ASPECTS OF GROWTH AND REPRODUCTION

OF THE HAIRY HANDED CRAB,

HEMIGRAPHS CRENULATUS

(BRACHYURA : GRAPSIDAE).

A thesis submitted in
partial fulfilment of
the requirements for the
Degree of
Master of Science in Zoology
at the
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By
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Abstract.

Aspects of the growth and reproductive biology of the grapsid brachyuran *Hemigrapsus crenulatus* (H. Milne Edwards 1837) have been studied in the Avon-Heathcote Estuary (43° 33’S, 172° 44’E), Christchurch, New Zealand.

Relative growth analysis revealed that males became mature between 13.0 and 15.0mm CW whilst females achieved maturity between 9.0 and 11.0mm CW which coincided with the size of the smallest ovigerous females found in the field. Differences in the allometric growth of secondary sexual characters were also apparent.

Monthly size frequency distributions over a two year sampling period revealed that the population consisted mainly of crabs in the middle size classes (12.0mm to 20.0mm CW) which maintained a 1:1 sex ratio however, males dominated all other size classes.

The breeding season, based on the occurrence of ovigerous females, spanned 8-9 months from June to January/February. This extended breeding season could be attributed to some females producing successive broods with or without an intervening moult. Females were capable of transmoult sperm retention and ovarian and embryological development was cyclic.

Laboratory studies indicated that a minimum incubation time of 45 days was required although lower salinities increased incubation period and egg size. Attempts at rearing *H.crenulatus* zoeae in various combinations of temperature and salinity were unsuccessful due to their apparent inability to feed.

Absolute growth analysis indicated that males have larger growth increments and reached maturity in a year in their 11th instar, whereas females became mature in their 9th instar after only 290 days. A seasonal incidence of moulting was apparent with observed peaks occurring before and after the breeding season.
Plate 1. Male *Hemigrapsus crenulatus*.

Plate 2. Ventral view of male (top left) showing first pleopods and deep sternal depression and female (top right) with wide abdomen and pleopods and ovigerous female with recently extruded brood.
GENERAL INTRODUCTION

The decapod fauna of New Zealand is relatively low in species diversity and richness when compared to a similar area in the Indo-Pacific. The New Zealand brachyuran fauna consists of 77 species in 52 genera in 16 families (McLay, pers comm). The fauna is ‘unbalanced’ with each genus represented by only one or two species (Dell 1968). This is well illustrated by the family Grapsidae which, in New Zealand, includes six genera but comprises only nine species, Leptograpsus variegatus, Plagusia chabrus, Hemigrapsus edwardsi, Hemigrapsus crenulatus, Helice crassa, Cyclograpsus insularum, Cyclograpsus lavauxii, Planes cyaneus and Planes marinus.

Along with the relative paucity of crab species in New Zealand, is the paucity of information regarding their ecology. The ecological requirement and population structure of only one of the grapsids, Helice crassa, have been well studied (Nye 1977, Jones 1980) as well as investigations of behavioural, structural and physiological adaptations (Hawkins and Jones 1982, Hawkins Jones and Marsden 1982, Jones 1977a, 1981, Jones and Simons 1982, Shumway and Jones 1981). Other grapsids have received less attention despite their common occurrence in the intertidal zone.

One example is the grapsid Hemigrapsus crenulatus, commonly known as the Hairy Handed crab (Plate 1), which is adapted to both marine and estuarine conditions (Morton and Miller 1973).

The ability of Hemigrapsus crenulatus to live in brackish water has been attributed to the maintenance of blood sodium concentration above that of the external medium. This is achieved by the active uptake of sodium ions from the medium (Ayers 1968, Hoskins 1966 and Jackson 1976). Field and laboratory work by Jones (1976) on factors limiting distribution have indicated that H. crenulatus is euryhaline and can extend into the more
dilute areas of the Avon-Heathcote estuary. Phillips (1968) and Hicks (1973) have drawn similar conclusions from salinity tolerance studies in *H. crenulatus* from different parts of New Zealand. Bloomfield (1982) investigated the importance of salinity acclimation in determining improved tolerance of *H. crenulatus* to dilute sea water.

