CHAPTER FIVE

Reproductive Biology

5.1 Introduction

Studies on the reproductive biology of decapod crustaceans are well documented, particularly in brachyurans. Several methods have been used to define the reproductive cycle in crabs including gonad indices (e.g. Boolootian et al., 1959 and Du Preez & MacLachlan, 1984c), incidence of ovigerous females (e.g. Fielding & Haley, 1976, Pillay & Ono, 1978 and Jones, 1980) and histological examination of the gonads (e.g. Hartnoll, 1972, Haefner, 1976, 1977, Gemmell, 1979 and Simon & Jones, 1981). The general applications of these methods on invertebrate reproduction have been reviewed by Giese & Pearse (1974) and Grant & Tyler (1983a, b), whilst the ecological aspects of crustacean reproduction were reviewed by Sastry (1983).

Despite the abundance of literature on brachyuran reproduction, there have been very few published accounts on the reproductive biology of the Matutinae, particularly in *Matuta lunaris*. Previous studies have been limited to observations on the incidence of ovigerous females (Pillay & Nair, 1976) and notes on larval development (Rajabai, 1959). Both these studies, however, were restricted to populations in India. The aim of this study therefore is to investigate several aspects of the reproductive biology of *M. lunaris*, namely 1) gonad structure and development, 2) size at sexual maturity, 3) brood size and egg number, 4) pair formation and mating behaviour and 5) the annual reproductive cycle.

5.2 Materials and Methods

The crabs used in this study were collected from Site 'B' (Plate 2.1), Pallarenda Beach, Townsville, in January and February, 1984 and between April, 1984 and May, 1985. Sampling was conducted using a large 25 mm mesh, 10 m x 1 m seine net following the methods described in Chapter 2. All crabs were brought alive to the laboratory for analyses and were dissected within three days of capture.

A preliminary study of the size at sexual maturity in *M. lunaris* was undertaken on individuals collected between January 4 and February 1, 1984. In both sexes, a minimum of five individuals from each 5-mm full carapace width (FCW) size class were dissected. Each crab was weighed to the nearest 0.1 gm and the full carapace width was measured to the nearest 0.1 mm. Crabs were then anaesthetized in ice and dissected in seawater, under a stereomicroscope. The gonads were removed and fixed in Bouin's fluid for histological analyses. The details of the histological procedure are summarized in Appendix II. Sections were then stained in Harris Haematoxylin and Eosin and mounted in DPX. Photomicrographs were taken of representative sections from sexually mature individuals to demonstrate gonad structure.

In order to investigate the annual reproductive cycle of *M. lunaris*, sampling was carried out at monthly intervals from April, 1984 to May, 1985, within two days of the full moon of each month and always on a midfalling tide. During these samplings, the sex and full carapace width of each crab was recorded and a note was made of the numbers of paired crabs in precopulatory position

(described in this chapter) and ovigerous females. A minimum of 10 mature males (>48.5 mm FCW) and 12 mature females (>40.0 mm FCW) were dissected each month. The gonads were removed and processed following the procedure described above. In addition, the female ovaries were weighed to the nearest 0.0001 g using an electric analytical balance and the gonad index was determined using the equation:

$$\text{Gonad Index} = \frac{\text{wet weight of ovary (in grams)}}{\text{wet weight of crab (in grams)}} \times 100 \quad ,$$

following Giese & Pearse (1974) and Grant & Tyler (1983a).

In ovarian sections, the sizes of oocytes/ova were measured using an eyepiece graticule calibrated against a stage micrometer. Only those oocytes/ova sectioned through the nucleus were measured. One hundred oocytes/ova were measured in each crab and grouped into 4 size classes based on cell diameter, following the methods of Grant & Tyler (1983b). The mean frequency of oocytes in each size class were then calculated for each month. As there was a recognizable 'spent' stage in the gonads of female *M. lunaris*, sections of spent ovaries were classified as such, without attempting to measure the oocytes.

In testis sections, the cells within 50 random lobes in each crab were classified based on the four stages of spermatogenic development, *i.e.* spermatogonia, spermatocytes, spermatids and spermatozoa. The mean frequency of lobes containing a particular cell type were then calculated for each month.

Ovigerous females were collected during the reproductive sampling and the ecological survey on Site 'B' (Appendix III). In each female, the full carapace width was measured and the colour of the egg mass was noted. All ovigerous females were brought alive to the laboratory and dissected within one day of capture. The ovaries were removed, weighed and processed following the procedure described above. The egg mass was removed from the abdomen and weighed to the nearest 0.0001 g. A sub-sample was then taken from the egg mass, weighed to the nearest 0.0001 g and the number of eggs counted. The number of eggs per egg mass was then calculated using the equation:

$$\text{total no. of eggs per egg mass} = \frac{\text{no. of eggs in subsample}}{\text{weight of subsample (g)}} \times \text{total weight of egg mass}$$

following Fielding & Haley (1976) and Du Preez & McLachlan (1984c).

Pairs of male and female *M. lunaris* in the precopulatory position were collected during the population (Chapter 7) and reproductive samplings at Sites 'A' and 'B', respectively. Those collected from Site 'A' were measured and returned to the surf zone after capture whilst those from Site 'B' were measured and brought alive to the laboratory. Pairs were maintained in a closed water system in separate 12 liter aquaria with a sandy substratum. Crabs were fed with pieces of fish every other day. Three or four days after copulation, the females were removed and placed in a separate tank, whilst the males were returned to the main holding tank for further observation.

5.3 Results

5.3.1 Gonad structure

5.3.1.1 Male gonad

The testes of mature male *M. lunaris* are smaller than the ovaries of mature females. As in the gonads of most male brachyurans, the testis is a narrow H-shaped, convoluted organ which is found in the dorsomedial region of the body cavity. It consists of two anterior branches which lie dorsolateral to the stomach and often extend to the anterolateral margins of the carapace, a small medial commissure which lies posterior to the stomach and two short posterior branches which extend ventral to the pericardium and are confluent with the tightly coiled vasa deferentia (Fig. 5.1A).

Histologically, the testis consists of lobes filled with cells in various stages of spermatogenesis. In transverse section, the lobes are surrounded by a thin layer of connective tissue and are arranged around a thick layer of connective tissue which serves as a central axis. In some sections, a spermatic duct may be visible in the lateral border furthest from the central axis. A typical transverse section of a male testis is shown in Plate 5.1b.

The cell stages within the lobes vary from one lobe to another, even in the same individual. Four types of cells may be distinguished based on their stage of development: 1) spermatogonia, 2) primary and secondary spermatocytes, 3) spermatids and 4) mature sperms. Spermatogonia are spherical cells with relatively large nuclei containing a slight chromatin network and peripheral chromatin granules (Plate 5.1a). In most sections, spermatogonia were present in the periphery of the lobes or at the
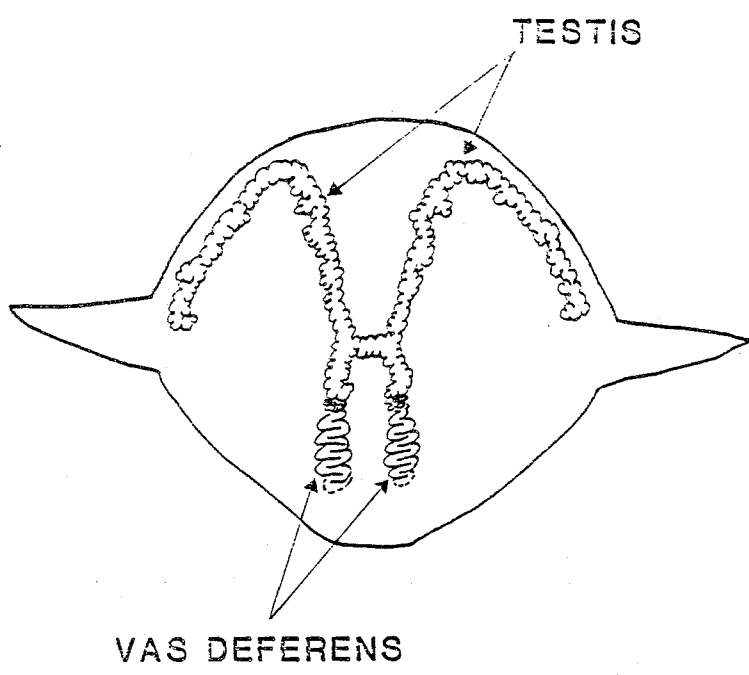
Figure 5.1

Schematic diagrams showing the location of the
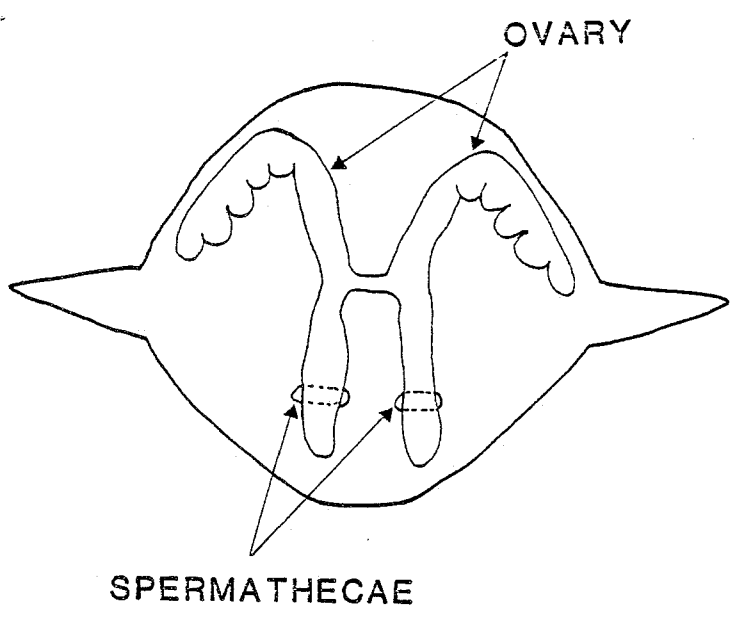
gonads in *M. lunaris*.

5.1A   Male testis

5.1B   Female ovary

TESTIS

A

VAS DEFERENS

B

OVARY

SPERMATHECAE

medial borders of several adjoining lobes. Occasionally, however, lobes were completely filled with spermatogonia (Plate 5.1a). Spermatocytes in general have smaller and more dense nuclei than spermatogonia, and are often in the same stage of development within a lobe. Primary spermatocytes have small nuclei with a heavily stained chromatin network, whilst secondary spermatocytes have nuclei which are distinctly smaller than those of the primary spermatocytes (Plate 5.1b, c). Both types of spermatocytes were often found in meiotic stages within the lobes (Plate 5.2a). Spermatids are smaller than the spermatocytes (Plate 5.2a). These possess smaller amounts of cytoplasm and the nuclei are typically dense. As the spermatids develop into mature sperms, the nuclear chromatin network appears compacted to one side of the nucleus giving the nucleus a 'ringed' appearance. Mature sperms are typically small, spherical (1-2μm in diameter) and strongly basophilic. Unlike the spermatogonia and spermatocytes, mature sperms were only found within the lumen of the spermatic ducts (Plate 5.2a).

The vas deferens is a tightly coiled tube which arises from the posterior end of the testis and extends ventrally through the thoracic cavity. The lumen is lined with a compact layer of cuboidal epithelial cells which are attached to a layer of connective tissue. In most specimens examined, the lumen of the vas deferens was filled with dense clusters of sperms, *i.e.* spermatophores (Plate 5.2b).

Plate 5.1

Histological sections of the male reproductive

organs of *M. lunaris*.

a. A transverse section of a testis lobe,

showing spermatogonia (SPG) and

mature spermatozoa (S).

b. A typical transverse section of the testis,

showing the orientation of the lobes

containing primary spermatocytes (SPC1)

around the connective tissue (CT) which

serves as a central axis.

c. A transverse section of the testis,

showing lobes containing secondary

spermatocytes (SPC2), around a central

axis of connective tissue (CT).
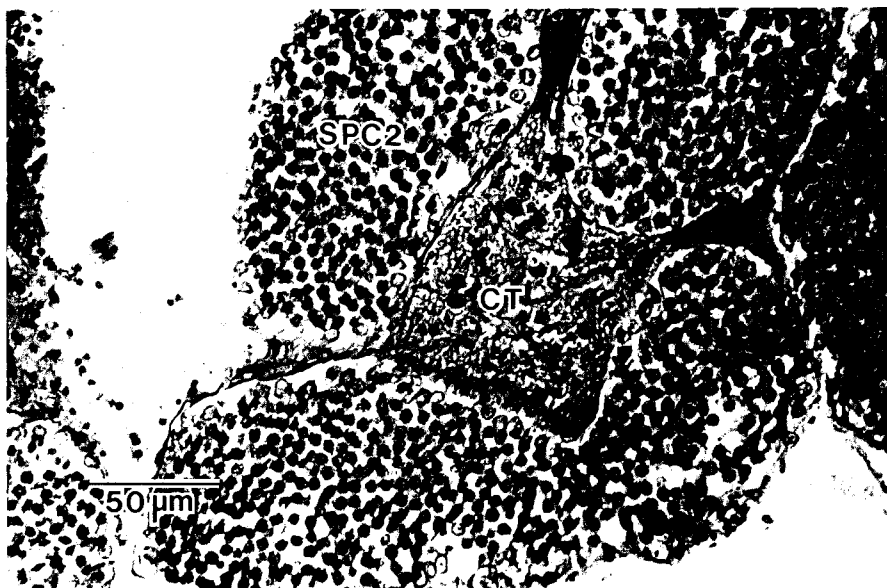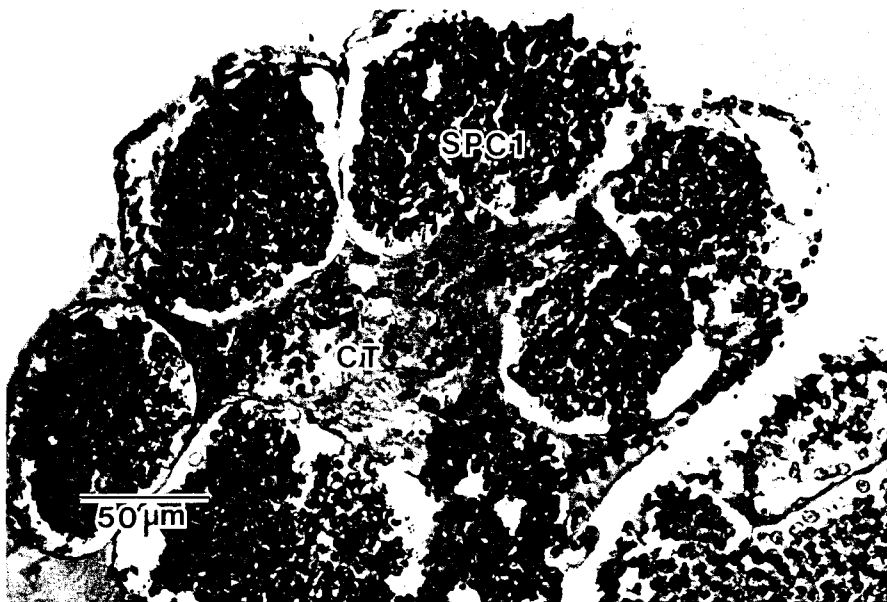
SPG
S
20 µm

SPC1
CT
50 µm

SPC2
CT
50 µm

Plate 5.2

Histological sections of the male reproductive

organs of *M. lunaris*.

a. A transverse section of testis lobes

    showing the location of the spermatic

    duct (SD) and the development of

    secondary spermatocytes (SPC2) into

    spermatids (SPT). Note the meiotic

    stages of the secondary spermatocytes

    in the central lobe. Mature sperms (S)

    may be seen inside the spermatic ducts.

b. A transverse section of the vas deferens

    (VD) filled with spermatophores (SPR).
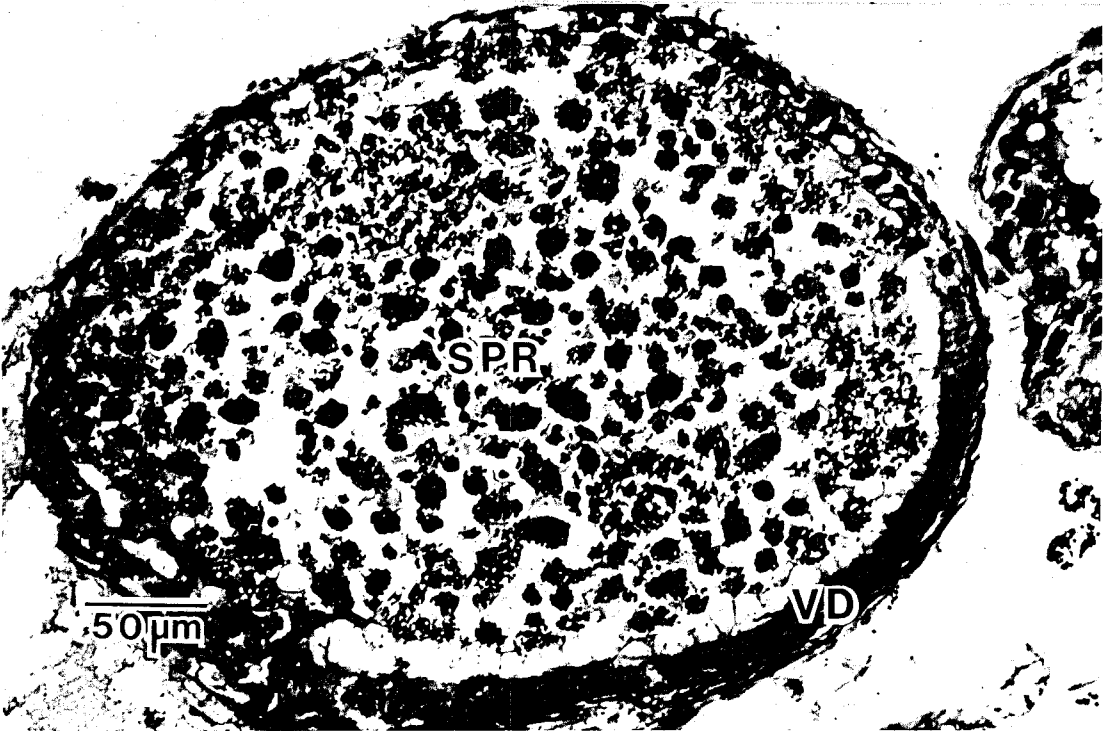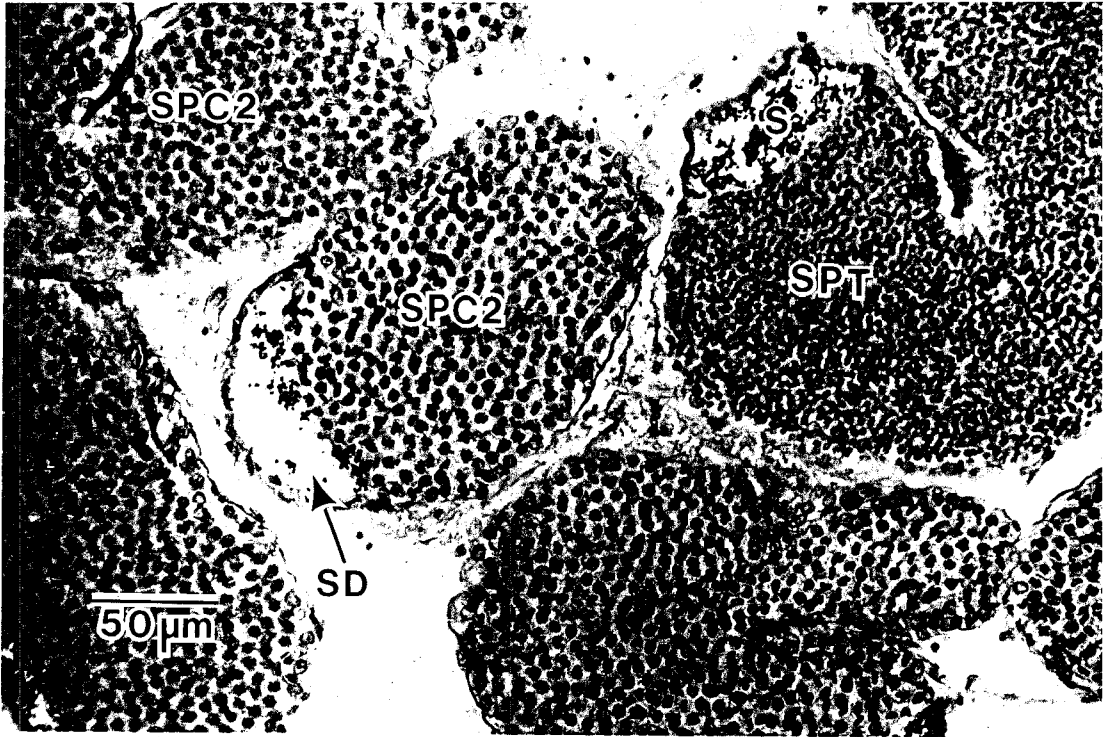
In this study, it was difficult to define the maturation stages of the testes *per se*. Unlike the ovaries, the testes of *M. lunaris* do not show marked colour and size changes during development. Mature males were only considered to have fully developed testes when histological evidence of mature sperms were recorded in the spermatic ducts and vas deferens.

