ASPECTS OF THE BIOLOGY OF THE FRESHWATER CRAYFISH EUASTACUS SPINIFER (HELLER) (DECAPODA: PARASTACIDAE)

BY

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A large, mature female *E. spinifer* from the study area (carapace length 96 mm).

DECLARATION

This thesis contains no material that has been accepted for the award of any other degree or diploma at any University, and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

[redacted]

S. P. TURVEY

11/6/1980

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SUMMARY

A study of the freshwater crayfish *Euastacus spinifer* (Heller) was conducted near Sydney, N.S.W., with the aim of describing the reproduction, growth, population structure and feeding habits of this crayfish under natural conditions.

Reproductive mechanisms were compared to those of other parastacids, and seasonal reproductive activity was determined from changes through time in the breeding condition of wild females. The gonopore setae of females and the genital papillae of males were established as field indicators of maturity by comparison with laboratory determinations of gonad weight and maturity, and, in the case of females, with breeding condition in the wild population. Sizes at the attainment of maturity were thus determined for both males and females, and two reproductive types of males were described.

Population structure was interpreted from the composition of catches, according to the results of tests for equal average catchabilities of crayfish in different sex- and size-groups based on recapture data.

Studies of growth were based primarily on the resuls of mark-recapture studies, and a number of statistical methods were combined to form a routine for the extraction of information on moult increments from mark-recapture data. Annual moult frequencies were deduced from total annual growth and/or information on moult increments, and the seasonality of moulting activity was inferred from mark-recapture records

and moult stages. The moult increments of crayfish of the two sexes, of different sizes and from two locations were compared for different seasons using analysis of covariance. Variation in the moult increments of individual crayfish, independent of size, location, and moulting season, was investigated further using multiple regression analysis. The observed types of variation in moult increment were used to categorise the types of environmental factors that may have been responsible for variation in the growth rates of wild *E. spinifer*.

Sizes at age were estimated from annual growth rates, and checked for reliability by comparison with size classes in the populations of crayfish at the commencement of study.

The feeding habits of wild *E. spinifer* were inferred from stomach contents and observations of feeding behaviour both in the laboratory and in the field. Two laboratory experiments were conducted with the aim of establishing the protein concentration of the gastric fluid and the fat content of the hepatopancreas of *E. spinifer* as indicators of feeding success, in an attempt to determine whether variations in feeding success were likely to have been responsible for the wide variations in growth rate among wild crayfish. The results of these experiments were inconclusive, and procedures were suggested for obtaining more conclusive results in future experiments.

CHAPTER ONE

GENERAL INTRODUCTION

Freshwater crayfish of the genus *Euastacus* and all other indigenous freshwater crayfishes of the southern hemisphere belong to the family Parastacidae (Riek, 1972). This family has undergone its most extensive speciation on the Australian mainland, and representatives are found over much of the continent.

The genus *Euastacus* has been treated extensively in taxonomic works (Clark, 1936, 1941; Riek, 1951, 1956, 1969, 1972; Monroe, 1977). The distribution of the genus includes much of Victoria and southern New South Wales, extending northwards through eastern New South Wales into South-eastern Queensland. Species become more restricted to mountainous areas in the north, and an isolated extension of the distribution occurs in the mountains of north-eastern Queensland (Riek, 1969). Of the eighty seven parastacid species recorded for the Australian mainland, twenty nine belong to the genus *Euastacus*, including the majority of the large species (Clark, 1941; Riek, 1969, 1972, Monroe, 1977). According to Riek, (1969) the genus may be subdivided into four species groups. The *'spinifer'* group contains fourteen species, including all those which attain a large size. *E. spinifer* is central to the relationships among these species in terms of both taxonomic morphology and distribution. The species inhabit the larger, permanent streams of river systems throughout the range of the genus, although they may also be found in less permanent headwaters.

E. spinifer is the large, spiny freshwater crayfish of the Sydney region, having been recorded from the Nepean, Parramatta, Georges, Shoalhaven (Riek, 1969) and Hacking Rivers, and from the vicinity of Gosford (Clark, 1941). It forms a conspicuous and characteristic element of the fauna of rocky streams in this region, and in contrast to representatives of several other parastacid genera in other parts of Australia, little is known of its biology. Ryder (1972) made some observations on the breeding season and size at maturity of female *E. spinifer*, and outlined the breeding season, size at maturity of females, embryonic development and the appearance and attachment of eggs and juvenile stages for a small sympatric species, tentatively identified as *E. austral asiensis*. Clark (1937) described the breeding season and appearance and attachment of eggs and juveniles for the large Victorian species *E. kershawi*, while Johnson (1979) recorded the breeding season and numbers of eggs produced by the large *E. armatus* from southern inland New South Wales. To date, there has been no information provided on the growth rates or population structures of any species of *Euastacus*. Also, the information on reproduction is incomplete, particularly with respect to size at maturity, and several of the results obtained by Clark (1937) for *E. kershawi* are anomolous in the context of results for other parastacids.

The aim of the present study was to describe the reproduction, growth, population structure and feeding habits of *E. spinifer* in a wild population. In the absence of information for similar animals under comparable conditions, the study has been largely exploratory, with the intention of providing a general framework for the study of this and related species. Experimental testing for the effects of

temperature, photoperiod and food availability on growth rates was also attempted under laboratory conditions. However, successful maintenance of *E. spinifer* under laboratory conditions was not achieved, and results have been obtained primarily from mark-recapture studies of wild crayfish. Attempts have been made wherever possible to erect hypotheses to explain observed trends and thus provide direction for further research.

There has been some interest during recent years in the large species of *Euastacus* as subjects for aquaculture. Relevant information on growth rates and size and age at maturity has not been available, and it is hoped that this study may partially fill this need.

The study is presented in two parts. Part A deals with aspects of the biology of *E. spinifer*, while in Part B a routine method is developed for the interpretation of decapod growth from mark-recapture data. This method was developed in view of the inadequacy of existing methods, and forms the basis of the studies of the growth of *E. spinifer* in Part A.

CHAPTER TWO

GENERAL METHODS

2.1 SELECTION AND DESCRIPTION OF STUDY SITE

The study was conducted on the Loddon River near Bulli, south of Sydney, New South Wales, during the years 1976-1979. The Loddon River is the most easterly tributary of the Nepean River system and forms part of the catchment of the Cataract Dam under the jurisdiction of the Metropolitan Water Sewerage and Drainage Board. Public access to the study area was thus restricted, and the greater part of the catchment was in natural condition.

The Loddon River originates on the plateau behind the Illawarra escarpment in a shallow basin, with an area of approximately 13 square kilometers at an elevation of approximately 360-380 m. in the region immediately north-west of Sublime Point. The river commences as a series of small, semi-permanent channels draining the extensive sedge swamp which overlies the Hawkesbury sandstone of the area and covers much of the basin. According to Davis (1936), this swamp is maintained by a combination of high rainfall and a high water table resulting from slow evaporation rates, the local soil structure and vegetation, and the presence of furrows at intervals of "one or two yards" at right angles to the normal drainage slope. Davis suggested that these furrows may have resulted from the burrowing activities of crayfish (*Euastacus kierensis*), which are abundant in the swamp. The swamp is characterised by a

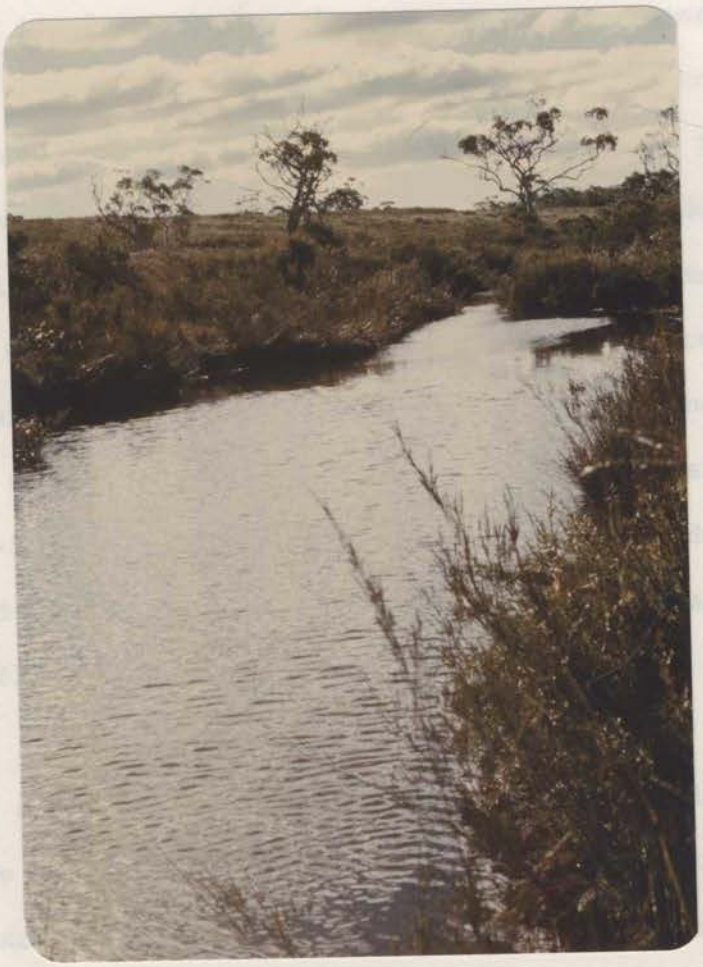


Figure 2.1: Part of the Study Area on the Loddon River. Pool 7, looking downstream, with sedge-swamp to the sides and in the distance.

deep soil layer (up to five metres) with an acid pH and a high humus content, and Davis considered that the swamp had been in its present state for a "very long period".

The Loddon River proper commences abruptly in the south-western sector of the swamp as a series of large pools connected by shallow ripples and channels. The study area consisted of the first eight of these pools, extending along approximately 500m of the river with little downstream gradient. The pools ranged from 30-100m in length and were up to 30m wide, consisting of channels excavated down through the sedge swamp to the bedrock at depths of up to 4m or more. One of the smaller pools (Pool 7) and its surrounds are illustrated in Figure 2.1. The banks of the pools were characteristically almost vertical, extending from generally less than one metre above water level to depths of up to 3m, and flow rates in the pools were negligible except during times of flood.

The study area was selected for its accessibility and the permanence of the stream, and for the large resident populations of *E. spinifer* in the pools. The habitat may be considered typical for *E. spinifer* in that the bed of the stream consisted of sandstone in the form of flat shelves, irregular outcrops and boulders, interspersed with areas of sand and gravel. The bed of the stream was typically clean in appearance; plant debris was sparsely and patchily distributed, with substantial accumulations occurring only in restricted areas. The aquatic flora was typical of *E. spinifer* habitats in that the only conspicuous vegetation consisted of dense but narrow stands of the aquatic angiosperm *Triglochin procera*

("ribbon weed") along the edges of some pools with less steeply sloping banks. Apart from an apparently normal assemblage of aquatic insects, the conspicuous macroscopic invertebrate fauna included the freshwater shrimp *Paratya australiense* and another, much smaller species of *Euastacus* (*E. kierensis*, maximum carapace length approximately 40mm) which was primarily found in the shallow areas, semi-permanent drainage channels and the adjacent sedge-swamps.

The study area may also be considered atypical in several respects. While flooding did occur, the terrain was flat and the study area was near the source of the river. Hence it is likely that flooding had less impact than on the typical sandstone streams of the Sydney region which are characteristically steep and flow through rocky gullies. It is also likely that the adjacent swamps and high rainfall provided a more continuous input of water than is generally experienced in streams around Sydney, and that the deep pools were not subject to the considerable relative decreases in water level normally associated with sandstone streams during periods of low rainfall. The hydrologic environment of *E. spinifer* in the study area may thus have been abnormally constant over an annual time scale, and the comment of Davis (1936) on the long-term stability of the sedge swamp suggests similar long-term stability in the condition of the stream. Also, the fish fauna of the Loddon River in the study area was depauperate, consisting of two species, the climbing galaxias *(Galaxias brevipinnis)* and the Macquarie perch *(Macquaria australasica)* (after Lake, 1978). Eleotrid gudgeons were absent and eels *(Anguilla* sp.) were observed on only two occasions, and were considered to be extremely rare relative to their observed

abundances in other streams in the Sydney region.

2.2 CAPTURE METHODS

Crayfish were captured using baited drop-nets (= hoop-nets) operating on a similar principle to those used widely in inland New South Wales, and those described by Morrissy (1970). The nets were designed specifically for this study so that they could be thrown accurately from the bank of a stream and carried in large numbers to inaccessible sites. The construction of a net is illustrated in Figure 2.2. The frame of each net consisted of two circular hoops of 4mm galvanised steel wire approximately 42cm in diameter. The two hoops were connected by a vertical wall of four ply nylon fishing net with a bar length of 7mm, which was continuous across the lower hoop to form the floor of the net; and a hauling rope with a cork was attached at four points to the upper hoop. Baits of fresh fish were enclosed in 'half inch' galvanised wire mesh to prevent rapid removal by the crayfish, and attached to the centre of the floor of the net by a wire hook.

When nets were in place on the bed of a stream, the upper hoop rested on the lower hoop allowing free access to the bait, and the cork served to keep the hauling ropes from fouling the crayfish. When nets were hauled for periodic inspections, the majority of crayfish that entered the net were captured while still attempting to remove the bait from its container, and prevented from escaping during hauling by the combination of water flow and the walls of the net.

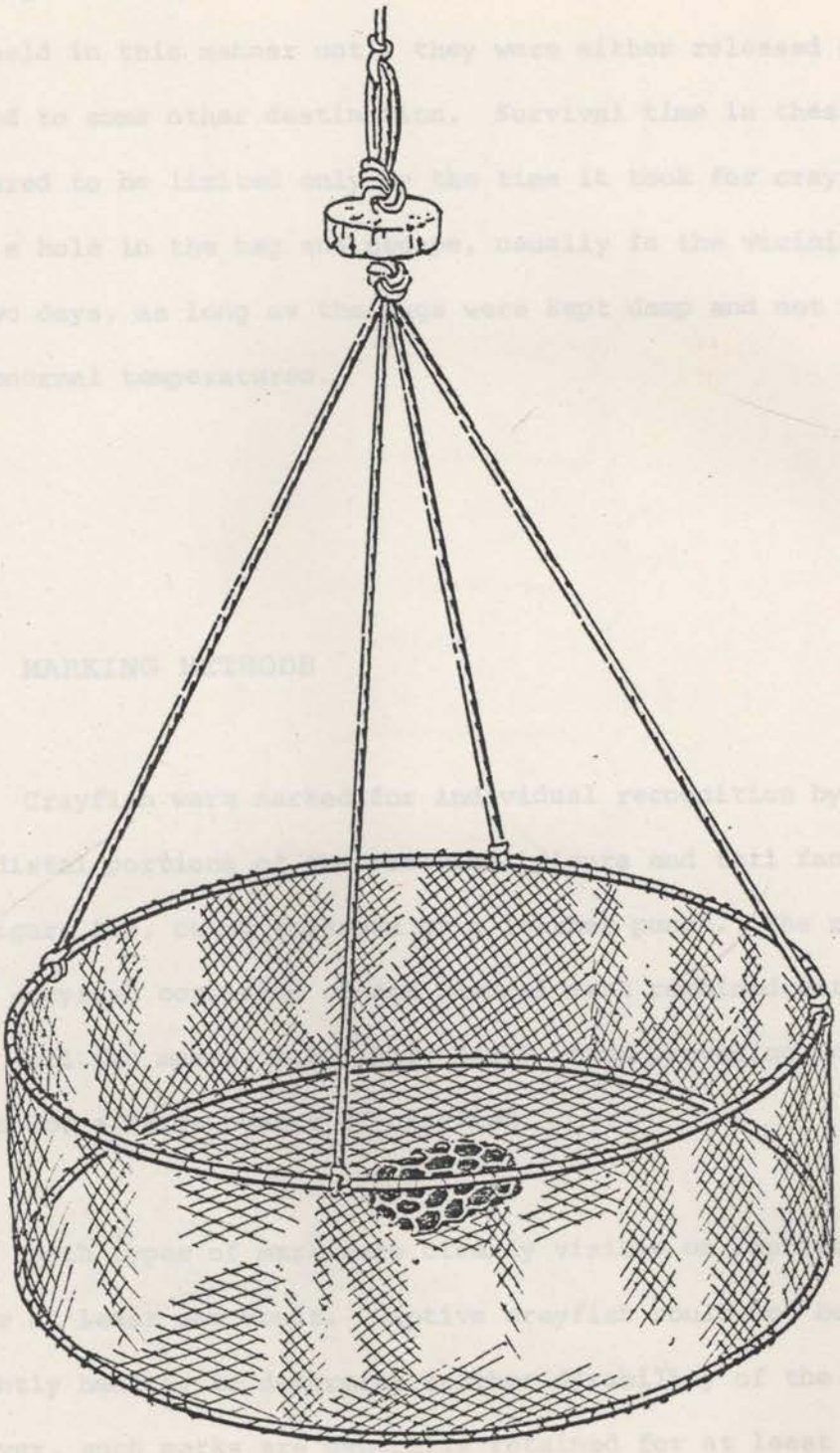


Figure 2.2: Construction of the Drop-nets used in this Study.

Upon capture, crayfish were placed into individual wet hessian bags bearing labels for the recording of relevant catch data, and held in this manner until they were either released or transported to some other destination. Survival time in these bags appeared to be limited only by the time it took for crayfish to chew a hole in the bag and escape, usually in the vicinity of one to two days, as long as the bags were kept damp and not subjected to abnormal temperatures.

2.3 MARKING METHODS

Crayfish were marked for individual recognition by removing the distal portions of the abdominal pleura and tail fan, as shown in Figure 2.3, using scissors or a leather punch. The mark for each crayfish consisted of one pleural mark combined with up to four tail fan marks, e.g., A13, I4679, thus providing individual marks for a large number of crayfish.

Both types of mark were clearly visible on captive *E. spinifer* after at least one moult. Captive crayfish could not be kept sufficiently healthy to determine further durability of the marks. However, such marks are generally retained for at least two moults in a number of reptant decapods (Wilder, 1953; Thomas, 1958; Simpson, 1961; Hepper, 1967; Cooper, 1970) and for three or more moults in others (Hopkins, 1967a; Momot, 1967; Chittleborough, 1970). Hence a conclusion that an individual *E. spinifer* had retained its mark for more than one moult would be reasonable (see Part B of this thesis).

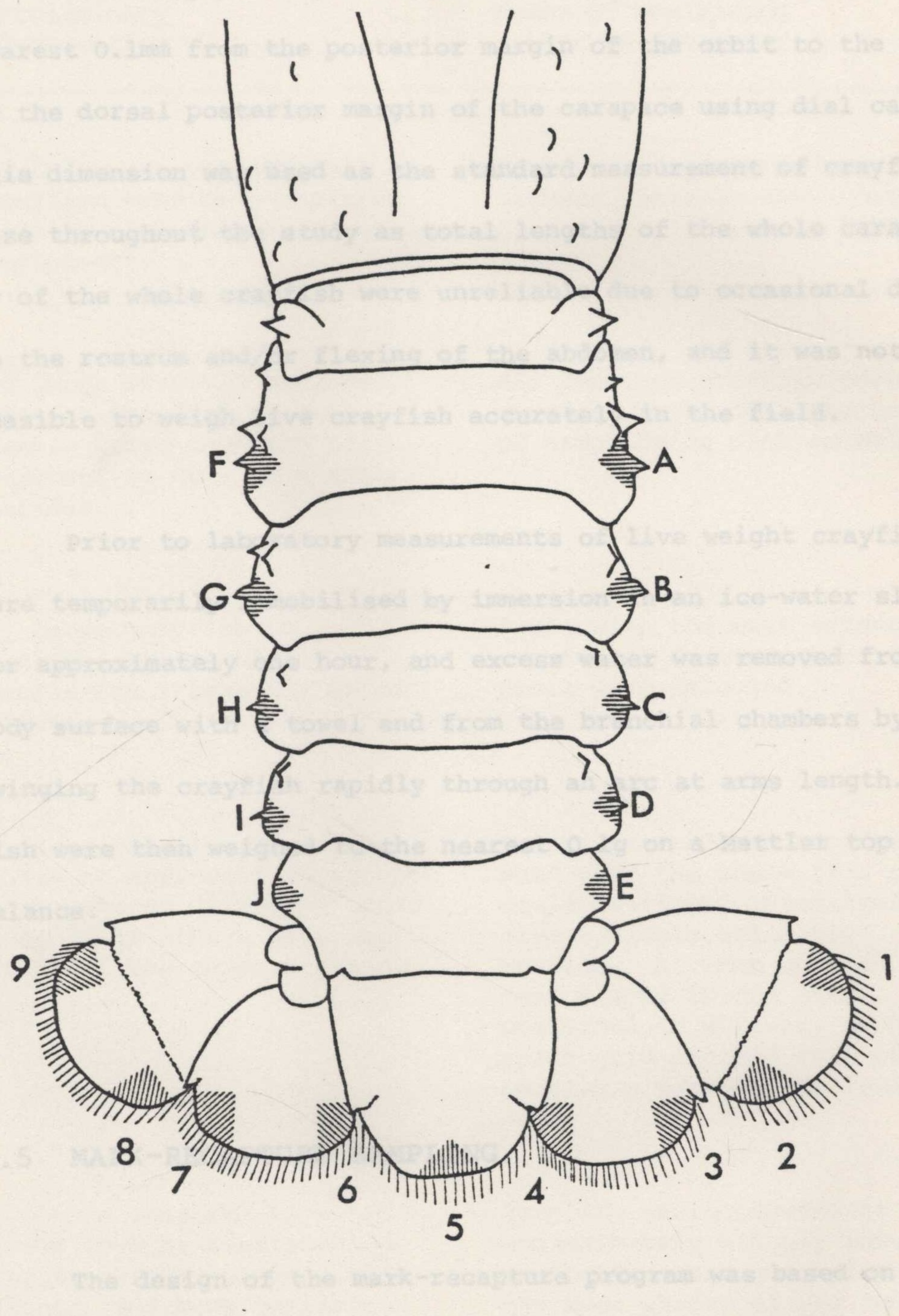


Figure 2.3: Crayfish abdomen showing positions of marks. Shaded areas denote the sections removed during marking.

2.4 MEASUREMENT OF CRAYFISH SIZE

The carapace length of each crayfish was measured to the nearest 0.1mm from the posterior margin of the orbit to the middle of the dorsal posterior margin of the carapace using dial calipers. This dimension was used as the standard measurement of crayfish size throughout the study as total lengths of the whole carapace or of the whole crayfish were unreliable due to occasional damage to the rostrum and/or flexing of the abdomen, and it was not feasible to weigh live crayfish accurately in the field.

Prior to laboratory measurements of live weight crayfish were temporarily immobilised by immersion in an ice-water slush for approximately one hour, and excess water was removed from the body surface with a towel and from the branchial chambers by swinging the crayfish rapidly through an arc at arms length. Crayfish were then weighed to the nearest 0.1g on a Mettler top pan balance.

2.5 MARK-RECAPTURE SAMPLING

The design of the mark-recapture program was based on a series of preliminary observations of *E. spinifer* in the study area during 1976 (Table 2.1). Two pools were selected for the mark-recapture sampling, referred to as Pools 3 and 7 (of the eight pools in the study area numbered towards the source of the river), with lengths of 54m and 40m and approximate areas of 770 and 320m^2

Table 2.1

Preliminary Observation	Means of maximising recapture frequency
1. Large numbers of small crayfish were only captured using a high density of net deployment.	Nets were deployed at the highest possible densities.
2. Large crayfish excluded smaller crayfish from the nets. Large crayfish often returned to nets soon after release.	All captured crayfish were retained until the completion of sampling on each occasion.
3. Large crayfish in particular were highly mobile and frequently moved between pools.	Pools with the most extensive separation from adjacent pools were selected.
4. Some crayfish were captured at different locations in a pool on different occasions while others were captured at the same location.	Pools were selected for size such that the whole of a pool could be fished intensively over a single collection session. At each session nets were placed in the same positions in a fixed, rectangular grid pattern that could be easily reproduced.
5. There appeared to be a trend towards consistently larger catches on moonless nights, and more variable catches on moonlight nights.	Sampling was conducted at approximately monthly intervals during the last quarter and new moon phases of the lunar cycle when the moon was not visible in the night sky.

respectively. Net stations were permanently marked with pegs at equal intervals along the length of each pool, at intervals of 6m for Pool 3 and 5m for Pool 7, and the number of nets deployed at each station was roughly proportional to the measured width of the pool at that point. Forty nets were used in Pool 3, approximating the maximum number of nets that could be hauled, checked and returned by two workers during the period of thirty minutes between hauls. Twenty two nets were used in Pool 7, providing a sampling intensity somewhat higher than that in Pool 3, and approaching the maximum number of nets that could be comfortably handled in an area of that size.

Sampling was conducted at monthly intervals at each pool, commencing between one and two hours after dark, from May 1977 to December 1978. The nets at each station were placed at equal intervals across the width of the stream and hauled in, checked, and returned to their original positions at intervals of thirty minutes, for five hours. Upon capture, crayfish were placed in individual wet hessian bags bearing the time and position of capture and the number of the haul, and retained until the completion of sampling. Crayfish were then measured and marked, or partially healed marks were renewed, and the time, haul number, position of capture, carapace length, mark number, sex, and condition of secondary sex characteristics (see Chapter 3) were recorded. Each crayfish was also allocated to a moult stage (Section 2.7), and note was made of any injuries or regenerating appendages. In the case of mature females, records were also kept of the presence of spermatophores, eggs, or first, second or third stage juveniles. Crayfish were then returned to the part of the pool from which they were captured.

2.6 MEASUREMENT OF WATER TEMPERATURE

It was considered that a small number of measurements of water temperature prior to sampling would not be representative of the range of variation over a pool on any given occasion due to the complex topography of the pools, and it was not feasible to construct a detailed temperature profile for each pool at each sampling. The hauling of nets for the duration of a sampling resulted in considerable mixing of the water in each pool. Hence average water temperatures were estimated from measurements taken at a specific point at the completion of each sampling. Temperature was measured approximately 0.5m below the surface using a laboratory thermometer graduated in degrees Celsius.

2.7 ALLOCATION OF CRAYFISH TO MOULT STAGES

Crayfish examined in the mark-recapture study were allocated to either of two moult stages as a record of the recent occurrence of moulting. These were 'early intermoult' (Ce), and 'intermoult' (C). Crayfish in stage Ce were characterised by branchiostegites that were flexible under light to moderate pressure from thumb and index finger, and by the absence or only slight development of reddish-brown deposits on the ventral surface. Crayfish in stage C were characterised by firm branchiostegites and slight to heavy deposits on the ventral surface.

2.8 DISSECTION TECHNIQUES

Crayfish were immobilised in a slush of ice and water for approximately one hour prior to dissection. The contents of the cephalothorax were exposed by gripping the dorsal posterior margin of the carapace medially and lifting it upwards and anteriorly until at an angle of approximately 45° to the long axis of the body. The midgut and hepatopancreas were then severed with sharp scissors at their junctions with the foregut, and the carapace, containing the foregut, was completely removed from the rest of the body by continuing the forward movement. Gonads were exposed by careful removal of the heart and dorsal blood vessels under a dissecting microscope, and, if necessary, by the removal of the dorsal walls of the first two abdominal segments. The hepatopancreas was exposed, where applicable, by the further removal of the gonads, and the foregut was removed from the isolated carapace by severing its extrinsic musculature when samples of its contents were required.

2.9 REMOVAL OF LARGE CRAYFISH FROM POOL 7

Twenty crayfish with carapace lengths greater than 70mm were removed from Pool 7 during 1976 for gonad studies prior to the decision to use this pool for mark-recapture studies of growth. The population in this pool had thus been altered, and considering the possibility that large crayfish interrupted the feeding activities of smaller crayfish (see Table 2.1), this might have affected the subsequent growth rates of the smaller individuals. Removal of

crayfish with carapace lengths greater than 70mm from Pool 7 was thus continued throughout the mark-recapture study in an attempt to maintain the altered condition of the population and facilitate the interpretation of any differences between the growth rates of crayfish in Pools 3 and 7.

Possible effects of this removal are discussed in Chapter 9. Crayfish used for subsequent gonad studies included those removed from Pool 7 and locations other than Pool 3.

PART A

ASPECTS OF THE BIOLOGY OF *E. SPINIFER*

CHAPTER THREE

REPRODUCTION

3.1 INTRODUCTION

The external reproductive morphology of parastacids, including *Euastacus*, has been previously described in numerous taxonomic works (e.g., Riek, 1951, 1956, 1969, 1972). The gonopores of females are located on the coxae of the third pereiopods, while the male gonopores are produced into papillae on the coxae of the fifth pereiopods. The sexual dimorphism seen in many decapods (Barnes, 1974) is not marked in parastacids, except that males may attain a slightly larger size, and have more robust chelae and a narrower abdomen.

Some aspects of the reproductive cycle have been described for species in most parastacid genera, e.g., *Euastacus kershawi* (Clark, 1937), *E. australasiensis* and *E. spinifer* (Ryder, 1972), *Cherax tenuimanus* (Shipway, 1951; Morrissy, 1970, 1975), *C. albidus* (Woodland, 1967), *C. destructor* (Johnson, 1979), *Paranephrops planifrons* (Hopkins, 1967b), *Parastacoides tasmanicus* (Lake and Newcombe, 1975), *Engaeus cisternarius* (Suter, 1977). Descriptions of the reproduction of *E. spinifer* in this study have been consequently limited to aspects that have not been described adequately for *Euastacus*, or to areas in which there are discrepancies in the literature.

As part of this study was concerned with variation in the growth rates of *E. spinifer* with sex and size, it was considered

necessary to develop methods for determining the state of maturity of individual live crayfish, apart from determining average size at maturity. Mature females of other decapods have been identified by the presence of attached eggs during the breeding season (e.g., Penn, 1943; Shipway, 1951; Heydorn, 1969; Berry, 1971; Chittleborough, 1976b), or by the presence of egg-bearing setae on the pleopods (e.g., Fielder, 1964b; Morrissy, 1970; Farmer, 1974b). Mature males have generally been outwardly unidentifiable except in instances where a specialised copulatory apparatus is present at maturity (e.g., Penn, 1943; Fielder, 1964b; Heydorn, 1969; Berry, 1971; Weagle and Ozburn, 1972; Farmer, 1974b; Stein,et. al., 1977).

Ryder (1972) observed that the gonopores of large female *Euastacus* were surrounded by setae, and on the basis of five mature specimens, two of *E. spinifer* and three of *E. australasiensis*, concluded that the presence of these setae indicated sexual maturity. The development of these setae has thus been compared with the development of egg-bearing setae, the occurrence of spawning, and ovarian maturity, in an attempt to establish a reliable field indicator of maturity in female *E. spinifer*.

Male parastacids lack specialised copulatory apparatus. However, Shipway (1951) noted that the genital papillae of male *Cherax tenuimanus* become more erect during the mating season. Variation in the degree of inflation of the genital papillae of male *E. spinifer* was noted early in this study, hence an attempt was made to establish whether the degree of inflation of the genital papillae could be used as an indicator of maturity for male *E. spinifer*.

3.2 METHODS

3.2.1 Spermatophores, Eggs and Juvenile Stages.

The appearance of spermatophore material was described before and after its attachment to females, and the structure of attached spermatophores was described from hand-cut sections of fresh spermatophore approximately 0.5mm thick.

Eggs, egg attachment, juvenile stages and juvenile attachment were briefly described, and any differences from accounts of other parastacids were noted. The maximum and minimum diameters of ten eggs from each of five crayfish collected in June 1978 were measured to the nearest 0.1mm, using dial calipers, to provide estimates of egg size. Also, the relationship between the number of eggs and the size of females was estimated as the linear regression of egg number on carapace length for ten crayfish with carapace lengths in the approximate range 70-105mm (Figure 3.1).

3.2.2 Events in the Annual Reproductive Cycle.

Events in the annual reproductive cycle of *E. spinifer* were described from the condition of mature females captured each month as part of the mark-recapture study of growth. These catches were taken from Pools 3 and 7 at the study site during the first week of each month for the period May 1977 to December 1978. Catches taken during the first week of both November and December were supplemented with further catches taken in late December 1977, late November 1978, and mid-December 1978, and additional information was obtained from

catches taken in mid June, early July, and mid-November in 1976. Mature females were identified upon capture using the characteristics described elsewhere in this chapter, and it was noted whether they were carrying spermatophores, eggs, or first, second or third stage juveniles. For the sake of brevity, such females will be described as being in a particular brooding state. Numbers of females in the different brooding states were tabulated for each period of each month (Table 3.1), and trends for the three years were compared using the raw data. The raw data were combined for the three years on the basis of this comparison, and the percentage of mature females in each brooding state was plotted against the time of capture for the period during which reproductive activity was apparent (Figure 3.2). Periods of the year during which spawning, hatching of eggs, and the moulting and departure of juveniles occurred were inferred from the relative abundances of females in the different brooding states.

Average water temperatures were recorded for each monthly sampling at Pools 3 and 7 for the period May 1977 to December 1978 (Chapter 2) and plotted against month (Figure 3.3). Periods during which mating, spawning and the departure of juveniles occurred were indicated on this graph, and compared with the annual water temperature regime.

3.2.3 Attainment of Maturity in Females.

Female *E. spinifer* with carapace lengths in the approximate range 20-100mm were collected from the study area in 1976, 1977, and 1978 during May and June, i.e., just prior to spawning. These

crayfish were returned to the laboratory, anaesthetised by chilling, and weighed to the nearest 0.1g (as in Chapter 2). Ovaries were removed under a dissecting microscope, after cutting away any adhering blood vessels and severing the oviducts at the points at which they turned ventrally around the lateral margins of the hepatopancreas. Ovaries were then drained briefly on a piece of tissue paper and weighed to the nearest 0.001g on a Mettler Type H6T digital analytical balance. The contribution of reproductive tissues to body weight was expressed for each crayfish as a 'gonosomatic index' of the form

[gonad weight/(body weight - gonad weight.)] x 100%

Gonosomatic index was plotted against body weight (Figure 3.7), and changes in gonosomatic index with body weight were noted. A carapace length scale was determined from the length-weight relationship in Appendix C and included in Figure 3.6 to facilitate comparison with other results in which carapace length was used as the measurement of body size.

The yolk of the eggs and mature oocytes of *E. spinifer* was a medium to dark maroon in colour. Hence white oocytes were classed as non-yolky, while maroon oocytes were classed as yolky. Colour thus provided a rapid means of assessing the state of development of ovaries for the purpose of identifying immature, incipiently mature and mature females, and histological examination of the ovaries was not undertaken. The size, location and shape of the ovary is illustrated (Figure 3.4) for a female with developing oocytes that was captured during December 1978, and the approximate size and position of a mature ovary prior to spawning is also indicated.

The left second pleopod and the coxa of the left third pereiopod (i.e., the coxa bearing the left gonopore) were also removed from each of the dissected females and fixed in formol-alcohol (Humason, 1972) for later examination. The second pleopod was selected as typical of the other pleopods in its arrangement of setae. The major types of setae surrounding the gonopores and on the pleopods were described (Figure 3.5), classified according to Thomas (1970), and the distributions of the setae were recorded for each crayfish after microscopic examination. Three patterns of setal distribution were proposed, termed Stages 0, 1 and 2 (Figure 3.6). Each crayfish was allocated to one of these stages from observation of the gonopore setae with the naked eye. This allocation was compared with the results of previous microscopic examinations, and the relationship between the stage of setal development and ovarian maturity was indicated for each crayfish on the graph of gonosomatic index vs. body weight (Figure 3.7).

More extensive information on changes with carapace length in the relative abundances of females in the different setal stages was obtained in the field. All females that were examined during the study were allocated to carapace length classes of 5mm width, commencing at 9.95mm, and the percentages of the crayfish in each size class that were in Stages 0, 1 and 2 were calculated. Ninety five percent confidence limits for values on repeated sampling were estimated for each percentage using a normal approximation for samples of more than thirty crayfish in each size class (Snedecor and Cochran, 1967), or from tables based on the binomial distribution (Crow, 1956) for smaller samples. Percentages and confidence limits were plotted against the midpoint of each size class (Figure

3.8), and changes with carapace length in the relative abundances of crayfish in Stages 0, 1 and 2 were noted.

Twenty additional females in Stages 1 and 2 were captured and examined in the laboratory during November to early December in 1976 and 1978. Numbers of crayfish in each stage with developing, yolky oocytes and immature oocytes were determined (Table 3.2), in order to predict which crayfish were likely to spawn in the following season. Stage 1 was further sub-divided to determine whether variations in setal development within the stage were related to future spawning.

The laboratory results for the relationship between the setal stages and maturity were extended using field data. Females examined in the field were by definition mature if, from capture records, they were known to have spawned, hence the setal stages to which these crayfish had been allocated on capture were noted. Additional information on the reliability of the allocation of immature females to the setal stages was provided by the mark-recapture records of individual crayfish. Setal stages were compared between captures for each individual female that was captured more than once, and any inconsistencies in allocations that could not be explained by transitions at moulting from Stage 0 to Stage 1 or from Stage 1 to Stage 2 were noted.

The maximum diameter of the left gonopore was measured for each of a series of females to the nearest 0.1mm with dial calipers, and plotted against carapace length (Figure 3.9) for comparison with the results of Ryder (1972). Ryder's results were in the form of

the relative area of the gonopore, i.e., the area of the gonopore of each female divided by the smallest area encountered. Data for the comparison were measured from Figure 32. in Ryder (1972) and plotted as the square root of relative gonopore area (Figure 3.10) to convert the areas to a linear scale comparable with the measurements of gonopore diameter obtained in this study. Ryder's original scale was also included, and the state of maturity of both Ryder's crayfish and crayfish in the present study were indicated. In both studies, the crayfish were captured just prior to spawning and mature females were identified by the yolkiness of the ovaries.

3.2.4 Attainment of Maturity in Males.

Male *E. spinifer* with carapace lengths in the approximate range 20-90mm were collected from the study area in 1976, 1977 and 1978 during May, i.e., during the mating period. These crayfish were returned to the laboratory, anaesthetised by chilling, and weighed to the nearest 0.1g. Gonads, including both testis and vasa deferentia, were removed under a dissecting microscope after cutting away any adhering blood vessels and severing the vasa deferentia close to the gonopores. Gonads were drained briefly on a piece of tissue paper, weighed to the nearest 0.001g on a Mettler Type H6T digital analytical balance, fixed for one hour in alcoholic Bouin (Humason, 1972), and stored in 70% alcohol. Gonosomatic indices were calculated as for females, and plotted against body weight (Figure 3.12). Mature males were identified by the presence of sperm in open spermatic cysts and ducts in the testis. Sperm were identified in 10µm paraffin sections that were taken at several places along the posterior lobes of the testis and stained

in Delafield's hematoxylin and eosin by the regressive method (Humason, 1972).

Male crayfish were allocated to one of three groups depending upon whether the genital papillae were uninflated, inflated, or highly inflated (Figure 3.11) and the degrees of gonad development typical of crayfish in each of these three groups is illustrated (Figure 3.13). The condition of the genital papillae and state of maturity of each male crayfish examined in the laboratory were indicated on the graph of gonosomatic index vs. body weight (Figure 3.12), and relationships between inflation of the genital papillae, maturity and gonosomatic index were noted.

All male crayfish captured during the period of study were allocated to carapace length classes of 5mm width commencing at 9.95 mm, and percentages of crayfish in each size class with uninflated, inflated and highly inflated genital papillae were calculated with 95% confidence limits as for females. Percentages and confidence limits were plotted against the mid-points of the size classes (Figure 3.14), and trends with carapace length in the relative abundances of males with uninflated, inflated and highly inflated genital papillae were described.

Records of marked crayfish that were captured more than once were also examined to check for variability through time in the degree of inflation of the genital papillae of individual crayfish (Table 3.3). It was also noted that occasional crayfish had aberrant numbers of gonopores. Records of crayfish of this type were tabulated (Table 3.4), and two such individuals were examined in the laboratory. The results of these examinations are included in Figure 3.12.

3.3 RESULTS

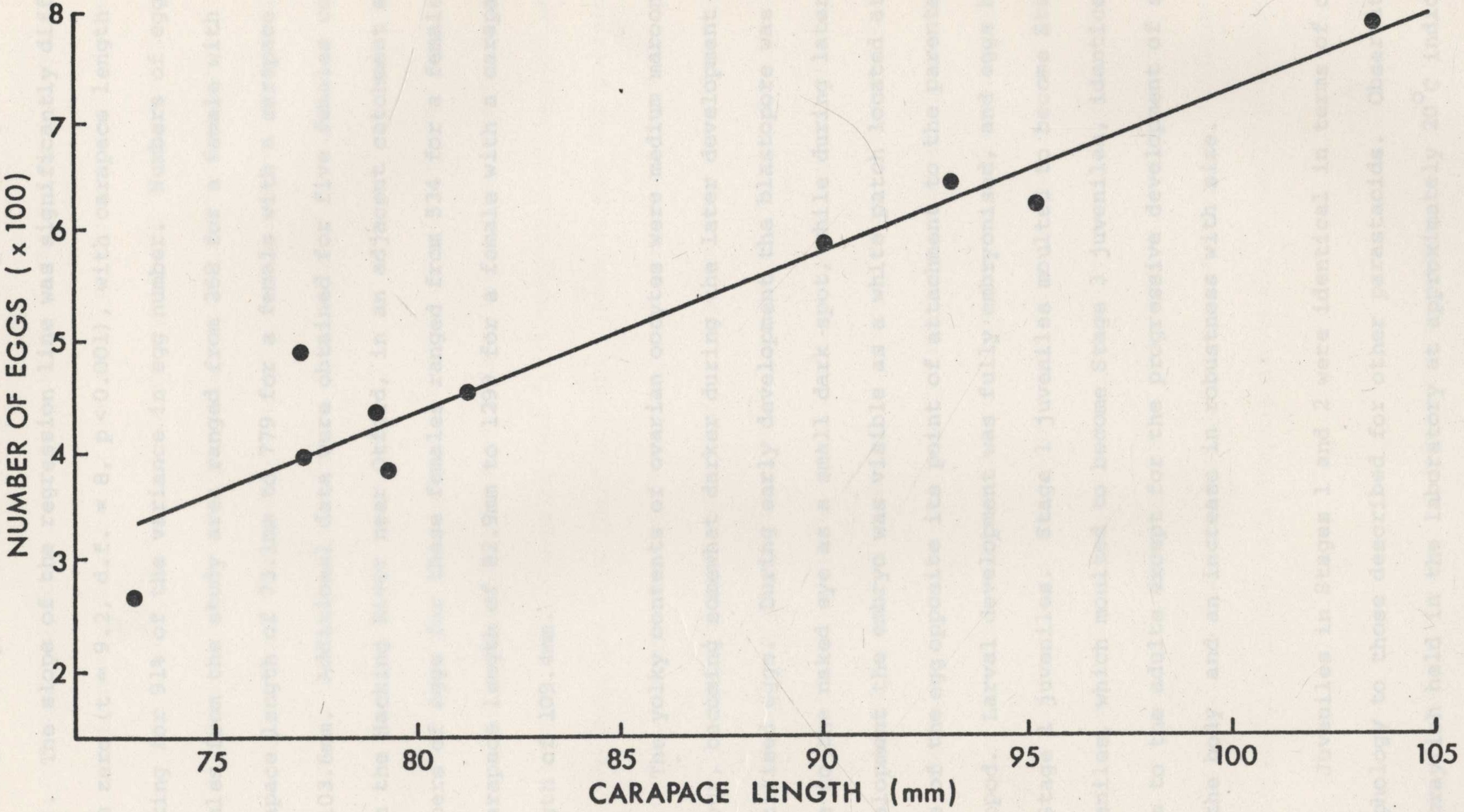
3.3.1 Spermatophores, Eggs and Juvenile Stages.

Spermatophores appeared as translucent, grey-white, irregularly-shaped masses of tough, gelatinous material distributed patchily over the coxae of the fourth and fifth pereiopods and the adjacent sternal plates of large females. In section, each spermatophore consisted of an amorphous matrix containing an irregularly distributed, highly convoluted tubule, approximately 0.05mm in diameter, in which the sperm were located. Spermatophore material obtained from the distal vasa deferentia of mature male crayfish was white in colour, of thick but plastic consistency and extremely adhesive, setting rapidly after release from the vasa deferentia.

Eggs were ellipsoid in shape, and maximum and minimum diameters of individual eggs fell in the respective ranges 3.2-3.9mm and 2.4-2.9mm, with mean diameters of 3.5 and 2.7mm. Two types of setae, termed plumose setae and oosetae, were present on the pleopods of mature females (see Section 3.3.3). Eggs were attached, either individually or in bunches, to the medial margins of the basipodites, to all margins of the endopodites except for the very tips, and to the proximal lateral margins of the exopodites of all pleopods by cords consisting of several oosetae twisted together. This distribution of eggs corresponded to the distribution of long oosetae, with the majority of the eggs carried on the endopodite. Eggs were never observed to be attached to plumose setae.

The relationship between the number of eggs and carapace length

Figure 3.1: Relationship Between the Number of Eggs and the Carapace Lengths of Female *E. spinifer.*


NUMBER OF EGGS (x 100)
8
7
6
5
4
3
2
75
80
85
90
95
100
105
CARAPACE LENGTH (mm)

for female *E. spinifer* was well described by a straight line (Figure 3.1). The slope of the regression line was significantly different from zero ($t = 9.2$, d.f. = 8, $p < 0.001$), with carapace length accounting for 91% of the variance in egg number. Numbers of eggs for females from the study area ranged from 268 for a female with a carapace length of 73.1mm to 779 for a female with a carapace length of 103.6mm. Additional data were obtained for five females collected from the Hacking River near Otford, in an adjacent catchment area. Numbers of eggs for these females ranged from 534 for a female with a carapace length of 82.9mm to 1299 for a female with a carapace length of 109.4mm.

The yolky contents of ovarian oocytes were medium maroon in colour, becoming somewhat darker during the later development of fertilised eggs. During early development the blastopore was visible to the naked eye as a small dark spot, while during later development the embryo was visible as a white patch located at the pole of the egg opposite its point of attachment to the parental pleopod. Larval development was fully embryonised, and eggs hatched as Stage 1 juveniles. Stage 1 juveniles moulted to become Stage 2 juveniles, which moulted to become Stage 3 juveniles, identical in form to the adults except for the progressive development of spines on the body and an increase in robustness with size.

Juveniles in Stages 1 and 2 were identical in terms of overall morphology to those described for other parastacids. Observations of crayfish held in the laboratory at approximately 20°C indicated that Stage 1 juveniles were attached to the egg membrane by a thread running from the telson for a period of at least several hours after

hatching. Approximately one day after hatching the telson threads were no longer apparent, and both Stage 1 and Stage 2 juveniles were subsequently attached to the setae of the parental pleopods by recurved hooks, one extending proximally in the medial plane from the distal end of the dactylus of each of the fourth and fifth pereiopods. These hooks closed onto a series of serrations on the body of the dactylus, providing a firm grip on the setae.

When brooding females were held in captivity they began to devour their offspring after periods ranging from several days to several weeks, and offspring had difficulty in hatching and moulting when detached from their mother. Hence it was not possible to describe with any accuracy the timing of stages in the development of offspring in a particular brood. Observations of offspring at different stages of development on several different females indicated that the development of all offspring on a given female was synchronised to within a period of several days. Observations of two females held in the laboratory indicated that all Stage 3 juveniles departed voluntarily from the parent female over periods of three to four days, although prior to this many Stage 3 juveniles made short excursions over and away from the parent. For the sake of brevity in other parts of this thesis, the voluntary departure of juveniles from the parent has been termed the 'release of juveniles', although this description is not strictly accurate.

3.3.2 Events in the Annual Reproductive Cycle.

Numbers of females captured at monthly samplings were often small, in spite of sampling efforts, and not all months for the

Table 3.1: Seasonal Changes in the Numbers of Mature Female *E. spinifer* in Different Brooding States.

*Month	Brooding State						
	O	S	S+E	E	J_1	J_2	J_3
YEAR - 1976							
June - mid		4					
July		1	1				
November - mid						6	
YEAR - 1977							
May	6						
June	2	1					
July			2				
August			1	4			
September		1	2	6			
October				10			
November				2	4		
December	1						5
December - late	6						
YEAR - 1978							
January	9						
February	2						
March	3						
May	1						
June		8					
July				1			
August		1	2				
September				3			
October				6			
November	1				4		
November - late						6	
December	*1						2
December - mid	4						

* Month - sampling during first week of month unless otherwise stated.

*[1] O - not brooding; S - carrying spermatophore only; S+E - carrying spermatophore and eggs; E - carrying eggs only; J_1, J_2, J_3 - carrying juveniles in Stages 1, 2 or 3.

*[2] Juveniles recently released.

period 1976-78 were represented (Table 3.1). Nevertheless, the available data indicated that trends through time in the numbers of females in different brooding states were similar for the three years. These trends were summarised in Figure 3.2. The majority of mature females mated during May, and were observed to be carrying spermatophores in early June. Spawning typically occurred during June, and the majority of mature females carried eggs in early July. The percentage of females that retained spermatophores after spawning was initially high at around 60% in early July, declining to zero by early October. Mark-recapture data indicated that, for at least one female, the spermatophore was retained for at least one month after spawning. Two immature females mated but did not lay eggs, and spermatophores were retained for at least two months after mating.

A small percentage of mature females captured in July, August and September carried spermatophores but not eggs. The value of 20% for July was from a single female, captured in 1976, for which no further records were available. Hence it could not be determined if this female spawned subsequently. The percentage for August was from a single female that was first observed to be carrying a spermatophore in June 1978, and was not carrying eggs in November 1978, i.e., it did not spawn, although it had spawned successfully during the previous year, while the percentage for September was from a single female captured in 1977 that carried eggs during October and November of that year. The small percentages of females carrying spermatophores but not eggs during July to September thus resulted from successful mating followed by both delayed spawning and failure to lay eggs.

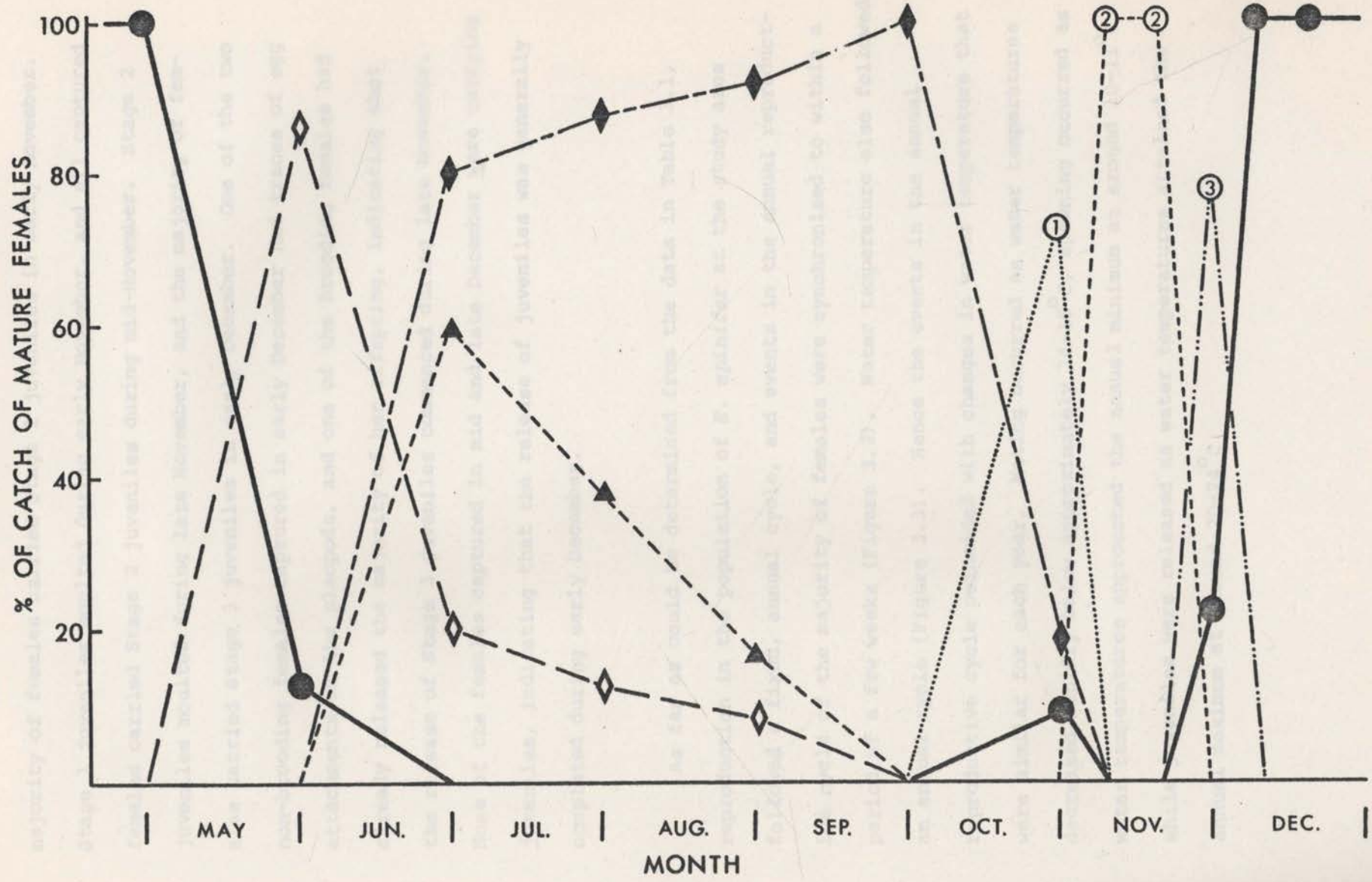
Figure 3.2: Seasonal Changes in the Numbers of Mature Female *E. spinifer* in Different Brooding States, Combined for 1976-78.

Key:-

● - not brooding; ◊ - carrying spermatophore only;

▲ - carrying spermatophore and eggs; ♦ - carrying eggs only;

① ② ③ - carrying juveniles in Stages 1, 2 or 3.


100
80
60
40
20
% OF CATCH OF MATURE FEMALES
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
MONTH
1
2
2
3

The eggs of most females hatched during October and the majority of females carried Stage 1 juveniles in early November. Stage 1 juveniles moulted during early November, and all captured females carried Stage 2 juveniles during mid-November. Stage 2 juveniles moulted during late November, and the majority of females carried Stage 3 juveniles in early December. One of the two non-brooding females captured in early December had traces of egg attachments on the pleopods, and one of the brooding females had already released the majority of her offspring, indicating that the release of Stage 3 juveniles commenced during late November. None of the females captured in mid and late December were carrying juveniles, indicating that the release of juveniles was generally completed during early December.

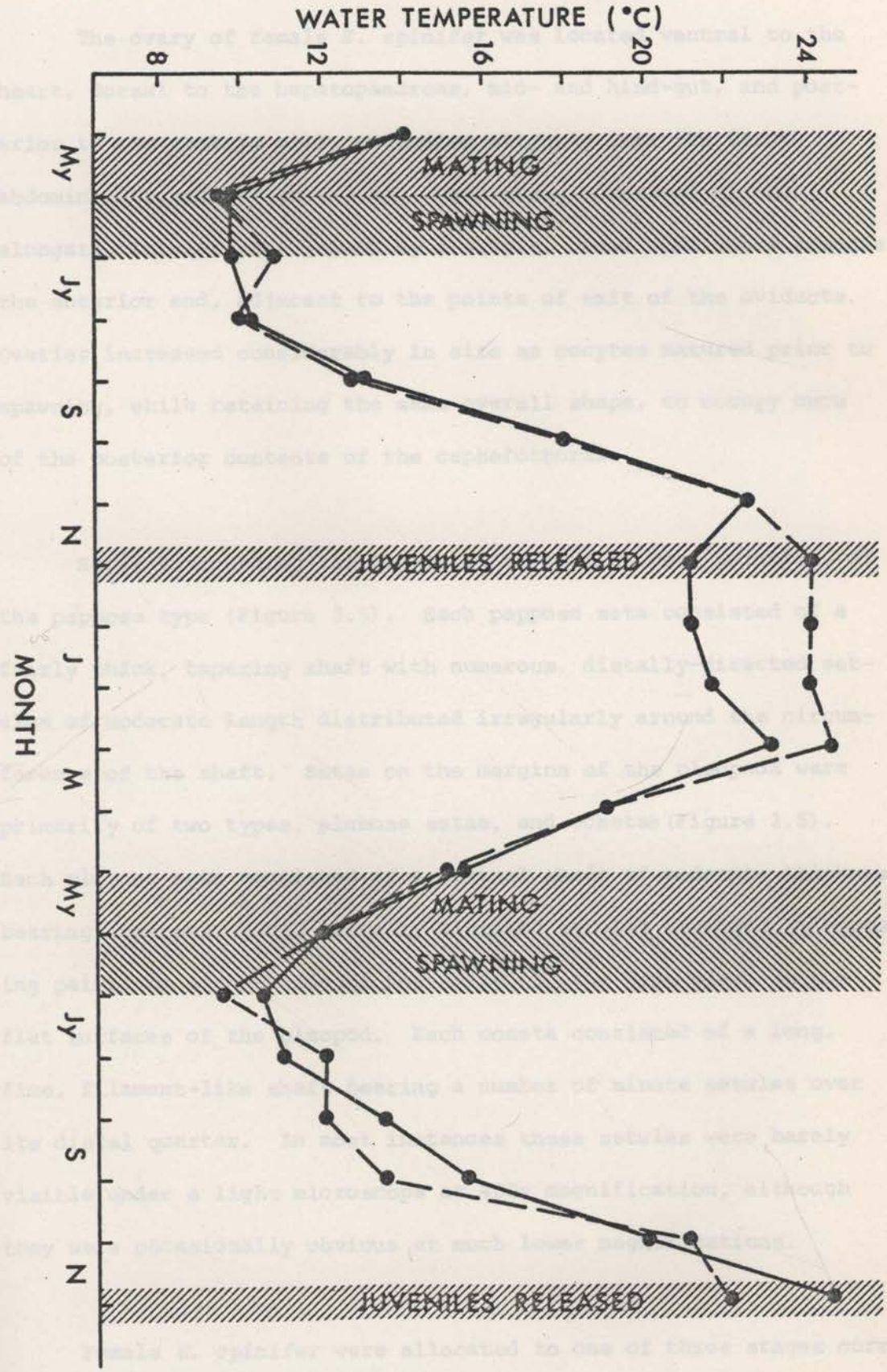
As far as could be determined from the data in Table 3.1, reproduction in the population of *E. spinifer* at the study area followed a fixed, annual cycle, and events in the annual reproductive cycle of the majority of females were synchronised to within a period of a few weeks (Figure 3.2). Water temperature also followed an annual cycle (Figure 3.3). Hence the events in the annual reproductive cycle coincided with changes in water temperature that were similar for each year. Mating occurred as water temperatures decreased rapidly below approximately 14-15^{o}C, spawning occurred as water temperatures approached the annual minimum at around 10-11^{o}C, while juveniles were released as water temperatures attained the annual maximum at around 20-24^{o}C.

Figure 3.3: Relationship Between the Annual Water Temperature Regime and the Annual Reproductive Cycle of *E. spinifer*.

Key:-

— — - Pool 3

——— - Pool 7


WATER TEMPERATURE (°C)
8
12
16
20
24
MONTH
My
Jy
S
N
J
M
MATING
SPAWNING
JUVENILES RELEASED
MATING
SPAWNING
JUVENILES RELEASED

3.3.3 Attainment of Maturity in Females.

The ovary of female *E. spinifer* was located ventral to the heart, dorsal to the hepatopancreas, mid- and hind-gut, and posterior to the gastric mill, extending slightly into the first abdominal segment (Figure 3.4). Each ovary consisted of two elongate, tubular sacs joined by a single, broad commissure towards the anterior end, adjacent to the points of exit of the oviducts. Ovaries increased considerably in size as oocytes matured prior to spawning, while retaining the same overall shape, to occupy much of the posterior contents of the cephalothorax.

Setae surrounding the gonopores of females were primarily of the pappose type (Figure 3.5). Each pappose seta consisted of a fairly thick, tapering shaft with numerous, distally-directed setules of moderate length distributed irregularly around the circumference of the shaft. Setae on the margins of the pleopods were primarily of two types, plumose setae, and oosetae (Figure 3.5). Each plumose seta consisted of a central shaft of moderate thickness bearing numerous, long, distally-directed setules arranged in opposing pairs, on either side of the shaft, in the same plane as the flat surfaces of the pleopod. Each ooseta consisted of a long, fine, filament-like shaft bearing a number of minute setules over its distal quarter. In most instances these setules were barely visible under a light microscope at 400x magnification, although they were occasionally obvious at much lower magnifications.

Female *E. spinifer* were allocated to one of three stages corresponding to three observed patterns of setae around the gonopores (Figure 3.6). Crayfish in Stage 0 were characterised by an absence

Figure 3.4: Anterior Dorsal View of a Mature Female *E. spinifer* Showing the Ovary *in situ*.

Key:-

O - ovary (developing), broken lines show the approximate extent of the ovary prior to spawning;

OD - oviduct;

G - gastric mill;

I - hind-gut;

H - hepatopancreas.

N.B. The dorsal walls of the carapace and first abdominal segment, the heart and dorsal blood sinuses, and the posterior dorsal musclature of the gastric mill have been removed.