The micro distribution of *H. crenulatus* at low tide reflects the presence of pools of water and rocks. It's photonegative response in both air and water (Jones 1976) and it's use of soft substrates for burying enable it to avoid detrimental conditions of temperature and salinity (Hicks 1973, Kinr 1967). However, *H. crenulatus* has also been found in permanent burrows (Woc 1968) and has been described as a burrowing crab (Bennett 1964, Cockayne 1906). Distribution at low tide yields an interesting pattern with a predominance of females at the lowest ebb (Walker 1970) or subtidally (Jackson 1976). Such a distribution could be a seasonal phenomenon and requires clarification. *Hemigrapsus crenulatus* has been seen actively migrating up the shore to feed on organic matter with the incoming tide and retreating again as the tide recedes (Walker 1970).

The physiological and ecological requirements of *H. crenulatus* have been studied previously, however little is known regarding other aspects of its biology. This study involved an investigation into the population structure of *H. crenulatus* and the breeding biology, based on observations on the incidence of ovigerous females. The gonad index method, accompanied by macroscopic and histological examination of the ovaries and testes also helped to achieve a greater understanding of the reproductive cycle. A study of the brood biology including changes in the egg during ontogeny, length of incubation period in a range of salinities, fecundity and the potential for multiple ovipositions with or without remating or moulting was also investigated. It's apparent ease of survival under laboratory
conditions allowed an investigation into absolute growth and moulting. Wherever possible, throughout this study, questions concerning the biology of *H. crenulatus* resulting from direct observation in the field, have been followed up by experimental procedures in the laboratory.

Chapter One is an investigation into the relative growth and general morphometry of *H. crenulatus*. The purpose of this exercise was to establish the size at which both sexes "moult to maturity" and become sexually mature. This information was pertinent for all subsequent chapters. Chapter Two contains information concerning changes in the population structure over a two year period. Tagging crabs in the field was not possible so a laboratory study of absolute growth was initiated (Chapter Three) while the incidence of moulting in the field provided clues on the timing of the mating season (Chapter Four). A study of the reproductive biology of *H. crenulatus* is described in Chapter Five and includes an investigation into the effect of salinity on egg incubation time. An attempt at rearing zoeae through to the first crab instar in the laboratory has also been included in Chapter Six although this was not particularly successful.

THE STUDY AREA

The study area used in this investigation was the Avon-Heathcote estuarine (43° 33′ S, 172° 44′ E) which is located 16 kilometres from the centre of Christchurch. It's shape is roughly that of an equilateral triangle with the Heathcote river entering the southwest corner, the Avon river entering the northern corner and the permanent outlet to the Pacific Ocean in the southeast (Fig. 1). It is flanked on its three sides by areas of completely different morphological character: to the South, the volcanic mass of Banks Peninsula, to the West, the flat swampy areas of alluvial
silt, peat and dune remnants of the Canterbury Plains and to the East, the sandy Brighton Spit separating the estuary from the South Pacific Ocean (Stephenson 1981).

It is a shallow bar-built estuary enclosing an area of approximately 8 km² which drains a total catchment of 190 km² comprising suburban and industrial land. At low tide, 80% of the estuary is tidal mudflats dissected by river channels (Webb 1972). Hydrology is weather (wind) dominated and well mixed with over 56% tidal exchange per tide (Knox and Kilner 1973, Macpherson 1978).