5.3.1.2 Female gonad

The ovary of female *M. lunaris* is an H-shaped organ which occupies the anteromedial region of the body cavity. It consists of two anterior branches which extend up to the margin of the carapace, a central commissure which lies posterior to the stomach and two posterior branches which pass ventral to the pericardium and extend along the medial edges of the endophragmal skeleton (Fig. 5.1B). Similar to the male testis, the female ovary is typically lobed, with 5-7 rounded lobes on each anterior branch. Each posterior branch has a ventral protrusion which connects with a spermatheca, *i.e.* a seminal receptacle.

Cells within the ovary, as revealed by histological sections, are of three general types: 1) oogonia, 2) developing oocytes and 3) accessory/follicular cells. Unlike the spermatogonia of the testis, oogonial cells are found in a central germinative zone (CGZ) which extends along the length of the ovary including the lobes in the anterior branches (Plate 5.3b, c). Oogonial cells are typically small (3.9μm in diameter), each with a large spherical nucleus and very little cytoplasm (Plate 5.3b). These oogonial cells give rise to oocytes which, in transverse sections, radiate from the central germinative zone in progressive stages of development, with the

newly formed oocytes being proximal to the germinative zone (Plate 5.3c). The early oocytes have large spherical pale nuclei with prominent nucleoli. The cytoplasm of these cells is compact and typically basophilic, with the nuclear:cytoplasmic diameter ratio (N:C ratio) being 1:0.5. The average cell diameter of early oocytes is 23μm. Eosinophilic yolk droplets begin to accumulate in the cytoplasm of oocytes at about 43μm in diameter. The N:C ratio decreases during development as the cytoplasm increases in volume. In the early stages of vitellogenesis, the nucleus remain large and pale with a distinct nuclear membrane and a prominent nucleolus. The N:C ratio in these stages is 1:2.6. In the later stages of vitellogenesis, the whole cytoplasm is filled with yolk droplets and the diameter of the oocytes increases to approximately 103.7μm. The nucleus becomes slightly basophilic but continues to have a prominent nucleolus and a distinct nuclear membrane (Plate 5.4a). The N:C ratio in these latter stages is 1:4.9. The fully formed mature ova is a large yolk-filled cell measuring approximately 200μm in diameter (Plate 5.4b). The nucleus is a small basophilic central structure with a prominent nucleolus and a diffused nuclear membrane. The N:C ratio at this stage is particularly low at 1:7.5.

The accessory/follicular cells are found in the matrix surrounding the developing oocytes or mature ova. In the early stages of vitellogenesis, the follicular cells are loosely distributed in the interstitial spaces between the oocytes. The nuclei of these cells are typically small (1-3μm), round and basophilic. The cell membranes are not distinct and the cytoplasm appears diffused. In the later stages of vitellogenesis and in ripe ovaries, follicular cells form a thin, single-cell layer which

Plate 5.3

Histological sections of the female reproductive

organs of *M. lunaris*.

a. A longitudinal section showing the

    spermatheca (STA) of a newly moulted

    postpuberty female. The spermatheca

    at this stage is filled with sperms

    whilst the ovary is essentially

    immature or early developing (imO).

b. A transverse section of a developing ovary,

    showing the developing oocytes (OC) arising

    from the central germinative zone (CGZ)

    which contains oogonia (OG). Accessory

    cells (AC) are found between the oocytes.

    (nu = nucleus, nl = nucleolus)

c. A typical transverse section of a developing

    ovarian lobe showing the orientation of the

    central germinative zone (CGZ) and the

    oocytes (OC). Yolk droplets (yd) begin to

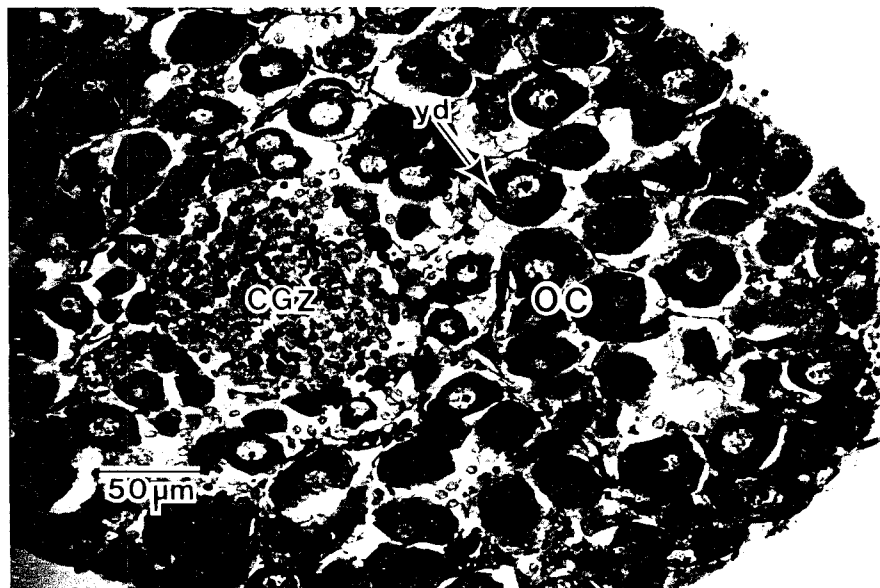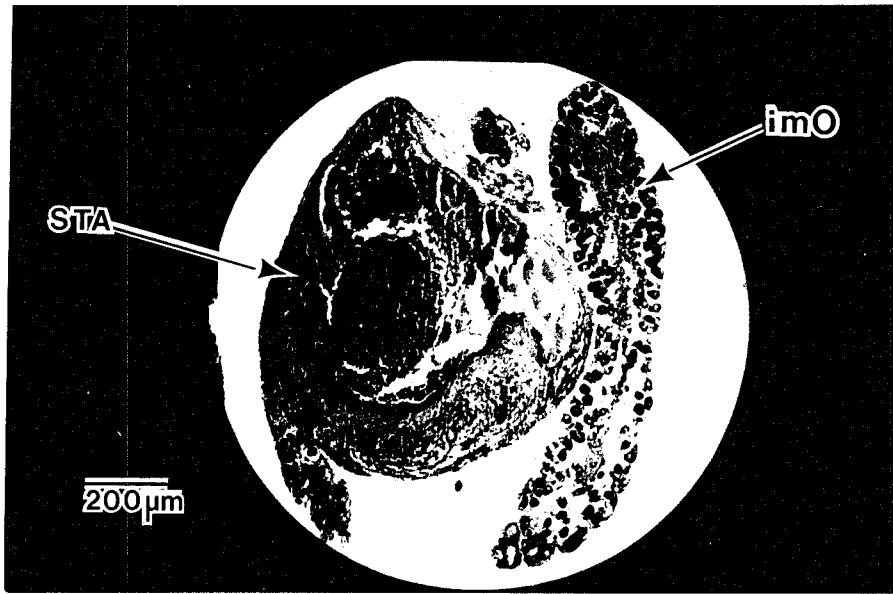   appear in the cytoplasm of larger oocytes.

Plate 5.4

Histological sections of the female reproductive
organs of *M. lunaris*.

a. A transverse section of an ovary in an

    advanced stage of vitellogenesis,

    showing the typical appearance of

    early oocytes (eOC) compared to the

    yolked or advanced oocytes (aOC).

b. The typical appearance of a mature ovum

    (OV), showing the yolk droplets (yd) and

    the arrangement of accessory cells (AC)

    around the ovum.

c. A transverse section of a spent ovary,

    showing the empty spaces previously occupied

    by ova and the few oocytes (OC) filling

    these spaces. An atretic ova (aOV) may

    be seen in the bottom right corner.

    Accessory cells (AC) are found lining

    the empty spaces.
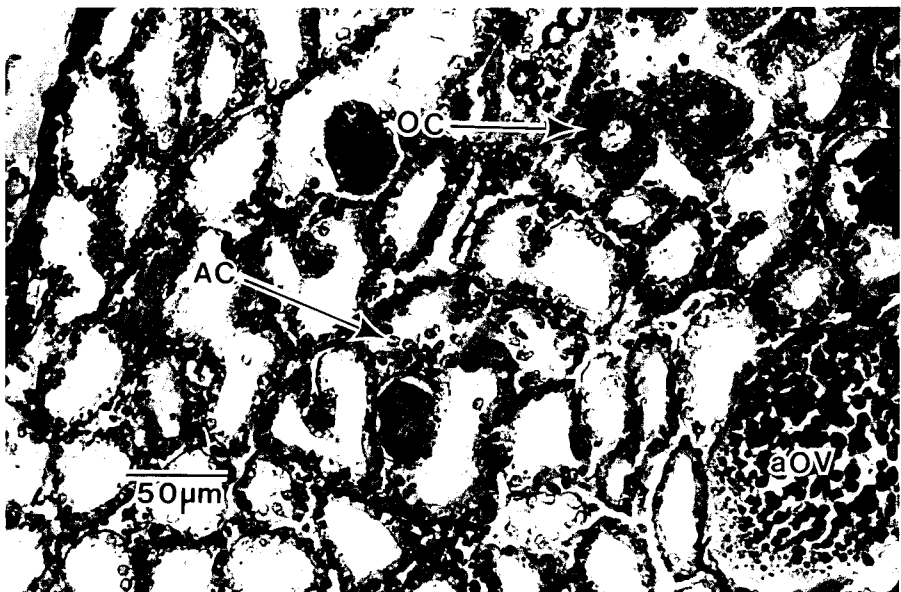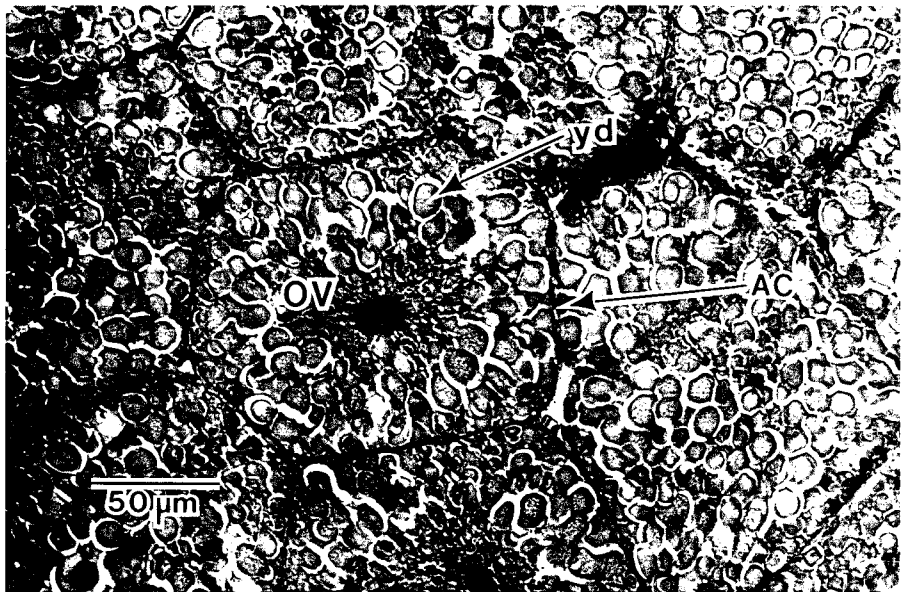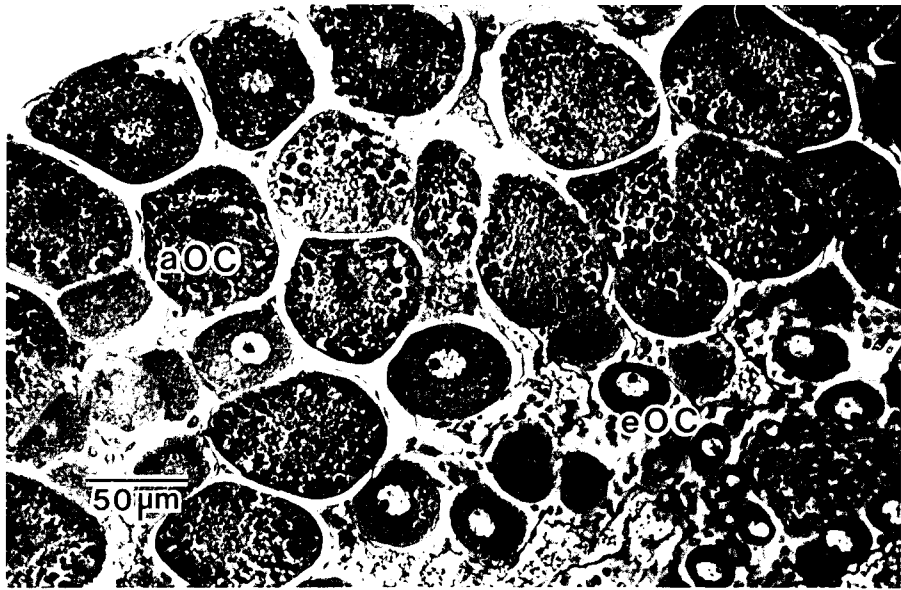
tightly surround each oocyte/ovum. At this stage, the nuclei of the follicular cells are typically elongate or ellipsoidal and are strongly basophilic. In spent ovaries, the follicular cells are loosely arranged in a single cell layer around the empty spaces previously occupied by each ovum. The nuclei of these cells at this stage are similar to the follicular cells surrounding the oocytes in the early stages of vitellogenesis.

Female *M. lunaris* have a pair of spermathecae which open to the exterior on the sternite of the sixth thoracic segment. The typical appearance of a full spermatheca is shown in Plate 5.3a . It is interesting to note that in all mature females examined, the spermathecae were invariably full.

In considering the macroscopic and histological appearances of the ovary, five reproductive phases may be recognized in female *M. lunaris*: immature, early developing, late developing, ripe and spent. These phases and the corresponding macroscopic and histological characteristics are summarized in Table 5.1. All females <29.0 mm FCW in size were found to have immature ovaries whilst prepuberty (29.0 - 39.9 mm FCW) and newly moulted postpuberty (>40.5mm) females have early developing ovaries. The only difference between the ovaries of prepuberty and newly moulted postpuberty females was the evidence of septa between groups of oocytes in the ovaries of the former. Late developing, ripe and spent ovaries were only found in sexually mature females.

Table 5.1   A summary of the reproductive phases of female *M. lunaris*.

Reproductive Phase	Size range and Sexual state	Macroscopic appearance and Histological stage of gonads
1. immature	immature females (<31.5 mm FCW)	ovaries: small, flaccid, fragile and transparent, contains large amounts of amorphous-like material, probably intersitial cell nuclei(?), no oogonia nor oocyte present, spermatheca is minute and appears undifferentiated.
2. early developing	prepuberty females (between 31.5-38.5 mm) newly molted postpuberty females (>40.5 mm FCW)	ovaries: thin, translucent or opaque, often white, lobes become apparent, contains a germ layer which occupies a large portion of the ovarian sections abundance of oogonia in the germ layer, few newly formed oocytes with deeply basophilic cytoplasm and no yolk vacuoles, cell size range: 1-50 μm, spermathecae of newly molted females: full.
3. late developing	postpuberty females (>40.7 mm FCW)	ovaries: enlarged, equal to the size of hepatopancreas, distinct lobulation in anterior branches, color ranges from yellow to orange, depending on the amount of yolk accumulation, central germ layer with oogonia is present, oocytes in varying stages of vitellogenesis, cell size range: 55-150 μm, follicular cells begin to form thin layers around yolked oocytes.
4. ripe	postpuberty females	ovaries: very large and distended, occupies the whole body cavity, covering the hepatopancreas, bright orange color, very thin ovarian wall, contains mature ova, >150 μm in size, follicular cells tightly packed around each ova.
5. spent	ovigerous females particularly those with stage I and III egg masses	ovaries: thin and flaccid, opaque, white or dull yellow, with few bright orange residual ova, in histological sections, demonstrate many empty spaces previously occupied by ova, follicular cells loosely outline these spaces, oocytes begin to occupy some of these spaces, few atretic ova are found, central germinative zone with oogonia is apparent.

5.3.2 Size at sexual maturity

In this study, the criterion for sexual maturity in males was the presence of mature sperms in the spermatic ducts of the testis, whilst in females, it was the presence of mature ova in the ovary. The size at sexual maturity based solely on gonad states, therefore, was expressed as the minimum carapace width at which mature gametes were observed in the gonads (*cf.* size at sexual maturity based on morphometric data, Chapter 1). The results of this study are summarized in Tables 5.2a, b. The smallest sexually mature male recorded in this study was 43.5 mm FCW, whilst the smallest sexually mature female was 41.0 mm FCW.

5.3.3 Annual reproductive cycle

The annual reproductive activity of male *M. lunaris* was estimated using monthly histological examinations of the gonads. The results of this study are summarized in Figure 5.2. From these results, there appears to be no distinct seasonality in the male reproductive cycle. Spermatogonia, spermatocytes and mature spermatozoa were consistently found in the lobes of the testis throughout the study period. Although there was a significant difference between months in the mean number of lobes containing spermatogonia (one-way ANOVA, $F=4.793$, d.f.$=13$, 119, $p<0.01$) and spermatids (one-way ANOVA, $F=2.365$, d.f.$=13$, 119, $p<0.05$), there was no significant difference between months in the mean number of lobes containing primary and secondary spermatocytes (one-way ANOVA, $F=1.340$, d.f.$=13$, 119, $p>0.05$) and mature spermatozoa (one-way ANOVA, $F=1.944$, d.f.$=13$, 119, $p>0.05$). The frequency of mature males with testis containing mature spermatozoa was relatively high

Table 5.2a The size at sexual maturity of male *M. lunaris* based
on the presence of mature sperms in the testis.

Carapace width (5-mm size classes)	Number of males with testis containing:	
	no mature sperms	mature sperms
25.0 - 29.9	5	0
30.0 - 34.9	6	0
35.0 - 39.9	5	0
40.0 - 44.9	3	2
45.0 - 49.9	3	4
50.0 - 54.9	1	5
55.0 - 59.9	0	5
60.0 - 64.9	0	5
65.1 - 69.9	0	3

Table 5.2b The size at sexual maturity of female *M. lunaris* based
the presence of mature ova in the ovaries.

Carapace width (5-mm size classes)	Number of females with ovaries containing:	
	no mature ova	mature ova
25.0 - 29.9	7	0
30.0 - 34.9	6	0
35.0 - 39.9	4	0
40.0 - 44.9	0	5
45.0 - 49.9	0	6
50.0 - 54.9	0	5
55.0 - 59.9	0	5
60.0 - 64.9	0	3

Figure 5.2

A summary of the monthly frequency distribution

of testicular cell types, expressed as the mean

percent of lobes containing each cell type,

using the equation:

$$\text{frequency} = \frac{\text{no. of lobes containing a cell type}}{\text{total no. of lobes examined}} \times 100$$

Vertical bars = 95% C.I.

Spermatogonia

Spermatocytes

MEAN FREQUENCY (% of total)

Spermatids

Spermatozoa

A M J J A S O N D J F M A M

CALENDAR MONTHS (1984-85)
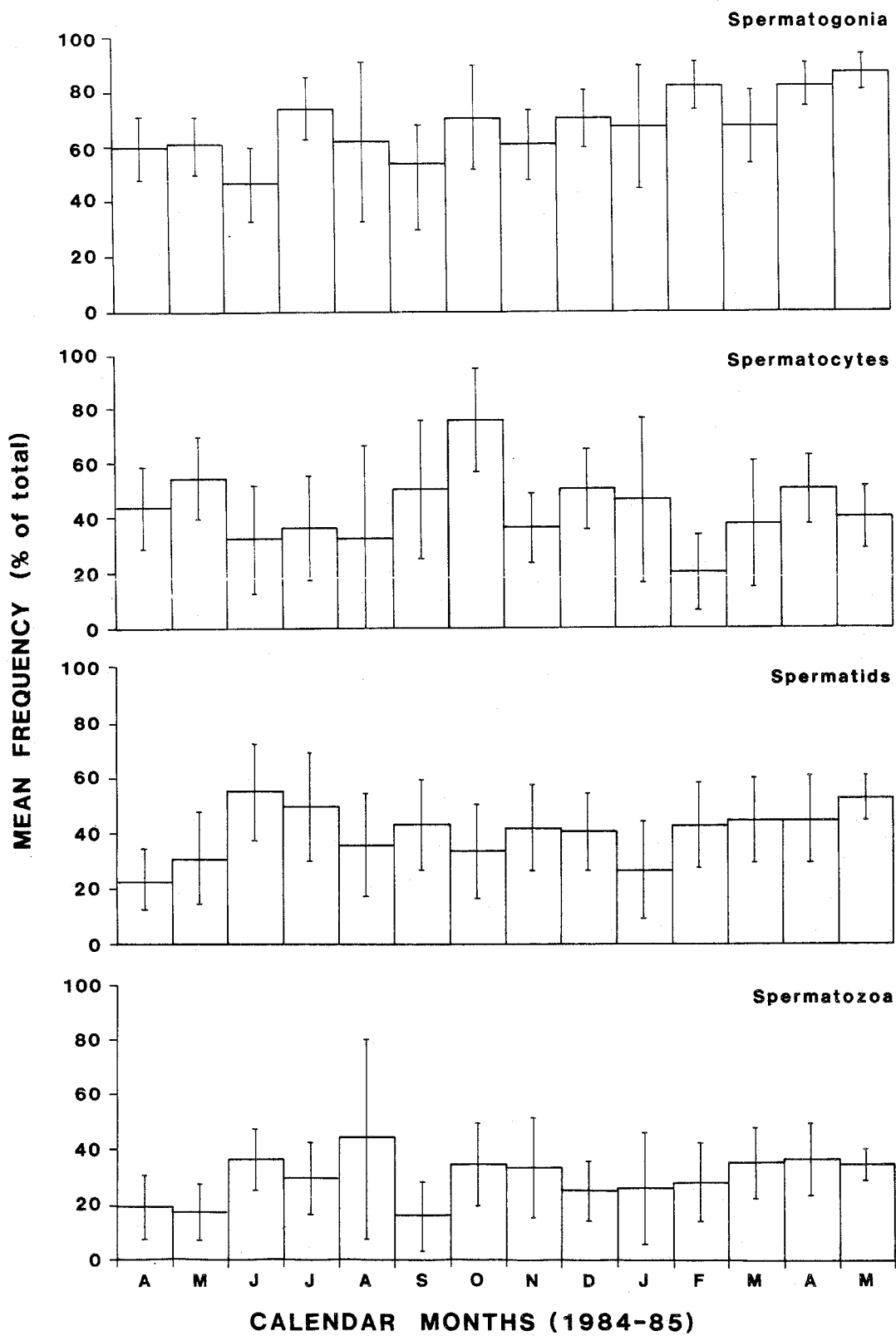
throughout the study period, ranging from 62.5% to 100% of the mature males in a sample and with a mean of 94.4% (±2.45 C.I.).