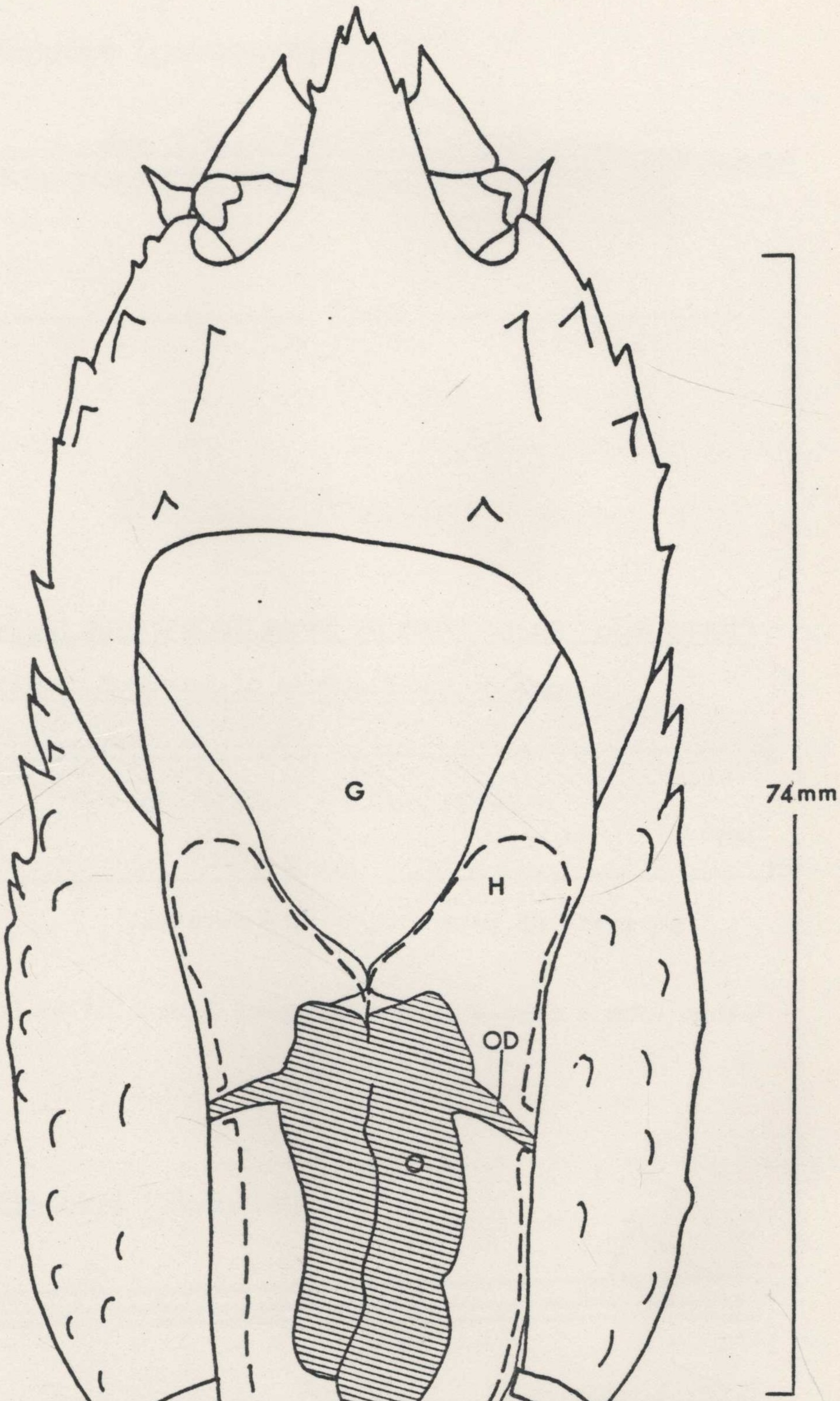

G
H
OD
74mm

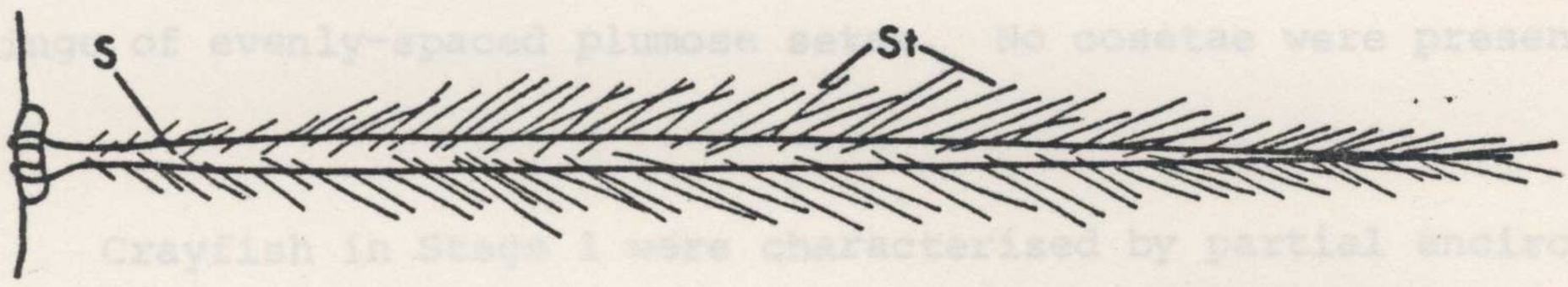
Figure 3.5: Major Types of Setae Surrounding the Gonopores and on the Pleopods of Female *E. spinifer.*

Key:-

S - shaft of seta ; St - setules.

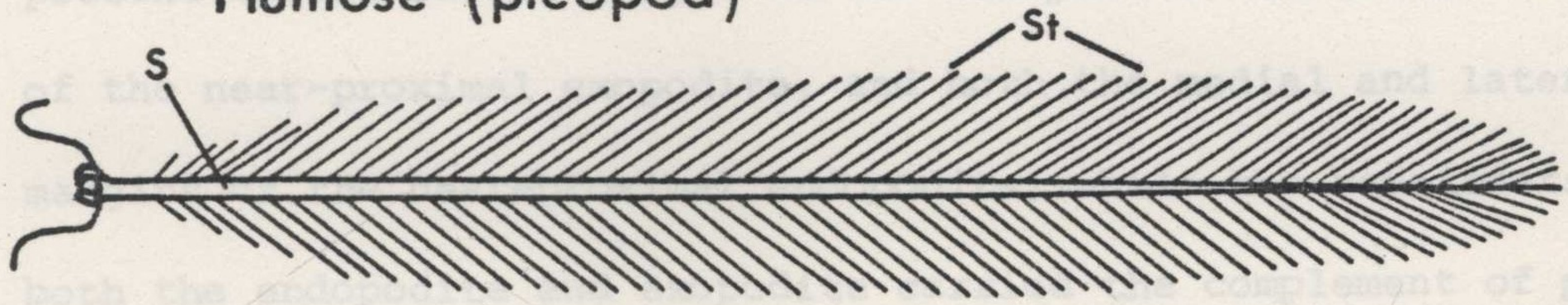
Setae from a female with a carapace length of 89.0mm.

Pappose (gonopore)



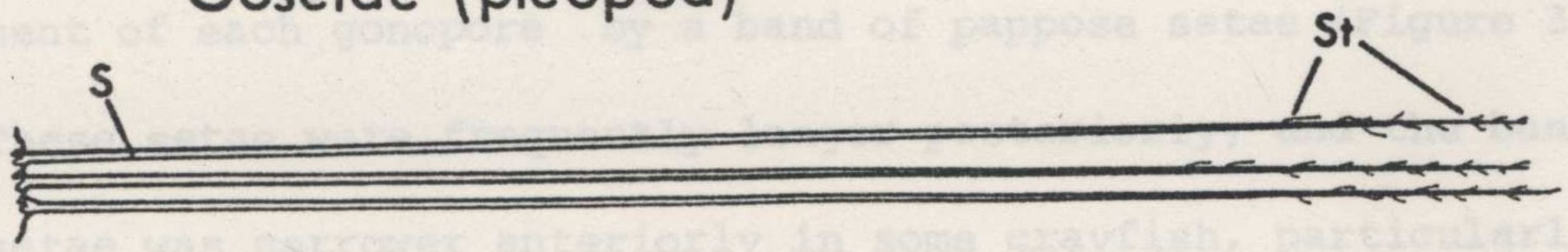
3mm

Plumose (pleopod)



3mm

Oosetae (pleopod)



7mm

of obvious setae around the gonopores. The basipodites of the pleopods of these females were free of obvious setae, while all margins of both endopodites and exopodites carried a continuous fringe of evenly-spaced plumose setae. No oosetae were present.

Crayfish in Stage 1 were characterised by partial encirclement of each gonopore by a narrow band of pappose setae (Figure 3.6). Setae were typically distributed sparsely within this band, although in some individuals setae were arranged in dense but narrow clumps for short intervals, particularly around the posterior margins of the gonopores. Both plumose setae and oosetae were present on the pleopods of Stage 1 crayfish. Oosetae and a few plumose setae were present on the medial margin of the basipodite, the lateral margin of the near-proximal exopodite, and both the medial and lateral margins of the near-proximal endopodite, while the remainders of both the endopodite and exopodite carried the complement of plumose setae typical of Stage 0 females. The presence of oosetae generally corresponded to a substantial but incomplete reduction in the number of adjacent plumose setae in Stage 1 females, and oosetae were typically about the same length as plumose setae.

Stage 2 females were characterised by the complete encirclement of each gonopore by a band of pappose setae (Figure 3.6). These setae were frequently longer posteriorly, and the band of setae was narrower anteriorly in some crayfish, particularly among smaller individuals. Otherwise, setae were densely packed to form a continuous band up to several millimetres wide around each gonopore, and in large Stage 2 females, a dense felt of setae frequently extended anteriorly over the surface of the coxa, as illustrated in

Figure 3.6: Patterns of Setae Around the Gonopores and on the Pleopods of Female *E. spinifer*.

Ventral view of the coxa of the left third pereiopod and anterior view of the left second pleopod.

Key:-

C - coxa of left third pereiopod;
A - anterior articulation of coxa with basis;
P - posterior articulation of coxa with basis;
S - articulation of coxa with sternum;
G - gonopore;
Pa - pappose setae;
B - basipodite of left second pleopod;
En - endopodite of left second pleopod;
Ex - exopodite of left second pleopod;
O - oosetae;
Pl - plumose setae.

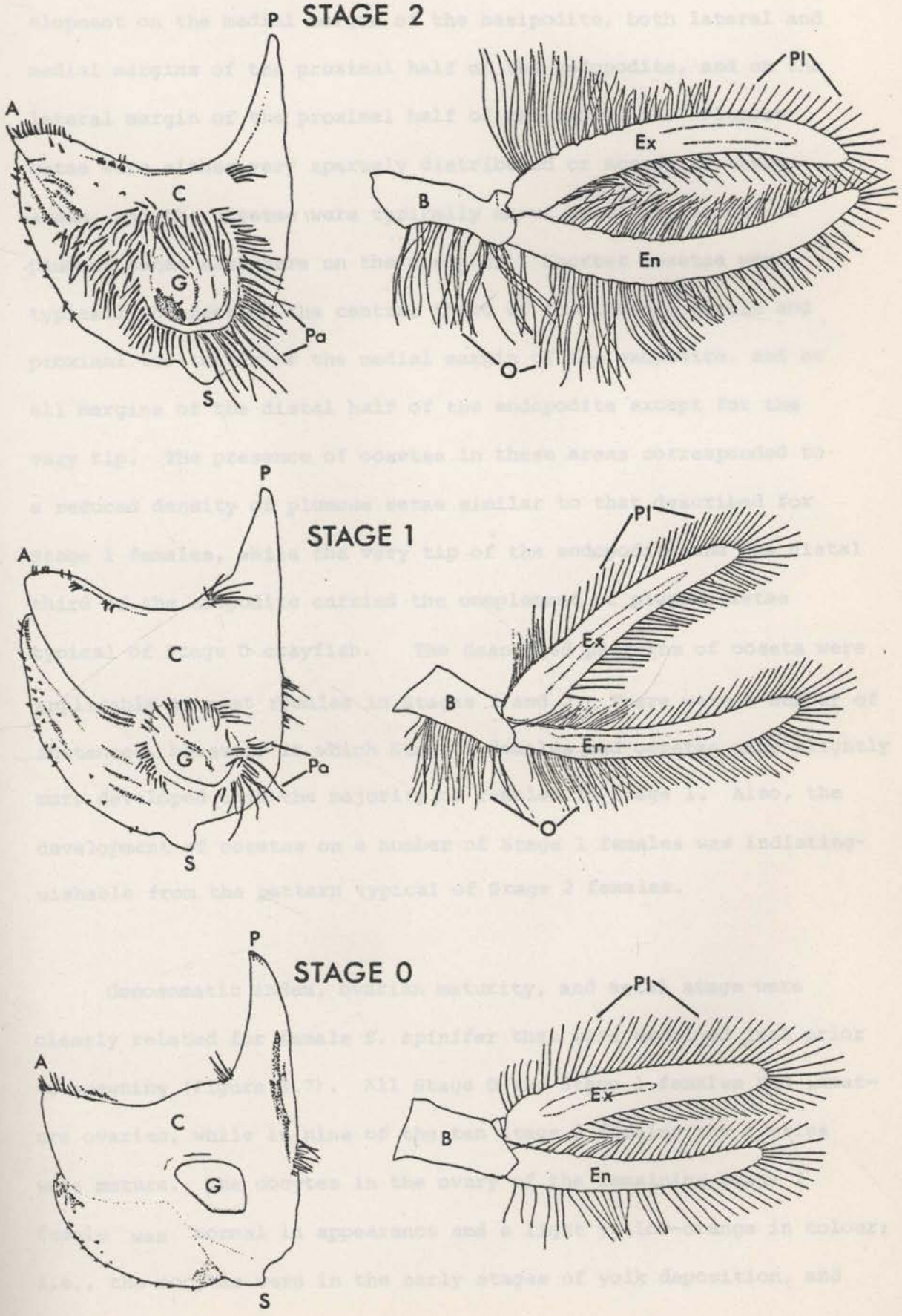

STAGE 2
P
A
C
G
Pa
S
Pl
Ex
B
En
O
STAGE 1
P
A
C
G
Pa
S
Pl
Ex
B
En
O
STAGE 0
P
A
C
G
S
Pl
Ex
B
En

Figure 3.6. Dense beds of long oosetae were immediately obvious on the pleopods of Stage 2 females. Oosetae showed greatest development on the medial margin of the basipodite, both lateral and medial margins of the proximal half of the endopodite, and on the lateral margin of the proximal half of the exopodite. Plumose setae were either very sparsely distributed or absent in these areas, and the oosetae were typically about twice the length of plumose setae elsewhere on the pleopods. Shorter oosetae were typically present on the central third of the lateral margin and proximal two-thirds of the medial margin of the exopodite, and on all margins of the distal half of the endopodite except for the very tip. The presence of oosetae in these areas corresponded to a reduced density of plumose setae similar to that described for Stage 1 females, while the very tip of the endopodite and the distal third of the exopodite carried the complement of plumose setae typical of Stage 0 crayfish. The described patterns of ooseta were applicable to most females in Stages 1 and 2. There were a number of instances, however, in which Stage 2 females had oosetae only slightly more developed than the majority of females in Stage 1. Also, the development of oosetae on a number of Stage 1 females was indistinguishable from the pattern typical of Stage 2 females.

Gonosomatic index, ovarian maturity, and setal stage were clearly related for female *E. spinifer* that were examined just prior to spawning (Figure 3.7). All Stage 0 and Stage 1 females had immature ovaries, while in nine of the ten Stage 2 females the ovaries were mature. The oocytes in the ovary of the remaining Stage 2 female was normal in appearance and a light yellow-orange in colour; i.e., the oocytes were in the early stages of yolk deposition, and

Figure 3.7: Relationship Between Gonosomatic Index, Ovarian Maturity, Setal Stage and Body Weight for Female *E. spinifer.*

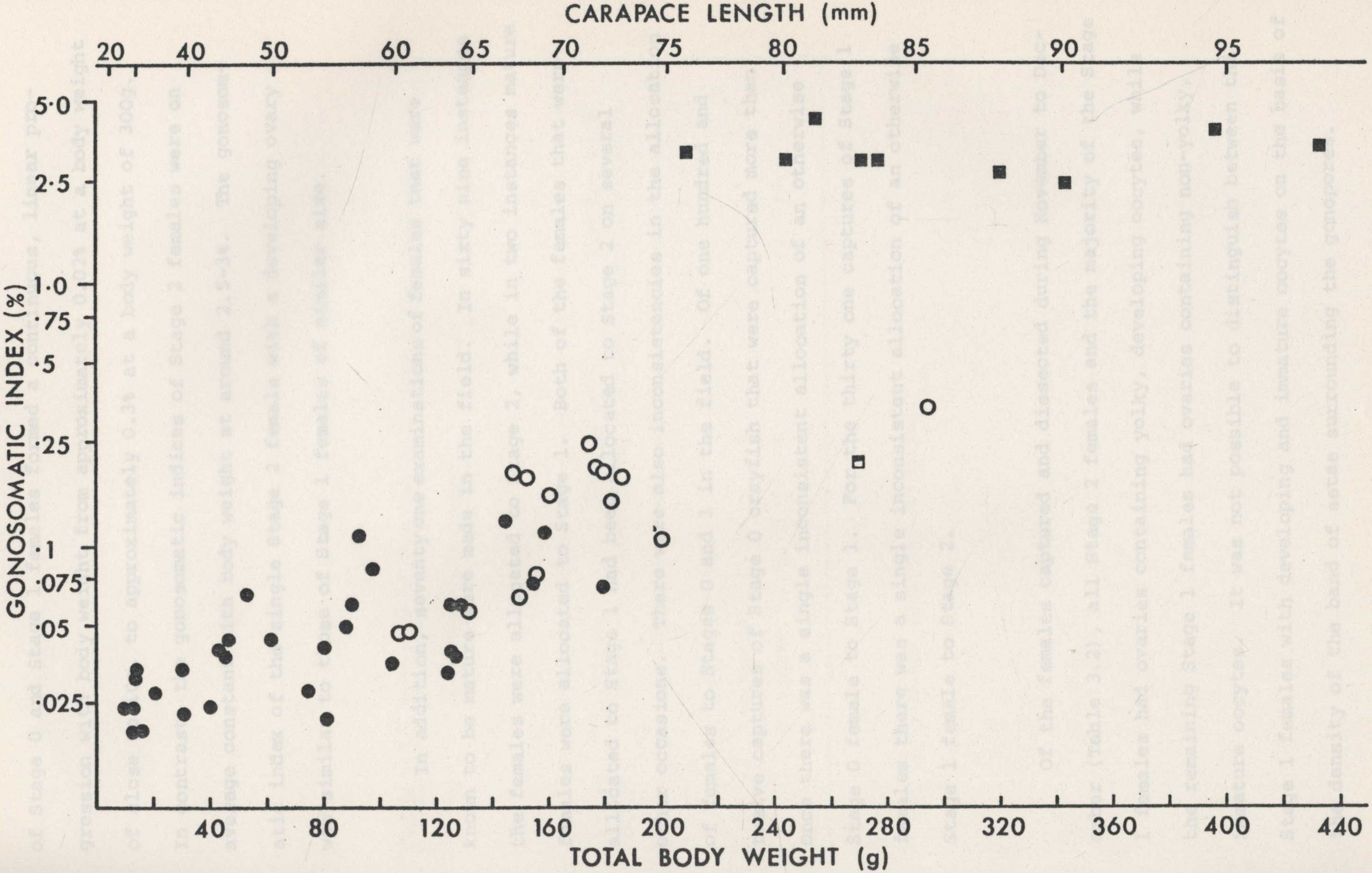
Key:-

● - Stage 0, ovary immature;

○ - Stage 1, ovary immature;

▬ - Stage 2, developing ovary;

■ - Stage 2, mature ovary.


CARAPACE LENGTH (mm)
20
40
50
60
65
70
75
80
85
90
95
GONOSOMATIC INDEX (%)
5·0
2·5
1·0
·75
·5
·25
·1
·075
·05
·025
40
80
120
160
200
240
280
320
360
400
440
TOTAL BODY WEIGHT (g)

the ovary was classified as developing. The gonosomatic indices of Stage 0 and Stage 1 females formed a continuous, linear progression with body weight from approximately 0.02% at a body weight of close to 10g, to approximately 0.3% at a body weight of 300g. In contrast, the gonosomatic indices of Stage 2 females were on average constant with body weight at around 2.5-3%. The gonosomatic index of the single Stage 2 female with a developing ovary was similar to those of Stage 1 females of similar size.

In addition, seventy one examinations of females that were known to be mature were made in the field. In sixty nine instances the females were allocated to Stage 2, while in two instances mature females were allocated to Stage 1. Both of the females that were allocated to Stage 1 had been allocated to Stage 2 on several other occasions. There were also inconsistencies in the allocation of females to Stages 0 and 1 in the field. Of one hundred and twelve captures of Stage 0 crayfish that were captured more than once there was a single inconsistent allocation of an otherwise Stage 0 female to Stage 1. For the thirty one captures of Stage 1 females there was a single inconsistent allocation of an otherwise Stage 1 female to Stage 2.

Of the females captured and dissected during November to December (Table 3.2), all Stage 2 females and the majority of the Stage 1 females had ovaries containing yolky, developing oocytes, while the remaining Stage 1 females had ovaries containing non-yolky, immature oocytes. It was not possible to distinguish between the Stage 1 females with developing and immature oocytes on the basis of the density of the band of setae surrounding the gonopores.

Table 3.2: Maturity of Females in Stages 1 and 2 during November to December.

Setal Stage	Gonopore Setae	Females with developing oocytes	Females with immature oocytes
2	dense	8*	-
1	moderate	4	-
1	sparse	4	4

* all individuals carrying offspring.

All eight of the Stage 2 females listed in Table 3.2 were carrying offspring, and the maturity of the gonads indicated that they would probably spawn again in the following season. Further evidence of spawning in successive years was obtained from mark-recapture records. Of the six Stage 2 females with capture records in the brooding periods of 1977 and 1978, five carried eggs in both years. The remaining individual carried eggs in 1977, but in 1978 carried only a spermatophore (see Section 3.3.2).

Body weights of Stage 0 and Stage 1 females examined in the laboratory (Figure 3.7) overlapped in the approximate range 110-180g (approximately 60-72mm carapace length) while Stage 1 and Stage 2 females overlapped in the approximate range 200-300g (approximately 75-85mm carapace length). Similar trends were evident when frequencies of capture of females in Stages 0, 1 and 2 were combined for all catches taken during the period of study (Figure 3.8), confirming the overlap in the sizes of Stage 1 and 2 females suggested by the laboratory results in Figure 3.7. Substantial numbers of females in both stages occurred in the approximate range of carapace lengths 70-95mm, with the relative abundance of Stage 1 females decreasing with carapace length.

In contrast to the results for gonosomatic index, ovarian maturity and setal stage, there was no change in the relationship between gonopore diameter and carapace length for females with carapace lengths in the range 50-90mm (Figure 3.9). Gonopore diameters increased linearly with carapace length throughout the entire range, and considerable variability about the average trend was evident. In view of this variability, the results obtained in this

Figure 3.8: Changes with Carapace Length in the Relative Abundances in Catches of Females in Stages 0, 1 and 2.

Key:-

○ - Stage 0

△ - Stage 1

▲ - Stage 2

Percentages with 95% confidence limits.

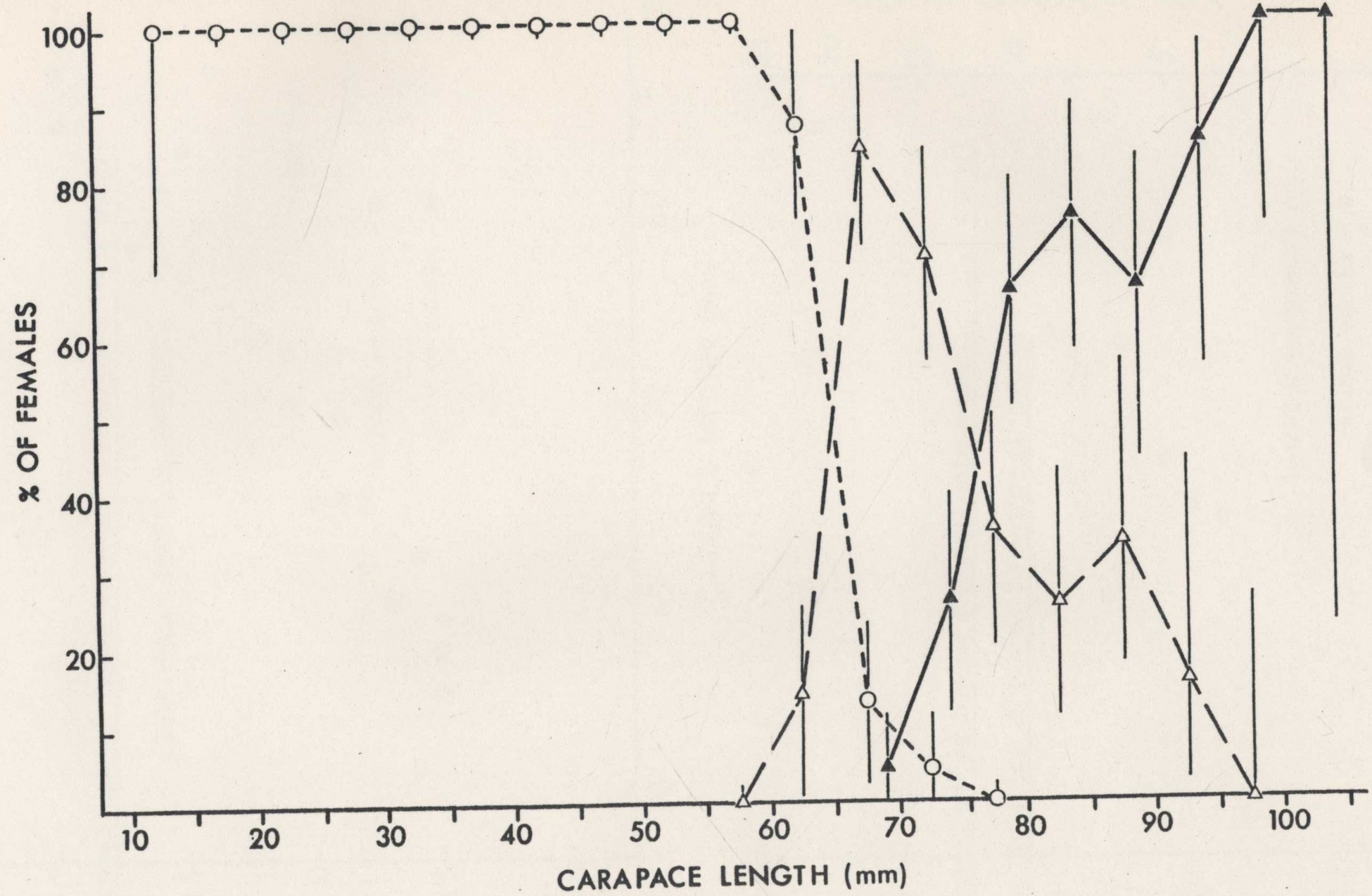

100
80
60
40
20
% OF FEMALES
10
20
30
40
50
60
70
80
90
100
CARAPACE LENGTH (mm)

Figure 3.9: Changes with Carapace Length in the Diameters of Gonopores of Female *E. spinifer*.

Key:-

o - immature ovaries;

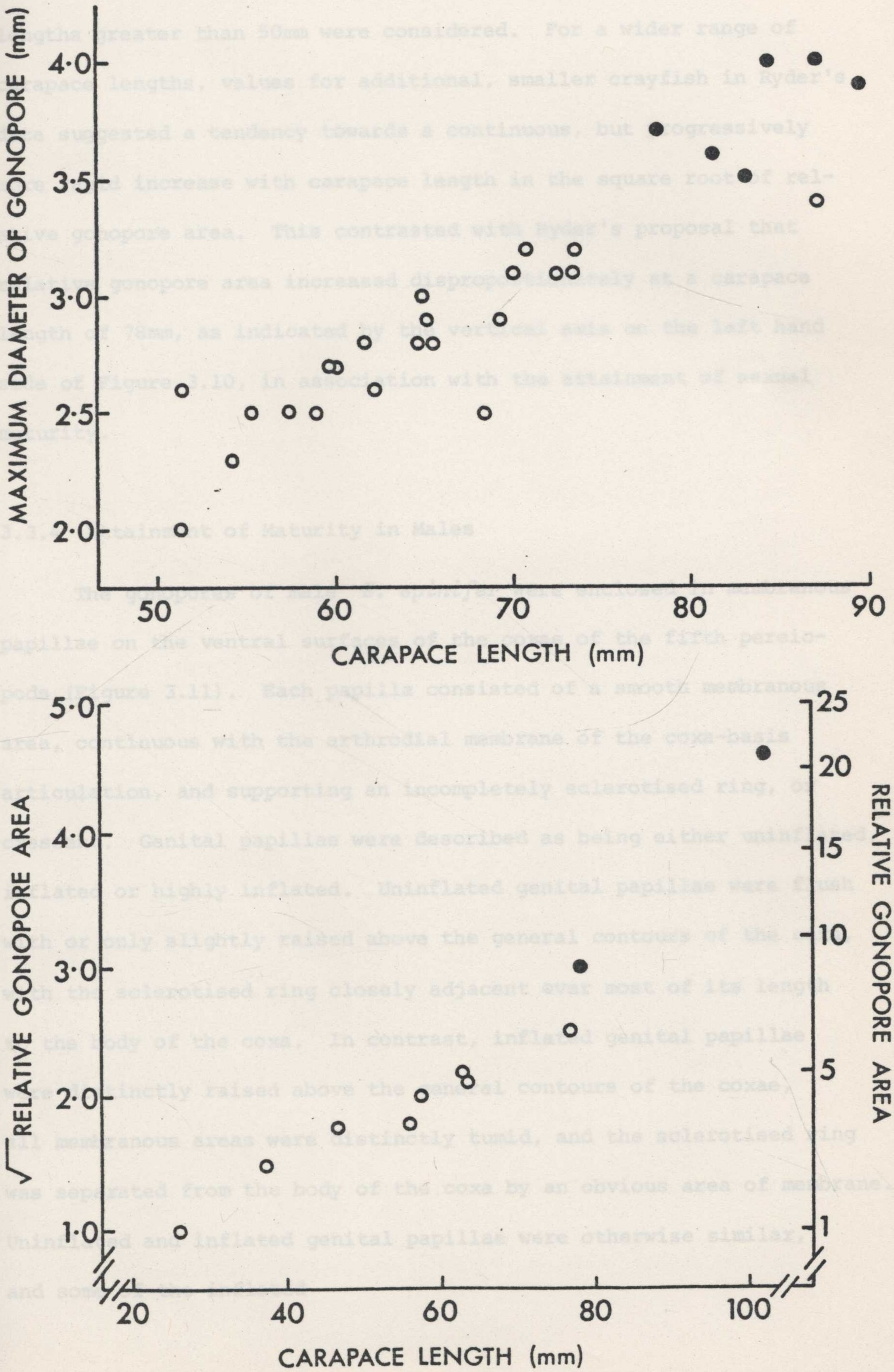
● - mature ovaries.

Figure 3.10: Changes with Carapace Length in the Dimensions of the Gonopores of Female *E. spinifer*, after Ryder (1972).

Key:-

o - immature ovaries;

● - mature ovaries.


MAXIMUM DIAMETER OF GONOPORE (mm)
4·0
3·5
3·0
2·5
2·0
50
60
70
80
90
CARAPACE LENGTH (mm)
√ RELATIVE GONOPORE AREA
5·0
4·0
3·0
2·0
1·0
20
40
60
80
100
RELATIVE GONOPORE AREA
25
20
15
10
5
1
CARAPACE LENGTH (mm)

study were essentially identical to those obtained by Ryder (1972) for gonopore area, when square roots of the areas were plotted against carapace length (Figure 3.10) and crayfish with carapace lengths greater than 50mm were considered. For a wider range of carapace lengths, values for additional, smaller crayfish in Ryder's data suggested a tendency towards a continuous, but progressively more rapid increase with carapace length in the square root of relative gonopore area. This contrasted with Ryder's proposal that relative gonopore area increased disproportionately at a carapace length of 78mm, as indicated by the vertical axis on the left hand side of Figure 3.10, in association with the attainment of sexual maturity.

3.3.4 Attainment of Maturity in Males

The gonopores of male *E. spinifer* were enclosed in membranous papillae on the ventral surfaces of the coxae of the fifth pereiopods (Figure 3.11). Each papilla consisted of a smooth membranous area, continuous with the arthrodial membrane of the coxa-basis articulation, and supporting an incompletely sclerotised ring, or crescent. Genital papillae were described as being either uninflated, inflated or highly inflated. Uninflated genital papillae were flush with or only slightly raised above the general contours of the coxa, with the sclerotised ring closely adjacent over most of its length to the body of the coxa. In contrast, inflated genital papillae were distinctly raised above the general contours of the coxae, all membranous areas were distinctly tumid, and the sclerotised ring was separated from the body of the coxa by an obvious area of membrane. Uninflated and inflated genital papillae were otherwise similar, and some of the inflated

Fig. 3.11A. Ventral views of male *E. spinifer*, showing genital papillae.

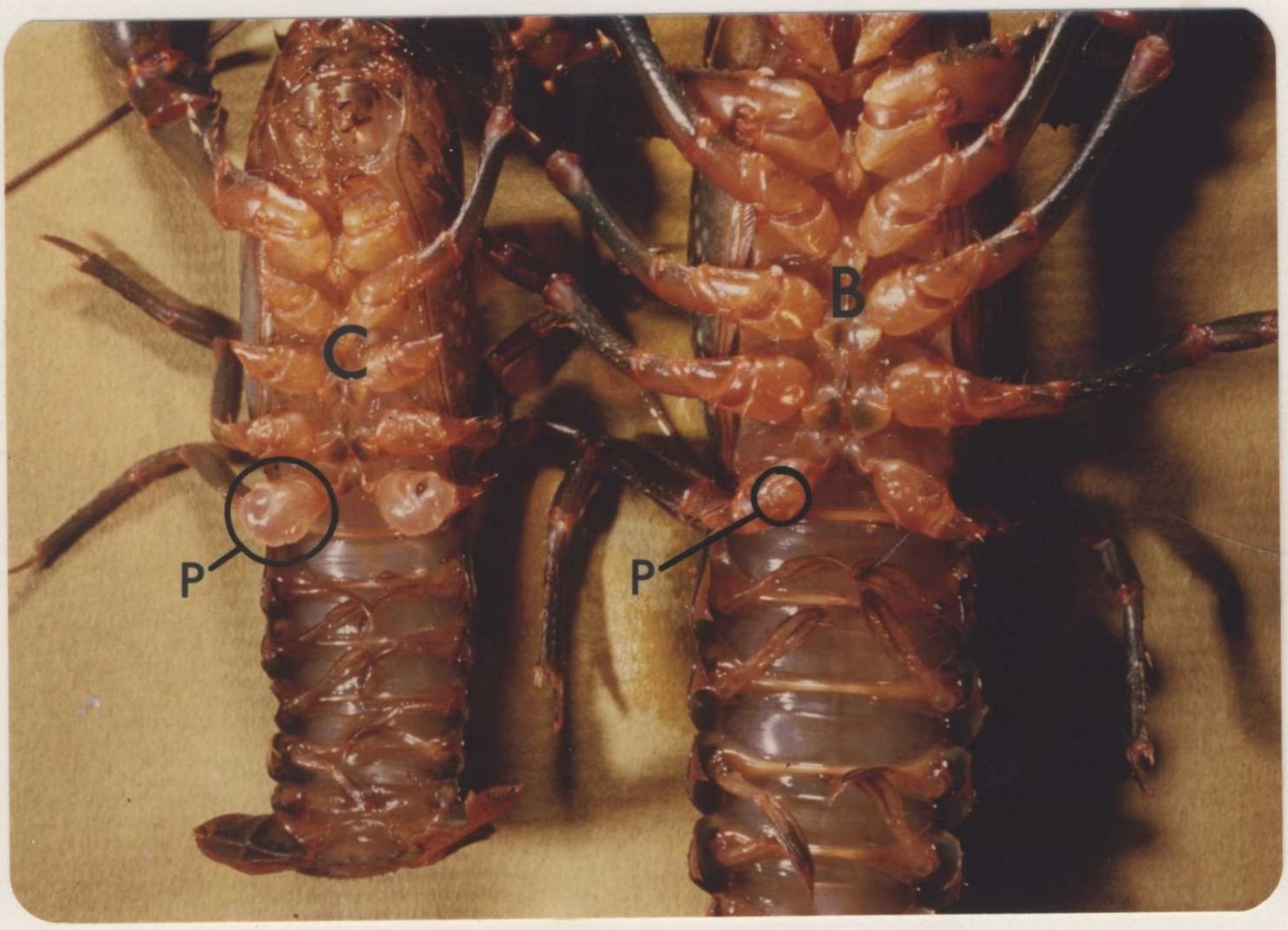
A Inflated genital papillae (normal mature)

B Uninflated genital papillae (normal immature)

C Highly inflated genital papillae (precociously mature).

Key

P - genital papilla.


B
C
P
P

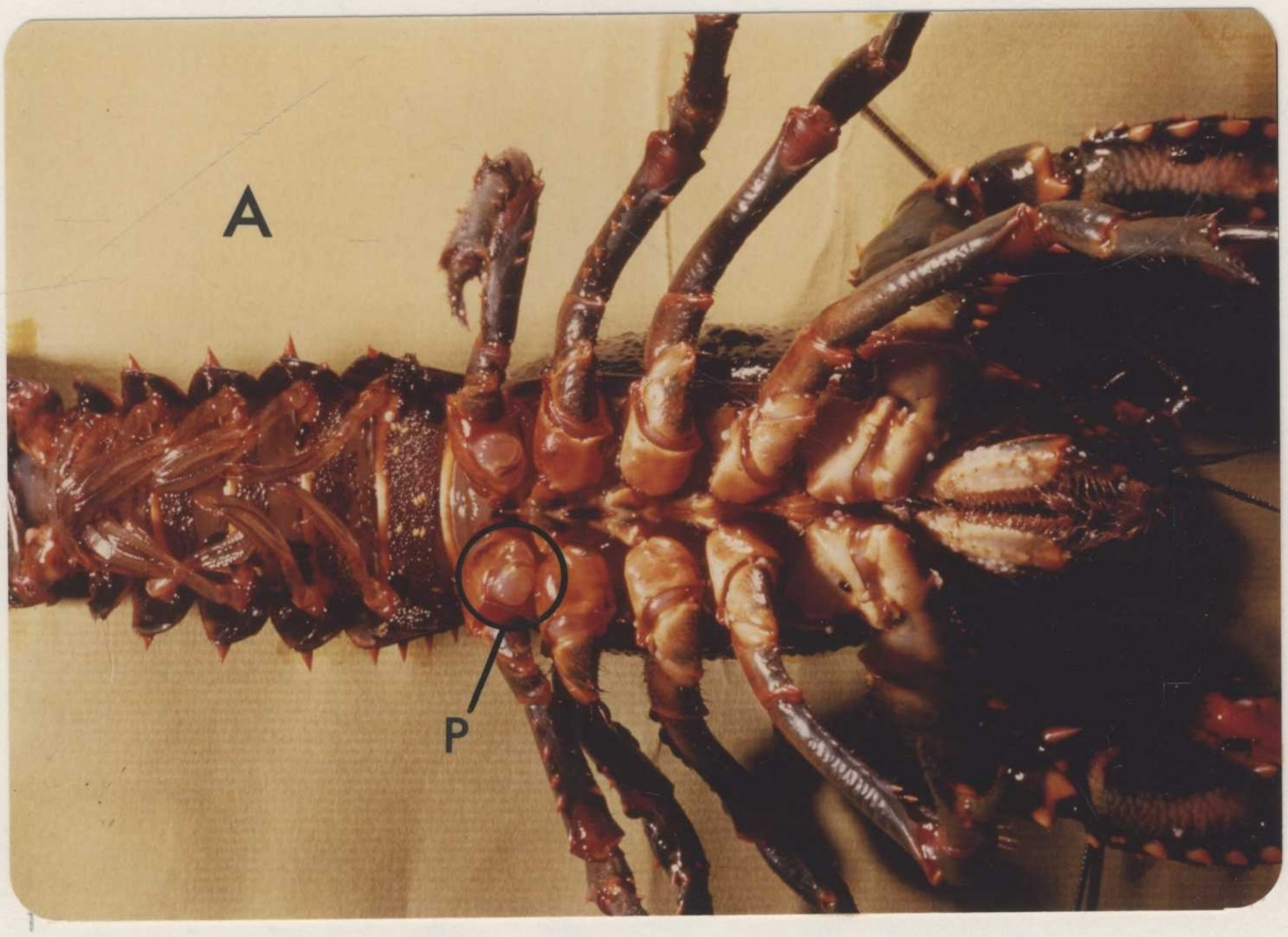

A
P

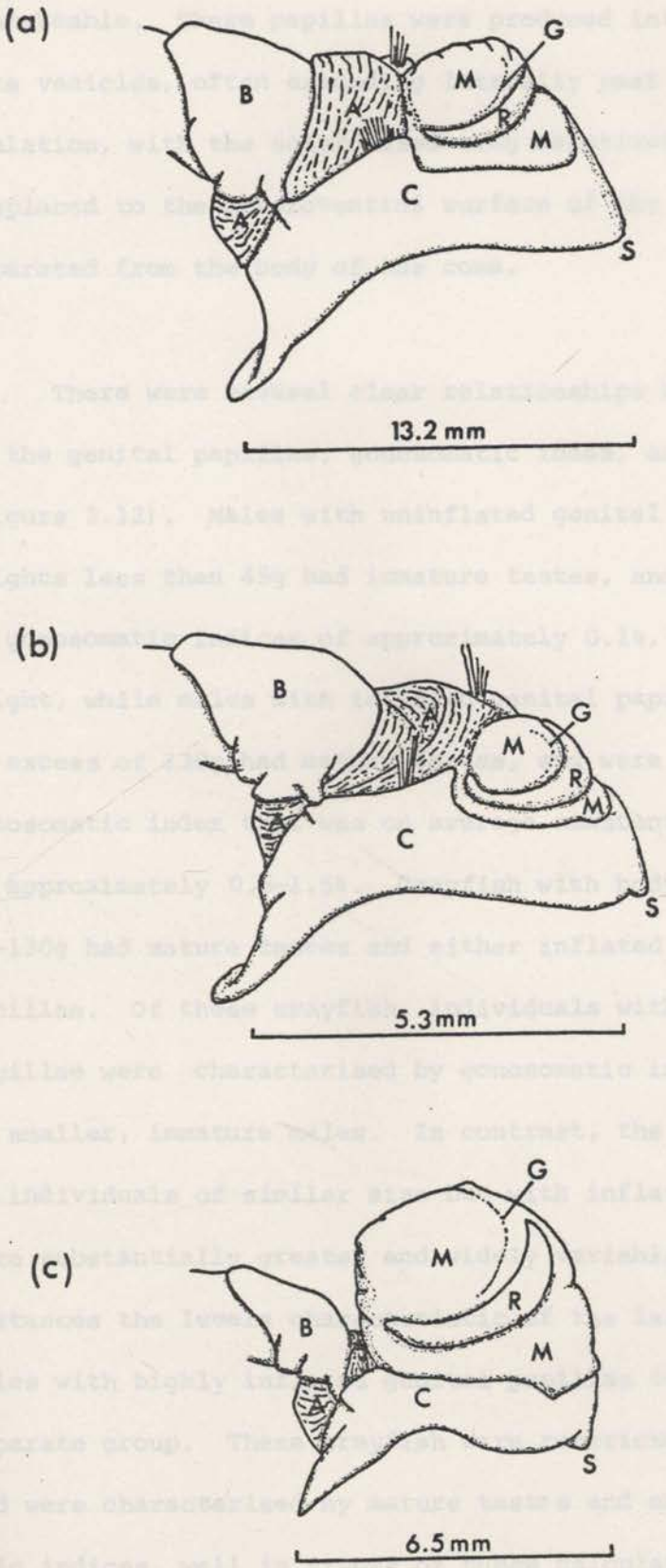
Figure 3.11B: Degrees of Inflation of the Genital Papillae of Male *E. spinifer*.

Coxa of the left fifth pereiopod, anterior view.

a) Inflated (from crayfish with carapace length 74.3mm).
b) Uninflated (from crayfish with carapace length 36.6mm).
c) Highly inflated (from crayfish with carapace length 24.6mm).

Key:-

C - coxa; B - basis; A - arthrodial membrane of the coxa-basis articulation; S - articulation with sternum; G - gonopore; M - membranous part of papilla; R - sclerotised ring.


(a)
B
G
M
R
M
C
S
13.2 mm
(b)
B
G
M
R
M
C
S
5.3 mm
(c)
G
M
R
B
M
C
S
6.5 mm

papillæ that were less tumid approached the uninflated papillae in form. Highly inflated genital papillae were striking and unmistakeable. These papillae were produced into turgid, balloon-like vesicles, often extending laterally past the coxa-basis articulation, with the sclerotised ring relatively small in size, displaced to the anteroventral surface of the papilla, and well-separated from the body of the coxa.

There were several clear relationships between the inflation of the genital papillae, gonosomatic index, and testicular maturity (Figure 3.12). Males with uninflated genital papillae and body weights less than 45g had immature testes, and were characterised by gonosomatic indices of approximately 0.1%, regardless of body weight, while males with inflated genital papillae and body weights in excess of 130g had mature testes, and were characterised by a gonosomatic index that was on average constant with carapace length at approximately 0.5-1.5%. Crayfish with body weights in the range 45-130g had mature testes and either inflated or uninflated genital papillae. Of these crayfish, individuals with uninflated genital papillae were characterised by gonosomatic indices similar to those of smaller, immature males. In contrast, the gonosomatic indices of individuals of similar size but with inflated genital papillae were substantially greater and widely variable, attaining in some instances the levels characteristic of the larger mature crayfish. Males with highly inflated genital papillae formed an entirely separate group. These crayfish were restricted to weights below 25g, and were characterised by mature testes and extremely high gonosomatic indices, well in excess of those calculated for mature crayfish. In instances where testes were not examined, or where aberrant female

Figure 3.12: Relationships Between Gonosomatic Index, Testicular Maturity, Inflation of the Genital Papillae and Body Weight for Male *E. spinifer*.

Key:-

○ - genital papillae uninflated, testis immature.

● - genital papillae uninflated, testis mature.

◒ - genital papillae uninflated, testis not examined.

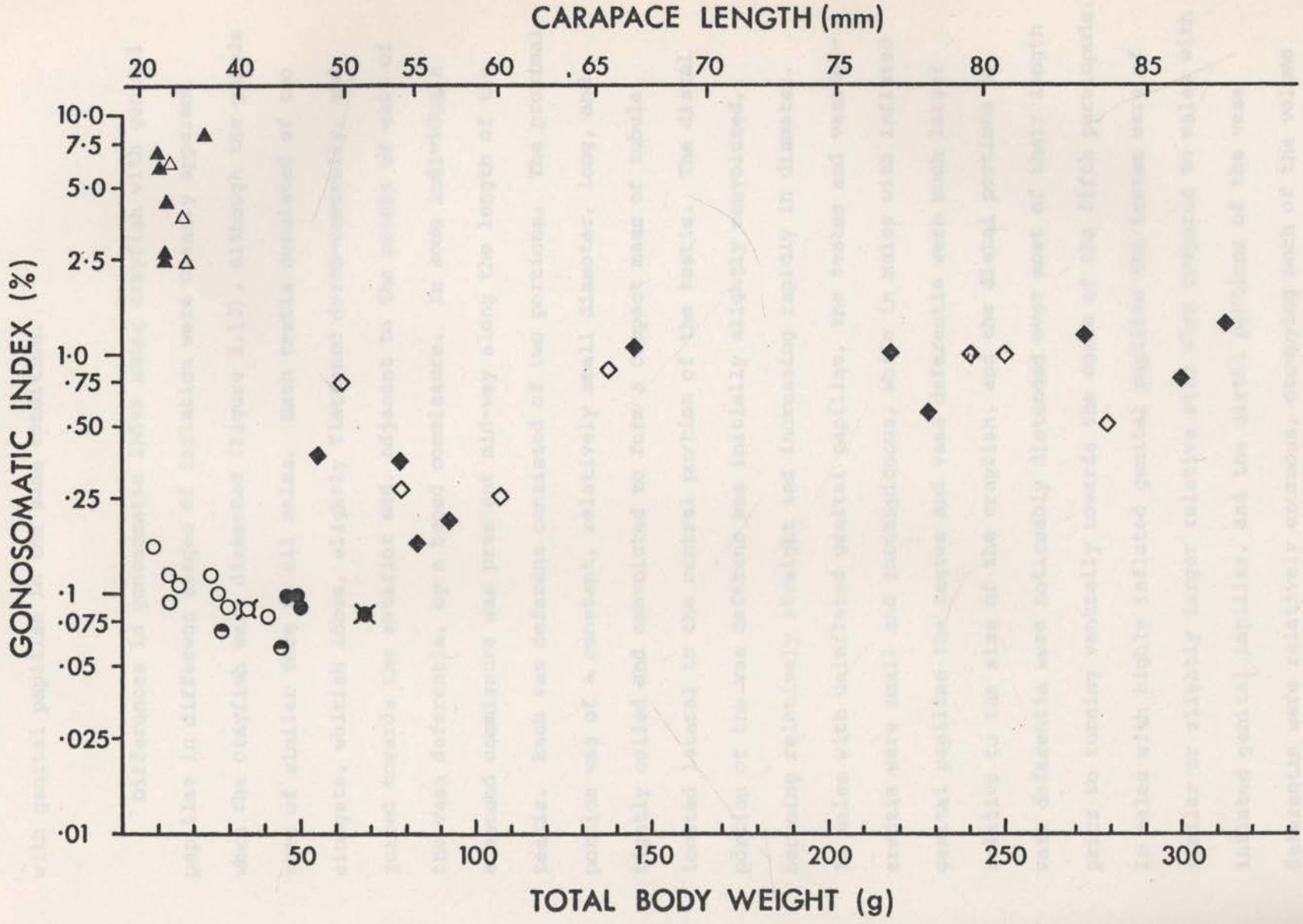
◆ - genital papillae inflated, testis mature.

◇ - genital papillae inflated, testis not examined.

▲ - genital papillae highly inflated, testis mature.

△ - genital papillae highly inflated, testis not examined.

⚤ - each individual with two male and two female gonopores.


CARAPACE LENGTH (mm)
20
40
50
55
60
65
70
75
80
85
GONOSOMATIC INDEX (%)
10·0
7·5
5·0
2·5
1·0
·75
·50
·25
·1
·075
·05
·025
·01
50
100
150
200
250
300
TOTAL BODY WEIGHT (g)

gonopores were present for a particular crayfish, the gonosomatic indices conformed to those of other crayfish of similar size and with genital papillae in the same condition.

Differences in gonosomatic index among crayfish with genital papillae in different stages of inflation were clearly apparent when the crayfish were dissected (Figure 3.13), although the gonads were of similar form in all males. Each testis consisted of two elongate, whitish lobes, slightly flattened dorso-ventrally, and joined towards the anterior end, adjacent to the points of exit of the vasa deferentia, by a broad commissure. In some individuals a second commissure was present mid-way along the length of the testis. Each vas deferens consisted of two portions. The proximal portion was of a constant, relatively small diameter, long, and tightly coiled and convoluted to form a compact mass of tubule located lateral to the central portion of the testis. The distal portion of the vas deferens was initially slightly convoluted, becoming relatively straight and increasing rapidly in diameter. In males with uninflated genital papillae, the testes and vasadeferentia were small and inconspicuous, while in males with inflated genital papillae the testes and vasa deferentia were much larger relative to the size of the crayfish, and the distal portions of vasa deferentia were noticeably distended over most of their length prior to turning ventrally towards the coxae of the fifth pereiopods. In males with highly inflated genital papillae the testes were of similar or slightly larger relative size when compared to males with inflated genital papillae, and the distal portions of the vasa deferentia were relatively enormous, occupying much of the volume of the posterior half of the cephalothorax. In males with either

Figure 3.13: Anterior Dorsal Views of Male *E. spinifer* with Uninflated, Inflated and Highly Inflated Genital Papillae showing Testes and Vasa Deferentia *in situ*.

(a) Inflated genital papillae (normal mature);

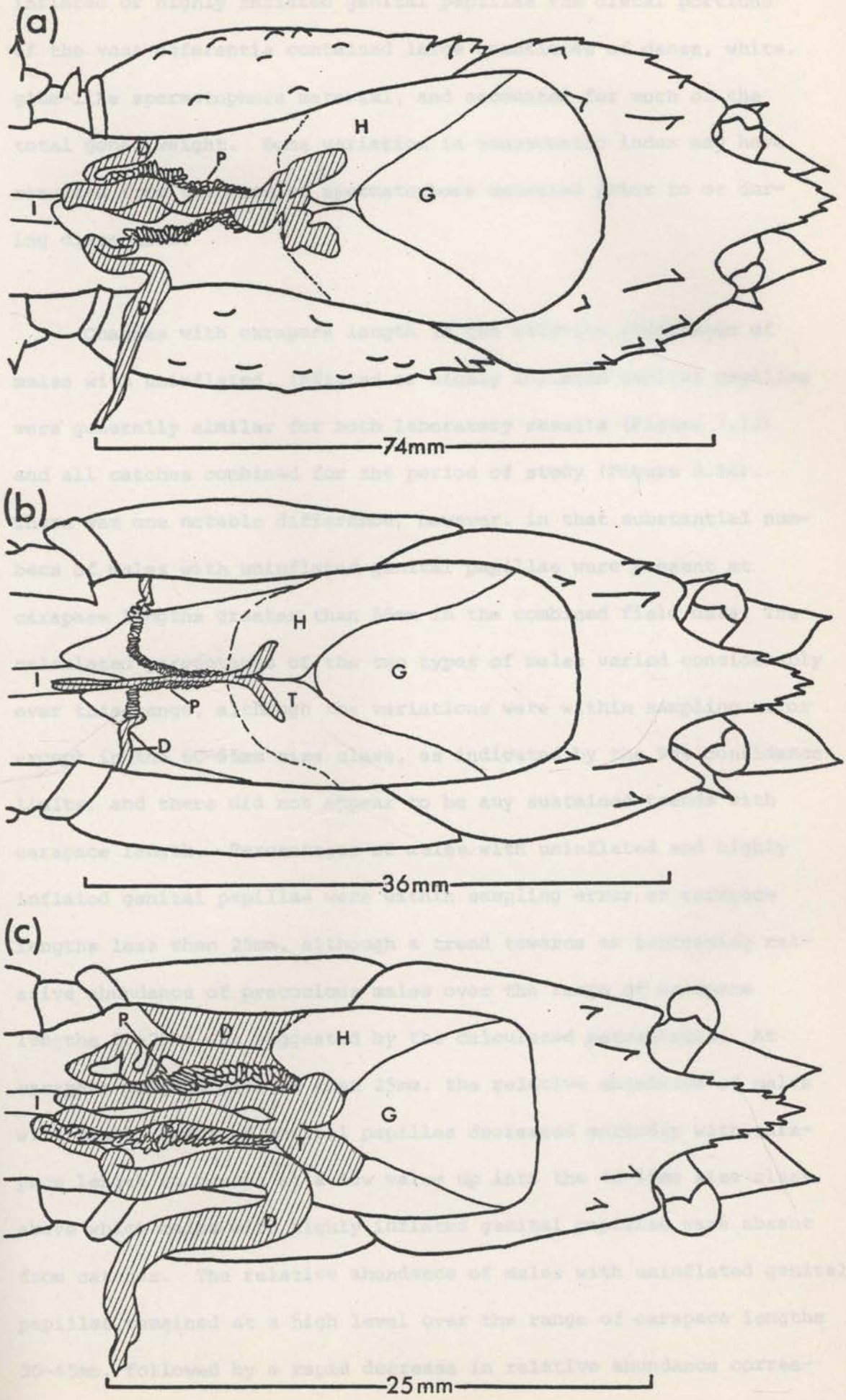
(b) Uninflated genital papillae (normal immature);

(c) Highly inflated genital papillae (precociously mature).

Key:-

T - testis; P - proximal vas deferens; D - distal vas deferens; G - gastric mill; H - hepatopancreas; I - hind-gut.

N.B. The dorsal walls of the carapace and abdominal segments, the heart and dorsal blood sinuses, and the posterior dorsal musclature of the gastric mill have been removed.


(a)
H
P
G
I
D
74mm
(b)
H
G
I
P
T
D
36mm
(c)
P
D
H
I
G
T
D
25mm

inflated or highly inflated genital papillae the distal portions of the vasa deferentia contained large quantities of dense, white, glue-like spermatophore material, and accounted for much of the total gonad weight. Some variation in gonosomatic index may have resulted from the loss of spermatophore material prior to or during dissection.

Changes with carapace length in the relative abundances of males with uninflated, inflated or highly inflated genital papillae were generally similar for both laboratory results (Figure 3.12) and all catches combined for the period of study (Figure 3.14). There was one notable difference, however, in that substantial numbers of males with uninflated genital papillae were present at carapace lengths greater than 55mm in the combined field data. The calculated percentages of the two types of males varied considerably over this range, although the variations were within sampling error except in the 60-65mm size class, as indicated by the 95% confidence limits, and there did not appear to be any sustained trends with carapace length. Percentages of males with uninflated and highly inflated genital papillae were within sampling error at carapace lengths less than 25mm, although a trend towards an increasing relative abundance of precocious males over the range of carapace lengths 10-25mm was suggested by the calculated percentages. At carapace lengths greater than 25mm, the relative abundance of males with highly inflated genital papillae decreased markedly with carapace length to remain at a low value up into the 40-45mm size class, above which males with highly inflated genital papillae were absent from catches. The relative abundance of males with uninflated genital papillae remained at a high level over the range of carapace lengths 30-45mm, followed by a rapid decrease in relative abundance corres-

Figure 3.14: Changes with Carapace Length in the Relative Abundances in Catches of Males with Uninflated, Inflated and Highly Inflated Genital Papillae.

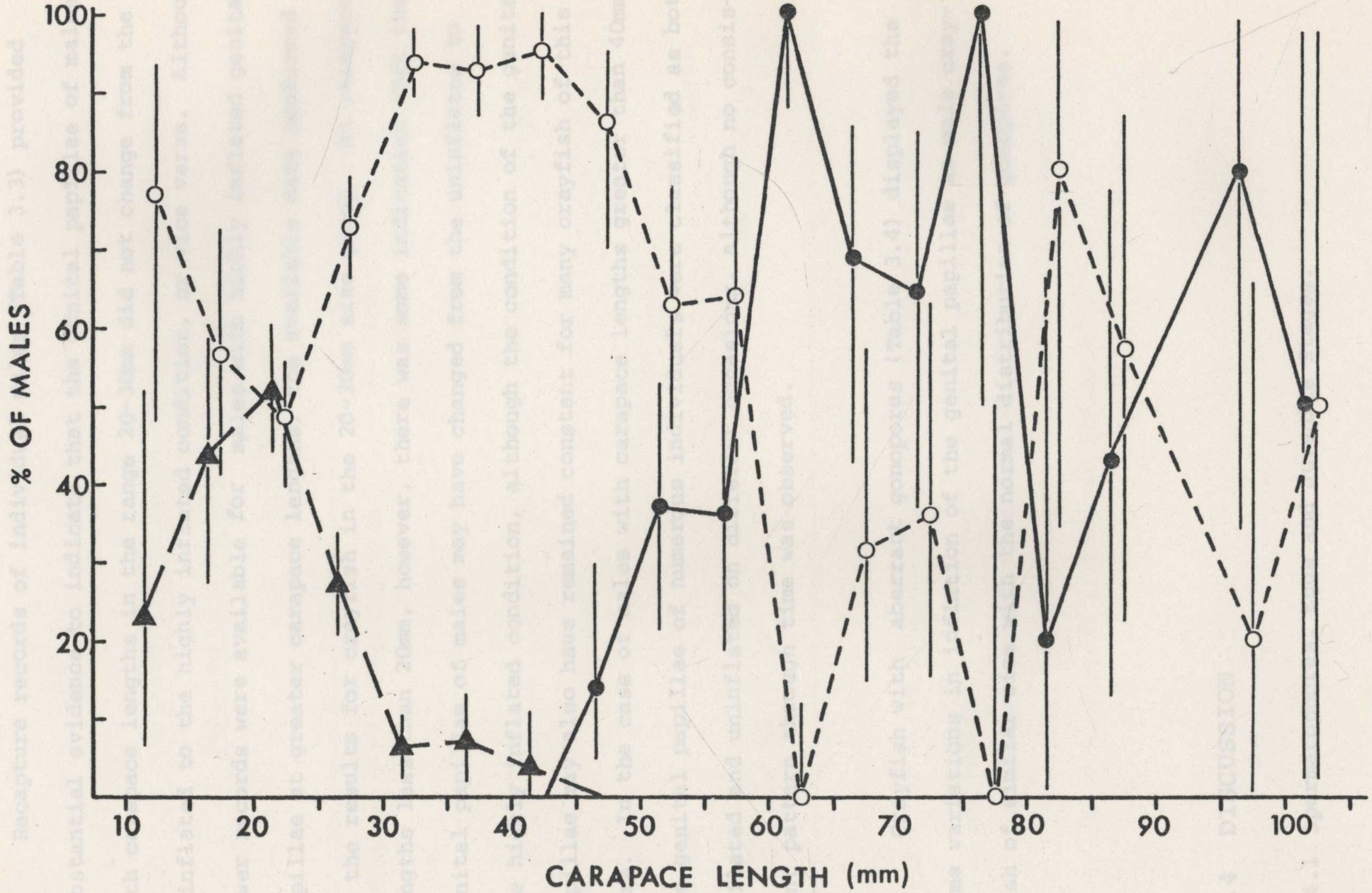
Key:-

O - uninflated genital papillae.

