-
Energy (joules/ egg)	12.72	11.88	11.65	11.13	11.11	10.86	10.77	9.94	8.59	6.03

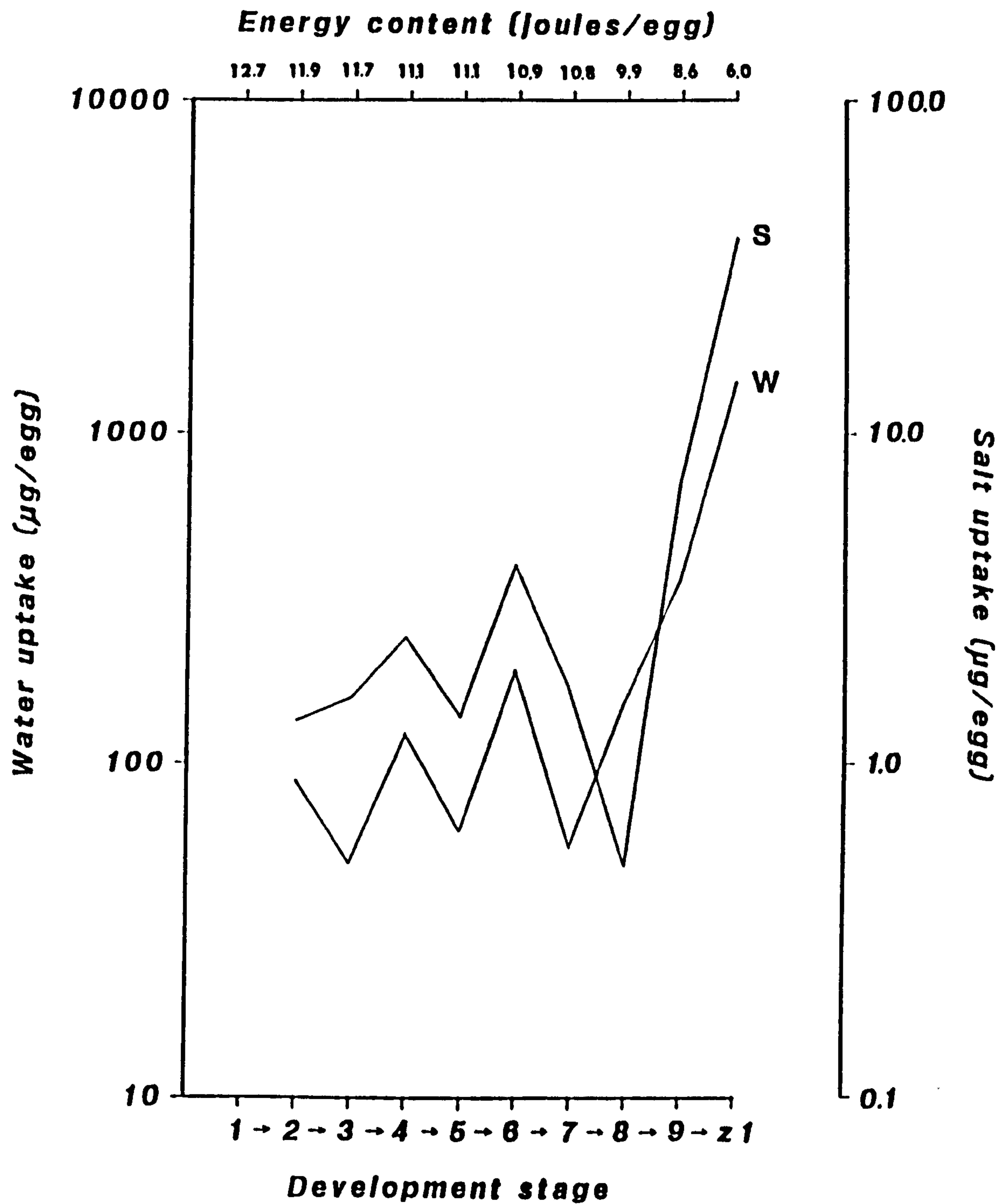
+ The protein values are calculated from the derived estimates (Table 7.3) which include non-protein nitrogen.



### **Figure 7.2**

**Changes in the water and salt uptake of a single egg during development. The graphs illustrate the difference in water and salt uptake between successive stages. The energy content for a single egg at each stage of development is shown.**

**z1 = newly hatched 1st stage zoea.**



#### 7.3.4. Effects of hatching time

##### 7.3.4.1. Release of larvae

The ovigerous females participating in this investigation were observed until they no longer retained any eggs. At the end of the incubation period larvae were hatched on successive nights over periods of 1 - 5 days (Table 7.7). The total values obtained for successive hatching nights for all 6 females were heavily biased by one animal (No. 3). This was the only female which appeared to have a full complement of eggs (see Chapter 1) and may therefore have been the most representative animal. From the total values, most larvae (834) were hatched on the 2nd night. The numbers of larvae hatched during the following 3 nights declined progressively. This trend was demonstrated by 4 of the 6 females. In previous unquantified observations the hatching period for some females was longer but very small numbers of larvae were produced after 5 days.

##### 7.3.4.2. Changes in composition and energy content

The effects of hatching time upon dry weight, percentage water and ash composition and energy content within newly hatched larvae are presented in Table 7.8. It has already been shown that egg diameter (see Chapter 1), egg dry weight (stage 2) (Figure 7.1a) and larval size (Figure 7.1b) were not directly related to the size of the female. It also appears that no direct relationship exists between size of female and dry weight or energy content of the larvae. The sample size was too small, however, to allow a statistical analysis to be performed on the data. For each female there was an increase in the



**Table 7.7**

**Number of larvae hatched on successive nights.**

Female no.	Carapace length (mm)	No. of larvae hatched on successive nights					Total no. larvae
		1	2	3	4	5	
1	41	45	134	78	12	-	269
2	41	64	140	40	32	10	286
3	42	32	250	490	378	430	1580
4	43	43	-	-	-	-	43
5	44	55	210	36	81	23	405
6	47	36	100	12	-	-	148
Totals =		275	834	656	503	463	2731
% =		10.1	30.5	24.0	18.4	17.0	

Table 7.8

Changes in the dry weight, percentage water and ash composition and energy content of larvae. Larvae were removed from the water between 0900-1000h after night time hatching. The brood stock were maintained under natural lighting.

Female	Carapace length	Night larvae hatched on	No. of larvae	Mean dry weight of 1 larvae (µg)	Water content (%)	Ash content (%)	Energy content		
							joules g <sup>-1</sup> dry weight	joules g <sup>-1</sup> dry organic substance	joules/larva
A	44	1st	55	384	88.2	16.9	15,025	18,064	5.78
		2nd	110	385	88.6	19.4			
		3rd	36	392	87.8	19.1			
		4th	81	398	88.8	20.2		17,250	5.28
		5th	23	409	88.6	21.3			
B	41	1st	80	361	88.0	15.8	15,282	17,382	5.52
		2nd	64	361	88.9	17.3			
		3rd	40	363	89.0	17.9		16,150	5.18
		4th	32	403	87.9	20.2			
C	47	1st	36	411	88.2	16.9			
		2nd	100	430	88.9	20.0			
D	41	1st	45	383	88.1	14.6	17,325	20,213	6.86
		2nd	84	396	88.0	18.0			
		3rd	78	421	88.0	20.7			
E	41	2nd	43	470	89.0	18.8	14,298	16,993	6.72
F	45	2nd	50	407	88.5	17.9	14,670	17,882	5.97
G	43	5th	65	452	88.8	23.2	15,280	19,890	6.91
Mean values		1st			88.1	16.0			
		2nd			88.7	18.6			
		3rd			88.3	19.2			
		4th			88.3	20.2			
		5th			88.7	22.3			

average dry weight of larvae hatched on successive nights. This increase in dry weight was paralleled by an increase in the ash content. After the hatching of the first batch of larvae, the embryos to be hatched on subsequent days rapidly absorbed salt. Between the first and fifth days the mean value increased by 6.3% from 16.0 to 22.3%.

In two cases (A and B in Table 7.8) direct comparisons between larvae hatched on day 2 and day 4 suggested a decline in energy content. Table 7.8 indicates some variability between females in the energy content of their larvae hatched on the same day.

Table 7.9 presents the results obtained for an individual ovigerous female which was maintained under conditions of artificial light/dark cycles with hatching synchronized by darkness (simulating sunset). After having observed that no larvae had been hatched during the period of illumination (green light of  $2 - 5 \mu\text{Es}^{-1}\text{m}^{-2}$  intensity) the light was switched off at 9.00am for 1h during each 24h period. After several minutes of total darkness the female was observed under conditions of ruby light (see Chapter 5) for the remainder of this period. All larvae were hatched and removed from the water for analysis within this 1h period. The trend of increasing dry weight and ash content of hatching larvae on successive days, as recorded previously (Table 7.8), did not occur in this case. The ash content was also much lower for all four successive days of hatching than that recorded previously. The water content (87.4 - 89.0%) was similar to that recorded for the larvae of the other ovigerous females (Table 7.8) but the energy content was slightly higher. Because this ovigerous female had been exposed to surface daylight during capture



Table 7.9

Changes in the dry weight, percentage water and ash composition and energy content of larvae which were liberated on successive days and removed from the water within 1h of hatching. All larvae were produced from one female (carapace length 42mm) which was maintained under an artificial light/dark cycle (23 : 1 LD). Hatching was synchronized during each 24h period by the onset of darkness (0900h).

Morning larvae hatched on	No. of larvae	Dry wt. of one larva ( $\mu$ g)	Water cont. (%)	Ash cont. (%)	Energy Content		
					joules g <sup>-1</sup> dry wt.	joules g <sup>-1</sup> organic subst.	joules/ larva
1st	150	389	87.4	13.2	19,312	21,695	7.51
2nd	280	353	88.6	9.5	18,251	20,121	6.44
3rd	178	357	89.0	9.8	17,674	19,844	6.31
4th	230	380	88.7	11.7	17,664	20,169	6.71

(creels) it will have suffered severe rhabdomal damage (Shelton et al., 1985). However, these authors suggest that peripheral regions of the eye may still be functional. It is also known that the sixth abdominal ganglion functions as a photoreceptor in a number of decapod crustaceans (Wiersma et al., 1982). The means by which the ovigerous female perceived light was not investigated but it was evident that a circadian hatching rhythm had been synchronized by the light/dark cycle.