The temporal pattern of reproductive activity in female *M. lunaris* was determined using the gonad index method, histological analysis of the ovaries and the incidence of ovigerous females, mating pairs and females with ripe ovaries within the population. The results of this study are summarized in Figures 5.3 and 5.4A, B, C, D). As in male *M. lunaris*, there appears to be no strong seasonality in the female reproductive cycle. Marked ovarian activity was observed in most months of the year, with the single exception of December 1984 where the mean gonad index was relatively low and no mature ova nor females with ripe ovaries were recorded. The frequency of ovarian cells measuring 1-50μm varied significantly between months (one-way ANOVA, F=2.240, d.f.=13, 157, p<0.02) but the frequencies of oocytes measuring 51-100μm and 101-150μm did not (one-way ANOVA, F=0.915, d.f.=13, 157, p>0.05 and F=0.722, d.f.=13, 157, p>0.05, respectively). Although there was a significant difference between months in the mean gonad indices (one-way ANOVA, F=2.239, d.f.=13, 152, P<0.02) and in the frequency of mature ova in ovarian sections (one-way ANOVA, F=1.883, d.f.=13, 157, p<0.05), this appeared to be a result of the atypical data in December 1984, as there was no significant difference between the other months (Student-Newman-Keuls [SNK], p>0.05). The percentage of mature females with ripe ovaries also varied slightly throughout the year, with an increase in May and September 1984 and no female with ripe ovaries in December 1984. Ovigerous females were only recorded between August, 1984 and March, 1985, whilst mating pairs were found throughout most months of the year. In both

Figure 5.3

A summary of the monthly frequency distribution of

ovarian cell sizes, expressed as the mean percent

of the total number of cells examined in each

size class, using the equation:

$$\text{frequency} = \frac{\text{no. of cells in each size class}}{\text{total no. of cells examined}} \times 100$$

Vertical bars = 95% C.I.

CALENDAR MONTHS (1984-85)
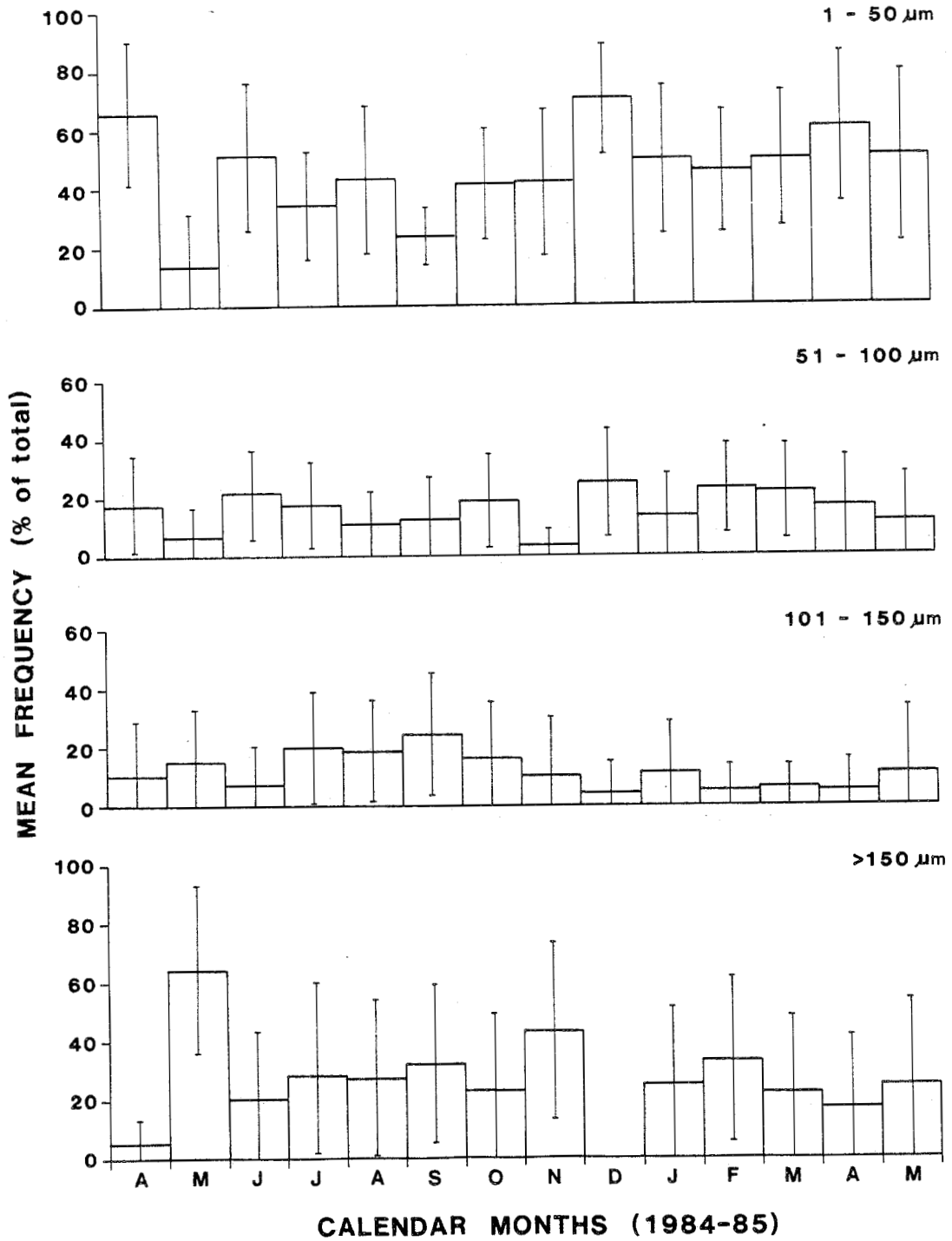
Figure 5.4

The annual reproductive activity of *M. lunaris*,

expressed in terms of the following:

5.4A   Mean gonad index ± 95% C.I.

$$\text{Gonad Index} = \frac{\text{gonad weight}}{\text{total weight}} \times 100$$

5.4B   Relative frequency of adult females with

ripe ovaries, expressed as a percent of

the total number of adult females

examined.

5.4C   Relative frequency of ovigerous females,

expressed as a percent of the total number

of adult females in the population.

5.4D   Relative frequency of mating pairs,

expressed as a percentage of the total

number of crabs caught.

A.

B.

C.

D.

CALENDAR MONTHS (1984-85)
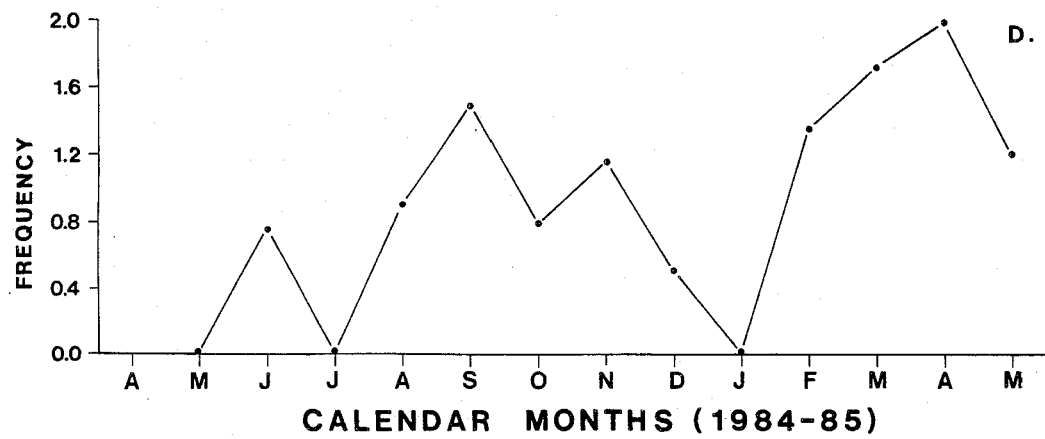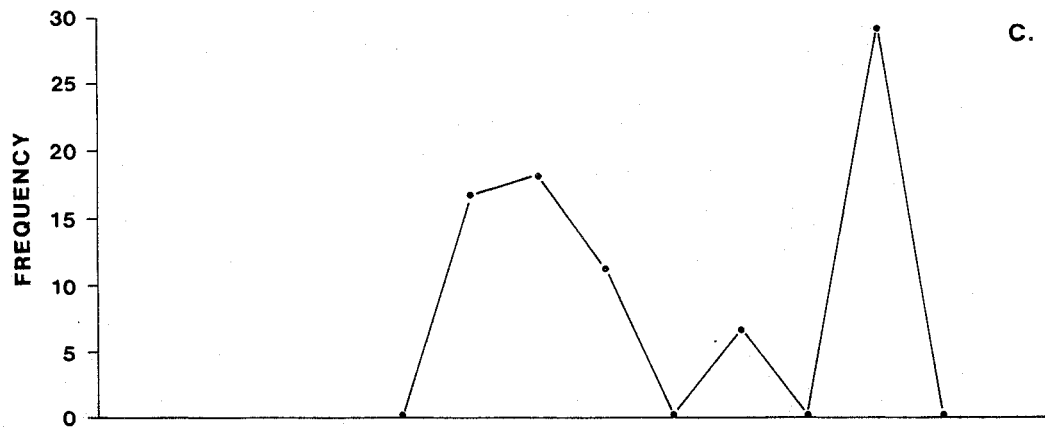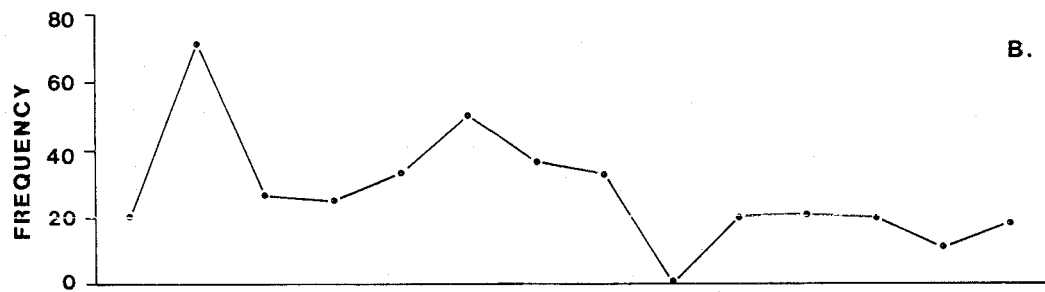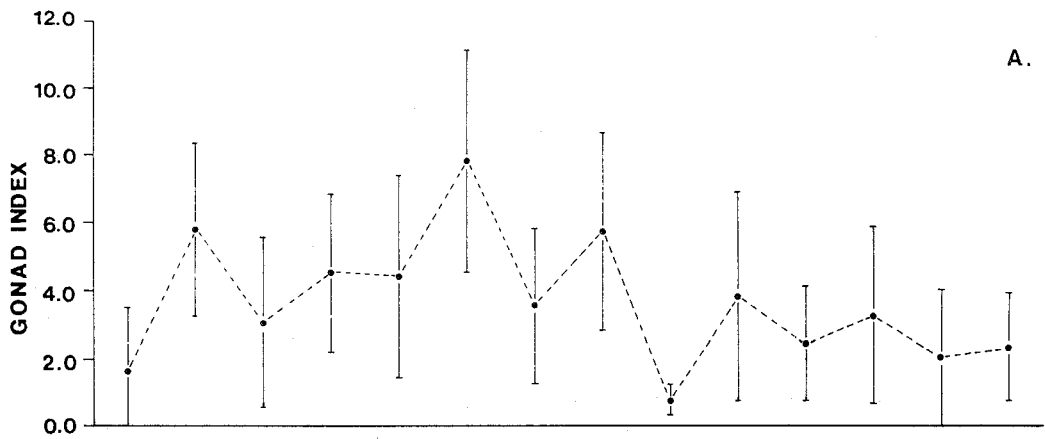
parameters, however, no distinct patterns were apparent.

In addition, there was no apparent relationship between the monthly mean gonad indices and either the number of ovigerous females or mating pairs in the population. These observations and the large degree of within-sample variability in the individual gonad indices and ovarian cell frequencies each month reflect the absence of reproductive synchrony among the individuals in the population.

5.3.4 Biology of ovigerous females

A total of 26 ovigerous females was collected from the study area between August, 1984 and March, 1985. The smallest egg-bearing female recorded in this study was 40.7 mm FCW in size. From these individuals, three stages of brood development were recognized, namely, stage I-newly laid, II-developing and III-mature. The results of this study, expressed separately for each of these stages are summarized in Table 5.3.

Histologically, it appears that mature female *M. lunaris* are capable of producing more than one brood. Marked ovarian activity was observed in most ovigerous females with the exception of those with ovaries in the 'spent' phase. In all individuals, oogonia were present in considerable numbers. All the females with stage II egg masses and 83.3% of females with stage III egg masses had ovaries exhibiting advanced stages of vitellogenesis (Table 5.3). In addition, all the ovigerous females in this study had spermathecae filled with sperms.

Table 5.3 A summary of results pertaining to the biology of ovigerous females.

Stage	I	II	III
A. Reproductive State			
No. of individuals examined	5	3	18
Minimum carapace width (mm)	43.5	41.9	40.7
Mean carapace width (mm)	46.1 ± 3.8	43.4 ± 3.9	46.5 ± 1.3
Mean body weight (g)	12.6 ± 1.6	10.7 ± 4.7	12.4 ± 1.0
Mean gonad index	1.55 ± 1.36	3.42 ± 2.79	3.46 ± 0.84
Mean % of ovarian cell stages			
1 — 50μm	55.2 ± 29.2	22.0 ± 15.5	36.3 ± 11.1
51 — 100μm	28.8 ± 29.2	8.3 ± 18.3	5.4 ± 6.8
101 — 150μm	14.6 ± 40.5	69.7 ± 29.6	56.9 ± 14.3
> 151μm	2.0 ± 5.6	0.0 ± 0.0	1.4 ± 2.6
% with full spermatheca	100	100	100
% exhibiting spent phase	60	0	17
% exhibiting advanced vitellogenesis	20	100	83
B. Reproductive Effort			
No. of individuals examined	4	2	4
Mean brood weight (g)	1.66 ± 0.42	1.21 ± 0.36	1.26 ± 0.47
Mean egg size (μm)	291 ± 2.08	279 ± 0.93	274 ± 3.19
Mean egg number	86990 ± 30837	44450 ± 41763	49270 ± 13756

all mean values with 95% C.I.

N.B. Stage I — newly laid, no cleavage, clear, bright orange yolk
      II — developing, eye pigment visible in some embryo, orange-brown or tan egg mass
      III — mature, zoea larvae visible with distinct pigmentation, black-brown egg mass

The number of eggs per brood ranged from 40,000 to 100,000 eggs with a mean of approximately 65,000 eggs. Although both the number of eggs per brood and brood weight did not vary significantly with carapace width ($r=-0.06$, $f=0.0310$, $p>0.05$ and $r=-0.08$, $f=0.0548$, $p>0.05$, respectively), a significant difference was found between the mean number of eggs per brood and the stage of development (one-way ANOVA, $f=9.542$, $d.f.=2$, 7, $p<0.05$). From the results in Table 5.3, it appears that the mean number of eggs per brood decreased markedly in stage II and III egg masses. This may, however, be a result of the restricted sample size of ovigerous females used in this study ($n=10$). Both the mean brood weight and egg diameter did not change markedly with development.

5.3.5 Notes on Reproductive Behaviour

The reproductive behaviour of *M. lunaris* can be divided into three stages: a) precopulatory attendance, b) copulation and c) postcopulatory attendance. These stages are summarized in Figure 5.6.

Precopulatory attendance in *M. lunaris* typically involves a postpuberty male grasping the chela of a prepuberty female (Plate 5.5). This has been observed in a total of 35 pairs of male and female *M. lunaris*, both in the field (27) and in the laboratory (8). The sizes of the individuals in these pairs are shown in Figure 5.5. There was no correlation between the size of the postpuberty male and that of the prepuberty female in a pair ($r=0.358$). There was also no apparent preference in the use of either the left or right chelae for grasping.

Plate 5.5

The typical position of *Matuta lunarts* in

precopulatory attendance.

a. The large post-puberty male grasping the

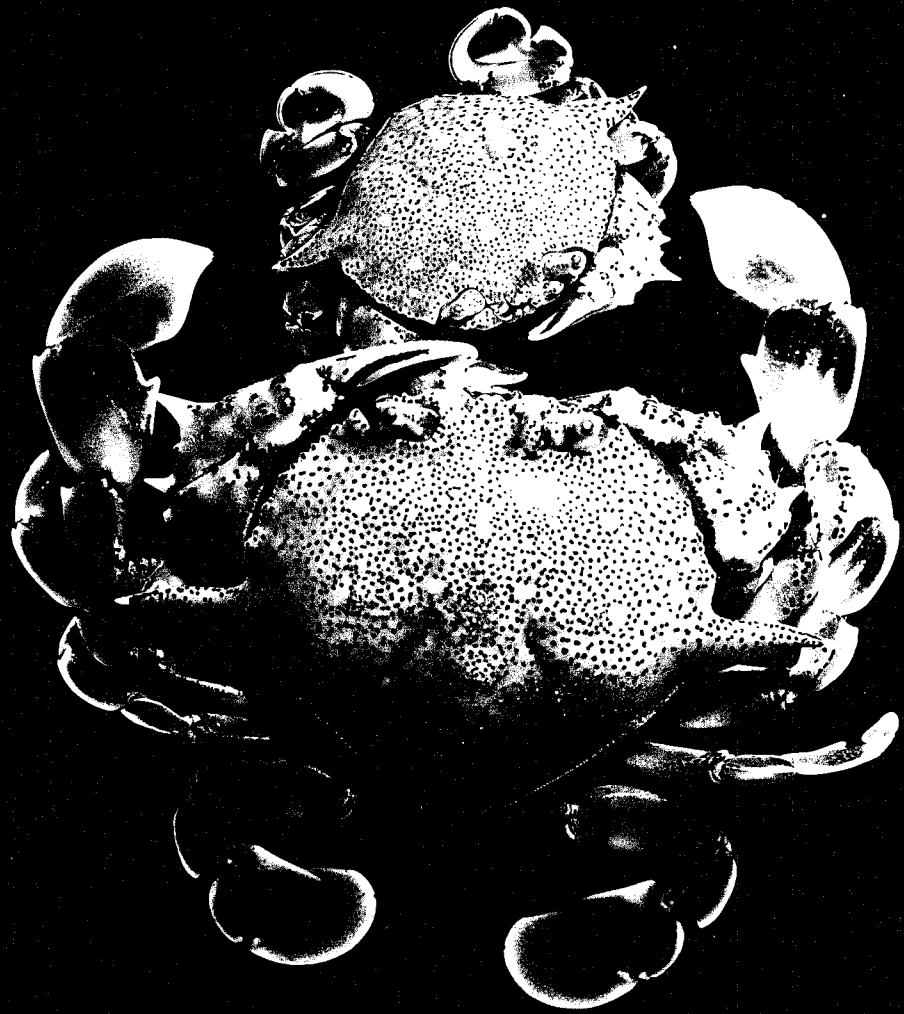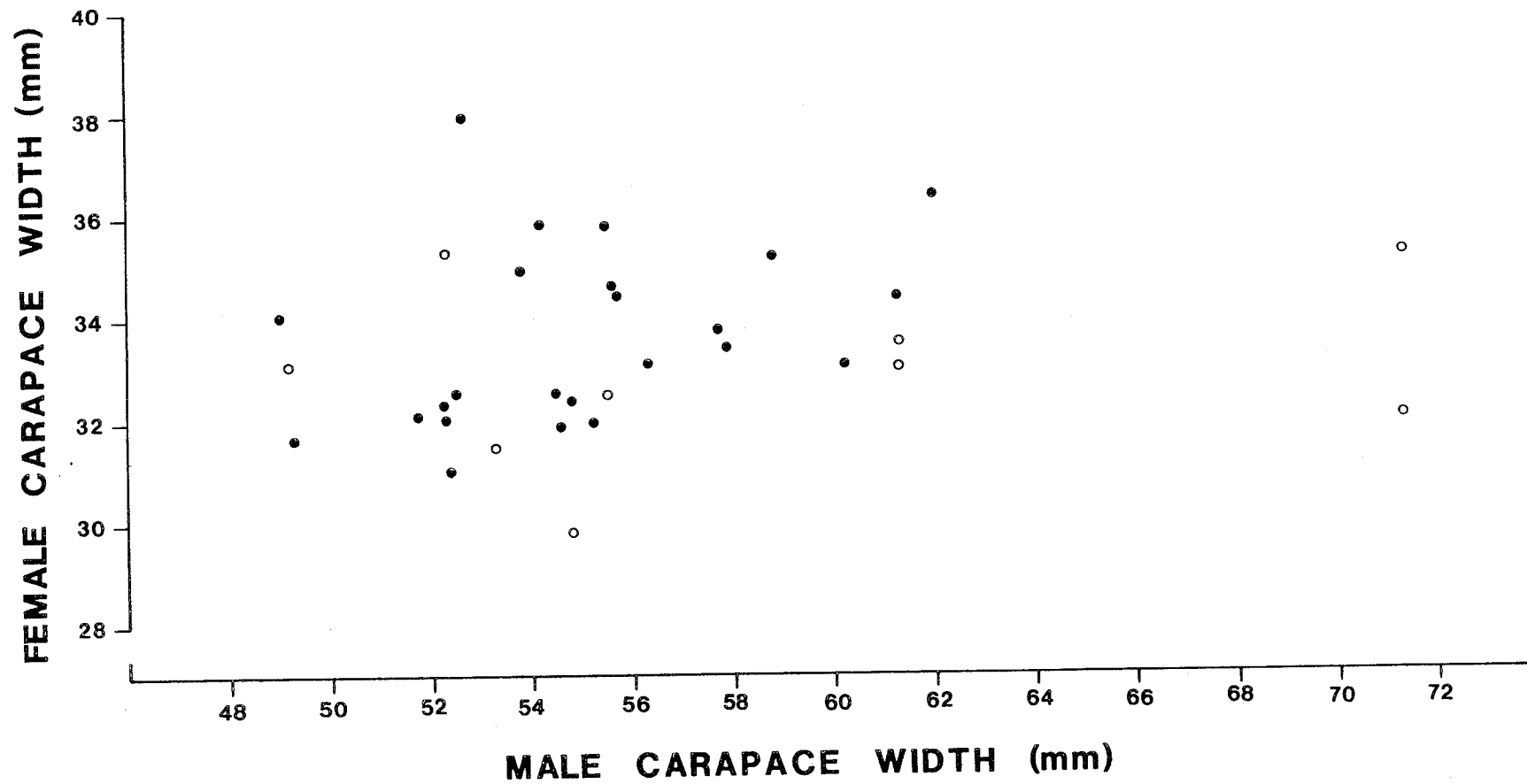chela of the small prepuberty female.

b. Detail of the grasping position.