● - inflated genital papillae.

▲ - highly inflated genital papillae.

Percentages with 95% confidence limits.


100
80
60
40
20
% OF MALES
10
20
30
40
50
60
70
80
90
100
CARAPACE LENGTH (mm)

ponding to the appearance of males with inflated genital papillae.

Recapture records of individual males (Table 3.3) provided substantial evidence to indicate that the genital papillae of males with carapace lengths in the range 20-30mm did not change from the uninflated to the highly inflated condition, or vice versa. Although fewer records were available for males with highly inflated genital papillae at greater carapace lengths, the available data conformed to the results for crayfish in the 20-30mm size group. At carapace lengths less than 20mm, however, there was some indication that the genital papillae of males may have changed from the uninflated to the highly inflated condition, although the condition of the genital papillae may also have remained constant for many crayfish of this size. In the case of males with carapace lengths greater than 40mm, the genital papillae of numerous individuals were classified as both inflated and uninflated on different occasions, although no consistent pattern through time was observed.

Crayfish with aberrant gonopores (Table 3.4) displayed the same variations in inflation of the genital papillae as male crayfish of similar size with the normal distribution of gonopores.

3.4 DISCUSSION

3.4.1 Spermatophores, Eggs and Juvenile Stages.

The spermatophores of *E. spinifer* were of typical macruran form (Green, 1961), and extremely similar in the details of structure to those described for *Cherax destructor* (Johnson, 1979). In both

Table 3.3: Variation in the Degree of Inflation of the Genital Papillae of Individual Male *E. spinifer.*

Carapace Length (mm)	Mean Captures per male	Number of Males O	HI	I	O/I/HI
<20	2.8	2	6		1(O/HI)
20-30	2.5	69	29		
30-40	3.4	63	3		
40-60	4.1	21	1	4	8 (O/I)
60+	3.1	2		12	6 (O/I)

* Number of males with multiple recapture records of the types:

O - genital papillae not inflated at all captures.

I - genital papillae inflated at all captures.

HI - genital papillae highly inflated at all captures.

O/I/HI - mixture of records as indicated.

Table 3.4: Records of Crayfish with Aberrant Numbers of Gonopores.

*Aberration	Carapace Length (mm)	*[1]Genital Papillae
L3	62.1 - 67.0	I
L3	26.3 - 36.6	O
L3	29.4 - 35.7	O
R3	20.7 - 24.4	HI
R3	29.9 - 37.8	O
R3	29.9	O
R3	90.2	O
R3	21.2 - 23.0	HI
R3	29.0	HI
L3 + R3	47.3 - 51.7	O
L3 + R3	24.6 - 25.5	O
L3 + R3	40.2 - 50.6	O
L3 + R3	25.3 - 38.9	O
L3 + R3	20.5	HI
L3 + L4	66.6 - 71.6	I

* Female gonopores in addition to male gonopores, e.g., an additional female gonopore on the coxapodite of the right third pereiopod of a crayfish with typical male gonopores = R3.

*[1]Papillae of typical male form located on the coxae of the fifth pereiopods only.

C. destructor (Johnson, 1979) and *C. tenuimanus* (Shipway, 1951), and spermatophores are placed on the sternal plates posterior to the female gonopores which are located on the coxae of the third pereiopods, while in *E. spinifer* spermatophores were placed irregularly over the coxae of the fourth and fifth pereiopods. This difference may reflect the relatively long genital papillae of male *Cherax* (Riek, 1972), allowing more accurate, central placement of the spermatophore than the short, well-separated genital papillae of *E. spinifer*. Mason (1970) considered that sperm were released by dissolution of the spermatophore during spawning in the astacid *Pacifastacus trowbridgii*. Spermatophores were also deposited externally in this species, and it is presumed that fertilisation of eggs in *E. spinifer* occurred by the same mechanism, except that dissolution of the spermatophore during spawning may not always have been complete (Section 3.3.2).

As in other freshwater crayfishes, *E. spinifer* produces large yolky eggs which hatch at a late stage of development. The eggs of *E. spinifer* were similar in shape and approximate size to those of other parastacids (*E. australasiensis*, Ryder, 1972; *Cherax albidus*, Woodland, 1967; *C. destructor*, Johnson, 1979; *C. tenuimanus*, Shipway, 1951; Morrissy, 1970; *Paranephrops planifrons*, Hopkins, 1967b), and an approximately linear increase with carapace length in the number of eggs carried by females has also been described for other parastacid species (*P. planifrons*, Hopkins, 1967b; *C. tenuimanus*, Morrissy, 1970, Figure 10; *C. destructor*, Johnson, 1979). Clark (1937) recorded 1000-1200 eggs for female *Euastacus kershawi*. Although the carapace lengths of these females were not stated, large female *E. spinifer* from the Hacking River carried similar numbers of eggs. Numbers of eggs carried by female *E. spinifer* from the study area on the Loddon River fell in the approximate range of 250-800 for females with carapace lengths of approx-

imately 70-105mm. Johnson (1979) recorded approximately 800 eggs for female *E. armatus*, while Morrissy (1970, Figure 10) recorded approximately 200-700 eggs for female *Cherax tenuimanus* with carapace lengths in the range 70-90mm. Hence the numbers of eggs carried by female *E. spinifer* were comparable with those of other large parastacids.

The means of attachment of eggs to pleopods in *E. spinifer* was similar to that described for other parastacids, given that the oosetae of *E. spinifer* were similar to the egg-bearing setae described by other authors (Appendix E).

The colour of the yolk in the eggs of parastacids has been found to change during development for *Paranephrops planifrons* (Hopkins, 1967b), *Euastacus austral asiensis* (Ryder, 1972), and *Cherax destructor* (Johnson, 1979). This was not the case in *E. spinifer*, except for a slight darkening of the initial maroon colour, and no reason for this difference can be suggested.

The embryonisation of larval development and presence of three juvenile stages in *E. spinifer*, as well as the overall morphology and means of attachment of juveniles, were typical of parastacids in general, e.g., *Euastacus kershawi* (Clark, 1937), *Cherax tenuimanus* (Shipway, 1951), *C. albidus* (Woodland, 1967), *Euastacus australasiensis* (Ryder, 1972), *Paranephrops planifrons* (Hopkins, 1967b), *Engaeus cisternarius* (Suter, 1977), *Cherax destructor* (Johnson, 1979), if it is assumed that the description of the third, adult-like juvenile was omitted by Clark (1937). Johnson (1979) noted, however, that descriptions given by Clark (1937) for *E. kershawi* implied that a telson thread was not involved in the initial attachment of Stage

1 juveniles in this species. Given the presence of a telson thread in *E. australasiensis* (Ryder, 1972), *E. kierensis* (personal observation), *E. spinifer*, and crayfish in other genera for which details of juvenile attachment were available, the absence of a telson thread in *E. kershawi* is anomalous. The brief period for which juvenile *E. spinifer* were attached by a telson thread suggests that the telson thread may have been overlooked by Clark, although further attention to the attachment of juvenile *Euastacus* is indicated.

Johnson (1979) also noted that the hooks on the chelae and antennal scales of juvenile *E. kershawi* (as described by Clark, 1937) were not apparent in *Cherax destructor*, nor in published descriptions of other Australian parastacids, although similar hooks were present in the South American parastacid *Parastacus pilimanus* (Gurney, 1942). The terminal teeth of the chelae and spines of the antennal scales of juvenile *E. spinifer* in Stages 1 and 2 may have been similar to the 'hooks' described by Clark (1937) for *E. kershawi*, although these were not obvious features of *E. spinifer* juveniles, as implied for *E. kershawi* by Clark. Terminal, incurved teeth were apparently also present on the chelae of Stage 1 juveniles of *Paranephrops planifrons* (Hopkins, 1967b, Figure 5) and Stage 1 and 2 juveniles of *Engaeus cisternarius* (Suter, 1977, Figures 2,3). Hence their presence is not restricted to *Euastacus*. In neither *E. spinifer* nor *E. kershawi* were the 'hooks' on the chelae and antennal scales used for attachment, and for *E. spinifer* it is suggested that they merely represented the initial stages of development of teeth and spines that were normally present in the adult.

Development of the offspring on individual female *E. spinifer* was synchronous to within a few days up to and including the departure of juveniles from their mother. A similar observation was made by Johnson (1979) for *Cherax destructor* while Hopkins (1967b) stated that juvenile *Paranephrops planifrons* departed over a period of 'many days'. Johnson suggested that the variation in the development times of juvenile *P. planifrons* may have arisen simply as the result of a long development time. The incubation period of *P. planifrons* was primarily from May to December (Hopkins, 1967b), which was only one month longer than that of *E. spinifer* (Section 3.4.2). Hence it is likely that the considerable variation in the development times of juvenile *P. planifrons* resulted from causes other than just the long incubation period.

3.4.2 Events in the Annual Reproductive Cycle.

Annual reproductive cycles of parastacids fall into two groups, those with a relatively short incubation period during the warmer months, and those with a long incubation period over winter. The first group includes *Cherax destructor*, which may spawn several times over the period October to March (Johnson, 1979), *C. tenuimanus*, with a single spawning and incubation period during October to January (Shipway, 1951; Morrissy, 1970), and *Engaeus cisternarius*, which breeds during the period October to April, with an estimated incubation time of four months (Suter, 1977). *Cherax albidus* (Woodland, 1967) and *Engaeus fossor* (Suter, 1977) also breed during summer. Crayfish in the second group include *Parastacoides tasmanicus*, with an incubation period from April to February-March (Lake and Newcombe, 1975), *Paranephrops planifrons*, with an incubation period from April

to December (Hopkins, 1967b) and *Euastacus armatus* (Johnson, 1979). The results of this study indicated that *E. spinifer* from the study area on the Loddon River belonged to the second group.

The author has also observed female *Euastacus* carrying eggs in early stages of development (i.e., no embryonic structures visible to the naked eye except for the blastopore in some instances) during the month of may for *E. spinifer* in the Hacking River, in a catchment some ten miles from the study area, for *E. kierensis* from the study area, for *E. australasiensis* near Gosford, NSW, for *E. valentulus* from the Richmond River near Lismore in Northern NSW, and for *E. hystricosus* from the upper Mary River near Maleny, in southern Queensland. These observations suggest that species of *Euastacus* typically spawn in late autumn throughout much of the eastern coastal range of the genus, and the observation for *E. armatus* by Johnson (1979) suggests that this may also apply to *Euastacus* in southern inland NSW. However, *E. spinifer* in the Georges River near Campbelltown, in the adjacent catchment north-west of the study area, spawned during early September in both 1975 and 1978 (personal observation). No reason can be given for this difference between areas. It is evident, however, that there may be substantial and consistent differences between neighbouring areas in the time of spawning for a given species underlying trends which appear to be typical of the genus over much of its range. Hence the statement of Clark (1937) that the Victorian species *E. kershawi* spawns in spring is consistent with results for other members of the genus.

Female *E. spinifer* incubated eggs for approximately 110-140 days over winter prior to hatching. Eggs spawned by *Cherax destructor* in

the early spring took approximately 80-90 days to hatch, while eggs spawned during mid-summer took only 21 days to hatch (Johnson, 1979). *Engaeus cisternarius*, a summer spawner from Tasmania, incubated eggs for 120 days (Suter, 1977). Hence the lengthy incubation of eggs by *E. spinifer* when compared with other species of crayfish that spawned during summer may have been accounted for by the effect of low temperatures in retarding development rate. Estimates of the duration of the juvenile stages of *E. spinifer* from field data were necessarily limited in precision by the lengths of the periods between samplings. Based on the earliest and latest records of females carrying first and third stage juveniles, the total period between the hatching of juveniles and their departure from the mother was probably between four and ten weeks. Attempts to obtain more accurate estimates from captive crayfish were unsuccessful, as females began to eat their offspring after a short period in captivity.

The majority of mature female *E. spinifer* were observed to begin carrying spermatophores in early June and eggs in early July. Hence spermatophores may have been carried for up to a month, or slightly longer, between mating and egg-laying. The presence of numerous females carrying both eggs and spermatophores shortly after the main spawning period indicated that spermatophores were not completely removed at spawning, while recapture records indicated that spermatophores could be retained for up to one month after spawning, and possibly for longer periods if spawning did not occur. *E. spinifer* thus differed considerably from *Cherax destructor* (Johnson, 1979), in which spawning commenced within a few hours of mating, and spermatophores were present for no more than a few days. The apparent durability of the spermatophores of *E. spinifer* may be an adaptation to prevent loss of the exposed spermatophores by abrasion against the

normally rocky substratum. This does not, however, explain the relatively long period of time between mating and spawning that would require such a durable spermatophore.

Control of reproductive cycles by temperature and/or photoperiod has been proposed for numerous organisms, including freshwater crayfishes; e.g., Stephens (1952), Lowe (1961), Aiken (1969a), Johnson (1979). Events in the annual reproductive cycle of *E. spinifer* in the study area were closely associated with the annual water temperature regime. However, since reproduction followed a fixed annual cycle, reproductive events were also closely associated with the annual photoperiod regime, and with any other environmental variables showing annual periodicity. Conclusive results could only be obtained by subjecting *E. spinifer* to a set of independently combined levels of temperature and photoperiod under controlled conditions. Such experiments were considered to be impractical for *E. spinifer* due to difficulties in maintaining this species in captivity (see Chapter 8). Some information could possibly have been provided by comparing water temperature regimes between areas in which the timing of reproductive activity differed, e.g., the Loddon and Georges Rivers, as previously described.

3.4.3 Attainment of Maturity in Females.

The ovaries of *E. spinifer* were of typical decapod form (Barnes, 1974), although the tubular, anterior extensions of the ovaries to form additional lobes lateral to the cardiac stomach in *Cherax tenuimanus* (Morrissy, 1970) and *C. destructor* (Johnson, 1979) were not observed. Gonosomatic indices of mature female *E. spinifer* collected

in May-June were in the range 2.3-4.2%. Johnson (1979) calculated similar gonosomatic indices in the range 0.9-3.5% for *C. destructor* with ovaries in the later stages of maturation, while for *C. destructor* with mature ovaries just prior to spawning, the gonosomatic indices were greater than 3.5%, with a maximum value of around 5%. Hence *E. spinifer* and *C. destructor* were similar in terms of the relative allocation of body tissues to reproduction, although the ovaries of *C. destructor* were extended to form additional lobes.

Allocation of female *E. spinifer* to Stages 0, 1 and 2 according to the development of setae around the gonopores during May-June resulted in complete segregation of mature and immature females. All mature females were allocated to Stage 2, and were characterised by complete encirclement of the gonopores by dense bands of setae, while all immature females were allocated to Stages 0 and 1, and were characterised by the respective absence of gonopore setae and partial encirclement of the gonopores by a band of setae. The immature condition of the ovaries and the single, linear progression in gonosomatic indices for females in Stages 0 and 1 indicated that the initial appearance of setae around the gonopores was not related to any immediate change in reproductive condition.

Observed inconsistencies in the relationships of Stages 0 and 1 with immaturity and Stage 2 with maturity may have arisen from two sources. The setal stages may not always have been associated with maturity in this way, or the observer may have been unable to allocate females with the same development of setae to the same stage consistently due to a gradation in setal development between stages, i.e., allocations were subjective.

The single Stage 2 female that was captured during May-June with a developing ovary (Figure 3.7) was not considered to be an example of inconsistent allocation, since the ovary of this individual differed from the ovaries of all Stage 1 females that were captured at the same time, and was similar to ovaries of all other Stage 2 females, in that yolk deposition, although incomplete, had occurred. Incomplete yolk deposition in the oocytes of this female may have been associated with delayed spawning, or with failure to spawn, both of which were recorded for mature females in the study area (Section 3.3.2).

Of the remaining 9 Stage 2 females that were examined in the laboratory in May-June, all were mature. Also, of the 71 field examinations of females that were known to be mature, there were 69 allocations of mature females to Stage 2, and 2 allocations to Stage 1. Hence mature females were allocated to Stage 1 rather than Stage 2, on 2 out of a total of 81 occasions. Combining the laboratory results for Stage 1 females with results from multiple recaptures, there was a single instance out of 46 allocations when an otherwise Stage 1 female was allocated to Stage 2.

Each of the crayfish for which the inconsistent stages were recorded had been allocated to the normal stage on several other occasions, and none of the inconsistencies were related to changes in setal development at moulting. Hence the inconsistencies implied that there was a degree of subjectivity involved in the allocation of females to Stages 1 and 2, and not that some females departed from the initial premise that Stage 2 indicated maturity while Stage 1 indicated immaturity.

Combining the allocations of females to Stages 1 and 2, there were thus 3 inconsistencies among a total of 127 allocations. Inconsistent allocations of Stage 2 females to Stage 1 and *vice versa* occurred with similar frequencies. Hence the allocation of an immature female to Stage 2 rather than to Stage 1, or of a mature female to Stage 1 rather than Stage 2, would be expected to have occurred approximately once for every forty examinations of females with gonopore setae as a result of subjective allocation. Inconsistencies occurring at this rate would have had little effect on conclusions concerning the relative numbers of mature and immature females. Where it was necessary to determine the state of maturity of individual, live females, however, determinations were based on several independent allocations of each female to the setal stages; e.g., for the interpretation of growth data.

It is likely that the single inconsistent allocation of an otherwise Stage 0 female to Stage 1 out of 112 allocations of females to Stage 0 was an error in recording, since the distinction between no gonopore setae and some gonopore setae is precise. The low frequency of such errors would have had little effect on conclusions.

The preceding discussion of the maturity of Stage 1 females has been based on the laboratory results for females collected during May-June. The ovarian maturity of females collected during November -December indicated, however, that a substantial proportion of females in Stage 1 were likely to spawn at the next spawning season; i.e., these females were incipiently mature. Females with carapace lengths greater than 55mm, including all females in Stages 1 and 2, were found to moult once per year, during March-April (Chapter 5). It is concluded that incipiently mature females in Stage 1 moulted during March-

April and assumed Stage 2 characteristics prior to their first spawning, since no Stage 1 females were observed to be carrying eggs.

It was not possible, however, to distinguish between immature and incipiently mature females in Stage 1 on the basis of setae around the gonopores. This has several implications for the use of the setal stages to identify females that either will spawn, or are likely to have spawned, during any given year. If crayfish are examined during the period between the annual moult and the commencement of spawning, then all females that are likely to spawn during that year will be in Stage 2. Similarly, for crayfish captured at any time outside the period between moulting and spawning, all females that spawned during the previous season will be in Stage 2. However, given the high proportion of Stage 1 females that were incipiently mature, numbers of Stage 2 females taken at any time outside the period between the annual moult and spawning would potentially provide a gross underestimate of the numbers of females likely to spawn during the next spawning season.

This problem does not apply to estimates of size at the attainment of maturity. Females allocated to Stage 1 at any time of the year had not spawned previously. Also, these females moulted only once per year, and in incipiently mature individuals this involved a change to Stage 2. Hence the size of a Stage 1 female at any time of the year was the size at which it was immature during the previous spawning season, regardless of whether the female was immature or incipiently mature when measured. Changes with carapace length in the relative abundance among females of females in Stages 1 and 2 thus provided a reliable estimate of the range of carapace lengths at

which different females attained maturity.

The overlap in the ranges of carapace lengths of females in Stages 1 and 2 (Figure 3.8) indicated that there was considerable variation in the size at which females attained maturity. Females began to mature at carapace lengths in the range 65-70mm, although only a single mature female of this size was encountered, and the vast majority of females of this size were immature. Substantial numbers of mature females were present in the 70-75mm size class, and the percentage of females that were mature increased rapidly above this carapace length, although 100% maturity was attained only among crayfish with carapace lengths greater than 95mm; i.e., only among the relatively few very large females. Considerable variability in size of females at the attainment of maturity has also been noted for the parastacids *Cherax tenuimanus* (Shipway, 1951; Morrissy, 1975) and *C. destructor* (Johnson, 1979), for the cambarid *Orconectes propinquus* (Stein et.al., 1977), and for the spiny lobsters *Jasus lalandei* (Fielder, 1964b), *Panulirus homarus* (Heydorn, 1969; Berry, 1971), and *P. longipes cygnus* (Chittleborough, 1974, 1976b). Thus maturity in many decapods, including *E. spinifer*, is not simply a function of size. Chittleborough (1974) concluded that variation in the size of female *P. longipes cygnus* resulted from variation in growth rates, with maturity being attained at a specific age. The same may have applied to *E. spinifer*, as growth rates were variable, resulting in a wide range of estimated sizes at any given age (Chapter 7). It was not feasible, however, to determine the ages of individual females. Hence there was no evidence to contradict variation in both size and age at the attainment of maturity in female *E. spinifer*.

The only other parastacid that attained a size comparable with

E. spinifer, and for which the minimum size at maturity was known, was *Cherax tenuimanus*. This species matured at a minimum carapace length of approximately 30mm (Morrissy, 1975). Hence the minimum size at maturity for female *E. spinifer* was much larger in comparison. This difference is discussed further in the context of age at maturity in Chapters 7 and 9.

Four out of five females for which records were available spawned in both 1977 and 1978, while the fifth individual spawned in 1977 only. In addition, of the eight females captured in November-December that were carrying juveniles, all had developing ovaries, indicating that they would probably have spawned again in the following spawning season. Hence it is likely that the majority of female *E. spinifer* spawned each year after the attainment of maturity. A similar conclusion was drawn by Morrissy (1970) for female *Cherax tenuimanus* that were known to have spawned previously. Morrissy considered, however, that a large percentage of eligible females of a wide range of sizes did not spawn for reasons other than immaturity. The eligibility of these females was apparently determined by the presence of egg-bearing setae on the pleopods. In a later publication Morrissy (1975) found that these setae appeared on females prior to maturity. Hence the 1970 results may have indicated variation in size at maturity rather than a low frequency of spawning among mature females. In contrast, Lake and Newcombe (1975) found that, of female *Parastacoides tasmanicus* larger than the minimum size at maturity, only 40% carried eggs, and that this resulted from the fertilisation of relatively few females.

The presence of setae around the gonopores of large female *E. spinifer* and *E. australasiensis* has been noted previously by Ryder

(1972), whose observations, in conjunction with the author's own, provided the initial stimulus to link gonopore setae with maturity. The author has also observed gonopore setae similar to those of *E. spinifer* in large female *E. armatus*, *E. valentulus*, *E. hystricosus*, and *E. kierensis*, and in each instance the full development of gonopore setae (i.e., Stage 2) has been associated with females carrying eggs. Although a detailed analysis of the relationship between gonopore setae and maturity in these species was not undertaken, the observations suggest that Stages 0, 1 and 2 in the development of gonopore setae, as described for *E. spinifer*, may be used to indicate the respective immaturity, incipient maturity and maturity of female *Euastacus* in general. Gonopore setae are also present in *Cherax destructor* (Johnson, 1979), although changes in the patterns of gonopore setae at different stages of maturity were not mentioned.

The oosetae of *E. spinifer* were similar to the egg-bearing setae of other macrurans (Appendix E), and found in similar locations on the pleopods to those described for other parastacids, e.g.,*Cherax tenuimanus* (Morrissy, 1975), *C. albidus* (Woodland, 1967), and *C. destructor* (Johnson, 1979), with the exception that short oosetae were also found on the medial margins of the exopodites in *E. spinifer*. The distributions of eggs were correspondingly similar, with the majority of eggs carried on the endopodites.

The pleopods of immature females bore only plumose setae, while the development of a partial, and then full complement of oosetae on the pleopods of incipiently mature and mature females corresponded to partial and full development of gonopore setae. There was, however, considerable overlap in the degree of development of oosetae of females in Stages 1 and 2. Also, the precise degree of development of oosetae

could only be determined by microscopic examination, while the degree of development of the gonopore setae was clearly visible to the naked eye. Hence gonopore setae alone were used as indicators of maturity. Nevertheless, oosetae must be considered a primary sex characteristic, since they performed the function of attaching eggs to the pleopods and were present only on mature or incipiently mature females, while in the absence of any observed function, the gonopore setae must be considered a secondary sex characteristic. Both types of setae appeared at an incomplete state of development before the attainment of maturity. Similar observations have been made for the egg-bearing setae of the parastacid crayfish *Cherax tenuimanus* (Morrissy, 1975), the astacid crayfish *Austropotamobius pallipes* (Thomas, 1970), and the spiny lobsters *Jasus lalandei* (Fielder, 1964b) and *Panulirus longipes cygnus* (Chittleborough, 1976b), while the egg-bearing setae of the lobster *Nephrops norvegicus* appeared shortly before maturity (Farmer, 1974b). The development of egg-bearing setae prior to maturity is logical, since these setae would be expected to have attained full development in preparation for spawning.

There was no relationship between gonopore diameter and maturity for *E. spinifer* examined in this study. Also, when the relative gonopore areas used by Ryder (1972) were transformed to square roots, these behaved in a manner similar to the gonopore diameters in that there was no substantial sudden increase at maturity. Given that the linear dimensions of gonopores increased approximately linearly with carapace length and gonopores remained the same shape, the areas of gonopores would be expected to describe an upwardly inflected branch of a parabola with increasing carapace length. Ryder's conclusions

were based on relative gonopore areas; i.e., the area of the gonopores of each crayfish divided by the smallest area encountered. Hence the carapace length at which the slope of the relationship between relative gonopore area and carapace length increased at the maximum rate was arbitrary, being dependent upon the carapace length of the smallest crayfish included in the sample. The relationship between the rapid increase in relative gonopore area and maturity found by Ryder was thus coincidental.

3.4.4 Attainment of Maturity in Males.

When male *E. spinifer* were examined during the mating season and degrees of inflation of the genital papillae were compared with testicular maturity and gonosomatic index, two separate assemblages of males were apparent (Figure 3.12). An isolated group of small males with mature testes and high gonosomatic indices were exclusively characterised by highly inflated genital papillae. These males will be termed 'precocious males' in the ensuing discussion, to distinguish them from the remaining 'normal males'.

Among normal males there was a progressive acquisition of mature characteristics with increasing carapace length. Normal males were initially characterised by a lower gonosomatic index, testicular immaturity, and uninflated genital papillae. The first sign of incipient maturity was the maturation of the testes, without any substantial increase in gonosomatic index, or inflation of the genital papillae. This was followed by a simultaneous acquisition of inflated genital papillae and increase in gonosomatic index to a level which was then maintained with increasing carapace length.

In males with high gonosomatic indices and inflated genital papillae, the distal vasa deferentia were turgid with large quantities of spermatophore material, and contributed a substantial proportion of the total gonad weight, while in males with low gonosomatic indices and uninflated genital papillae the distal vasa deferentia were relatively small (Figure 3.13). Hence the increase in gonosomatic index was considered to indicate the commencement of spermatophore production, and the associated turgor of the distal vasa deferentia was reflected in the inflation of the genital papillae. Only those males that produced spermatophore material could be considered reproductively functional. Hence the normal males with mature testes and uninflated genital papillae were considered to be functionally immature, and inflated genital papillae were concluded to be indicative of functional maturity.

The carapace lengths of functionally mature and immature normal males overlapped in the approximate range of body weights 55-70g (approximately 45-55mm carapace length). Hence it is concluded that normal males become functionally mature over this range. The gonosomatic indices typical of large males were generally attained at body weights in excess of approximately 140g (about 65mm carapace length), indicating that full spermatophore production may not have been achieved until males became considerably larger than the size at which they initially became functionally mature.

The gonosomatic indices of precocious males were considerably greater than those recorded for large, mature, normal males (Figure 3.12), and their vasa deferentia were extremely large (Figure 3.13) and filled with spermatophore material. Hence precocious males were considered to be functionally mature at a size considerably less than

that of the smallest functionally mature normal male. Apart from the highly inflated genital papillae, precocious males retained the appearance of small *E. spinifer* of both sexes, showing no outward signs of maturity in terms of body proportions or development of spines (Figures 3.13, 3.4). The gonads of all males were also similar in form, apart from differences in proportions, and were similar in overall form to those of other astacuran decapods, e.g., *Cherax destructor* (Johnson, 1979), *Nephrops norvegicus* (Farmer, 1974a).

Multiple recapture records of the condition of the genital papillae of individual males (Table 3.3) indicated that the highly inflated condition of the genital papillae was fixed once it had been attained. Precocious males could thus be reliably identified by the presence of highly inflated genital papillae, and, having become precociously mature, they remained in that condition. Based on similar evidence, immature normal males did not assume the precocious condition at carapace lengths greater than approximately 20mm. Hence at carapace lengths greater than approximately 20mm male *E. spinifer* were dimorphic. They were in either the fixed, precociously mature condition, or they were initially immature, with maturity being attained at carapace lengths in the range 45-55mm. Increases with carapace length over the range 10-20mm in the relative abundance in catches of males with highly inflated gential papillae suggested that immature normal males may have assumed the precocious condition over this range of carapace lengths. Only a small number of individual crayfish of this size were captured more than once during the study, and the genital papillae of one individual were known to have changed from the uninflated to the highly inflated condition. Thus the conclusion that males assumed the

precocious condition at carapace lengths of 10-20mm is considered justified. It must be noted, however, that the smallest precocious male that was captured during the study had a carapace length of 12.0mm, and this was among the smallest crayfish captured. Hence males may have been precociously mature at carapace lengths considerably smaller than this.

Records of the inflation of the genital papillae varied through time for numerous individual normal males with carapace lengths greater than the minimum carapace length at functional maturity (Table 3.3). This variation may have arisen in two ways. Recording of the degree of inflation may have been inconsistent for males with inflated genital papillae due to variation among males in the degree of inflation. Alternatively, the degree of inflation of the genital papillae of individual males may have varied through time, as in *Cherax tenuimanus* (Shipway, 1951). Such variation may have reflected varying turgor of the distal vasa deferentia, due to production or loss of spermatophore material. In either instance, it may be concluded that while records of inflated genital papillae were reliable indicators of functional maturity (Figure 3.12), records of uninflated genital papillae were not reliable as indicators of the immaturity of individual males.

There were, however, no systematic changes through time in the records of uninflated and inflated genital papillae for males at any carapace length. Hence changes with carapace length in the relative abundances in catches of males with inflated genital papillae may be used to indicate minimum size at functional maturity. These results were compared with similar results for males with highly

inflated genital papillae (Figure 3.14) and clearly illustrated the dimorphic nature of maturity in the male population during the period of the study. Changes through time in the relative abundances of the different types of males are considered in Chapter 4, and hypotheses are presented to account for the observed patterns.

The contribution of precocious males to the successful mating of the much larger mature females was not determined for wild crayfish. Several pairs of crayfish were maintained in captivity during each of the years 1977, 1978 and 1979 in an attempt to obtain information on mating habits. Only two matings occurred, between a normal male and a mature female, and between a precocious male and a mature female. Hence precocious males were capable of mating with mature females in the absence of other males in captivity. Also, precocious males captured during the mating season ejected considerable quantities of spermatophore material if they were handled at all roughly. Hence it is likely that precocious males were in an advanced state of preparation for mating under natural conditions.

Examples of male size dimorphism have been recorded for copepods (Haq, 1965, 1972) and fish (Briggs, 1953; Shapovalov and Taft, 1954). Other types of unusual male reproductive phenomena among crustaceans include the complemental males of some barnacles (Barnes, 1974) and the alternating reproductive and non-reproductive forms of male cambarid crayfishes (e.g., Hobbs, 1974). However, the simultaneous presence of two types of mature males of different sizes does not appear to have been described previously for decapods. The possible significance of these two male forms in the biology of *E. spinifer* is discussed in Chapter 9, in the context of information

on relative abundances and growth rates from other chapters.

Riek (1951) considered that individuals with three or four gonopores were not uncommon among Australian parastacids, that when three gonopores were present they were almost invariably two male and one female, and that the female and male gonopores were typically located in the normal positions on the coxae of the third and fifth pereiopods respectively. *E. spinifer* conformed to this pattern, although the single individual with female gonopores on the coxae of the left third and fourth pereiopods was unusual. Johnson (1979) found that *Cherax destructor* with one female and two male gonopores were typically normal, functional males, and Woodland (1967) reached a similar conclusion for *C. albidus*. Similarly, *E. spinifer* with aberrant gonopores displayed characteristics typical of males, and have been treated as such elsewhere in this study.

CHAPTER FOUR

POPULATION STRUCTURE

4.1 INTRODUCTION

Sizes at maturity of female, normal male and precocious male *E. spinifer* were described in the previous chapter, although their numerical contributions to populations were not considered. An attempt is made in this chapter to describe the sex and size structures of populations of *E. spinifer* in the study area. Relative abundances in populations of females and normal and precocious males of different sizes have been inferred from their abundances in catches, on the basis of tests for equal average catchability. Where possible, general trends in the structures of populations of crayfish in the study area have been characterised, particularly with respect to the occurrence of precocious males. Two hypotheses have been presented to account for the observed abundances of these crayfish, and the nature of additional information required to evaluate the hypotheses is discussed.

4.2 METHODS

4.2.1 Variation in Average Catchability.

Average catchabilities of crayfish were estimated from monthly catches taken from Pools 3 and 7 during the mark-recapture study. From mark-recapture records, crayfish were considered to be available

for capture at all samplings during a particular period if they were captured on or before the first sampling of the period, and on or after the last sampling of the period. On this basis, proportions of actual captures of crayfish among total numbers of opportunities for capture were used to estimate average catchabilities of crayfish in selected sex and size groups. Estimates of this type were obtained separately for females and normal males in the carapace length classes 20-30mm, 30-40mm, 40-50mm, and 50-75mm from Pool 3, and for crayfish from Pool 7 in the carapace length classes 20-40mm and 40-70mm, for the periods November 1977 to February 1978, and May to August 1978. These two periods were selected as a compromise between the availability of data, and the need to cover as much of a single year as possible in order to include any seasonal differences in the average catchabilities of crayfish of different sex and size. The periods were restricted to four months as data for longer periods were scarce, and size classes were made as narrow as possible while still retaining sufficient data for analysis.

Results were analysed separately for each period, for each location (Tables 4.1-2). Initially, the average catchabilities of normal males and females within each size class were tested for significant differences ($p < 0.05$) using values of chi-squared calculated from 2 x 2 contingency tables (Table 4.1). The difference between the overall catchabilities (i.e., combined for size classes) of males and females was tested for significance in a similar manner, and the relationship between the catchabilities of males and females was tested for heterogeneity among the size classes. A comparison between the catchabilities of male and female crayfish with carapace

lengths of 75-100mm was also conducted for the period February to May, 1978. Average catchabilities were recalculated for the combined data for normal males and females in each size class in instances where, for a particular period and location, there were no significant results in the preceding analysis. The combined average catchabilities of normal males and females were then tested for heterogeneity with respect to size class, separately for each period and location, using a variance test for homogeneity of the binomial distribution (Snedecor and Cochran, 1967) (Table 4.2). Average catchabilities of females from Pool 3 with carapace lengths in the range 75-100mm were also included in this analysis. In instances where this overall test indicated that there were significant differences among the catchabilities of crayfish in the different size classes, the size class with the most obviously different catchability was removed, and the test was recalculated.

An attempt was also made to compare the catchabiliites of precocious and normal males. This test was confined to crayfish with carapace lengths of 20-25mm, due to the virtual absence of multiple recapture records for precocious males of other carapace lengths. Data were collected for the periods July to October 1977, November 1977 to February 1978, and March to June 1978. Since the data for each period were in general inadequate for separate testing, the periods were combined. In order to prevent bias in the results due to possible differences in the catchabilities of precocious and normal males during the different periods, random samples of normal males were selected such that the ratio of normal males to precocious males in each period was fixed for each of the two locations (Table 4.3). Also, sample sizes were insufficient to allow testing for heterogeneity among the periods in the relationship

between the catchabilities of precocious and normal males. A visual inspection was thus made,before combining the periods, of the differences between the observed catches and the catches expected under the null hypothesis that there was no difference between the catchabilities of the two groups of crayfish for each period, to check if such differences were likely to have occurred. The combined data were then used to test for significant differences between the catchabilities of precocious and normal males for each of the two locations (Table 4.3) using 2 x 2 contingency tables.

4.2.2 Sex Structure of Populations.

Catches were analysed initially to determine whether there had been any changes in the relative abundances of female and male crayfish of different sizes over the duration of the mark-recapture study. Female and male crayfish were allocated to the carapace length classes 20-30mm, 30-40mm, 40-50mm, 50-70mm, and 70-100mm, and separate distributions of percent of catch vs. month were constructed for males and females in each size class, for each location (Figure 4.1). Differences between the distributions for males and females were tested for significance using a Kolmogorov-Smirnov test (Siegel, 1956). All data were used in these tests in instances where the total catches for both males and females exceeded forty. For smaller total catches, tests were made using samples of equal numbers of males and females selected randomly from the total catches.

Changes through time in the numbers of precocious males were not comparable with those of other crayfish. To provide additional

insight into these differences, another approach was taken. Normal and precocious males were allocated to the carapace length classes <20mm, 20-25mm, 25-30mm and 30-35mm, for each of the two locations. Total catches of males with carapace lengths less than 35mm were tabulated for the periods May to August 1977, September to December 1977, January to April 1978, May to August 1978, and September to December 1978, and the percentage of each catch attributable to precocious or normal males in each size class was calculated. The resulting percentages were plotted as frequency histograms for each period (Figure 4.2), and trends through time in the relative numbers and sizes of precocious and normal males were noted.

Average sex structures of the populations of crayfish in Pools 3 and 7 (Figure 4.3) were determined for the period of the mark-recapture study, and interpreted on the basis of the results for catchabilities and variation through time in the relative abundances of females, and normal and precocious males. The total catch taken in each location during the mark-recapture study was divided into 5 mm carapace length classes, commencing at 10mm. Percent frequencies of male and precocious male crayfish in the total catch for each size class were calculated, and relationships between these frequencies and carapace lengths were constructed for both locations. Ninety-five percent confidence limits for the mean frequencies with repeated sampling were also plotted, based on tables by Crow (1956) for sample sizes less than or equal to thirty, or on a normal approximation for samples of larger size (Snedecor and Cochran, 1967). This procedure was also applied to combined catches for June, July, August, and November 1976 from Pool 7, for January and June 1978 from Pool 8, and for a single catch taken from Pool 6 during December 1977. These catches from Pools 6, 7 and 8 were taken using nets

spaced at considerably larger intervals than used in the mark-recapture study, with the aim of maximising the numbers of large crayfish in catches.

4.2.3 Size Structure of Populations

Monthly catches from the mark-recapture study were combined for the periods May to August 1977, September to December 1977, January to April 1978, May to August 1978, and September to December 1978, for each of the two sampling locations. Frequency histograms were constructed for the carapace lengths of crayfish in each combined catch (Figure 4.5) and changes through time in the size structure of the catches were noted for the two populations.

4.3 RESULTS

4.3.1 Variation in Average Catchability.

There were no significant differences ($p > 0.05$) in the average catchabilities of male and female *E. spinifer* for the periods of testing, either within any particular size class, or when size classes were combined to provide estimates of overall catchabilities (Table 4.1). Also, there were no instances of significant heterogeneity ($p > 0.05$) among the size classes in the relationships between the catchabilities of male and female crayfish. When average catchabilities were combined for males and females and tested for homogeneity with respect to size class, there was significant heterogeneity for

Table 4.1: Comparisons of Average Catchabilities of Female and Normal Male Crayfish.

Location & Period	Size Class (mm)	Female *P	Female N	Male P	Male N	χ^2 (1 d.f.)	p
Pool 3: Nov. 1977 - Feb. 1978	20-30	0.48	104	0.50	104	0.08	>0.25
	30-40	0.31	32	0.42	36	0.79	>0.25
	40-50	0.55	40	0.34	44	3.70	>0.05
	50-75	0.39	36	0.47	32	0.44	>0.50
	Overall	0.45	212	0.45	216	0.01	>0.90
	Heterogeneity χ^2 = 5.02 (3 d.f.)						>0.10
Pool 3: May 1978 - Aug. 1978	20-30	0.21	52	0.15	40	0.57	>0.25
	30-40	0.41	132	0.37	112	0.47	>0.25
	40-50	0.18	28	0.13	16	0.22	>0.50
	50-75	0.29	24	0.22	32	0.39	>0.50
	Overall	0.33	236	0.28	200	1.10	>0.25
	Heterogeneity χ^2 = 0.55 (3 d.f.)						>0.90
Pool 7: Nov. 1977 - Feb. 1978	20-40	0.18	44	0.14	56	0.28	>0.50
	40-70	0.18	76	0.25	16	0.36	>0.50
	Overall	0.18	120	0.17	72	0.09	>0.50
	Heterogeneity χ^2 = 0.56 (1 d.f.)						>0.25
Pool 7: May 1978 - Aug. 1978	20-40	0.61	64	0.57	44	0.18	>0.50
	40-70	0.41	68	0.63	16	2.38	>0.10
	Overall	0.51	132	0.58	60	0.95	>0.25
	Heterogeneity χ^2 = 1.61 (1 d.f.)						>0.10
Pool 3: Feb. 1978 - May 1978	75-100	0.14	36	0.08	12	0.25	>0.50

*P is the proportion of captures among the total number of opportunities for capture (N) of crayfish known to be present during the given period.

Table 4.2: Comparisons of Average Catchabilities of Crayfish in Different Size Classes, Combined for Females and Normal Males.

Location	Size Class	PERIOD Nov '77 - Feb '78 * P	N	May '78 - Aug '78 P	N
Pool 3:	20-30	0.49	208	0.18	92
	30-40	0.37	68	0.39	244
	40-50	0.44	84	0.16	44
	50-75	0.43	68	0.25	56
	[1]75-100	0.18	28	0.19	36
	Overall	0.43	456	0.30	472
		Overall Homogeneity		Overall Homogeneity	
		[2]χ^2_c = 11.4, d.f. = 4		χ^2 = 21.9, d.f. = 4	
		0.025 > p > 0.01		p < 0.005	
		Minus 75-100		Minus 30-40	
	Adjusted	0.45	428	0.20	228
		Remaining Homogeneity		Remaining Homogeneity	
		χ^2_c = 3.4, d.f. = 3		χ^2 = 1.5, d.f. = 3	
		p > 0.25		p > 0.50	
Pool 7:	20-40	0.16	100	0.59	108
	40-70	0.20	92	0.45	84
	Overall	0.18	192	0.53	192
		Overall Homogeneity		Overall Homogeneity	
		χ^2_c = 0.21, d.f. = 1		χ^2 = 3.19, d.f. = 1	
		p > 0.50		0.1 > p > 0.05	

* P is the porportion of captures among the total number of opportunities for capture (N) of crayfish known to be present during the given period.

[1]Female crayfish only.
[2]Chi-squared corrected for continuity.

both periods in Pool 3 (Table 4.2). Removal of the 75-100mm size class from the November 1977 to February 1978 series rendered the catchabilities of the remaining size classes homogeneous ($p > 0.25$), while similar results were obtained for the period May to August 1978 by removing the 30-40mm size class ($p > 0.50$). The catchabilities of each of these size classes were aberrant in only one of the two periods. There was no significant heterogeneity ($p > 0.05$) with respect to size in the catchabilities of crayfish from Pool 7 for either period.

There was a substantial difference between the observed and expected catches of precocious and normal male crayfish from Pool 3 for the period November 1977 to February 1978 (Table 4.3). Separate testing using a 2 x 2 contingency table indicated that this difference was not significantly different from zero (χ^2 corrected for continuity = 1.42, d.f. = 1, $0.25 > p > 0.1$), and the observed and expected catches were extremely close for all other periods, for both locations. On this basis, the data for the three periods were combined for each location, and no significant differences were detected between the catchabilities of precocious and normal males (Table 4.3).

4.3.2 Sex Structure of Populations.

Female and male crayfish in both locations generally showed similar overall trends in capture frequency during the mark-recapture study (Figure 4.1), although short-term differences were evident in many instances. The only evidence of a change through time in the relative capture frequencies of males and females was for crayfish

Table 4.3 Comparisons of Average Catchabilities of Precocious and Normal Males with Carapace Lengths of 20-25 mm.

Male Type	Separate Periods								
	Jul-Oct.'77			Nov.'77-Feb.'78			Mar.-June '78		
	*C	E	N	C	E	N	C	E	N
Location - Pool 3.									
Precocious	1	1	4	7	4.8	12	3	2.5	8
Normal	3	3	12	12	14.2	36	7	7.5	24
Location - Pool 7.									
Precocious	6	5	16	3	4	16	1	1.5	8
Normal	4	5	16	5	4	16	2	1.5	8

Location	Combined Periods					
	Precocious		Normal		x_e^2	P.
	P	N	P	N	(1d.f.).	
Pool 3	0.46	24	0.31	72	1.23	> 0.25
Pool 7	0.25	40	0.28	40	0	≃ 1

* C is the observed number of captures among the total number of opportunities for capture (N) of crayfish known to be present during the given period. E is the expected value for C under the null hypothesis that there is no difference between the frequency of capture of precocious and normal males that are known to be present. As in previous tables, P = C/N.

Figure 4.1: Changes During the Mark-Recapture Study in the Capture Frequencies of Male and Female Crayfish at the Two Locations.

Key:-

● - Female crayfish

○ - Male crayfish

◉ - Female + Male Crayfish

Size classes 20-30mm, 30-40mm, 40-50mm, 50-70mm, and 70-100mm.

Total catches of female and male crayfish are included in each graph.

Pool 3

Pool 7

% OF TOTAL CATCH OF MALES OR FEMALES

20-30

20
10

F = 259
M = 224

14
7

F = 116
M = 140

30-40

12
6

F = 271
M = 209

16
8

F = 136
M = 67

40-60

13
6·5

F = 81
M = 78

17
8·5

F = 86
M = 24

50-70

15
7·5

F = 48
M = 74

13
6·5

F = 117
M = 45

70-100

18
9

F = 74
M = 28

29
14·5

F = 41
M = 17

My Jy S N Ja M My Jy S N

My Jy S N Ja M My Jy S N

MONTH

in the 20-30mm size class in Pool 7 (Table 4.4). In this instance the difference between males and females just failed to reach significance at the 5% level (D = 0.168, critical value of D at the 5% level = 0.171), suggesting a trend towards an increasing number of males relative to females in the later part of the study.

The size frequency distributions of precocious and normal males (Figure 4.2) indicated that in Pool 7 there was a substantial increase in the numbers of precocious males relative to normal males in the 20-25mm size class during the period May 1977 to April 1978. This occurred simultaneously with substantial increases in the relative numbers of smaller, normal males, and was followed by a striking increase in the relative numbers of precocious males in the 25-30mm size class during May to August 1978. Relative numbers of normal and precocious males were, however, approximately equal during September to December 1978, in all size classes except the 30-35mm class, from which precocious males were absent. An increase in the relative numbers of precocious males also occurred in Pool 3 over the same period. Normal males were initally much more abundant in the 20-25mm and 25-30mm size classes, while approximately equal numbers of precocious and normal males were present in the 30-35mm size class. At the end of the mark-recapture study the numbers of precocious and normal males were approximately equal, except in the 30-35mm size class in which normal males were considerably more abundant. The relative abundances of precocious and normal males differed considerably between periods twelve months apart in both locations, indicating that the overall changes were not the result of some type of annual cycle.

Table 4.4 Results of Kolmogorov-Smirnov Tests Comparing Trends Through Time in the Capture Frequencies of Male and Female Crayfish.

Size Class	*	Pool 3				Pool 7		
	F	M	D	p	F	M	D	p.
20-30	259	224	11	>0.1	116	140	17	≈0.05
30-40	271	209	11	>0.1	136	67	12	>0.1
40-50	81	78	19	>0.1	24	24	17	>>0.05
50-70	48	74	23	>0.1	117	45	11	>0.1
70-100	28	28	39	>>0.05	17	17	24	>>0.05

F = total catch of females.

M = total catch of males.

O = maximum difference between the cumulative distributions of percent frequency of capture vs. month for males and females.

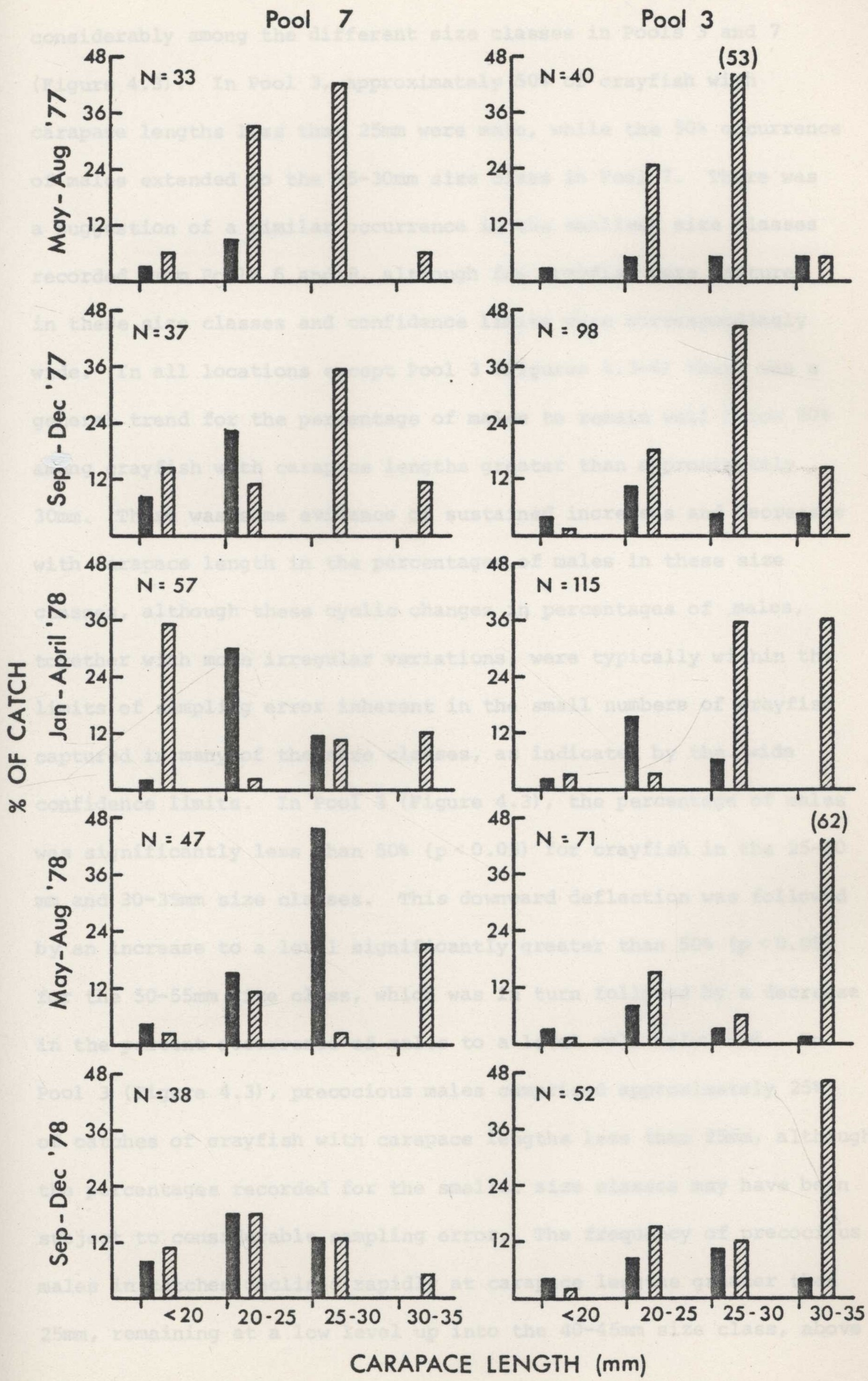
p = the probability of obtaining a difference equal to or greater than the observed difference D purely by chance, given that the two distributions were from the same population.

Figure 4.2: Changes During the Mark-Recapture Study in the Relative Capture Frequencies of Precocious and Normal Male Crayfish in the Smaller Size Classes.

Key:-

Size classes < 20mm, 20-25mm, 25-30mm, 30-35mm.

The total catch of males in all of the included size classes (N) is listed in each graph.


Pool 7
Pool 3
May-Aug '77
Sep-Dec '77
Jan-April '78
May-Aug '78
Sep-Dec '78
N = 33
N = 40
(53)
N = 37
N = 98
N = 57
N = 115
N = 47
N = 71
(62)
N = 38
N = 52
48
36
24
12
% OF CATCH
<20
20-25
25-30
30-35
CARAPACE LENGTH (mm)

Abundances in catches of males relative to females varied considerably among the different size classes in Pools 3 and 7 (Figure 4.3). In Pool 3, approximately 50% of crayfish with carapace lengths less than 25mm were male, while the 50% occurrence of males extended to the 25-30mm size class in Pool 7. There was a suggestion of a similar occurrence in the smallest size classes recorded from Pools 6 and 8, although few crayfish were captured in these size classes and confidence limits were correspondingly wide. In all locations except Pool 3 (Figures 4.3-4) there was a general trend for the percentage of males to remain well below 50% among crayfish with carapace lengths greater than approximately 30mm. There was some evidence of sustained increases and decreases with carapace length in the percentages of males in these size classes, although these cyclic changes in percentages of males, together with more irregular variations, were typically within the limits of sampling error inherent in the small numbers of crayfish captured in many of the size classes, as indicated by the wide confidence limits. In Pool 3 (Figure 4.3), the percentage of males was significantly less than 50% ($p < 0.05$) for crayfish in the 25-30 mm and 30-35mm size classes. This downward deflection was followed by an increase to a level significantly greater than 50% ($p < 0.05$) for the 50-55mm size class, which was in turn followed by a decrease in the percent occurrence of males to a level well below 50%. In Pool 3 (Figure 4.3), precocious males comprised approximately 25% of catches of crayfish with carapace lengths less than 25mm, although the percentages recorded for the smaller size classes may have been subject to considerable sampling error. The frequency of precocious males in catches declined rapidly at carapace lengths greater than 25mm, remaining at a low level up into the 40-45mm size class, above

Figure 4.3: Changes with Carapace Length in the Frequency of Capture of Males Relative to Females, Averaged Over the Mark-Recapture Study for Pools 3 and 7.

Key:-

● - Normal males.

O - Precocious males.

| - 95% confidence limits for the mean percentage on repeated sampling.

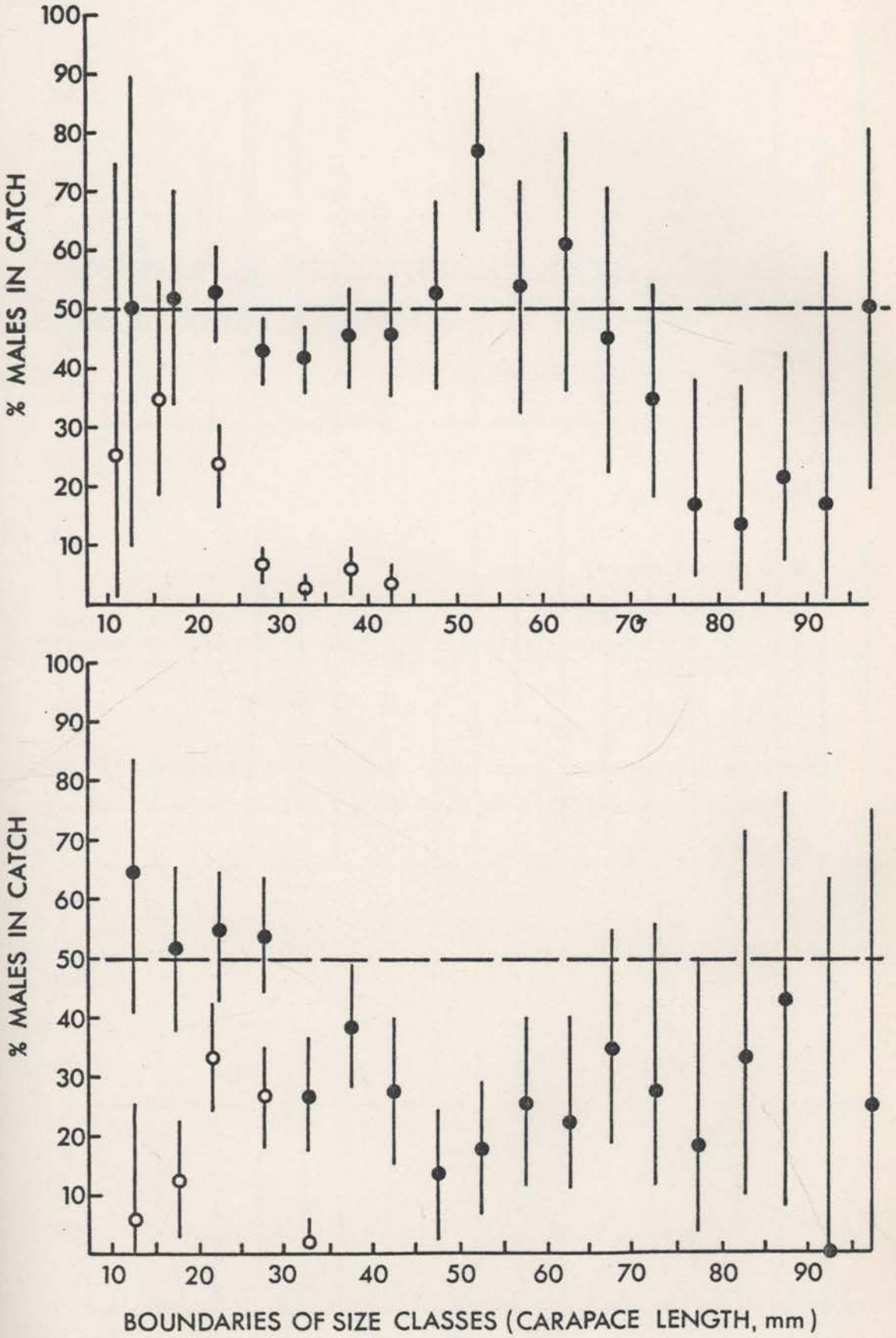

% MALES IN CATCH
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
% MALES IN CATCH
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
BOUNDARIES OF SIZE CLASSES (CARAPACE LENGTH, mm)

Figure 4.4: Changes with Carapace Length in the Frequency of Capture of Males Relative to Females for Catches taken in Pools 6, 7 and 8.

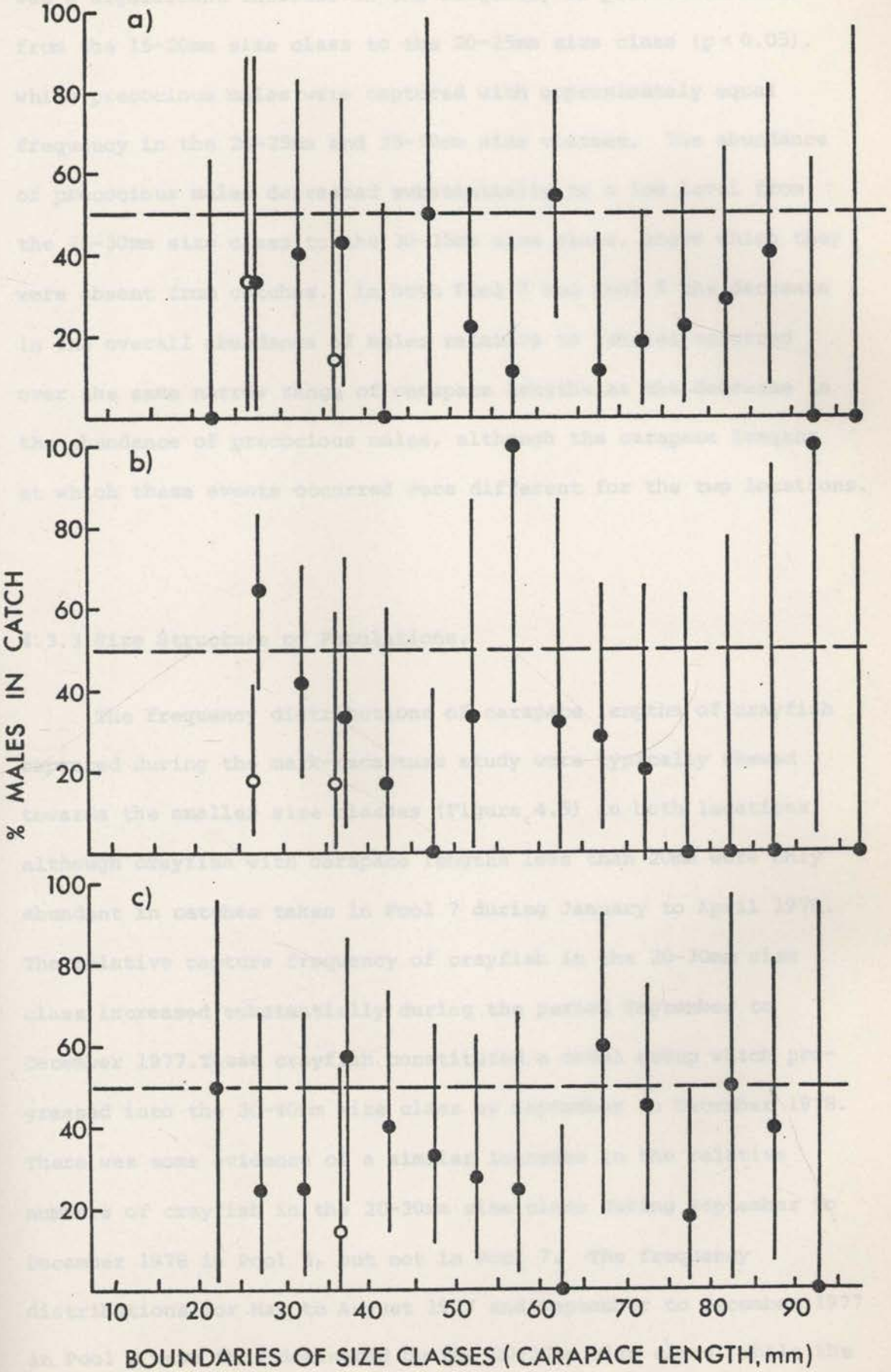
Key:-

● - Normal males.

o - Precocious males.

| - 95% confidence limits for the mean percentage on repeated sampling.