#### 7.3.5. Events of eclosion

Immediately prior to hatching the female moved out from the sanctuary of its shelter (length of plastic piping positioned at the corner of its tank) and occupied a position at the centre of the tank. It then raised itself on the tips of its last four pairs of pereopods and spread its claws (1st pereopods) anteriorly at an acute angle. The abdomen was raised and the telson and uropods were fanned out horizontally. The female then beat its pleopods vigorously (with a stronger stroke appearing to occur in the backwards direction) and larvae were flushed posteriorly from under the abdomen, effectively removing them from the vicinity of the female.

The released larvae were in the prezoeal stage (Plate 4.1), with their thoracic appendages immobilised within a cuticle on either side of the abdomen. They swam upwards, while moulting, through the water column by means of rapid flexions of the abdomen in a 'whiplashing' movement. This resulted in the larvae concentrating at the water surface. Within 5min (most requiring less than 30s) all prezoeae (approximately 200 in each observation) had shed their larval cuticles

and moulted into first stage zoeae.

During the hatching process some embryos at various stages of development were released prematurely before they had reached the prezoeal form. These appeared incapable of further development and remained motionless at the bottom of the tanks. When it became apparent that no further development was going to occur in these embryos they were removed for microscopic examination.

The embryos of Nephrops immediately before hatching are surrounded by 3 discrete membranes. In this study the outer, middle and inner membranes refer to the outer membrane, inner membrane and prezoeal exoskeleton, respectively, of Farmer's earlier investigation (Farmer, 1974b). The outer membrane is a 3 layered trichromatic membrane and, according to Cheung (1966), the other 2 membranes are formed after spawning during the embryo stage prior to the appearance of eye spots (stage 3 in this study). Some of the embryos released prematurely were enclosed within all 3 membranes. When attempts were made to remove the outer membrane (using dissecting needles) it was found that the middle membrane was attached to it in the region of the embryonic eye, so both could be removed as one entity. The outer membrane was much thicker and more robust than the middle membrane and both were transparent. These embryos were now in the prezoeal stage surrounded only by the inner membrane which formed a thin, partially transparent, milky white layer. The inner membrane, which is now the prezoeal exoskeleton, is not strictly a true membrane since it is the larval cuticle (Davis, 1964). The prezoeae are completely devoid of setae and abdominal spines, with their thoracic appendages incapable of independent movement. As reported by Farmer (1975), the prezoeae



generally remained in their embryonic position with their abdomens curled ventrally, though these were less closely apposed to their thoracic regions than when in the egg.

Other prematurely released embryos were found to have shed their outer membranes. They had not managed complete liberation from their middle membrane, however, and were bound within this structure to varying degrees. After these embryos were artificially released from their middle membranes, most of the prezoeae succeeded in shedding their larval cuticles and consequent development into first stage zoeae was observed. In contrast, further development appeared to be arrested and death resulted in embryos which had been artificially liberated from both the outer and middle membranes.

On liberation from the middle membrane the larval cuticle (ie. inner membrane) was shed as in any other ecdysis. A slit appeared over the dorsal part of the carapace. This may have occurred at a point of attachment between the middle membrane and the prezoeal exoskeleton with rupture of the larval cuticle occurring on final removal of the middle membrane. As the cuticle was slowly worked down the thoracic region the pereopods were released and aided completion of the moult. The emerging first stage zoeae became less curled up as the ecdysis proceeded and the flexions of the abdomen became increasingly vigorous. The cuticle was finally flicked off as one complete entity from the tip of the telson, although it was occasionally liberated from the extremities of the first pereopods and rostrum at the same instance.

The time taken for completion of ecdysis to the first zoeal stage in those prezoeae which had been artificially released from their inner membranes was very variable (5min to several hours). Some died within their prezoeal exoskeleton having been unable to shed their cuticle. Similar mortality was also recorded when Davis (1964) attempted to artificially hatch larvae of H.americanus. He attributed this to poor conditions of egg ventilation in the laboratory in contrast to conditions affecting eggs on the pleopods of ovigerous females in the sea.

The ovigerous female Nephrops which had been maintained under artificial conditions of light/dark cycles with hatching synchronized by sunset (ie. lights off), moulted while still carrying several hundred eggs (Plate 7.1). The eggs discarded with the cast exoskeleton were all viable and by slowly agitating the cast in a beaker of water, prezoeae were hatched out successfully. Ecdysis into first stage zoeae followed rapidly.



Plate 7.1

Moulted exoskeleton of an ovigerous female, with some eggs still attached to the pleopods.





## 7.4. Discussion

### 7.4.1. Chemical changes associated with egg development

Throughout development the decrease in energy content was closely paralleled by depletion in the fat reserves, clearly establishing fat as the principal food source for the developing embryo. This is not unexpected having been reported previously for the embryonic development of other crustaceans, e.g. H.gammarus, H.americanus, L.oceanica (Pandian, 1967, 1970a,b,), P.bernhardus (Pandian and Schumann, 1967) Pollicipes cornucopia and Chthamalus stellatus (Achituv and Barnes, 1978). In marine non-cleidoic eggs, such as those of Nephrops, the utilization of fat as the principal food source provides the embryo with not only the most concentrated energy supply available but also the most water conserving. Oxidation of 1g of fat, carbohydrate and protein produces 39,348, 17,372 and 23,651 joules and 1.07, 0.56 and 0.41g of water, respectively (Pandian, 1970a). In aquatic non-cleidoic eggs of fresh water animals water is readily available and the strategy of conservation is unimportant. These eggs rely upon protein as the principal energy source (Needham, 1950) and can provide the considerable amounts of water required for removal of ammonia, the principal waste product of protein metabolism. In their reliance on fat metabolism the eggs of Nephrops and other marine Crustacea are more akin to terrestrial cleidoic eggs than non-cleidoic eggs of fresh water animals (Needham, 1950). Lipids also appear to play an important role in the life of adult Nephrops (N.Bailey, pers. comm.).

The energy requirements of the developing embryo increase rapidly after egg stage 7. Over the 9 arbitrary egg developmental stages 79% of the energy derived from the oxidation of the food reserves was used in metabolic processes, with the remainder (1.04 joules) contributing to embryonic development. During this period 60% of the initial fat reserves were oxidised (Table 7.6) providing almost 99% of the total energy requirements of the embryo. In H.gammarus fat oxidation provides the main source of energy for the final developmental stage from egg to newly hatched larva (Pandian, 1970a). If this is also the case for Nephrops then a large portion of the remaining fat reserves (40%) will be oxidised during this the most highly energy dependent period of development. During this period, 38% of the total energy required for complete development is utilized (Table 7.6). This would therefore result in the Nephrops embryo utilizing a considerably larger portion of its initial fat reserves during complete development than that recorded for the other marine Crustacea (Table 7.10).

Depletion of initial energy reserves was also higher in Nephrops than the other examples of marine Crustacea (Table 7.10) and would account for the expected continued heavy depletion of fat reserves during this period (ie. beyond 59.7%).

Over this final developmental stage, and included in the 38% of the total energy utilized over this period, the egg membrane was sloughed off. This would account for 15% of this value if similar in structure to that of H.gammarus (Pandian, 1970a).

From the initial energy content of 12.72 joules in newly spawned eggs 6.31 joules (6.69 - 0.38 joules) was expended during embryonic

Table 7.10

Percentage utilization of fat and energy content in demersal eggs of some marine crustaceans during embryonic development. References as cited in Table 7.11 (adapted from Pandian (1970b)).

Species	Energy Content (joules g <sup>-1</sup> dry weight)		Percentage of respective initial values utilised during embryonic development	
	eggs	larvae	energy	fat
Nephrops norvegicus	24805	14750	40.5	59.7 +
Homarus americanus	27778	17966	35.3	-
Homarus gammarus	25836	18937	26.7	52.6
Crangon crangon	24760	19021	23.2	66.4
Pagurus bernhardus*	25338	20009	21.0	65.0
Ligia oceanica	24932	17476	29.9	42.2
Mean	25575	18027	29.5	57.2

+ Minimum value taken for eggs in final stage of development prior to larval hatching (i.e. stage 9, see Table 7.3).

\* As Eupagurus bernhardus in Pandian's work.



development. The remaining 6.03 joules (approximately 47%) was retained within the newly hatched first stage zoeae.

#### 7.4.2. Water and salt metabolism

Nephrops embryos are surrounded immediately after spawning by a single three layered trichromatic membrane (Cheung, 1966). Pandian (1970a) suggests that this membrane controls permeability to water and salts during embryonic development since the two membranes which are later synthesised between it and the embryo (ie. the middle and inner membrane) are freely permeable.

In H.gammarus, Pandian (1970a) recorded greater variation in the water content of eggs analysed from representative samples over the first developmental stage than in all subsequent egg stages (50.5 - 58.6%). He attributed this to progressive attainment of the gastrula stage, after which, the membrane became almost impermeable to water and salts until attainment of his stage 3 (which corresponds to stage 5 of this study). It is evident (Table 7.2) that the variation within stage 1 eggs of Nephrops was not particularly high over this period. The period of incubation by the female after spawning was unknown. Nevertheless, the low variability may be because all the eggs analysed had already reached the gastrula stage (yolk cleavages occur a few days after egg laying (Farmer, 1974b) and the outer membrane has become impermeable).