Figure 5.5

A summary of the sizes of post-puberty males and

pre-puberty females in precopulatory attendance.

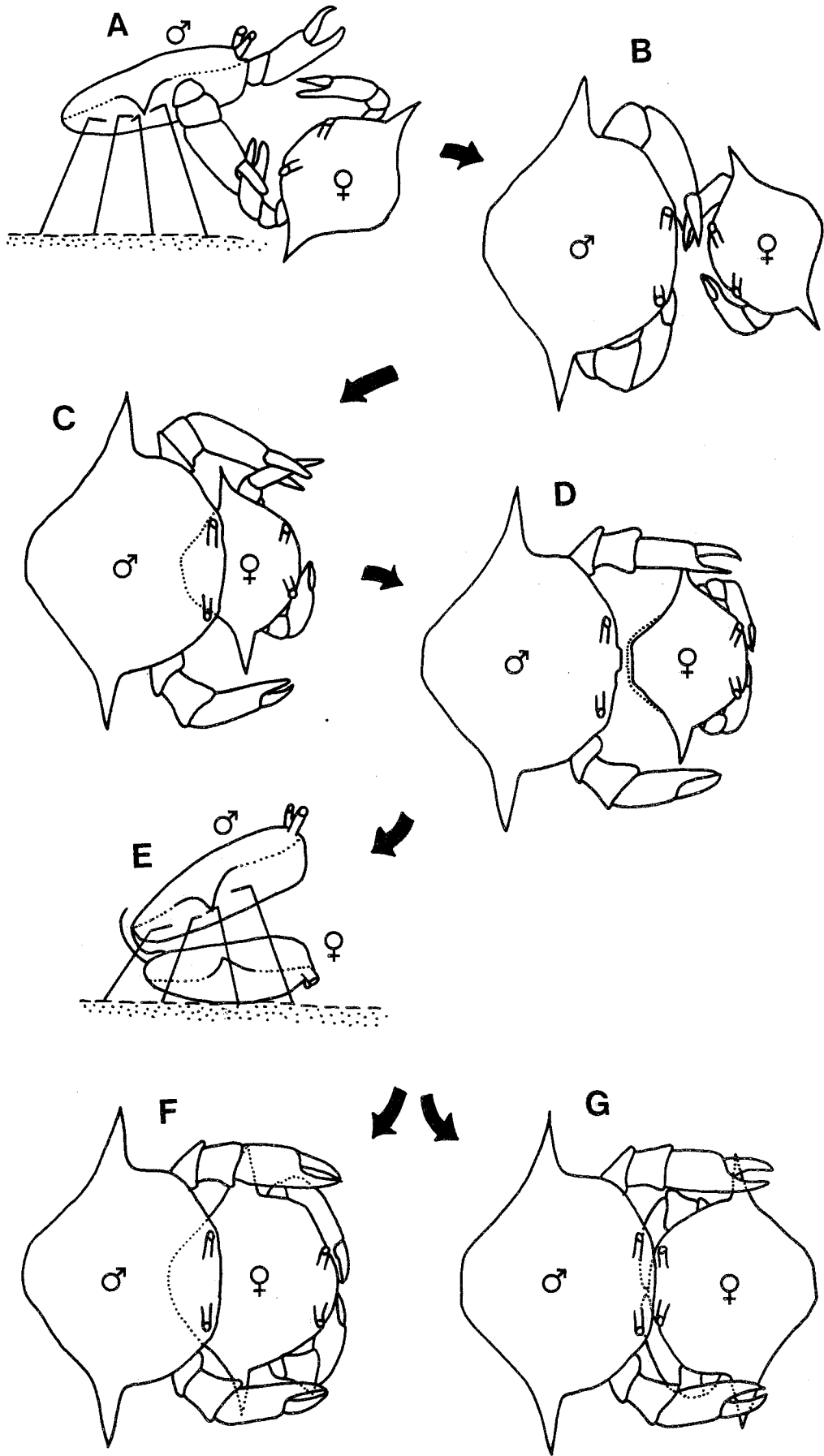● field observations

O laboratory observations

The following is an account of pair formation leading to precopulatory attendance, observed in the laboratory. These observations are based on five attempts at pair formation, two of which were successful. The postpuberty male approached the prepuberty female and grasped her chela using one of his chelae. The female showed signs of resistance and struggled to escape (in 3 out of the 5 observed cases, the female managed to escape). At this point, the male was observed to raise its body by extending his walking legs until only the tips were in contact with the substratum, and remained in this position whilst holding down the chela of the struggling female (Fig. 5.6A). This position was maintained until the female became subdued and a typical precopulatory position was assumed (Fig. 5.6B). Up to this point, the male was observed to alternate the use of either the left or right chelae in grasping the female. At one point, the male was observed to use both chelae at the same time. Once the precopulatory position was established, however, alternation in the use of the left or right chelae was no longer observed.

In the laboratory, the duration of precopulatory attendance ranged from 4 to 30 days with a mean of 15.4 days (± 8.1, 95% confidence limits, n=5). Of those mating pairs collected in the field and maintained in the laboratory, however, precopulatory attendance lasted from 1 to 18 days with a mean of 7.1 days (± 3.9, 95% confidence limits, n=14). Throughout this period, the male continued to grasp the chela of the female. The grasping pairs typically remained buried in the substratum, although, when disturbed, they would often swim around the aquarium in tandem. Most grasping pairs maintained in the laboratory fed until the day before

Figure 5.6

Schematic diagrams showing the sequence of
reproductive behaviour in *M. lunaris*.

A. Pair formation — the postpuberty male grasps
   the chela of a prepuberty female. The
   female struggles but the male raises
   its body and remains in this position
   until the female is subdued.

B. Precopulatory attendance — the male maintains
   its hold on the female's chela. This stage
   extends for several days until the female
   moults.

C. Shortly before female ecdysis, there is a
   change in grasping position, with the
   male grasping the female's chela
   from the rear.

D. Female ecdysis — the female undergoes its
   puberty moult whilst encircled by the
   male's chelae. The male may assist
   in the moulting process.

E. Copulation — shortly after female ecdysis,
   the pair assumes a sternum-to-sternum
   position with the male uppermost. The
   abdomen of the female overlaps that
   of the male. The pleopods of the
   male are inserted in the female's
   genital openings.

F. & G. Postcopulatory attendance — the
   female remains encircled by the male's
   chelae for several hours following
   copulation.

A ♂ ♀

B ♂ ♀

C ♂ ♀

D ♂ ♀

E ♂ ♀

F ♂ ♀

G ♂ ♀

copulation.

The following is an account of the copulatory and postcopulatory behaviour in *M. lunaris*, based on laboratory observations of two copulating pairs on two separate occasions. In both cases, copulation occurred between a hard-shelled male and a soft, recently moulted female. In both observed cases, moulting and copulation occurred late in the evening. The sequence of events was as follows:

1) after several days of precopulatory grasping, the male changed its position and grasped the female by its chelae from the rear (Fig. 5.6C).

2) shortly thereafter, the male released the female but continued to encircle her with his chelae (Fig. 5.6D).

3) the female then moulted whilst completely encircled by the male's chelae, with the male occasionally assisting in the moulting process by pushing or lifting off the female's old carapace using his chelae.

4) copulation occurred shortly after the female completed her moult (approximately within one hour), in a sternum to sternum position with the male uppermost. In this position, the abdomen of the female was extended and overlapped that of the male (Fig. 5.6E). The male's pleopods were inserted into the female's genital openings.

5) after copulation, the female assumed a normal upright position, *i.e.* with the sternum against the substratum, whilst remaining completely encircled by the male's chelae, either facing towards or away from the male (Figs. 5.6F, G).

Postcopulatory attendance in this position was maintained for at least 3 hours and, in both cases, had ceased within 13 hours of copulation.

Pairing and copulation was also observed in aquaria between a male *M. granulosa* and a female *M. lunaris*. In this pair, the reproductive behaviour followed essentially the same sequence as described above.

In this study, male polygamy was observed in *M. lunaris* maintained in aquaria. On numerous occasions, postpuberty males formed precopulatory pairs with successive prepuberty females. Successful precopulatory attendance with subsequent copulation, however, was only observed in 2 postpuberty males: one, 3 times within 40 days and the other, twice within 9 days. When dissected, all the females involved possessed distended spermathecae.

5.4 Discussion

Reproduction in most crustaceans follows a fixed sequence of events. These are: 1) activation of gametogenesis, 2) gamete production, 3) mating and pair formation and 4) copulation, 5) ovulation, 6) oviposition and 7) brood incubation. The ecological aspects of these events have been reviewed by Sastry (1983) and in Brachyura, in particular, by Hartnoll (in press). In both reviews, considerable variation in the chronology, timing and duration of these events have been reported between different species and between individuals or populations of the same species (in: Sastry, 1983). These variations constitute the reproductive pattern which is characteristic of a particular species in a given environment.

The results of this study show that reproduction in *M. lunaris* follows the typical sequence of crustacean reproductive events. The different aspects of reproductive biology considered in this study will, therefore, be discussed in relation to these events.

5.4.1 Reproduction in individual *M. lunaris*.

The general structure of the reproductive organs in *M. lunaris* is similar to those described in other brachyuran species, such as *Callinectes sapidus* (Cronin, 1947), *Portunus sanguinolentus* (Ryan, 1967a, b) and *Chionoecetes opilio* (Kon & Honma, 1970a, b). There were only a few dissimilarities which were apparent from the histological sections. In *P. sanguinolentus*, for example, oogonial cells were not found in the lobes of the ovary but were contained in a central hollow shaft running through the length of the ovary (Ryan, 1967b). This was not observed in the ovaries of female *M. lunaris* as oogonial cells were found in a central germinative layer which extended the length of the ovary including the lobes of the anterior branches.

In the testis of male *M. lunaris*, the spermatic duct does not serve as a central axis around which the lobes are located, as found in *P. sanguinolentus* (Ryan, 1967a). In this study, the spermatic ducts were found in the lateral border of the lobes furthest from the central axis and was observed only in some sections. This is similar to the form in *C. sapidus* (Cronin, 1947), wherein the spermatic duct does not have a central location and only feeds directly into some lobes. The complete route of the spermatic duct and the physiology of the testis lobe in brachyurans, however, remains unclear as there have been few studies of spermatogenesis in

brachyurans *per se*.

The activation of gametogenesis in *M. lunaris* begins in the prepuberty phase or instars of the two sexes. In males, this was marked by a proliferation of the spermatogonial cells in the testis of prepuberty individuals, whilst in females, it is marked by the development of the germinative layer and the occasional presence of newly formed oocytes in the ovary. In both sexes, however, gametogenesis does not proceed to maturity until after the puberty moult. This pattern of gonad maturation has also been reported in several species of spider crabs (Hartnoll, 1963), female *P. sanguinolentus* (Ryan, 1967b), *Corystes cassivelaunus* (Hartnoll, 1972), *C. sapidus* (Johnson, 1980) and *Trapezia ferruginea* (Finney & Abele, 1981). The physiological and ecological advantages of gonadal development in final prepuberty instars is unknown, although it has been suggested that it may be an induced side-effect of the hormonal changes that bring about the puberty moult (Hartnoll, in press, in: Sastry, 1983).

The puberty moult in *M. lunaris* occurs at different size ranges in the two sexes, resulting in considerable variation between the sexes in the sizes of sexually mature individuals. In males, the puberty moult occurs approximately between 43.0 to 50.0 mm FCW, whereas in females, it is approximately between 37.0 to 40.0 mm FCW. The significance of the puberty moult and the size variation between adult individuals is discussed in relation to relative and absolute growth patterns in Chapters 3 and 6, respectively. In relation to reproduction, however, the puberty moult is particularly significant in two ways: 1) it marks the onset of gonad maturity for both sexes

and most importantly, 2) it is associated with distinct behavioural patterns associated with reproduction.

The mating behaviour of *M. lunaris* follows the typical brachyuran pattern involving a hard-shelled male and a soft-shelled female. This pattern has often been reported in the Cancridae and the Portunidae (*e.g.* Edwards, 1966, Ryan, 1967b, Elner & Stasko, 1978, Du Preez & McLachlan, 1984c, review by Hartnoll, 1969), although it has also been found to occur in some species belonging to the other brachyuran families, such as the Grapsidae (Hiatt, 1948) and the Majidae (Hartnoll, 1963 and Watson, 1970, 1972). In all these species, the mating process is prolonged and often involves pre- and post-copulatory attendance of the female by the male (Hartnoll, 1969), as in *M. lunaris*.

The position of the individuals during the precopulatory period varies between species, although in most cases, it involves the male holding the female under his sternum (*e.g.* Berrill & Arsenault, 1982 and Elner *et al.*, 1985, see also Hartnoll, 1969). *M. lunaris*, however, is unique in that the male grasps the prepuberty female by her chelae using either his left or right chelae and carries her around in this position. In this position, the female remains relatively free to feed, swim and bury under the substratum using her other appendages. This grasping position appears to be a unique characteristic of the genus *Matuta* as it was also observed between a male *M. granulosa* and a prepuberty female *M. lunaris*. The only published report of precopulatory grasping in brachyurans was in the spider crab, *C. opilio* (Watson, 1972), where the male grasps the walking legs of the female.

The duration of precopulatory attendance in *M. lunaris* ranged from 1 to 30 days with a mean of 15.4 days (± 8.1) for those individuals which paired in the laboratory. This was comparable with observations of cancrid species (*e.g.* Edwards, 1966, Elner & Stasko, 1978, Berrill & Arsenault, 1982 and Elner *et al.*, 1985), although in other brachyuran species such as *Ovalipes punctatus* (Du Preez & McLachlan, 1984c, review by Hartnoll, 1969), the duration of precopulatory attendance were considerably shorter (*e.g.* 10 to 20 seconds in *O. punctatus* [Du Preez & McLachlan, 1984c]).

Copulation in *M. lunaris* occurs immediately after the female puberty moult and involves a male superior sternum-to-sternum position. This position is similar to that observed in *C. opilio* (Watson, 1972) and several species in the Cancridae (Hartnoll, 1969), including *Carcinus maenas* (Berrill & Arsenault, 1982) and *Cancer borealis* (Elner *et al.*, 1985). In considering these species, there appears to be a degree of correlation between the copulatory position and the state of the female at the time of mating. It has been suggested that the position adopted during copulation depends upon the strength and activity of the female, as in species where the female is soft and vulnerable during copulation, there is a tendency for males to be uppermost (Hartnoll, 1969).

The duration of copulation in *M. lunaris* was approximately 5 to 30 minutes, after which the male releases the female. Post-copulatory attendance was of a relatively short duration when compared to the precopulatory attendance. Unlike most species where the post-copulatory position is similar to the precopulatory one (*e.g.* *C. maenas* [Berrill & Arsenault, 1982] and *Cancer magister*

[Snow & Nielsen, 1966]), male *M. lunaris* no longer grasped the chelae of the newly moulted female. A post-copulatory position similar to that in *M. lunaris* has also been observed in *C. opilio* (Watson, 1972).

It is not known why in some species, including *M. lunaris*, mating is restricted to the period immediately after female ecdysis, although it has been suggested that it may be related to the shape of the female genital duct (Hartnoll, 1969) or in some species such as *Corystes casstvelaunus* (Hartnoll, 1968), to the presence of a rigid operculum in the opening of the vulvae which only becomes flexible for a short period after ecdysis. In general, however, the mating behaviour which involves the male attending to the female before and after ecdysis, has considerable survival value to the species. As a crab is exceptionally vulnerable to predation during ecdysis and while in a soft-shelled state, it would be expected to seek shelter or conceal itself during these periods. For a female undergoing a puberty moult, however, this would reduce the chances of a male finding her or vice-versa. Pair formation between a hard-shelled male and a prepuberty female, therefore, could be beneficial to both sexes in several ways. Firstly, it ensures the presence of a male at the exact time when the female is capable of copulation. Secondly, it improves the chances of the female surviving the vulnerable moulting period thus insuring the male's genetic investment against the death of the female from predation and cannibalism. And finally, as observed in lobsters and isopods (Parker, 1974, Manning, 1975 and Atema *et al.*, 1979), it probably ensures that the male in attendance is the sole parent of a female's brood. These advantages may also be of adaptive value in the

environment in which the species is found (Sastry, 1983). In considering the surf zone environment of *M. lunaris*, therefore, it is possible that similar advantages may be gained from pre- and post-copulatory attendance and that the specific problems in this region may account for some of the unusual aspects of the mating behaviour of *M. lunaris*.

The results of this study show that female *M. lunaris* do not ovulate immediately after copulation, unlike some majids such as *C. opilio* (Watson, 1970, 1972) and *Inachus* sp. (Hartnoll, 1963) where the females generally laid eggs within 24 hours of mating. The ovaries of newly-moulted females immediately after copulation are essentially at an early developing phase similar to the ovaries of the prepuberty instars. The spermathecae are, however, engorged with sperms. From the preliminary investigation of gonad development in the laboratory, it appears that several weeks are required for oocyte growth prior to ovulation and oviposition. This period is primarily characterized by intensive oocyte production and vitellogenesis. These observations are comparable with those recorded in several brachyuran species such as *C. pagurus* (Edwards, 1966), *P. sanguinolentus* (Ryan, 1967b) and *C. sapidus* (Johnson, 1980).

Although none of the females which copulated in the laboratory were kept long enough to observe ovulation and oviposition, histological analysis of the ovaries of ovigerous females from the field indicate that at each ovulation, most of the mature ova are discharged and the ovaries undergo a 'spent' phase. This is often characterized by the migration of newly formed oocytes into the

lumen of the ovary, probably for growth for the next ovulation.

There is evidence to suggest that female *M. lunaris* are capable of producing more than one batch of eggs from a single copulatory event. Firstly, the spermathecae of all ovigerous females examined in this study were found to be full of sperm even after the extrusion of the previous egg batch. Sperm storage and retention in brachyurans is not unusual and has been reported in several species including *Chionoecetes bairdii* (Adams & Paul, 1983), *C. sapidus* (Tagatz, 1968), *Halicarcinus australis* (Lucas & Hodgkin, 1970) and *P. sanguinolentus* (Ryan, 1967b). In all cases, the females were observed to produce multiple egg batches within the breeding period without subsequent copulation. It has been suggested that the amount of sperms remaining in the spermathecae of ovigerous females were sufficient to fertilize these additional egg batches (Adams & Paul, 1983). Secondly, marked ovarian activity characterized by high gonad indices and advanced stages of vitellogenesis was observed in most ovigerous females. There was a characteristic proliferation of oogonia and newly formed oocytes into the empty spaces previously occupied by ova in the ovaries of those individuals in the 'spent' phase. The occurrence of ripening ovaries simultaneous with brooded eggs has been reported in several species of hermit crabs (Ameyaw-Akumfi, 1973 and Varadarajan & Subramoniam, 1982), *Portunus pelagicus* (Pillay & Nair, 1971) and *P. sanguinolentus* (Ryan, 1967b) and has been interpreted as an indication of the potential to produce a second brood during the same breeding season (Pillay & Nair, 1971). In the present study, both lines of evidence described above strongly suggest that female *M. lunaris* produce at least two egg batches during a single breeding

season.