Catches taken during June, July, August and November 1976 in Pool 7, during December 1977 in Pool 6, and during January and June 1978 in Pool 8.


a)
b)
c)
100
80
60
40
20
% MALES IN CATCH
10
20
30
40
50
60
70
80
90
BOUNDARIES OF SIZE CLASSES (CARAPACE LENGTH, mm)

which they were absent from catches. In Pool 7 (Figure 4.3) there was a significant increase in the frequency of precocious males from the 15-20mm size class to the 20-25mm size class ($p < 0.05$), while precocious males were captured with approximately equal frequency in the 20-25mm and 25-30mm size classes. The abundance of precocious males decreased substantially to a low level from the 25-30mm size class to the 30-35mm size class, above which they were absent from catches. In both Pool 3 and Pool 7 the decrease in the overall abundance of males relative to females occurred over the same narrow range of carapace lengths as the decrease in the abundance of precocious males, although the carapace lengths at which these events occurred were different for the two locations.

4.3.3 Size Structure of Populations.

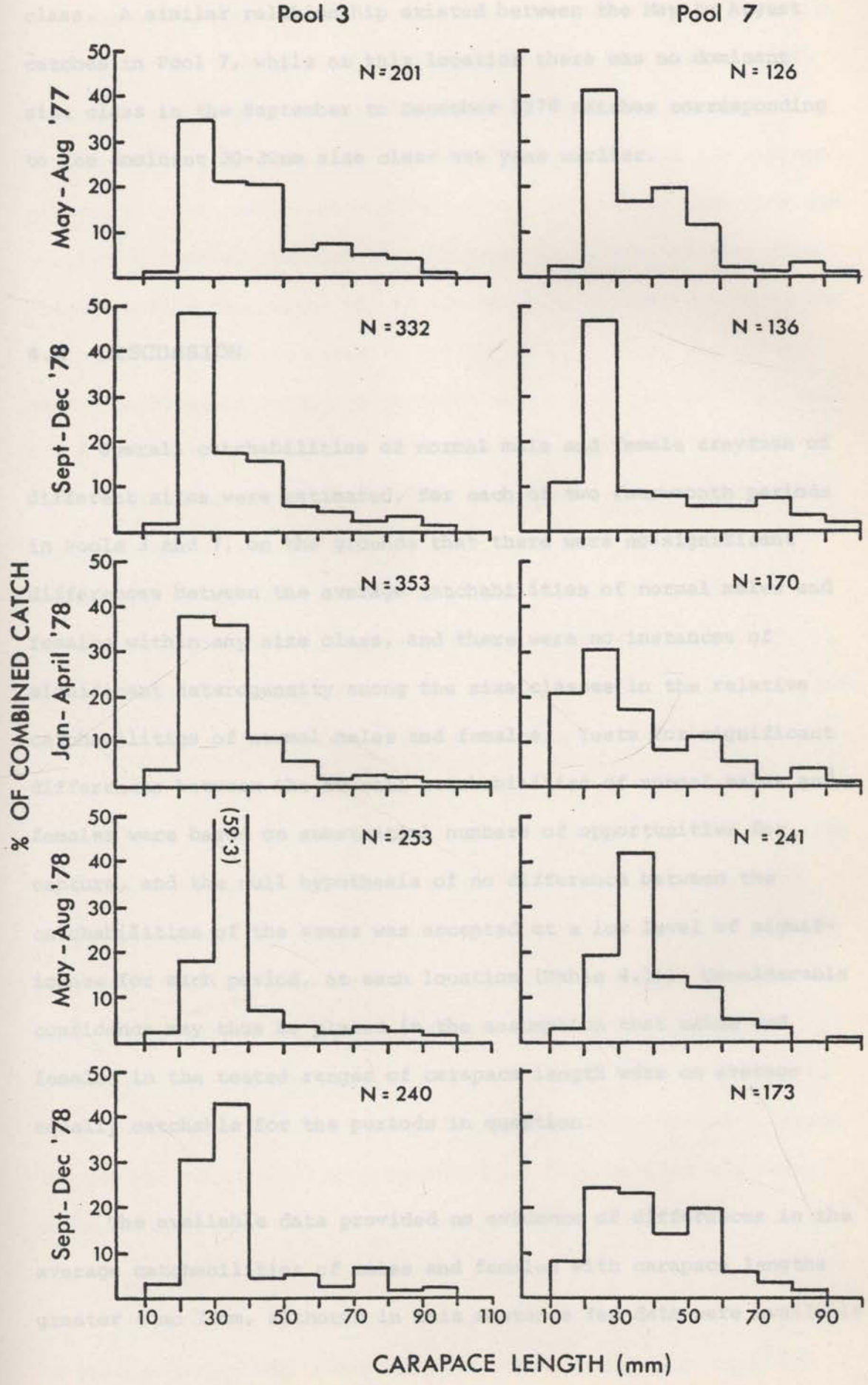
The frequency distributions of carapace lengths of crayfish captured during the mark-recapture study were typically skewed towards the smaller size classes (Figure 4.5) in both locations, although crayfish with carapace lengths less than 20mm were only abundant in catches taken in Pool 7 during January to April 1978. The relative capture frequency of crayfish in the 20-30mm size class increased substantially during the period September to December 1977.These crayfish constituted a modal group which progressed into the 30-40mm size class by September to December 1978. There was some evidence of a similar increase in the relative numbers of crayfish in the 20-30mm size class during September to December 1978 in Pool 3, but not in Pool 7. The frequency distributions for May to August 1977 and September to December 1977 in Pool 3 were thus dominated by the 20-30mm size class, while the

Figure 4.5: Size Frequency Distributions of Captures Taken Over Five Periods During the Mark-Recapture Study in Pools 3 and 7.

Key:-

N = Total number of captures for the period.

( ) = Percentage extending off graph.


Pool 3
Pool 7
May - Aug '77
Sept - Dec '78
Jan - April '78
May - Aug '78
Sept - Dec '78
N = 201
N = 126
N = 332
N = 136
N = 353
N = 170
N = 253
N = 241
N = 240
N = 173
(59.3)
% OF COMBINED CATCH
CARAPACE LENGTH (mm)
10
30
50
70
90
110

same periods one year later were dominated by the 30-40mm size class. A similar relationship existed between the May to August catches in Pool 7, while at this location there was no dominant size class in the September to December 1978 catches corresponding to the dominant 20-30mm size class one year earlier.

4.4 DISCUSSION

Overall catchabilities of normal male and female crayfish of different sizes were estimated, for each of two four-month periods in Pools 3 and 7, on the grounds that there were no significant differences between the average catchabilities of normal males and females within any size class, and there were no instances of significant heterogeneity among the size classes in the relative catchabilities of normal males and females. Tests for significant differences between the overall catchabilities of normal males and females were based on substantial numbers of opportunities for capture, and the null hypothesis of no difference between the catchabilities of the sexes was accepted at a low level of significance for each period, at each location (Table 4.1). Considerable confidence may thus be placed in the assumption that males and females in the tested ranges of carapace length were on average equally catchable for the periods in question.

The available data provided no evidence of differences in the average catchabilities of males and females with carapace lengths greater than 75mm, although in this instance few data were available

for males (Table 4.1). Males of this size were found to be similar to males of smaller size in terms of both reproductive capabilities (Chapter 3) and annual growth patterns (Chapter 5). Females with carapace lengths greater than 70mm may have been reproductively mature while smaller females were not (Chapter 3), and the average catchability of females with carapace lengths greater than 75mm was similar to that of crayfish of both sexes in several smaller size classes during the period May to August 1978, including much of the period of reproductive activity for that year. Also, the annual growth patterns of females with carapace lengths greater than 70mm did not differ substantially from those of crayfish with carapace lengths in the range 55-70mm. Thus given the apparent absence of likely causes of changes with carapace length in the relative catchabilities of males and females, and the similarities observed in the scant data available for the large crayfish (Table 4.1), it seems reasonable to assume that the conclusions for smaller crayfish also hold for crayfish with carapace lengths greater than 75mm.

Although few data were available for precocious males for any four-month period, there was no substantial evidence that their average catchability differed from that of normal males of similar size when the data were combined for periods spanning one year. Also, the observed catches of precocious and normal males were extremely close to the catches expected under the null hypothesis of no difference in catchability for most of these periods. Hence the assumption of similar catchabilities of precocious and normal males at different times during the year was considered justified.

Extension of the assumption of equal average catchabilities to females and normal and precocious males in any size class of

crayfish throughout the study area, and for the duration of the period of study, involves an element of uncertainty. However, considering the strength of the conclusions for the majority of female and normal male crayfish over eight months of a year for two locations, together with the results for precocious males and the probability that large crayfish conformed to the patterns observed for smaller crayfish, there are reasonable grounds for accepting the general assumption of equal average catchabilities within size classes as a realistic working approximation. Hence the composition of catches taken during the study may be used to provide a realistic indication, within the limits set by sample sizes, of the relative abundances of female, normal male, and precocious male crayfish in populations at the study site.

Since the relative capture frequencies of male and female crayfish within the different size classes did not change significantly over the duration of the mark-recapture study (Figure 4.1, Table 4.4), the relative abundances of males and females were adequately summarised by calculating the percent frequency of males in the total catch for each size class over the duration of the mark-recapture study. These results indicated that for crayfish in Pool 7, the percentage of males was typically well below 50% at carapace lengths in the approximate range 30-100mm (Figure 4.3). Similar results were obtained for the catches taken from Pool 7 during the previous year (1976), and for catches taken during 1977 and 1978 in Pools 6 and 8 respectively (Figure 4.4). Also, while there was an increase in the percentages of male crayfish at intermediate carapace lengths in Pool 3, the trend towards percentages of males below 50% was apparent for both larger and smaller crayfish

(Figure 4.3). A scarcity of males relative to females at carapace lengths greater than approximately 30mm may thus be considered typical of the population of crayfish at the study site during the period of study, while the superabundance of males at intermediate carapace lengths in Pool 3 may be considered aberrant. This is in contrast to the similar abundances of males and females in the smaller size classes in both Pool 3 and Pool 7 (Figure 4.3). Precocious males were also abundant in these size classes, and the carapace length at which the relative abundance of males decreased coincided exactly with the virtual disappearance of precocious males from catches. Although the smaller size classes were poorly represented in other catches (Figure 4.4), precocious males were occasionally included in them, indicating that an association between an absence of precocious males among the larger crayfish and percentages of males below 50% was probably general throughout the study area.

Before an attempt is made to clarify this association, several points require further discussion. According to results in Chapter 3, it was unlikely that any males changed from the precocious to the normal condition, and that while a change in the reverse direction was unlikely at carapace lengths greater than 20mm, this may have occurred at smaller sizes. This suggestion may be further supported by the simultaneous increases in the abundances of normal males with carapace lengths less than 20mm and larger precocious males during the period September 1977 to April 1978 in Pool 7 (Figure 4.2). Hence increases with carapace length in the percentages of precocious males in size classes up to a carapace length of 20mm may have been due to normal males assuming the precocious

condition. It is unlikely, however, that decreases in percentages of precocious males at carapace lengths greater than 20mm were due to precocious males assuming the normal condition. It also follows that normal males with carapace lengths greater than 20mm were unlikely to have been either previously or incipiently precocious. Precocious males were abundant in the 20-25mm size class in both Pool 3 and Pool 7 during the mark-recapture study (Figure 4.3). Hence the contribution of precocious males to the high percentages of males in the smaller size classes in Pools 3 and 7 during the mark-recapture study may be investigated by comparing the percentage of normal males in the 20-25mm size class in Pools 3 and 7 with percentages of larger males in size classes from which precocious males were typically absent. If it is assumed that the unusually high percentages of males in the intermediate size classes in Pool 3 (Figure 4.3) were due to a chance aggregation of males in this location, then the best estimate of the percentage of males among crayfish with carapace lengths greater than 30mm in the study area in general is given by the combined percentage of males in catches from the other locations. This percentage was compared to the percentage of normal males among crayfish with carapace lengths of 20-25mm, combined for Pools 3 and 7. The respective percentages were calculated as 28% and 26%, with ninety-five percent confidence limits for the mean values after repeated sampling of ±3% and ±5% respectively. The close agreement of these estimates strongly suggests that the general low abundance of males among crayfish with carapace lengths greater than 30mm was a direct consequence of similar relative abundances of normal males of smaller size. Similarly, it is likely that the higher overall abundances of males among the smaller crayfish in Pools 3 and 7 during the mark-recapture study were accounted for exclusively by the presence of precocious males.

This result may be viewed in either of two ways, generating two hypotheses for the origins of the observed trends in relative abundances of males (Table 4.5).

The first of the two hypotheses assumes that the observed relative abundances of females, precocious males and normal males from Pools 3 and 7 (Figure 4.3) were characteristic, when averaged over the duration of the mark-recapture study, of previous generations of crayfish throughout the study area, and is included as the simplest explanation of the observed trends. If this hypothesis is to be acceptable, it is necessary to invoke some factor to prevent most precocious males from attaining a carapace length greater than 25-30mm. A cessation of growth alone is unlikely, since in the absence of excessive mortality it would have resulted in a persistent accumulation of precocious males at this carapace length, and this was not indicated by changes in the abundance of precocious males through time (Figure 4.2). There was also some evidence that the growth rates of precocious males were similar to those of other crayfish (Chapter 7). Hence if Hypothesis 1 holds, then it is implicit that initially abundant precocious males underwent close to 100% mortality over the range of carapace lengths 25-30mm. This hypothesis is supported to some extent by the dramatic decrease in the relative abundance of precocious males in the 25-30mm size class in Pool 7 between the periods May to August 1978 and September to December 1978 (Figure 4.2).

The second hypothesis (Table 4.5) implies that during previous generations females were consistently more abundant than males at all sizes, and that the observed equal abundances of males and females in the smaller size classes in Pools 3 and 7 during the

Table 4.5 Hypotheses for the Origins of the Observed Trends in the Abundances of Male Crayfish Relative to Females.

Hypothesis 1.	The decrease with increasing carapace length in the abundance of males relative to females resulted from the failure of initially abundant precocious males to attain a larger size.
Hypothesis 2.	The increase in the abundance of males relative to females with decreasing carapace length indicated that numbers of precocious males had only recently entered the population, and had not had time to attain a larger size.

mark-recapture study were due to the recent inclusion of numerous precocious males in the populations, in addition to normal males and at the expense of females. This hypothesis is supported by the considerable increase in the abundance of precocious males during the mark-recapture study in Pool 7 (Figure 4.2), in association with a possible decrease in the relative abundance of small females (Table 4.4; Figure 4.1), and by a less substantial increase in the relative abundance of precocious males in Pool 3 (Figure 4.2).

It is not possible to exclude either of the above hypotheses on the basis of the available data, since they are not mutually exclusive when applied to data collected over a relatively short period; i.e., the observed changes through time in the relative abundances of precocious males may have been typical of previous generations when averaged over the duration of the mark-recapture study. Based on this argument, both Hypothesis 2 and Hypothesis 1, if in the latter instance precocious males did not always suffer excessive mortality, may account for the high percentages of males among crayfish in the intermediate size classes in Pool 3 (Figure 4.3). The assumption that these high percentages represented a chance aggregation of males is considered reasonable. Nevertheless, it may represent an increase in the proportion of males among offspring during previous generations, thus implying that the type of event described by Hypothesis 2 may not have been an isolated occurrence, having occurred previously. Also, there was some evidence of cyclic variations with carapace length in the relative abundances of males in all locations apart from Pool 3 (Figures 4.3-4). These cycles were different for the different locations, and although they may have been accounted for by sampling error, they may have reflected

the irregular occurrence of numerous precocious males during the previous histories of the populations of crayfish in the different locations. Such an occurrence could be explained simply by the hypothesis that precocious males were produced by only some females, since mature females were sparsely distributed through the study area and were highly mobile.

Variations in sex ratios with size are widespread among crustaceans, although apart from well-documented instances of sequential hermaphroditism, the origins of these variations are generally unknown (Wenner, 1972). It has been established with reasonable certainty that for *E. spinifer* differences between the approximate 1:1 sex ratio among small crayfish in Pools 3 and 7 and the general relative scarcity of males among larger crayfish were due to the respective presence and absence of precocious males. The long-term numerical contributions of precocious males to populations of *E. spinifer* remain unclear, however. The exact nature of these contributions could only be established by following changes in the relative abundances of precocious males, normal males and females in successive year classes of crayfish in different locations, in conjunction with sampling experiments designed to provide information on size-specific mortality rates. A study of the relative abundances of the three reproductive types among the offspring of individual females is also indicated.

Although the catches of crayfish in both Pool 3 and Pool 7 were characterised by a high percentage of small crayfish over the duration of the mark-recapture study (Figure 4.5), the size composition of catches over this period was by no means stable. Some of this

variability in catch composition would have been due to differences in the average catchabilities of crayfish of different carapace lengths. For example, the high percentage of crayfish in the 30-40 mm size class in catches taken during the period May to August 1978 (Figure 4.5) would have been due in part to an average catchability for these crayfish approximately twice that of crayfish in other size classes during this period (Table 4.2). However, given that the average catchabilities of crayfish in most size classes were similar within each of the two periods for which catchabilities were calculated, and that differences in average catchabilities with carapace length were not sustained over both of these periods, it is likely that the major trends through time in catch composition were indicative of real trends in the size structures of the populations of crayfish.

The size frequency distributions of the populations of crayfish in both locations were characteristically skewed towards the smaller size classes. This differed considerably from catches of *E. spinifer* taken from nearby catchments using the same trapping gear and similar trapping intensities. Catches from the Georges River near Campbelltown during 1976 and 1978, and catches from the Hacking River at Otford during 1978 and 1979, consisted entirely of crayfish with carapace lengths in the range 50-110mm. Eels (*Anguilla* sp.) were frequently observed in both of these locations, while only two eels were sighted in the study area on the Loddon River, both just prior to the end of the study. It is thus suggested that the high relative abundance of small crayfish at the study site may have reflected a low level of predation by eels.

The increases in the relative numbers of crayfish in the 20-30 mm size classes in Pools 3 and 7 during the period September to December 1977 (Figure 4.5) are taken to represent recruitment of previously smaller crayfish into this size class, since the majority of these crayfish had recently moulted at the commencement of growth after winter (see Chapter 5). This recruitment was neither prece ded by, nor simultaneous with, catches of similar numbers of smaller crayfish, indicating that the catchability of crayfish with carapace lengths less than 20mm was considerably less than that of larger crayfish, and that their poor representation in catches was not indicative of population structure. Crayfish in the 20-30mm size class dominated the two populations during the early part of the study. This domination was maintained into the 30-40mm size class until the end of the study in Pool 3, and until May to August 1978 in Pool 7, with resulting changes in population structure through time (Figure 4.5). A further pulse of recruitment into the 20-30mm size class may have commenced during the September to December 1978 period in Pool 3, while there was no evidence of this in Pool 7. It is thus apparent that while the catchable populations were characterised by relatively large numbers of small crayfish during the period of study, the size-frequency distributions of the smaller crayfish were not stable through time, and were largely dependent upon variable recruitment. Given the duration of the mark-recapture study, this may indicate that the overall size structures of the populations were in a state of flux.

In Chapter 3 it was considered that precocious males were capable of mating with mature females. In view of this, and on the grounds that the size frequency distributions of catches generally reflected the size structure of the crayfish populations, an attempt

has been made to estimate the relative abundances of mature females, and both precociously and normal mature males in the intact population in Pool 3, with the aim of describing the potential contributions of the two types of males to the reproductive output of the population. Of the total number of different individual crayfish captured in Pool 3 during the mark-recapture study, approximately 3% were mature females, 6% were normal mature males, and 9% were precocious males. The true percentage of precocious males in the population was likely to have been higher than the calculated percentage, since these males were known to mature at carapace lengths considerably less than 20mm, and crayfish of this size were less catchable than larger crayfish. Nevertheless, the calculated percentages indicate that there was a substantial surplus of males with respect to females, and suggest that precocious males may have contributed significantly to successful mating of mature females, and hence to the overall reproductive output of the population. A similar conclusion would undoubtedly have applied to Pool 7 if the population of large crayfish had been left intact.

CHAPTER FIVE: GROWTH

MOULT FREQUENCY

5.1 INTRODUCTION

As noted by numerous authors, the overall growth rates of crustaceans are the result of two fundamental components. These are the amount by which an individual increases in size at moulting, i.e., moult increment, and moult frequency. Variation in overall growth rate may result from variation in either or both of these components. Hence in this study, as in others (e.g., Wilder, 1953; Kurata, 1962; Hancock and Edwards, 1967; Berry, 1971; Bennett, 1974; Mauchline, 1977), the contributions of moult increment and moult frequency to overall growth rate have been analysed separately.

It was found in this study that information on moult frequency formed a necessary basis for the interpretation of temporal variations in moult increment (Chapter 6). Moult frequency has thus been treated first, while moult increment is treated in the following chapter (Chapter 6). The joint expression of moult frequency and moult increment in terms of annual growth rate has been treated, along with other material, in Chapter 7.

Descriptions of moult frequency and increment were confined to crayfish with carapace lengths greater than 20mm, since the low catchabilities of crayfish with carapace lengths less than 20mm (Chapter 4) resulted in the collection of few data for wild crayfish of this size. It would have been possible to estimate the moult frequencies and increments of these crayfish from the relationship

between the pre- and post-moult lengths of larger crayfish (e.g., Farmer, 1973), but this was not attempted, and descriptions were confined to those crayfish for which field data were available. Data were also insufficient for the description of moult frequencies of precocious male crayfish to be undertaken, although some information on the moult increments and overall growth rates of these crayfish is provided in Chapters 6 and 7 respectively. The present chapter thus deals exclusively with the moult frequencies of female and normal male crayfish.

While the frequency of moulting was of primary interest in terms of a contribution to overall growth rate, the time of year during which moulting occurred was also of interest in this study, in that it may have reflected the effects of environmental factors on growth, or changes in the growth patterns of crayfish in different parts of the life cycle. This chapter is thus concerned with variation in both the frequency and seasonality of moulting with respect to the sex, size and capture location of individual *E. spinifer*, and an attempt is made to relate the observed seasonality of moulting to changes in water temperatures. The analysis is based on the mark-recapture sampling conducted in Pools 3 and 7 at the study site during the period May 1977 to December 1978.

5.2 METHODS

5.2.1 Annual Moult Frequency

Total annual growth was estimated for crayfish with carapace lengths in the range 20-55mm as the difference between measurements of the carapace lengths of individual marked crayfish taken twelve months apart. These annual growth increments were subdivided into a series of moult increments based on the results of Part B of this thesis, and the number of moults per year was thus determined for each crayfish as the number of consecutive moult increments. Where the annual growth increments could not be divided into a series of consecutive moult increments, the contributing number of moults was estimated as follows. Average moult increments were determined for crayfish in size classes 5mm in width, commencing at a carapace length of 19.95mm, separately for each sex and location. For each individual crayfish, the average moult increment from the relevant size class was then added to the carapace length recorded at the beginning of the twelve month period to provide a new carapace length, with which the process was repeated, until the annual moult frequency could be estimated as the number of average moult increments providing the final carapace length closest to that recorded at the end of the twelve month period. It was concluded (Appendix D) that general trends in moult frequencies determined using the two methods provided essentially similar conclusions. Hence results from the two methods were combined. However, in accordance with the results of Appendix D, conclusions about the lowest moult frequencies recorded for crayfish in a particular group were based only on actual, and not estimated, moult frequencies. 'Annual' moult frequencies of crayfish with carapace lengths greater than 55mm were determined from numbers of consecutive moult increments only, over periods of twelve to fifteen months duration. Annual moult frequencies of all crayfish

were plotted against initial carapace length (Figure 5.1). Male and female crayfish from the two locations were distinguished by different symbols, and trends were interpreted, where possible, directly from the graph, since the data were inadequate for statistical evaluation. The range of carapace lengths was divided into three intervals, 20-35 mm, 35-55mm, and 55-100mm, subsequently referred to as small, medium and large, such that the crayfish in each interval were characterised by a particular set of moult frequencies.

5.2.2 Seasonality in Moulting Activity.

Separate criteria were used to determine the commencement and termination of periods during the year in which the moulting activities of crayfish were concentrated. The initiation of moulting activity was determined as follows. Crayfish captured in Pools 3 and 7 were classified into the moult stages Ce (early intermoult) or C (intermoult) at each monthly sampling of the mark-recapture study, as described in Chapter 2, and percentages of the crayfish in each catch that were in moult stage Ce were used to indicate the extent of recent moulting activity. For the sake of brevity, these percentages will be referred to subsequently as 'percentages of post-moult crayfish'. Percentages of post-moult crayfish were calculated separately for small, medium and large crayfish of each sex, from each of the two locations. These percentages were plotted against month of sampling, and the onset of moulting activity was taken to occur during the month in which the percentage of post-moult crayfish increased markedly from a previously low value. Percentages for males and females were plotted on the same graphs for small and medium sized crayfish, while the two locations were treated separately (Figures

5.2-5). However, trends in the percentages of post-moult crayfish obviously differed between large males and females. Hence percentages for large crayfish of the same sex were plotted on the same graph to facilitate comparisons between the two locations, while the sexes were treated separately (Figures 5.6-7).

It was found that crayfish could remain in moult stage Ce for several months after moulting, thus rendering constant and decreasing percentages of post-moult crayfish inaccurate as indicators of the respective continuation and cessation of moulting activity. An alternative method was thus used to indicate decreasing moulting activity, and moulting seasons were defined as the period between the initial increase and final decrease in moulting activity.

From mark-recapture records and the results of Part B of this thesis, it could be determined whether or not a crayfish had moulted during the period between any two captures. It was not possible to obtain adequate data for captures only one month apart. However, if a particular crayfish did not moult during a period of several months between captures, then it obviously did not moult during the month between any pair of consecutive samplings within this period. In contrast, if a crayfish did moult during a period between captures that extended over several monthly samplings, then it was not possible to determine during which particular month moulting had occurred. For crayfish that were captured before and after any particular month, there were thus two types of mark-recapture records available, records stating that crayfish definitely did not moult, and records stating that crayfish may or may not have moulted. The latter records could not be used in any meaningful way, except as a contribution to the total number of records of crayfish for a particular

month. Also, when the frequency of records of crayfish that definitely did not moult was calculated as a percentage of the total number of records, the contribution of the indefinite records to the total would have rendered the absolute value of this percentage inaccurate as an indicator of moulting activity, and any decrease in the percentage would have occurred prior to any real increase in moulting activity. However, the percentage of crayfish that definitely did not moult during any given month would be expected to increase immediately after any decline in moulting activity. Hence a substantial increase in this percentage was used to indicate decreasing moulting activity. For the sake of brevity, this percentage will be referred to subsequently as 'the percentage of non-moulting crayfish', although the description is not strictly correct. These percentages were calculated for the groups of crayfish that were used in the calculation of percentages of post-moult crayfish, and the results were plotted on the same graphs (Figures 5.2-7).

5.2.3 Relationship Between Moulting Activity and Water Temperatures.

Water temperatures were recorded for each sampling at Pools 3 and 7 as described in Chapter 2, and plotted against the month of sampling (Figure 5.8). The months during which each moulting season commenced and ceased were obtained for small crayfish, and indicated on Figure 5.8. Possible relationships between temperature and moulting activity were then deduced from the graph.

5.3 RESULTS

5.3.1 Annual Moult Frequency

Crayfish with carapace lengths of 20-35mm (small crayfish, Figure 5.1) were characterised by a moult frequency of three per year, with smaller numbers of crayfish moulting from one to six times per year. Crayfish with carapace lengths of 35-55mm (medium crayfish) typically moulted twice per year, with occasional individuals moulting once or three times, while crayfish with carapace lengths greater than 55mm (large crayfish) moulted only once per year, with the exception of a single individual which moulted twice. All moult frequencies of one per year were based on the actual numbers of moult increments recorded for the crayfish, and not on estimated moult frequencies. There were no apparent differences in annual moult frequencies with respect to either sex or location for small and medium crayfish. Moult frequencies of large male and female crayfish from Pool 3 were similar to each other, and to the single value from Pool 7. All crayfish with records for a period of twelve months moulted at least once.

5.3.2 Seasonality in Moulting Activity.

Trends in the percentages of post-moult and non-moulting crayfish of small and medium size were similar for males and females and for the two locations (Figures 5.2-5), while trends for large males and females were distinctly different (Figures 5.6-7). Variations of percentages about the main trends were obvious in many instances, particularly where percentages were calculated from small samples of crayfish, e.g., percentages of

Figure 5.1: Annual Moult Frequency vs. Initial Carapace Length.

Key:-

○ - Pool 7, Male.

● - Pool 3, Male.

△ - Pool 7, Female.

▲ - Pool 3, Female.

Small - carapace lengths 20-35mm.

Medium - carapace lengths 35-55mm.

Large - carapace lengths 55-100mm.

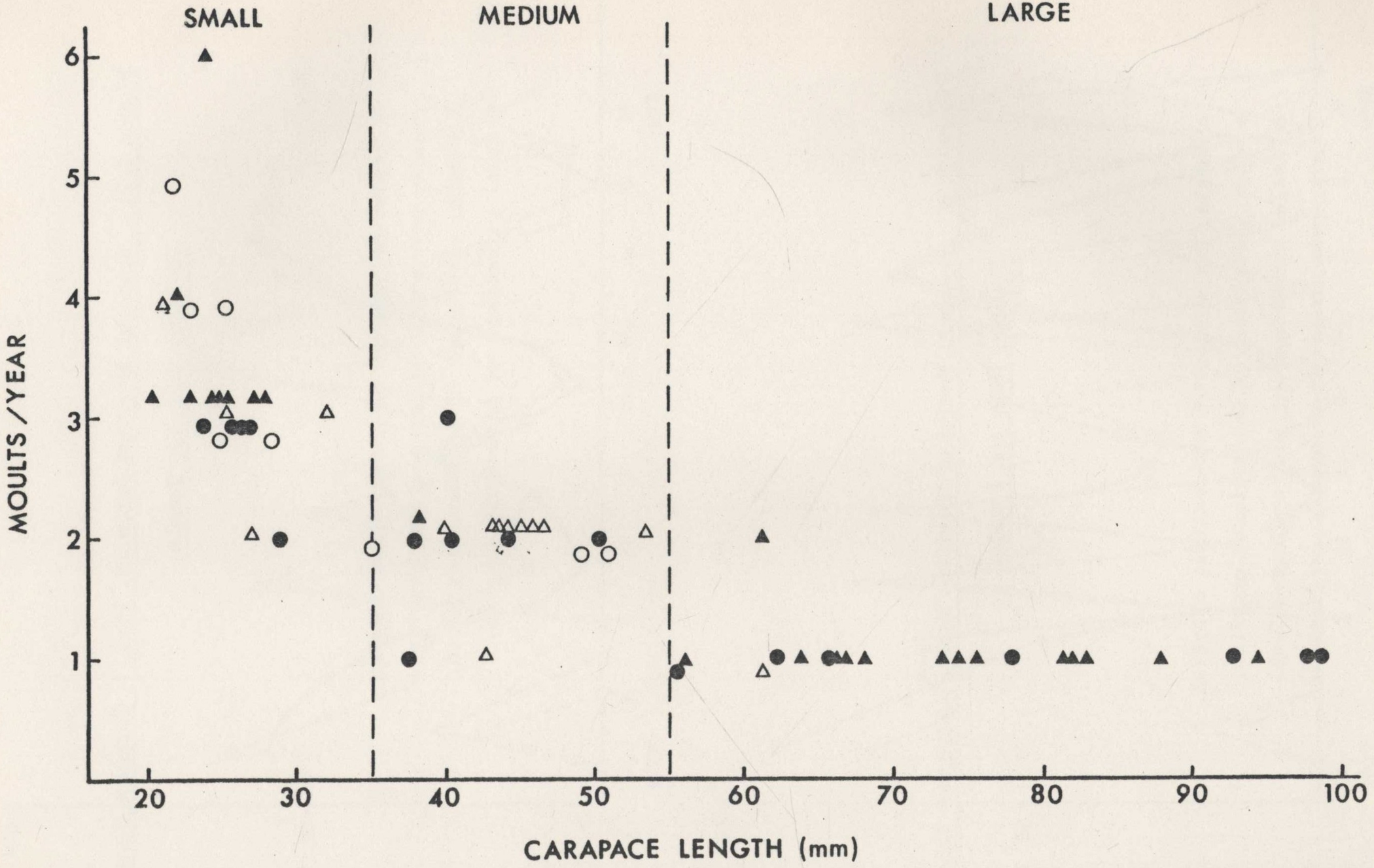

SMALL
MEDIUM
LARGE
MOULTS / YEAR
6
5
4
3
2
1
20
30
40
50
60
70
80
90
100
CARAPACE LENGTH (mm)

Figure 5.2: Seasonal Moulting Activity, Pool 3, Carapace Lengths 20-35mm, Male and Female.

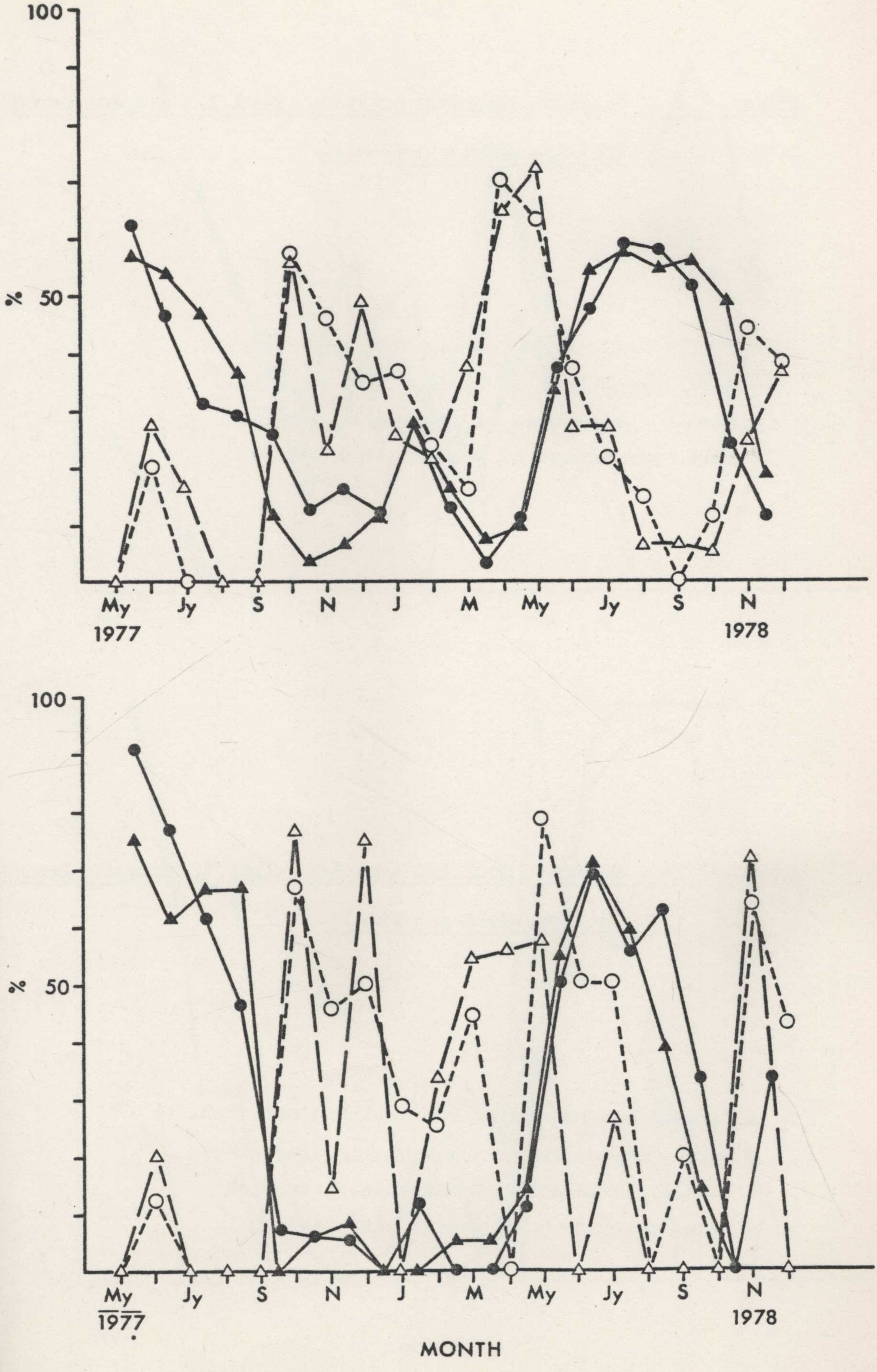
Key:-

▲ Female, percentage of non-moulting crayfish.
● Male, percentage of non-moulting crayfish.
△ Female, percentage of post-moult crayfish.
○ Male, percentage of post-moult crayfish.

Figure 5.3: Seasonal Moulting Activity, Pool 7, Carapace Lengths 20-35mm, Male and Female.

Key:-

▲ Female, percentage of non-moulting crayfish.
● Male, percentage of non-moulting crayfish.
△ Female, percentage of post-moult crayfish.
○ Male, percentage of post-moult crayfish.


100
% 50
My
Jy
S
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J
M
My
Jy
S
N
1977
1978
MONTH

Figure 5.4: Seasonal Moulting Activity, Pool 3, Carapace Lengths 35-55mm, Male and Female.

Key:-

▲ Female, percentage of non-moulting crayfish.

● Male, percentage of non-moulting crayfish.

△ Female, percentage of post-moult crayfish.

○ Male, percentage of post-moult crayfish.

Figure 5.5: Seasonal Moulting Activity, Pool 7, Carapace Lengths 35-55mm, Male and Female.

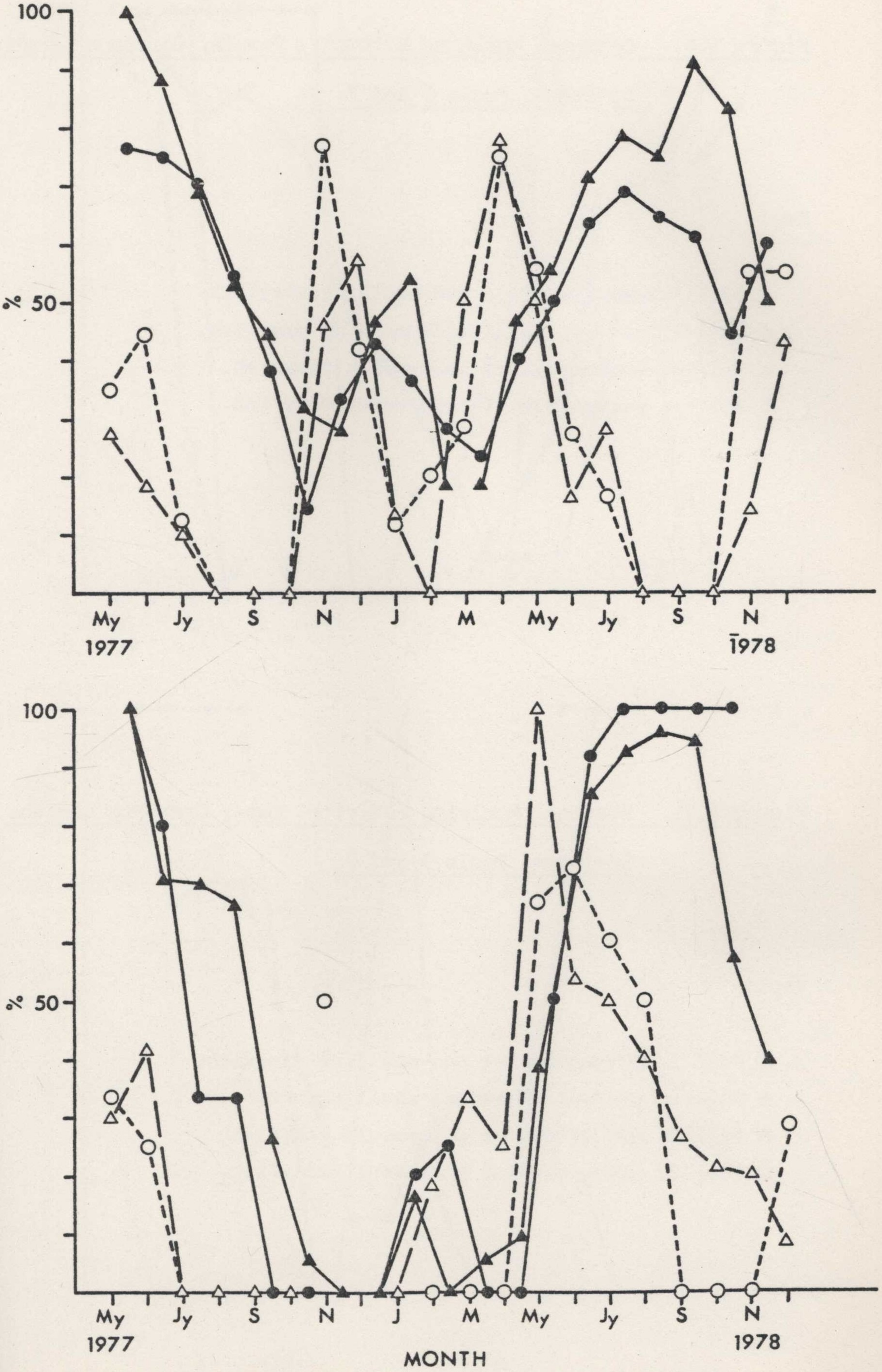
Key:-

▲ Female, percentage of non-moulting crayfish.

● Male, percentage of non-moulting crayfish.

△ Female, percentage of post-moult crayfish.

○ Male, percentage of post-moult crayfish.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My
Jy
S
N
1977
1978
MONTH

Figure 5.6: Seasonal Moulting Activity, Female, Carapace Lengths 55-100mm, Pools 3 and 7.

Key:-

▲ Pool 3, percentage of non-moulting crayfish.

● Pool 7, percentage of non-moulting crayfish.

△ Pool 3, percentage of post-moult crayfish.

○ Pool 7, percentage of post-moult crayfish.

Figure 5.7: Seasonal Moulting Activity, Male, Carapace Lengths 55-100mm, Pools 3 and 7.

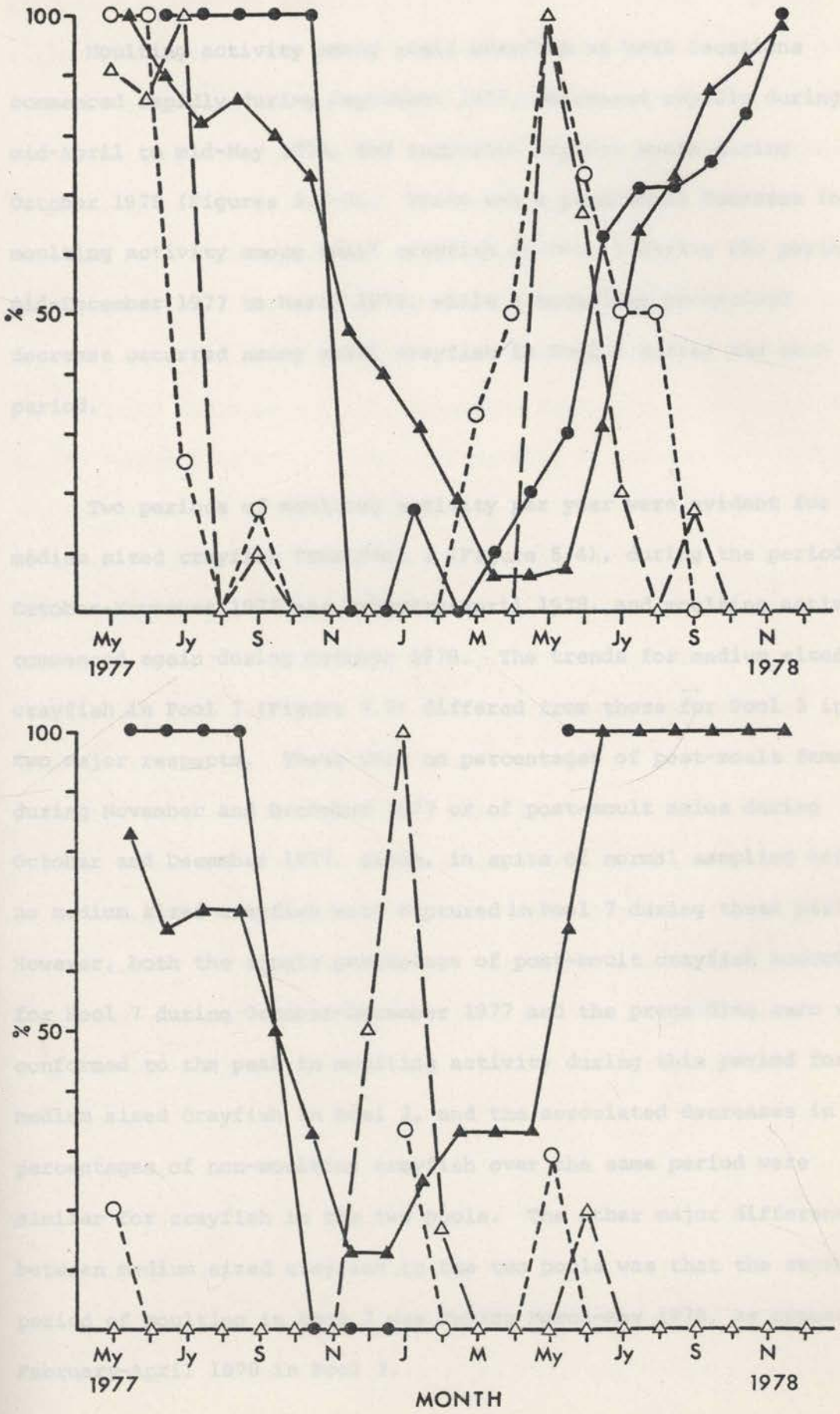
Key:-

▲ Pool 3, percentage of non-moulting crayfish.

● Pool 7, percentage of non-moulting crayfish.

△ Pool 3, percentage of post-moult crayfish.

○ Pool 7, percentage of post-moult crayfish.


100
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S
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1977
1978
100
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J
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My
Jy
S
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1977
1978
MONTH

post-moult crayfish in Figure 5.3.

Moulting activity among small crayfish at both locations commenced rapidly during September 1977, decreased rapidly during mid-April to mid-May 1978, and increased rapidly again during October 1978 (Figures 5.2-3). There was a pronounced decrease in moulting activity among small crayfish in Pool 3 during the period mid-December 1977 to March 1978, while a much less pronounced decrease occurred among small crayfish in Pool 7 during the same period.

Two periods of moulting activity per year were evident for medium sized crayfish from Pool 3 (Figure 5.4), during the periods October-November 1977 and February-April 1978, and moulting activity commenced again during October 1978. The trends for medium sized crayfish in Pool 7 (Figure 5.5) differed from those for Pool 3 in two major respects. There were no percentages of post-moult females during November and December 1977 or of post-moult males during October and December 1977, since, in spite of normal sampling efforts, no medium sized crayfish were captured in Pool 7 during these periods. However, both the single percentage of post-moult crayfish recorded for Pool 7 during October-December 1977 and the prece ding zero values conformed to the peak in moulting activity during this period for medium sized crayfish in Pool 3, and the associated decreases in the percentages of non-moulting crayfish over the same period were similar for crayfish in the two pools. The other major difference between medium sized crayfish in the two pools was that the second period of moulting in Pool 7 was during March-May 1978, as opposed to February-April 1978 in Pool 3.

Trends in the percentages of large, non-moulting females (Figure 5.6) were of similar configuration for the two locations, taking into account the small samples used to calculate the percentages for some months. In Pool 7, however, the increase in the percentage of non-moulting crayfish marking the end of the moulting season occurred in two steps, commencing slowly during March-May 1978, followed by a sharp increase in June, while in Pool 3 a single, sharp increase occurred during June 1978. The major peak in the percentage of post-moult crayfish occurred in May 1978 in both locations. In contrast, moulting activity commenced during April in Pool 3, and during February in Pool 7. The percentages of post-moult crayfish for the March and April 1978 samplings were from two crayfish with carapace lengths of 53.0 and 58.9mm respectively. The remaining percentages of post-moult crayfish in the May 1978 peaks for both locations were from a mixture of crayfish with carapace lengths both greater and less than 70mm. It was ascertained from recapture records that the small peaks in percentages of post-moult crayfish for September 1977 and 1978 in Pool 3 were from crayfish that had not moulted since the previous major peaks of moulting activity. No prior records were available for the single crayfish responsible for the September 1977 peak in Pool 7. The high percentages of both post-moult and non-moulting crayfish during May-June 1977 indicated that a period of moulting activity had occurred just prior to this in both locations.

The trends in the percentages of large, non-moulting male crayfish from the two locations (Figure 5.7) appeared to be of similar configuration, and related in the same general manner as those described for females of similar size, although the data for Pool 7

were incomplete. Changes through time in the percentages of both post-moult and non-moulting crayfish were, however, quite different from those described for large females. In Pool 3, the initial increase in percentages of large, non-moulting males indicated the finish of the major period of moulting activity during January 1978, while moulting was initiated during November 1977. Although percentages of large, post-moult males in Pool 7 were absent for the initial part of this period, the values for October 1977 and January 1978 indicated that large male crayfish had also moulted in Pool 7 during November-January.

The May 1977 peak in percentages of large, post-moult males in Pool 7 resulted from two crayfish with carapace lengths of 59.4mm and 63.4mm. It was ascertained from recapture data that both of these crayfish had moulted during the period January-May 1978, and previously during late 1977. The carapace lengths of the crayfish prior to the first of these moults were 49.1mm and 50.7mm respectively. The May 1977 peak for Pool 7 was from a crayfish with a carapace length of 57.6mm, while the June 1978 peak for Pool 3 was from a crayfish with a carapace length of 60.7mm that had moulted during the period January-June 1978, from an initial carapace length of 55.4mm. The major peak in moulting activity during November 1977-January 1978 resulted from crayfish with carapace lengths both greater and less than 70mm.

Two crayfish did not conform to the apparent general pattern of moulting activity. One, with an initial carapace length of 21.9mm, moulted during June 1977 in Pool 3. The other, with an initial carapace length of 34.9mm, moulted during July 1978 in Pool 7.

FIGURE 5.8: Relationship Between Water Temperature and Moulting Activity.

Key:

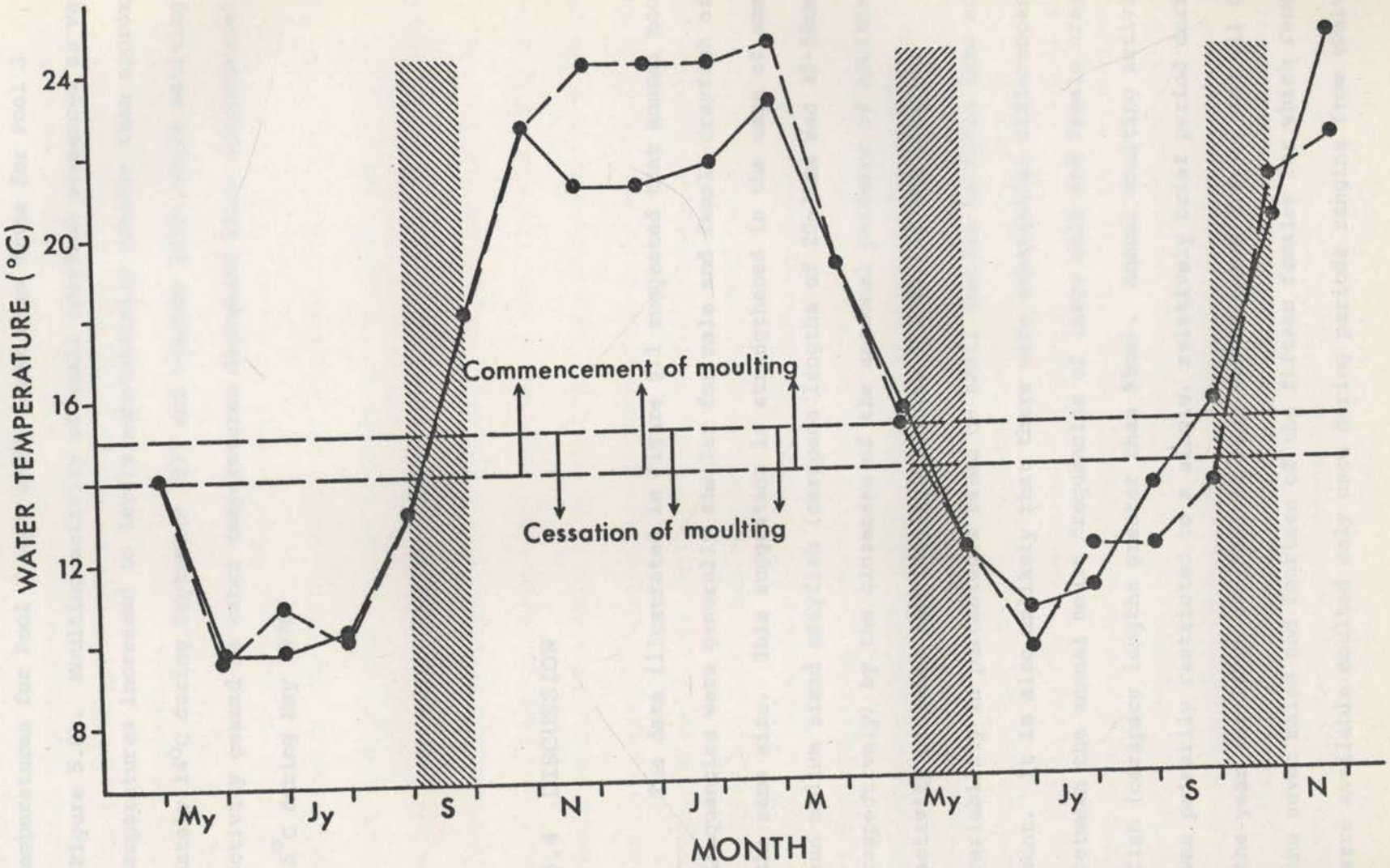
— — Monthly water temperature for Pool 3.

——— Monthly water temperature for Pool 7.

*Month for commencement of moulting activity.

*Month for cessation of moulting activity.

Results for crayfish with carapace lengths of 20-35mm for both sexes and locations.


WATER TEMPERATURE (°C)
24
20
16
12
8
Commencement of moulting
Cessation of moulting
My
Jy
S
N
J
M
My
Jy
S
N
MONTH

5.3.3 Relationship Between Moulting Activity and Water Temperature.

Variations in water temperature were strongly seasonal and similar for the two pools, with the exception that the summer 1977-78 temperatures for Pool 7 were 2-3°C lower than those for Pool 3 (Figure 5.8). Moulting activity of small crayfish commenced as water temperatures increased to levels substantially greater than approximately 14°C during September 1977 and October 1978, while moulting activity ceased as water temperatures decreased below approximately 15°C during May 1978.

5.4 DISCUSSION

The data illustrated in Figure 5.1 suggested that annual moult frequencies were generally similar for male and female crayfish of the same size. This suggestion is strengthened in the case of small and medium sized crayfish (carapace lengths of 20-35mm and 35-55mm respectively) by the closeness of the seasonal patterns of moulting activity of males and females (Figures 5.2-5), if the inherent variability in percentages based on small samples is taken into account. It is also unlikely that there were substantial differences between the annual moult frequencies of large male and female crayfish (carapace lengths greater than 55mm). Annual moulting activity was primarily restricted to a single, relatively brief period during the year for large crayfish of each sex (Figures 5.6-7), and all of the seven males and fourteen of the fifteen females for which records were available moulted only once during periods ranging from twelve

to fifteen months (Figure 5.1). Hence a single, annual moult was probably typical of large crayfish of both sexes. Normal male *E. spinifer* became sexually mature over the range of carapace lengths 45-55mm while, in contrast, females matured at carapace lengths greater than approximately 70mm (Chapter 3). Hence it is likely that annual moult frequencies were independent of both sex and state of maturity.

Records of moult frequencies of juvenile decapods are relatively scarce. As for *E. spinifer*, juveniles of both sexes have been found to moult with similar frequencies for *Orconectes virilis* (Weagle and Ozburn, 1972), *Paranephrops planifrons* (Hopkins, 1967a), and *Panulirus longipes* (Chittleborough, 1975). Considering the different taxa to which these examples belong, the similarity among juveniles may be a general phenomenon. In contrast, mature decapods appear to show two different patterns of moulting activity. In the lobster *Homarus americanus* (Wilder, 1963; Stewart and Squires, 1968), the cambarid crayfishes *Procambarus hayi* (Payne, 1972) and *Orconectes virilis* (Weagle and Ozburn, 1972), the parastacid crayfish *Paranephrops planifrons* (Hopkins, 1967a), and the crab *Cancer pagurus* (Hancock and Edwards, 1967; Bennett, 1974), males moult more frequently than females, while moult frequencies of males and females are similar for the spiny lobsters *Panulirus elephas* (Hepper, 1977) and *Jasus lalandii* (Newmann and Pollock, 1974), and the astacid crayfish *Astacus astacus* (Abrahamsson, 1966). *E. spinifer* thus appears to show an affinity with the spiny lobsters and astacid crayfish in terms of adult moult frequency, although the discrepancy between *E. spinifer* and the other parastacid *Paranephrops planifrons* is puzzling, and no satisfactory explanation can be given.

Although the annual moult frequencies of *E. spinifer* were probably independent of sex and state of maturity, there was clearly a relationship with carapace length. Decreases in moult frequency with size have been recorded for many large decapods apart from *E. spinifer*, and are probably typical of crustaceans in general (Kurata, 1962). Records for some species, e.g., *Homarus americanus* (Stewart and Squires, 1968; Cooper and Uzmann, 1971), *Cancer pagurus* (Bennett, 1974), indicate that this decrease may be continuous over the whole size-range of a species, resulting in frequencies of less than one moult per year in mature individuals. Mauchline (1977), in an extensive review of the literature, proposed models describing a continuous increase in intermoult period, i.e., decrease in moult frequency, with size, and concluded that such models may be generally applicable to the larger decapods. This clearly was not the case with *E. spinifer*, since moulting occurred at a constant frequency of once per year over much of the upper part of the size-range of the species. Similar results have been obtained elsewhere for another parastacid crayfish *Paranephrops planifrons* (Hopkins, 1967a), and for the spiny lobster *Panulirus elephas* (Hepper, 1977).

Seasonal patterns of moulting activity differed for small, medium and large *E. spinifer* in accordance with differences in annual moult frequency. Hence small crayfish typically moulted three times annually with a range of one to six moults per year, and moulting activity in the field population was continuous, albeit with some fluctuations in intensity, from early spring to late autumn. The majority of medium sized crayfish moulted twice per year and moulting activity was largely confined to two discrete periods, one in spring and one in autumn, while large crayfish moulted almost exclusively once per year, and moulting activity was generally confined to a single period, during

early summer for males, and during late autumn for females. These generalisations were based on the allocation of crayfish to size classes covering discrete ranges of carapace length, i.e., 20-35mm for small crayfish, 35-55mm for medium crayfish, and 55+mm for large crayfish. In contrast, average moult frequencies and hence seasonal patterns of moulting activity, probably changed continuously with carapace length until the establishment at a large size of a single, annual moult. Departures from the generalised patterns of moulting activity may thus have arisen from this discrepancy. The moulting activity of small crayfish decreased in intensity during summer in both Pool 3 and Pool 7, indicating that some small crayfish may have adopted the bi-annual pattern of moulting activity described for medium sized crayfish. It was also found that the subsidiary peaks in moulting activity that were described for large males resulted from the inclusion in the large size class of several crayfish that had moulted twice during the year from an initial carapace length of less than or approximately equal to 55mm. These peaks are best interpreted as artefacts of the method, however, since they would not have been present if the crayfish had been allocated to the size classes according to their carapace lengths at the time of the annual commencement of growth. In the case of large females, two of the subsidiary peaks in moulting activity resulted from crayfish that had remained in moult stage Ce for a considerable period of time since the previous annual moulting season, and were thus not indicative of moulting activity at all, and the third subsidiary peak may have arisen from a similar cause. The only evidence of a continuation of the bi-annual pattern of moulting activity into the large size class was the single female, with a carapace length of approximately 60mm, which moulted twice in one year (Figure 5.1). Hence the description of different annual patterns of moulting activity for

medium and large crayfish, separated at a carapace length of 55mm, was probably a realistic interpretation. Also, while the description of different annual patterns of moulting activity for small and medium crayfish may not have been totally accurate, the arbitrary separation of crayfish in these size classes at a carapace length of 35mm was profitable in that it provided some insight into changes in patterns of moulting activity with size.