Between stages 1 - 8 the egg remained relatively impermeable to water and salts and if development is similar to that recorded for H.gammarus (Pandian, 1970a), 2 chitinous membranes were formed

internal to the trichromatic membrane during this period, corresponding to Cheung's 4th and 5th layers (Cheung, 1966). As these membranes are both protein based their synthesis may account for the slight increase in protein recorded within the egg over this period of development (Table 7.6). After stage 8 the Nephrops eggs appeared to become much more permeable to water and salts. This was accompanied by an apparent increase in the energy demands of the embryo (Figure 7.2). Pandian (1970a) observed a similar change in the later embryonic stages of development in H.gammarus and recorded an increase in membrane weight over this period. He suggested alteration of the outer trichromatic membrane as a possible reason for the increased permeability of the eggs. Whether or not denaturing of the astaxanthin-protein complex is directly responsible for the change in permeability is unknown. It was evident, however, that the resultant change in egg colour from green to orange-brown coincided with the increased rate of permeability. Ovoverdin has been shown to be the pigment responsible for the dark green colouration in the eggs of H.americanus (Stern and Solomon, 1938), H.gammarus (Pandian, 1970a) and Nephrops (Farmer, 1974b). It is an astaxanthin-protein complex and during development it is progressively denatured. The egg consequently becomes paler and a red colour gradually appears due to the formation of chromatophores and the general pigmentation of the embryo by free astaxanthin (Farmer, 1974b). As no attempt was made to determine the changing selectivity of the trichromatic membrane it is unknown what proportion, if any, of the 58% total energy budget expended over this period of development (ie. after stage 8) is directed towards maintaining the integrity of the egg against diffusion gradients of solutes from the external environment.

The dependence of the developing embryo upon the environment for salt and water supply varies considerably in different species of marine demersal Crustacea (Table 7.11). As expected, Nephrops eggs most closely resemble those of Homarus spp. in accumulating considerable quantities of water and salts during their development, while those of shrimps and hermit crabs require relatively small quantities. The possible reasons for such variation and the advantages which may be derived by the egg and/or larva in having a high water content has been discussed by Pandian (1970a). These include ; a high internal osmotic pressure for hatching purposes, decreased specific gravity for flotation (attached eggs carried by the female and released larvae) and greater ability for thermal adjustment to changes in the surrounding water temperatures (planktonic organisms may be subjected to quicker temperature changes than demersal forms (Pandian, 1970a)).

#### 7.4.3. Events associated with hatching

##### 7.4.3.1. The effects of hatching time

During the hatching period no apparent change in water content occurred but there was a progressive depletion in the energy content which was paralleled by a continued increase in salt (= ash) content (Table 7.8). As found by Pandian (1970b) for H.americanus, the salt accumulated appeared to be deposited in the exoskeleton, causing those larvae hatched on later days to have a harder cuticle.

Between stage 9 and the first batch of newly hatched larvae, ash content increased from 8.2 to 16.0%. It increased further from 16.0 to



Table 7.11

Water and salt requirements of some marine demersal eggs of crustaceans for successful completion of their embryonic development. Values based on 1mg wet weight per egg. (Adapted from Pandian (1970b)).

Species	Required quantity of		Authors
	water (mg)	salt (µg)	
Nephrops norvegicus	2.20	51.5	Smith (this study)
Homarus americanus	2.12	61.1	Pandian (1970b)
Homarus gammarus	1.32	50.4	Pandian (1970a)
Crangon crangon	0.84	5.5	Pandian (1967)
Pagurus bernhardus	0.81	15.7	Pandian & Schumann (1967)
Ligia oceanica	0.07	67.5	Pandian (1970b)

22.3% over the hatching period. These changes in the rate of salt uptake may have resulted from modifications in the trichromatic membrane at the onset of both periods of increased salt metabolism. In a similar study Anger and Püschel (1986) reported that newly hatched 1st stage zoeae had a mean ash content of 35.7%. If salt uptake continues after hatching a delay in the removal of the larvae from the water in which they were liberated would cause an increase in the ash content. This may provide one possible explanation for the higher ash contents reported by Anger and Püschel (1986). However, all studies conducted by these authors were performed on larvae produced from a single ovigerous female.

The heavy depletion in energy content of approximately 10% per day during the period of hatching was of a similar level to that recorded by Pandian and Katre (1972) for Macrobrachium idae. If future oxidation of the depleted energy reserves was similar in Nephrops larvae to that recorded for M. idae larvae, then the rate of oxidation of the reserve yolk will be slower in the later hatched individuals, resulting in reduced swimming speeds being attained. Since the larvae are dependent upon swimming speed for catching food organisms and avoiding predators the daily reduction in food reserves could considerably reduce the chances of survival.

Although the effects of starvation upon survival of larvae hatched on different days from the same female were not investigated, the overall effects of initial periods of starvation upon survival are presented and discussed in Chapter 3.

#### 7.4.3.2. Control of hatching

The hatching behaviour demonstrated by the ovigerous female in this study complements the accounts given by Farmer (1974b) and Moller and Branford (1979). This behaviour implies that in the field hatching occurs outside the burrow, which has been previously suggested (Moller and Branford, 1979) and appears to be reflected in the greater availability of ovigerous females in creel catches at the end of the incubation period (Chapter 1).

All ovigerous females which were maintained under normal diel conditions hatched larvae during the night. Night time hatching has been widely reported for the Nephropidae (Ennis, 1973b, 1975 ; Farmer, 1974b ; Branford, 1978 ; Moller and Branford, 1979) and it is generally accepted that the onset of darkness is the appropriate cue. This was also observed for the ovigerous female maintained under artificial light/dark cycles. Here, hatching was synchronized by artificial sunset (ie. 'lights-off') and all larvae were liberated within the 1h period of darkness on successive nights (Table 7.9). Moller and Branford (1979) observed similar hatching patterns when ovigerous Nephrops were maintained under artificial light/dark cycles, reporting hatching to occur during a few minutes 2 to 3h after dusk on 5 to 20 successive days. The hatching periods were much shorter in this present study (1 to 5 days) than those observed by Moller and Branford (1979). This can probably be attributed to egg loss. Although large numbers of larvae were produced by the female maintained under artificial light/dark cycles, for the reasons discussed below, it cannot be considered as a typical hatching rhythm.



For adult Nephrops, endogenous circadian rhythms have been implicated in locomotor activity (Atkinson and Naylor, 1976), retinal shielding pigment activity (Aréchiga and Atkinson, 1975) and neurohumoral activity (Aréchiga et al, 1980). Moller and Branford (1979) established that an endogenous component was involved in the hatching rhythm and suggested that the rhythm was of a circadian nature. They were unable, however, to establish whether the endogenous component resided with the female or the egg. As a circadian timing system is available to the female it has been suggested that endogenous control through exploitation of this system would constitute an economical solution for accurate hatching (Moller and Branford, 1979). In H.gammarus the endogenous component of the hatching rhythm has been attributed to the female (Branford, 1978).

In the case of the female Nephrops where hatching was artificially synchronized by light/dark cycles and larvae were removed for analysis within 1h of hatching, it was evident that embryonic development had been curtailed since ash contents were lower and energy contents higher than those recorded for all other larvae released under natural light conditions (Table 7.9). This same female moulted before hatching of all its larvae. This has also been recorded previously for H.americanus and H.gammarus females (Aiken and Waddy, 1980) and partly attributed to conditions of unusual photoperiod. It is evident, therefore, that the presence of eggs on the pleopods of the female Nephrops did not inhibit premoult development. Recent studies involving the inspection of pleopods have confirmed this observation (C.J.Chapman, pers. comm.). The apparent premature release of larvae and the moulting of the female before completion of hatching implies a breakdown in co-ordination between

the mechanisms controlling the two processes (ie. hatching and ecdysis). As mentioned above this may have been caused by the unusual photoperiod conditions (23:1 LD). The female observed by Moller and Branford (1979) during its hatching period (16:8 LD) moulted 9 days after the last hatch. It is unknown whether the presence of eggs inhibits moulting. As reported by Moller and Branford (1979), the female is clearly aware of the state of the eggs, as her hatching behaviour, in the form of increased locomotor activity, coincides with the hatching period. They conclude that this information may be communicated to the female by means of the last pair of pereopods, which perpetually probe and groom the egg mass throughout the incubation period.

Through the manipulation of different artificial light/dark regimes Moller and Branford (1979) demonstrated that the hatching process in Nephrops is affected by the timing of dawn and dusk. They also suggested that the 'hatch interval' (period between perception of dusk (lights-off under artificial light/dark regimes) and hatching) is shorter under warmer temperatures. The hatching behaviour observed in the present study for the female maintained under the artificial light/dark cycle (23:1 LD) supports the findings of Moller and Branford (1979). Hatching occurred within several minutes of 'dusk' at a temperature of approximately 15°C. In H.gammarus the endogenous component of the hatching rhythm is influenced by temperature, daylength and photoperiod experience (Branford, 1978).

Having implicated photoperiod as a controlling influence upon the endogenous component associated with hatching the immediate mechanisms which cause hatching are less clearly defined. Davis (1964) suggested

that hatching in H.americanus was facilitated by intake of water which brought about bursting of the egg membrane from the resulting internal pressure. In this study although the water content did not appear to change over the hatching period the internal solute concentration progressively increased. The osmotic pressure within the eggs must therefore have increased over the hatching period without bursting the egg membrane. This therefore implies that the immediate cause of hatching in Nephrops involves more than a simple osmotic mechanism.

Katre and Pandian (1972) suggested that the hatching mechanism in decapods was mechanical rather than osmotic. Consequently, they suggested that it would be possible to simultaneously hatch on the first night all developing eggs carried by M.idae by using artificial agitation. In this one observation for Nephrops, although simulated pleopod beating was successful in liberating some of the larvae retained on the ovigerous females moulted exoskeleton, more vigorous agitation only succeeded in displacing from the pleopods embryos that were not fully developed. These remained trapped within their membranes and were unable to complete development into free swimming larvae.

Some embryos were observed which were still attached by their broken outer membranes to the ovigerous setae and pleopods of the female. These had only managed to partially escape from their inner membranes. It seems likely, therefore, that osmotic pressure may cause bursting of the outer membrane along a weakened line in the cephalic region of the trichromatic membrane, as reported for H.americanus (Davis, 1964) but that mechanical agitation, as provided by the pleopod beating of the female, is required to liberate the embryos



from the remnants of their inner membrane. This supports the evidence of previous investigations (Farmer, 1974b ; Moller and Branford, 1979). It is known that in H.americanus the outer membranes of those eggs from which larvae will be released at any one time burst throughout the period following the previous larval release (Ennis, 1975). This may also be the case for Nephrops and implies that if hatching is dependent upon a circadian hatching rhythm some larvae will be ready for liberation for periods up to 24h prior to release. It is unknown what stimulates the female to perform the hatching behaviour.