In the present study, the number of eggs produced per egg batch by *M. lunaris* varied widely, ranging from 40,000 to 100,000 eggs with an approximate mean of 65,000 eggs. This is notably higher than the estimated number of eggs per brood recorded from *M. lunaris* in India (*cf.* 1100 - 2580 eggs, Pillay & Nair, 1976). The mean egg diameter, however, was comparable with those recorded by Rajabai (1959) and Pillay & Nair (1976). These observations are consistent with the general observations of Bliss (1968) that swimming crabs and lower intertidal crabs produce a high number of small eggs compared to other species of brachyurans. It is also interesting to note that, in this study, the mean number of eggs per brood decreased markedly in stage II and III egg masses. Whether this is related to egg mortality during incubation, asynchronous hatching or other factors such as underestimation resulting from changes in egg volume, is unclear and requires further investigation.

5.4.2 The annual reproductive cycle

In most studies of crustacean reproduction, the qualitative and quantitative investigation of the reproductive events, *i.e.* gamete production, reproductive behaviour, oviposition and brood incubation, often provides information on the period and amplitude of the reproductive activity in a population (*e.g.* Pillay & Nair, 1971, Nye, 1977, Pillay & Ono, 1978, Swartz, 1978, Jones, 1980 and Du Preez & McLachlan, 1984c) with the temporal pattern of the reproductive activity being reflective of the reproductive cycle of the species (*e.g.* Cox & Dudley, 1968, Kon & Honma, 1970a, b; reviewed by Sastry, 1983). In this study, the annual reproductive

cycle of *M. lunaris* was investigated using both qualitative and quantitative observations of male and female gonadal activity, in addition to the incidence of ovigerous females and mating pairs in the population.

The results of this study show that the annual reproductive cycle of *M. lunaris* is primarily of a continuous nature, *i.e.* uninterrupted breeding throughout the year. This is evidenced by the following:

For males,

1) spermatogonia, spermatocytes, spermatids and mature spermatozoa were consistently found in the lobes of the testis throughout the study period with no significant difference between months in the mean number of lobes containing a particular cell stage, and

2) the frequency of mature males with testis containing mature spermatozoa remained relatively high throughout the year.

For females,

1) marked ovarian activity was observed in most months of the year with no apparent patterns of seasonality in the fluctuations of the gonad indices,

2) mature females with ripe ovaries were found in 13 out of 14 months during the study period, and

3) mating pairs were found in most months of the year with no distinct pattern of seasonality in their occurrence.

It is interesting to note that ovigerous females were only found between the months of August, 1984 and March, 1985. The relatively low catch rate of ovigerous females in this study, however, limits the use of this parameter in assessing the reproductive cycle of

M. lunarts. It is possible that the low catch rate of ovigerous females may be affected by changes in their behaviour, *t.e.* off-shore migration similar to *Scylla serrata* (Hill, 1975) and *C. sapidus* (Steele, 1979), thereby affecting their catchability in the surf zone or by lunar periodicities in their reproductive activity. These aspects, however, were not considered in this study and therefore require investigation.

Although it was generally believed in the past that tropical invertebrate species breed continuously throughout the year (Semper, 1881 in: Pillay & Nair, 1971, Stephenson, 1934 and Giese & Pearse, 1974), recent studies have reported seasonal periodicities in the reproductive cycles of many tropical groups such as corals (*e.g.* Harrison *et al.*, 1984, Babcock *et al.*, in press), polychaetes (*e.g.* Caspers, 1984), echinoderms (*e.g.* Holland, 1981 and Kubota, 1981) and molluscs (*e.g.* Nagabhushanam & Deshpande, 1982 and Braley, 1982). Among the crustaceans, several tropical species have also been reported to demonstrate seasonal reproduction, for example, *Panulirus argus* (Kanciruk & Herrnkind, 1976), *Crypotodromia hilgendorft* (McLay, 1982), *S. serrata* (Hill, 1975) and *P. pelagicus* (Potter *et al.*, 1983). A great majority of the tropical crustacean species, however, continue to be reported to breed for an extended period or even continuously throughout the year, including most species of anomurans (Ahmed & Mustaquim, 1974, Ameyaw-Akumfi, 1975, Subramoniam, 1979 and Varadarajan & Subramoniam, 1982) and brachyurans (Ryan, 1967b, Pillay & Nair, 1976, Du Preez & McLachlan, 1984c and Gotelli *et al.*, 1985, reviewed by Sastry, 1983). In considering the results of this study, therefore, *M. lunarts* appears to be a typical tropical brachyuran species in that it demonstrates

a continuous and almost uninterrupted reproductive pattern.

There exists a substantial literature dealing with the proximate and ultimate factors influencing the reproductive cycles of marine invertebrates. These have been reviewed extensively in invertebrates by Giese & Pearse (1974) and in Crustacea, by Sastry (1983). The determinant factors of a continuous reproductive cycle are unclear. It has been suggested that it is generally a response to environmental conditions which do not fluctuate markedly throughout the year (Sastry, 1983), for example, in some areas of the tropics and in the deep sea. In such areas where there is little or no seasonal variations in environmental condition, there would be little if any selective pressure favouring reproduction at one time of the year (Giese & Pearse, 1974). Upon close examination, however, the reproductive activities of most continuously breeding tropical species are often not of the same intensity throughout the year, and rather show periods of more intense reproduction. Examples of such species include the hermit crab, *Clibanarius clibanarius* (Varadarajan & Subramoniam, 1982) and such brachyurans as *P. sanguinolentus* (Ryan, 1967b) and *O. punctatus* (Du Preez & McLachlan, 1984c). In this respect, *M. lunaris* is unusual in that the fluctuations in its reproductive intensity are slight and show no significant patterns of peak activity.

The environmental factors which affect the reproduction of *M. lunaris* are beyond the scope of the present study and it is difficult to form conclusive statements as to the environmental cues which control the initiation and duration of its reproductive cycle. It is interesting to note, however, that whilst, in this study,

M. lunarts from Pallarenda beach reproduces continuously throughout the year, the breeding pattern of *M. lunarts* in India as reported by Pillay & Nair (1976), is seasonal. Such intraspecific variability in the reproductive patterns of geographically separate populations of a single species has also been observed in other brachyurans, such as *P. pelagicus* (Rahaman, 1967, Ryan, 1967b and Pillay & Nair, 1971) and *Helice crassa* (Nye, 1977 and Jones, 1980) and have often been explained in terms of geographic differences in temperature, salinity and food availability. These observations support the theory of Sastry (1983) that the reproductive cycle of an organism is a genotypic response to the environment. According to Sastry (1983), the environment is an important factor in determining the reproductive pattern of a population but the extent of such environmental influence is limited by the genetically predetermined character traits of the species.

In conclusion, therefore, the reproductive pattern of *M. lunarts* is typical of a tropical brachyuran species. It is characterized by the following trends: 1) a year-round production of gametes, 2) asynchrony in gonad development between individuals, 3) an almost year-round occurrence of ovigerous females, 4) aseasonal mating activity, 5) multiple egg batches per female, 6) relatively small egg sizes and 7) high numbers of eggs per brood.

CHAPTER SIX

Absolute Growth

6.1 Introduction

There have been numerous studies of absolute growth in crustaceans, particularly brachyurans (*e.g.* Hartnoll, 1965b, Poole, 1967, Bennett, 1974, Klein Breteler, 1975a, b Hogarth, 1975, Haefner, 1977 and Du Preez & McLachlan, 1984b; reviews by Kurata, 1962 and Hartnoll, 1982). In most cases, however, some degree of difficulty has been experienced because of the discontinuous nature of crustacean growth. These include: 1) difficulty in recovering exuviae together with newly moulted individuals in the field, 2) loss of tags during ecdysis and 3) distinguishing post-larval instars. The limitations of growth studies in crustaceans have been discussed by Hartnoll (1982). These problems in crustacean research are appropriately summarized by Wilder (1953): "with an animal...which has neither scales, otoliths, vertebrae, nor fin rays, which at each moult loses all hard parts...and which over the size range normally captured does not fall into recognizable size or age groups, the estimation of growth and age is particularly difficult".

In the Matutinae, particularly *Matuta lunaris*, there have been no published studies of absolute growth. Although the larval development of *M. lunaris* has been described (Rajabai, 1959), information on postlarval growth is lacking. This study, therefore, is a preliminary investigation of postlarval growth and moult stages in *M. lunaris*.

6.2 Materials and methods

There are two aspects in the study of absolute growth in crustaceans: 1) the study of the increase in size during ecdysis—the moult increment, and 2) the study of the intervals separating successive moults—the intermoult period. These two aspects are essentially discrete and often exhibit very different responses to intrinsic and extrinsic factors (Hartnoll, 1982). Moult increment data may be obtained using the following methods: 1) collection of exuviae in the field together with the newly moulted individuals (Hiatt, 1948), 2) analysis of the modes of a size frequency histogram (Klein Breteler, 1975a, Childress & Price, 1978 and Diaz, 1980), 3) tagging experiments (Bennett, 1974 and Hill, 1975) and 4) monitoring of growth in captivity (Turoboyski, 1973, McLay, 1982 and Du Preez & McLachlan, 1984b). Data on intermoult periods may be obtained using the following methods: 1) tagging experiments (Bennett, 1974), 2) determination of the proportion of moulting crabs in a field population and correlating the data with observations of captive specimens (Warner, 1967) and 3) observation of multiple moults in captivity (Turoboyski, 1973 and McLay, 1982). These methods have been reviewed extensively by Hartnoll (1982).

In this study, no tagging experiments were undertaken as no suitable tagging technique was available and the extremely large population and variable catch rate could not ensure adequate recovery of tagged individuals. The use of growth data from captive specimens was avoided in order to eliminate the effects of captivity on moult increments and intermoult period as have been found in

other crustacean growth studies (e.g. Hiatt, 1948 and Childress & Price, 1978). In addition, of the crabs maintained in the laboratory, no crab moulted more than once during the study period, thus intermoult periods could not be followed. Consequently, the present investigation of growth in *M. lunaris* will be based mainly on one aspect of absolute growth, that is, the moult increment.

The data used in this study were obtained using the methods described below. Firstly, a size frequency histogram based on 2-mm full carapace width (FCW) size classes was generated for each sex, based on the size measurements of all individuals used in the morphometric, feeding, reproduction and population aspects of this study (see Chapter 2 for details pertaining to collection methods and study sites). Moult increment data were then calculated from the modes of the histograms. In addition, the sex ratio within 5 mm FCW size classes was calculated from the same data set. Secondly, newly moulted individuals were collected together with their exuviae in the field and within 5 days of capture, in the laboratory. The pre-moult and post-moult sizes were measured and the moult increments determined.

There are several methods of analysing crustacean moult increment data. In this study, two methods were employed:

1) A Hiatt diagram of pre-moult and post-moult sizes was constructed and fitted by a linear regression, following Kurata (1962). The relationship between pre-moult and post-moult sizes was then expressed as: $L_{n+1} = a + bL_n$, where L_n is the pre-moult size and L_{n+1} is the post-moult size. A t-test was used to determine the significant deviation of the

slope (b) from a standard of 1, following Bennett (1974).

Kurata (1962) referred to b as the growth coefficient and

defined three patterns of growth on the basis of its value:

$\qquad$ b > 1 progressive geometric growth

$\qquad$ b = 1 arithmetic growth

$\qquad$ b < 1 retrogressive geometric growth

The theoretical considerations of the Hiatt diagram have been

discussed by Mauchline (1976) and Hartnoll (1982).

2) The relative or percent increment was calculated using the

following equation, based on Mauchline (1976) and Hartnoll

(1982):

$$\text{relative increment (\%)} = \frac{\text{postmoult size} - \text{premoult size}}{\text{premoult size}} \times 100$$

The relationship between percentage increment and premoult

size was subsequently expressed as the linear regression of

log percent increment on premoult size, following Hartnoll

(1980).

6.3 Results

6.3.1 Size Frequency Distribution and Sex Ratio

The size frequencies of male and female *M. lunaris* measured in

this study are shown in Figure 6.1. Considerable differences

between male and female size distributions are apparent. From the

histogram of females, there are three discrete modes which appear to

correspond to moult stages or instars. No such modes are

distinguishable from the histogram of males. In both sexes, the

puberty moult is represented by a well defined gap in the histogram,

although the size classes at which this gap occurred varied between

Figure 6.1

    The size distribution, based on 2-mm size classes,

    of all the individuals measured in this study

    from January, 1984 to May, 1985.
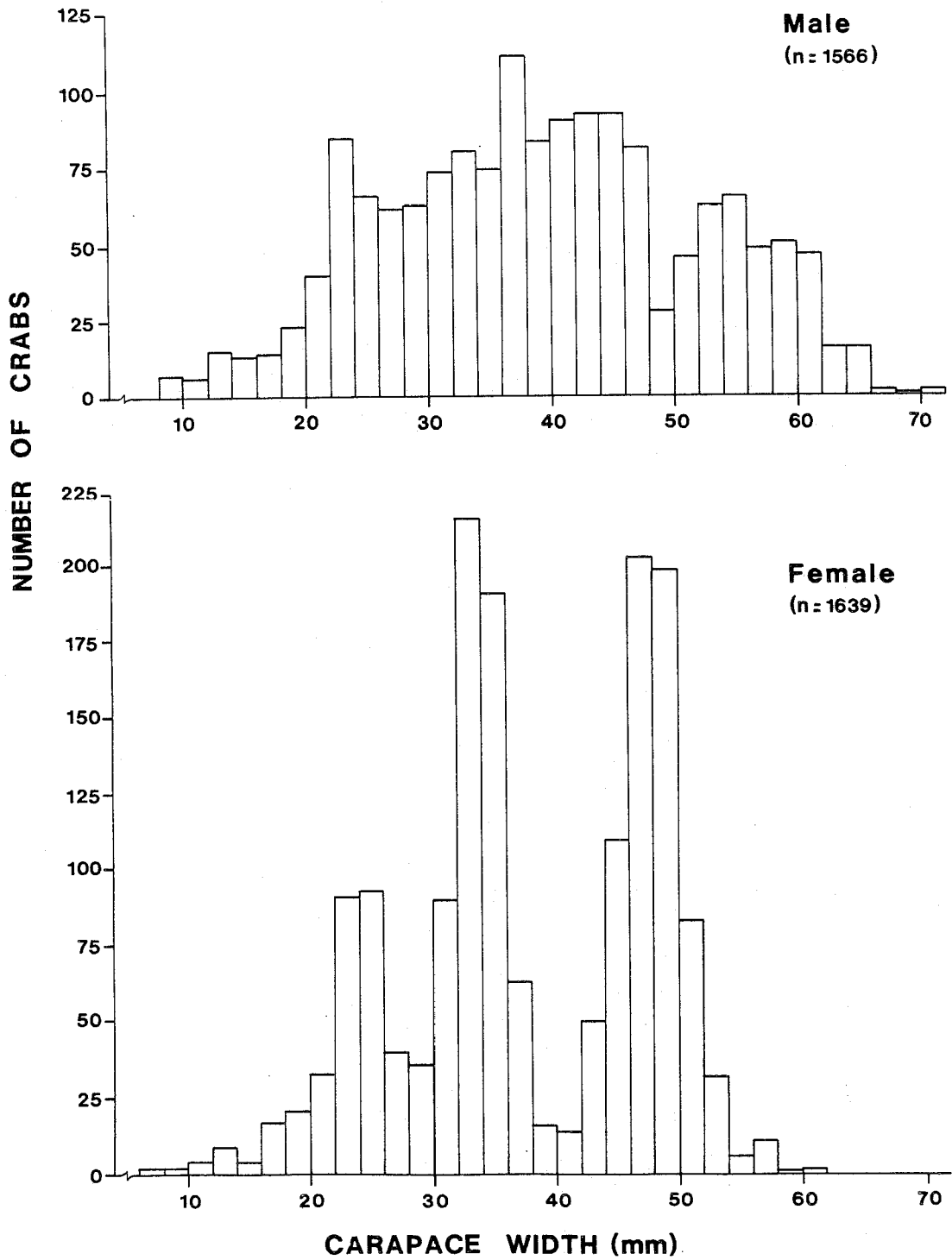
the sexes. In females, the gap is found between 38.1 and 42.0 mm FCW whereas in males, the gap is found between 48.0 and 50.0 mm FCW. The maximum sizes attained by both sexes in this study also differed. The maximum carapace width attained by males was 71.1 mm FCW whereas that of females was 61.3 mm FCW.

Although, in general, the proportion of males to females in this study did not differ significantly from a 1:1 ratio (χ^2 = 1.66, p>0.05), the sex ratio within each 5 mm FCW size classes varied considerably (Fig. 6.2). This variability may have been the result of sample size in the smaller size classes. However, in the intermediate size classes (20.0 - 50.0 mm FCW), it is probably a result of the differences in the relative abundances of the two sexes. Within this size range, the males consistently occurred in relatively high numbers whereas the females showed distinct peaks in abundance (Fig. 6.1). The size classes at which the females were most abundant corresponded with the size range over which the proportion of females exceeded that of the males. It is also interesting to note that the proportion of females in this study decreases markedly at sizes greater than 55.0 mm FCW. At sizes greater than 65.0 mm FCW, the sex ratio was 100% males.

6.3.2 Moult increment study

Some difficulty was experienced in obtaining moult increment data both in the field and in the laboratory. In the field, very few exuviae of *M. lunaris* were recovered together with newly moulted individuals. In the laboratory, most of the usable (*i.e.* within 5 days of captivity) moult increment data were obtained from prepuberty females undergoing a puberty moult prior to copulation.

Figure 6.2

      The relationship between sex ratio and size:

      the relative proportions of male and female

      M. lunaris within 5-mm size classes.
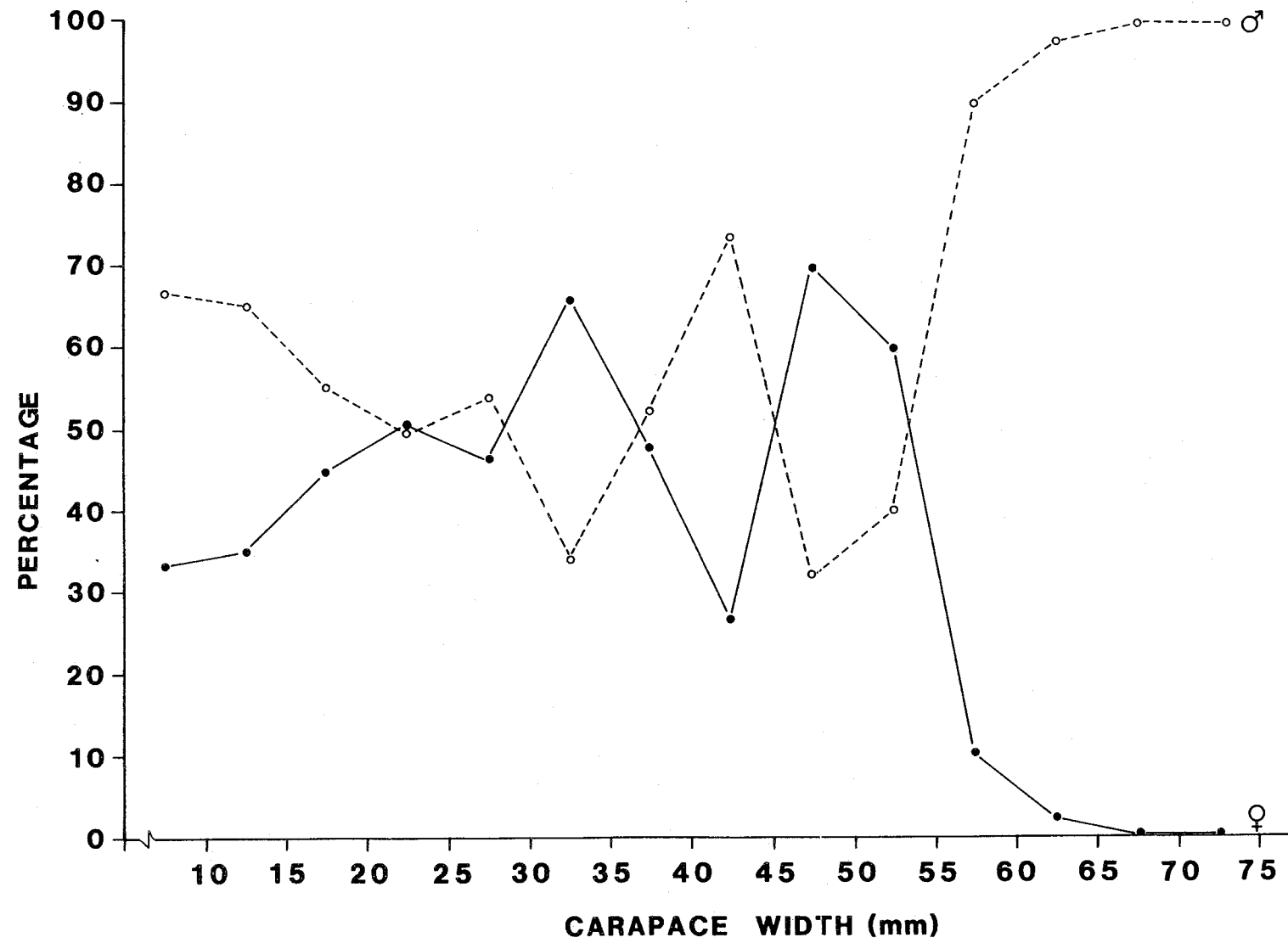
Moulting records were not obtained for most sizes.