The different moulting seasons of large male and female *E.spinifer* contrasted with the similar moulting seasons of smaller males and females, and with annual moult frequencies *per se*, which were independent of sex and state of maturity. In other species, males may moult at various times of the year, while females typically moult either just after the release of juveniles or just before mating and oviposition (e.g., Hopkins, 1967a,b; Payne, 1972; Weagle and Ozburn, 1972; Farmer, 1973; Ansell and Robb, 1977; Hepper, 1977). In mature female *E. spinifer* moulting occurred prior to mating in May, rather than after the release of juveniles in November-December (Chapter 3), with sufficient time elapsing for the exoskeleton to harden prior to mating. This is also the case in the other parastacids *Paranephrops planifrons* (Hopkins, 1967b), and *Cherax destructor* (Johnson, 1979), the astacid *Austropotamobius pallipes* (Ingle and Thomas, 1974), and the spiny lobsters *Panulirus homarus* (Berry, 1971) and *Panulirus longipes cygnus* (Chittleborough, 1974). In all of these species fertilisation, i.e., spermatophore deposition, is external. In contrast, mating occurs shortly after the female moults and is still soft in the lobsters *Homarus americanus* (Dunham, 1979) and *Nephrops norvegicus* (Farmer, 1974a), and in cancrid and portunid crabs (Barnes, 1974), and in each instance fertilisation is internal. These findings suggest that the hard- or soft-shelled condition of

the female at mating, and hence the relationship between moulting and mating, may be related to the need for external or internal fertilisation respectively. This point has been made previously by Chittleborough (9174) and Johnson (1979), and is strengthened by the findings of Silberbauer (1971) that the females of the spiny lobster *Jasus lalandii* mate while soft and probably require internal fertilisation, in contrast to other spiny lobsters. However, moulting of mature females of the cambarid crayfishes of North America occurs after the release of juveniles (Payne, 1978), rather than at mating, and fertilisation is internal. Thus the true relationship between moulting activity and mode of fertilisation is not clear. The close proximity of moulting to mating and oviposition in female *E. spinifer* may be related to the need for pleopodal setae to be in good condition for egg attachment. This suggestion is applicable to other species in which moulting occurs prior to mating, and also to those species in which moulting occurs after the release of juveniles, since the interval between release and mating does not usually appear to be great. The need for proximity of moulting and mating in mature females may contribute partly to differences in the seasonal moulting activities of mature male and female *E. spinifer*. The long periods during which females carry eggs, and hence cannot moult, may also be a contributing factor, as may be the need to accumulate large stores of assimilated nutrients for egg production.

There was some evidence of differences between the moult frequencies of small crayfish from Pools 3 and 7. The spans and timing of the moulting seasons were similar for crayfish from the two locations, indicating that the ranges of moult frequencies were probably also similar. In contrast, the decrease in moulting activity midway through the season was much more pronounced in Pool 3 (Figures 5.2-3), indicating that the distribution of moult frequencies in

Pool 3 may have been biased towards a lower value. Although the distribution of moult frequencies for Pool 7 contained only a small number of values (Figure 5.1), comparison of the frequencies for the two locations tended to confirm this difference. It was also noted in the results for large female crayfish that the increase in percentages of non-moulting crayfish at the end of the moulting season in Pool 7 occurred one month prior to that for Pool 3. The percentages were based on recapture records for crayfish with carapace lengths of 55-100mm for Pool 3, but this range was reduced to 55-70mm for Pool 7, since all larger crayfish were removed from the Pool (Chapter 2). These smaller crayfish may have temporarily continued to moult in the pattern described for medium sized crayfish, and percentages of non-moulting crayfish of this size increased in Pool 7 during May 1978. The inclusion of a greater number of such individuals among the large crayfish from Pool 7 would thus have caused an initial bias in the increase in percentages of non-moulting crayfish towards May. This is identical to the result described for Pool 7, indicating that the rapid increase in percentages of non-moulting crayfish in Pool 7 during June 1978 may be considered equivalent to that for Pool 3. Consequently, the apparent differences between the dates of termination of moulting activity for the two pools probably resulted from the difference in the average sizes of the crayfish used to provide the data. The same argument may be applied to the earlier increase in percentages of large, post-moult females in Pool 7, and to the earlier decreases in percentages of non-moulting crayfish for large males and females in Pool 7 (Figures 5.6-7). There was thus no evidence of differences between the two locations in the moulting seasons of large crayfish. The above argument may not, however, be applied to the results for medium sized crayfish, since the crayfish used to calculate the percentages were

of similar size for both pools. The later increases in percentages of both post-moult and non-moulting crayfish in Pool 7 during autumn 1978 (Figures 5.4-5) thus indicated that the autumn moult of medium sized crayfish in Pool 7 was delayed when compared with Pool 3.

An attempt was made to link seasonal moulting activity to water temperature using the moulting activity of small crayfish. Small crayfish moulted more frequently than larger crayfish, hence changes in their patterns of moulting activity would be expected to provide a more sensitive indication of changes in metabolic responses to environmental parameters. The results for this investigation suggested that there was a strong association between growth processes and annual water temperature cycles, with growth being initiated above a temperature of 14-15^{o}C. From this it is implicit that growth was also closely associated with the annual photoperiod cycle. However, the commencement of moulting in October in 1978 as opposed to September in 1977 departed from the expected schedule if photoperiod was the controlling factor, while it remained in agreement with the trends in temperature. Attempts to maintain crayfish in the laboratory for crucial experiments to test the joint effects of temperature and photoperiod on growth were unsuccessful. The regulation of growth activity by temperature is thus accepted as the best explanation of the observed trends, in the absence of information on other variables showing comparable seasonal cycles. This conclusion is at variance with the findings of Aiken (1969b) and Armitage et al (1973) who concluded that the seasonal growth patterns in the North American crayfish *Orconectes virilis* were regulated by photoperiod rather than temperature. Other crustaceans may, however, have less dependence upon photoperiod (Aiken, 1969b), although there appears to be little information available for decapods. Increases in moult

frequency with increasing temperature have been described for various decapods by numerous authors (e.g., Penn, 1943; Kurata, 1962; Fielder, 1964a; Hopkins, 1967a; Berry, 1971; Chittleborough, 1975). Taken in this context, the delay in the autumn 1978 period of moulting activity for medium sized *E. spinifer* in Pool 7 may have resulted from summer water temperatures 2-3°C lower than those for Pool 3. If this explanation is accepted, then the decrease in the moulting activity of small *E. spinifer* in Pool 3 during the same period must have been due to factors other than temperature alone. Possible solutions are discussed in the following chapter in conjunction with variation in moult increment. Hopkins (1967a) found that moult frequencies decreased during winter for the New Zealand parastacid crayfish *Paranephrops planifrons*, but moulting activity did not cease. If low temperature affected moulting activity for this species, then it must have done so in a quantitative manner, rather than as a threshold below which growth could not occur. The same conclusion may be drawn for *E. spinifer*, since at least two individuals moulted outside the normal season, with ambient temperatures well below the 14-15°C above which moulting typically occurred.

Some evidence has been presented to suggest that temperature is the major factor regulating growth processes in *E. spinifer*. In the absence of reproductive constraints, it is likely that the patterns of moulting activity described for males and immature females resulted from a combination of the effects of annual temperature cycles and a decrease in moult frequency with increasing body size. Trends in percentages of both post-moult and non-moulting crayfish during the first few and last few months of the study for all groups of crayfish indicated that the cessation of moulting activity at the end of the previous growth season, and the commencement of moulting

activity at the beginning of the next growth season, occurred at similar times during the year to those described for the growth season covered by the period of study. Given the general similarity of seasonal patterns of moulting activity of crayfish in two separate locations, there is some evidence to suggest that the patterns of moulting activity observed during this study were typical of *E. spinifer* in the study area.

CHAPTER SIX: GROWTH

MOULT INCREMENT

6.1. INTRODUCTION

As noted in the previous chapter, an adequate interpretation of the growth of a decapod crustacean requires a knowledge of the nature of increases in size at moulting, i.e., moult increment. Part of this chapter takes the form of a survey of variation in moult increment, with the aim of outlining the types of factors that might have affected the growth of *E. spinifer* during the period of study. From preliminary examination of the data it was apparent that moult increments were highly variable for crayfish of any given size. An initial attempt was made to determine whether this size-independent variation in moult increment was related to the more obvious factors of sex, the season during which moulting had occurred, or location of capture. These factors accounted for only a portion of the total variation in moult increment. An attempt was then made to determine whether this remaining variability was likely to have resulted from consistent differences between individual crayfish, in combination with the effects of pre-moult carapace length, to errors in measurement, or to other, unidentified factors, independent of season and location.

The remainder of the chapter is devoted to an interpretation of variation in moult increment with body size, based on the results for size-independent variation.

Moult increments were extracted from the mark-recapture data for

Pools 3 and 7 at the study site using the method developed in Part B of this thesis.

6.2 METHODS

6.2.1 Variation in Moult Increment with respect to Sex, Season and Location.

Moult increments were tabulated separately for males and females at each of the two locations. These data were further sub-divided into classes based on the pre-moult carapace lengths of the individual crayfish, for each of several seasons of moulting activity, according to the results of Chapter 5. The period during which each moult had occurred was located by the dates of the captures on which the pre- and post-moult measurements of carapace length were taken. Moult increments of crayfish with carapace lengths of 20-35mm were compared for the periods May-October 1977 (Spring '77), October 1977-February 1978 (Summer '77/78), February-August 1978 ("Autumn '78") and May-October 1978 ("Spring '78"). Moult increments of crayfish with carapace lengths of 35-55mm were compared for the periods May-November 1977 ("Spring '77"), December 1977-August 1978 ("Autumn '78"), and May-November 1978 ("Spring '78").

The moult increments of the crayfish in the above classes were compared by calculating the linear regression of moult increment on pre-moult carapace length for each class, and comparing the slopes and elevations of the regression lines using analysis of covariance (Snedecor and Cochran, 1967). In instances where slopes were not significantly different, differences between the elevations of the regressions were used to indicate differences in average moult increment

independent of any effects of pre-moult carapace length. Significant differences among the elevations of regressions were located using a Student Newman Keuls test for elevations (Zar, 1974). Comparisons were conducted separately for each size-class. Initial comparisons were made between males and females within each season and location. Where regressions for the sexes were not significantly different ($p > 0.05$), the data for the two sexes were combined and compared between seasons, separately for each location. Where regressions for seasons were not significantly different ($p > 0.05$), the data for similar seasons were combined, and the regressions for the sets of similar seasons were compared between locations.

The results of the analyses of covariance for all comparisons have been summarised in Appendix B. Final results have been presented graphically in Figures 6.1-4. In instances where there were no significant differences among both slopes and elevations of the regressions for a number of groups of crayfish, the regression line for the combined data has been illustrated. In instances where elevations differed significantly but slopes did not, regression lines with the pooled slope for all groups (Snedecor and Cochran, 1967) have been plotted through the means of each different group.

It was necessary to allow seasons to overlap to some extent to make use of the available data. Adjacent autumn and spring seasons were allowed to overlap for the period May-August, since crayfish with carapace lengths of 20-55mm rarely moulted during this period (Chapter 5). Other seasons were allowed to overlap by one month for the 20-35mm size-class. The possible implications of this will be considered during the discussion of the results.

Seasonal comparisons were not conducted for crayfish with carapace lengths greater than 55mm, since normally only a single, annual moult was recorded for crayfish of this size (Chapter 5). Comparisons between males and females with carapace lengths of 55-70 mm were treated in the manner described for the smaller crayfish. Differences between the moult increments of males and females with carapace lengths greater than 70mm have been treated in connection with variation in moult increment with size (Section 6.2.3) and not in the present section.

The males considered in the above comparisons were of the normal type. Comparisons were also made between the moult increments of precocious males, normal males and females for the range of carapace lengths 20-25mm during Summer 1977-78 in Pool 3. The scarcity of data for precocious males precluded further comparisons.

Data were inadequate to allow comparison between the sexes for some seasons. In such instances, data for the sexes were combined on the basis of results for the other similar comparisons, and the combined data were then used for comparison between seasons. The scarcity of data for Spring 1978 in Pool 3 made it necessary to exclude this season from the set of comparisons.

6.2.2 Contributions of Pre-Moult Carapace Length, Consistent Differences between Individual Crayfish, Measurement Error, and Unidentified Factors to Variation in Moult Increment.

Moulting seasons were defined as in the previous section. Mark-recapture records were examined for instances in which moult increments had been recorded in consecutive seasons for individual

crayfish. The data were arranged into classes based on the initial carapace lengths of the crayfish, locations of capture, and the two seasons involved. Multiple regressions of moult increment for the second season (Y) on pre-moult carapace length for the second season (X_1) and moult increment for the first season (X_2) were calculated for each class. These variables were subsequently referred to respectively as moult increment, pre-moult carapace length, and previous increment. In this context, any correlation between moult increment and previous increment would include an estimate of the extent to which the sizes of consecutive moult increments of individual crayfish were related. The extent of this relationship may also be interpreted as an estimate of the contribution to variation in moult increment of consistent differences between the moult increments of different individual crayfish. Separation of the effects of previous increment and pre-moult carapace length on moult increment was not possible, however, as any correlation between moult increment and pre-moult carapace length would also have implied a correlation between pre-moult carapace-length and previous increment, and hence between previous increment and moult increment. The error variance from the multiple regression was thus used to estimate the variability in moult increment that was independent of the combined effects of pre-moult carapace length and consistent differences between individual crayfish by expressing it as a percentage of the total variance of the moult increments for each group of crayfish.

The variability in moult increment that resulted from measurement error was estimated for each group of crayfish, independently of the multiple-regression analysis, using the variance of the measurement errors that were isolated from the mark-recapture data in Part B of this thesis. The measurement error variance was then expressed as

a percentage of the error variance from the multiple regression for each group of crayfish.

Size-classes, seasons, capture locations, and results of calculations are listed in Table 6.1.

6.2.3 Relationship between Moult Increment and Pre-Moult Carapace Length.

The relationship between moult increment and pre-moult carapace length was illustrated using the final results (Figures 6.1-6.3) of the analysis of variation in moult increment with respect to sex, season and location. Regression lines and 95% confidence belts for the linear regressions of moult increment on pre-moult carapace length for each of the final sets of seasons for each size-class were plotted on the same graph (Figure 6.5), together with the individual moult increments of crayfish with carapace lengths greater than 70mm.

Mauchline (1977), in a review of the relationships between moult increment and pre-moult carapace length in various crustaceans, found that the use of moult increment *per se* did not produce useful relationships. Instead, moult increment was expressed as a percentage of pre-moult carapce length, providing a so-called 'growth factor'. The linear and $\log_{10}$-linear regressions of growth factors on pre-moult carapace length were then determined. This method was also applied to *E. spinifer*, to allow comparison with Mauchline's (1977) and other studies. Crayfish with moult increments recorded from seasons of maximum growth at moulting were allocated to a series of size-classes of 5mm width, commencing at 19.95mm. Random samples of a maximum of three crayfish were taken from each size-class to provide an approxi-

mately even spread of values over the whole range of carapace lengths, thus preventing the excessive representation of the smaller size-classes from causing any substantial distortion in the calculated relationships. Growth factors were calculated for each crayfish, and the linear regressions of growth factor and $\log_{10}$ growth factor on pre-moult carapace length were determined (Figure 6.6) for the whole range of carapace lengths. The correlation coefficients for each relationship were also determined, and tested for significant departures from zero and each other (Snedecor and Cochran, 1967). The same procedure was applied to the combined data for crayfish with carapace lengths greater than 70mm and crayfish with moult increments recorded from seasons of minimum growth at moulting, with the exception that random samples of a maximum of two crayfish were taken from size-classes in the range 20-70mm carapace length.

6.3 RESULTS

6.3.1 Variation in Moult Increment with Respect to Sex, Season and Location.

No significant differences were detected among the slopes of the regressions of moult increment on pre-moult carapace length in any of the comparisons for sex, season or location (Appendix B).

Regressions of moult increment on pre-moult carapace length for males and females were compared within the size-classes, seasons and locations listed in Table 6.1. No significant differences were detected between the sexes in these comparisons.

Table 6.1: Size-Classes, Seasons and Locations for the Moult Increments of Male and Female Crayfish that were Compared Using Analysis of Covariance.

* Comparison	Size-Class (mm)	Season	Location
1	20-35	Spring 1977	Pool 3
2	20-35	Summer 77/78	Pool 3
3	20-35	Autumn 1978	Pool 3
4	20-35	Spring 1977	Pool 7
5	20-35	Autumn 1978	Pool 7
6	35-55	Spring 1977	Pool 3
7	55-70	Annual	Pool 3
'8	20-35	Summer 77/78	Pool 3

* Numbers refer to the results of the analysis of covariance listed in Appendix B.

' Comparison between moult increments of precocious males, normal males and immature females.

Regressions of moult increment on pre-moult carapace length were compared, separately for each location and size-class, for the seasons listed in Table 6.2. No significant differences were detected among seasons for crayfish in Pool 7. Significant differences were present, however, among the seasonal regressions for crayfish with carapace lengths in the range 20-35mm in Pool 3 ($p < 0.005$). The Student Newman Keuls test indicated that the elevations of the regressions for Spring '77 and Autumn '78 were similar ($p > 0.05$), and significantly greater than the elevation of the Summer '77/78 regression ($p < 0.001$). In the case of crayfish with carapace lengths of 35-55mm in Pool 3, the elevation of the Spring '77 regression was significantly greater than that of the Autumn '78 regression ($p < 0.005$).

When seasonal regressions of moult increment on pre-moult carapace length were compared between locations, significant differences were detected among elevations for both size-classes ($p < 0.005$). For the 20-35mm size class (Figure 6.1), the elevations of the regressions for all seasons in Pool 7 and Spring '77 and Autumn '78 in Pool 3, were similar ($p > 0.50$), and significantly greater than the elevation of the regression for Summer '77/78 in Pool 3 ($p < 0.001$). For the 35-55mm size-class (Figure 6.2), the elevations of the regressions for all seasons in Pool 7 and Spring '77 in Pool 3 were similar ($p > 0.20$), and greater than the elevation of the regression for Autumn '78 in Pool 3 ($p < 0.005$).

The elevation of the regression of annual moult increment on pre-moult carapace length for crayfish with carapace lengths of 55-70 mm in Pool 7 was significantly greater than that for Pool 3 (Figure 6.3, $p < 0.005$).

Table 6.2: Size-classes and Locations for Moult Increments Compared Between Seasons, Within Each Location, Using Analysis of Covariance.

*Comparison	Size-Class (mm)	Location	Seasons
9	20-35	Pool 3	Spring '77, Summer '77/8 Autumn '78.
10	35-55	Pool 3	Spring '77, Autumn '78.
11	20-35	Pool 7	Spring '77, Summer '77/8 Autumn '78, Spring '78.
12	35-55	Pool 7	Spring '77, Autumn '78, Spring '78.

* Numbers refer to the results of the analyses of covariance listed in Appendix B.

Figure 6.1: Regressions of Moult Increment on Pre-Moult Carapace Length for Crayfish with Carapace Lengths of 20-35mm.

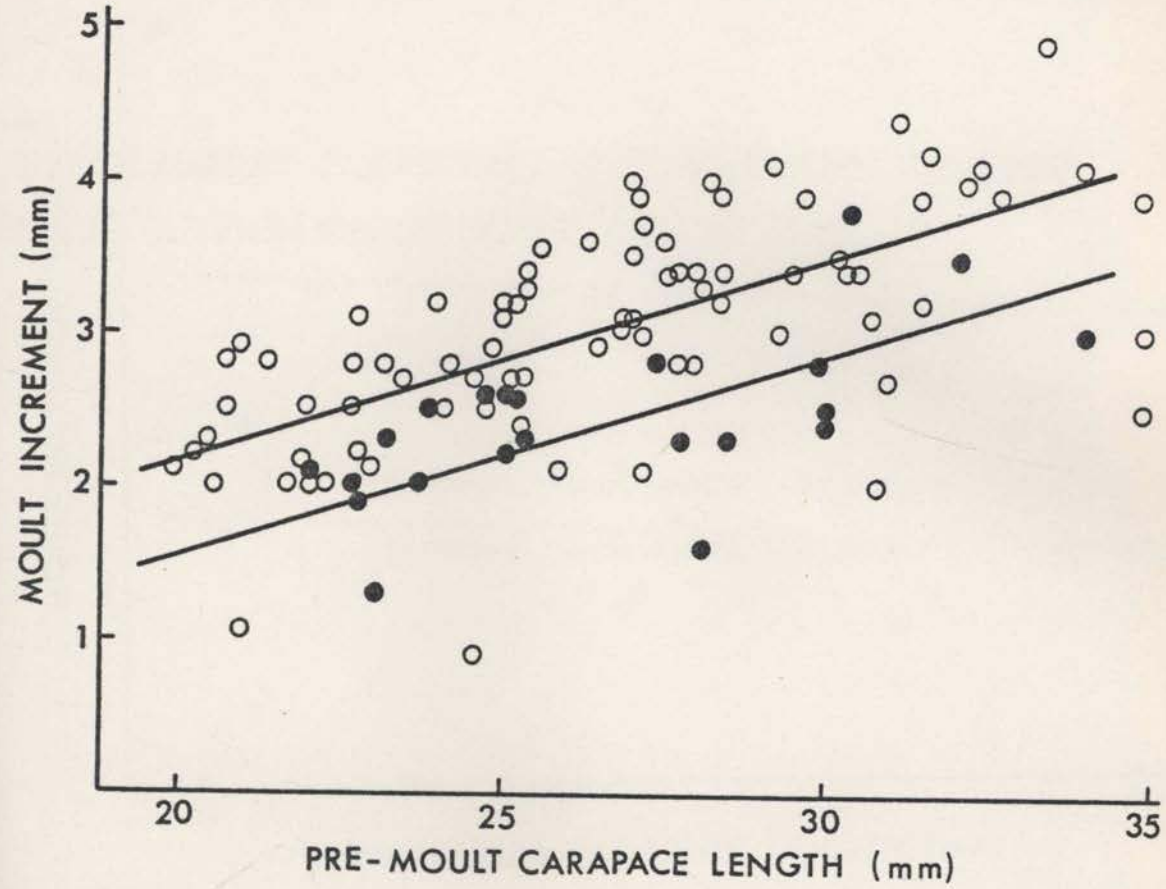
Key:-

- o Pool 3, Spring '77 and Autumn '78 and Pool 7, Spring '77 and Summer '77/78 and Autumn '78 and Spring '78.
- ● Pool 3, Summer '77/78.

Figure 6.2: Regressions of Moult Increment on Pre-Moult Carapace Length for Crayfish with Carapace Lengths of 35-55mm.

Key:-

- o Pool 3, Spring '77 and Pool 7, Spring '77 and Autumn '78 and Spring '78.
- ● Pool 3, Autumn '78.


MOULT INCREMENT (mm)
5
4
3
2
1
20
25
30
35
PRE-MOULT CARAPACE LENGTH (mm)

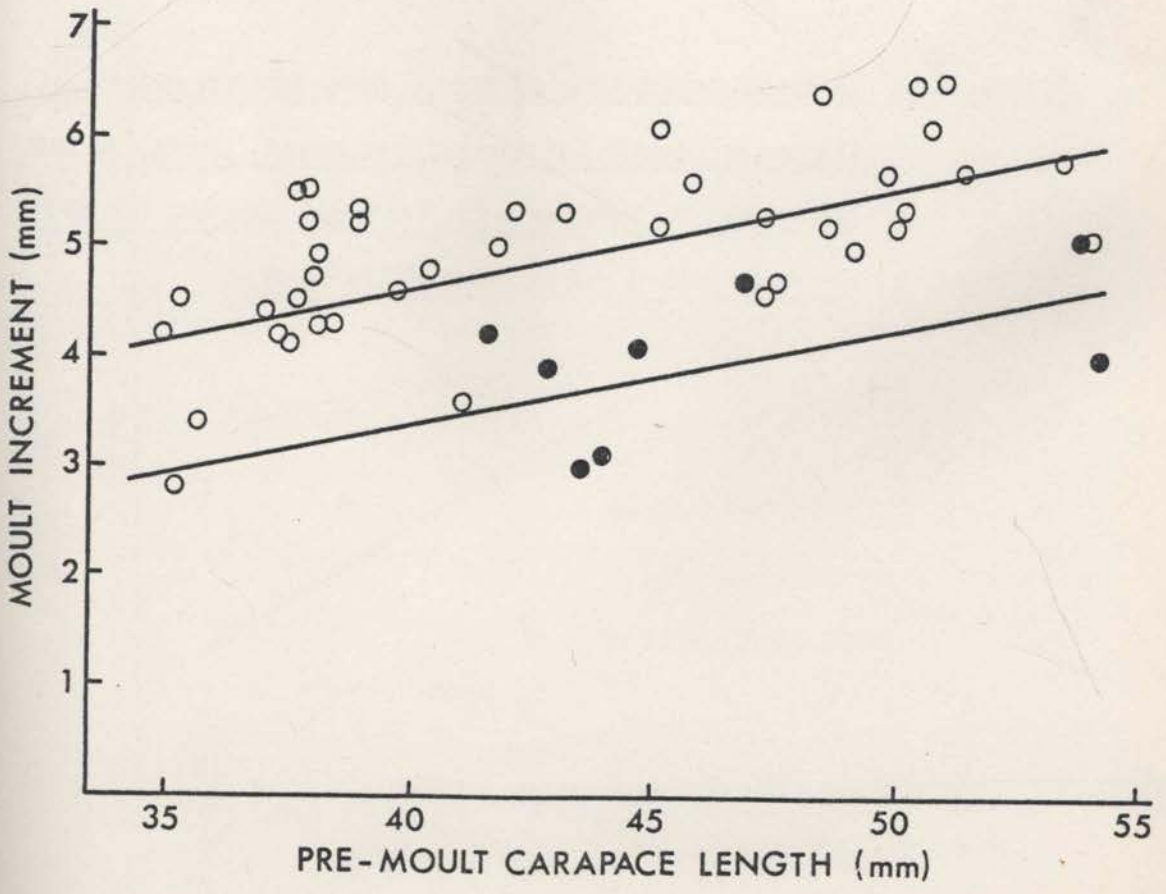

MOULT INCREMENT (mm)
7
6
5
4
3
2
1
35
40
45
50
55
PRE-MOULT CARAPACE LENGTH (mm)

Figure 6.3: Regressions of Moult Increment on Pre-Moult Carapace Length for Crayfish with Carapace Lengths of 55-70mm.

Key:-

○ Pool 7 Annual Moult Increment

● Pool 3 Annual Moult Increment

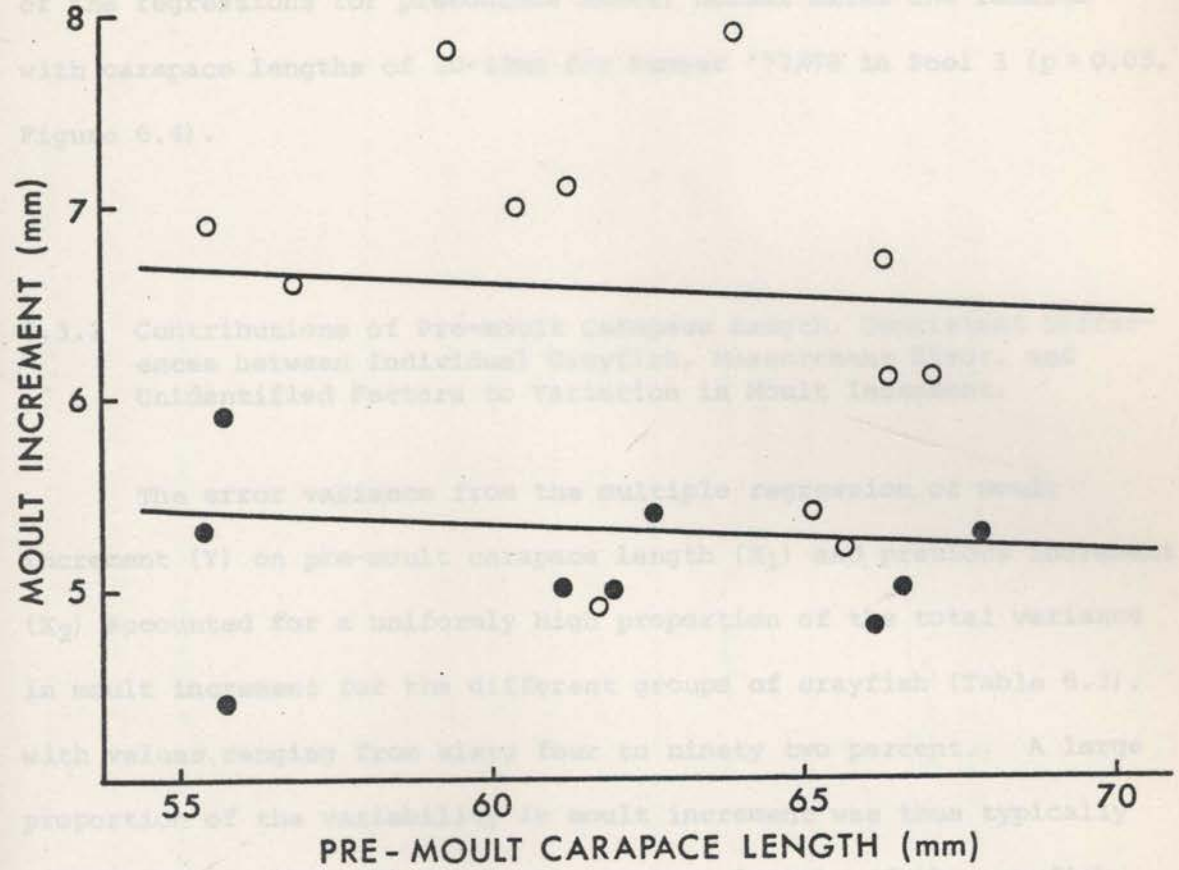
Figure 6.4: Overall Regression of Moult Increment on Pre-Moult Carapace Length for Precocious Males, Normal Males, and Immature Females with Carapace Lengths of 20-25 mm from Pool 3 during Summer 1977-78.

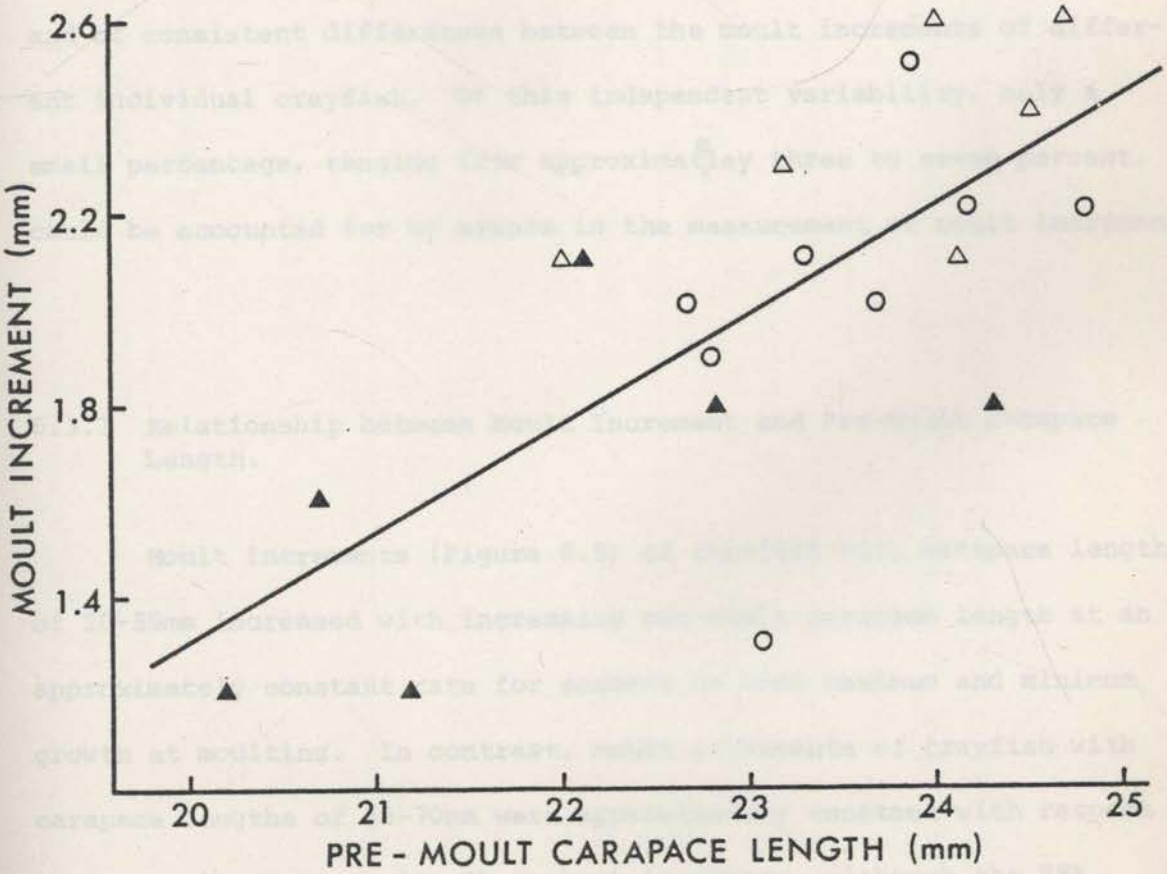
Key:-

○ Immature Female

△ Normal Male

▲ Precocious Male


MOULT INCREMENT (mm)
8
7
6
5
55
60
65
70
PRE - MOULT CARAPACE LENGTH (mm)


MOULT INCREMENT (mm)
2.6
2.2
1.8
1.4
20
21
22
23
24
25
PRE - MOULT CARAPACE LENGTH (mm)

No significant differences were detected among the elevations of the regressions for precocious males, normal males and females with carapace lengths of 20-25mm for Summer '77/78 in Pool 3 ($p > 0.05$, Figure 6.4).

6.3.2 Contributions of Pre-moult Carapace Length, Consistent Differences between Individual Crayfish, Measurement Error, and Unidentified Factors to Variation in Moult Increment.

The error variance from the multiple regression of moult increment (Y) on pre-moult carapace length (X_1) and previous increment (X_2) accounted for a uniformly high proportion of the total variance in moult increment for the different groups of crayfish (Table 6.3), with values ranging from sixty four to ninety two percent. A large proportion of the variability in moult increment was thus typically independent of both the pre-moult carapace lengths of the crayfish, and of consistent differences between the moult increments of different individual crayfish. Of this independent variability, only a small percentage, ranging from approximately three to seven percent, could be accounted for by errors in the measurement of moult increment.

6.3.3 Relationship between Moult Increment and Pre-moult Carapace Length.

Moult increments (Figure 6.5) of crayfish with carapace lengths of 20-55mm increased with increasing pre-moult carapace length at an approximately constant rate for seasons of both maximum and minimum growth at moulting. In contrast, moult increments of crayfish with carapace lengths of 55-70mm were approximately constant with respect to pre-moult carapace length in both locations, although the 95%

Table 6.3: Contributions of Measurement Error and Unidentified Factors to Variation in Moult Increment, based on Multiple Regressions of Moult Increment (Y) on Pre-Moult Carapace Length (X_1) and Previous Increment (X_2).

Site	Source of Regression: Size-Class (mm)	Y Season	X_2 Season	*Estimates of Variability: Total (T)	Error (E)	%E/T	Measurement (M)	%M/E
Pool 3	20-35	Summer '77/8	Spring '77	0.46 (9)	0.32 (7)	69	0.010 (66)	3.1
Pool 3	20-35	Autumn '78	Summer '77/8	0.30 (20)	0.20 (18)	64	0.010 (66)	5.1
Pool 3	35-65	Autumn '78	Spring '77	0.63 (8)	0.56 (6)	89	0.038 (179)	6.8
Pool 7	35-55	Autumn '78	Spring '77	0.56 (7)	0.52 (5)	92	0.019 (92)	3.6

* Total (T) variance in moult increment, with degrees of freedom (d.f.).

Error (E) variance from the multiple regression of Y on X_1 and X_2, with degrees of freedom (d.f.).

Measurement (M) error variance in Y, estimated from independent data, with degrees of freedom (d.f.).

Figure 6.5: Variation in Moult Increment with Pre-Moult Carapace Length for All Crayfish.

Key:-

Seasons of Maximum Growth at Moulting.

A. Pool 7 all seasons and Pool 3 Spring '77 and Autumn '78.

C. Pool 7 all seasons and Pool 3 Spring '77.

E. Pool 7 annual moult.

Seasons of Minimum Growth at Moulting.

B. Pool 3 Summer '77/8.

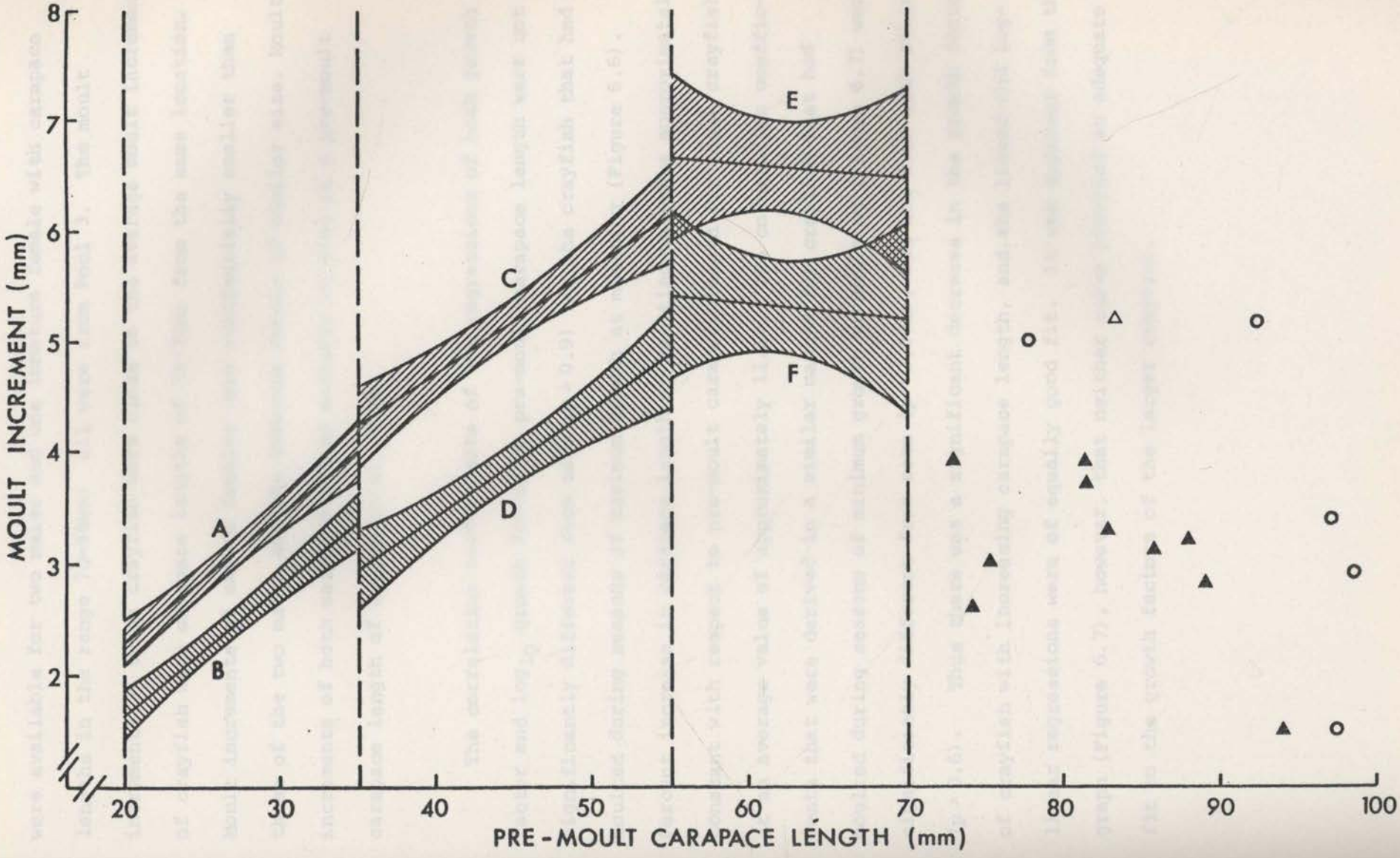
D. Pool 3 Autumn '78.

F. Pool 3 annual moult.

O - Male, Pool 3, annual moult.

Δ - Immature female, Pool 3, annual moult.

▲ - Mature female, Pool 3, annual moult.


MOULT INCREMENT (mm)
8
7
6
5
4
3
2
A
B
C
D
E
F
20
30
40
50
60
70
80
90
100
PRE-MOULT CARAPACE LENGTH (mm)

confidence belts for the slopes of the regressions were wide. Data were available for two males and one immature female with carapace lengths in the range 70-95mm; all were from Pool 3. The moult increments of these crayfish were close to the average moult increment of crayfish with carapace lengths of 55-70mm from the same location. Moult increments of mature females were substantially smaller than those of the two males and the immature female of similar size. Moult increments of both sexes decreased markedly in size at a pre-moult carapace length of approximately 95mm.

The correlation coefficients of the regressions of both growth factor and $\log_{10}$ growth factor on pre-moult carapace length were not significantly different from zero ($p > 0.9$) for the crayfish that had moulted during seasons of maximum growth at moulting (Figure 6.6). Percent increase in carapace length at moulting was thus approximately constant with respect to pre-moult carapace length for these crayfish, at an average value of approximately 11.5%. The correlation coefficients that were derived in a similar manner for crayfish that had moulted during seasons of minimum growth at moulting (Figure 6.7) were significantly different from zero ($p < 0.001$), but not from each other ($p > 0.6$). Thus there was a significant decrease in the growth factors of crayfish with increasing carapace length, and the linear and log-linear regressions were of equally good fit. It was apparent from the graph (Figure 6.7), however, that neither curve provided an adequate fit to the growth factors of the larger crayfish.

Figure 6.6: Variation in Growth Factor with Pre-Moult Carapace Length, for Male and Immature Female Crayfish with Carapace Lengths of 20-70mm, during Seasons of Maximum Growth at Moulting in Pools 3 and 7.

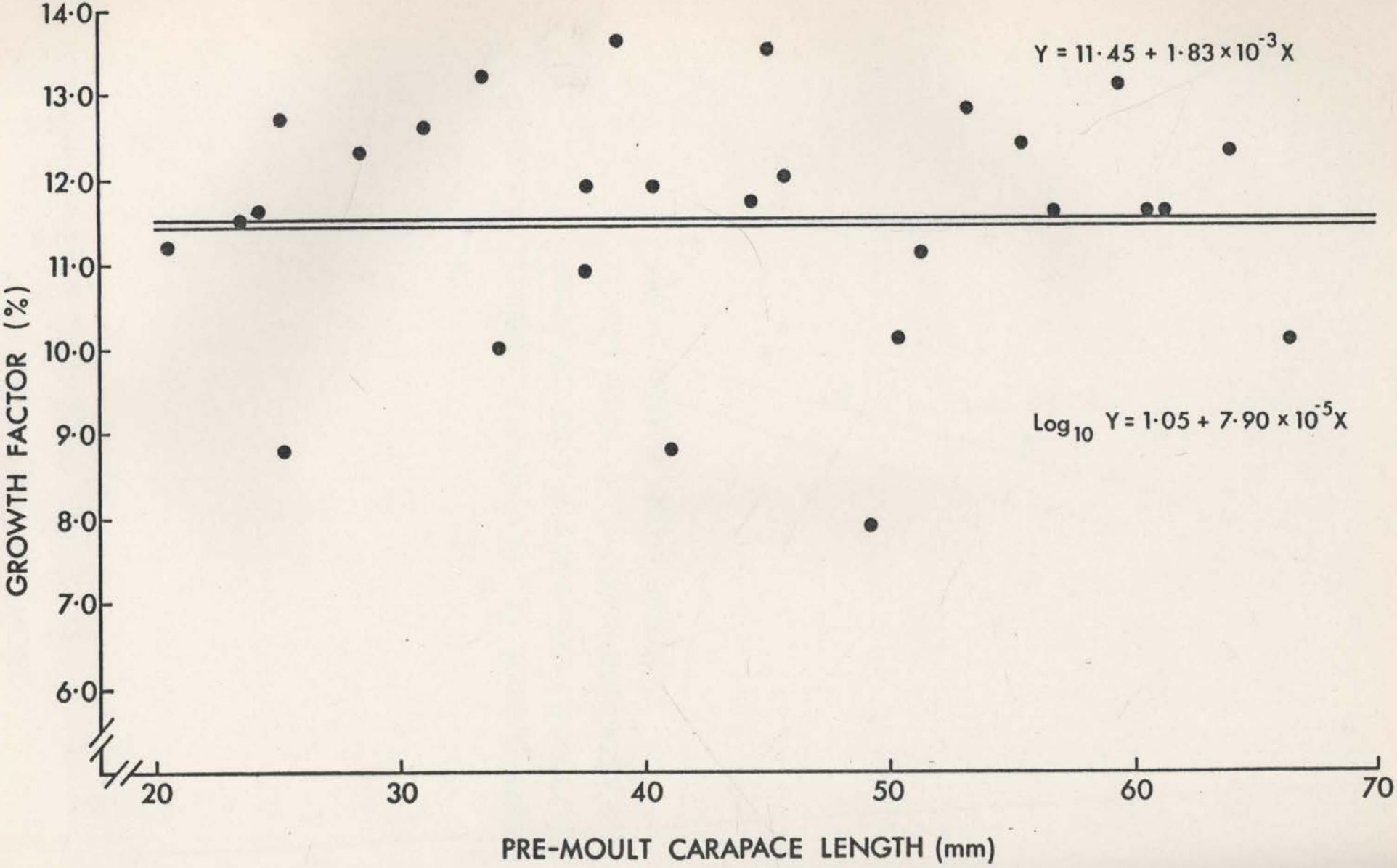
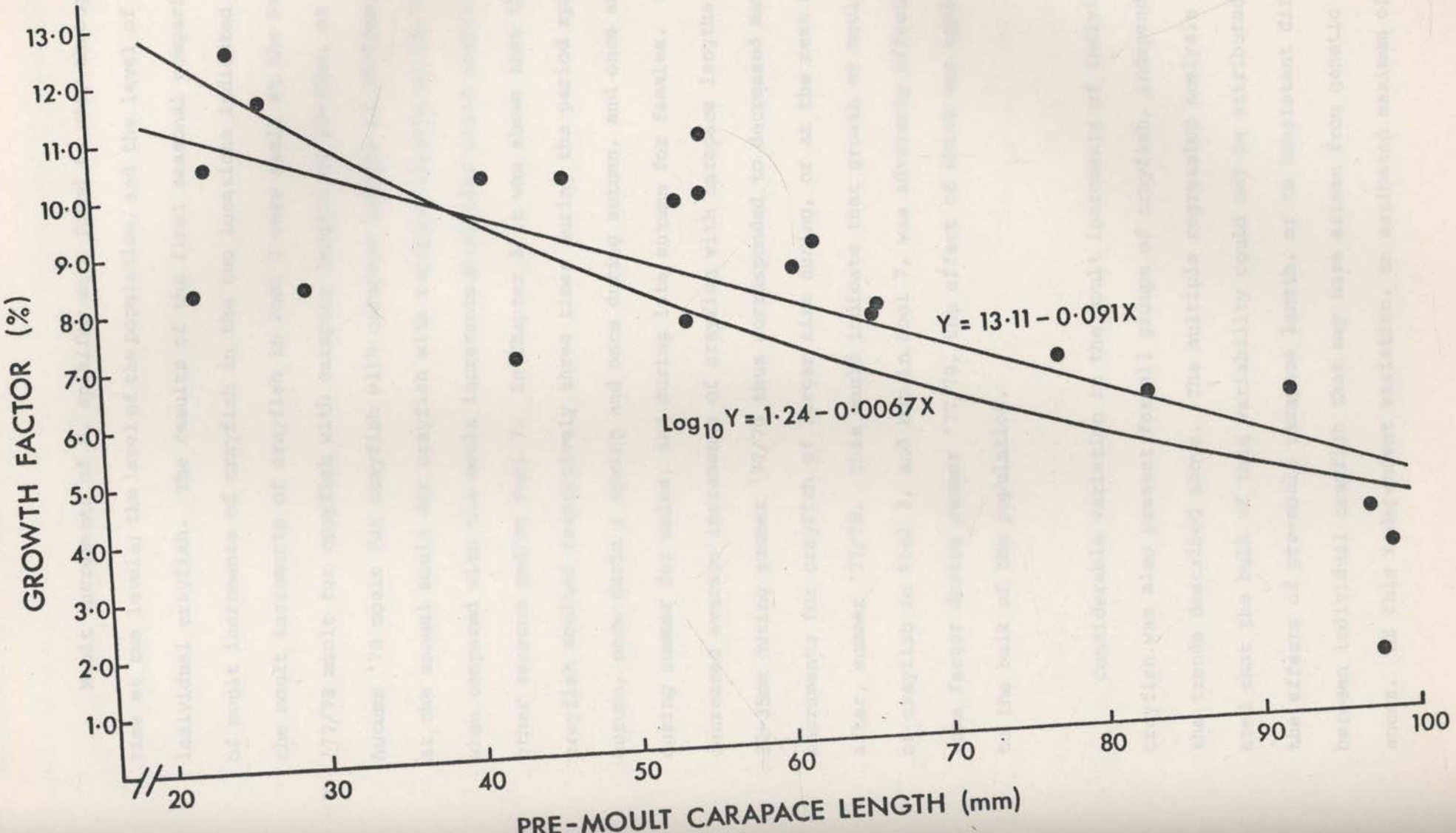

14·0
13·0
12·0
11·0
10·0
9·0
8·0
7·0
6·0
GROWTH FACTOR (%)
Y = 11·45 + 1·83 × 10⁻³ X
Log10 Y = 1·05 + 7·90 × 10⁻⁵ X
20
30
40
50
60
70
PRE-MOULT CARAPACE LENGTH (mm)

Figure 6.7: Variation in Growth Factor with Pre-Moult Carapace Length, for Male and Immature Female Crayfish with Carapace Lengths of 20-100mm, during Seasons of Minimum Growth at Moulting in Pool 3.


GROWTH FACTOR (%)
13·0
12·0
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
20
30
40
50
60
70
80
90
100
PRE-MOULT CARAPACE LENGTH (mm)
Y = 13·11 – 0·091X
Log10Y = 1·24 – 0·0067X

6.4 DISCUSSION

Moult increments of *E. spinifer* were found to vary through time at two levels; the level of the population, and the level of the individual crayfish. The results of the final seasonal comparisons of moult increments of crayfish in the two locations indicated that the moult increments of crayfish in Pool 3 were small at the Summer '77/78 moult for crayfish with carapace lengths of 20-35mm, at the Autumn '78 moult for crayfish with carapace lengths of 35-55mm, and at the annual moult for crayfish with carapace lengths of 55-70mm, when compared with the moult increments of similar sized crayfish from other seasons and/or Pool 7. In Chapter 5 it was shown that these crayfish moulted respectively three times during the period spring--autumn, once durin g spring and once during autumn, and once annually; during summer for males, and during late autumn for females. The decreased average increments of crayfish with carapace lengths of 20-35mm during summer '77/78 thus corresponded to decreased moult increments for crayfish of larger size during, or at the next moult after, summer '77/78. This would indicate that growth at moulting of crayfish in Pool 3, and not in Pool 7, was adversely affected by some factor during summer '77/78, the effect of which was applied to the bulk of the population.

Considerable variation in the moult increments of individual crayfish was also present for all groups of crayfish, independent of the trends described above. The multiple regression analysis indicated that the bulk of this variability could not be attributed to the effects of pre-moult carapace length, or to consistent differences between individual crayfish that may have arisen from genetic differences. Of this independent variation, an estimated maximum of

approximately 7% could have been attributed to errors in the measurement of moult increment. Errors in the measurement of pre-moult carapace length and previous increment could have further contributed only a proportion of the variability estimated for measurement errors in moult increment, leaving at the minimum approximately 50-80% of the total variability in moult increment unaccounted for. Potential causes of such variability would need to fit the requirements of fluctuation over a time-scale not exceeding the period between moults, with fluctuations independent in time for different crayfish, and have an effect on the whole population. A possible explanation could be inherent variability in the mechanism of increase in size at moulting. Although this was not demonstrated for *E. spinifer*, and there appears to be no reference to such a mechanism in the literature, the possibility of its occurrence cannot be excluded.

Variation in moult increment has been linked with the availability of food in the spiny lobsters *Panulirus longiper cygnus* (Chittleborough, 1975, 1976a) and *Jasus lalandii* (Newman and Pollock, 1974), and some indirect evidence of variation in feeding success has been presented for *E. spinifer* in this study (Chapter 8). The major component of the diet of *E. spinifer* was found to be decaying plant material of terrestrial origin (Chapter 8). Substantial input of such material into the pools in the study area would have occurred only during 'flooding', which occurred at the most only two or three times per year, at irregular intervals, over the period of study, and the distribution of such material over the bed of the stream was typically in the form of small isolated deposits. Also, a combination of bushfires and brief but heavy rain resulted in the entry of a considerable amount of plant debris into Pool 7 during December 1977 but not into Pool 3 and flooding did not occur until the following

March, while during the remainder of the study, conditions in the two pools appeared to be similar. Thus variation in food availability may have been responsible for variation in the moult increments of *E. spinifer* at the level of both the individual crayfish and the population respectively.

There were no significant differences between the moult increments of immature female and normal male crayfish up to a carapace length of 70mm, and the moult increments recorded for the two males and one immature female with carapace lengths in the range 70-95mm were of similar size. Normal males matured reproductively at carapace lengths of approximately 45-55mm, while females commenced maturing at approximately 70mm carapace length (Chapter 3). Also, in the only instance in which data were adequate for testing, there were no significant differences among the moult increments of precocious males, normal males and immature females that could not be accounted for by differences in carapace length. There was thus a general similarity between the moult increments of males, regardless of state of maturity, and immature females. In contrast, the moult increments of mature females were substantially smaller than those of the two males and the immature female of comparable size. For *E. spinifer* it is thus concluded that reproduction acts to decrease the size of moult increments in females, but not in males. This effect may be general among the larger decapods, having been recorded for another parastacid *Paranephrops planifrons* (Hopkins, 1967a), the lobsters *Homarus americanus* (Wilder, 1963) and *Homarus vulgaris* (Thomas, 1958; Hepper, 1967, 1972; Ennis, 1972), the spiny lobsters *Jasus tristani* (Pollock and Roscoe, 1977) and *Panulirus homarus* (Berry, 1971), and the crab *Cancer pagurus* (Bennett, 1974), among others.

Growth factors for *E. spinifer*, i.e., moult increments expressed as a percentage of pre-moult carapace length, ranged from 14% for juveniles to 6-1% for large adults, covering a range similar to those recorded for two other parastacids, *Cherax tenuimanus* (Shipway, 1951), and *Paranephrops planifrons* (reinterpreted from Hopkins, 1967a, Figure 1; see in this thesis, Part B, Conclusion). These growth factors showed no major departures from the values recorded for other large decapods.

The absolute values of the average moult increments of *E. spinifer* of any given carapace length varied with both season and location. However, the slopes of the relationships between moult increment and pre-moult carapace length within any given size-class were unaffected by such variation (Figure 6.5). Hence changes in the slopes of these relationships are likely to be fundamental to the patterns of growth of *E. spinifer* in the area, rather than effects of variable external factors. The change from an increase in moult increment with pre-moult carapace length for crayfish with carapace lengths of 20-55mm to a moult increment which was constant with carapace length for crayfish with carapace lengths of 55-70mm is thus considered to be both real and general, although the confidence limits for the slopes of the latter regressions were wide (Figure 6.5), since the average moult increment of the latter group of crayfish in Pool 3 was extremely close to the moult increments of the male and immature female crayfish with carapace lengths of 70-95mm, also recorded from Pool 3. There was no evidence of curvature of the relationship between moult increment and pre-moult carapace length adjacent to this change in slope for crayfish in the 35-55mm size-class (Figure 6.2). Such curvature, if present, must have been restricted to the 55-70mm size-class (Figure 6.3). The considerable variability in moult increments for

these crayfish may in part have reflected such curvature, although it also obscured any obvious relationship. It was found that moult frequency changed from typically twice per year to once per year at a carapace length of approximately 55mm (Chapter 5). Hence there is a strong suggestion of an association between the onset of a constant moult increment and the adoption of a frequency of moulting of once per year. A similar conclusion may be drawn for another parastacid, *Paranephrops planifrons*, if the data of Hopkins (1967a, Figure 1) are re-interpreted to account for the possible inclusion of growth increments that resulted from more than one moult (see Part B, Conclusions). This pattern of growth may thus be typical of parastacid crayfish. Kurata (1962) described variation in moult increment with pre-moult carapace length by plotting post-moult carapace length against pre-moult carapace length, providing what he termed a"Hiatt growth diagram". This method has been subsequently used by numerous authors, but was criticised, on justifiable grounds, by Mauchline (1976) and, as a result, has not been used in this study. Kurata (1962) found, however, that the Hiatt growth diagrams for numerous crustaceans were characterised by abrupt changes in slope, which he termed 'inflexions'. These inflexions were frequently independent of gonad maturation and, as such, may have had a similar origin to that proposed for the change in slope described for *E. spinifer*. Although the data on moult frequencies were not available to allow the testing of this hypothesis on Kurata's data, the inference of Mauchline (1976) that these inflexions should be removed by fitting a single curve to such data must be of doubtful value. This has not been done in the present study, as significant biological information may have been obscured for the sake of expediency.

The equations given by Mauchline (1977) relating growth factors

and pre-moult carapace length indicate an increase in moult increment with carapace length over at least the lower portion of the size-range of each species for the caridean shrimps *Crangon crangon* and *Palaemon serratus*, for the crabs *Cancer magister* and *Cancer pagurus*, and for the lobster *Homarus americanus*. Similar results have been obtained elsewhere for the lobster *Nephrops norvegicus* (Farmer, 1973) and for the cambarid freshwater crayfishes *Procambarus hayi* (Payne, 1972) and *Orconectes virilis* (Weagle and Ozburn, 1972). Constant or decreasing moult increments over the middle and/or upper parts of the size range are indicated by Mauchline's (1977) equations for the spiny lobster *Panulirus interruptus*, the lobsters *Homarus americanus* and *Homarus vulgaris*, and for the crab *Cancer pagurus*, and similar results have been obtained for the spiny lobsters *Jasus lalandei* (Newman and Pollock, 1974) and *Jasus tristani* (Pollock and Roscoe, 1977), and for the lobster *Homarus vulgaris* (Hepper, 1967, 1972). The relationship between moult increment and pre-moult carapace length for *E. spinifer* thus appears to conform to patterns of growth which are fairly general among decapods, in that an increase in moult increment with body size may be followed by a constant, or decreasing, moult increment. The results for *E. spinifer* differ from those for the spiny lobsters *Jasus lalandii* (Fielder, 1964a) and *Panulirus homarus* (Berry, 1971), and the cambarid freshwater crayfish *Cambarus clarkii* (Penn, 1943), in which moult increments were constant from a small size, and from the spiny lobster *Panulirus argus* (from the equation given by Mauchline, 1977), in which moult increments decreased with carapace length from a small size. This may indicate either a fundamental difference between the patterns of growth of panulirids and other decapods, or a difference that is due to other factors. The latter possibility is feasible, since the relationship between moult increment and carapace length has been found to differ between captive and wild *Panulirus longipes cygnus*

(Chittleborough, 1976a), and between different locations for the lobsters *Homarus vulgaris* and *Homarus americanus*, as reviewed by Ennis (1972). The difference between *Cambarus clarkii* and the other cambarids may be of a similar nature.

Although parts of the relationship between moult increment and pre-moult carapace length for *E. spinifer* conform to general patterns seen in other decapods, the equations relating growth factors (moult increment as a percentage of pre-moult carapace length) and pre-moult carapace length proposed for decapods by Mauchline (1977) do not adequately describe this relationship in *E. spinifer*, either in part or in total (Figures 6.6-6.7), for moults undergone by crayfish during growing seasons of similar clemency. When the dependent variable is converted to moult increment, the equations given by Mauchline (1977) for growth factors may describe increasing, constant or decreasing moult increments with size. Such a curve is likely to provide a reasonable fit to the moult increments from any portion of the size-range of a species, regardless of whether the curve can be fitted to data for the whole size-range. For this reason, the lack of fit of Mauchline's (1977) equations to the growth factors of *E. spinifer* does not imply that *E. spinifer* differs fundamentally from other decapod taxa in its growth patterns. Rather, it implies that the equations may not adequately describe the relationship between growth at moulting and size over the greater part of the growth history of a species.

It has been previously noted that the absolute size of moult increments of *E. spinifer* may vary for different seasons, and crayfish of different sizes moult at different times, while the slope of the relationship between moult increment and pre-moult carapace length

remains constant for any given carapace length. It is also possible that slope of the relationship between moult increment and pre-moult carapace length was related to changes in moult frequency. For these reasons it is concluded that the relationship between moult increment and pre-moult carapace length, when calculated separately for crayfish moulting at different times and in different places, provides the most profitable description of the relationship between growth at moulting and the body size for *E. spinifer*.