#### 7.4.3. Eclosion

Embryos are released in the prezoeal form and are still retained within their larval cuticle with both the outer and middle membranes retained on the ovigerous setae of the females pleopods. The larval cuticle is the inner of the two chitinous membranes synthesised once spawning has occurred and corresponds to Cheung's 5th layer (Cheung, 1966) and Burkenroad's embryonic moult skin (Burkenroad, 1947).

The larval cuticle constricts the prezoeae into a spherical shape with the abdomen curled ventrally and all setae, spines and thoracic appendages located within. This transient prezoeal form may be a protective strategy to minimise the risk of mechanical damage occurring to the larvae during release into the water column.

The prezoeal stage is very short lived and most moult into the first free swimming stage (ie. first stage zoeae) within a few minutes of hatching.

## CHAPTER 8

### General discussion and stock assessment

Although adult Nephrops have been extensively researched in recent times (Farmer, 1975 ; Chapman, 1980) the early stages have been relatively neglected. The purpose of this study was to redress this situation and, using both field and laboratory based studies, provide a comprehensive investigation into the biology of larval and juvenile Nephrops.

In ovigerous females ovary and egg development occur simultaneously as two discrete reproductive cycles. The final phase of oocyte maturation (secondary vitellogenesis) seems to be delayed until after hatching of the previous brood. In the late summer and early autumn ripe oocytes are extruded and most females possess newly spawned eggs. Immediately after spawning Nephrops eggs are an undifferentiated mass of dark green yolk material contained within a trichromatic membrane. During embryonic development, which takes approximately 9 months in Scottish waters (Farmer, 1975), there is a progressive change in the biochemical composition of the egg. This is mainly associated with a depletion in the lipid content, the principal energy reserve. Similar studies have identified lipids as the major yolk material utilized during embryonic development in the non-cleiodic eggs of other marine crustaceans (Pandian, 1967, 1970a,b ; Pandian and Schumann, 1967). As well as providing a more concentrated energy supply than either proteins or carbohydrates, lipid metabolism is more water conserving and this may be the principal reason for its

adoption as the major yolk reserve. Towards the end of the incubation period embryonic development proceeds at a faster rate and a corresponding decrease in the energy content is registered. During this period there appeared to be an increase in the permeability of the egg membrane which caused an increase in the water and salt content and the colour of the egg changes from green to orange-brown. Similar changes have been reported during the later stages of embryonic development in Homarus spp. (Stern and Solomon, 1938 ; Pandian, 1970a).

In Nephrops sunset appears to synchronise a circadian hatching rhythm (Moller and Branford, 1979) and batches of larvae are released at night over a period of 5 to 20 days. Over this hatching period there is some evidence for a continual depletion in the energy reserves. This may adversely affect the viability of the larvae released in the later batches. For Nephrops it is uncertain whether the endogenous component which controls hatching activity resides with the female or the developing embryo. In Homarus gammarus the endogenous component resides with the female (Branford, 1978) and this is also probably true for Nephrops. The immediate mechanisms which control hatching in Nephrops include a preliminary osmotic bursting of the outer trichromatic membrane followed by night time mechanical agitation provided by the pleopod beating of the female (Farmer, 1974b). Nephrops larvae emerge from the egg as a short-lived prezoea which, after discarding the larval cuticle, become 1st stage zoeae.

Fecundity studies for Nephrops have revealed that during the period of incubation a progressive egg loss occurs (Farmer, 1975 ; Chapman and Ballantyne, 1980). According to Morizur (1981) this loss



is mainly caused by predation and may account for the regional differences which seem to exist ; 75% in Portuguese waters (Figueiredo and Nunes, 1965) ; 45 - 50% in the Bay of Biscay (Morizur et al., 1981) ; 32 - 51% in the Moray Firth (Chapman and Ballantyne, 1980). A corresponding value of 18% was reported for Firth of Clyde Nephrops in the present study. Almost a fifth of the spawning population appeared to have suffered additional egg loss, probably caused at the time of spawning by failure of the eggs to adhere to the pleopods.

After the exclusion of the individuals which seemed to have suffered additional egg loss the data obtained for stage 8 eggs (1984 and 1985 combined, see Chapter 1) was used to estimate the effective fecundity. A highly significant relationship was obtained between fecundity and carapace length (Figure 8.1). Although the individuals which had suffered additional egg loss were excluded from Figure 8.1, their contribution towards larval production must be included in an estimate of mean potential fecundity. A highly significant relationship was obtained between fecundity and carapace length for these individuals (Figure 8.2). As expected, the larger Nephrops were more fecund. For each carapace length the proportion of individuals which were judged to have suffered additional egg loss was calculated. The results are plotted in Figure 8.3 and a highly significant relationship was obtained. The proportion of the population which seem to have suffered additional egg loss increased with female size. For each carapace length the mean effective fecundity was calculated using the 3 relationships presented in Figures 8.1, 8.2, and 8.3.

In order to determine the spawning stock size for the Lower Firth of Clyde (L.F.C.) from the larval survey data the mean effective

### Figure 8.1

Relationship between effective fecundity (Y) and carapace length (X) after individuals which had suffered abnormal egg loss had been excluded (1984 and 1985 data combined, see Chapter 1). The best fit log-log relationship was :

$$\text{Log Y} = -1.8036 + 3.2305 \text{ Log X}$$

number of observations = 64, correlation coefficient = 0.7325,  
(P < 0.001).