An extensive ecological survey of the estuary (Knox and Kilner 1973) and other unpublished theses (Estcourt 1962, Voller 1973) describe the abiotic and biotic features of the estuary. Some general trends are apparent from these studies. Salinities within the estuarine triangle are influenced daily by river and tidal flows and seasonally by rainfall and variable discharge. A salinity gradient extends from the mouth to the head of the estuary with salinities being reduced upstream from the mouth by mixing with river water. An intertidal salinity gradient also exists such that during the rising and receding tides, the lower tidal areas experience lower salinities than the high tide regions. Finally, organic matter generally increases from the mouth to the head of the estuary and decrease up the intertidal zone.

Preliminary investigations were undertaken to determine the distribution and abundance of H. crenulatus at various sites within the estuary. This exercise indicated that the source of greatest crab abundance was a small intertidal beach at Rockhouse point, at the beginning of Beachville road (Plate 3).
Figure 1. Map of the Avon-Heathcote estuary. Sampling site is indicated by a star.

Plate 3. Sampling site at low tide at the Avon-Heathcote estuary.
Boulders and stones are scattered along the margins of the mudflats on a more or less uniform substrate which consists of a variable mixture of mud, silt and fine sand. Further up the shore, the muddy substrate gives way to sand which is covered by small stones of a fairly uniform size (6.0 cm x 10.0 cm). Breaking waves are a rare occurrence in this location, and tidal currents are slight so that marine invertebrates require rocks simply for protection from dessication and predators.

Owing to the soft nature of the mud, crabs are able to burrow to a limited extent alongside the rocks or within small ponds of muddy water beneath the rocks.

**PHYSICAL DATA**

Air and water temperature recordings were obtained from Mr. D. Carver (Christchurch Drainage Board) from April 1985 to January/February 1987.

Air temperature was recorded at sewage oxidation pond No. 6 (Fig.1). A temperature probe which is permanently fixed in a channel near my study site continuously records water temperature.

Figures 2 and 3 show that there is a marked but regular seasonal variation in both water and ambient temperature although the ranges are not as extreme as in the air. The maximum mean air and water temperature was recorded in January and minimum temperatures recorded in July.
Figure 2. Monthly air temperature recordings (Carver 1987).

Figure 3. Monthly water temperature recordings (Carver 1987).
Monthly ambient temperature.

Monthly water temperature.
Chapter 1.

Relative Growth.

Introduction.

In the Crustacea, growth occurs intermittently by a series of moults or ecdyses which separate each instar. During the growth process, it is usual for certain body dimensions to increase at rates different from others so that ultimately there is a change in relative proportions in addition to a change in size; this phenomenon has been termed relative or allometric growth (Huxley 1932, Hartnoll 1972, 1978, 1982).

The Brachyura in particular have proved to be excellent subjects for relative growth analysis for the following reasons: the rigidity of the crab exoskeleton is conducive to greater accuracy of measurement, the process of ecdysis enables clear subdivision of ontogeny and finally there are usually wide differences in growth rates between sexes. Although the change in shape is usually progressive through a series of ecdyses, sometimes a particularly marked change can occur at a single moult. Particularly relevant is the moult separating the last immature from the first mature instar, which Perez (1928) designated the pubertal moult, which indicates the size of sexual maturity. Such an estimate is a valuable and necessary tool in the management of commercially exploited species as it specifies the size at which copulation and egg bearing in females is physically possible (Haley 1969, Finney and Abele 1981, Carroll 1982 and Jewett et al 1985).

Morphometric analysis is used to study growth and form (Thompson 1917, Cott 1929) and to compare differences between sexes (Kwei 1978 and Haefner 1985). It is a powerful tool used for detecting maturity of some crabs since it’s onset is marked by appreciable changes in allometric growth which evokes secondary sexual characteristics (Warner 1977,
Hartnoll 1978). The use of the chelae and pleopod allometry for estimating male size at maturity is common, whereas a pronounced change in the abdomen width permits an estimate of physical maturity in females.

Analysis.