CHAPTER 7

ANNUAL GROWTH AND SIZE AT AGE

7.1 INTRODUCTION

The difficulties of determining the ages of crustaceans have been noted by numerous authors. In the apparent absence of ageing structures, growth rates may be determined by identifying age classes in the size frequency distributions of catches, or by following the growth of marked individuals. Size at age can be estimated directly by the former method, as has been done for several relatively short-lived species, e.g., *Orconectes virilis* (Momot, 1967; Weagle and Ozburn, 1972), *Paranephrops planifrons* (Hopkins, 1966, 1967a). In larger and more long-lived species, age classes after the first few years may be obscured by variable and/or low average growth rates (e.g., Farmer, 1973; Bennett, 1974; Chittleborough, 1976a), and in such instances, information on the age of larger individuals in wild populations may only be obtained through marking experiments. This type of information is complicated by the discontinuous nature of growth. The typical solution to this problem is to derive estimates of size at age by combining the mathematical relationships to body size of moult increment and moult frequency (e.g., Wilder, 1953; Kurata, 1962; Hancock and Edwards, 1967; Berry, 1971; Bennett, 1974; Macuhline, 1977). Such a method involves errors in the fitting of curves to two sets of data, and is unrealistic if the relationships for moult increment and moult frequency cannot each be fitted by a single, continuous curve, as in the case of *E. spinifer* in this study (Chapters 5 and 6).

In the present study, annual growth rates have been estimated from the linear relationship between final carapace length after a twelve month period and initial carapace length, averaged for a number of individual crayfish of different initial sizes. This method was developed independently and extended in this study, but was first proposed by Manzer and Taylor (1947) for the lemon sole. The method always provides a reliable estimate of the true growth rate in the stock for the year in question, providing that the data are drawn from a number of age groups (Hancock, 1965), since the curves fitted to the data represent the growth of the surviving individuals (Ricker, 1975). This estimate of true growth rate may be used to provide an estimate of the mean size at any age for the surviving individuals, given that the mean size at one age covered by the calculated relationship is known. The reliability of estimates of size at age drawn from these relationships depend upon the assumption that the observed growth rates were typical of growth rates in previous years. In order to test the validity of this assumption, estimates of mean size at age obtained in the above manner have been compared with the arrangement of size-classes in the size-frequency distributions of catches of *E. spinifer* taken during the early part of the study.

Since field data were unobtainable for crayfish with carapace lengths less than twenty millimetres, the growth of crayfish over this size range was estimated from the growth of captive, newly released juveniles, and compared with the results for the other methods.

Also, adequate comparisons of the moult increments and frequencies of precocious males with those of other crayfish of similar size

was not possible in the previous chapters (5 and 6). Due to the scarcity of data for precocious males, an attempt has been made in this chapter to compare the total growth of precocious males and other crayfish over varying periods, to determine whether the size-age relationship determined for other crayfish may also be extended to precocious males.

The aim of this chapter is to provide a series of estimates of size at age that can be used as a temporal context for the life cycle of *E. spinifer*.

7.2 METHODS

7.2.1 Annual Growth Rates of Newly Released Juveniles.

Mature female *E. spinifer* were collected from the study site during November 1977, just prior to the release of juveniles, introduced into a new farm dam, and provided with shelter and leaf litter. Twenty juvenile crayfish were collected from this dam during March 1978 and maintained in an outdoor tank with a floor area of 2 x 1 metres, and a depth of 1 meter. Juveniles were supplied with shelters, leaf litter, and detritus. The tank was aerated, the supply of decomposing material maintained, and small pieces of fish were added occasionally to supplement the diet of the crayfish. The crayfish were only disturbed three times during this period of captivity, and the tank was subjected to the ambient temperature and photoperiod regimes, with the aim of maintaining the crayfish in

conditions approximating those in the wild.

Failure in the aeration system in early November 1978 resulted in the death of most of the crayfish. Carapace lengths of the survivors and intact carcases, a total of eight individuals, were measured to the nearest 0.1mm. The mean carapace length of these crayfish at the time of their release from the parent females was estimated from a random sample of twenty juveniles that were measured before they had moulted, after they were released from females that had been maintained briefly in aquaria. The relationship between final carapace length after approximately one year and initial carapace length that was obtained in this manner was then compared with the relationship obtained for wild crayfish.

7.2.2 Estimation of Annual Growth Rate and Size at Age of Crayfish in the Field Populations.

Annual growth increments were obtained for crayfish at the study site as described in Chapter 5, Section 5.2.1. Carapace lengths after one year were plotted against initial carapace lengths for the annual growth increments of females and normal males of different sizes from Pools 3 and 7 (Figure 7.1). No distinction was made between the data for males and immature females, since their moult increments and annual moult frequencies did not differ (Chapters 5 and 6), while data for mature females were distinguished on the graph by the use of different symbols. Linear regressions of final carapace length after one year on initial carapace length were calculated separately for crayfish with initial carapace lengths above and below 55mm. The regressions were calculated separately for the

following reason. Crayfish with carapace lengths just below 55mm typically moulted twice per year, while crayfish with a carapace length greater than 55mm almost exclusively moulted only once per year (Chapter 5). Since the moult increments of crayfish with carapace lengths just below and for a considerable distance above 55mm were similar (Chapter 6), this resulted in an abrupt and sustained decrease, by a factor of approximately one half, in the annual growth rates of crayfish at this point (Figure 7.1). Ninety five percent confidence limits for the population of final carapace lengths at any given initial carapace length were calculated for each regression line, and plotted as confidence belts. The regression line and confidence belts for crayfish with carapace lengths of 20-55mm were then extrapolated backwards to the estimated initial carapace length of the captive juveniles, and compared with the final carapace lengths of these crayfish after approximately one year of growth.

From the type of graph described above, mean carapace length at age one year may be estimated by taking mean carapace length at age 0 as the mean carapace length of newly released juveniles, and reading from the regression line the corresponding final carapace length after one year of growth. Mean carapace length at age 2 years may be estimated in a similar manner from the calculated mean size at age 1 year, and so on. If the regression line is linear, i.e., it is of the form,

$$L_{t+1} = a + bL_t$$

where L_{t+1} = final carapace length after one year, L_t = initial carapace length, then the process of estimating consecutive sizes at age from the regression line generates a relationship of the form,

$$L_n = \frac{a(1 - b^n)}{1 - b} + b^n L_0$$

where L_n = mean carapace length after n years, L_0 = mean carapace length at age 0, providing that $b \neq 1$ (adapted from Kurata, 1962, p. 44). Separate equations of this form were constructed from the regression lines for crayfish with carapace lengths below and above 55mm. The former equation was applied up to and including the first mean carapace length at age that exceeded 55mm, and the latter from this point onwards (Figure 7.2). The same procedure was applied to straight lines fitted to the upper and lower 95% confidence limits for the population of final carapace lengths at any initial carapace length, for both regressions, and used to estimate approximately size at age for crayfish sustaining respectively the estimated maximum and minimum observed annual growth rates (Figure 7.2).

Also, the maximum carapace length attainable by a crayfish growing at a particular rate may be predicted from the regression line as the point at which final carapace length is equal to initial carapace length, assuming that the relationship between these two variables remains unchanged. Maximum attainable carapace lengths were thus determined from the regression line and upper and lower 95% confidence limits calculated for crayfish with carapace lengths greater than 55mm, to provide estimates of carapace length at age for crayfish growing at the mean and sustained maximum and minimum annual growth rates respectively. These estimates of attainable size and the relationship between mean carapace length and age were compared with the maximum carapace length recorded for crayfish at the study site (Figure 7.2).

7.2.3 Relationship Between Estimated Size at Age and Size Classes in Catches at the Commencement of the Study.

Size classes were determined for the combined catches taken in May, June, July and August 1977 from Pools 3 and 7, i.e., for the first four samplings in the mark-recapture study at each location. These catches were selected for two reasons. Crayfish rarely moulted during this period (Chapter 5), therefore the catches could be combined to provide a larger sample and more sensitive and reliable analysis. Also, the characteristics used to determine this lack of moulting were independent of size classes in the catches, and size classes were determined from catches taken prior to the occurrence of any growth that was used to estimate annual growth rates from the mark-recapture data. Hence the arrangement of size classes used in this analysis was free of possible effects of the sampling program on growth rates, and sizes at age indicated by the arrangement of size classes were completely independent of sizes at age estimated from mark-recapture data, thus allowing meaningful comparisons to be made between the two.

A single size frequency distribution with carapace length classes of width 1 mm was constructed for the combined catches. Points of overlap of its constituent size classes were located using the probability paper method of Harding (1949) and, where feasible, the mean carapace length of the crayfish in each size class was calculated. The size frequency distribution was plotted as a frequency histogram, and the spacings and mean carapace lengths of the contributing size classes were compared with the estimates of mean carapace length at age that had been derived from the mark-recapture data (Figure 7.3).

7.2.4 Annual Growth rates of Precocious Male Crayfish.

Growth over a period of twelve months could be determined for only a single precocious male. In order to provide at least some information on the relationship between the growth rates of precocious males and other crayfish, growth increments of precocious males were determined for periods ranging from eleven to fourteen months. These growth increments were paired with growth increments of normal males or immature females so that the increments in each pair were recorded over periods of identical duration, from crayfish with initial carapace lengths as similar as possible (Table 7.1). The significance of the mean difference between the increments of the precocious males and normal males or immature females was then tested using a paired t-test.

7.3 RESULTS

7.3.1 Annual Growth Rates of Newly Released Juveniles.

The carapace lengths of the captive juveniles after approximately fifty weeks of growth from the time of release from their mother were 10.8mm, 12.8mm, 12.8mm, 13.4mm, 14.4mm, 15.5mm, 17.0mm and 20.6mm. The mean carapace length of juveniles at the time of release was estimated to be 4.46mm with 95% confidence limits of ±0.02mm.

7.3.2 Estimation of Annual Growth Rate and Size at Age in the Field Population.

The majority of the final carapace lengths of the juveniles reared in captivity were grouped about the regression line for the group of smaller crayfish (Figure 7.1), and contained within the 95% confidence limits for the population of final carapace lengths at that point. The final carapace length of a single captive juvenile was located just above the upper confidence limit.

The relationship between carapace length after one year and initial carapace length (Figure 7.1) was closely approximately by a straight line for both size groups, as were the 95% confidence belts. There was considerable variability about the regression line in both instances, although only a small number of the final carapace lengths of crayfish in each size group were located outside the 95% confidence limits. For crayfish with initial carapace lengths greater than 70mm, the final carapace lengths of females were located on or just below the regression line, while the final carapace lengths of males and the single immature female were located above the regression line, with the single exception of the final carapace length of one of the largest males, which was below the regression line. The estimated maximum carapace lengths attainable by crayfish growing at the mean and sustained maximum and minimum annual growth rates were 123mm, 159mm, and 89mm respectively.

Estimated mean carapace lengths increased exponentially with age up to a carapace length of 60.4mm at an age of six years (Figure 7.2). The yearly increase in annual growth increment for these crayfish was slight, however. For crayfish with ages greater than

Figure 7.1: Relationship of Carapace Length After One Year to Initial Carapace Length.

Key:-

△ - Juveniles raised in captivity.
○ - Wild male or immature female crayfish.
□ - Wild mature female crayfish.

95% confidence limits are for the population of carapace lengths after one year at any given initial carapace length.

Separate regressions have been fitted for crayfish with carapace lengths above and below 55mm.

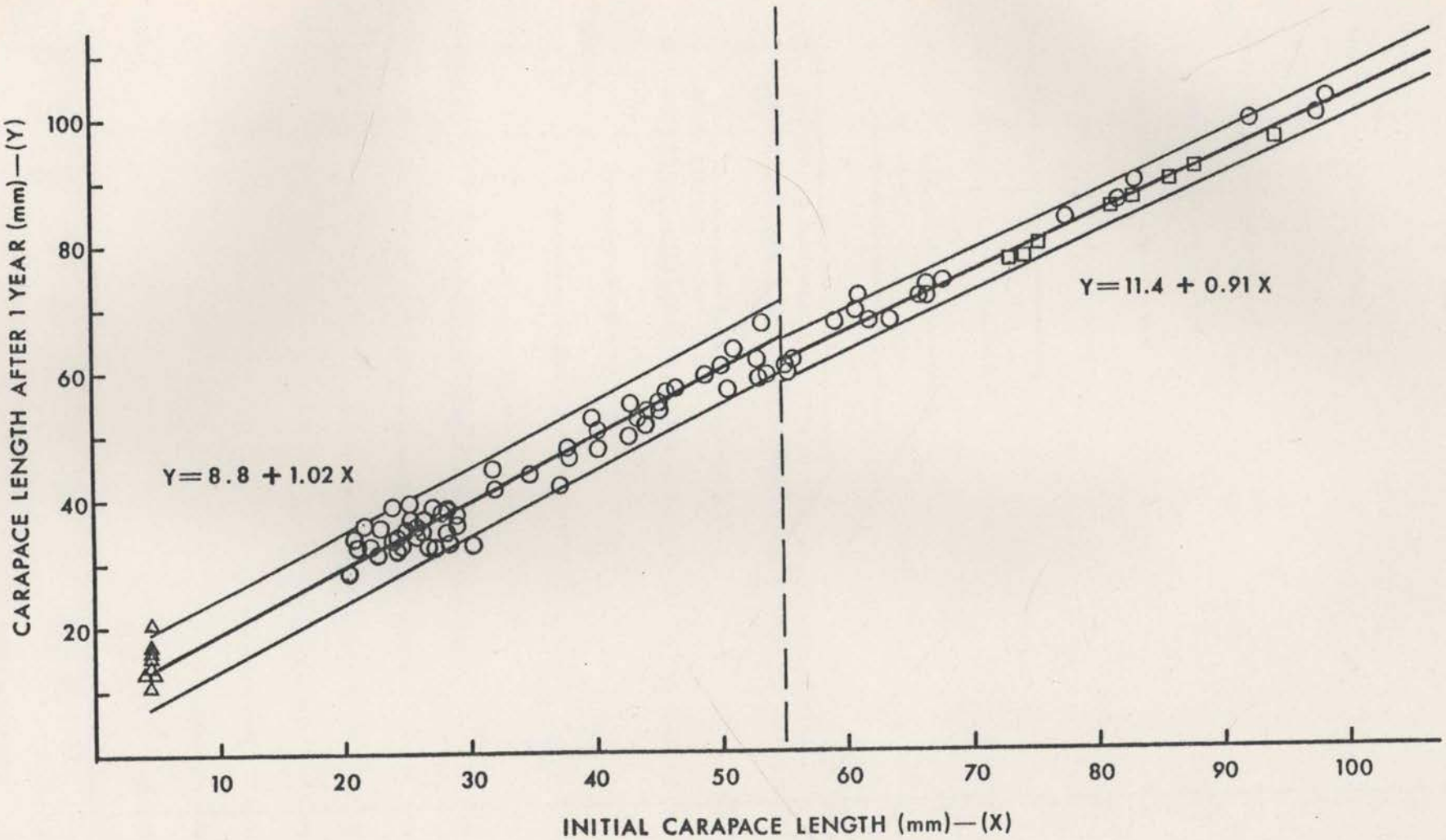
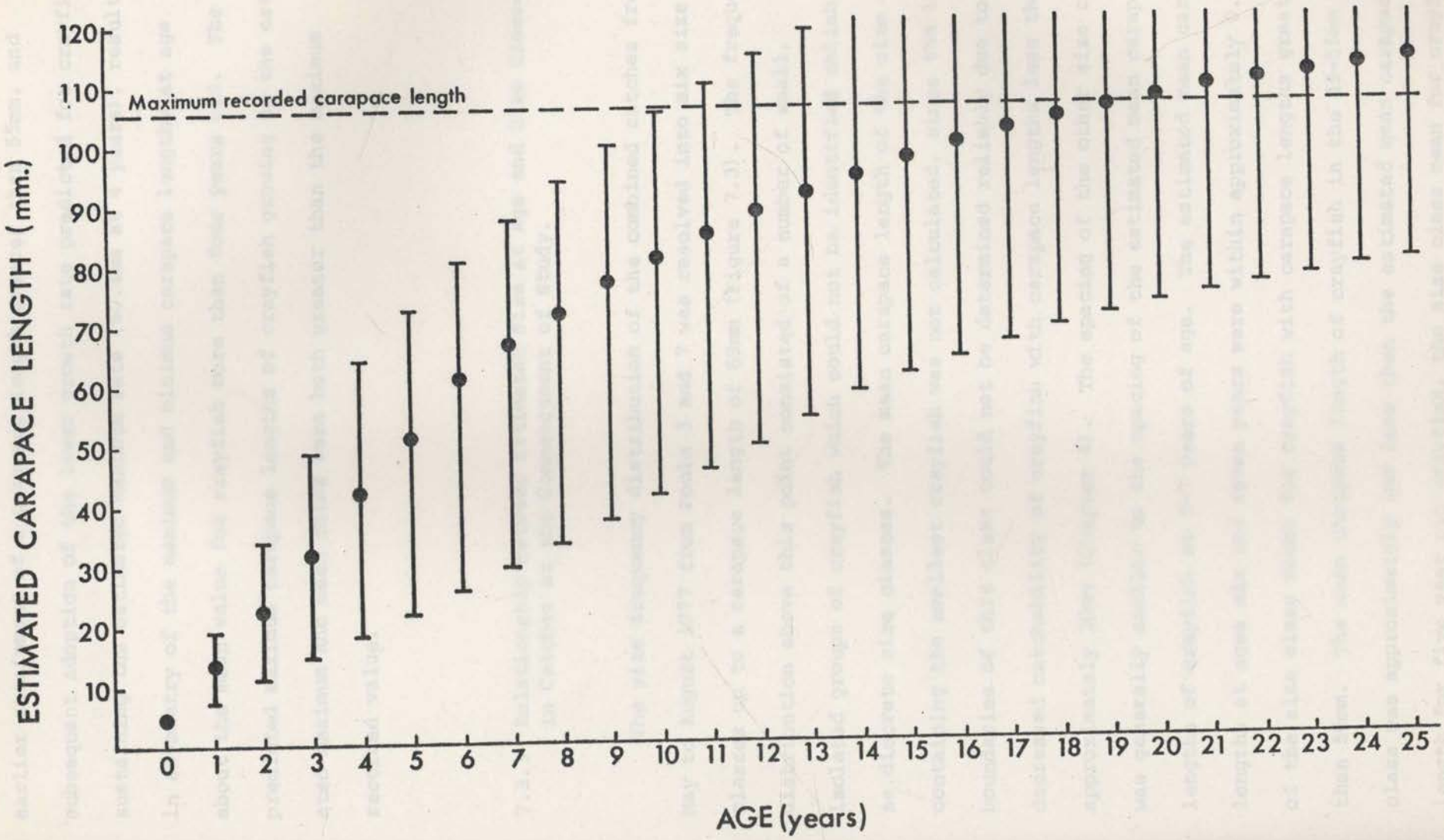

CARAPACE LENGTH AFTER 1 YEAR (mm)—(Y)
100
80
60
40
20
Y=8.8 + 1.02 X
Y=11.4 + 0.91 X
10
20
30
40
50
60
70
80
90
100
INITIAL CARAPACE LENGTH (mm)—(X)

Figure 7.2: Estimated Carapace Length at Age.

Key:-

● - Estimated carapace length at age for crayfish growing at the mean annual rate.

T
⊥ - Estimated carapace length at age for crayfish sustaining the estimated maximum or minimum annual growth rate.


Maximum recorded carapace length
ESTIMATED CARAPACE LENGTH (mm.)
120
110
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (years)

six years, annual growth increments decreased each year. The earlier attainment of a carapace length greater than 55mm, and subsequent adoption of the lower growth rate predicted for crayfish sustaining the estimated maximum rate (60.4mm at 4 years), resulted in asymmetry of the maximum and minimum carapace lengths at age about the mean value for crayfish more than four years old. The predicted maximum carapace lengths of crayfish growing at the estimated maximum and mean rates were both greater than the maximum recorded value.

7.3.3 Relationship Between Estimated Size at Age and Size Classes in Catches at the Commencement of Study.

The size frequency distribution of the combined catches from May to August 1977 from Pools 3 and 7 was resolved into six size classes up to a carapace length of 69mm (Figure 7.3). The frequency distribution above this point consisted of a number of small, isolated groups of crayfish which could not be identified reliably as discrete size classes. The mean carapace length of the size class containing the smallest crayfish was not calculated, since the lower boundaries of this class could not be determined reliably due to the decreased catchability of crayfish with carapace lengths less than approximately 20mm (Chapter 4). The spacing of the other size classes was generally similar to the spacing of the estimated mean carapace lengths of crayfish at 3-7 years of age. The estimated mean carapace lengths at ages six and seven years were within approximately 0.5mm of the size class means for crayfish with carapace lengths greater than 56mm. The mean carapace length of crayfish in the 45-55mm size class was approximately 1mm less than the estimated mean carapace length for five year old crayfish, the size class mean for crayfish

Figure 7.3: Relationship Between Estimated Mean Carapace Length At Age and Size Classes of Crayfish in Catches.

Key:-

● - Mean carapace length of crayfish in the size class directly below.

Alternate size classes are distinguished by hatching. The size class containing the smallest crayfish is incomplete.

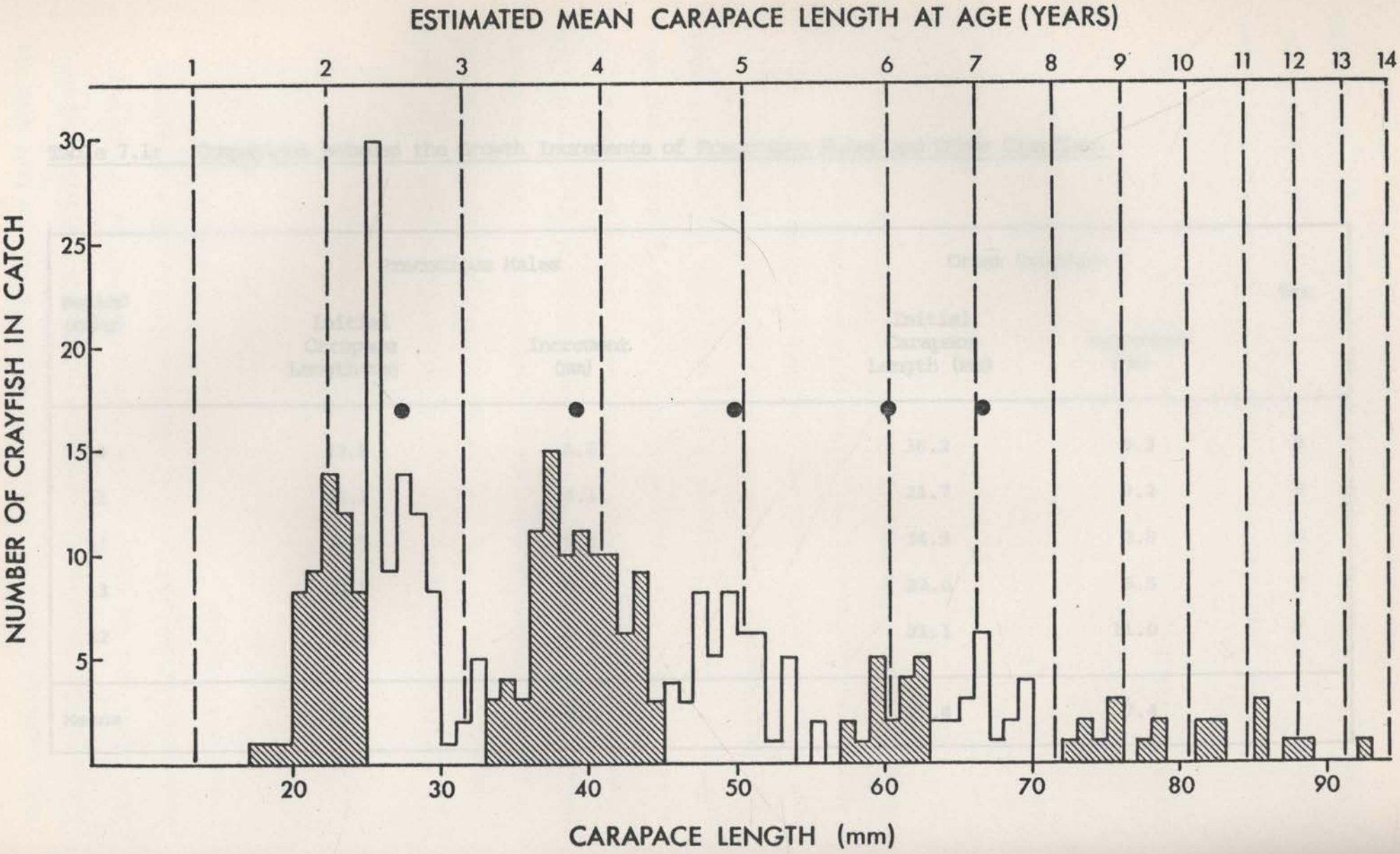

ESTIMATED MEAN CARAPACE LENGTH AT AGE (YEARS)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
NUMBER OF CRAYFISH IN CATCH
30
25
20
15
10
5
20
30
40
50
60
70
80
90
CARAPACE LENGTH (mm)

Table 7.1: Comparison Between the Growth Increments of Precocious Males and Other Crayfish.

Period (mths)	Precocious Males		Other Crayfish		Sex
	Initial Carapace Length (mm)	Increment (mm)	Initial Carapace Length (mm)	Increment (mm)	
14	33.8	6.6	36.2	6.3	M
11	22.1	4.1	21.7	9.2	M
11	33.9	5.1	34.9	3.8	M
13	20.5	7.5	23.0	6.5	F
12	20.9	8.4	21.1	11.0	F
Means	26.2	6.3	27.4	7.4	

with carapace lengths of 33-44mm was approximately 2mm less than the estimated mean carapace length for four year old crayfish, and the size class mean for crayfish with carapace lengths of 25-33mm was approximately 4mm less than the estimated mean carapace length for 3 year old crayfish.

7.3.4 Annual Growth Rates of Precocious Male Crayfish.

Data were available for only five precocious males, with initial carapace lengths ranging from the approximate minimum catchable size of 20mm to a final carapace length of approximately 40mm, the latter being roughly the maximum carapace length recorded for these crayfish (Table 7.1). Data for other crayfish were adequate to allow the pairing of precocious males with other crayfish of similar carapace length, for the exact periods of time over which the growth increments had been measured. Although the mean growth increment for the precocious males was 1.0mm less than that for other crayfish, the mean difference between the increments in each pair was not significantly different from zero at the 5% level ($t = 0.82$, d.f. = 4, $p > 0.40$).

7.4 DISCUSSION

It was found that the carapace lengths attained by the captive juvenile crayfish after approximately one year of growth were generally in close agreement with the values predicted by the relationship

between carapace length after one year and initial carapace length that had been determined for wild crayfish with carapace lengths of 20-55mm. The occurrence of crayfish that attained carapace lengths in excess of that predicted by the 95% confidence limits for the population of final carapace lengths at any given carapace length was not restricted to the captive juveniles, and variability of the final carapace lengths of the juveniles about the regression line was similar to that of the larger, wild crayfish. Considering the conditions under which the captive juveniles were maintained, the similarities of both the average values and variability of the final carapace lengths of the juveniles to the predictions based on the results for the larger crayfish were considered to be adequate justification for the assumption that the relationship between carapace length after one year and initial carapace length for the 20-55mm crayfish could be extended to juvenile crayfish from the time of their release into the population. On this basis, mean carapace length at age 0 was estimated as the mean carapace length of juveniles at the time of their release, and subsequent carapace lengths at age were estimated from the calculated relationship between carapace length after one year and initial carapace length.

When the mean carapace lengths at age were calculated and compared with the identifiable size classes of crayfish in the catches, it was found that the spacing between the size classes and the estimated mean carapace lengths at ages 3-7 years were similar. However, the mean carapace lengths of crayfish in the 25-32mm, 33-44 mm, and 45-55mm size classes were less than the mean estimated carapace lengths for 3, 4 and 5 year old crayfish by approximately 4, 2 and 1mm respectively, while the mean carapace lengths of crayfish in the 55-62mm and 63-69mm size classes were essentially ident-

ical to the estimated mean carapace lengths for crayfish aged 6 and 7 years respectively. This pattern of differences in means conforms to that expected if the growth rates observed during the study were typical of the growth rates of previous years, for the following reasons. Juveniles were released by the parent females into the population during the period late November to early December each year (Chapter 3). Hence the compilation of each growth year, in the context of the estimates of mean carapace length at age, occurred at this time.

Based on the results for moult frequency and moult increment (Chapters 5 and 6), by the end of the 1977 growth year, most of the crayfish in the 25-32mm size class would have moulted at least once, resulting in an upward displacement of the mean carapace length for the size class by approximately 3-5mm. The mean carapace lengths of crayfish in the 33-44mm and 45-55mm size classes would have been displaced upwards by smaller amounts due to the later onset and lower frequency of moulting, while for the 55-62mm and 63-69mm size classes, only males moulted during late 1977, and moulting commenced in the vicinity of the end of the growth year, which would have resulted in little change, if any, in the mean carapace lengths of the size classes prior to the end of the growth year. This indicates that if the difference between the time of sampling and the end of the growth year was taken into account, the mean carapace lengths at age estimated from the growth of crayfish during the study coincided remarkably well with the mean carapace lengths of crayfish in the constituent size classes of the catches. Since this coincidence was present for crayfish up to an estimated seven years of age, it is concluded that the annual growth rates observed during the study provided an accurate assessment of the annual growth rates of crayfish

for at least seven years previous to the commencement of the study. This conclusion applies to the growth rates estimated separately for crayfish with carapace lengths both greater and less than 55mm (as in Figure 7.1), since the agreement between size class means and size-age estimates was present for crayfish in both size ranges. The conclusion has two implications. It implies that mean carapace length at age can be estimated, with a reasonable degree of confidence, for crayfish up to an age of at least eight years. It also implies that the effects of environmental factors on growth were, on an annual average over the two locations, approximately constant over a period of at least eight years up to the completion of the mark-recapture study. The significance of this latter finding is discussed in the context of the life history tactics of *E. spinifer* in Chapter 9.

The carapace lengths at age calculated from the 95% confidence limits provide estimates for crayfish sustaining the approximate maximum and minimum growth rates likely to have been attained by crayfish in any one year during the study. It was found in Chapter 6 that the sizes of consecutive moult increments were generally not substantially correlated for any particular crayfish. Hence it is unlikely that any crayfish maintained the growth rates estimated from the 95% confidence limits for any great period of time. The sizes at age calculated from these growth rates must thus be interpreted as potential maximum and minimum values that are unlikely to have been attained by crayfish in the study area, although they may have been attained by crayfish in other areas, as discussed later in this section.

Discussion of age with respect to events in the life cycle of

E. spinifer in the study area will thus be concerned with estimated mean carapace length at age. The spread of carapace lengths about the mean carapace lengths for the size classes illustrated in Figure 7.3 indicated that the carapace lengths of most crayfish in each size class were within approximately ±5mm of the mean. The range of likely ages for a crayfish of any given carapace length is thus likely to cover approximately one year either side of the age indicated by the mean growth rate for crayfish with carapace lengths up to approximately 60mm; e.g., age at a carapace length of 50mm ≃ 5±1 years (Figure 7.2). This range of ages is likely to increase with increasing carapace length due to variability in annual growth rates. The size of such an increase is unlikely to be great, however, due to growth compensation (as defined by Ricker, 1975) since the higher growth rates of smaller individuals in a group that resulted from the decreasing annual growth rate with increasing carapace length (Figure 7.1) would cause a decrease in the spread of carpace lengths about the mean. The type of variability outlined above is assumed in all further discussion of the age of crayfish at any given carapace length. It was also found that the average annual growth rates of mature females were typically smaller than those of males and the immature female of comparable size (Chapter 6 and Figure 7.1). No attempt has been made to provide separate estimates of mean carapace length at age for these two groups, since the size at which females attained maturity varied considerably (Chapter 3). The strongest conclusion that may be drawn is that age at carapace length may be slightly above the estimated average for females with carapace lengths greater than the minimum size at maturity, and slightly below average for males. This conclusion is also implicit in all further discussion.

Assuming that the relationship between final carapace length after one year and initial carapace length remained constant for very large crayfish; i.e., that there was an asymptotic approach to some theoretical maximum size, it was predicted that the maximum carapace length attainable by crayfish growing at the mean annual rate was approximately 123mm. This was considerably greater than the carapace length of the largest crayfish, a female with a carapace length of 105mm, that was measured during the study. Several larger individuals were captured at the study site, however, with carapace lengths estimated to be in the range 110-120mm, hence the predicted maximum size was probably realistic. Based on the mean annual growth rate, crayfish with carapace lengths of 110 and 120mm would be approximately 22 and 39 years of age respectively, while a crayfish with a carapace length of 100mm would be 16-17 years of age. These ages must, however, be viewed with some caution. Information on the constancy of growth rates more than seven years prior to the commencement of the study was not available, and although the constancy of growth rates over a period of this length suggests that growth rates were probably similar during previous years, an element of uncertainty must be implicit in the estimated ages. It was also observed that the annual moult increments of crayfish of both sexes decreased rapidly with carapace length at a carapace length of approximately 95mm (Chapter 6). This may have indicated that the approach to a maximum size was direct rather than asymptotic, while the variability among crayfish in the magnitude of the decrease in moult increment, coupled with the similarity between observed and predicted maximum sizes of crayfish indicated that, if a direct approach to a maximum size did occur, then it probably occurred at different carapace lengths for different crayfish. Further, given that there was a probability of dying during any year for crayfish of any size, it is likely that the crayfish surviving to

a large size were the more rapidly growing individuals. Although this effect would be counteracted to some extent by growth compensation, it may have resulted in the general overestimation of the ages of large crayfish. The sizes and directions of possible departures of estimated ages from the true ages of large crayfish are thus not clear. Nevertheless, crayfish with carapace lengths greater than 110mm were present at the study site, and crayfish with carapace lengths of approximately 100mm were not uncommon. While their exact ages cannot be estimated reliably, it is unlikely that they all sustained growth rates for the whole of their life-span in excess of the maximum rate estimated during the study, since mean growth rates were approximately constant for the preceding seven years. Hence the lower limits to the ages of crayfish with carapace lengths of 100mm and 110mm may be reasonably estimated from the sustained maximum growth rate as ten and twelve years respectively (Figure 7.2). It is thus reasonable to conclude that *E. spinifer* at the study site attained ages of at least ten years with some regularity. Also, while the considerably greater ages estimated for larger crayfish from the mean annual growth rate may have been unreliable, the considerable duration of the period of constant average growth rates prior to the study suggests that these ages, in the range 20-40 years, may not have been totally unrealistic.

According to Shipway (1951), another large parastacid, *Cherax tenuimanus*, may attain a carapace length of 165mm, and the growth rates of this species in natural streams (Morrissy, 1970) appear to be similar to those recorded for *E. spinifer* in this study. Wilder (1953) estimated that the lobster *Homarus americanus* may attain a total length of nine inches in approximately eight years (similar to *E. spinifer*), while the growth rates of small to medium sized

Nephrops norvegicus described by Farmer (1973) were similar to those for *E. spinifer*, and this species attained a carapace length of approximately 90mm. The ages attained by *E. spinifer* may thus be similar to those of other large decapods. These ages are, however, considerably in excess of those estimated for smaller parastacid crayfish, e.g., 3-4 years for large *Cherax albidus* (Woodland, 1967), a maximum of four years for *Paranephrops planifrons* (Hopkins, 1966, 1967a), and 3-4 years for *Engaeus cisternarius* (Suter, 1977), and of the three years estimated for the cambarid crayfishes *Orconectes virilis* (Momot, 1967; Weagle and Ozburn, 1972) and *Procambarus hayi* (Payne, 1972).

The minimum carapace length at maturity of female *E. spinifer* was approximately 70mm, while normal males matured over the range 45-55mm. These carapace lengths indicated ages at maturity of approximately 8 and 5 years respectively. In contrast, Morrissy (1975) found that female *Cherax tenuimanus* became mature at a carapace length of approximately 30mm, during their second year. Farmer (1973) found that female *Nephrops norvegicus* also matured during their second year, at a carapace length of 19-20mm. As noted above, the maximum sizes and annual growth rates of these two species were similar to those of *E. spinifer*. Also, Berry (1971) found that female *Panulirus homarus*, a spiny lobster with a maximum size similar to that of *E. spinifer*, matured at a carapace length of approximately 54mm during their third year. It thus appears that male, and particularly female, *E. spinifer* do not mature until they attain both an unusually large relative size and unusually great age. From personal observations, using the secondary sex characteristics described in Chapter 3, females of the closely related (Riek, 1969) species *E. armatus*, *E. hystricosus* and *E. valentulus* also do not

mature until they attain considerable size. Hence this pattern of late maturity may be characteristic of the larger members of the genus *Euastacus*.

The samples of crayfish used to test for differences between the growth rates of precocious males and other crayfish were small. However, in view of the small difference between the mean increments of the two groups, the lack of significance of the mean difference between the increments in each pair ($p > 0.4$), and the degree to which pairing was achieved, the conclusion that the growth rates of the two groups were similar is considered to be justified, for the purposes of estimating size at age, on the grounds that any real average differences must have been small. Also, the range of the carapace lengths of the crayfish involved in the comparison allows this conclusion to be applied to precocious males up to their maximum recorded carapace length of approximately 40mm. This provides further justification for the conclusion drawn in Chapter 4 that the disappearance of precocious males from catches at a small carapace length may have been due to mortality rather than to a cessation of growth. Based on the results of Chapter 4 and the mean annual growth rate of other crayfish, it is concluded that precocious males matured at a minimum carapace length not greater than approximately 12mm and a minimum age not greater than one year, and began to disappear from catches at carapace lengths in the range 25-30mm and ages of 2-3 years, with very few further captures up to a maximum carapace length of approximately 40mm and an age of 4 years.

The author has received verbal reports of *E. spinifer*, captured in other catchments near the study site, which regularly attain weights of 'about four pounds'. Although these reports have not been authent-

icated to date, they are considered to be fairly reliable. Based on the length-weight relationship given in Appendix C, crayfish of this weight would have a carapace length of approximately 160mm. This is close to the maximum attainable size that was predicted for crayfish sustaining growth at the maximum annual rate estimated for crayfish at the study site. Also, the average maximum size of *E. spinifer* in catches has been observed to vary considerably between locations. Hence it is not unreasonable to assume that *E. spinifer* attains the weights indicated by the reports. Such a weight would rank *E. spinifer* among the largest known species of freshwater crayfish in the world, behind *Astacopsis gouldii* (8 pounds) and *E. armatus* (6 pounds) (after Francois, 1960).

The considerable sizes and ages attained by *E. spinifer* in the study area, as well as the sizes and ages at maturity of precocious males, normal males, and females, are further discussed in Chapter 9 in the context of the life-history tactics of *E. spinifer*.

CHAPTER 8

DIET AND FEEDING SUCCESS

8.1 INTRODUCTION

The diets of astacid and cambarid freshwater crayfishes of the northern hemisphere have been described by numerous authors (e.g., Abrahamsson, 1967; Lorman and Magnuson, 1978), and accounts have also been given for Australian parastacid freshwater crayfish in several genera (*Cherax:* Shipway, 1951; Woodland, 1967; Johnson, 1979; *Engaeus:* Suter and Richardson, 1977; *Parastacoides:* Lake and Newcombe, 1975). It appears that the only published account of the natural diet of *Euastacus* is that given by Clark (1936), who stated that *Euastacus,* together with *Cherax* and *Engaeus,* "swallow mud and debris of the rivers, etc., in which they live." An attempt has been made in this study to provide a more detailed account as a background to the studies of growth.

It was apparent early in this study that the growth rates of wild *E. spinifer* were very variable and variation in feeding success was suggested as a possible cause by the patchy distribution of plant debris in the stream. Crayfish could only be captured in numbers by using baits, and since the action of a bait involves interrupting the normal feeding pattern, the volume of stomach contents could not be used as a measure of feeding success. Also, the need for some estimate of average feeding success over a longer period was indicated.

In previous studies it had been found that the amount of food consumed over a period by various decapods affected the dry weight of the hepatopancreas (Dall, 1974), the lipid content of the hepatopancreas

(Stewart, et.al., 1967; Armitage, et.al., 1972), and the neutral fat content of the hepatopancreas (Heath and Barnes, 1970). Also, Dall (1975) found that the concentration of protein in the gastric fluid could be used as a reliable and convenient field index of nutritional state.

An attempt has thus been made to establish a relationship between the amount of food consumed over a period of time and both the fat content of the hepatopancreas and protein concentration of the gastric fluid for *E. spinifer*, with the aim of investigating the hypothesis that variations in growth rate resulted from differences in feeding success. Although this attempt was unsuccessful, the results provided some insight into the growth and feeding of *E. spinifer*, and several problems involved in conducting laboratory experiments with this crayfish were exposed. The results of the experiments have thus been treated in some detail. The extent of variation in feeding success among wild *E. spinifer* and its relationship with variation in growth rate is discussed within the limitations of the results, and methods are suggested for increasing the likelihood of obtaining meaningful results from experiments with this, or similar species.

8.2 METHODS

8.2.1 Diet.

Crayfish that were to be used for examination of gut contents were fixed immediately upon capture in 10% formalin to prevent continuing trituration of ingested material by the gastric mill. An initial sample of crayfish was collected from several locations at

the study site in June 1976. The contents of the cardiac proventriculus were removed from each crayfish, and combined for crayfish in each of the carapace length classes 20-30mm, 30-40mm, 40-50mm, and 50+mm. Each combined sample of stomach contents was separated into particle-size fractions by sieving through 0.5mm mesh to facilitate observation of the constituents. Coarse material was examined using a dissecting microscope while finer material was examined under higher magnification, and the constituents were qualitatively described.

Further samples of stomach contents were examined for crayfish captured during May and December 1978, to determine whether any obvious changes in diet occurred during the period between these two samplings, or since the time of the initial sample. The stomach contents of each of ten crayfish from each of the two samplings were sieved through 1mm mesh, since the larger mesh and smaller samples provided essentially similar results to the previous methods and were easier to use, and both of the resulting fractions were examined as described above. Samples of the contents of the hindgut of five of these crayfish were treated in the manner described for stomach contents. Maximum dimensions of some of the smaller particles from both stomach and hindgut were measured using a graduated microscope eyepiece. Results of the above analysis were compared with the observed feeding behaviour of captive *E. spinifer* and other species of *Euastacus*.

8.2.2 Feeding Experiment 1.

An experiment was commenced in February 1978 to investigate the effects of differences in food consumption on the concentration of protein in the gastric fluid and fat content of the hepatopancreas for crayfish of different sex and size. Six normal males and six sexually immature females with body weights from 20-240g. were allocated to each of two feeding groups such that there were no significant differences among either the means or variances of the weights of crayfish in each of the four resulting groups. Individual crayfish were allocated randomly to separate glass aquaria, with capacities of approximately 45 litres. Aquaria were aerated, the floor of each was covered with sand collected at the study site, and each crayfish was provided with a plastic flower pot for shelter. All aquaria were subjected to the ambient temperature and photoperiod regimes. All crayfish were fed *ad.lib.* with pieces of fish, prawns, or specially prepared pelletized food (after Balazs, et.al., 1973) on three or four occasions per week for a period of three months, with the aim of negating any effects of variation in the previous feeding history of the crayfish. The amount of food consumed at each feeding was determined, for each crayfish, as the difference between the weight of food introduced into the tank each evening and the weight of food remaining the following morning. This difference was adjusted for the uptake of water using results from food placed in tanks that did not contain crayfish. Since the quantity of food consumed by each crayfish varied widely at different feedings, it was decided that the only feasible means of varying the food consumption of the crayfish was to vary the frequency of feeding, while feeding *ad.lib.* on each occasion to account for differences in the amounts of food required by crayfish of

different sizes. The different frequencies of feeding used in this and the following experiment (Section 8.2.3) are referred to subsequently as 'feeding levels'. Crayfish in one feeding group were then fed once every three days, while crayfish in the other group were fed once every nine days. The tanks of all crayfish were cleaned, and particles of uneaten food were removed the morning after each feeding of the three-day group. All crayfish were thus disturbed with the same regularity, and food was available only for the night of feeding, regardless of the feeding rate.

The experiment was terminated after three months, during August 1978. Several crayfish died during the course of the experiment, while the remainder were in obviously poor condition, and not comparable with wild crayfish. Hence no attempt was made to estimate fat content of the hepatopancreas or protein concentration of the gastric fluid. A quick check for any major effects of feeding level was made by comparing the relationships between hepatopancreas dry weight and total body weight for the remaining crayfish in the different feeding groups. The hepatopancreas and carcass, the latter including all fluids released during dissection, were dried in an oven at 105°C for twenty-four hours. Linear regressions of hepatopancreas dry weight on total body dry weight were calculated separately for male and female crayfish in each feeding group (Figure 8.1), and compared using analysis of covariance (Snedecor and Cochran, 1967) (Table 8.2). Moult increments undergone by crayfish during the experiment were also compared with moult increments undergone by wild crayfish. The experimental crayfish were paired with wild crayfish of the same sex and similar carapace length, and the mean of the differences between the moult increments of the crayfish in each pair was tested for significance using a paired t-test (Table 8.3).

8.2.3 Feeding Experiment 2.

A second feeding experiment was commenced during early January 1979. Eighteen normal male and immature female crayfish with carapace lengths in the range 20-30mm were installed in glass aquaria with capacities of approximately 45 litres. Each crayfish was provided with a flower pot for shelter, and all aquaria were aerated, and subjected to the ambient temperature and photoperiod regimes. All crayfish were fed with earthworms and leaf litter on five nights per week for the first two weeks, with the aim of negating, to some extent, any variations in previous feeding history. Crayfish were then allocated randomly to two feeding groups. Crayfish in one group were fed one night per week, while crayfish in the other group were fed on five or six nights per week, for the next eight weeks. Uneaten food was removed daily and the tanks of all crayfish were cleaned the morning after the weekly feeding of the one-day group. All crayfish were fed on the last night of the experimental feeding period, then starved for three days until most of the ingested material had been eliminated from the digestive tract.

Crayfish were then immobilised by placing them in an ice-water slush for approximately thirty minutes, and a sample of 0.05 - 0.1ml of gastric fluid was collected from the cardiac proventriculus of each crayfish by applying gentle suction to a glass canula which had been inserted between the mandibles, along the oesophagus, and into the cardiac proventriculus (after Vonk, 1960). Each sample of gastric fluid was centrifuged at 3000 rpm for two minutes to settle any suspended material. Two 0.01 ml sub-samples of supernatant were collected from each initial sample of gastric fluid using 'Microcap' micropipettes and diluted in separate 0.99ml volumes of distilled water. The protein

concentration of each of the resulting 1% solutions of gastric fluid was determined spectrophotometrically using the Biuret method (Layne, 1957), based on a standard curve of absorbance vs. mg/ml of crystalline bovine serum albumin in distilled water. Four readings were taken for each sample and converted to the protein concentrations of the original gastric fluid. Differences in protein concentration between the samples for each crayfish, between crayfish within each feeding group, and between feeding groups, were analysed using a three-factor analysis of variance. Mean protein concentrations were calculated for all crayfish, and 95% confidence limits for these means were determined using the 'samples' mean square in the analysis of variance (Snedecor and Cochran, 1967) (Table 8.4). Percentages of the total variability in protein concentration due to differences between feeding levels, crayfish within feeding levels, and samples for each crayfish were calculated using the method given by Winer (1971). The moult stages of all crayfish were also noted. The universal moult stage notation (e.g., Passano, 1960) has been generalised to some extent for practical reasons. The relationship between the present notation, the moult cycle, and physical characteristics of the crayfish, is given in Table 8.1.

Protein concentrations of the gastric fluid were also determined for a sample of wild crayfish captured at the study site shortly after the termination of the experiment. These protein concentrations were compared with those of crayfish in the experiment, and an attempt was made to determine whether the different moult stages of crayfish at the end of the experiment were likely to have affected the results. Again, the crayfish were starved for three days until most of the ingested material had been eliminated, and samples of the gastric fluid were analysed in the manner described for the experimental crayfish, with

Table 8.1: Moult Stage Notation.

Notation	Position in Moult Cycle	Physical Characteristics
Ce	Early intermoult.	Only branchiostegites deformable under light pressure, no gastroliths.
C	Intermoult.	Exoskeleton firm all over, no gastroliths.
De	Early premoult.	Exoskeleton firm all over, gastroliths present, new exoskeleton not obvious, or slight.
Dl	Pre-moult.	Branchiostegites deformable under light pressure, large gastroliths, new exoskeleton obvious and well-formed.

the exception that, in the analysis of variance, feeding levels were replaced by moult stages (Table 8.5).

A small sample of the hepatopancreas of each of the experimental crayfish was fixed in Bouin's fixative (Humason, 1967) at the termination of the experiment, in the event of histological examination being necessary. The remainder of each hepatopancreas was frozen in liquid nitrogen and stored in a deep-freeze in preparation for freeze drying and subsequent determination of fat content. These latter samples were lost due to a failure in refrigeration. Fat determinations were thus conducted on the small, fixed samples. These samples were soaked in water until there was little further loss of fixative (approximately 48 hours) and vacuum-dried to constant weight. The quantity of fat in each sample was estimated as the decrease in dry weight after refluxing with diethyl ether in a Quickfit 'Soxhlet' fat extraction apparatus for sixteen hours. Fat content was expressed as a percentage of the initial dry weight of each sample, and the difference in mean fat content between the groups of crayfish that had been fed at the two rates was tested for significance using a t-test (Table 8.6). The correlation coefficient for the relationship between fat content of the hepatopancreas and concentration of protein in the gastric fluid of the experimental crayfish was calculated and tested for significant departure from zero. The fat content of the hepatopancreas was also determined for five wild crayfish, all in moult stage De, that were collected from the study site in August 1978. The lobe of the hepatopancreas from one side of each crayfish was vacuum dried fresh, while the other lobe was fixed in Bouin's fixative and treated as described above, and the fat content of each sample was determined. The mean difference in estimated fat content due to fixation was estimated for each of the five crayfish, and tested for significance using a paired t-test

(Table 8.7). Estimations of fat content for the experimental crayfish were interpreted in the light of this difference, and comparisons were made between the experimental and wild crayfish. Further comparisons were made with wild crayfish to determine whether differences in moult stage were likely to have affected the results of the experiment. Four crayfish in moult stage Ce and four in moult stage De were collected from Pool 7 during January 1980. Separate fat determinations were conducted for the unfixed lobes of the hepatopancreas on each side of each crayfish, as described above. Differences in fat content between moult stages and between crayfish within moult stages were analysed using a two-factor analysis of variance (Table 8.8).

Moult increments undergone by crayfish during the experiment were compared with the moult increments of wild crayfish of similar carapace length. Linear regressions of moult increment on pre-moult carapace length were calculated for the experimental crayfish, for crayfish in the field population in Pool 3 that moulted during summer 1977-78 (Chapter 6), and for crayfish that moulted during other seasons in pool 3 and during any season in Pool 7 (Figure 8.2). Differences among the slopes and elevations of the regressions were tested for significance using analysis of covariance (Snedecor and Cochran, 1967) (Table 8.9). Significant differences among the elevations were located using a Student Newman Keuls test for elevations (Zar, 1974).

8.3 RESULTS

8.3.1 Diet.

The contents of the proventriculus contained particles with maximum dimensions up to approximately 5mm. Particles ranging from 1-5mm consisted of pieces of wood, bark, and twigs, with occasional severely decomposed pieces and/or fibres of the leaves of the aquatic macrophyte *Triglochin procera* ('ribbon weed'). Fragments of wood, bark and twigs were either weathered, discrete particles, or they had obviously been torn, chewed or broken from some larger object. These particles were typically blackened, and in a state of partial decomposition.

The bulk of all stomach contents consisted of particles not retained by the 0.5 or 1.0mm meshes. The maximum dimensions of individual particles ranged down to a typical size of 2-10 μm although small quantities of much finer material were present. A small proportion of particles down to approximately 5 μm could be identified as elements of woody plant tissue in all samples. Apart from very occasional diatoms, no algal material was obvious, and the remainder of the fine material was unidentifiable, except for occasional small grains of sand with maximum dimensions up to approximately 0.1mm.

Samples of hindgut contents were not obviously different from the stomach contents, except for the absence of particles with maximum dimensions greater than 1.0mm. The size of the overwhelming majority of the particles corresponded to the 2-10 μm determined for the typical small particles among the stomach contents.

There was no obvious variation in gut contents with respect to either the size of the crayfish, or between the samples taken at different times.

8.3.2 Feeding Experiment 1.

All foods were at first eagerly accepted by the crayfish, but in all instances feeding activity declined over the first two months until individual crayfish fed only occasionally, and generally showed little interest in food.

Prior to the termination of the experiment five crayfish had died during or shortly after moulting, while of the remainder, four had not moulted, three had moulted normally, and ten had moulted but failed to harden their new exoskeleton after a considerable period of time. Some of the moulted crayfish were an abnormal colour, either blue or varying shades of light grey-brown, and all surviving individuals were lethargic. The hepatopancreases of all surviving crayfish were extremely fragile and abnormally coloured when compared with those of wild crayfish.

Sufficient crayfish survived to allow calculation of linear regressions of hepatopancreas dry weight on total body dry weight for female crayfish in both feeding groups,and for males in the nine-day feeding group (Figure 8.1). There were no significant differences among either the slopes ($p > 0.25$) or elevations ($p > 0.25$) of these regressions (Table 8.2), indicating that feeding level had no discern-

Figure 8.1: Relationships Between Hepatopancreas and Total Body Dry Weights for Crayfish in First Feeding Experiment.

Key:-

● female, fed once every three days.

○ female, fed once every nine days.

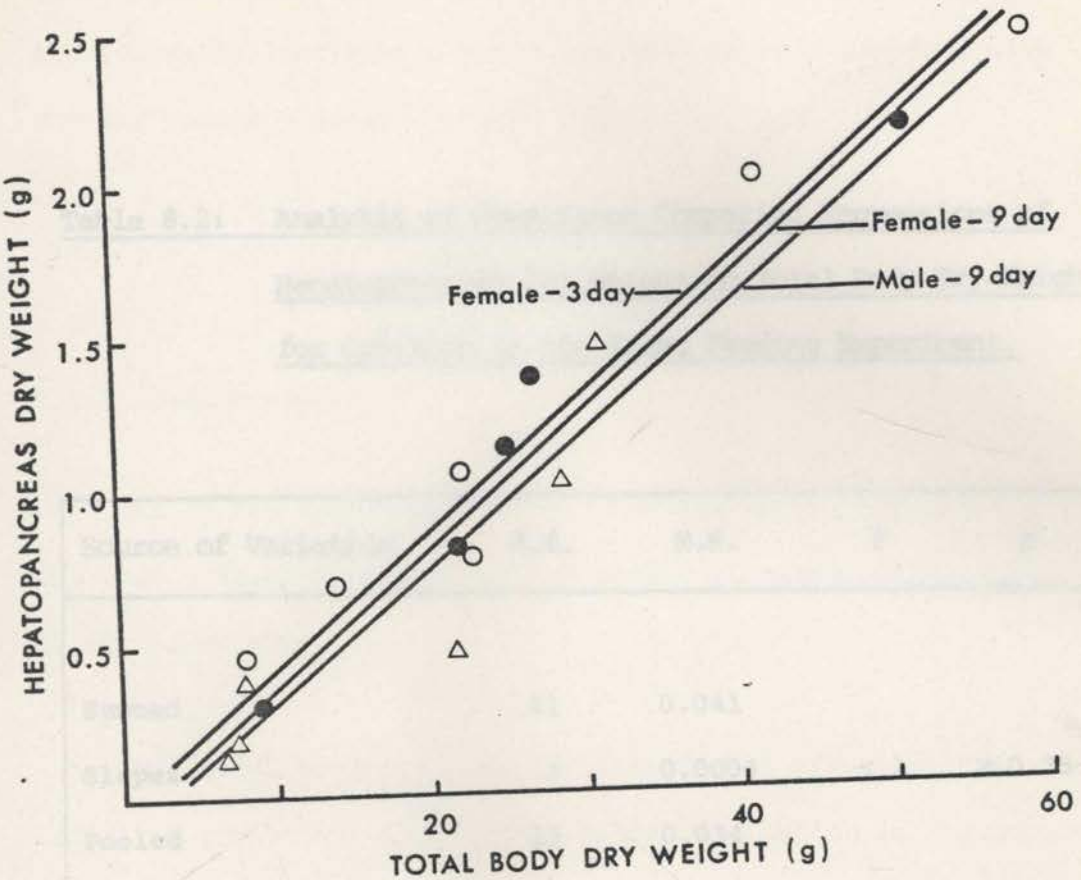
△ male, fed once every nine days.

Figure 8.2: Relationships Between Moult Increment and Pre-moult Carapace Length for Experimental and Wild Crayfish.

Key:-

● - experimental; ○ - wild; Pool 3 Summer 1977-78.

▲ - wild; other seasons in Pools 3 and 7.


HEPATOPANCREAS DRY WEIGHT (g)
2.5
2.0
1.5
1.0
0.5
Female – 9 day
Male – 9 day
Female – 3 day
20
40
60
TOTAL BODY DRY WEIGHT (g)

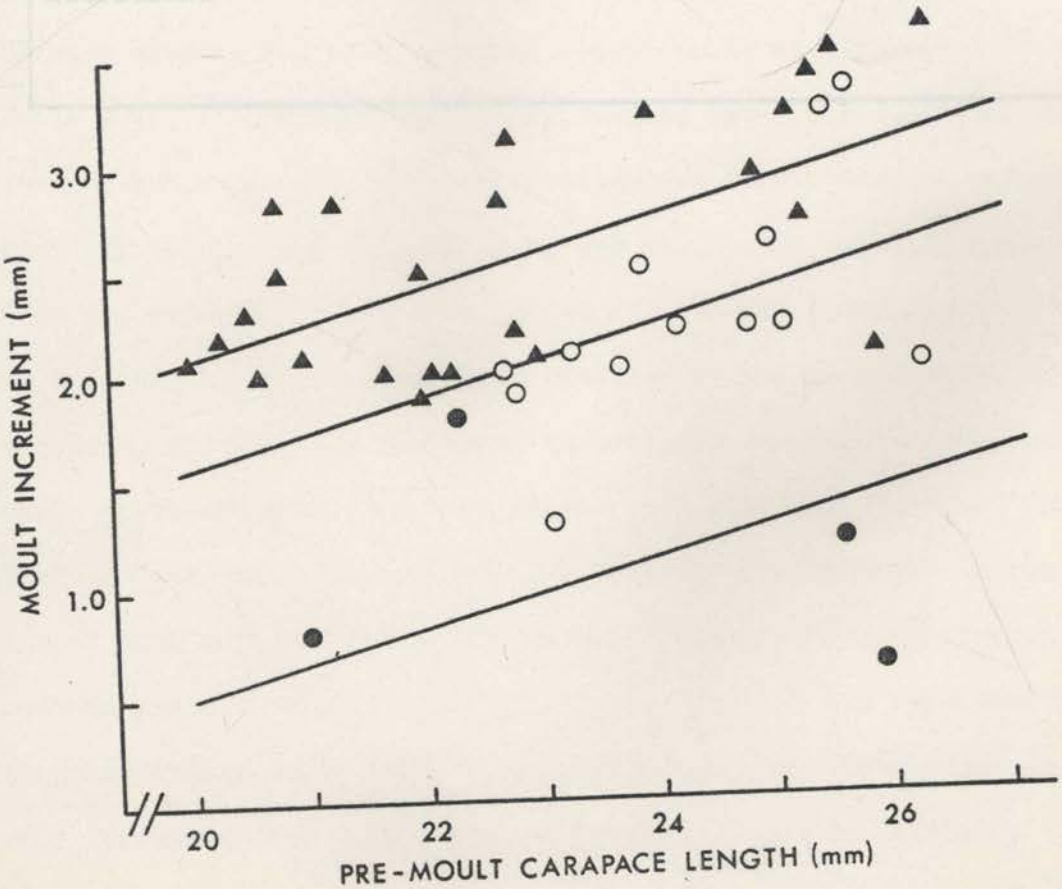

MOULT INCREMENT (mm)
3.0
2.0
1.0
20
22
24
26
PRE-MOULT CARAPACE LENGTH (mm)

Table 8.2: Analysis of Covariance Comparing Regressions of Hepatopancreas Dry Weight on Total Body Dry Weight for Crayfish in the First Feeding Experiment.

Source of Variation	d.f.	M.S.	F	p
Summed	11	0.041		
Slopes	2	0.0004	< 1	$\gg 0.25$
Pooled	13	0.034		
Elevations	2	0.018	< 1	$\gg 0.25$

able effect on total hepatopancreas solids, independent of crayfish size. The moult increments of crayfish in the experiment were significantly less than moult increments of wild crayfish with similar carapace lengths (Table 8.3).

8.3.3 Feeding Experiment 2.

As in the first experiment, food was eagerly accepted at first, but food consumption declined and became irregular during the course of the experiment.

The level of feeding had no significant effect on the concentration of protein in the gastric fluid of crayfish in the experiment, while differences among crayfish within each feeding group, and between samples for each crayfish, were highly significant ($p<0.005$, Table 8.4). Since the F-ratio for feeding level was less than one, the percentage of the total variability due to differences between crayfish within each feeding level was calculated from the mean square from the analysis of variance pooled for feeding level and crayfish. On this basis, differences among crayfish within each feeding level accounted for 99.2% of the total variability in protein concentration, while differences between samples for each crayfish accounted for a further 0.4%, and the remainder was attributable to error in the reading of each sample. There did not appear to be any relationship between protein concentration and moult stage for the experimental crayfish (Table 8.4), while for the wild crayfish protein concentrations increased from moult stage Ce to moult stage De, although this increase was not significantly different from zero at the 0.05 level

Table 8.3: Comparison Between the Moult Increments of Crayfish in the First Feeding Experiment and Wild Crayfish.