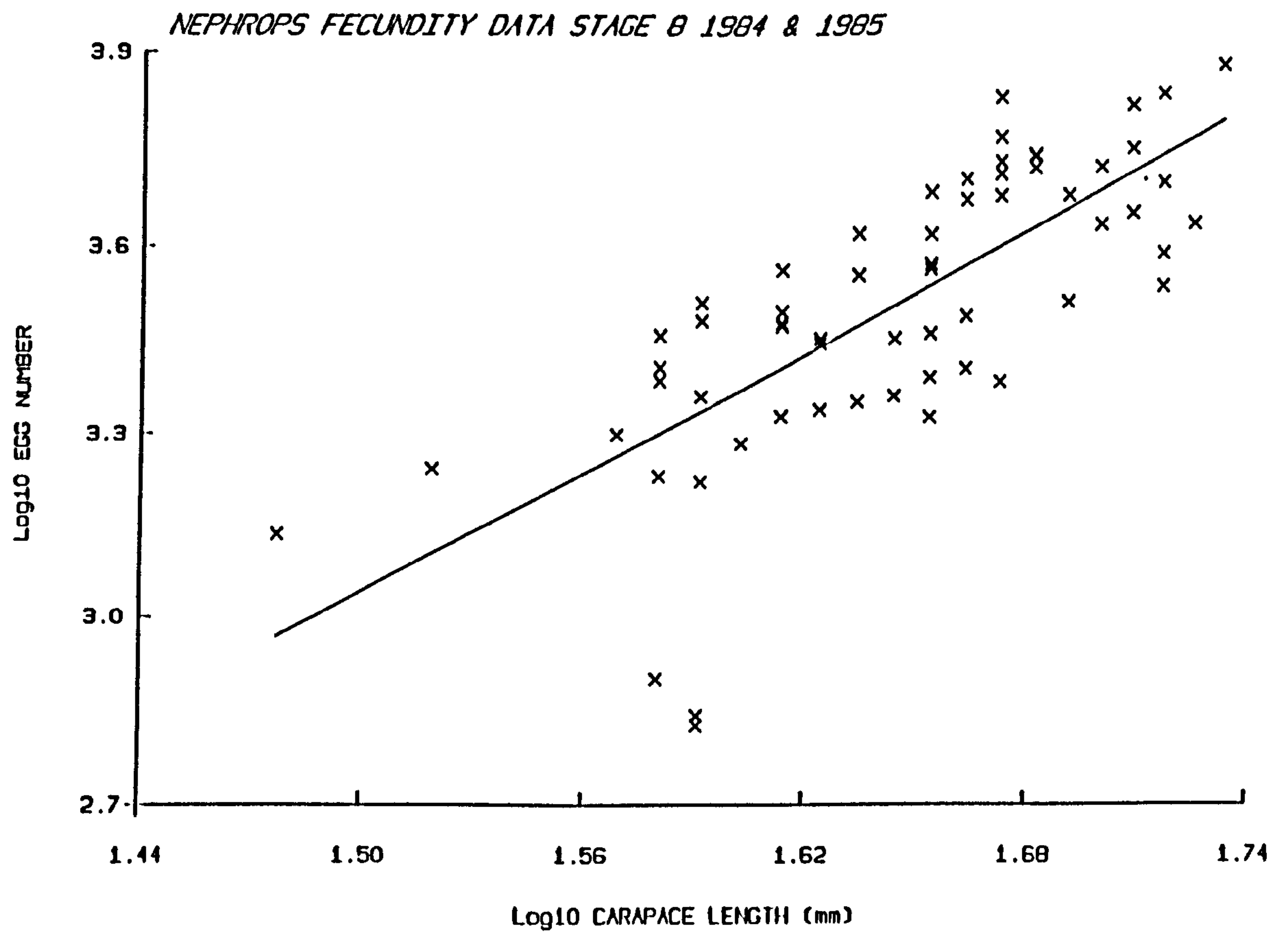




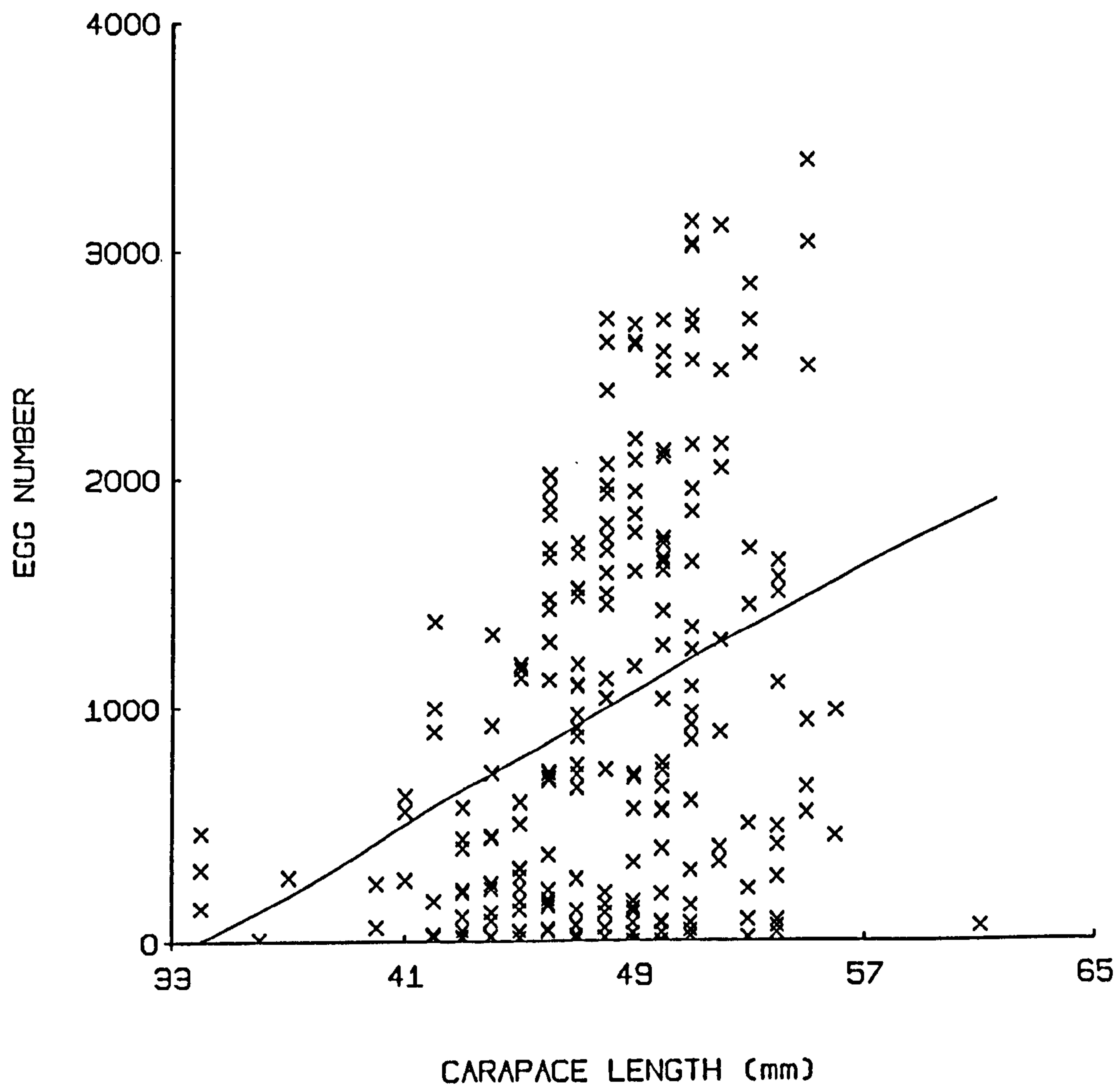
Figure 8.2

Relationship between fecundity (Y) and carapace length (X) for individuals which were judged to have suffered abnormal egg loss and had been excluded for purposes of estimating incubation loss in Chapter 1. The best fit linear relationship was :

$$Y = -2407.6729 + 70.9068 X$$

number of observations = 222, correlation coefficient = 0.3242,  
(P < 0.001).

NEPHROPS FECUNDITY EXCLUDED DATA



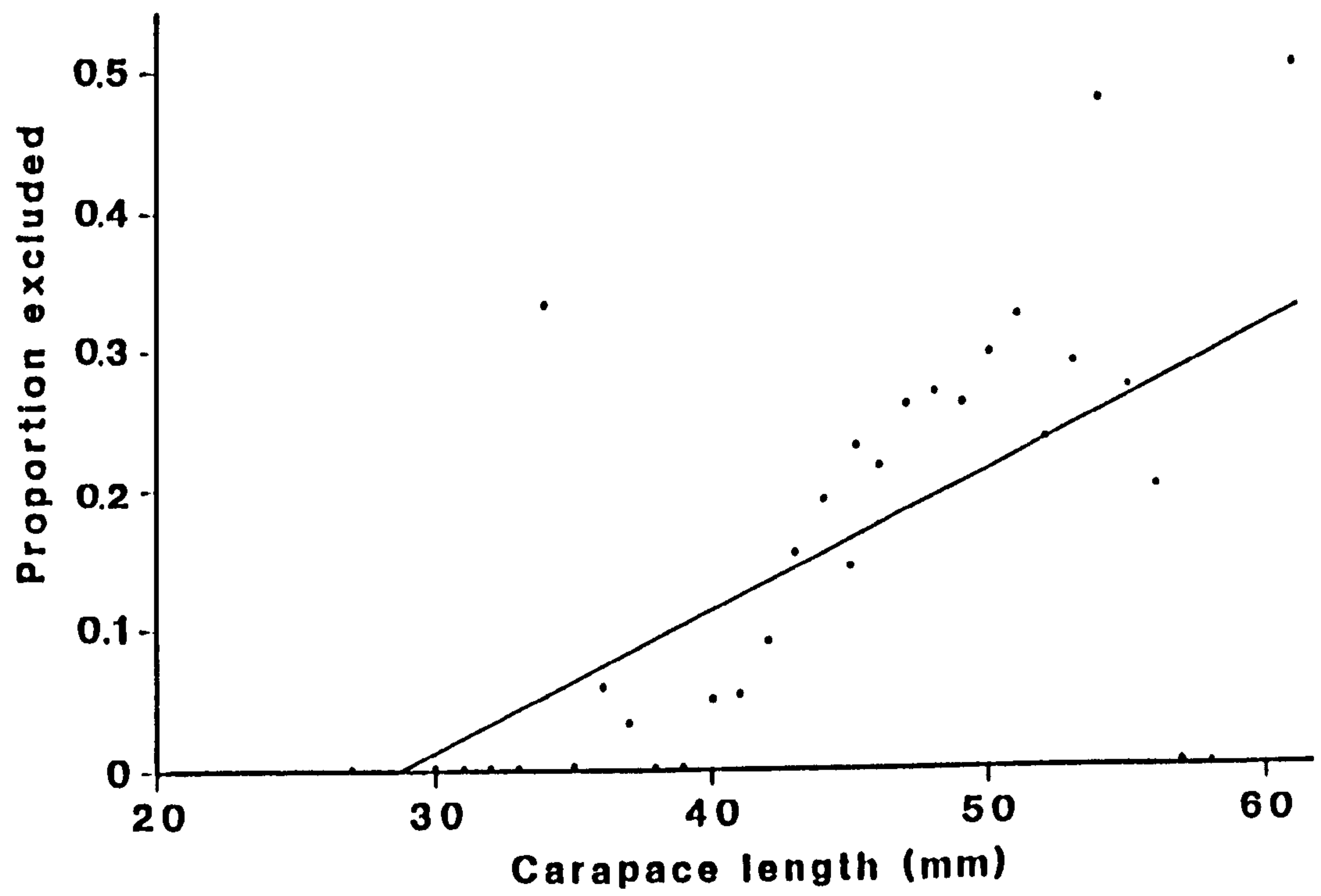
### Figure 8.3

Relationship between the proportion of females which were judged to have suffered abnormal egg loss (Y) and carapace length (X). The best fit linear relationship was :

$$Y = -0.2910 + 0.0101 X$$

number of observations = 33, correlation coefficient = 0.6083,  
(P < 0.001).





fecundity for all spawning females within the region had to be calculated. It was therefore necessary to determine the length frequency distribution for L.F.C. female Nephrops. For a number of different reasons, some of which have been discussed by Bailey et al. (1986), it is difficult to obtain accurate length frequency distributions for Nephrops populations. These include, gear selectivity, the influence of diel and tidal variations on the emergence behaviour of different sized animals, seasonal variations in the size composition and spatial changes in the size composition over relatively short distances.

It has been suggested that Nephrops growth may be somehow influenced by the characteristics of the sediment they inhabit (Bailey et al., 1986). These authors have indicated that the finer mud substrata tend to support low densities of large fast growing Nephrops. An extensive silt-clay facies extends throughout most of the Nephrops grounds in the Firth of Clyde (B.G.S., 1985) north of Lat. 55°24.26'N, the southern limit of my larval surveys. If the substratum type does influence the size composition of the resident Nephrops population, which is as yet unproven, it seems likely that the relatively uniform composition of the silt-clay facies (90 - 100% silt/clay) may cause a similar uniformity in the size composition of the Nephrops population. At the periphery of the silt-clay facies in the Firth of Clyde there are a range of other mud types (B.G.S., 1985). Bailey et al. (1986) suggest that this may explain why observations of biological characteristics made for Nephrops at discrete locations throughout the Firth of Clyde show some variations. These authors conclude that overall, the Nephrops population within the Firth of Clyde appears to possess the attributes associated with a

fine mud substratum ie. low densities of large fast-growing individuals.