Growth of a part of the body (y) relative to another part of the body (x) is best shown graphically. There may be a constant difference between the growth rates of x and y producing a progressive change in shape with growth. This relationship can be described by the power function \( y = ax^b \) which has been termed the allometric growth equation (Huxley 1932). The resulting graph is an exponential curve and growth is positively allometric when \( b > 1.0 \) (y develops faster than x), negatively allometric when \( b < 1.0 \) (y develops slower than x) and when \( b = 1.0 \), growth is isometric (both dimensions increasing at the same rate) (Gould 1966, Hartnoll 1982).

The log transformation of the growth equation is

\[ \log y = \log a + \log x \]

producing a linear relationship which is a straight line relationship when data are plotted on a log-log graph. The transition between the immature and mature phase is accompanied by significant changes in the relative growth of the secondary sexual characters and is marked by the moult to puberty. This critical moult is represented by a sudden change in slope or discontinuity on the growth curve although such a change is not always obvious (Teissier 1960).

This chapter is concerned with an investigation of the general morphometrics and relative growth patterns of *Hemigrapsus crenulatus* to show changes of form with onset of sexual maturity, the size at sexual maturity and to define growth patterns and sexual dimorphism of males and females. Carapace width was used as the reference dimension.
Materials and Methods.

Crabs used in the relative growth and morphometric analysis were collected from the estuary between June 1985 and April 1987 as part of the quantitative population sampling program.

For ease of measurement, the majority of crabs were killed in 10% sea water formalin and sexed in the laboratory. Supplementary morphometric data were obtained from the exuviae of crabs which were used in the absolute growth experiment (Chapter 3). Dimensions larger than 10.0mm CW were measured with manual vernier calipers while an eyepiece micrometer in a stereomicroscope was necessary to measure smaller dimensions. All measurements were accurate to the nearest 0.1mm.

The maximum width of the carapace (CW) was chosen as the reference dimension but other skeletal characters were also measured. These included

CL. Carapace Length. Distance between the frontal margin to the extreme posterior margin of the carapace excluding curvature of the carapace.

RPD LPD. Right and Left Propodus Depth. Distance from the propodus/dactyl (PD) joint to the base of the propodus.

RLD LDL. Right and Left Dactyl Length. Distance from the PD joint to the tip of the dactylus.

RPL LPL. Right and Left Propodus Length. Distance from the PD joint to the propodus/carpus (PC) joint.

MAW FAW. Male and Female Abdomen Width of segment number 5, the penultima segment.

MPL. Male Pleopod Length. Length of first pleopod from point of articulation with the first abdominal segment to the distal tip of
the organ.

FPL. Female Pleopod Length. Length of the exopodite of the second pleopod from point of articulation to the distal tip of the organ. The degree of setation on the exopodite and endopodite was noted.

These positions of measurement are shown in Fig.1.1. Only undamaged body dimensions of intermoult crabs were used in the relative growth analysis.

Data Analysis.

Measurements were grouped into 1mm CW size classes and plotted against carapace width on arithmetic coordinates to determine which relationships were linear. Linear relationships were expressed by the least squares regression \( y = a + bx \).

Relative growth of body parts was quantitatively studied by applying the formula \( y = ax^b \) to the morphometric data. The constant of allometry \( (b) \) was determined for each dimension by logarithmic transformation of all data and subsequent computation of log-log regressions (Simpson, Roe and Lewontin 1960).

In order to determine whether growth was positively or negatively allometric, the slope \( (b) \) was tested against the slope standard of 1.0 by student's t-Test.

Where an inflection in the curve was evident, separate regression lines were fitted to either side and a student t-Test used to determine if the slopes of the two lines were significantly different (Jones 1978).

Male and female regressions of the transformed data were compared by covariance analysis using the BMDP 1v and 1r packages to determine whether there were significant differences in growth of various body dimensions (Haefner 1985).
Figure 1.1 Dimensions (↔ →) measured to analyse relative growth.

A Carapace width.

B Cheliped.
1 Propodus length.
2 Dactylus length.

C Male abdomen width.