Experiment		Wild		Experiment		Wild	
C.L.*	I.*	C.L.	I.	C.L.	I.	C.L.	I.
61.8	4.7	61.7	4.9	49.4	2.6	49.1	5.0
44.6	2.8	44.4	5.2	49.1	1.3	49.3	4.9
78.2	2.7	74.3	2.6	47.5	3.1	48.6	5.2
53.7	5.9	54.0	5.1	63.7	3.7	64.0	7.9
51.5	5.2	51.4	5.7	73.8	2.9	77.9	5.0
71.5	4.2	73.0	3.9	67.5	3.9	67.1	6.1
60.4	3.9	61.3	7.1				

Paired t-test: $t = 3.76$; d.f. = 12; $p < 0.005$.

Mean difference Wild-Experiment = 1.7mm with 95% confidence limits of ± 1.0mm.

* Pre-moult carapace length (C.L.) and moult increment (I.) in mm.

Table 8.4: Effect of Feeding Level on the Protein Concentration of the Gastric Fluid of Crayfish in the Second Feeding Experiment.

Results for Individual Crayfish:			
5 days/week feeding		1 day/week feeding	
Moult Stage	Mean Protein Concentration*	Moult Stage	Mean protein Concentration*
Ce	166 ± 14	Dl	33 ± 14
Ce	110 ± 14	De	204 ± 14
De	52 ± 14	De	48 ± 14
De	77 ± 14	De	371 ± 14
De	102 ± 14	De	73 ± 14
Mean	101 ± 109	Mean	145 ± 109

Analysis of Variance Summary

Source of Variation	d.f.	M.S.	F	p
Feeding Level	1	39117	< 1	n.s.
Crayfish within Level	8	89152	267	< 0.005
Samples within Crayfish	10	335	8.1	< 0.005
Readings within Sample	60	41.3		

* Protein concentration expressed as mg/ml of crystalline bovine serum albumin, with 95% confidence limits.

($0.25 > p > 0.1$, Table 8.5). As for the experimental crayfish, the major portion of the total variability in protein concentration was attributable to differences among individual crayfish, in this instance within each moult stage, at a level of 79%. The difference between moult stages accounted for 13.7% of the total variability in protein concentration, assuming that this difference may have been real, while the differences between the samples for each crayfish accounted for a further 5.1%. Again, differences among crayfish within each moult stage and between samples for each crayfish were highly significant ($p<0.005$, Table 8.5).

The mean fat content of the hepatopancreas of crayfish that had been fed on five days per week was somewhat greater than the mean for crayfish that had been fed on only one day per week, although this difference was not significant at the 0.05 level ($0.4 > p > 0.2$, Table 8.6). Several negative estimates of fat content were obtained, indicating that considerable error was involved in at least some estimates. The estimated fat content of the hepatopancreas of one crayfish in the five-day feeding group was considerably lower than the general range of estimates for other crayfish in that group while the reverse applied to the group of crayfish that had been fed once per week (Table 8.6). There did not appear to be any relationship between moult stage and fat content, except in so far as crayfish in moult stages Ce and C were only present in the five-day feeding group. The fixing of hepatopancreas in Bouin's fixative resulted in overestimation of fat content (Table 8.7). The fat content of the hepatopancreas of wild crayfish (Table 8.8) increased both substantially and significantly ($p < 0.005$) from moult stage Ce to moult stage De, although, as for the trends in previous comparisons, there were significant differences among crayfish within each of the moult stages ($p < 0.005$).

Table 8.5: Effect of Moult Stage on the Protein Concentration of the Gastric Fluid of Wild Crayfish, April 1978.

* Mean Protein Concentrations for Individual Crayfish	
Moult Stage Ce	Moult Stage De
93 ± 21	99 ± 21
49 ± 21	132 ± 21
141 ± 21	152 ± 21
160 ± 21	197 ± 21
81 ± 21	138 ± 21
Mean 105 ± 42	Mean 144 ± 42

Analysis of Variance Summary

Source of Variation	d.f.	M.S.	F	p
Moult Stage	1	30968	2.33	0.25 > p > 0.1
Crayfish within Stage	8	13318	19.7	< 0.005
Samples within Crayfish	10	678	18.3	< 0.005
Readings within samples	60	37		

* Protein concentration expressed as mg/ml of crystalline bovine serum albumin, with 95% confidence limits.

Table 8.6: Effect of Feeding Level on the Fat Content of the Hepatopancreas of the Experimental Crayfish.

Results for Individual Crayfish			
5 days/ week feeding		1 day/week feeding	
Moult Stage	Fat Content (% dry weight)	Moult Stage	Fat Content (% dry weight)
C	8.5	Dl	- 2.9
Ce	6.7	De	5.8
Ce	7.1	De	- 2.8
Dl	- 5.7*[1]	De	- 6.4
De	5.2	De	14.8*[2]
Ce	8.2	De	5.4
De	2.1	De	3.4
De	11.2		
Mean:	5.4	Mean:	2.5

t-test for means: $t = 0.919$, d.f. = 13, $0.4 > p > 0.2$.

*[1] Value considerably lower than others.

*[2] Value considerably higher than others.

Table 8.7: Effect of Fixing in Bouin's Fixative on the Estimated Fat Content of Hepatopancreas for Wild Crayfish in Moult Stage De, August, 1979.

Treatment	Fat Content for Individual Crayfish (% dry weight)				
Fresh	31.8	2.4	20.4	36.7	23.3
Bouins	42.8	9.6	24.8	40.2	29.4

Paired t-test: $t = 4.94$, d.f. = 4, $p < 0.01$.

Mean increase due to Bouins= 6.5%, with 95% confidence limits of ±3.6%

Table 8.8: Effect of Moult Stage on Fat Content of the Hepatopancreas of Wild Crayfish, January 1980.

Mean Fat Content for Individual Crayfish (% dry weight), with 95% Confidence limits of ±1.4%

Moult Stage Ce				Moult Stage De			
43.3	46.3	50.5	42.3	60.5	61.8	71.5	70.9

Analysis of Variance Summary

Source of Variation	d.f.	M.S.	F	p
Moult Stage	1	1710	37	< 0.005
Crayfish within stage	6	46.2	67	< 0.005
Determinations	8	0.688		

Table 8.9: Analysis of Covariance Comparing Regressions of Moult Increment on Pre-Moult Carapace Length for Experimental and Wild Crayfish.

Source of Variation	d.f.	M.S.	F	p
Summed	36	0.20		
Slopes	2	0.54	2.75	$0.1 > p > 0.05$
Pooled	38	0.22		
Elevations	2	4.57	21.2	< 0.005

The fat content of the hepatopancreas was significantly correlated ($r = 0.672$, d.f. = 9, $0.025 > p > 0.01$) with the concentration of protein in the gastric fluid of the experimental crayfish.

There were highly significant differences among the elevations of the regressions of moult increment on pre-moult carapace length (Figure 8.2) for the experimental and wild crayfish ($p < 0.005$, Table 8.9), indicating that moult increments for the different groups of crayfish differed significantly independent of any effects of differences in pre-moult carapace length. The Student Newman Keuls test indicated that moult increments of the experimental crayfish were significantly less than the moult increments of wild crayfish in Pool 3 that moulted during summer 1977-78 ($p < 0.005$), while the latter increments were in turn significantly less than the moult increments of crayfish that moulted in Pool 3 during other seasons, and in Pool 7.

8.4 DISCUSSION

8.4.1 Diet.

Since there was no obvious variation in the stomach contents of *E. spinifer* with respect to either the time of sampling or the size of crayfish, and considering the nature of the stomach contents together with the effect of the gastric mill in triturating and homogenising ingested material, no attempt was made to accurately quantify the stomach contents in terms of either types of constituents or particle sizes.

The stomach contents of wild crayfish examined in this study indicated that the natural diet of *E. spinifer* consisted mainly of decomposing plant material, and that at least the larger particles of food consisted almost exclusively of woody plant tissues of terrestrial origin. Similar observations have been made for semi-terrestrial Tasmanian parastacids *Parastacoides tasmanicus* (Lake and Newcombe, 1975) and *Engaeus fossor* and *Engaeus cisternarius* (Suter and Richardson, 1977). Although the aquatic macrophyte *Triglochin procera* was abundant at several sites from which crayfish were collected for examination of stomach contents, there was no evidence that this plant was used as a food source, except occasionally when in a decomposed state. This result conforms to aquarium observations in which *E. spinifer* only consumed living plant material *(Egeria densa,* filamentous green algae) after having been starved for several weeks, and then only a small quantity was consumed by occasional individual crayfish.

In contrast, the European astacid freshwater crayfish *Astacus astacus* (Abrahamsson, 1966) and both astacid and cambarid freshwater crayfishes in North America (Lorman and Magnuson, 1978) have been found to consume large quantities of living aquatic macrophytes and algae, in addition to decomposing material. Also, Shipway (1951) considered that the West Australian parastacid freshwater crayfishes *Cherax tenuimanus, C. quinquecarinatus* and *C. preissii* were particularly destructive to aquatic macrophytes. The diet and nutritional requirements of *E. spinifer* may thus differ fundamentally from those of freshwater crayfish in the same and other families, in that only plant material that is in a state of decomposition is used. Observations on the feeding behaviour of captive specimens of *Euastacus armatus, E. australasiensis, E. hystricosus, E. kierensis* and *E.*

sulcatus concerned with those recorded for *E. spinifer*. Hence, the dietary preferences of *E. spinifer* may be typical of the genus.

The presence of fragments of woody plant tissue among the small particles of the stomach contents, when considered in the context of the function of the gastric mill, indicates that at least a portion of the finer material resulted from the mechanical breakdown of larger particles similar to those that were actually observed. The gastric mill of *E. spinifer* is large and robust, and well-suited for this purpose. It is possible, however, that much of the finer material may simply have been ingested as fine, particulate detritus. Repeated attempts to feed captive *E. spinifer* using different types of food have indicated that these crayfish are capable of utilising food particles of virtually any size, from most types of substratum. Pieces may be bitten, scraped, or torn from large pieces of flesh or plant debris using the mandibles, which are extremely powerful. Smaller particles may be picked from the substratum using the chelae of the second and third pairs of walking legs and passed to the mouth parts, while fine sediments may be scraped from a surface using the mouth parts.

A further mode of feeding was observed for captive crayfish in aquaria that had a floor-covering of sand from the study site containing small fragments of blackened, decomposing wood and charcoal. The sand had been seived previously through 1mm mesh, in order to remove larger food particles that crayfish could pick up using their chelae. After several days of starvation, crayfish began to 'bulldoze' the sand. This process involved a crayfish pressing its mouth and the anterior part of the cephalothorax onto the sand, and piling further sand against the anterior part of the cephalothorax using the second

and third pairs of walking legs. The crayfish then pushed the pile of sand forwards with its mouth buried, propelled by the fourth and fifth pairs of walking legs, and holding the sand in place using the second and third pairs of walking legs and the great chelae. Crayfish were observed to release faeces consisting of fine, black material, after indulging in this activity, and it was concluded that particles of wood and charcoal had been removed from the sand during the bulldozing process. *E. spinifer* was observed to use the same bulldozing technique when excavating shelters, except in the above instances there was no attempt to remove sand from any particular part of the tank, and the activity appeared to be randomly directed. It must be noted, however, that before both bulldozing and scooping techniques were initiated, the crayfish made numerous, abortive attempts to pick the food particles up using the chelae, and that bulldozing was preceeded by a period of starvation.

Hence it may be inferred that *E. spinifer* feeds preferentially on particles of a size that may be manipulated with the chelae. In this context, the conclusion that much of the stomach contents had been originally ingested as fine detritus would suggest that the preferred food was in short supply. This suggestion is supported by the absence of obvious macroscopic plant debris in many parts of the stream and the presence of small grains of sand among the stomach contents, while it is to some extent contradicted by the almost complete absence of diatoms and other algal material.

The origin of a considerable portion of the stomach contents of *E. spinifer* is thus not clear. However, considering the preference of *E. spinifer* for large food particles, the nature of the large particles in the stomach contents, and the abundance of crayfish, it is probable

that *E. spinifer* contributed significantly to the breakdown of terrestrial plant debris entering the stream. Further evidence of this was the clean, well-chewed appearance of logs and branches on the bed of the stream. Measurements of the size of particles in the contents of the hindgut of several crayfish indicated that most of the ingested material was reduced by the gastric mill to a fine sludge, with particles typically having maximum dimensions in the range of 2-10 μm . *E. spinifer* would thus have made incoming plant debris available to smaller detritivores, and greatly increased the surface area for microbial action. The energy budget of many streams is based largely on incoming terrestrial plant debris (Cummins, 1974), hence *E. spinifer* may play an important role in the nutrient dynamics of its habitat.

The attracting power of baits of fresh fish, meat and other animal material indicate that *E. spinifer* is not wholly detritivorous, and may act as a scavenger. Also, *E. spinifer* were occasionally observed to actively hunt and capture tadpoles at the study site. At such times, the actions of the crayfish were obviously predatory, and the capture of tadpoles was not accidental. Movements of the crayfish were tense and stealthy, while the substratum was searched cautiously with the antennae. When a tadpole was encountered, the crayfish lunged forward with considerable speed, assisted by a rapid extension of the abdomen, and the tadpole was captured using the great chelae. Captive crayfish were observed to show similar responses to fish, earthworms and, occasionally, the smaller crayfish. In addition, wild *E. spinifer* have been observed on two occasions to be in the process of consuming small, live frogs. *E. spinifer* must thus be considered an opportunistic feeder in the broadest sense, although its normal feeding role at the study site could be described as that of a detritivore, a role to which it is well adapted.

8.4.2 Feeding Success.

The poor condition of the crayfish at the end of the first experiment must be considered indicative of severe physiological stress. In particular, the general condition of the hepatopancreas, the inability of several crayfish to re-build their exoskeletons, and the decline through time in the feeding activity of crayfish, suggest that this stress may have had a nutritional basis, and that the diet of prepared food and flesh, initially selected for convenience in determining food consumption, may not have been adequate. The absence of any significant difference in total hepatopancreas solids between the crayfish in the two feeding groups is, in this context, not surprising. As a result, crayfish in the second experiment were fed a more natural diet of leaf litter, supplemented with earthworms, and the experiment was run over a shorter period of time.

In the second feeding experiment, the crayfish were in outwardly normal condition, and an attempt was made to establish a relationship between level of feeding and the two potential indices of feeding success. In the case of the protein concentration of the gastric fluid, any effect of feeding level was completely overwhelmed by the considerable variability among crayfish within each feeding group (99.2% of the total variability). In the case of the fat content of the hepatopancreas, considerable variation among individuals within each feeding group was also apparent, due at least partly to substantial errors involved in making determinations for samples of such a small size, and again there was no significant effect of feeding level. Corresponding variability among wild crayfish was determined for protein concentrations (79% of total variability, Table 8.5), and for fat content, particularly for one individual in the sample of crayfish

collected in August 1978 (Table 8.7). In view of this, the protein concentration of the gastric fluid and the fat content of the hepatopancreas may have varied considerably among the experimental crayfish at the beginning of the experiment.

It was noted that the estimated fat content of the hepatopancreas of one crayfish in each of the two feeding groups differed markedly from the other estimates for each group. The t-test comparing fat content of the hepatopancreas for the two feeding groups has been recalculated with these two crayfish omitted (Table 8.10), with the result that the mean fat content for the group of crayfish fed five times per week was significantly greater ($0.025 > p > 0.01$) than the mean fat content for crayfish fed only once per week. This result cannot be used as a basis for concluding that a causal relationship existed between feeding level and fat content. However, for the purpose of interpreting why such a conclusion may not have been reached, the result is relevant in so far as it conforms to the hypothesis that effects of feeding level on fat content of the hepatopancreas may have been obscured by the considerable departure of individual crayfish from the general level as a result of conditions experienced before capture.

Although the protein concentrations of the gastric fluid and the fat content of the hepatopancreases of the experimental crayfish showed no obvious relationship with moult stage, it was found that the fat content of the hepatopancreas of wild crayfish increased substantially and significantly ($p < 0.005$, Table 8.8) from moult stage Ce to moult stage De, while a similar increase in protein concentration of the gastric fluid may have been real, but was obscured by differences between crayfish (Table 8.5). The fact that crayfish in moult stage

Table 8.10: Effect of Feeding Level on the Fat Content of the Hepatopancreas of Experimental Crayfish: Extreme Values Removed.

Results for Individual Crayfish			
5 days/week Feeding		1 day/week Feeding	
Moult Stage	Fat Content (% dry Weight)	Moult Stage	Fat Content (% dry Weight)
D1	- 5.7 - EXTREME VALUES -	De	14.8
C	8.5	D1	-2.9
Ce	6.7	De	5.8
Ce	7.1	De	-2.8
De	5.2	De	-6.4
Ce	8.2	De	5.4
De	2.1	De	3.4
De	11.2		
Means	7.0		0.42

t-test for Means: $t = 2.932$, d.f. = 11, $0.025 > p > 0.01$.

Ce or C were restricted to the five-day feeding group may thus have obscured differences in the results for the two feeding groups, in addition to the possible effects of variation among crayfish at the beginning of the experiment.

While mean concentrations of protein in the gastric fluid of the experimental and wild crayfish were similar, the estimates of fat content of the hepatopancreas of the experimental crayfish were considerably below those of wild crayfish, with the exception of a single individual in the August 1977 sample. This difference was even more pronounced than indicated by the estimates, due to the effect of Bouin's fixative in increasing the estimates for the experimental crayfish. These low values of fat content for crayfish in the second experiment corresponded to the poor condition of crayfish at the end of the first experiment, indicating that in both experiments crayfish were under stress, and that the condition of the crayfish deteriorated with time in captivity, regardless of the type of food they were given. This conclusion is consistent with the decrease in food consumption through time and the small moult increments relative to wild crayfish for crayfish in both experiments.

Combining the conclusions for the deterioration in the condition of crayfish through time and the possible effects of previous feeding history and moult stage, it is obvious that several modifications to the experimental design are necessary if a causal relationship between feeding success and either fat content of the hepatopancreas or protein concentration of the gastric fluid are to be demonstrated. Given that both the rate of food consumption and the condition of crayfish deteriorated through time regardless or feeding level, and the condition of crayfish may have varied widely at the commencement of the experiment,

it is unlikely that feeding all crayfish *ad.lib.* for a period of time at the start of the experiment would overcome this initial variation. Demonstration of any effects of feeding level could only be achieved by using a much larger sample of crayfish for the determinations at the end of the experiment. Also, the moult stage of crayfish is a function of both time and success in obtaining sufficient nutrients for growth. To avoid confounding the effects of moult stage and feeding level, it would be necessary to run the experiment for a shorter period of time, commence the experiment with crayfish all in the same moult stage, and reject crayfish that moulted during the experiment from the final analysis. A suitable period of time would be within the initial period of captivity prior to any substantial decrease in feeding activity.

In terms of the techniques for estimating fat content, the use of the whole fresh lobes of the hepatopancreas on either side of the crayfish as replicates produced narrow confidence limits for the mean fat content of the hepatopancreas of each crayfish, when confidence limits were based on the replicates (= determinations) mean square from the analysis of variance (Table 8.8), and this technique would appear to be adequate. The small samples of hepatopancreas available for the experimental crayfish produced some negative estimates and were clearly inadequate, while fixing the hepatopancreas in Bouin's fixative resulted in a highly variable increase in estimated fat content (Table 8.7), and should be avoided in further work. In the case of protein concentration, significant differences existed between samples for individual crayfish in both sets of determinations (Tables 8.4 and 8.5), indicating that the measurement and dilution of the small sub-samples was inaccurate. More accurate dilutions could have been achieved by using larger sub-samples of gastric fluid. These were not

always obtainable, and in view of this, the small contribution of sample differences to the total variability in protein concentration may be accepted as unavoidable.

Armitage et.al. (1972) found that the lipid content of the hepatopancreas of the cambarid freshwater crayfish *Orconectes nais* varied in the region of 25-75% dry weight for wild crayfish, values similar to those obtained for wild *E. spinifer* in this study, and that lipid was the major nutrient reserve. Stewart et.al. (1967) found that the lipid content of the hepatopancreas of the American lobster *Homarus americanus* was greatly reduced by starvation, while Heath and Barnes (1970) described a similar occurrence for levels of neutral fat in the hepatopancreas of the crab *Carcinus maenas*. Hence there is some apparent generality among decapod crustaceans in the use of hepatopancreatic lipid as a nutrient reserve, and estimates of fat content for wild *E. spinifer* were similar, in terms of both absolute size and variability, to estimates obtained for another crayfish in which this process is known to occur. Dall (1974) found that hepatopancreas solids of the spiny lobster *Panulirus longipes* decreased during starvation, and in a later study of the same species found that the protein concentration of the gastric fluid decreased with decreased feeding, and was a reliable indicator of nutritional state. The results for *E. spinifer* were similar to the findings of Dall in so far as the fat content of the hepatopancreas was significantly positively correlated with the protein concentration of the gastric fluid, despite considerable errors in the former estimates, and the concentrations of protein in the gastric fluid of wild *E. spinifer* were similar, in terms of both absolute size and extent of variability, to the estimates Dall obtained for wild *Panulirus longipes*.

Heath and Barnes (1970) also noted that the neutral fat content of the hepatopancreas of *Carcinus maenas* increased between moult stages that were equivalent to stages Ce and De in the present study, while Dall (1975) found similar changes in the protein concentration of the gastric fluid of *Panulirus longipes*. Similar results were obtained for *E. spinifer* for fat content, and although the significance of the increase in protein concentration from moult stage Ce to moult stage De could not be demonstrated for *E. spinifer*, the presence of such an increase suggested that there may have been a pattern similar to that described by Dall (1975) underlying the wide variation among individual crayfish. The results obtained for the fat content of the hepatopancreas and the protein concentration of the gastric fluid of *E. spinifer* thus agreed in many respects with results obtained for decapods in which these two aspects of physiological state were found to be related to food consumption. Also, it was found that there was considerable variation in the protein concentration of gastric fluid and fat content of the hepatopancreas among wild *E. spinifer*, independent of effects of moult stage. Hence there is some evidence that there was considerable variation in the feeding success of wild *E. spinifer*.

This evidence is not conclusive, since a relationship between feeding success and the two aspects of physiological state could not be established. On the other hand, the evidence is not contradicted, since the inability to establish such a relationship may have been largely due to variation in the feeding success of crayfish prior to the commencement of experimentation.

It was found that the moult increments of crayfish in both experiments were substantially below those of wild crayfish. In the

case of the first experiment, crayfish moulted even when they were incapable of completing the moult successfully, indicating that the initation of moulting was not entirely dependent on a state of physiological well-being. These crayfish were in obviously poor condition, and there was some evidence to suggest that this may have had a nutritional basis. In the second experiment, depressed moult increments were associated with low fat content of the hepatopancreas. These results suggest that size-independent variation in moult increments in wild crayfish (Chapter 6) may have been related to variation in feeding success. Similarly, the difference in the fat content of the hepatopancreases of wild crayfish captured in August 1979 and January 1980 may indicate seasonal variations in feeding success corresponding to observed seasonal variation in the average size of moult increments of crayfish in different locations (Chapter 6).

Further consideration of the response of *E. spinifer* to captivity is required. In contrast to the experiments described in this chapter, juvenile crayfish that were raised in captivity for growth studies (Chapter 7), appeared to grow normally over the period of one year for which they were maintained, successfully undergoing several moults during this time. The conditions under which these crayfish were maintained differed from those in the experiments in two respects. The juvenile crayfish were disturbed only a few times during the year, and an actively decomposing bed of detritus from diverse sources was maintained in the tank. This may indicate that *E. spinifer* is metabolically sensitive to repeated disturbance, or that some component of a complex, intact detrital system is necessary for adequate maintenance of physiological condition.

CHAPTER NINE

CONCLUSIONS TO PART A: GENERAL DISCUSSION OF THE BIOLOGY OF E. SPINIFER

Specific aspects of the biology of *E. spinifer* have already been discussed in the relevant chapters. The aim of the present discussion is to draw together some of the major conclusions, providing an overall view of the life history of *E. spinifer*.

The reproductive mechanisms of *E. spinifer* follow taxonomic lines in that eggs and juvenile stages were of typical parastacid form, as were the means by which they attach to the pleopods of females. Spermatophores were of typical macruran form, and were extremely similar to those in the only other detailed description of a parastacid spermatophore (*Cherax destructor;* Johnson, 1979). The annual reproductive cycle also corresponded to patterns of reproductive activity observed among other parastacids (Chapter 3). Growth rates were similar to those of several other macrurans attaining a similar size under natural conditions (Chapter 7), although patterns of growth departed from general models in terms of changes with increasing crayfish size in both moult frequency (Chapter 5) and moult increment (Chapter 6).

The major differences between the results of *E. spinifer* and other macrurans were in terms of size and age at maturity (Chapter 3 and 7), and of these differences, the occurrence of precocious males was striking. The presence of precocious males in other populations of *E. spinifer* has not been established, since in spite of efforts, few small *E. spinifer* have been captured in other areas (Chapter 4).

The same has applied to collections of the similarly large and spiny *E. valentulus* and *E. hystricosus*, and there are no references to precocious males in the literature on *Euastacus*. Hence the occurrence of precocious males in populations of large *Euastacus* outside of *E. spinifer* in the study area can currently only be speculated upon.

The major aspects of the life history of *E. spinifer* relating to growth, maturity, and relative abundance of the three reproductive types are summarised in Table 9.1. The attainment of maturity in females was both delayed and occurred at a larger size in comparison with normal males. It is possible that this difference may be accounted for by the greater cost of reproduction to females in terms of reduced growth rate (Chapters 6 and 7), assuming that females must be large to survive and reproduce successfully. Nevertheless, both females and normal males mature relatively late and at a relatively large size (Chapter 7), and both are probably capable of reproducing annually for a considerable number of years after the attainment of maturity. In contrast, precocious males matured much earlier, and at a much smaller size. If the decrease in the abundance of precocious males at carapace lengths in the range 25-30 mm was due to mortality (Chapter 4), then they were probably restricted to only one or two seasons of mating. If, on the other hand, the observed size distributions of precocious males resulted from their recent inclusion in the population (Chapter 4), then precocious males may subsequently have attained a larger size than observed, with the result that mating in many more seasons may have been possible. This issue could only be clarified by further research along the lines indicated in Chapter 4.

Some information is available on the environmental context of the life history of *E. spinifer*. Davis (1936) suggested that the swamps

Table 9.1. Summary of the Major Aspects of the Life History of *E. spinifer* for Females, Normal Males and Precocious Males.

Life History Trait	Female	Normal Male	Precocious Male
Weight at attainment of maturity (g).	160-400	50-80	1 - 5
Carapace Length at attainment of maturity (mm)	70-95	45-55	12 -20
Attainable Carapace Length (mm).	100 +	100 +	40?
*Age at attainment of maturity (years).	8 +	5 +	1 - 2
Repeated annual Breeding	Yes	Probably	?
*^{1}% of population.	3	6	9

* based on the average annual growth rate (Chapter 7).

^{1}* for Pool 3 during the mark-recapture study (Chapter 4).

that feed the Loddon River and surround the study area had been stable over a long period of time. Also, the hydrologic environment of *E. spinifer* in the Loddon River was probably fairly stable on an annual basis (Chapter 1), and this may have been reflected in the constancy of annual average growth rates over a period of at least eight years up to and including 1978 (Chapter 7). In the short-term, growth rates of individual crayfish varied considerably both between seasons in different locations (Pools 3 and 7) and within any given season for a particular location (Chapter 5). This suggests that the environment of *E. spinifer* was predictable in the long-term, but showed considerable short-term variability both temporally and spatially.

It is unlikely that either type of variability resulted from the removal of large crayfish from Pool 7 (see Chapter 2), for two reasons. Removals were conducted regularly over the period of study, whereas differences between the growth rates of crayfish in Pools 3 and 7 were not consistent through time. Also, the removals did not result in any substantial reduction in catches of large crayfish from Pool 7 during the growth studies (Chapter 4, Fig. 4.5). On the other hand, food availability was suggested as an environmental factor that fitted the pattern of variability described for growth rates (Chapter 5), and the considerable variation among wild crayfish in both the protein concentration of the gastric fluid and the fat content of the hepatopancreas provided some circumstantial evidence linking variation in growth rates and feeding success (Chapter 8).

The types of life history traits exhibited by female and normal male *E. spinifer* on the one hand and by precocious male *E. spinifer* on the other have frequently been interpreted as belonging to separate strategies designed to cope with different sets of environmental

conditions (Stearns, 1976). So-called "r- and K-selection" may be considered as an example (after Stearns, 1976). K-selection is aimed at maximising ability to compete and avoid predation, tending to maintain the size of the population at the carrying capacity (K) of the habitat, in constant and/or predictable environments, and its associated life-history traits include delayed reproduction, large size at maturity, brood care, and potentially long life. Based on the available results, K-selection seems applicable to *E. spinifer* in terms of both life history traits and long-term (i.e. greater than one year) environmental constancy, if precocious males are disregarded. In contrast, r-selection favours increased reproductive output in fluctuating environments, tending to maximise the ability of the population to increase rapidly in size (r), and its associated life history traits include early reproduction and small size at maturity. Thus r-selection fits the available results for precocious males. It is difficult to see how explanations that are based on a dichtomy of the environmental contexts for sets of life history traits can account for the occurrence of two sets of traits in the one population. It is possible that precocious males were an adaptation to short-term variability in the environment, although it is difficult to see how. Females only matured at a considerable age, hence the number of potential mates for the precocious males could not have responded to short-term environmental variations. It seems that more likely explanations may be provided by sexual selection, i.e., selection acting specifically on the means of procuring a mate.

Gadgil (1972) suggested that a dimorphism of structures used by males in competing for females, e.g. the antlers of deer, cephalic horns of some beetles, could be selected for in a population. He reasoned that development of such devices proceeds until the cost, in terms of

survival prior to mating, of investing in the device renders individuals with and without the device equally fit in terms of ability to survive and procure a mate. An analogous situation may apply to *E. spinifer* in that, in terms of numbers of successful matings, the relatively high numbers of precocious males resulting from earlier maturity may balance the possibility for normal males of a superior ability to compete for mates and a larger number of years of maturity during which mating can occur. That is, the cost that is balanced between the two male forms of *E. spinifer* derives from different investments in size rather than in devices for competition.

A further possibility is provided by Ghiselin (1974, pp. 126-7), who suggested that random mortality in a small, local population might regularly result in a scarcity of large males, and that in such instances a small mature male could gain a considerable advantage, even though selection might normally favour large size in males. This argument was used by Ghiselin in a different context. Nevertheless, it might also apply to the occurrence of precocious males in *E. spinifer*, as large individuals are relatively sparsely distributed, and the streams inhabited by the species throughout much of its range consist of small pools connected by long, shallow riffles which are frequently reduced to a trickle, and are not normally inhabited by large crayfish.

These explanations are not intended to be exhaustive, and others are probably available. Critical examination of such explanations would require considerable information in addition to that presented in this thesis, as discussed in the chapters on reproduction, population structure, and feeding. Also, the responses of *E. spinifer* to its environment have not been considered quantitatively and in detail, nor have variations in

environmental parameters that may have affected the results of this study. Such information would be essential to a more comprehensive interpretation.

PART B

A METHOD FOR INTERPRETING DECAPOD GROWTH FROM MARK-RECAPTURE DATA.

SECTION 1

INTRODUCTION AND REVIEW

In field studies, data on the growth rates of the larger decapod crustaceans are normally collected by following the increases in size of marked or tagged individuals. This is generally the only method available, since age determination using growth marks is not possible, and the identification of year classes as modes in the size-frequency distributions of catches can, in general, be applied to only the first few years of growth (e.g., Shipway, 1951; Hopkins, 1967a; Momot, 1967; Farmer, 1973; Morrissy, 1975a; Chittleborough, 1976a).

Methods for treating crustacean growth data, once they have been collected, have been extensively developed by Kurata (1962), with more recent reviews by Mauchline (1976, 1977). Since measured growth is essentially incremental, these methods have been based on the use of growth at moulting (moult increment) and moult frequency as joint descriptors of growth rate. There has, however, been little attempt to develop a general method for extracting information on moult increment and moult frequency from mark-recapture data. This task was considered to be impossible for the extensive and variable growth data collected for *E. spinifer* unless a systematic routine could be developed. A number of authors have provided partial solutions to the problem, as described below. The present work is aimed at combining some of these solutions with new material to form a systematic routine of general application.

In order to tackle the problem adequately, the nature of the data provided by mark-recapture studies must be clearly defined. Mark-recapture data for growth consists of differences between measurements of the size of individual animals taken on consecutive capture occasions. Also, measured growth is incremental, occurring only at moulting. Consequently, between any two capture occasions, an individual may not moult, and any differences in measured size will be due to measurement error. Alternatively, an individual may moult once between any two capture occasions, and increase in size by a given amount. Such an increase in size will be termed a simple moult increment in the ensuing discussion. The third alternative is that an individual may moult more than once between any two captures, and increase in size by some multiple of the increment for a single moult. These increases in size will be termed compound moult increments. The result of this is that data obtained for a group of individuals over a particular period of time will consist of either measurement errors, of simple, or compound moult increments, or of a combination of the three.

These three types of data may superficially be distinguished by either of two methods. If the number of moults undergone by each individual could be determined for the period between captures, then simple moult increments could be identifieid, or estimated from compound moult increments. In a study of *Nephrops norvegicus*, Farmer (1973) tested a method initially designed for determining the instars of species of Amphipoda and Isopoda. This method was not successful for *N. norvegicus*, and not other methods for non-larval decapods have been found in the literature. An exception to this is the special case of distinguishing between individuals that have moulted and those that have not. This is dealt with in depth in Section 3.

The alternative approach is to identify simple and compound moult increments and measurement errors using the size of the increment as the distinguishing criterion. This is the approach taken in numerous studies of commercially harvested decapods, and it is the approach taken in this study.

In studies of *Homarus americanus* (Wilder, 1953), *Jasus lalandii* (Newman and Pollock, 1974), *Panulirus longipes cygnus* (Chittleborough, 1976a),and *Panulirus elephas* (Hepper, 1977), animals have been marked and released prior to a known season of moulting. Recaptures have then been made during or shortly after that period, and any changes in size taken to be simple moult increments. This method requires that the moulting of all individuals in the population is highly synchronised, and infrequent relative to recapture rate. These requirements are probably fulfilled adequately in many studies in which this method is used, since the animals under consideration are either mature or approaching maturity, with the result that moulting may be infrequent. Nevertheless, studies of *Homarus vulgaris* (Simpson, 1961; Hepper, 1972) and *Homarus americanus* (Wilder, 1963) were conducted in the same manner, but reached the conclusion that both simple and compound moult increments had been collected. In the latter study, Wilder estimated that 86.4% of the 110 lobsters captured during the second half of the growing season had moulted twice. Mixtures of simple and compound moult increments have also been obtained for studies in which recaptures have been taken for a period extending over several growing seasons. Results of this type have been obtained for *Cancer pagurus* (Hancock and Edwards, 1967; Bennett, 1974), *Homarus vulgaris* (Hepper, 1967), *Homarus americanus* (Ennis, 1972), and *Jasus tristani* (Pollock and Roscoe, 1977). Further, if the intermoult period is variable and/or is, on average, similar to the period of

time between captures, then the data obtained for a group of individuals will almost certainly consist of a variable mixture of simple and compound moult increments and measurement errors. Clearly, if a method of interpreting mark-recapture data is to be both reliable and of general usefulness, then it must function to distinguish between these three classes of events, regardless of the period over which the data have been collected.

It has been concluded in several studies that isolated increments were compound on the grounds that they were separated from the main distribution of increments, and were of a considerably larger size (Simpson, 1961; Hancock and Edwards, 1967; Ennis, 1972; Hepper, 1972). In such instances the numbers of suspected compound increments were small. Presumably, similar grounds were used in other studies in which no method was given (Bennett, 1974; Pollock and Roscoe, 1977). Wilder (1963) released marked lobsters belonging to a series of narrow size-classes, then plotted the size-frequency distributions separately for the recaptured lobsters from each of the initial size-classes. In each instance this resulted in a bimodal frequency distribution, the first mode being attributed to simple moult increments, the second to compound moult increments. Maximum growth per moult was determined from distributions in which the modal groups were completely separate, and then used to split distributions in which the modal groups overlapped. Hepper (1967), taking a different approach, separated size-frequency distributions of growth increments into normally distributed components attributed to 1, 2 or more moults, using the probability paper method of Harding (1949) and Cassie (1954).

All of the methods outlined above assume that the distribution

of simple moult increments is unimodal, symmetrical, and continuous. It has been found that some individuals of the spiny lobsters *Panulirus interruptus* (Lindberg, 1955, cited in Mauchline, 1977), *Panulirus elephas* (Ansell and Robb, 1977; Hepper, 1977), and *Jasus tristani* (Pollock and Roscoe, 1977) undergo moult increments that are indistinguishable from measurement error on the basis of size alone. Also, given that the size of a simple moult increment may be affected by environmental factors, it is quite possible that different parts of a population may be subject to different conditions, with the result that the size-frequency distribution of simple moult increments for the whole population is polymodal, or at least non-normal. Further complications will arise if the variability in size of the simple moult increment is large with respect to the mean value, resulting in considerable overlap between the distributions of simple and compound moult increments. If any of these situations were to arise and remain undetected, then serious inaccuracies in estimates of growth rates might result.

An attempt has been made in the following sections to present a set of techniques that will allow reliable interpretation of growth from mark-recapture data. The method of Hepper (1967) has been used as the basis for generating hypotheses concerning the nature of the data. Methods for evaluating these hypotheses are presented, and all steps of the procedure have been applied to the mark-recapture data for *E. spinifer*.

SECTION 2

SEPARATION OF DATA INTO COMPONENTS

2.1 METHODS

Differences between the measurements of carapace length taken on each pair of successive captures were calculated for each crayfish by subtracting, in each instance, the earlier measurement from the later. These differences were allocated to a series of categories according to the initial carapace length, capture location, and sex of each crayfish, and, in the case of males, state of maturity. Keys for the identification of these categories are provided prior to the presentation of the relevant data in Figures 2.1-7 and Appendix A, and in Table 4.4. A frequency distribution of measurement differences was then constructed for each category, and plotted on arithmetic probability paper according to the method of Harding (1949). In most instances, points of inflexion on these curves were obvious, e.g., Figures 2.1-3, 5-7, and Figues 1, 3-4, 6-9, 12-22 in Appendix A. In some instances, e.g., Figure 2.4 and Figures 2, 5, 10, 11 in Appendix A, a number of points could be chosen in the general vicinity of the inflexion. Where this occurred, the percent cumulative frequencies for each of the points in the component below the inflexion were converted to probits. Separate linear regressions of probit on size of measurement difference were then calculated for values in the component up to, and including, each of the possible points of inflexion. The point resulting in the smallest standard deviation about the linear regression was then used as the point of inflexion (Underwood, 1975).

The extreme values of each component were marked with arrows, and, if subject to doubt, with an asterisk. If the method of regression fitting was used, arrows were also marked with a circle.

Of the twenty-nine graphs produced in this manner, seven were selected to represent the range of variation in the form of the distributions. These seven graphs are illustrated in Figures 2.1-7. Components of the seven distributions were extracted using the method of Cassie (1954). The new values of these extracted components were represented by open circles on Figures 2.1-7, while the values recorded originally are represented by solid circles. A detailed examination of this representative selection was then used to develop the ideas presented in this, and the following two sections. The remaining graphs were placed in Appendix A, to be used as a source of data in sections 5 and 6.

2.2 RESULTS

A representative selection of graphs of percent cumulative frequency against size of measurement difference is given in Figures 2.1-7.

In each instance there is a component centred about zero, labelled 'A', separated by both a distinct inflexion and a marked discontinuity in the recorded distribution of measurement differences from a second component, labelled 'B', which is displaced a variable but substantial distance above zero. In Figures 2.2-5 a third component, labelled 'C', is present, displaced a variable distance above

FIGURES 2.1-7: Graphs of Percent Cumulative Frequency vs. Measurement Difference.

Key to Graphs

Graph	Pool*	Sex	C.L.	Graph	Pool	Sex	C.L.
2.1	3	F	35-40	2.5	7	PM	20-25
2.2	7	F	20-25	2.6	7	F	35-40
2.3	3	F	25-30	2.7	7	M	50-70
2.4	7	F	40-50				

Pool*: Capture location.

Sex: F = female, M = male, PM = precocious male.

C.L.: Range of carapace lengths (mm).

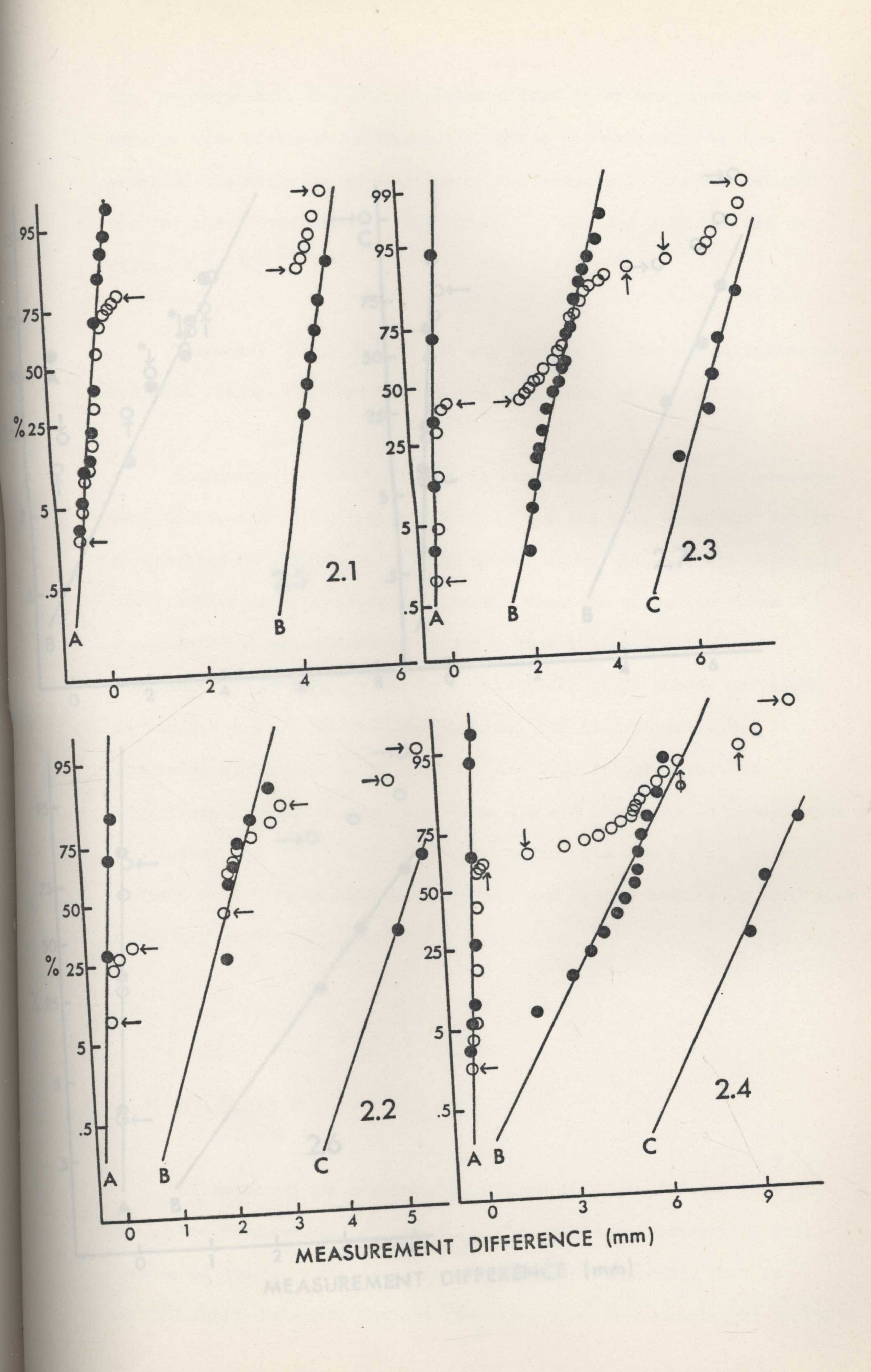
SYMBOLS:

○ = Original values; ● = Extracted values.

↑ = Extreme value of component; inflexion determined by eye.

↑ = Extreme value of component; inflexion determined by method of regression fitting.

*↑ = Extreme value of component, inflexion uncertain.


95
75
50
% 25
5
.5
A
B
0
2
4
6
2.1
99
95
75
50
25
5
.5
A
B
C
0
2
4
6
2.3
95
75
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% 25
5
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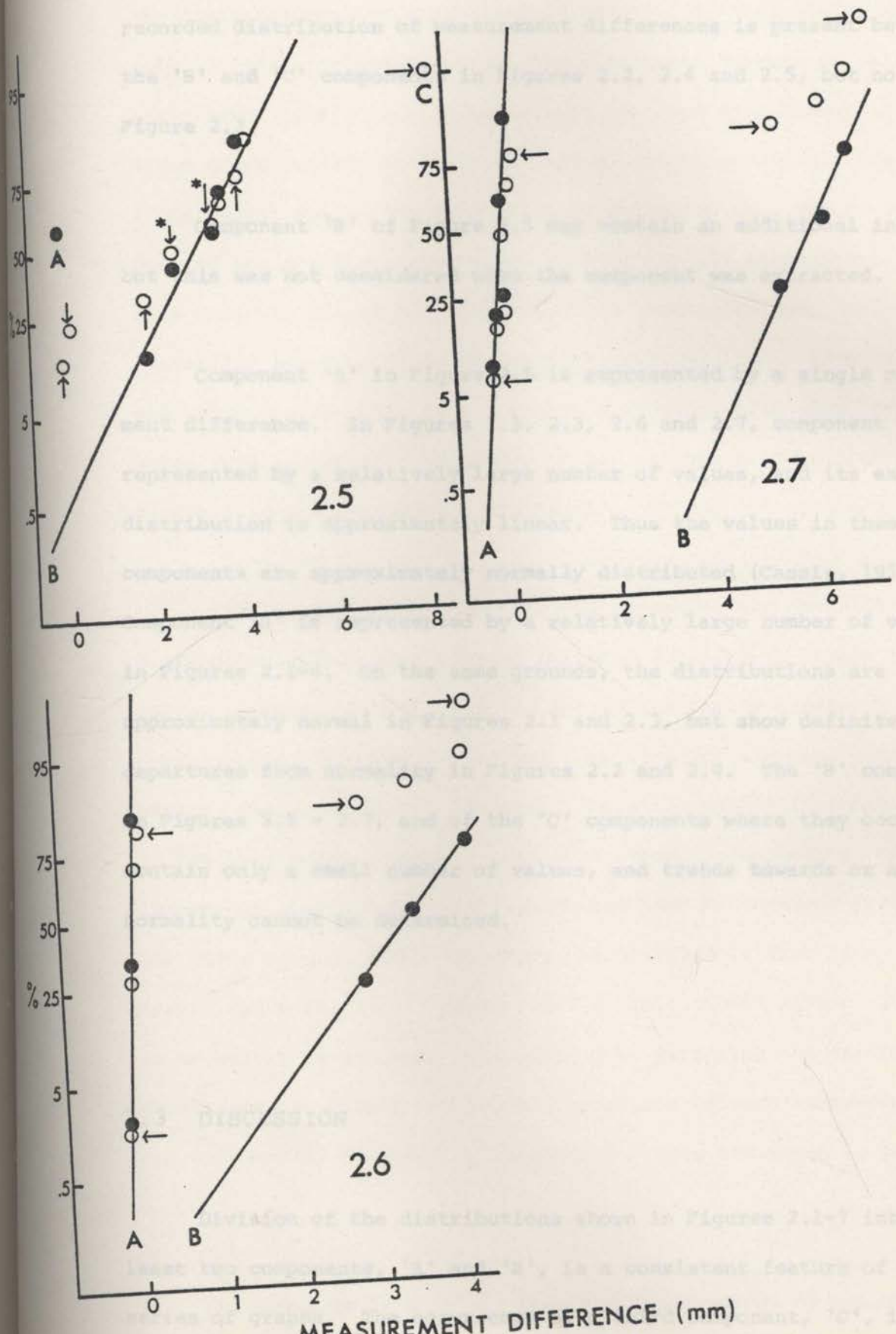
A
B
C
0
1
2
3
4
5
2.2
95
75
50
25
5
.5
A
B
C
0
3
6
9
2.4
MEASUREMENT DIFFERENCE (mm)


95
75
50
% 25
5
.5
A
B
2.5
0
2
4
6
8
C
75
50
25
5
.5
A
0
2
4
6
2.7
B
95
75
50
% 25
5
.5
A
B
2.6
0
1
2
3
4
MEASUREMENT DIFFERENCE (mm)

the 'B' component, and distinguishable from it by the presence of a more or less distinct inflexion. A marked discontinuity in the recorded distribution of measurement differences is present between the 'B' and 'C' components in Figures 2.2, 2.4 and 2.5, but not in Figure 2.3.

Component 'B' of Figure 2.5 may contain an additional inflexion, but this was not considered when the component was extracted.

Component 'A' in Figure 2.5 is represented by a single measurement difference. In Figures 2.1, 2.3, 2.6 and 2.7, component 'A' is represented by a relatively large number of values, and its extracted distribution is approximately linear. Thus the values in these components are approximately normally distributed (Cassie, 1954). Component 'B' is represented by a relatively large number of values in Figures 2.1-4. On the same grounds, the distributions are approximately normal in Figures 2.1 and 2.3, but show definite departures from normality in Figures 2.2 and 2.4. The 'B' components in Figures 2.5 - 2.7, and of the 'C' components where they occur, contain only a small number of values, and trends towards or away from normality cannot be determined.

2.3 DISCUSSION

Division of the distributions shown in Figures 2.1-7 into at least two components, 'A' and 'B', is a consistent feature of this series of graphs. The occurrence of a third component, 'C', is sufficiently common to warrant its inclusion as a consistent feature.

The subdivision of a frequency distribution using the method of Harding (1949) and Cassie (1954) assumes that the components of the distributions are approximately normal. In this study, component 'A' is approximately normal where adequate numbers of values are present, while this is not always the case with component 'B'. Since non-normality of distributions of growth increments may have a biological basis, the imposition of normality on a set of growth data is not a satisfactory means of defining its composition. In this context, the method of Harding and Cassie has been used as a systematic means of dividing the distributions into characteristic sub-units, rather than as a means of accurately splitting them into normally-distributed components. Other methods must be used for determining the composition of the 'A', 'B' and 'C' components.

The values in component 'A' are approximately normally distributed with a mean of zero (Figures 2.1-4, 6-7), while the values in components 'B' and 'C' are substantially greater. The most probable explanation is that component 'A' contains measurement errors, while components 'B' and 'C' contain growth increments. Since it has been found, however, that some decapods may undergo moult increments that are not distinguishable from measurement erros on the basis of size alone (Lindberg, 1955; cited in Mauchline, 1977; Ansell and Robb, 1977; Hepper, 1977; Pollock and Roscoe, 1977), it is necessary to use some other method to determine the partitioning of measurement errors and growth increments between component 'A' and components 'B' and 'C'. Methods for this are given in Section 3.

Although it is unlikely that the large values of measurement difference in components 'B' and 'C' could be due to anything other

than growth, one qualification must be made. If it is found that component 'A' contains measurement errors, i.e., that such errors are detectable with the scale of measurement that has been used, then each of the values in components 'B' and 'C' will have resulted partially from growth, and partially from measurement error. This qualification is implicit in all further discussion and interpretation of growth.

Three interpretations of the composition of component 'B' are realistic. Component 'B' may contain only simple moult increments, or only compound moult increments, i.e., it may be described as 'homogeneous', or it may contain a mixture of the two, in which case it may be described as 'heterogeneous'. Some information on the likelihood of each of these three alternatives may be deduced from the distributions of values in components 'A' and 'B'. This information is summarised below, in the form of a series of criteria.

Criterion 1: If component A contains a number of measurement errors, then it is unlikely that component B contains only compound moult increments.

This follows, since if measurement errors are present, then capture frequency must have been greater than moult frequency at certain times for at least some individuals, and simple moult increments would almost certainly have been recorded if compound increments were. It also follows that if component 'A' is absent, then the arrangement of components provides no evidence to imply that simple moult increments were available for measurement.

Criterion 2: If component A is absent, then the hypothesis that component B contains only compound moult increments cannot be rejected unless evidence to the contrary can be obtained from an independent source.

It is difficult to envisage the type of evidence required to negate the conclusion of Criterion 2. It might be concluded that component 'B' contains simple moult increments since the values are of similar size to those obtained for other groups of individuals, or in other studies. Such a decision would, however, render the data virtually useless, since it would invalidate comparisons with simple moult increments from these other sources. It appears that the only solution is to design sampling so that the requirements of Criterion 1 are fulfilled.

The likelihood that component 'B' is heterogeneous can be deduced, to some extent, from the range of values in component 'B'.

Criterion 3: If the range of values in component B is such that the largest value is less than twice the size of the smallest value, then it is unlikely that the component is heterogeneous.

This follows, since the size of the smallest compound moult increment would be expected to equal twice the size of the smallest simple moult increment. The converse, however, may also hold.

Criterion 4: If the largest value in component B is at least twice the size of the smallest

value, then the hypothesis that component B is heterogeneous cannot be rejected without additional information.

In Section 4, methods are presented for obtaining the information required to negate the conclusion of Criterion 4. The conclusions of Criteria 3 and 4 are approximations only, since the smallest recorded value in component 'B' may, as a result of sampling, be considerably larger than the smallest simple moult increments undergone by individuals in the population. For this reason, the criteria were used in conjunction with the methods of Section 4, as described in that section.

Hypotheses describing the contents of component 'C' are relevant only if it has been concluded that component 'B' contains simple moult increments. If this is the case, then the hypothesis that component 'C' contains simple moult increments may be tested against the alternative that it contains compound moult increments, as described in Section 5. The likelihood that component 'C' is heterogeneous may, to some extent, be deduced from the results of this test.

SECTION 3

PARTITIONING OF MEASUREMENT ERRORS AND GROWTH INCREMENTS BETWEEN COMPONENTS A AND B

3.1 METHODS

Several methods are available for determining whether or not an individual has moulted during the period between captures. Simpson (1961) identified *Homarus vulgaris* by both punching the tail fan and applying tags that were lost at moulting. If individuals with punched tail fans were recaptured without tags, it was concluded that moulting had occurred. Pollock and Roscoe (1977) used tags for *Jasus tristani* that were retained during moulting. It was found that these tags caused the exoskeleton to be deformed when soft immediately after a moult, and that this deformity became permanent as the exoskeleton hardened, thus allowing identification of moulted individuals.

It was also observed during this study that the partially healed marks of moulted *E. spinifer* were quite different in appearance from the marks of unmoulted individuals. If records of this had been kept for each capture, then distinguishing between increments for moulted and unmoulted crayfish would have been straightforward. Unfortunately, the need for this procedure was not realised until sampling had neared completion. An indirect method was subsequently adopted, as described below.

Capture records of all crayfish were examined for instances where, on two successive capture occasions, a crayfish was found to

be missing a claw, or to have it present only as a limb-bud, thus permitting the inference that the claw had not successfully regenerated during the period between captures. The difference between the measurements of carapace length for each such pair of occasions was then calculated.

The same procedure was repeated for instances in which a lost claw had regenerated, and for instances in which a lost walking leg had not regenerated.

A single frequency distribution was then constructed for all measurement differences obtained in this manner, and plotted on probability paper according to the method of Harding (1949) (Figure 3.1). Values of measurement difference for regeneration were distinguished from those for no regeneration by the use of different symbols. The resulting distribution of values was then compared to the distributions obtained in the previous section.

3.2 RESULTS

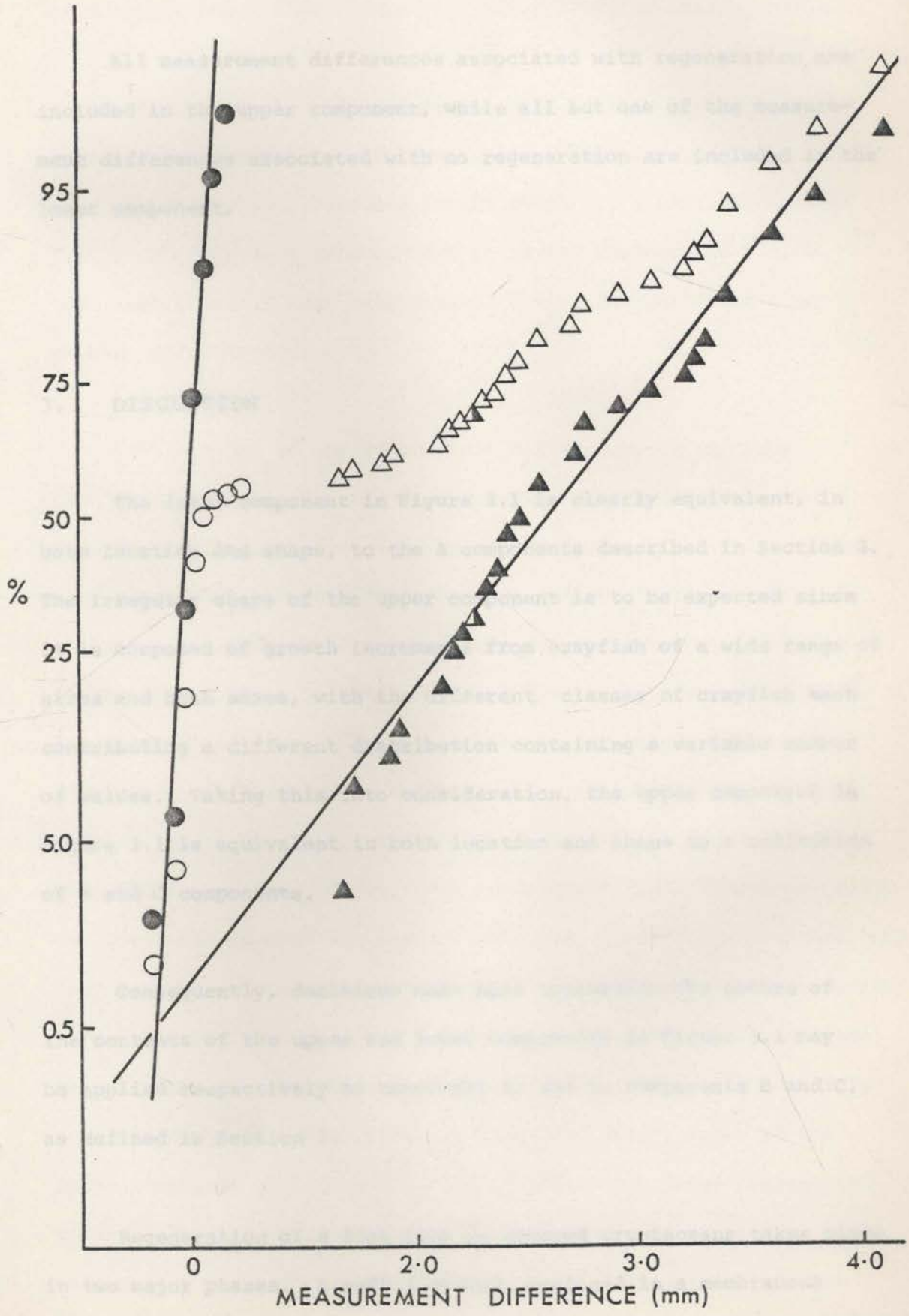
Eighty four measurement differences were collected and graphed. Two components are present in the resulting distribution (Figure 3.1). The lower component is centred about zero measurement difference, with a small variance, and includes forty seven of the observations. The remaining values are included in the upper component, which is displaced a substantial distance above zero, and is separated from the lower component by a distinct vertical inflexion and a marked discontinuity in the recorded distribution of measurement differences.

Figure 3.1: Percent Cumulative Frequency vs Measurement Difference for Injured Crayfish.

● ○ = Extracted and original values for crayfish showing no regeneration.

▲ △ = Extracted and original values for crayfish showing regeneration.

◭ = One crayfish regenerated, one crayfish not regenerated.


95
75
50
%
25
5.0
0.5
0
2·0
3·0
4·0
MEASUREMENT DIFFERENCE (mm)

The variance of the upper component is much greater than that of the lower, and it is much more irregular in shape.

All measurement differences associated with regeneration are included in the upper component, while all but one of the measurement differences associated with no regeneration are included in the lower component.

3.3 DISCUSSION

The lower component in Figure 3.1 is clearly equivalent, in both location and shape, to the A components described in Section 2. The irregular shape of the upper component is to be expected since it is composed of growth increments from crayfish of a wide range of sizes and both sexes, with the different classes of crayfish each contributing a different distribution containing a variable number of values. Taking this into consideration, the upper component in Figure 3.1 is equivalent in both location and shape to a collection of B and C components.

Consequently, decisions made here concerning the nature of the contents of the upper and lower components in Figure 3.1 may be applied respectively to component A, and to components B and C, as defined in Section 2.

Regeneration of a lost limb in decapod crustaceans takes place in two major phases. A soft limb-bud, enclosed in a membranous sheath, is produced at the site of loss during the intermoult period.