In this study, analyses were based on data from 22 individuals (7 males, 15 females) which were either observed moulting in the field or within 5 days of captivity in the laboratory. The results of the moult increment analysis using both the Hiatt diagram and relative increment, are summarized in Table 6.1 and Figures 6.3A, B. From the Hiatt diagram, the relationship between postmoult size and premoult size in males approximates a straight line (Fig. 6.3B). The restricted distribution of moult data in females, however, precluded a similar interpretation based on these data. From the analysis of the relative increments in both sexes, the slopes of the linear regression of log percent increment on premoult size were shallow and did not differ significantly from 0 (Table 6.1). There was no significant difference between the slopes of both sexes $(t = 0.00627, p>0.05)$.

Moult increment data were also calculated based on the modes of the size frequency histogram. However, this was only possible in the females as there were no discrete modes in the size frequency histogram of the males. The results of the moult increment analysis are shown in Table 6.1 and Figure 6.3. These are comparable with the individual moult increments obtained from the field, with the exception of the moult increment in small individuals which is relatively large.

Table 6.1 A summary of moult increment data using two methods of analysis.

I. Hiatt Diagram[†]

	n	Growth Equation	Growth Coeff.	Sig.	Growth Pattern
Male	7	$y = 0.5366 + 1.2245x$	1.23	ns	arithmetic
Female	15	$y = 2.6245 + 1.2870x$	1.29	ns	arithmetic
Female*	3	$y = 8.4339 + 1.1370x$	1.14	ns	arithmetic

II. Relative Increment[††]

	n	Mean % Increment	Slope	r	Sig.
Male	7	24.74 ± 1.463	-0.001	-0.2974	ns
Female	15	36.76 ± 1.762	-0.003	-0.1387	ns
Female*	3	(85.4, 41.1, 41.5)	-0.015	-0.8767	ns

(*) - data based on the modes of the size frequency histogram

[†] - following Kurata (1962) and Bennett (1974)

[††] - following Mauchline (1976) and Hartnoll (1982)

Figure 6.3

A summary of the approaches used to quantify the
relationship of moult increment to size.

6.3A   The relationship between percentage moult

increment and pre-moult size, expressed as the

linear regression of log percent increment

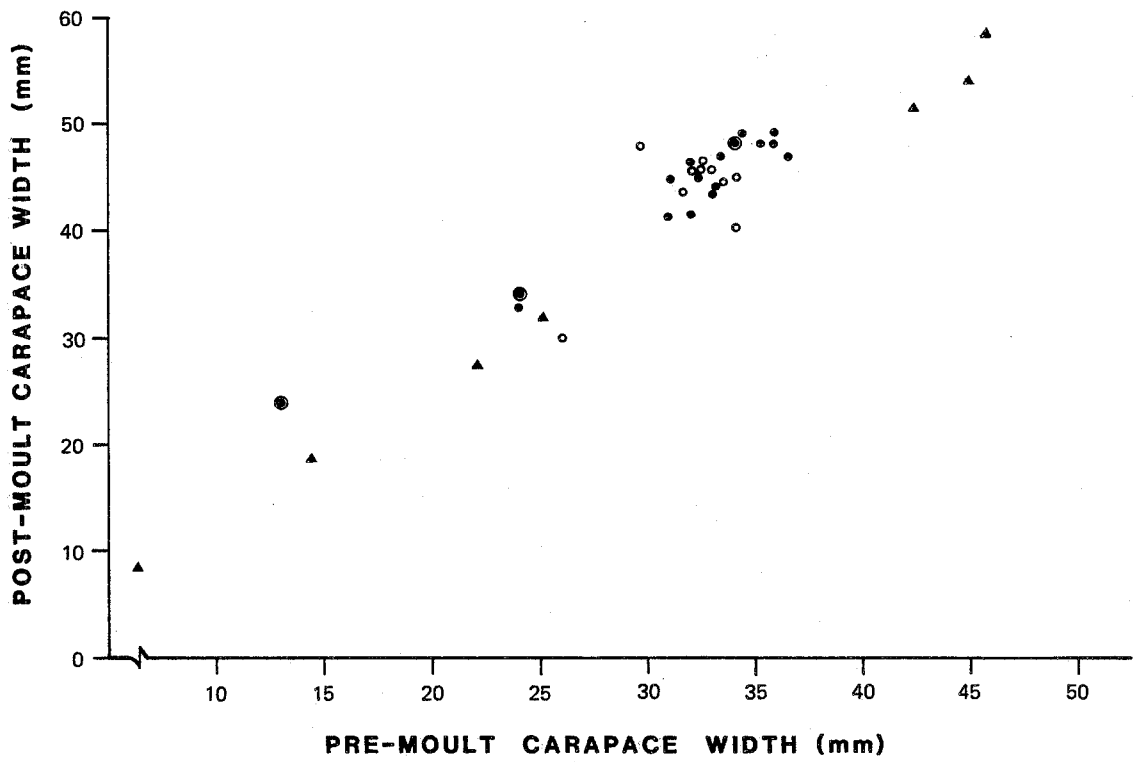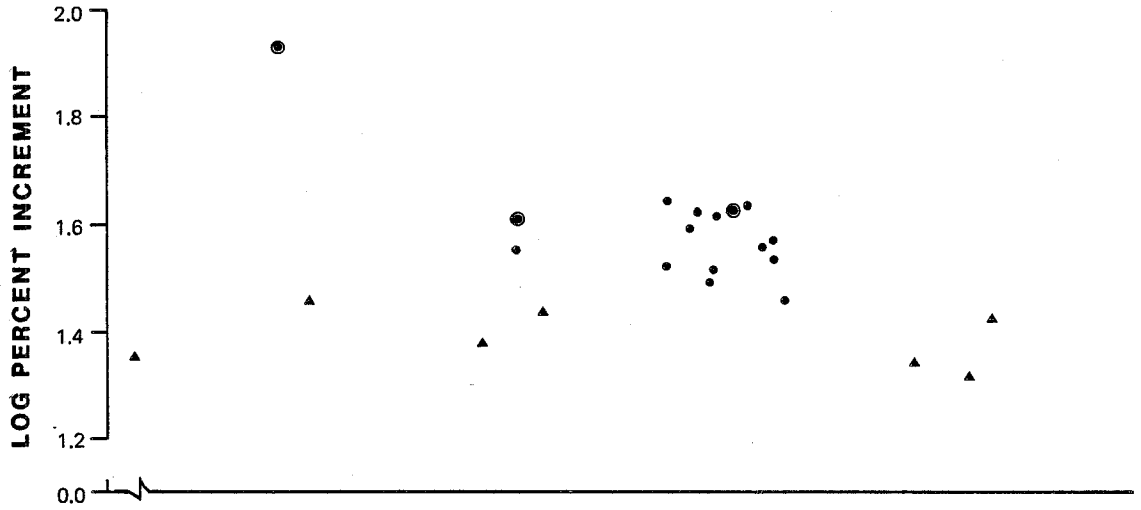on pre-moult carapace width.

▲ male

●○ female

◉ female*

6.3B   A Hiatt growth diagram of *M. lunaris*:

pre-moult size vs. post-moult size.

▲ male

●○ female

◉ female*

(*   — based on the modes of the size distribution
         in Fig. 6.1)

6.4 Discussion

Absolute growth is defined as the change in size with time, and in crustaceans, it is essentially a discontinuous process occurring in a series of moults or ecdyses. As a result, there are two basic patterns of absolute growth in crustaceans: 1) indeterminate growth and 2) determinate growth. These growth patterns have been extensively reviewed by Hartnoll (1980, 1982). Indeterminate growth is defined as 'unrestricted growth' where there are no terminal moults and a succession of moults continues until death from extrinsic factors intervenes. Determinate growth, however, is characterized by a terminal moult with growth occurring in a series of fixed or variable number of moults. There are four types of determinate growth patterns based on the number of resulting intermoult stages or instars and the relationship between sexual maturity and the terminal moult. These are: 1) growth with a variable number of instars and maturity occurring before the terminal moult, 2) growth with a fixed number of instars and maturity occurring before the terminal moult, 3) growth with a variable number of instars and maturity occurring after the terminal moult and 4) growth with a fixed number of instars and maturity occurring after the terminal moult. Examples of crustaceans exhibiting these types of determinate growth and indeterminate growth, are given in Table 6.2.

In this study, absolute growth in male and female *Matuta lunaris* have been investigated based on the size frequency distribution of a natural population and field moult increment data. The results of this study indicate that absolute growth in both

Table 6.2 A taxonomic list of examples of the growth patterns in Crustacea[*]

Examples	References	Indeterminate Growth	Determinate Growth		
			Instar Number Variable		Instar Number Fixed[**]
			Mature before final moult	Mature after final moult	Mature after final moult
Ostracoda					
Cyprinotus sp.	Kurata, 1962				+
Copepoda					
Calanoida	Kaestner, 1970				+
Harpacticoida	Kaestner, 1970				+
Cirripedia					
Thoracica	Kurata, 1962	+			
Mysidacea					
Gnathopausia ingens	Childress & Price, 1978	♂+			♀+
Amphipoda					
Microdeutopus gryllotapa	Myers, 1971	+			
Isopoda					
Dynamena bidentata	Boldich, 1968				+
Astacidea					
Nephrops norvigecus	Farmer, 1973	+			
Brachyura					
Grapsidae	Hartnoll, 1965	+			
Cancer pagurus	Bennett, 1974	+			
Neopanope sayt	Swartz, 1978	+			
Cryptocromia hilgendorfi	McLay, 1982	+			
Rhithropanopeus harrisii	Turoboyski, 1973	+			
Carcinus maenas	Klein Breteler, 1975a		+		
Portunus sanguinolentus	Ryan, 1967c		+		
Callinectes sapidus	Tagatz, 1968		♂+	♀+	
Callinectes arcuaus	Paul, 1982		♂+	♀+	
Callinectes toxotes	Paul, 1982		♂+	♀+	
Majidae	Hartnoll, 1963			+	
Maja squinado	Carlisle, 1957			+	
Corystes cassivelaunus	Hartnoll, 1972			+	
Halicarcinus australis	Lucas & Hodgekin, 1970			+	
Matuta lunaris	present study		♂+	♀+	

[*] - modified from Hartnoll (1982)

[**] - there are no published examples of crustaceans with fixed number of instars and maturity occurring before the final moult.

sexes is of a determinate nature, where both sexes appear to reach a state of terminal anecdysis at a particular size, as evidenced by the following observations:

1) both sexes have relatively discrete postpuberty size ranges,

2) postpuberty individuals of both sexes maintained in aquaria did not moult and showed no evidence of proecdysis when dissected, and

3) the slopes of the regressions of log percentage moult increment on size for both sexes are typical of species with determinate growth (in: Hartnoll, 1980).

However, marked differences between sexes have also been found. Each sex will therefore be considered separately below.

The absolute growth pattern of male *M. lunaris* is of a determinate nature, with the moult increments remaining constant with size. The size at which males settle from the megalopa stage is unknown, although it is probably smaller than the minimum male carapace width recorded in this study (*i.e.* 8.1 mm FCW). From the size frequency distribution, it is not possible to determine the number of instars from the time of settlement to the onset of sexual maturity as there were no distinguishable modes which appeared to correspond with intermoult stages or instars. The puberty moult of males is marked by a distinct gap in the size frequency distribution approximately between 48.0 to 50.0 mm FCW (Fig. 6.4), which corresponds with the morphological changes observed in Chapter 3 and the behavioural and histological observations in Chapter 5. It appears that this puberty moult is also the terminal moult in males, with the sexually mature individuals being found only in the final instar. The size range at which sexually mature males occur is

Figure 6.4

The size distribution, based on 2-mm size classes,

of all the individuals measured in this study,

showing the proposed growth stages in *M. lunaris*.
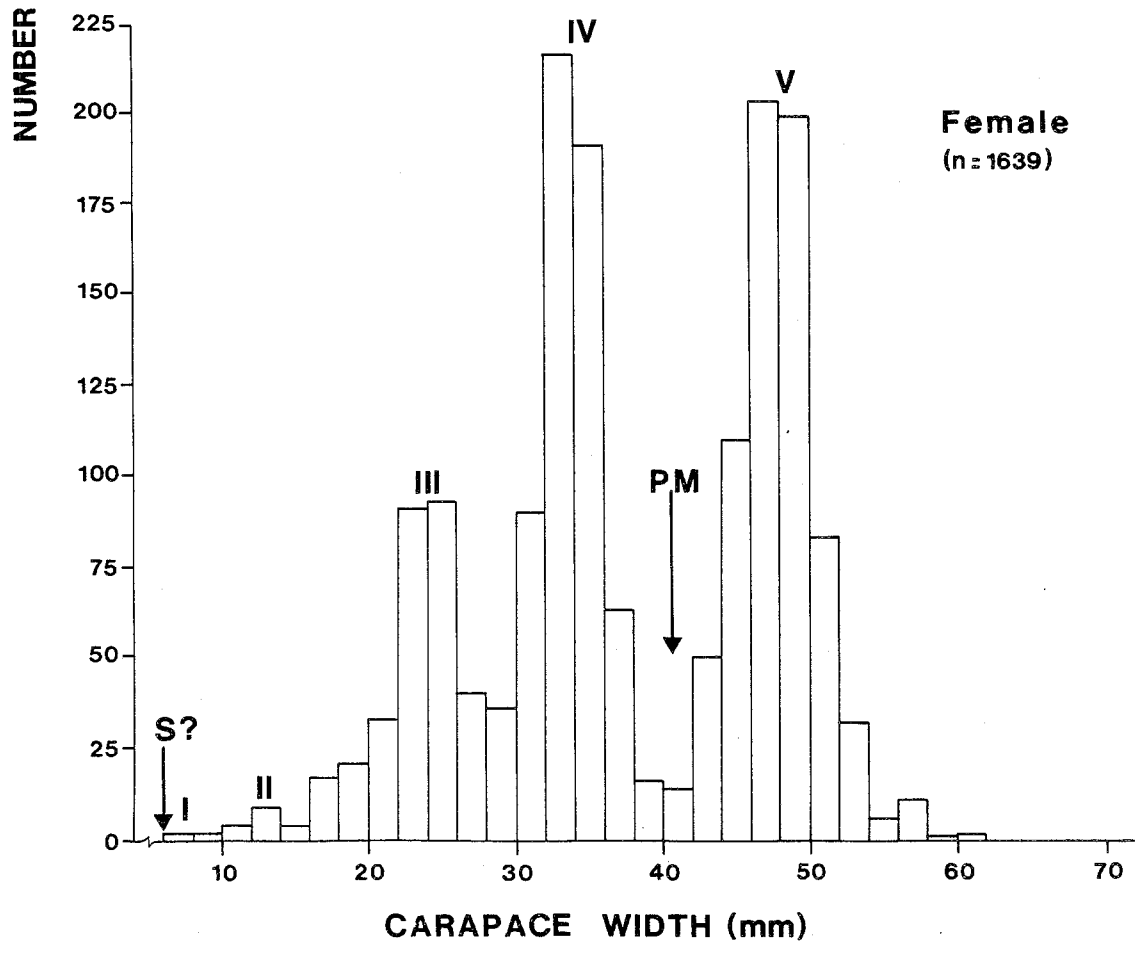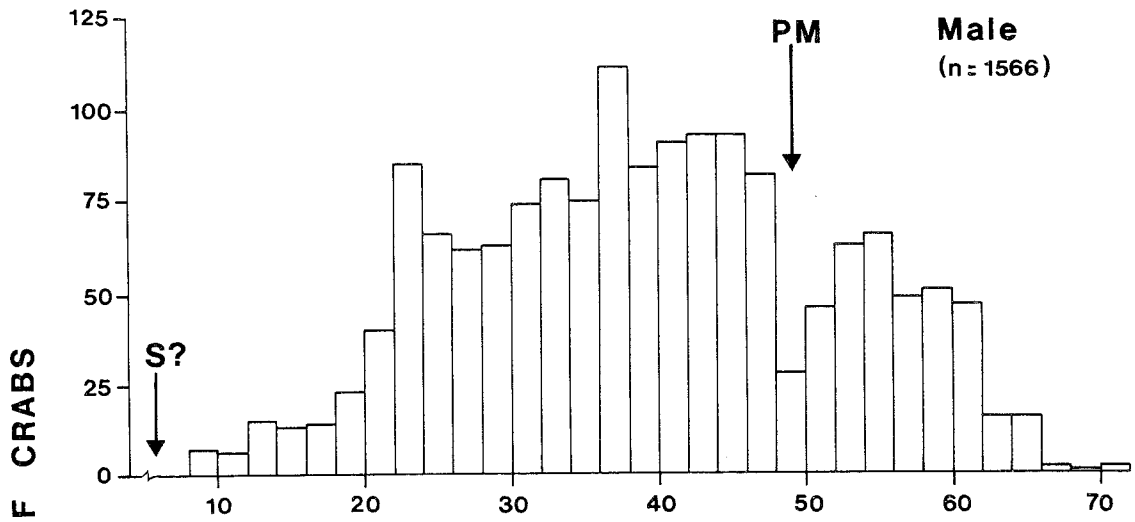
S?   possible size at settlement

PM   puberty moult

In this study, only the females showed distinct

growth stages or instars, namely:

Instars I, II, III, IV and V.

No distinct modes which correspond to growth

stages are apparent in the size distribution

of male *M. lunaris*.

relatively large (*i.e.* 50.0 to 71.1 mm FCW). There is evidence to suggest that at this size range, sexually mature males are in a state of anecdysis, namely, the absence of any developing epicuticle in all sexually individuals examined (Chapter 3). On the assumption of a constant percent moult increment (24.74% from Fig.6.1), it is possible to extrapolate the maximum prepuberty size given the maximum sexually mature size recorded in this study (*i.e.* 71.1 mm FCW), following Hartnoll (1972). The maximum prepuberty size possible, therefore, is 53.5 mm FCW. In this study, the largest immature male examined had a full carapace width of 51.8 mm (Chapter 3). This suggests that all the observed sexually mature males in this study may have resulted from a single moult at puberty.

In considering the results of the morphological studies in Chapter 1 and the observations in this study, therefore, two trends become apparent: 1) that the puberty moult in males does not occur at a specific size but rather at a particular size range, and 2) that there is considerable size variation in the resultant sexually mature males. Overall, the observations in this study suggest that male *M. lunaris* have a determinate growth pattern with maturity occurring after the terminal moult and an unknown and possibly variable number of instars. This type of growth pattern is similar to that reported in other brachyuran species, such as several species of majiids (Hartnoll, 1963, 1965a), *Corystes cassivelaunus* (Hartnoll, 1972) and *Halicarcinus australis* (Lucas & Hodgkin, 1970).

The growth pattern of female *M. lunaris* is fully determinate with a characteristic decrease in the moult increments with increasing size. The size at which female *M. lunaris* settle from

its planktonic larval stage is unknown, however, the minimum size of females collected in this study was 6.6 mm FCW. If this represents the size of the first post-larval instar then there appear to be five intermoult stages or instars before the onset of sexual maturity (Fig. 6.4). These instars are characterized by relatively large moult increments, with the increment between the 3rd and 4th instars being particularly high. Insufficient data on newly settled individuals, however, precluded the estimation of moult increments between the 1st, 2nd and 3rd instars, although considering the minimum size of females collected in this study and the mode which represents the 2nd and 3rd instars, one could predict the moult increments to be comparable with that between the 3rd and 4th instars. The 4th instar represents the final immature instar, prior to the puberty moult. It is interesting to note that the size range of the 4th instar corresponds with the size range at which female M. lunaris were observed to pair with male M. lunaris before copulation (Chapter 5). The puberty moult in females is well defined. It is marked by the distinct gap between 38.1 and 42.0 mm FCW in the size frequency histogram (Fig. 6.4). This corresponds with the morphological changes observed in Chapter 3, the gonadal maturation observed in Chapter 5 and the observed moulting events in the laboratory. The resultant 5th instar, therefore, is essentially sexually mature. In females, the puberty moult is also the terminal moult: all individuals are in a state of terminal anecdysis and are incapable of further moulting. This is supported by the absence of any females at sizes greater than 64.5 mm FCW, the absence of postpuberty exuviae in the field and in the laboratory and the absence of a developing epicuticle in all postpuberty specimens

examined (Chapter 5). It appears, therefore, that female *M. lunaris* have a fully determinate growth pattern with a fixed number of instars and sexual maturity occurring after the terminal moult. This type of growth pattern, however, is unusual for brachyurans. Similar growth patterns have been reported in copepods (Ivanova, 1973) and ostracods (Kurata, 1962) but have not been reported previously in brachyurans (Hartnoll, 1980, 1982).

The advantages of a determinate growth pattern in crustaceans as outlined by Hartnoll (1980) include the following: 1) faster growth to full size, 2) all resources can be concentrated on reproduction in the mature phase, 3) breeding is not interrupted by moulting events, and 4) decrease in the probability of moult-induced mortality. In this study, *M. lunaris* exhibits a fully determinate growth pattern. However, the differences observed between the two sexes suggest that male and female *M. lunaris* follow two distinct but disparate growth strategies. Male absolute growth appears to occur in a variable number of instars, each with a wide size range, and is characterized by constant but relatively small (24.7%) moult increments. In constrast, female absolute growth occurs in a fixed number of instars, each with a restricted size range, and is characterized by relatively large but variable (85-41%) moult increments. In addition, males grow to a larger maximum size than females. From these observations, several questions arise:

A. Why do males grow larger than females?

B. Why is female growth restricted to a fixed number of instars with restricted size ranges?

C. Why are there two different growth strategies?

Each of these questions will be considered separately below.