D Female abdomen width.

E Male pleopod length.

F Female pleopod length.
1 Exopodite.
2 Endopodite.
Results.

Arithmetic plots of the dactyl length in males and abdomen width in females against the reference measurement (CW), were the only dimensions which indicated an inflection in the slope of the line (Figures 1.2 and 1.3). For males, the inflection occurred between 13.0mm and 15.0mm CW. Separate regression equations were fitted for crabs < 15.0mm and > 13.0mm CW.

A discontinuity in the relative growth of the female abdomen occurred between 9.0mm and 11.0mm CW, therefore regression lines < 11.0mm and >9.0mm CW were fitted accordingly.

Linear constants of the regression equations for relative growth of male and female *H. crenulatus* are shown in Tables 1.1 and 1.2 respectively.

Allometry.

A summary of pre- and post-puberty growth equations, slopes, correlation coefficients and allometric status for male and female *H. crenulatus* are shown in Tables 1.3 and 1.4 respectively. Covariance analyses of these data is shown in Table 1.5.

Carapace Length - Carapace Width.

Males.

The complete relationship between carapace length and carapace width (N=136) was isometric indicating that carapace length grew at the same rate as carapace width. However, division of immature and mature crabs showed that pre-pubertal crabs had isometric growth, which manifested itself into an almost perfect circular shape but after the pubertal moult, the carapace adopted an oval shape due to faster growth in the width of the carapace.

Females.

The complete relationship between carapace length and carapace width
was negatively allometric which ultimately produced an oval shaped carapace.

Cheliped Morphology.

Males.

Of all the measurements, only the chelae dimensions, propodus length, propodus depth and dactylus length exhibited positive allometry both before and after the pubertal moult.

Females.

The rate of growth of the female dactyl length and propodus depth was constant relative to carapace width as is shown by the regression line not being significantly different from 1.0 but propodus length was positively allometric (Table 1.4).

Abdomen Width.

Males.

A regression of the entire data set (N=139) of abdomen width against carapace width revealed negative allometry. However, separation into pre and post puberty growth (< 13.0mm and > 15.0mm CW) indicated a tendency for the abdomen width to grow at the same rate as the carapace width before the pubertal moult. Following maturity, growth of the abdomen was significantly negatively allometric.

Females.

In contrast to the males, the relationship between abdomen width and carapace width was significantly positively allometric throughout development. This results in females having characteristically broad abdomens which completely cover the abdominal somites in mature females.

Pleopod Length.

Males.

Using the entire data set, the rate of growth of the pleopod length was slower relative to carapace width as is shown by the regression line slope being significantly less than 1.0 (Table 1.3). However division
into pre- and post-pubertal growth indicated that the pleopod grew isometrically before puberty and then grew at a significantly slower rate following maturity.

Females.

In contrast to the males, a regression of the entire data set of pleopod length against carapace width revealed significant positive allometry. Growth of the pleopod was rapid before puberty after which isometry prevailed.

Female pleopod length was a particularly difficult variable to measure due to change in the shape of the endopodite and exopodite through maturity. Immature females possessed straight endopodites and exopodites were sparsely setose. As maturity approached, the exopodites became longer, more curved and increasingly setose and the endopodites became more deeply curved (Fig.1.1).

Analysis of covariance showed significant differences in growth between the sexes of all the allometric variables examined. Males have greater allometric constants for all proportions of the chelae and females have a wider abdomen. Chelae and abdomen width were strong sexually dimorphic characters (Table 1.5).

Statistical package BMDPlr often yielded significant serial correlation of the residuals which implied that complex allometry with pre- and post-pubertal growth phases was occurring throughout growth. Furthermore, plots of the detrended normals indicated that the samples were heterogenous. Measurements of crabs with partially regenerated structures could account for this heterogeneity.
Table 1.1. Linear constants of the regression equations for relative grow of the chelae, abdomen, pleopods and legs in male *H. crenulatus*. 
\[ Y = Bx + a \] (B=Slope, a=Constant, \( r^2 \)=Correlation coefficient, 
N=Number of data points).