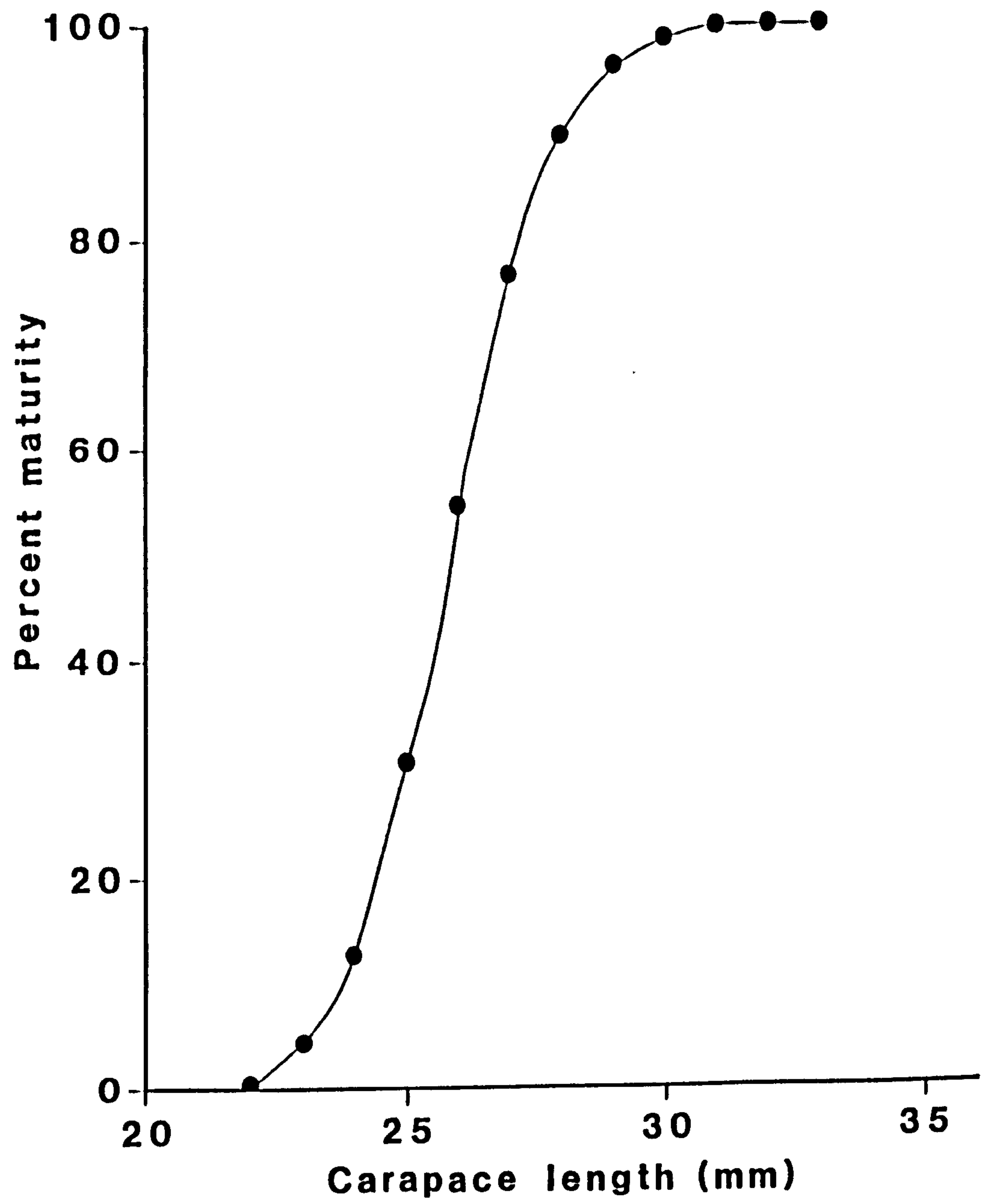
The size frequency distributions of female Nephrops obtained in an earlier investigation (N.Bailey and C.J.Chapman, pers. comm.) within the silt-clay facies of the larval survey area (L.F.C.) may provide a reasonable indication of the true population structure. These studies were conducted over a 3 year period (1980 - 1983) between July - September, when most mature females were in the non-ovigerous condition and more available to the sampling gear. Samples were obtained using 22 and 70mm mesh trawls. The smaller Nephrops tend to be under-represented in the 70mm mesh trawl catches and there is some evidence to suggest that the larger individuals are under-represented in the 22mm mesh trawl catches (N.Bailey, pers. comm.). The bias caused by gear selectivity is likely to be more serious in the catches of the 70mm mesh trawl. The catch data obtained from the 2 sets of gear were treated separately and 2 estimates of the female population structure were obtained for the larval survey area (L.F.C.).

From the results of an earlier study of Nephrops reproduction, also undertaken within the silt-clay facies of the L.F.C. (N.Bailey, pers. comm.), a maturity ogive was constructed (Figure 8.4). The maturity ogive was used to transform the female population structures obtained using the 22 and 70mm mesh trawls into length distributions of females contributing to the larval hatch (Figure 8.5). These length distributions were then used in conjunction with the various fecundity estimates (Figures 8.1, 8.2, 8.3) derived for each carapace length to determine the fecundity of an average female.



**Figure 8.4**

**Maturity ogive for the Firth of Clyde showing percentage of females with mature ovaries in each millimetre carapace length (data provided by N.Bailey).**

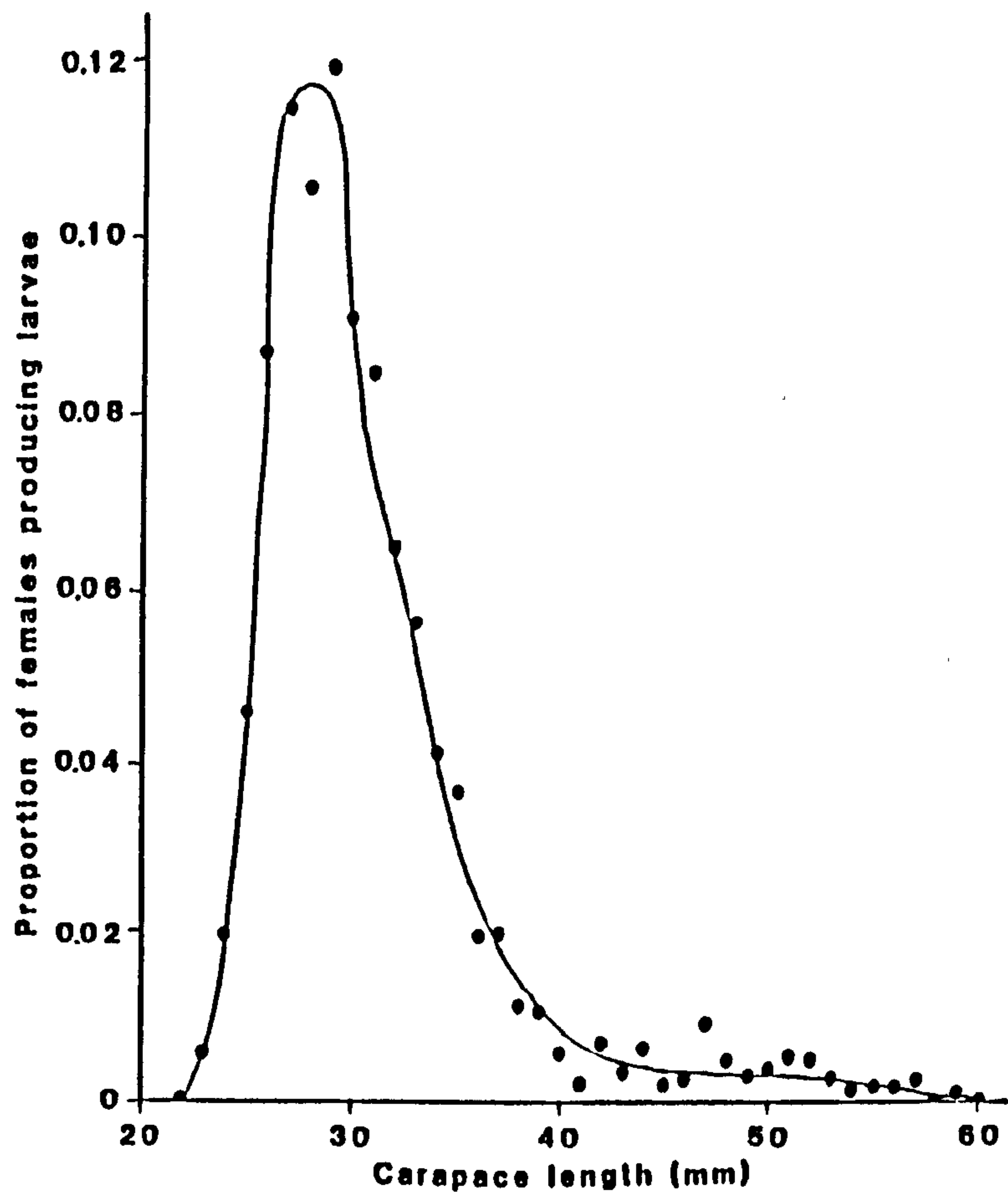


**Figure 8.5**

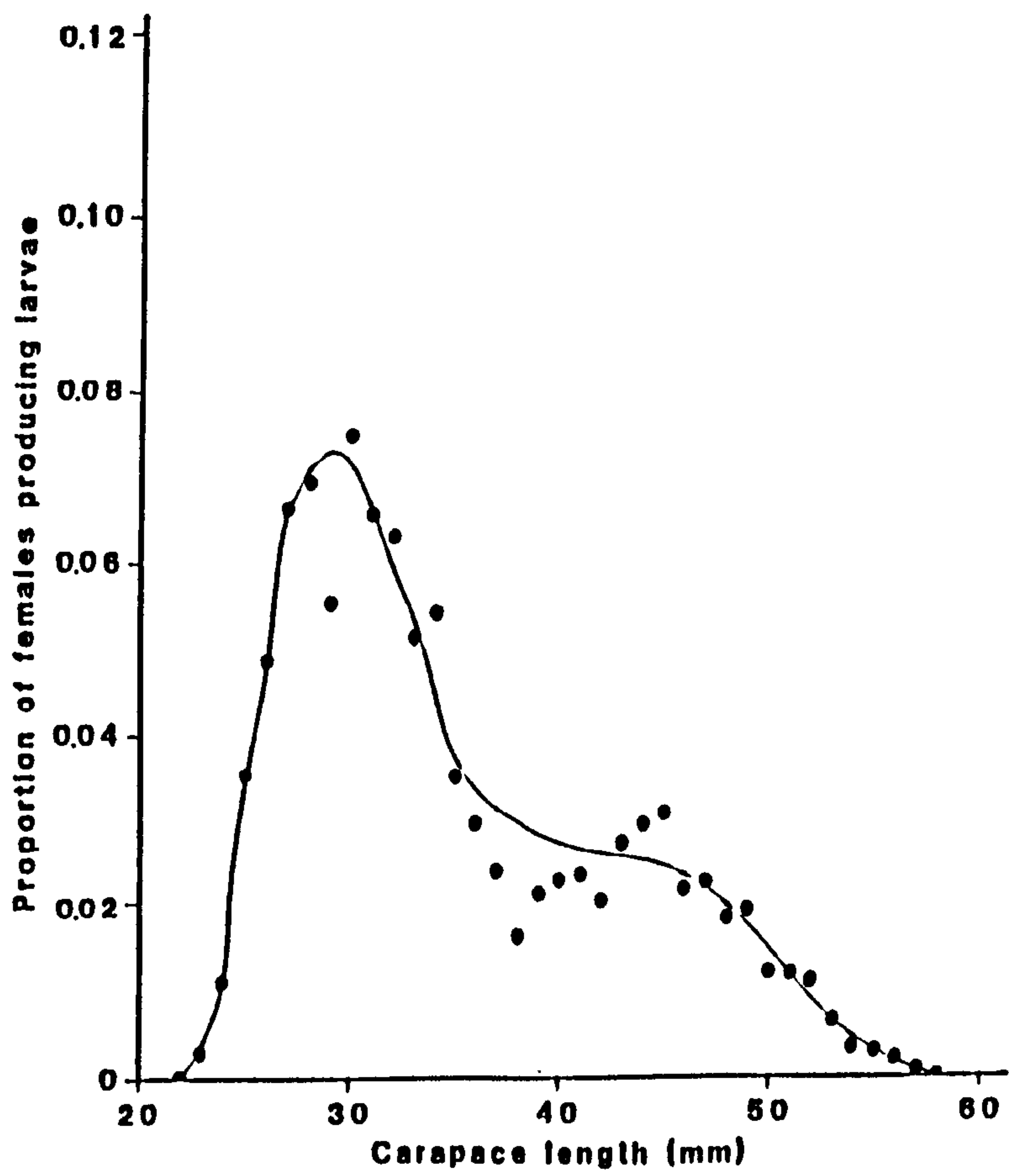
**Estimated length distribution for females contributing towards larval production in the Lower Firth of Clyde. The distributions were derived from information on the female population structure obtained using (a) 22mm mesh trawl and (b) 70mm mesh trawl (data provided by N.Bailey and C.J.Chapman).**