A. Why do males grow larger than females?

This question cannot be directly answered from the observations in this study. However, in considering the results from the studies on other aspects of the biology of *M. lunaris* (Chapters 3, 4 and 5), several possible explanations may be forwarded. Firstly, it may be advantageous for males to be larger than females at sexual maturity, to be able to provide adequate protection for the soft, newly moulted female during copulation, as noted in Chapters 3 and 5. Secondly, the existence of considerable size variation between sexes may be ecologically advantageous by decreasing niche overlaps, as noted by Hartnoll (1965b) in several species of spider crabs where the mean size of post puberty males was greater than that of the females. And finally, it may be the result of differential energy allocation, as males are able to put more energy into somatic growth than females which require more energy for egg production at sexual maturity. The phenomenon of males growing larger than females has been reported in other brachyurans such as *Ranina ranina* (Fielding & Haley, 1976), *C. cassivelaunus* (Hartnoll, 1972), *Lithodes aequispina* (Sloan, 1985) and several species of spider crabs (Hartnoll, 1965a).

B. Why is female growth restricted to a fixed number of instars with restricted size ranges?

In most brachyurans, reproductive fitness is directly related to female body size (Hines, 1982). It would therefore appear to be advantageous for a female to grow as large as possible. In *M. lunaris*, however, mature females have a restricted size distribution, the mean size of which is considerably smaller than that of the males. This suggests that there are strong selective pressures limiting the possible size distribution of mature females. It is probable that there is an optimal size at maturity for females and that the observed distribution of immature female instars in this study represents the most efficient strategy by which this optimal size may be attained. A possible advantage of this growth strategy is that it minimises the number of moults required to reach the mature size. As a consequence, the number of times an individual is exposed during the vulnerable moult period is reduced. The energy required for further moulting may therefore be allocated for reproduction. The selective forces which appear to limit the size distribution of mature females are not known, although these are likely to be related to the reproductive processes. A potential mechanism which controls within-instar size variations and the significance of such restricted size ranges at important stages in the life history of a crustacean have been discussed by Hartnoll & Dalley (1981).

In comparison to females, male *M. lunaris* do not appear to have an optimal size at maturity, as evidenced by the considerable size variation between the adult males in this study. In addition, males

do not demonstrate any discrete instars during the immature phase, as evidenced by the lack of distinguishable modes in the size frequency histogram. These observations suggest that the selective forces acting upon the growth pattern of males are different from those influencing the growth patterns of females. Unlike females, the observed size distribution of mature males do not appear to be directly related to their reproductive function. In this study, mature males at all sizes were observed to pair and copulate with immature females (Chapter 5). There were no apparent size-related differences in the frequency of pair formation and there was no evidence of a particular size being selected for. It has been suggested that a broad size distribution of mature males in the population decreases direct ecological competition between individuals, as noted by Hartnoll (1963, 1965a) in the Majidae where mature males occurred over a wide size range. This may also be the case in *M. lunaris*.

In male *M. lunaris*, there appears to be a considerable overlap of instar size ranges during the immature phase despite the relatively constant moult increment. It is interesting to note that in this study, the mean male moult increment (24.7%) is comparable with the 25-26% mean increment in crustaceans postulated in the Brook's and Pzibram's Rules (in: Rice, 1968 and Hartnoll, 1982). This suggests that male *M. lunaris* probably optimizes the moult increment at each moult rather than attempting to achieve an optimal reproductive size. If male *M. lunaris* settles over a wide size range, a constant moult increment throughout postlarval development would result in a wide size variation between individuals of the same instar. This may account for the observed growth pattern in

male *M. lunaris*.

C. Why are there two different growth strategies?

In brachyurans, there are very few records of differing growth strategies between the sexes of the same species. In most species, males and females invariably have similar growth strategies, with the differences recorded in some species being limited to the size at sexual maturity (*e.g.* *C. cassivelaunus* [Hartnoll, 1972]), the moult increment (*e.g.* *Callinectes sapidus* [Tagatz, 1968]) or the intermoult period after maturity (*e.g.* *Cancer pagurus* [Bennett, 1974]). *M. lunaris* is unusual in that males and females follow different growth strategies in addition to the observed differences in their size at sexual maturity. Because of the similarity of both sexes in the other aspects of their biology, *i.e.* feeding and ecology, it is likely that the observed differences in their growth strategies are a result of their disparate reproductive roles. However, because of the small sample size of some aspects of this study, these interpretations must remain tentative.

CHAPTER SEVEN

Population Biology

7.1 Introduction

Population studies have a vital part in the understanding of the biology of brachyurans. Information on the relative abundance, sex ratio, population structure and growth of commercial species such as *Scylla serrata* (Hill, 1975), *Cancer borealis* (Haefner, 1976, 1977), *Ranina ranina* (Fielding & Haley, 1976) and *Portunus pelagicus* (Potter *et al.*, 1983) were found to be directly applicable to fisheries work. Likewise, population studies of non-commercial species such as *Trapezia* spp. (Gotelli *et al.*, 1985), *Cryptodormia hilgendorfi* (McLay, 1982) and *Halicarcinus australis* (Lucas & Hodgkin, 1970) were invaluable in the understanding of the spatial and temporal distributions of these species.

In the Matutidae, population studies are completely lacking. Despite the widespread distribution of *Matuta lunaris*, there has been no previous work on its population biology. This study therefore was designed to investigate the population biology of *M. lunaris* at Pallarenda beach, Townsville and to provide preliminary information on its relative abundance, sex ratio, population structure and growth.

7.2 Methods

This study was conducted at Site 'A' in Pallarenda beach, Townsville (19°11.8'S, 146°46.6'E) between April 1984 to May 1985. Sampling was carried out at monthly intervals, with each sample being within two days of the full moon of each month and always on a

midfalling tide. *M. lunarts* was collected using a large 25 mm mesh, 10 m x 1 m seine net and a small 3.5 mm mesh net following the methods described in Chapter 2.

A minimum of five tows of the large net was carried out in each monthly sample, or until a minimum of 50 individuals was caught. After each tow, all crabs caught were placed in containers filled with sea water and a note was made of the number of crabs per tow. In addition, five tows of the small net were undertaken. All crabs were measured at the completion of the sampling period. The sex and full carapace width (FCW) of each crab was recorded and a note was made of its reproductive or moult condition, *i.e.* grasping, ovigerous or soft-shelled. After measurement, all crabs were returned to the surf zone in the area of capture.

In addition, the volume of detached macrophytes and detrital accumulation in the surf zone, *i.e.* macroalgae, seagrass and mangrove leaves, was estimated for each month and ranked using a scale of 5, with 0 representing no accumulation.

7.3 Results

7.3.1 Relative Abundance

The relative abundance of *M. lunarts*, based on the mean number of crabs caught in each tow of the large net, varied considerably between months. A summary of the monthly mean number of crabs per tow is given in Table 7.1. It appears, however, that the variation is related to catch efficiency rather than to actual changes in population abundance. For example, the amount of macrophyte accumulation in the surf zone affected the efficiency of the nets

Table 7.1 A summary of the monthly catch rates based on the
samples collected using the large net.

Month	Total no. of crabs caught using both nets	No. of crabs caught using only the large net	Mean catch rate ± 95% C.I. (No. per tow)
April	80	77	13.8 ± 15.26
May	51	51	10.2 ± 8.11
June	71	71	14.2 ± 9.55
July	64	64	12.8 ± 7.67
August	49	47	3.4 ± 1.83
September	25	25	4.2 ± 5.14
October	43	42	8.4 ± 5.86
November	251	250	50.4 ± 33.55
December	140	87	9.7 + 6.05
January	64	50	10.0 + 10.86
February	77	60	5.0 ± 1.82
March	219	207	41.4 ± 27.54
April	109	101	12.6 ± 5.91
May	91	77	8.5 ± 4.05

used in the study. The effect of the macrophyte accumulation on the monthly mean number of crabs per tow is shown in Figures 7.1A, B, C.

7.3.2 Sex Ratio

There was no significant difference in the proportion of male and female crabs in the population throughout the study period (χ^2 = 14.413, p> 0.05). In 13 out of 14 months, the sex ratio of *M. lunaris* did not deviate significantly from a 1:1 ratio, with the exception of October where the ratio of males to females approached 2:1. Table 7.2 summarizes the proportion of males and females in the population over the 14-month sampling period.

7.3.3 Population Structure

The mean size of male and female *M. lunaris* and the relative abundance of adults and juveniles in the population each month, are summarized in Figures 7.2A, B, C. From these figures, the following trends are apparent:

1) The mean size of both sexes in the population varied throughout the study period. In both sexes, however, the mean sizes were notably larger between May and November, 1984. This was most marked in males, with the largest mean size being recorded in September, 1984.

2) The relative abundance of adult males and adult females in the population also varied throughout the study period. Both sexes displayed a similar trend in their abundance with the highest numbers being recorded between the months of May and November, 1984.

Figure 7.1

A summary of the monthly abundance of *M. lunaris*,

expressed as the mean number of crabs per tow,

showing the effects of macrophyte accumulation

on the monthly catch rate.

7.1A   The relationship between catch rate and

macrophyte accumulation.

7.1B   The degree of macrophyte accumulation in

the area during the sampling period.

7.1C   The monthly abundance of *M. lunaris*, based

on the number of crabs caught per tow.

Macrophyte accumulation rank: 0 to 5,

where 0 = no macrophyte present

and 5 = large volumes of macrophyte

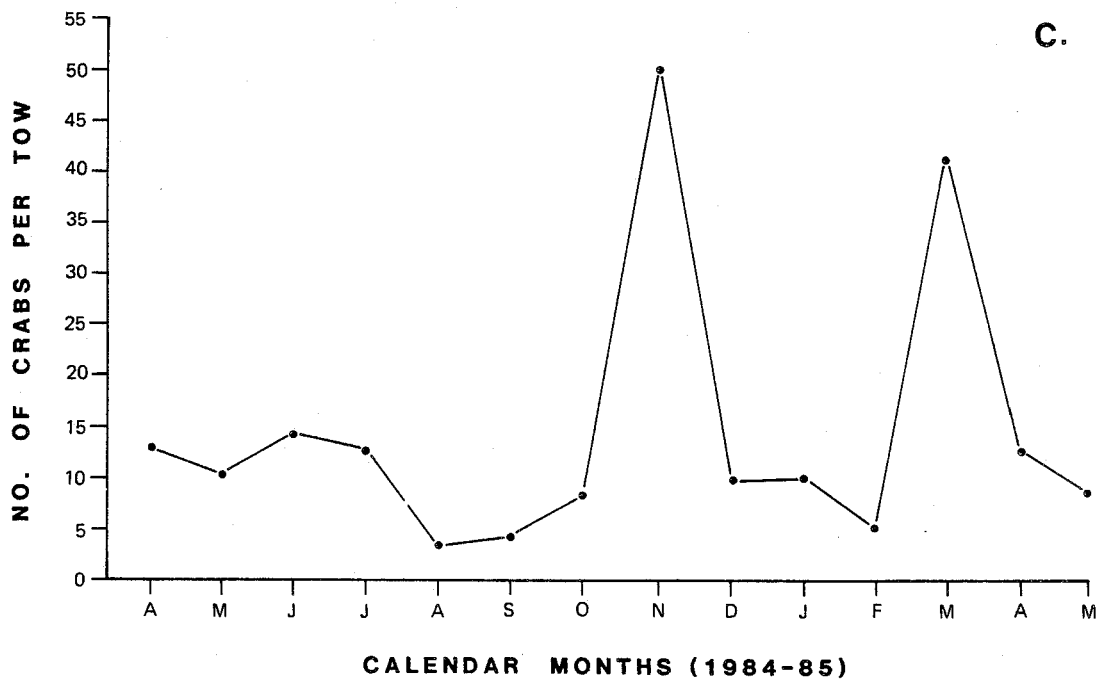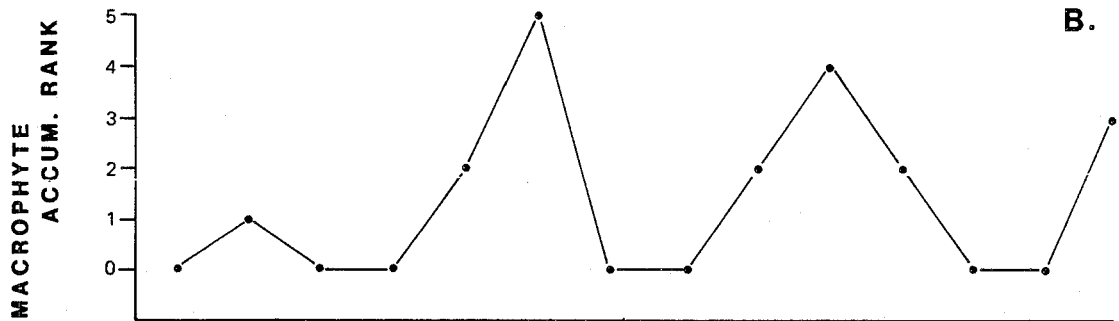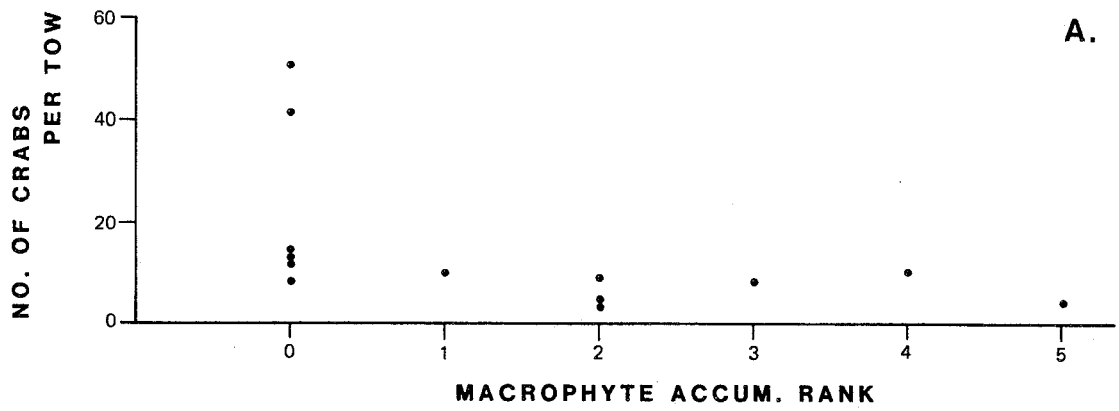above 5, sampling was discontinued.

Table 7.2 A summary of the monthly sex ratio of *M. lunaris*,
from April, 1984 to May, 1985.

Month	n	% male	% female	significance
April	80	50	50	ns
May	51	47	53	ns
June	71	59	41	ns
July	64	51	49	ns
August	49	45	55	ns
September	25	48	52	ns
October	43	65	35	**
November	251	48	52	ns
December	140	48	52	ns
January	64	65	35	ns
February	77	58	42	ns
March	219	50	50	ns
April	109	52	48	ns
May	91	47	53	ns

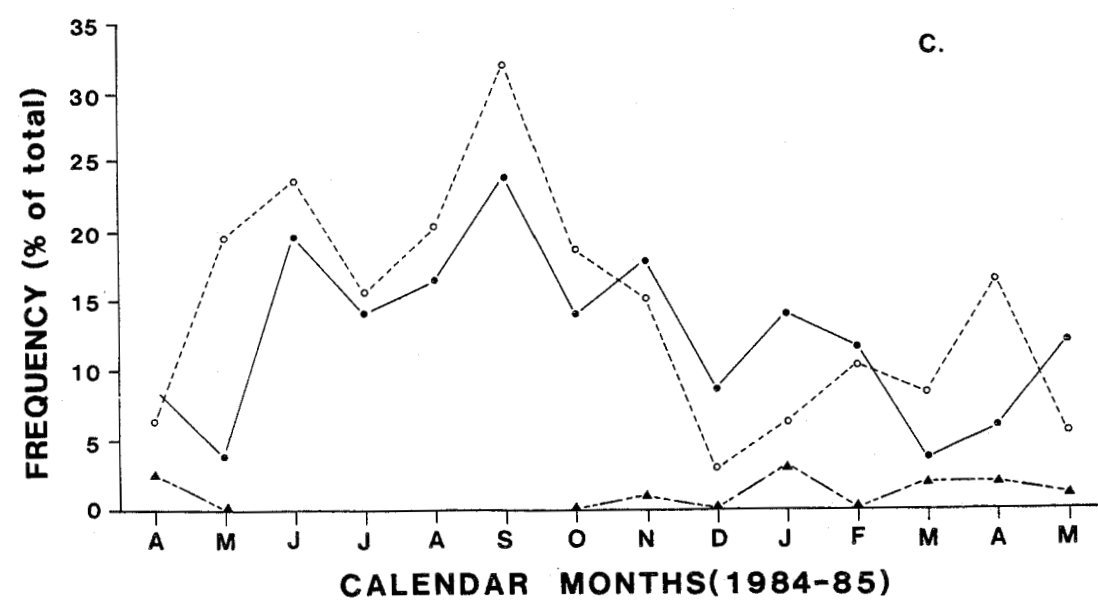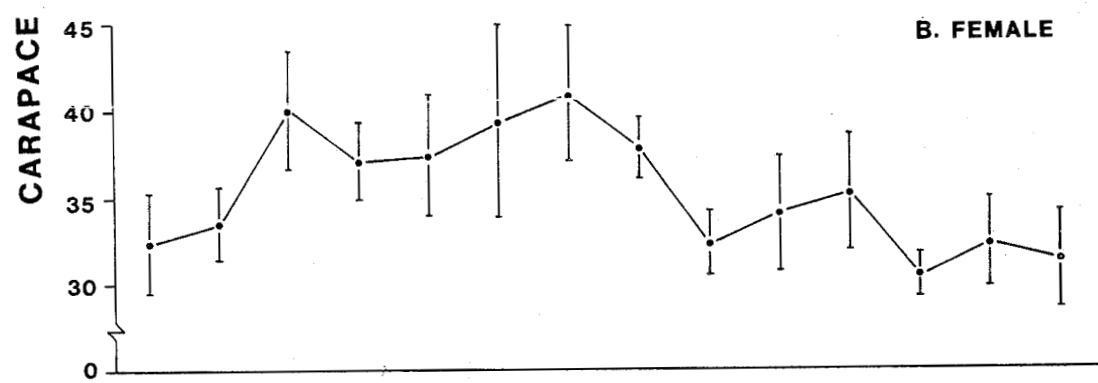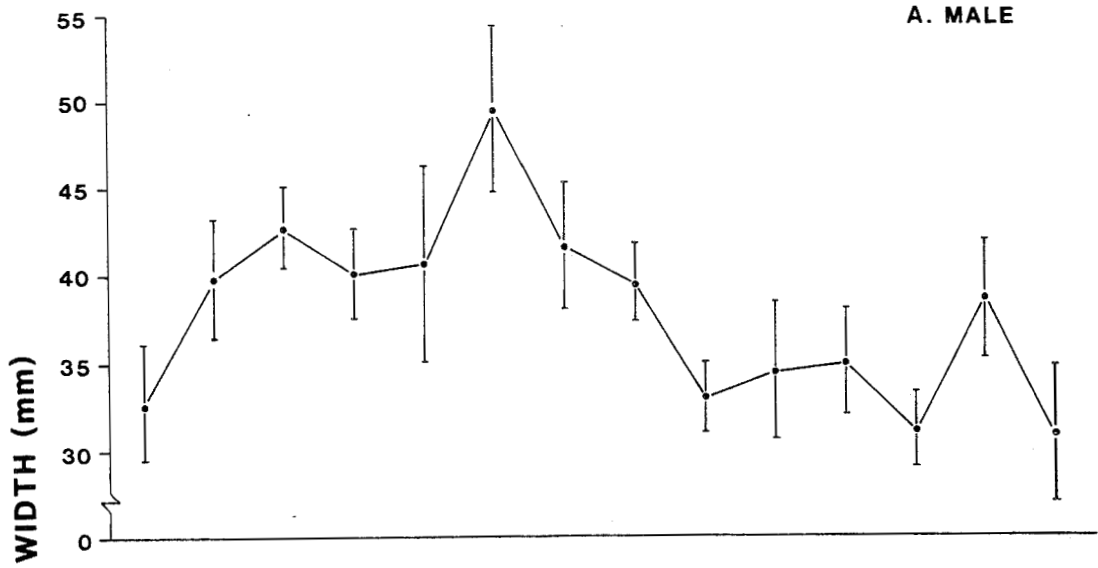* — based on the standard 1:1 sex ratio

** — $p < 0.05$

Figure 7.2

The mean sizes per month (± 95% C.I.) of male and

female *M. lunaris* and the proportion of adult

males, adult females and juveniles of both sexes

in the population each month.

A. Mean size of males per month.

B. Mean size of females per month.

C. Relative frequencies of adults and juveniles.

   ●————● adult male

   ○------○ adult female

   ▲— — –▲ juveniles of both sexes

A. MALE

B. FEMALE

C.

CARAPACE WIDTH (mm)

FREQUENCY (% of total)

CALENDAR MONTHS(1984-85)

3) The relative abundance of juveniles was generally very low. Juvenile *M. lunaris* were caught only in April, 1984 and from November, 1984 to May, 1985. No juveniles were caught between May and October, 1984.

The size frequency distributions of *M. lunaris*, at site 'A', from April, 1984 to May, 1985 are summarized in Figure 7.3. In general, all size classes were represented in the study area throughout the sampling period, although the relative abundance of the individuals in these size classes varied between months. The population of *M. lunaris* was often multimodal and no distinct year classes were apparent. From Figure 7.3, the following trends may be observed:

1) In males, most size classes were present in the study area throughout the year although the larger size classes were more abundant between the months of May and November, 1984. As in the results of Chapter 6, however, there were no distinct modes which appeared to correspond to moult stages or instars.