During the next moult the membranous sheath is lost, the limb-bud enlarges, and its exoskeleton hardens. Hardening of the exoskeleton of a regenerating limb occurs only after moulting (Bliss, 1960).

The definition of regeneration used in this analysis refers only to the second phase of regeneration, that is, the formation of a new limb with a hardened exoskeleton. If a particular crayfish is described in this context as having regenerated a limb, then that crayfish must have moulted during the period of time between measurements.

All but one of the measurement differences in the upper component of Figure 3.1 are from crayfish that had regenerated a limb during the period of time between measurements. This implies that these measurement differences are changes during moulting in the size of crayfish; i.e., they are growth increments. There was one observation in this component from a crayfish which showed no regeneration. This may have arisen in two ways. The crayfish from which the measurement difference was calculated may not have moulted during the period between measurements, in which case the observation would be classed as a measurement error. Alternatively, the crayfish may have moulted, regenerated the limb, and subsequently lost it again before the second capture, in which case the measurement difference would be classed as a growth increment. A choice cannot be made here between these two alternatives. However, since it is possible for only one observation among the thirty seven in the upper component of Figure 3.1 to be classed as a large measurement error, it may be concluded that the potential contribution of large measurement errors to components B and C is very small, and its effect on the estimate of mean growth increment in component B would

be negligible.

The lower component in Figure 3.1 contains only measurement differences from crayfish that have shown no regeneration of a lost appendage during the period between measurements. If a number of the crayfish represented in the lower component have moulted without increasing significantly in size, then in every case these idividuals must have lost a regenerated limb before the second measurement was taken. However, of the thirty seven crayfish represented in the upper component, there is only one possible instance of such an occurrence. It is therefore highly unlikely that a regenerated limb would be lost before recapture in every case where a crayfish had moulted without increasing significantly in size.

The conclusions drawn for *E. spinifer* from this analysis are that it is unlikely that component A contains anything apart from measurement errors, and that components B and C almost certainly contain only growth increments.

SECTION 4

EVALUATION OF THE COMPOSITION OF COMPONENT B

PART 4A: DEVELOPMENT OF THE METHOD

4A.1 METHODS

A simple moult increment may be measured after any interval of time that is greater than the period for which an individual is not catchable during moulting, and is less than the period between consecutive moults. This implies that if a component contains only simple moult increments, i.e., it is homogeneous, then the sizes of growth increments and the intervals over which they have been measured must be independent, that is, no correlation may exist between the two.

The minimum interval over which compound moult increments can be measured will obviously be greater than the period of time between two consecutive moults. If moult frequency is constant both within and between individual crayfish, then the interval over which a compound moult increment has been measured will always be greater than that for a simple moult increment. If moult frequency is variable, then some overlap may occur between the two. In either case, if a component contains both simple and compound moult increments, i.e., it is heterogeneous, then there may be a positive correlation between the sizes of growth increments and the intervals of time over which they have been measured.

The sensitivity of a correlation coefficient to the presence of compound moult increments in samples consisting mainly of simple moult increments will depend upon both the proportion of the sample that consists of compound moult increments, i.e., the level of heterogeneity, and the degree to which the intervals between measurements for the compound moult increments overlap those for the simple moult increments. These relationships were investigated by calculating the correlation coefficients for samples of known composition. The method of obtaining these samples is described below, followed by a description of the methods used to calculate the correlation coefficients.

Means and standard deviations were calculated for the values in the B components of six of the classes of crayfish examined in Section 2. Normally distributed samples of two hundred simple moult increments were then simulated for each class using tables of random normal variates (Dixon and Massey, 1969; Table A-24, p.552-560). Normally distributed samples of compound moult increments were simulated for each class by combining pairs of randomly selected simple moult increments. Intervals between measurements of 1, 2, 3 or 4 months were allocated randomly to the simple moult increments using a table of random numbers. These intervals were selected as being representative of the bulk of the values recorded for the six classes of crayfish. Intervals from the ranges 2-8 months, 3-8 months, 4-8 months and 5-8 months were allocated randomly to compound moult increments, to provide respective levels of 75%, 50%, 25% and zero overlap with the intervals for simple moult increments.

Samples of twenty simulated growth increments with levels of heterogeneity of 5%, 10%, 20% and 40% were then constructed for each

of the six classes of crayfish using the above increments, by including respectively 1, 2, 4 or 8 compound moult increments in each sample, together with the complementary number of simple moult increments. Compound moult increments were allocated to these samples so as to provide samples at all possible combinations of levels of overlap and heterogeneity for each of the six classes of crayfish. A single sample containing twenty simple moult increments, i.e., with 0% heterogeneity, was also constructed for each class.

Samples of twenty simulated growth increments were also constructued to approximate three non-normal distributions, for the same six classes of crayfish. The locations of these distributions were fixed by the maximum and minimum values of measurement difference recorded for the B component of each of the six classes, and the distributions from which the samples were drawn consisted of all values at 0.1mm intervals between these two extremes. Uniformly distributed samples of simple growth increments were drawn by taking random samples of these values, while skewed samples were taken as follows. Two samples of one hundred of the values were constructed for each class of crayfish such that the frequency of occurrence of each value in the sample either increased or decreased linearly with the size of the value, providing sets of values with negative and positive skew respectively. Skewed samples of simple moult increments were then drawn randomly from these sets of values. Compound moult increments were again simulated by combining pairs of randomly selected simple moult increments, and the samples of twenty increments were fixed at 5% heterogeneity and 75% interval overlap using the methods described for normally distributed samples.

Correlation coefficients were calculated for each sample of

twenty growth increments in the simulation, and tested for significant departure from zero. Results of these tests are summarised in Tables 4.2 and 4.3. The hypothesis that all correlation coefficients were from the same population was tested for the six classes of crayfish at each combination of levels of heterogeneity and overlap, for each type of distribution. The results of these tests are given in Table 4.1. The corrected average correlation coefficient was then calculated for each group of six classes, and tested for significant departure from zero by conversion to a standard normal deviate (Snedecor and Cochran, 1967, pp.184-187). The results of these calculations are also included in Tables 4.2 and 4.3.

4A.2 RESULTS

The hypothesis that all sample correlations were from the same population was accepted ($p > .05$) for all sets of samples except the normally distributed samples with 75% overlap and 10% heterogeneity ($0.025 > p > .01$), and the negatively skewed samples ($.05 > p > .025$) (Table 4.1).

None of the correlation coefficients from the six normal samples containing only simple moult increments were significantly different from zero ($p > .05$), and the corrected average correlation coefficient for these samples was also not significantly different from zero ($p > 0.20$) (Table 4.2).

All significant correlations were positive.

Table 4.1: Tests of hypothesis that all correlation coefficients were from the same population.

Type of Distribution	% overlap	% heterogeneity	χ^2	d.f.	p.
Normal	75	5	8.8	5	>0.10
Normal	75	10	13.6	5	.025>p>.01
Normal	75	20	4.4	5	>0.25
Normal	75	40	1.8	5	>0.75
Normal	50	5	8.0	5	>0.10
Normal	50	10	0.5	5	>0.99
Normal	50	20	5.6	5	>0.25
Normal	50	40	7.5	5	>0.10
Normal	25	5	2.1	5	>0.75
Normal	25	10	5.2	5	>0.25
Normal	25	20	1.6	5	>0.75
Normal	25	40	6.0	5	>0.25
Normal	0	5	10.0	5	>0.05
Normal	0	10	3.4	5	>0.50
Normal	0	20	4.3	5	>0.50
Normal	0	40	1.2	5	>0.90
Normal	0	0	3.0	5	>0.50
Uniform	75	5	9.4	5	>0.05
Positively skewed	75	5	4.3	5	>0.50
Negatively skewed	75	5	12.5	5	0.5>p>.025

Table 4.2: Values of p for correlation coefficients from samples of simulated growth increments based on the normal distribution.

Composition of sample		Number of sample correlation coefficients with given value of p				Value of p for corrected average correlat. coefficient
% Overlap	% Heterogeneity	p>.05	.05>p>.01	.01>p>.001	p<.001	
75	5	4	1	1		.01>p>.001
	10	4		2		.01>p>.001
	20	1	2	3		p<.001
	40	5	1			p<.001
50	5	3	1	2		p<.001
	10		3	3		p<.001
	20	1	2		3	p<.001
	40		1	2	3	p<.001
25	5	5	1			p<.001
	10	3		2	1	p<.001
	20			3	3	p<.001
	40			2	4	p<.001
0	5	1	1	2	2	p<.001
	10	1		4	1	p<.001
	20			1	5	p<.001
	40				6	p<.001
0	0	6				p>0.2

The levels of significance of sample correlation coefficients for the normally distributed samples containing compound moult increments showed a general tendency to decrease with decreasing heterogeneity and increasing overlap (Table 4.2). Correlation coefficients were significantly different from zero ($p < .05$) for all six samples in only six of the sixteen categories of heterogeneity and overlap; e.g. samples with 50%, 20% and 0% overlap, at 40% heterogeneity (Table 4.2). Such instances were largely confined to categories with high heterogeneity, and did not occur in categories with 75% overlap. The corrected average correlation coefficients were highly significant ($p < .001$) for all but two of these categories, regardless of the levels of significance of the correlation coefficients of the individual samples. For the categories with 75% overlap and 5% and 10% heterogeneity, both of which contained a high proportion of non-significant sample correlations, the corrected average correlation coefficients were significant, but at a lower level ($.01 > p > .001$).

The levels of significance of the sample correlation coefficients from the non-normal samples (Table 4.3) were similar to those calculated for the normal samples at the same levels of overlap and heterogeneity; i.e. 75% and 5% respectively (Table 4.2). Corrected average correlation coefficients for the sets of non-normal samples were all significantly different from zero ($p < .05$). The levels of significance for the two skewed samples were similar to the levels calculated for the normal samples, while the levels of significance for the set of uniformly distributed samples was the lowest ($.05 > p > .025$) of any in the simulation except for the samples containing only simple moult increments.

Table 4.3: Values of p for correlation coefficients from samples of growth increments based on non-normal distributions.

Type of Distribution	Number of sample correlation coefficients with given value of p			Value of p for corrected average correlation coefficient
	p>0.05	.05>p>.01	.01>p>.001	
Uniform	4	2		.05>p>.025
Positively skewed	3	3		p<.001
Negatively skewed	4		2	.01>p>.001

4A.3 DISCUSSION

This simulation was designed to assess the argument that if a sample contains both simple and compound moult increments, then there will be a positive correlation between the sizes of the increments and the periods of time over which they were measured. The lack of such correlation for samples containing only simple moult increments is consistent with this argument. However, samples containing compound moult increments may also show no significant correlation for a considerable range of levels of both overlap and heterogeneity. In only a small number of sets of samples with high levels of heterogeneity and low levels of overlap were all six sample correlations significantly different from zero (Table 4.2). Consequently, lack of significant correlation between size of increment and interval of time between measurements does not necessarily imply that a particular sample contains only simple moult increments. It is therefore concluded that the correlation coefficient of an individual sample of the size used in this simulation is a relatively insensitive indicator of the presence of compound moult increments, and is unreliable except at a combination of high levels of heterogeneity and low levels of overlap in intervals between measurements.

On the other hand, the corrected average correlation coefficient was non-significant for the set of samples containing only simple moult increments, and highly significant for all sets of normally distributed samples containing some compound moult increments, regardless of the levels of heterogenity or overlap. Results for the sets of non-normal samples indicate that departures from normality in the distributions of growth increments may result in corrected average correlation coefficients that are significant, although at a reduced level (Tables 4.2

and 4.3). These correlation coefficients were calculated for samples with the highest levels of overlap and lowest levels of heterogeneity used in the simulation. Results for normal samples indicated that significance levels increased with decreasing overlap and increasing heterogeneity, so it is assumed that corrected average correlation coefficients will also be significant for sets of non-normal samples at higher levels of heterogeneity and lower levels of overlap.

These results indicate that, under the conditions of the simulation, the corrected average correlation coefficient can be used reliably to detect levels of heterogeneity as low as 5%. This reliability is maintained when the intervals over which simple moult increments are measured overlap those for compound moult increments by as much as 75%, regardless of the forms of the underlying distributions of growth increments. If these levels of sensitivity and reliability are to be achieved when analysing other data, then both the extent of the data and the basic assumptions of the simulation must be approximated.

The standard error of a corrected average correlation coefficient calculated from $i = 1 \ldots\ldots a$ samples of size n_i is based on $1/\sqrt{\sum_{i=1}^{a}(n_i-3)}$ (Snedecor and Cochran, 1967, pp.185, 187). In this simulation, $\sum_{i=1}^{a}(n_i-3) = 6(20 - 3) = 102$ for all sets of samples. If this value is attained for independent data, then the sensitivity of the correlation coefficient should be at least as great as that obtained in the simulation. Such a conclusion must also rest on the assumption that the time between measurements for a simple moult increment is independent of the size of the increment, since this was used as the basis for allocating times to increments in the simulation. It must also be noted that the calculation of a corrected average correltion coefficient for a set of samples requires the acceptance of the null hypothesis that

all simple correlation coefficients are from the same population (Snedecor and Cochran, 1967, p.186).

This hypothesis was rejected at the 0.05 level of significance for two of the twenty sets of samples used in this simulation. The construction of the simulation implies that the underlying populations of the correlation coefficients for samples in any particular set will be similar. Also, the use of a significance level of 0.05 implies that the null hypothesis will be rejected incorrectly on average once in every twenty decisions, and in neither of the above instances could the null hypothesis be rejected at a high level of significance. On this basis, it is considered that the calculation and interpretation of corrected average correlation coefficients for all sets of samples is justifiable for the purposes of the simulation.

PART 4B: APPLICATION OF THE METHOD

4B.1 METHODS

B components from all classes of crayfish in the study (see Figs. 2.1-7, and Appendix A) were classified as being either homogeneous, or heterogeneous, using the criteria developed in Section 2. For each of these components, the correlation coefficient was calculated for the relationship between the sizes of growth increments and the intervals of time over which the growth increments were measured, and tested for significant departure from zero. The results of these calculations are listed in Table 4.4.

The hypothesis that all correlation coefficients were from the same population was tested (Snedecor and Cochran, 1967, p.186) for the class of all B components, and for the separate classes of homogeneous and heterogeneous B components. Where this hypothesis was accepted, the corrected average correlation coefficient was calculated for the class (Snedecor and Cochran, 1967, p.187), and tested for significant departure from zero by conversion to a standard normal deviate, as in Section 4, Part A. The results of these tests are given in Tables 4.5 and 4.6 respectively.

4B.2 RESULTS

Correlation coefficients for the relationship between size of growth increment and interval of time between measurements (Table 4.4) varied widely, including both positive and negative values, for both homogeneous and heterogeneous B components. The correlation coefficient was significantly different from zero for three classes of crayfish. In each case the coefficient was positive, and, in each case, the B component had been classified as heterogeneous.

The hypothesis that all correlation coefficients were from the same population was accepted for all classes of B components (Table 4.5). The corrected average correlation coefficient was not significantly different from zero for the class of homogeneous B components ($p > 0.7$), whereas this difference was highly significant for the class of all B components ($p < .003$), and for the class of heterogeneous B components ($p < .0005$) (Table 4.6).

Table 4.4: Correlation Between Size of Growth Increments and Time Interval Between Measurements for Growth Increments in B Components from Crayfish Data.

*Crayfish Class	Type of Component	n	r	*1 p	*2 Conclusion
3M 20-25	Heterogeneous	18	-.0565	>.50	n.s.
3F 20-25	Heterogeneous	18	.4568	>.05	n.s.
7M 20-25	Homogeneous	6	.1455	>.50	n.s.
7F 20-25	Homogeneous	12	.3581	>.20	n.s.
3M 25-30	Heterogeneous	35	.4193	<.02	sig.
3F 25-30	Heterogeneous	41	.4368	<.005	sig.
7M 25-30	Homogeneous	7	-.1944	>.50	n.s.
7F 25-30	Heterogeneous	11	-.2763	>.20	n.s.
3M 30-35	Homogeneous	20	-.0338	>.50	n.s.
3F 30-35	Heterogeneous	38	.0070	>.50	n.s.
7M 30-40	Homogeneous	7	.7500	>.05	n.s.
7F 30-35	Homogeneous	6	-.6578	>.10	n.s.
3M 35-40	Homogeneous	8	-.3278	>.20	n.s.
3F 35-40	Homogeneous	8	.0452	>.50	n.s.
7F 35-40	Homogeneous	4	.9254	>.05	n.s.
3M 40-50	Heterogeneous	12	.2873	>.20	n.s.
3F 40-50	Homogeneous	7	-.5601	>.10	n.s.
7M 40-50	Homogeneous	3	-.3273	>.50	n.s.
7F 40-50	Heterogeneous	15	.5641	<.05	sig.
3M 50-70	Homogeneous	11	.0319	>.50	n.s.
3F 50-70	Homogeneous	6	.4943	>.20	n.s.
7F 50-70	Homogeneous	17	-.1812	>.20	n.s.
3M 70-100	Homogeneous	4	.3070	>.50	n.s.
3F 70-100	Homogeneous	11	-.2619	>.20	n.s.
3PM 20-25	Homogeneous	11	.3308	>.20	n.s.
7PM 20-25	Homogeneous	7	.3853	>.20	n.s.
7F 15-20	Homogeneous	3	-.5000	>.50	n.s.

* Classes of crayfish described in Section 2.1. Pools 3, 7 - locations of capture; F = Female; M = Male; PM = Precocious Male. Range of carapace lengths, e.g., 70-100mm.

*1 two-tailed probability.

*2 0.05 significance level.

Table 4.5: Tests of Hypothesis that all Correlation Coefficients are from the Same Population.

Source of Coefficients	χ^2	d.f.	p	Conclusion about Hypothesis
All B Components	30.8	26	>0.1	Accept at 0.1 level of significance.
Homogeneous B components	15.5	18	>0.5	Accept at 0.5 level of significance.
Heterogeneous B components	11.3	7	>0.1	Accept at 0.1 level of significance.

Table 4.6: Tests of Hypothesis that the Corrected Average Correlation Coefficient is not Significantly Different from Zero.

Source of Coefficients	1 $\bar{r}_{corr}$	2 z	3 p	Conclusion about Hypothesis
All B Components	0.1812	2.9902	0.0028	Reject at 0.003 level of significance.
Homogeneous B Components	0.0322	0.3236	0.7463	Accept at 0.7 level of significance.
Heterogeneous B Components.	0.2732	3.5877	0.0004	Reject at 0.0005 level of significance.

1 $\bar{r}_{corr}$ = corrected average correlation coefficient.

2 z = standard normal deviate.

3 p = 2-tailed probability.

4B.3 DISCUSSION

The class of all B components was found to have a significant correlation, as was the class of heterogeneous B components. When the heterogeneous B components were removed from the class of all B components, the remaining class of homogeneous components was found to have no significant correlation. This implies that most, if not all, compound moult increments must have been contained in the components classified as heterogeneous. Considering the sensitivity demonstrated for the corrected average correlation coefficient in the previous section (4A), it may thus be reasonably concluded that the B components classified as homogeneous contained only simple moult increments.

It is still possible, however, that some components classified as heterogeneous may contain only simple moult increments, as this could not have been detected by the methods used in this section. It is also possible that the significant correlation for the class of heterogeneous components may have resulted from the inclusion of only a small number of compound moult increments in only a few of the components. Complete rejection of all heterogeneous B components may thus be wasteful of valuable data. Methods for overcoming this are presented in the following section (4C).

PART 4C: REINTERPRETATION OF HETEROGENOUS DATA

4C.1 METHODS

The heterogeneous B components described in the previous section (4B) were examined for instances where the values of both increment and interval of time between measurements were distinctly large. These values were removed, and the sample correlation coefficients were recalculated for the respective components. The results of these calculations are shown in Table 4.7. The corrected average correlation coefficient was then recalculated for the whole group of heterogeneous components, as described in the previous section.

4C.2 RESULTS

A total of six values was removed, with the result that none of the components showed a significant sample correlation ($p > 0.05$). The hypothesis that all sample correlations were from the same population was accepted ($\chi^2 = 9.6$, d.f. = 7, $.25 > p > 0.10$), and the corrected average correlation coefficient was not significantly different from zero ($r = 0.1465$, $p > 0.05$).

4C.3 DISCUSSION

Since the corrected average correlation coefficient was not significantly different from zero, it may be reasonably concluded that

Table 4.7: Sample Correlation Coefficients (r) for Adjusted Heterogeneous B Components.

*Component	Number of values removed	n	r	p
2M 20-25	0	18	-0.0565	> 0.50
2F 20-25	0	18	0.4568	> 0.05
2M 25-30	1	34	0.3112	> 0.05
2F 25-30	2	39	0.1663	> 0.25
2F 25-30	1	10	-0.5774	> 0.05
2F 30-35	0	38	0.0070	> 0.50
2M 40-50	1	11	0.0620	> 0.50
3F 40-50	1	14	0.4166	> 0.10

* As for Table 4.4.

the components contained only simple moult increments after the six pairs of values had been removed.

It is considered, however, that the methods used in this section require some qualification. Since there may be considerable variation in both size of increment and time between measurements due to natural causes, there is no justification for removing extreme pairs of values unless it has been shown that the extent of the variation is in excess of that expected through chance; i.e., unless there is a significant correlation between increment and time between measurements. Even if this has been demonstrated, the correlation may have been due to a small number of values located in a few sets of data, as in this section. If this is the case, then the removal of the extreme values from all sets of data may result in the loss of information. For this reason, the analysis has been divided into three steps. Initially, all data were combined and tested for the presence of a significant correlation. As the correlation was significant, the criteria of Section 2 were used in an attempt to isolate the compound moult increments in a smaller portion of the data. This was successful (Section 4B) allowing the smallest possible portion of the data to be subjected to post-hoc removal of values. Fortunately, in terms of the usefulness of the data, it was possible to remove the significant correlation by removing a small number of distinctive pairs of values. If this had not been successful, then the remaining data could not have been interpreted as simple moult increments.

SECTION 5

EVALUATION OF THE COMPOSITION OF COMPONENT C

5.1 METHODS

It has been argued previously (Section 4) that the size of a simple moult increment is independent of the interval of time over which it has been measured. If this argument holds, then the hypothesis that component C contains only simple moult increments may be rejected if it can be shown that the intervals of time between measurements for component C are significantly greater than those for component B.

Intervals between measurements for growth increments in component C were compared with those for component B using a Wilcoxan distribution-free rank sum test (Hollander and Wolfe, 1973), for each class of crayfish whose distribution of measurement differences contained a C component. In each comparison the null hypothesis of no difference between intervals was tested against the alternative that component C contained the longer intervals. Results of these comparisons are given in Table 5.1.

5.2 RESULTS

Intervals between measurements for component C were significantly greater ($p < 0.05$) than those for component B in nine of the seventeen classes of crayfish for which component C was present (Table 5.1).

Table 5.1: Wilcoxon Tests of the Null Hypothesis (H_O) that there is No Difference in Times Between Measurements for Components B and C, against the Alternative (H_A) that the Times Between Measurements are Greater for Component C.

*Crayfish Class	No. in B (m)	No. in C (n)	W or* W^1	p	Hypothesis accepted?
3M 20-25	18	3	W = 52.0	.031	H_A
3F 20-25	18	11	W^1 = 2.9	.0019	H_A
3M 25-30	35	10	W^1 = 3.0	.013	H_A
3F 25-30	40	7	W^1 = 2.7	.0032	H_A
3M 30-35	20	2	W = 37.5	.07>p>.05	H_O
3F 30-35	38	1	W = 30.0	>.18	H_O
3M 35-40	8	1	W = 8.0	.22	H_O
3M 40-50	13	2	W = 22.5	>.15	H_O
3F 40-50	7	2	W = 17.0	.028	H_A
7M 20-25	6	3	W = 22.0	.048	H_A
7F 20-25	12	2	W = 22.0	.132	H_O
7M 25-30	7	4	W = 38.0	.003	H_A
7F 25-30	11	4	W = 53.5	.001	H_A
7M 30-35	6	2	W = 14.0	.071	H_O
7F 30-35	6	3	W = 18.5	>.19	H_O
7F 40-50	15	4	W = 68.0	.001	H_A
7F 50-70	13	1	W = 11.0	.286	H_O

*Classes of crayfish described in Section 2.1. Pools 3, 7, locations of capture; F = female; M = male. Range of carapace lengths, e.g., 70-100mm.

* W = Wilcoxon statistic.

W^1 = Wilcoxon statistic for large sample approximation.

A significant result occurred in all cases where the number of observations in component C was greater than or equal to four. Two of the three classes of crayfish for which the C component contained three observations showed a significant difference, while of the five classes for which the C component contained two observations, one showed a significant difference. None of the three classes for which the C component contained a single observation showed a significant difference.

5.3 DISCUSSION

It has been previously noted (Section 4A) that some overlap may occur between the times between measurements for simple and compound moult increments. If a comparison is made between the two classes of intervals, and either of the classes contains a small number of observations, then this overlap may obscure differences that would be apparent had a larger number of observations been present.

Taking this into consideration, the pattern of occurrence of significant differences implies that the intervals of time between measurements for growth increments in a C component were significantly greater than those in the corresponding B component, wherever adequate numbers of observations were present. In turn, this implies that at least the majority of the increments in the C components were compound. In particular, the occurrence of significant differences in several instances where component C contained only two or three observations implies that the degree of overlap between the two classes of intervals was small. Thus it is concluded that simple moult increments were restricted to component B for *E. spinifer*.

SECTION 6

CONCLUSION

The method devised in the previous sections has allowed the majority of the simple moult increments to be extracted, with a reasonable degree of certainty, from the extensive and variable growth data collected for *E. spinifer* using mark-recapture techniques. These simple moult increments have been used elsewhere in this thesis in the description of the biology of *E. spinifer*. Where used as such, they have been referred to exclusively as 'moult increments', as is the normal practice.

The method has two major requirements. The relationship between recapture frequency and moult frequency must be such that both simple moult increments and measurement errors can be detected, in order to fulfil the requirements of Criterion 1, and negate the conclusion of Criterion 2 (Section 2.3). Secondly, the data may require division into smaller sections prior to analysis. There are two reasons for this. Subdivision must be undertaken so that the features of the distributions of increments that are subjected to dissection using probability paper result primarily from the presence of simple and compound moult increments and measurement errors, rather than from variability due to different groups of animals. The second reason is that if the intention is to compare between the simple moult increments of different groups within a population, then it is necessary to identify the increments separately for each group. Treating them collectively requires the initial assumption that the increments are similar, and later comparisons are invalidated.

The sets of data that result from such splitting may be small, and statistics calculated from them may be difficult to interpret on an individual basis. In the case of Section 4, if it can be shown that the correlation coefficients from several small samples belong to the same population, then the corrected average correlation coefficient can be calculated for the set of samples, thus allowing more reliable interpretation. This facility provides the method with considerable power.

It must be noted, however, that the conclusions based on the methods of Sections 4 and 5 are purely statistical. There is no absolute certainty that they are correct, and they involve an element of generalisation. Also, the conclusions must be considered flexible in the face of biological evidence. Possible sources of such evidence are not readily obvious, hence the need for statistical inference.

The method may have been applied profitably to several other instances in the literature where a mixture of simple and compound moult increments may have been collected (see Introduction). In particular, the distribution of 'moult increments' presented by Hopkins (1967a, Figure 1, p.466) for *Paranephrops planifrons* appears to be distinctly bimodal for smaller individuals. Further examination of these data may indicate the presence of both simple and compound moult increments, necessitating alterations in the conclusions that have been drawn.

The routine application of the method to field studies of decapod growth would provide a uniformity of interpretation that has been previously lacking, and facilitate reliable comparisons between the results of different workers.

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APPENDIX A

GRAPHS OF PERCENT CUMULATIVE FREQUENCY VS MEASUREMENT DIFFERENCE FOR CLASSES OF CRAYFISH NOT INCLUDED IN SECTION 2 OF PART B.

Key to Graphs

Graph	Pool	Sex*	C.L.	Graph	Pool	Sex	C.L.
A1	3	M	20-25	A12	3	F	40-50
A2	3	M	25-30	A13	3	F	50-70
A3	3	M	30-35	A14	3	F	70-100
A4	3	M	35-40	A15	7	M	20-25
A5	3	M	40-50	A16	7	M	25-30
A6	3	M	50-70	A17	7	M	30-40
A7	3	M	70-100	A18	7	M	40-50
A8	3	PM	20-25	A19	7	F	15-20
A9	3	F	15-20	A20	7	F	25-30
A10	3	F	20-25	A21	7	F	30-35
A11	3	F	30-35	A22	7	F	50-70

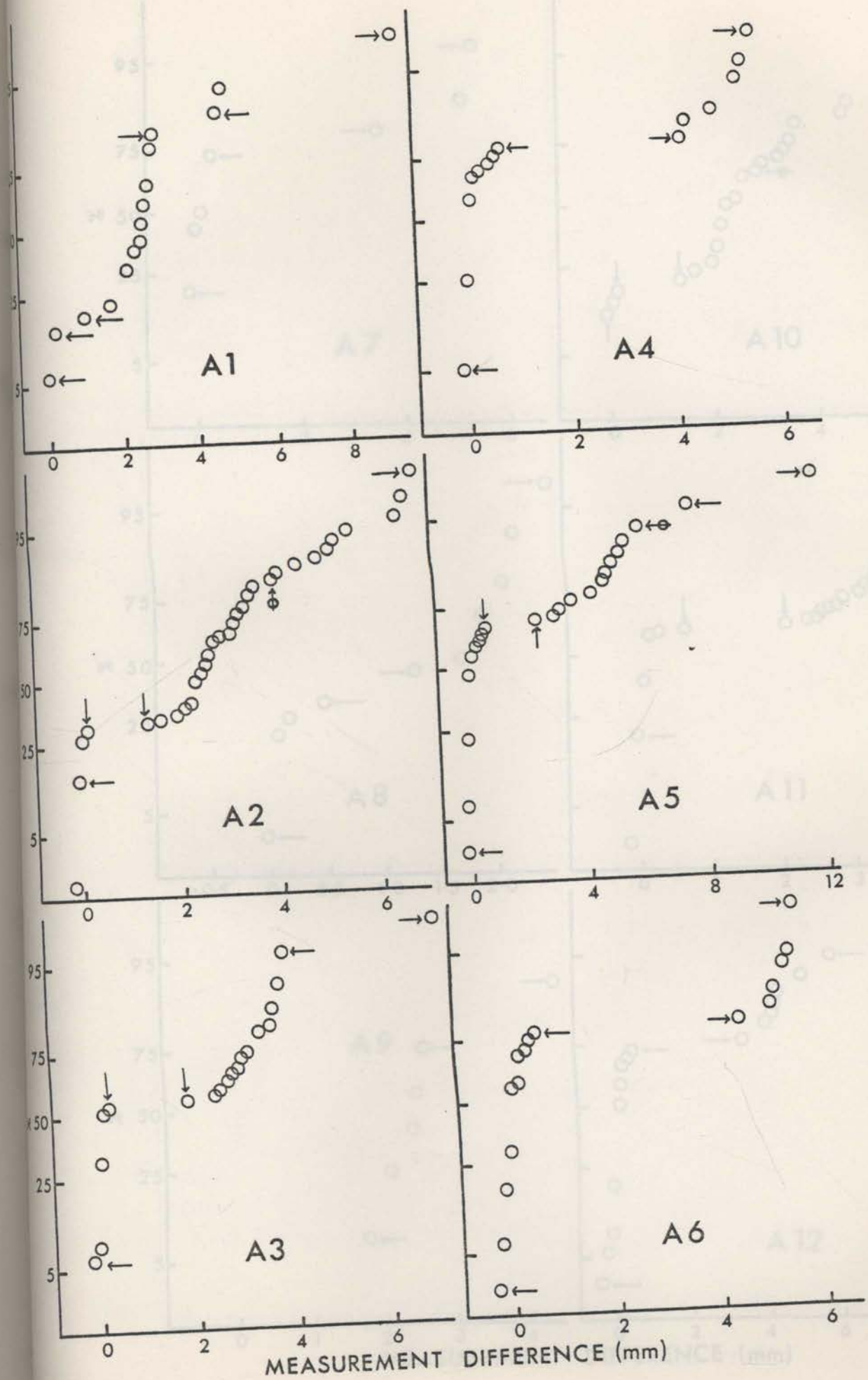
* Sex: F = female, M = normal male, PM = precocious male.

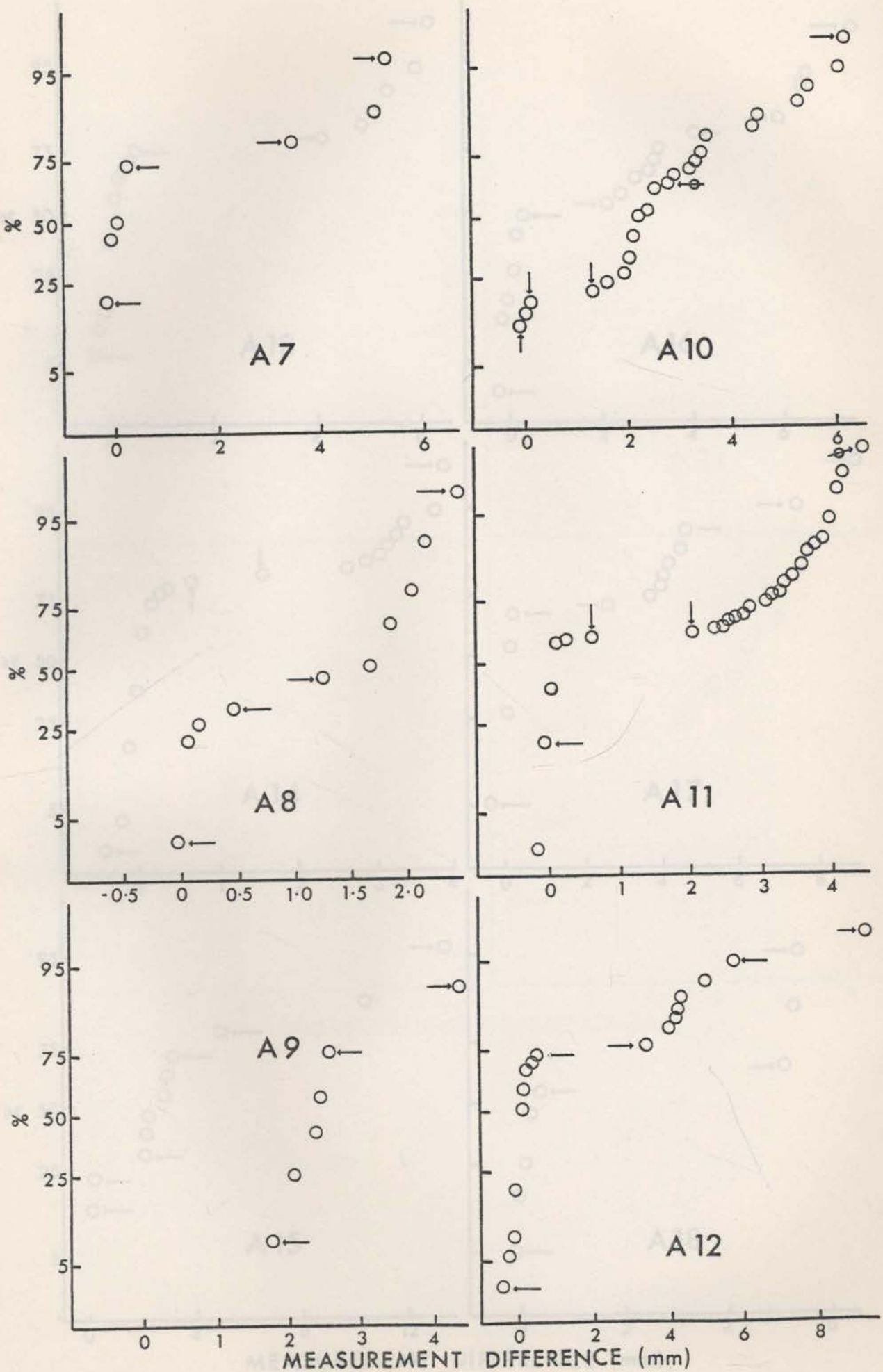
C.L. = Range of carapace lengths (mm).

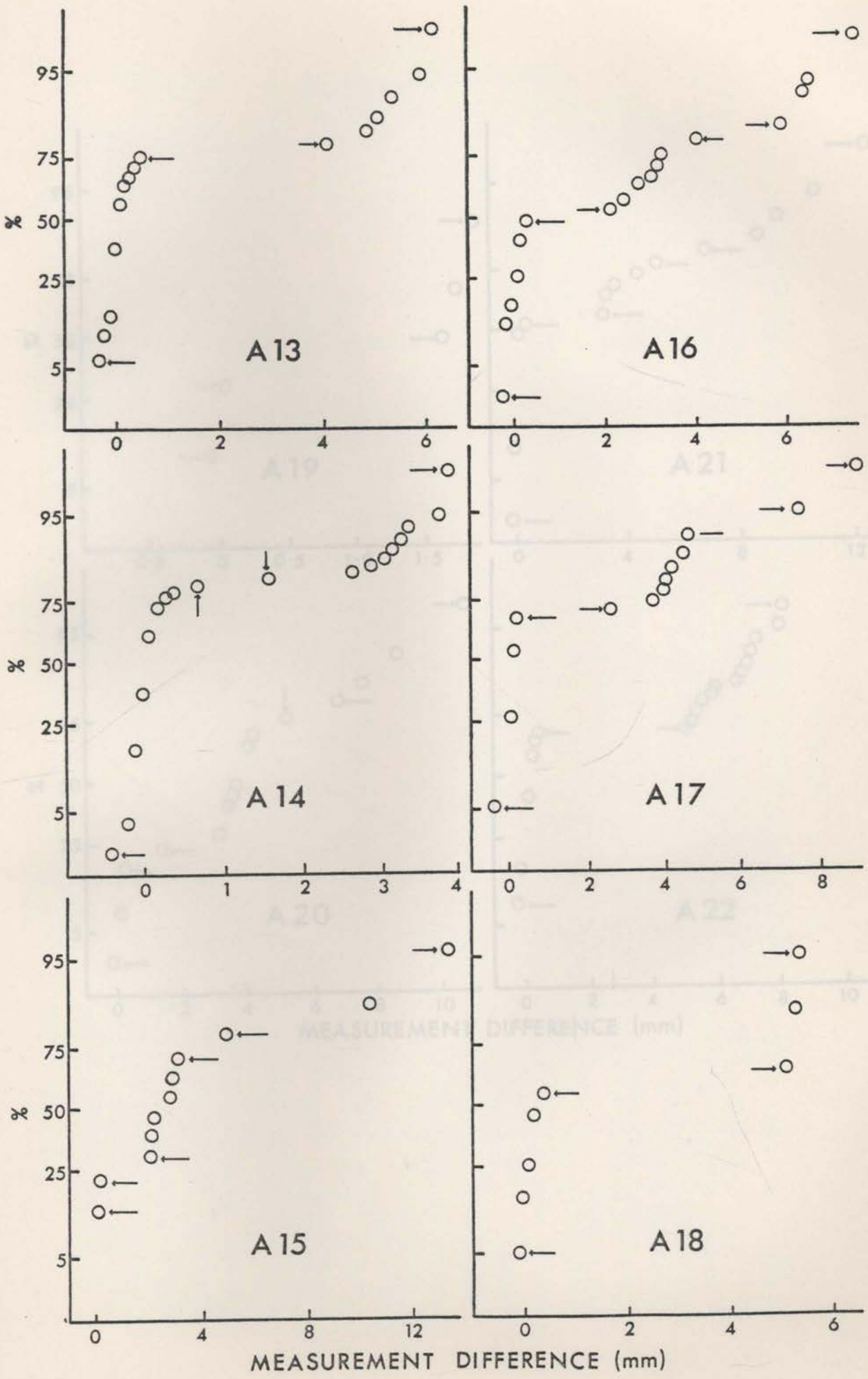
O = Original values for percent cumulative frequency.

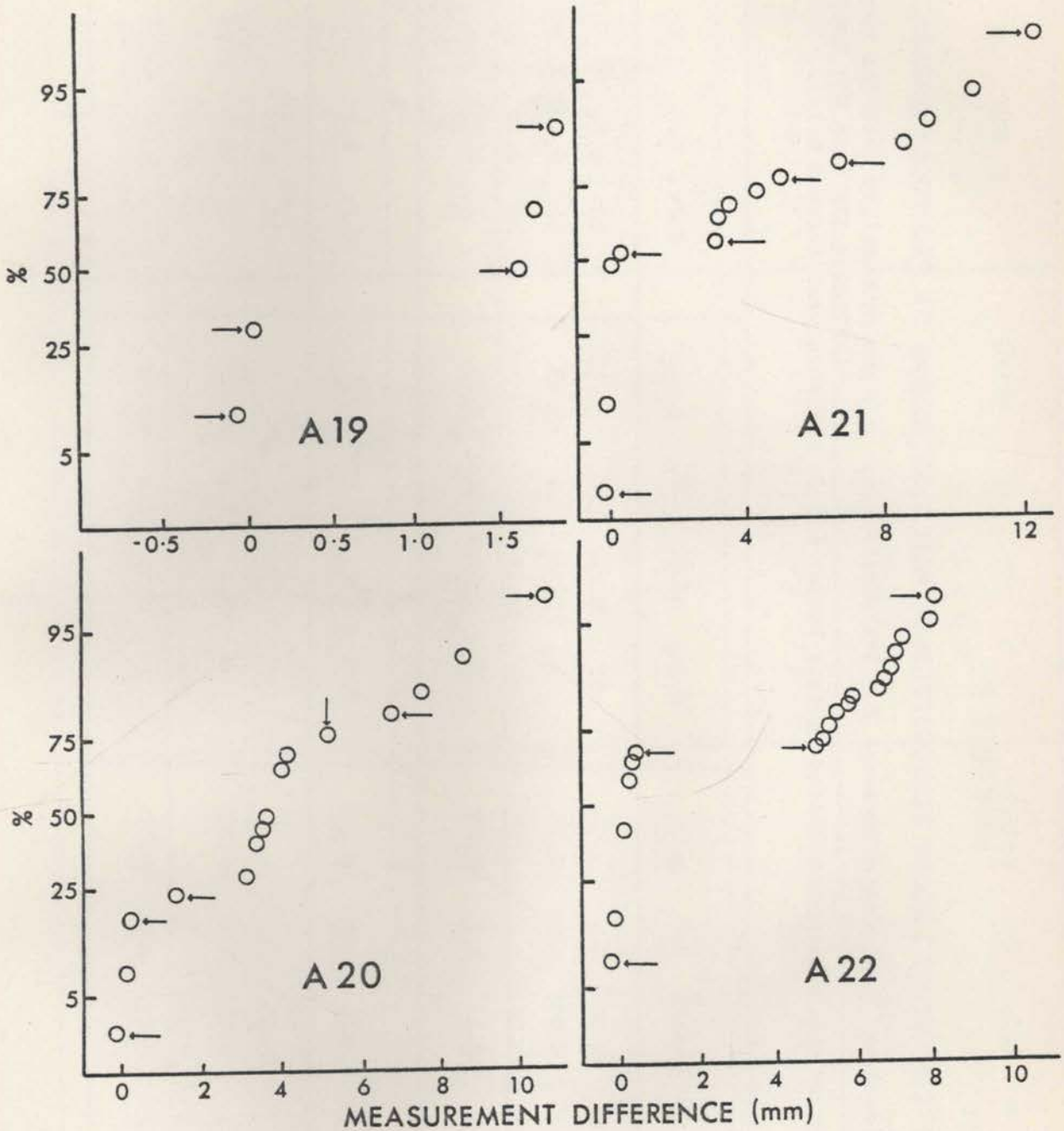
$\dagger$ = Extreme value of component; inflexion determined by eye.

ϕ = Extreme value of component; inflexion determined by method of regression fitting.


A1
A4
A2
A5
A3
A6
MEASUREMENT DIFFERENCE (mm)


A7
A10
A8
A11
A9
A12
%
95
75
50
25
5
MEASUREMENT DIFFERENCE (mm)


A 13
A 16
A 14
A 17
A 15
A 18
%
95
75
50
25
5
MEASUREMENT DIFFERENCE (mm)


A 19
A 21
A 20
A 22
%
95
75
50
25
5
-0·5
0
0·5
1·0
1·5
0
4
8
12
2
6
10
MEASUREMENT DIFFERENCE (mm)

APPENDIX B

ANALYSIS OF COVARIANCE COMPARING LINEAR REGRESSIONS OF MOULT INCREMENT ON PRE-MOULT CARAPACE LENGTH

Key:- d.f. = degrees of freedom; M.S. = Mean Square; F = variance ratio; p = the probability that the slopes or elevations are from the same populations.

Comparisons - numbers refer to the comparisons listed in text Tables 6.1-2, and with text Figures 6.1-3.

Comparison	*1*				*2*				*3*			
Source	*d.f.*	*M.S.*	*F*	*p*	*d.f.*	*M.S.*	*F*	*p*	*d.f.*	*M.S.*	*F*	*p*
Summed	18	0.066			51	0.22			30	0.24		
Slopes	1	0.006	0.09	>0.25	1	0.29	1.3	>0.25	1	0.009	0.04	>0.25
Pooled	19	0.063			52	0.23			31	0.23		
Elevations	1	0.14	2.3	>0.10	1	0.0004	0.001	>0.25	1	0.10	0.4	>0.25

Comparison	*4*				*5*				*6*			
Source	*d.f.*	*M.S.*	*F*	*p*	*d.f.*	*M.S.*	*F*	*p*	*d.f.*	*M.S.*	*F*	*P*
Summed	7	0.15			9	0.15			15	0.29		
Slopes	1	0.17	1.1	>0.25	1	0.02	0.2	>0.25	1	0.02	0.06	>0. 5
Pooled	8	0.16			10	0.14			16	0.27		
Elevations	1	0.13	0.8	>0.25	1	0.24	1.8	>0.10	1	0.22	0.8	>0.

Comparison Source	7 d.f.	M.S.	F	p	8 d.f.	M.S.	F	p	9 d.f.	M.S.	F	p
Summed	7	0.32			14	0.082			63	0.22		
Slopes	1	0.49	1.5	>0.25	2	0.016	0.2	>0.25	2	0.63	2.8	>0.05
Pooled	8	0.34			16	0.074			65	0.24		
Elevations	1	0.08	0.24	>0.25	2	0.22	3.0	>0.05	2	3.3	14	<0.005

Comparison Source	10 d.f	M.S.	F	p	11 d.f.	M.S.	F	p	12 d.f.	M.S.	F	p
Summed	23	0.38			29	0.29			16	0.37		
Slopes	1	0.29	0.8	>0.25	3	0.41	1.4	>0.25	2	0.53	1.4	>0.25
Pooled	24	0.31			32	0.30			18	0.38		
Elevations	1	4.3	14	<0.005	3	0.64	2.1	>0.10	2	0.55	1.4	>0.25

Comparison Source	13 d.f.	M.S.	F	p	14 d.f.	M.S.	F	p	15 d.f.	M.S.	F	p
Summed	100	0.27			43	0.35			17	0.68		
Slopes	2	0.34	1.2	>0.25	2	0.84	2.4	>0.10	1	1.2	1.7	>0.10
Pooled	102	0.28			45	0.37			18	0.71		
Elevations	2	3.6	13	<0.005	2	5.4	15	<0.005	1	8.0	11	<0.005

APPENDIX C

CARAPACE LENGTH - WEIGHT RELATIONSHIP

INTRODUCTION

It was not feasible to weigh live *E. spinifer* accurately in the field, hence carapace length was used as the standard measurement of crayfish size. Where it was necessary to estimate the body weights of crayfish, this was done using a single, calculated length-weight relationship.

METHODS

One hundred and two females and sixty three males were selected over the period of study such that males and females covered similar ranges of carapace lengths, and the carapace lengths were distributed as evenly as possible over this range when converted to $\log_{10}$. These crayfish were weighed to the nearest 0.1g, and the carapace lengths were measured to the nearest 0.1mm (as in Chapter 2). Separate, least squares linear regressions of the form

$$\log_{10} W = a + b \log_{10} L$$

where W = body weight (g); L = carapace length (mm).

were calculated for males and females, and compared using analysis of covariance (Snedecor and Cochran, 1967). The regression was recalculated for combined males and females on the basis of this comparison.

RESULTS

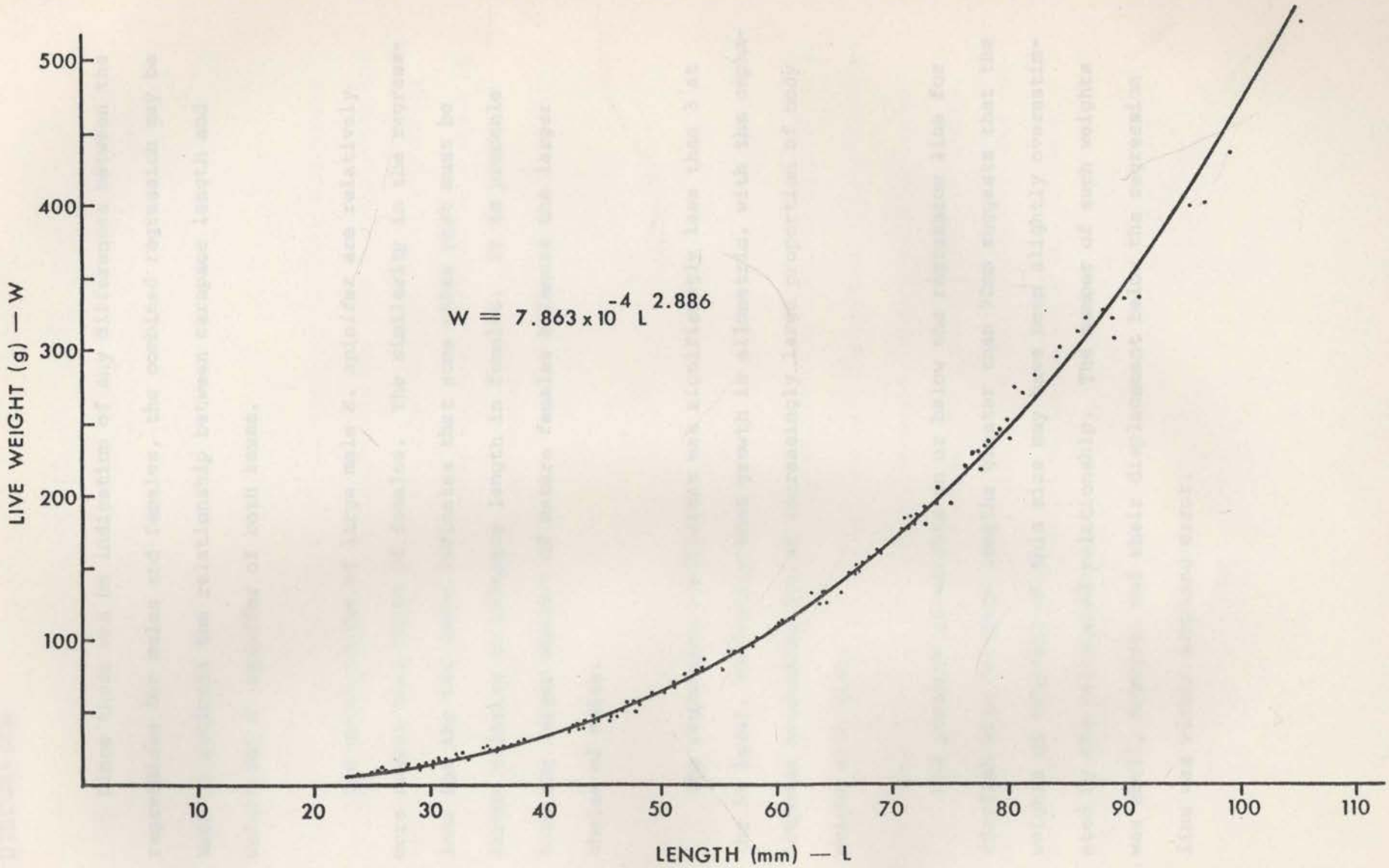
There were no significant differences between the slopes or elevations of the length-weight regressions for males and females (Table C.1).

The slope of the combined regression was estimated as b = 2.8860, with 95% confidence limits of ±0.0187. The combined regression provided a close fit to the data (correlation coefficient r = 0.9991) (Figure C.1), although the weights of crayfish with carapace lengths greater than 90mm were on or slightly below the regression line. Ninety-five percent confidence limits for the weight at any given carapace length within the recorded range were approximately ±9.5% of the estimated weight.

Table C.1: Analysis of Covariance Comparing the Length-Weight Regressions of Males and Females.

Source	d.f.	M.S.	F	p
Summed	161	4.29×10^{-3}		
Slopes	1	5.90×10^{-7}	$<< 1$	$\simeq 1$
Pooled	162	4.26×10^{-3}		
Elevations	1	4.10×10^{-6}	$<< 1$	$\simeq 1$

Figure C.1: Length-Weight Relationship for *E.spinifer.*


500
400
300
200
100
LIVE WEIGHT (g) — W
W = 7.863 x 10^-4 L^2.886
10
20
30
40
50
60
70
80
90
100
110
LENGTH (mm) — L

DISCUSSION

Since there was no indication of any differences between the regressions for males and females, the combined regression may be used to estimate the relationship between carapace length and weight for *E. spinifer* of both sexes.

The great chelae of large male *E. spinifer* are relatively more robust than those of females. The similarity in the regressions for the two sexes indicates that some other part must be larger relative to carapace length in females. It is probable that the larger abdomen of mature females balances the larger chelae of males.

The regression coefficient was significantly less than 3 at the 5% level, indicating that growth is allometric, with the cephalothorax accounting for an increasingly large proportion of body weight with size.

The presence of weights on or below the regression line for crayfish with carapace lengths greater than 90mm suggests that the weights of crayfish of this size may have been slightly overestimated by the calculated relationship. The number of such weights was small, however, and their displacement below the regression line was within sampling error.

APPENDIX D

COMPARISON OF ACTUAL AND ESTIMATED MOULT FREQUENCIES

INTRODUCTION

In Chapter 5 it was found necessary to estimate the number of moults undergone by crayfish during one year to supplement the small number of crayfish for which consecutive moult increments had been measured. This appendix has been included as a check on the reliability of the method of estimation.

METHODS

Measured growth increments of crayfish that had moulted more than once over any period of time were subdivided, wherever possible, into a series of consecutive moult increments based on the results of Part B of this thesis. The actual number of moults undergone by each of these crayfish was thus determined, and compared with the number of moults estimated using the method of Section 5.2.1. (Table D.1).

RESULTS

Consecutive moult increments were identified for a total of forty crayfish of varying initial carapace lengths (C.L.) (Table D.1). The estimated number of moults was identical to the actual number for thirty four of these crayfish. The actual numbers of moults undergone by the remaining six crayfish were in all instances underestimated by a single moult.

DISCUSSION

The method of estimating moult frequencies in Section 5.2.1 of Chapter 5 resulted in underestimation of actual moult frequency for approximately one in every seven crayfish. Considering that in each instance the actual number of moults was only underestimated by a single moult, it is unlikely that use of the method of estimation would result in substantially different conclusions about average moult frequencies when compared with conclusions based on the actual number of moults. Conclusions about the lowest moult frequencies of crayfish in any group should be based, however, on actual, and not estimated, numbers of moults.

Table D.1: Comparisons Between Actual and Estimated Numbers of Moults.

Initial C.L.(mm)	Moult Increments (mm)	Est. no Moults
	Female, Pool 3	
22.8	2.2, 2.0, 2.9	3
23.9	2.5, 2.9	2
26.4	3.6, 2.4, 4.1	3
27.3	3.7, 3.8, 3.8	3
27.6	3.2, 3.0, 2.3	3
25.0	2.6, 2.5	2
29.4	2.4, 2.6	2
27.8	3.0, 4.0, 3.3	3
27.4	2.8, 3.5, 3.3	3
30.4	2.0, 3.4	2
37.0	4.4, 4.2	2
38.0	4.7, 3.9	2
61.2	5.0, 4.8	2
	Male, Pool 3	
28.8	3.1, 3.9	2
26.8	1.3, 1.6, 2.2	2*
25.1	2.6, 2.4, 3.7	3
25.4	2.4, 3.4	2
26.8	1.6, 2.2, 2.8	2*
31.1	3.0, 3.4	2
31.8	1.8, 2.5	1*
36.2	4.2, 2.3	1*
37.9	5.2, 3.1	2
37.3	4.2, 4.2	2
41.8	4.7, 5.0	2
40.4	4.8, 2.7	2
50.2	5.3, 4.4	2
	Female, Pool 7	
27.0	1.3, 3.9	1*
29.7	3.9, 3.4	2
43.6	3.7, 5.3	2
43.0	6.4, 5.4	2
49.8	5.7, 6.9	2
45.8	4.6, 6.5	2
46.6	4.2, 6.1	2
45.1	4.9, 5.2	2
53.2	6.8, 1.7	1*
53.4	5.8, 7.8	2
	Male, Pool 7	
22.8	3.1,2.1,3.1,4.4	4
28.4	3.2, 3.9, 4.5	3
49.1	5.0, 5.3	2
50.7	6.1, 6.6	2

* Number of moults underestimated.

APPENDIX E

TERMINOLOGY OF PLEOPODAL SETAE

There appears to be considerable confusion in the terminology used to describe pleopodal setae, giving rise to statements such as "pleopodal setae of other parastacids differ markedly from that of *C. (Cherax) destructor*" (Johnson, 1979), while Thomas (1970) argued that the egg-bearing setae of the astacid *Austropotamobius pallipes* differed from those of other decapods.

The egg-bearing setae of *A. pallipes* described by Thomas (1970) were similar to those of *E. spinifer* in that each 'ooseta' consisted of a long, filament-like shaft bearing a number of small setules over its distal extremity. The other predominant type of setae on the pleopods of both species were termed 'plumose' by Thomas (1970). Each 'plumose' seta consisted of a more robust, tapering shaft with long setules arranged in opposing rows along its length. Oosetae were thus also described as 'part-plumose' by Thomas.

Farmer (1974b) made similar observations for the lobster *Nephrops norvegicus*. Farmer (1974b) also noted that the setules on the egg-bearing setae of *N. norvegicus* were gradually lost after moulting, probably due to abrasion, and suggested that this may have accounted for the absence of setules on the egg-bearing setae of another lobster *(Homarus vulgaris)* that had been described by Lloyd and Yonge (1940) and termed 'non-plumose'. In a similar vein, Thomas (1970) noted that while Yonge (1938) had described the egg-bearing setae of *Homarus* as 'non-plumose', there were a few setules

attached to the tips of these setae. This appears to contradict the prior statement made by Thomas that the egg-bearing setae of *Austropotamobius pallipes* were 'part-plumose' and thus differed from the 'non-plumose' egg-bearing setae of other decapods. In addition, Gurney (1942) stated that the eggs of most decapods were attached to specialised setae which are usually quite smooth.

Among parastacids, Morrissy (1970) described the egg-bearing setae of *Cherax tenuimanus* as 'plumose' (as opposed to other, non egg-bearing 'pinnate' setae = 'plumose' of Thomas, 1970), and noted that they were similar to those of *Paranephrops planifrons* (Hopkins, 1967b). In a later publication, Morrissy (1975) also stated that the so-called 'plumose' egg-bearing setae of *C. tenuimanus* were similar to the 'oosetae' described by Thomas (1970). Hopkins (1967b) described the egg-bearing setae of *Paranephrops planifrons* as 'non-pinnate', while examination of Hopkins' Figure 2.c indicated that these setae were essentially identical to the oosetae of Thomas (1970). Suter (1977) stated that the eggs of *Engaeus cisternarius* were attached to the pleopods by 'strands of plumose setae'. Johnson (1979) stated that the eggs of *Cherax destructor* were 'filamentous' as opposed to non-egg-bearing 'pinnate' (= 'plumose' of Thomas, 1970) setae, while Woodland (1967) stated that the egg-bearing setae of *C. albidus* were 'filamentous'.

Non-egg-bearing setae have thus been described as either 'plumose' or 'pinnate', while egg-bearing setae have been variously described as 'usually quite smooth', non-plumose', part-plumose', 'plumose', 'non-pinnate', 'filamentous', and 'oosetae'. The comments by Thomas (1970) and Farmer (1974b) indicated that the source

of the differences in terminology lay in whether or not setules had been observed on the tips of the egg-bearing setae. Farmer (1974b) noted that these setules may be lost some time after moulting, while in *E. spinifer* the setules were small and usually only visible at high magnification. Hence unless the egg-bearing setae were examined shortly after moulting, and/or under high magnification, the setules may not have been observed.

Wherever descriptions were adequate, the egg-bearing setae were presented as having a long, filament-like shaft, and in several instances the presence of setules was mentioned or illustrated, although this was not always incorporated into the terminology. Also, setules were present on the tips of the egg-bearing setae of representatives of the three superfamilies (Nephropoidea, Astacoidea, Parastacoidea) comprising the infraorder Astacidea (after Hobbs, 1974), and in all three families (Nephropidae, Astacidae, Parastacidae) included in this discussion. This suggests strongly that setules were probably present on the egg-bearing setae of species for which they were not mentioned, although more critical attention to the presence of setules on egg-bearing setae is indicated.

The available evidence thus suggests that all of the terms used to describe egg-bearing setae referred to the same basic structure. In view of this, it is suggested that the terminology of Thomas (1970) be adopted, and that the egg-bearing setae be termed 'oosetae'. This term is useful in that it implies the function of the setae, and avoids confusion with terms for other types of setae.