**a**



**b**



The average fecundity per female derived from the '22mm' length distribution was 1115 eggs and from the '70mm' distribution it was 1631 eggs. It is known that a proportion of the L.F.C. Nephrops population do not reproduce annually (Bailey et al., 1986). At present the composition and proportion of the population which fall into this category is unknown; however, biennial spawning seems to be more common in larger sized Nephrops (N.Bailey, pers. comm.). If biennial spawning was considered to occur only in individuals larger than 40mm carapace length and an arbitrarily chosen 50% of this larger size group were assumed to be biennial spawners, then the mean effective fecundity range for the '22mm' distribution would be adjusted to 985 - 1115 hatching eggs per female per year. For the '70mm' distribution it would be adjusted to 1207 - 1631 hatching eggs per female per year.

Two estimates were obtained for the total seasonal production of stage 1 zoeae in the L.F.C.. The first value of  $111.14 \times 10^8$  larvae was calculated from the 1st zoeal production curve (see Chapter 2, Figure 2.10) and is an underestimate because stage 1 production is not instantaneous. The zoeae may range in age from newly hatched to 14.5 days old at the mean sea temperature ( $8.9^{\circ}\text{C}$ ) calculated for this stage during the survey period. The other value of  $177.00 \times 10^8$  larvae is the intercept at zero time from the stage to stage mortality regression (see Chapter 2, Figure 2.11). The precision of this estimate depends upon adequate sampling of the 2nd stage zoeae. Laboratory based studies indicated that swimming speed was not markedly different between stages. It therefore seems unlikely that the 2nd zoeae were better able to avoid the sampling gear.

For the '22mm' distribution the mean fecundity range of 985 - 1115 hatching eggs per female per year gives a range of numbers of females participating from  $9.97 \times 10^6$  to  $11.28 \times 10^6$  for the lower larval production estimate and from  $15.87 \times 10^6$  to  $17.97 \times 10^6$  for the higher production estimate. For the '70mm' distribution the effective mean fecundity range of 1207 - 1631 hatching eggs per female per year gives a range of numbers of females participating from  $6.81 \times 10^6$  to  $9.21 \times 10^6$  for the lower larval production estimate and from  $10.85 \times 10^6$  to  $14.76 \times 10^6$  for the higher production estimate. Since the higher production estimate probably provides a better approximation of total 1st zoeal production than the lower estimate and, the 22mm mesh trawl probably gives a better indication than the 70mm mesh trawl of the true size frequency distribution of the population, the best available estimate of spawning stock size for the L.F.C. is probably  $15.87 \times 10^6$  to  $17.97 \times 10^6$  females. From the spawning length distribution constructed using the 22mm mesh trawl catch data the mean spawning length was found to be 32mm carapace length.

In a similar study conducted by Nichols *et al.* (1987) for an area approximately 20 times larger than the L.F.C. in the Western Irish Sea the number of spawning females participating in the hatch was estimated as between  $4.47 \times 10^8$  to  $8.13 \times 10^8$  and the mean length of the spawning females was 27.5mm carapace length. This indicates that the L.F.C. Nephrops stock is composed of a relatively less densely populated community of larger individuals than occur in the Western Irish Sea. If the sediment in some way influences the biological characteristics and structure of the Nephrops populations it supports, regional differences in the substratum may have caused these apparent



differences between the stocks (ie. the L.F.C. Nephrops may be supported on a finer mud sediment than the Western Irish Sea Nephrops). A muddy-sand substratum (10 - 50% silt/clay) was identified for the study area used by Patterson (1983) in the Western Irish Sea and recent work suggests that the Irish Sea Nephrops population may, as a whole, possess the attributes associated with a coarser mud sediment ie. smaller individuals and slower growth rates (Brander and Bennett, 1986).

From the size frequency data obtained using the 22mm mesh trawl it was estimated that the spawning stock represents approximately 42 - 48% of the total female population in the L.F.C.; this includes an allowance for the possible occurrence of biennial spawners. This produced an estimate of  $33.06 \times 10^6$  to  $42.79 \times 10^6$  for the total number of females available to the fishing gear. If a sex ratio of 1:1 is assumed for the L.F.C., which at present is the best available estimate although recent work indicates that it may depart considerably from this (Bailey, 1984), the size of the L.F.C. Nephrops stock (which is available to commercial fishing activities) is estimated as  $66.12 \times 10^6$  to  $85.58 \times 10^6$  Nephrops.

Nephrops usually occur at depths greater than 15m (Chapman, 1980) and where the silt/clay content of the sediment exceeds 30% (Chapman and Bailey, 1987). On this basis the Nephrops ground in the L.F.C. would be expected to extend over approximately 80% of this area ie.  $701\text{km}^2$ . This value can then be used to transform the L.F.C. stock estimate into a density estimate of approximately 0.094 - 0.122 Nephrops per  $\text{m}^2$ .

The population density of Nephrops in the Firth of Clyde has been estimated in earlier studies from both counts of Nephrops and their burrows using underwater television techniques (Bailey *et al.*, 1986). As discussed by these authors the density estimate obtained from Nephrops counts (which was 0.007 - 0.013 per m<sup>2</sup>) is likely to be an underestimate since not all animals emerge at the same time. Also, the density estimate obtained from counts of burrows (which was 0.268 - 1.400 per m<sup>2</sup>) overestimates density, since some are likely to be unoccupied. The intermediate density estimate obtained in this present study therefore seems to be reasonably acceptable.

The value of the Scottish Nephrops fishery has increased steadily since the early 1950's and in 1986 was valued at over £29m (DAFS, 1987). There are now signs that the fishery is being exploited to its maximum capacity (McIntyre, 1986). Any further increase in fishing effort may have a detrimental effect on yields. It is important therefore that the resource is effectively managed.

Due to the benthic habit of the postlarval and adult Nephrops larval surveys offer the best means of monitoring the size of the spawning stock. It is hoped that future larval surveys will be conducted in the L.F.C. so that any changes in the strength of the spawning stock will be detected. Using this information in conjunction with fisheries monitoring (eg. catch per unit effort) this may provide useful information on the state of the stocks in the L.F.C. fishery.

No dramatic diel changes were detected in the vertical distribution of Nephrops zoeae and any changes which did occur were subtle. During the hours of daylight most Nephrops larvae were

distributed between 6 - 38m depth with the greatest concentration in the region of the pycnocline. During darkness there was an increase in the number of larvae near the surface. Whether this was solely due to a nocturnal rise or whether it was contributed to by dispersion followed by overcompensation to sinking cannot be determined. Larvae appeared unresponsive to light at levels below approximately  $4 \times 10^{-3} \mu\text{Es}^{-1}\text{m}^{-2}$ . The diel surveys were conducted during the period of peak larval production and an upward migration of newly hatched larvae from the bottom of the water column may have contributed towards the night-time peak, which occurred at a shallower depth (5m) than the normal daytime position (16m). The increase in light intensity around dawn was accompanied by a general sinking of the Nephrops zoeae to the normal daytime depth.

The sampling interval between the nets was relatively large (11 - 12m) and small changes in larval distribution, possibly caused by tidal variables, would have been difficult to detect. It is uncertain whether the discontinuity layer exercises any influence over the depth distribution of the larvae although, during daylight hours, most seem to remain at depths immediately below it.

Laboratory based studies have shown that Nephrops zoeae are sensitive to pressure changes of between 0.3 to 0.5 ats, which corresponds to a change in depth of 3 to 5m. It was found that a pressure increase enhanced movement towards the light source and a pressure decrease caused reduced activity and consequent sinking in the photopositive individuals of the 1st, 2nd and early 3rd zoeal stages. Nephrops zoeae appear to be most sensitive to light within the same wavelength range as the adults (400 - 600nm).



Sometime during the 3rd zoeal stage the light response appears to change from photopositive to photonegative. This may cause the older 3rd zoeae to sink towards the bottom of the water column and metamorphosis into the 1st postlarval stage probably occurs near or on the sea bed. The most obvious changes which occur at metamorphosis include a general change in the shape of the carapace from subtriangular to subrectangular, development of an extremely long pair of antennae (larval antennae are comparatively short), an increase in the size of the pleopods into a fully functional form, reduction of the heavily setose thoracic exopods to a small residual form, removal of the large abdominal spines and the replacement of the furcate telson with a tail fan.