2) In females, most size classes were likewise present in the study area throughout the year. As in the males, there was an increase in the relative abundance of the larger size classes between the months of May and November, 1984. In addition, there were three distinct modes which persisted throughout the year. These modes correspond with instars III, IV and V, as described in Chapter 6.

Figure 7.3

The monthly size frequency distributions of *M. lunaris*

in Pallarenda beach, from April 1984 to May, 1985.

The sample sizes in each month are given in brackets.

MALE   FEMALE

FREQUENCY (% OF TOTAL)

CARAPACE WIDTH (mm)
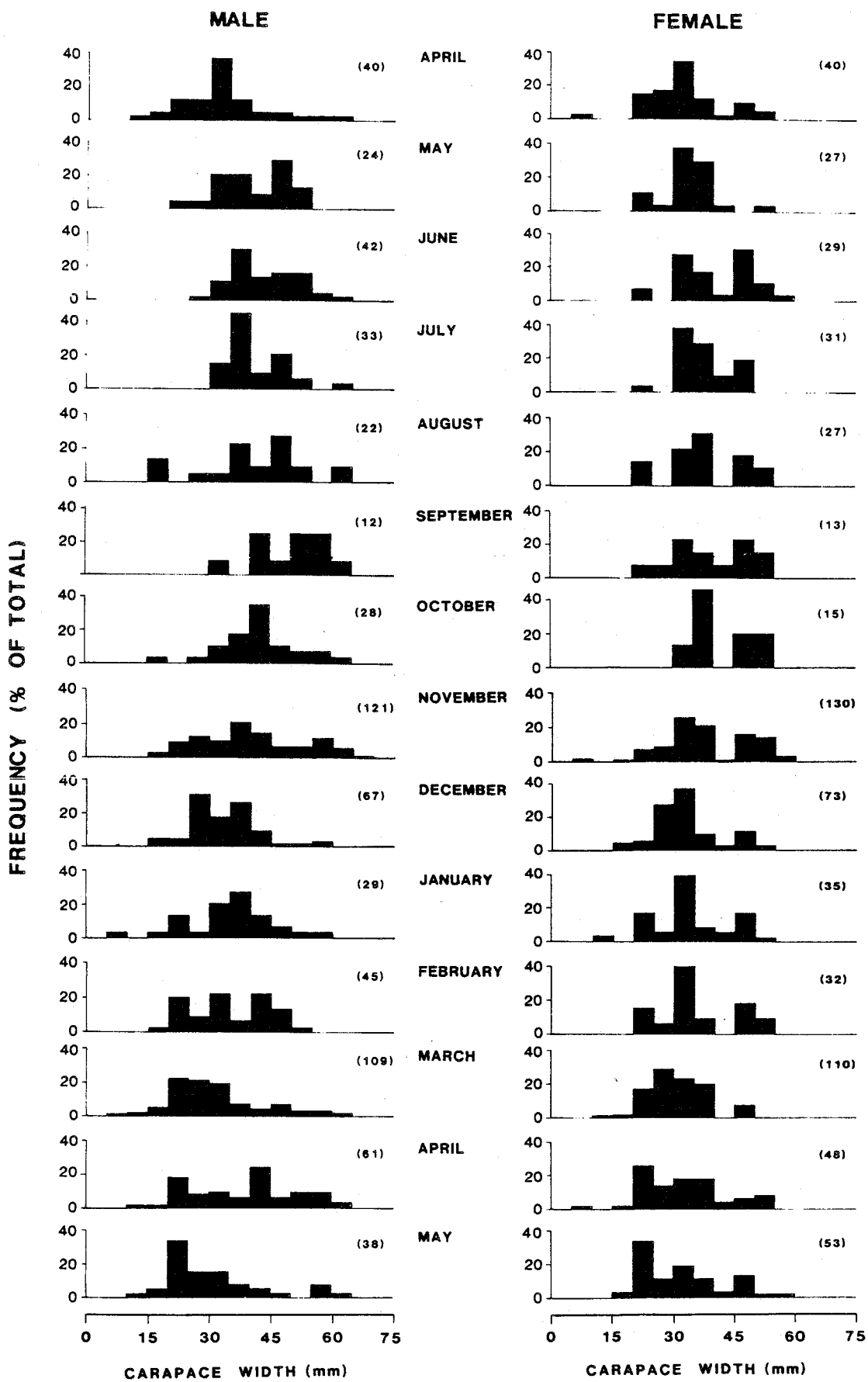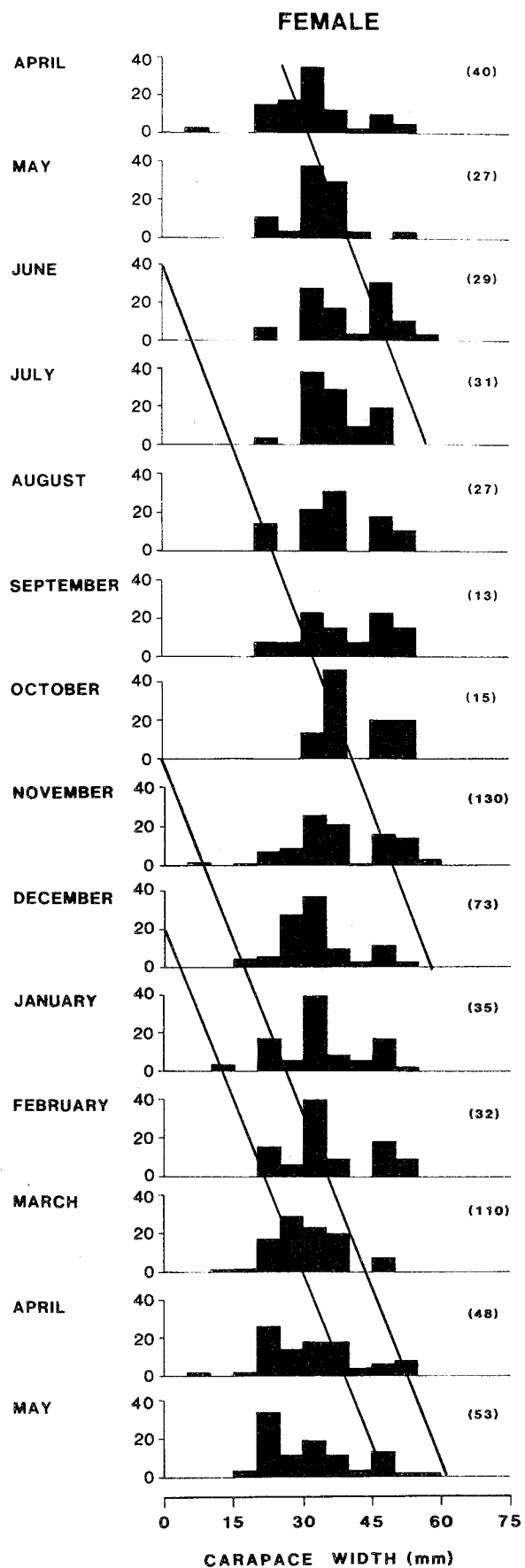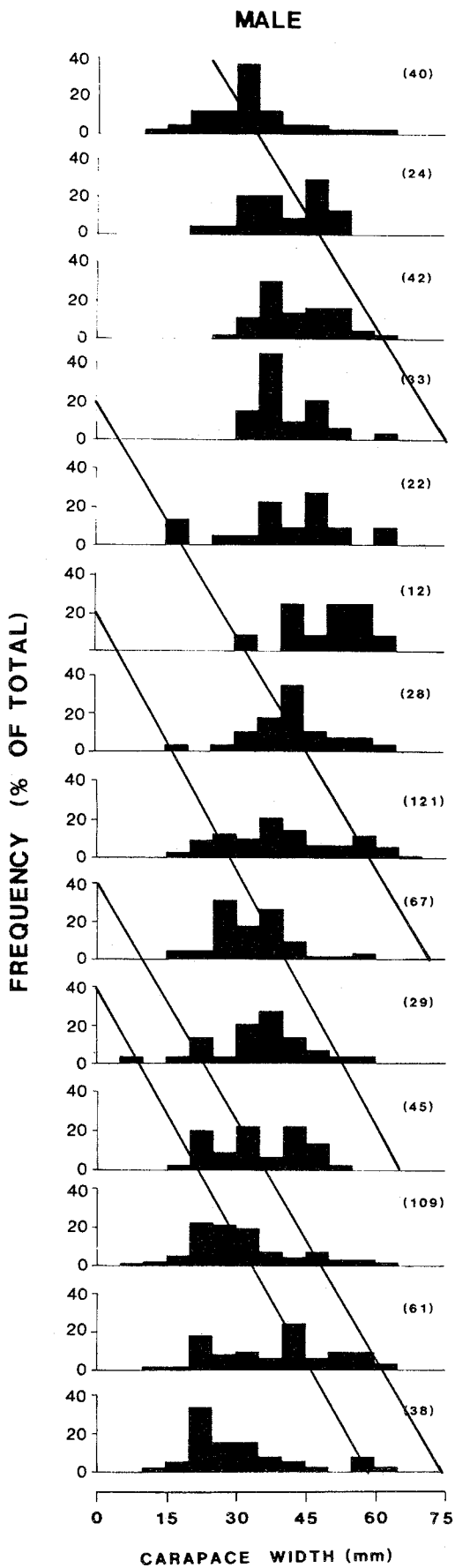
7.3.4 Notes on Population Growth

As growth in crustaceans is essentially of a discontinuous nature, *i.e.* occurring in a series of moults, the use of modal progression in determining population growth is limited. This is particularly the case in *M. lunaris*, where the population is often multimodal with most of the modes reflecting the moult stages rather than pulses of recruitment. In order to obtain some indication of the growth rate of *M. lunaris* through time, therefore, sequential changes in the relative abundance of various size classes were noted. In Figure 7.4, some of the more apparent sequential changes in relative abundance have been traced and connected. This, however, assumes a linear growth pattern which, from the results in Chapter 6 (Fig. 6.3B), is probably true for *M. lunaris*. If the connecting lines are extrapolated to the likely size at settlement in *M. lunaris*, an estimate of the total time from settlement to maturity can be made. In males, the time varies from 100 to 127 days ($\bar{x} = 119$, $n = 4$, S.E. = 6.36), and in females, from 154 to 160 days ($\bar{x} = 156$, $n = 3$, S.E. = 1.86). The length of life after maturity could not be assessed. In the laboratory, sexually mature individuals survived for up to four months.

7.4 Discussion

In this chapter, several aspects of the population biology of *M. lunaris* in Pallarenda beach were investigated. These aspects were: relative abundance, sex ratio, population structure and growth.

Figure 7.4

The monthly size frequency distribution of *M. lunaris*

in Pallarenda beach, showing the proposed trends of

population growth based on sequential changes in

the relative abundance of various size classes.

MALE

FEMALE

FREQUENCY (% OF TOTAL)

APRIL (40)
MAY (24)
JUNE (42)
JULY (33)
AUGUST (22)
SEPTEMBER (12)
OCTOBER (28)
NOVEMBER (121)
DECEMBER (67)
JANUARY (29)
FEBRUARY (45)
MARCH (109)
APRIL (61)
MAY (38)

(40)
(27)
(29)
(31)
(27)
(13)
(15)
(130)
(73)
(35)
(32)
(110)
(48)
(53)

CARAPACE WIDTH (mm)

CARAPACE WIDTH (mm)

Although some difficulty was experienced in obtaining absolute records of the abundance of *M. lunaris* in the study area, the results of this study indicate that *M. lunaris* is abundant throughout the year. The mean monthly abundance estimates varied from four individuals per 250 m^2 to 50 individuals per 250 m^2. These estimates were comparable with those obtained for *C. arcuatus* in a lagoon system on the Pacific coast of Mexico (Paul, 1982) but were greater than those obtained for *C. hilgendorfi* in Moreton Bay, Australia (McLay, 1982) and *S. serrata* in South Africa (Hill, 1975).

There appeared to be no seasonal pattern in the abundance of *M. lunaris*. This is contrast to other studies of brachyuran populations where a large degree of seasonal variation has been reported, as a result of seasonal migration and/or pulses of recruitment (*e.g. P. pelagicus* [Potter *et al.*, 1983]). In this study, the number of crabs caught per tow varied to some extent with the amount of macrophyte accumulation in the surf zone. However, whether this variation is directly related to the effect of macrophyte accumulation on the catch efficiency of the nets or on the crab population itself (*cf.* the study of Robertson & Lenanton [in press] on fish communities associated with macrophyte accumulations in the surf zone) is unclear.

In general, brachyuran populations tend to have a biased or skewed sex ratio. This is often a result of sex-related differences in movement patterns (*e.g. C. borealis* [Haefner, 1977], *P. pelagicus* [Potter *et al.*, 1983] and *C. sapidus* [Dittel *et al.*, 1985]), physiological tolerances (*e.g. C. arcuatus* and *C. toxotes* [Paul, 1982]), and behaviour (*e.g. H. australis* [Lucas & Hodgkin, 1970] and

C. hilgendorfi [McLay, 1982]). In this study, the sex ratio of *M. lunaris* in Pallarenda beach did not deviate signficantly from a 1:1 ratio throughout the study period. A consistent 1:1 sex ratio in brachyuran populations is unusual and has been previously reported in only a few species (*e.g. Macrophthalmus hirtipes* [Simon & Jones, 1981], *Pachygrapsus laevis* [Gemmell, 1979] and *Rhithropanopeus herbstii* [Turoboyski, 1973]). In *M. lunaris*, this is an indication that both sexes are present in the surf zone at the same time in similar proportions. It strongly suggests that both sexes have similar recruitment, movement and behavioural patterns and physiological tolerances.

The results of this study indicate that the population structure of *M. lunaris* in Pallarenda beach is primarily dominated by medium and large sized individuals in most months of the year. However, this may have been a result of the relatively low catch rate of juveniles and small individuals. The reasons for the low catch rate of juveniles in this study are unknown, although this may reflect the catch efficiency of the nets used in sampling. In other studies of benthic crustaceans, such as *Homarus gammarus* (Howard & Bennett, 1979) and *C. sapidus* (Dittel *et al*, 1985), the paucity of small individuals in the population has also been noted, and has been attributed to sampling bias. However, in a preliminary study of the effect of growth on the population structure of *Rhithropanopeus harrissii* and *Cancer anthonyi* (Hartnoll, 1978b), it has been suggested by Hartnoll (1982), that the scarcity of small individuals may be an actual feature of the population structure.

Despite the numerical dominance of relatively large individuals in the population, most size classes were present in the study area throughout the sampling period. This suggests that the rates of recruitment and mortality are relatively consistent throughout the year and reflects the continuous reproductive pattern described in Chapter 5. The slight increase in the mean sizes of individuals and in the relative abundance of adults between May and October, 1984, may be the result of slightly higher rates of recruitment and/or survival of juveniles in the earlier months. Whether this represents seasonality, however, remains to be established, as the observations in the present study were only made over a relatively limited sampling period, $i.e.$ over a single year.

The extrapolation of growth rates from size frequency data is particularly difficult in tropical species with continuous or protracted reproductive patterns (Dittel et $al.$, 1985). In $C.$ $sapidus$, for example, juveniles recruited into the population throughout the year, so that cohorts were difficult to distinguish (Dittel et $al.$, 1985). In this study, similar difficulties were experienced. The technique used in this study was also limited by the qualitative assessment of the modes in the size frequency distribution and by the small sample sizes during some months in the sampling period. The estimated growth rates of $M.$ $lunaris$ are therefore tentative. Nevertheless, the growth rate appears to be relatively fast, with the time between settlement and maturity being approximately 119 days in males and 156 days in females. This is comparable with the growth rates of other tropical brachyuran species, for example, $C.$ $hilgendorfi$ where the time between settlement and maturity is 70 days in females and 200 days in males

(McLay, 1982). Other tropical and subtropical brachyuran species with relatively rapid growth rates (*i.e.* maturity within one year) include *H. australis* (Lucas & Hodgkin, 1970), *P. pelagicus* (Potter *et al.*, 1983) and *C. arcuatus* (Paul, 1982).

The total life span of *M. lunaris* in the field is unknown, as the duration of life after maturity could not be determined. However, the lack of any large build-up of adult numbers in the monthly size frequency distribution and the lack of any individuals showing evidence of age (*e.g.* worn carapace or extensive epiphyte growth, as in *Corystes cassivelaunus* [Hartnoll, 1972]) suggests that the life span of adults in the field is not particularly long. In the laboratory, sexually mature individuals survived for more than three months. It is probable therefore that the total life span of *M. lunaris* is approximately more than six months. This is comparable with the life spans of other tropical brachyuran species, which range from eight months to slightly over one year (*e.g.* *C. sapidus* [Dittel *et al.*, 1985], *C. arcuatus* [Paul, 1982] and *H. australis* [Lucas & Hodgkin, 1970]). As most of these species exhibit a determinate growth pattern similar to that in *M. lunaris*, *i.e.* with a terminal moult, it would be interesting to compare the causative factors of mortality in these species.

Overall, the population of *M. lunaris* in Pallarenda beach is characterized by the following features: 1) numerical abundance throughout the year, 2) a consistent 1:1 sex ratio, 3) year-long presence of most size classes although the larger size classes tend to be more abundant, 4) year-round recruitment and probably consistent rates of mortality and 5) a rapid growth rate.

CHAPTER EIGHT

CONCLUDING DISCUSSION AND SUMMARY

The surf zone of tropical sandy beaches is a unique environment (McLachlan, 1983). Organisms living in this hydrodynamically active region of the beach must deal constantly with a variety of problems including food availability, exposure and wave action (Coull & Bell, 1983). The information to be gained from studies of the growth, feeding and reproduction of surf zone species are invaluable, yet the biology of these species remains one of the least studied topics in tropical marine biology. *Matuta lunaris* is one such species. In Pallarenda beach, Townsville, Australia, *M. lunaris* is a dominant member of the surf zone macrofauna. It is relatively unusual in that it spends all its postlarval life in the surf zone. The aim of the present study was to investigate several aspects of the biology of this species. These aspects were: relative growth, feeding, reproduction, absolute growth and population biology.

The results of the present study have shown that the biology of *M. lunaris* resembles that of many tropical and sub-tropical marine brachyuran species (e.g. *Portunus pelagicus* [Rahaman, 1967], *Callinectes sapidus* [Paul, 1981, 1982] and *Ovalipes punctatus* [Du Preez & McLachlan, 1984 a, b, c and Du Preez, 1983, 1984]). In terms of its feeding habits, it is a facultative scavenger and a predator of slow-moving benthic invertebrates. Its absolute growth pattern is of a determinate nature whilst its relative growth patterns follow the typical brachyuran pattern of allometry. It breeds continously throughout the year, and there is a marked asynchrony in the reproductive activity of individuals within the

population. Overall, therefore, there appeared to be no distinct features in the biology of *M. lunaris* which were uniquely adapted to its life in the surf zone.

It is possible, however, that the main character which enables *M. lunaris* to survive in the surf zone is its morphology. The primary requirement for surf zone animals is the ability to withstand the scouring action of the waves and cope with the receding tides (McLachlan, pers. comm.), and morphologically, *M. lunaris* possesses several features which fulfill this requirement. It has four pairs of well developed flattened swimming legs with paddle-like terminal joints which enable it to maintain its position within the surf zone by either swimming in the water column or burrowing in the substratum. Observations of individuals in the field and in aquaria have shown *M. lunaris* to be a strong swimmer and a fast back-burrower. As a result, it is able to avoid being exposed by receding tides or washed away by wave action.

The swimming and burrowing abilities of *M. lunaris* are facilitated by its possession of a relatively smooth and simple carapace which, apart from the two well developed lateral spines, is devoid of any major denticulations or protuberances. In the study of relative growth patterns, there were no marked changes in the growth patterns of the carapace dimensions with size, sex or sexual maturity. This reflected the consistency of the requirement throughout ontogeny for a protective covering which is also relatively smooth and simple to enable a highly mobile way of life. In addition, the modified frontal openings of the respiratory channels in *M. lunaris* allow individuals to remain burrowed for

extended periods of time (Garstang, 1897a, b).

However, these morphological characteristics are shared with other *Matuta* species, most of which have not been found to occur in the surf zone (*e.g.* *M. granulosa*, which was only recorded from deeper water in the Townsville region). From this study, therefore, it appears that *M. lunaris* has no unique characteristics which are specifically adapted to the surf zone.

This study has shown the value of morphological analyses in the assessment and interpretation of several aspects of the biology of *M. lunaris*. The study of the relative growth patterns was an invaluable aid in defining the puberty moult and size at sexual maturity, and provided a context within which the reproductive habits of *M. lunaris* could be investigated. In addition, the functional interpretations of the relative growth patterns have resulted in a better understanding of the mating behaviour of *M. lunaris* and the ontogenetic changes in its feeding habits.

The present study was a preliminary investigation into the biology of *M. lunaris*. As there have been few published studies on this species, the results of this study will hopefully provide the background information upon which further research may be based. This study has revealed many interesting aspects which require further investigation. These include: 1) the impact of the predatory activities of *M. lunaris* on the surf zone benthic communities, 2) the function of the stridulatory organs in the Matutinae and 3) the basis for the sex-related growth patterns in *M. lunaris*. Finally, in order to understand the factors which regulate the distribution and abundance of *M. lunaris*, there are two

areas of further study which may be prove to be particularly rewarding. These are: 1) the larval biology and behavioural basis of larval dispersal of surf zone species and 2) the physiological tolerances of various *Matuta* species and their possible relation to observed distributions.