Some other morphological changes which were primarily initiated during metamorphosis were not completed until a later postlarval stage. These included changes in the mouthparts (the development of the molar processes of the mandibles and discrete 'teeth' on the ischium of the 3rd maxillipeds), the development of the gills from simple lobes into the trichobranch type and the changes in the eye associated with the transition from apposition to superposition optics eg. the 'squaring-off' of the corneal facets. Superposition optics have been developed for its improved light gathering capacity under low ambient light conditions. In Nephrops an 8th retinula cell has now been located in both the apposition eyes of the larval stages (this study) and the reflecting superposition eyes of the adults (P.M.J.Shelton, pers. comm.).

The 1st postlarval stage seems to be a transitional stage between the planktonic and benthic environments. Individuals within the 1st

postlarval stage are probably engaged in extensive exploratory migrations over the substratum surface. Final settlement probably occurs sometime after moulting into the 2nd postlarval stage. It has been shown that the older juveniles (ie. beyond the 1st postlarval stage) are capable of constructing their own independent burrows and will actively select a mud substratum which supports the excavation of these structures. The laboratory based studies have shown that juveniles will enter the burrows of adult Nephrops through the surface openings and immediately excavate a tunnel in one of the walls of the adults burrow. This provides the juvenile with its own separate burrow and gives protection from the likely cannibalistic tendencies of the adults. Adult / juvenile associations are known to occur in the field (Rice and Chapman, 1974 ; Chapman, 1980 ; Atkinson, 1986) but independent juvenile burrows have not yet been found. Juveniles are likely to derive several benefits from an association with an adult burrow and future research may reveal whether recruitment to the benthic environment is usually achieved by direct entry into existing adult burrows. It has been suggested that following settlement the juveniles remain within their burrows during their first year until they have reached a size of 10 - 15mm carapace length (Chapman, 1980).

The aquaculture study has shown that Nephrops larvae can be reared using the same husbandry techniques as previously applied for the larval stages of Homarus spp.. Nephrops larvae seem to become more cannibalistic with development through the successive zoeal stages and survival can be improved by rearing each individual in isolation. Under the culture conditions of the present study 16°C was found to be the overall 'optimum' temperature for larval development, although there appeared to be some variation between the 3 zoeal stages.

Temperatures of around 20°C seemed to be lethal for both the larval (this study) and postlarval stages (Figueiredo, 1979). Initial periods of starvation in excess of 1 - 2 days led to an increase in mortality. This shows the need for hatching to be synchronised with the production and seasonal succession of organisms within the zooplankton community on which Nephrops larvae feed.

At present the commercial culture of Nephrops is unlikely to be feasible but the aquaculture techniques developed in this study may be useful for future investigations requiring a ready supply of larvae and postlarvae. These stages are difficult to obtain from the field in healthy condition. The culture facilities also provided valuable information on the influence of temperature on larval development for each zoeal stage. Information of this type is scarce for Nephrops and is required to transform larval abundance data into field estimates of production and larval mortality rates. It is hoped that the relationships formed between temperature and larval development in this study has at least removed one of the uncertainties in estimates of larval production which will be of value in future studies.



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## **Appendix**



The number of zoea captured per metre square of sea surface area for each stage and for all 3 stages during the 5 surveys.

Date of Survey	Zoeal Abundance												(Numbers of zoeae caught.m <sup>-2</sup> of sea surface)																	
	20-22/4/84						30/5-1/6/84						27-28/6/84						24-25/7/84						22-23/8/84					
	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ						
Survey Station																														
A1	1.189	-	-	1.189	3.606	0.949	0.380	4.935	4.244	0.326	-	4.570	0.704	0.563	1.830	1.379	0.125	0.125	1.629											
A2	-	-	-	-	6.688	0.936	0.535	8.159	0.379	-	0.190	0.569	0.618	0.247	0.989	0.845	-	-	0.845											
A3	-	-	-	-	9.140	0.751	-	9.891	2.656	0.625	-	3.281	1.475	0.105	1.685	0.244	-	-	0.244											
A4	0.440	-	-	0.440	1.300	-	-	1.300	0.173	-	-	0.173	0.478	-	0.478	1.081	0.154	-	1.235											
B1	3.132	0.392	-	3.524	0.425	0.142	-	0.567	1.293	0.485	0.162	1.940	0.192	0.096	-	0.288	-	-	0.515											
B2	-	-	-	-	4.587	1.223	-	5.810	3.769	0.314	0.628	4.711	1.765	0.980	-	2.745	-	-	0.940											
B3	0.354	-	-	0.354	8.965	3.362	0.747	13.074	0.738	0.492	0.492	1.722	3.155	0.901	0.225	4.281	0.159	-	0.940											
B4	-	-	-	-	0.322	-	-	0.322	0.796	0.159	-	0.955	2.246	0.173	0.346	2.765	-	-	0.318											
C2	-	-	-	-	1.707	-	0.213	3.627	1.819	0.202	-	2.021	1.625	0.711	0.609	2.945	-	-	0.369											
C3	0.407	-	-	0.407	2.170	0.241	0.964	3.375	0.124	-	-	-	1.838	0.836	-	2.674	0.381	-	0.381											
C4	-	-	-	-	3.773	0.444	0.222	4.439	0.124	-	-	0.124	1.714	0.685	-	2.399	0.316	-	0.316											
C5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-											
D2	0.692	-	-	0.692	2.057	0.229	0.457	2.743	1.240	0.620	0.207	2.067	1.602	1.424	0.178	3.204	0.179	-	1.614											
D3	0.405	-	-	0.405	4.195	1.259	-	5.454	0.525	-	-	0.525	2.732	0.182	0.364	3.278	-	-	0.179											
D4	-	-	-	-	3.151	0.210	0.210	3.571	0.525	-	-	-	2.296	1.879	-	4.175	-	-	0.378											
D5	0.284	-	-	0.284	1.761	0.271	0.135	2.167	0.665	-	-	0.665	1.420	1.420	-	1.840	-	-	0.196											
D6	-	-	-	-	7.856	1.647	-	9.503	0.665	0.078	-	-	-	0.627	0.125	0.752	-	-	0.150											
E2	0.310	-	-	0.310	-	-	-	-	0.078	0.078	-	-	-	-	-	-	-	-	-											
E3	0.573	-	-	0.573	1.525	0.436	-	1.961	0.229	-	-	0.229	0.657	0.657	0.164	1.478	0.195	0.195	0.390											
E4	-	-	-	-	2.225	2.003	0.445	4.673	0.229	-	-	-	0.331	0.166	0.166	0.663	0.316	0.158	0.474											
E5	1.151	-	-	1.151	5.070	1.472	1.308	7.850	0.163	-	-	0.163	1.447	1.808	0.723	3.978	0.150	-	0.150											
E6	0.809	-	-	0.809	6.025	3.244	0.927	10.196	0.298	-	-	0.298	2.160	2.160	0.432	4.752	0.657	0.821	1.642											
F2	0.308	-	-	0.308	0.786	-	-	0.786	1.568	1.121	-	-	1.689	0.138	0.152	0.276	-	-	0.182											
F3	0.470	-	-	0.470	4.996	1.303	0.652	6.951	0.261	-	-	0.261	0.762	0.152	0.152	1.066	0.182	-	0.182											
F4	0.352	-	-	0.352	1.043	0.782	0.261	2.086	1.239	-	-	1.593	1.063	-	1.063	1.063	0.512	-	0.512											
F5	1.458	-	-	1.458	6.205	0.931	0.155	7.291	0.521	0.174	-	0.354	1.254	1.411	0.157	2.822	-	-	-											
F6	0.416	-	-	0.416	6.013	2.053	0.440	8.506	0.521	-	-	0.695	1.063	3.659	0.203	7.927	-	-	0.512											
F7	-	-	-	-	0.721	0.120	-	0.841	-	-	-	-	4.065	-	-	-	0.081	-	-	0.081										
G2	0.296	-	-	0.296	0.823	0.206	0.206	1.235	0.236	-	-	0.236	0.138	0.138	-	0.276	0.088	0.088	0.088											
G3	-	-	-	-	5.018	2.400	0.655	8.073	0.255	-	-	0.255	0.623	0.311	0.156	1.090	0.185	-	0.185											
G4	1.192	-	-	1.192	2.025	0.900	0.675	3.600	0.140	0.281	-	0.421	0.491	0.327	0.818	0.201	-	-	0.201											
G5	0.474	-	-	0.474	0.940	-	-	0.940	0.687	-	-	0.687	0.867	0.693	0.347	1.907	-	-	-											
G6	-	-	-	-	8.220	2.202	0.440	10.962	0.147	-	-	0.147	0.274	0.684	0.274	1.232	0.146	0.146	0.292											
G7	-	-	-	-	0.616	0.176	-	0.792	0.093	-	-	0.093	-	-	-	-	-	-	-											
H2	-	-	-	-	4.106	0.513	0.513	5.132	0.200	-	-	0.200	0.935	0.624	-	1.559	-	-	-											
H3	0.285	-	-	0.285	3.763	0.896	0.358	5.017	0.517	-	-	0.620	0.774	0.929	-	0.997	0.462	-	0.462											
H4	2.766	0.395	-	3.161	3.861	1.679	0.839	6.379	0.517	0.103	-	0.676	1.382	1.580	0.592	3.554	0.199	-	0.199											
H5	2.565	-	-	2.565	2.508	0.148	0.590	3.246	0.676	-	-	-	-	-	-	-	-	-	-											
H6	-	-	-	-	0.171	-	-	0.171	-	-	-	-	-	0.085	-	0.085	-	-	-											
Σ	20.328	0.787	-	21.115	128.192	35.006	12.327	175.525	25.729	3.980	2.033	31.742	41.216	27.348	6.005	74.569	11.853	1.830	0.484	14.167										
Mean	0.521	0.020	-	0.541	3.287	0.898	0.316	4.501	0.660	0.102	0.521	0.814	1.057	0.701	0.154	1.912	0.304	0.047	0.012	0.363										

**Σ = Total**