<table>
<thead>
<tr>
<th>LINE.</th>
<th>B</th>
<th>a</th>
<th>( r^2 )</th>
<th>N</th>
</tr>
</thead>
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<td>CARAPACE LENGTH.</td>
<td>0.843</td>
<td>0.695</td>
<td>0.997</td>
<td>136</td>
</tr>
<tr>
<td>&lt;15.0mm</td>
<td>0.894</td>
<td>0.145</td>
<td>0.992</td>
<td>72</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>0.818</td>
<td>1.308</td>
<td>0.995</td>
<td>79</td>
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<tr>
<td>DACTYLUS LENGTH.</td>
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<td>-2.235</td>
<td>0.962</td>
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<td>-0.742</td>
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<td>72</td>
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<tr>
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<td>ABDOMEN WIDTH.</td>
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<td>&lt;15.0mm</td>
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<td>PLEOPOD LENGTH.</td>
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<tr>
<td>&gt;13.0mm</td>
<td>0.230</td>
<td>1.566</td>
<td>0.955</td>
<td>95</td>
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Table 1.2. Linear constants of the regression equations for relative growth of the chelae, abdomen, pleopods and legs in female *H.crenulatus*.

Y=Bx+a (B=Slope, a=Constant, r²=Correlation coefficient, N=Number of data points).

<table>
<thead>
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<th>LINE.</th>
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<th>a</th>
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<th>N</th>
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<td>0.235</td>
<td>-0.175</td>
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<td>-2.559</td>
<td>0.841</td>
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<td>&gt;11.0mm</td>
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</tr>
<tr>
<td>&lt;13.0mm</td>
<td>0.553</td>
<td>-2.128</td>
<td>0.951</td>
<td>24</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>0.397</td>
<td>-0.195</td>
<td>0.943</td>
<td>118</td>
</tr>
</tbody>
</table>
Table 1.3. Summary of allometric statistics for male *H. crenulatus*.

\[ Y = ax^b \]

(Se(b)=std.error of b, \( r^2 \)=correlation coefficient, N=sample size, A.S=Allometric status).

<table>
<thead>
<tr>
<th>LINE</th>
<th>b</th>
<th>a</th>
<th>Se(b)</th>
<th>( r^2 )</th>
<th>N</th>
<th>t</th>
<th>A.S statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARAPACE LENGTH.</td>
<td>0.960</td>
<td>-0.005</td>
<td>0.043</td>
<td>0.997</td>
<td>136</td>
<td>0.930</td>
<td>0</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>0.978</td>
<td>-0.044</td>
<td>0.015</td>
<td>0.986</td>
<td>58</td>
<td>1.466</td>
<td>0</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>0.932</td>
<td>0.080</td>
<td>0.009</td>
<td>0.993</td>
<td>65</td>
<td>7.55</td>
<td>-ve</td>
</tr>
<tr>
<td>DACTYL LENGTH.</td>
<td>1.301</td>
<td>-1.830</td>
<td>0.015</td>
<td>0.981</td>
<td>136</td>
<td>20.06</td>
<td>+ve</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>1.203</td>
<td>-1.615</td>
<td>0.036</td>
<td>0.952</td>
<td>58</td>
<td>5.638</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>1.450</td>
<td>-2.293</td>
<td>0.044</td>
<td>0.945</td>
<td>65</td>
<td>10.22</td>
<td>+ve</td>
</tr>
<tr>
<td>PROPODUS LENGTH.</td>
<td>1.452</td>
<td>-2.658</td>
<td>0.018</td>
<td>0.963</td>
<td>136</td>
<td>18.833</td>
<td>+ve</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>1.322</td>
<td>-2.387</td>
<td>0.076</td>
<td>0.843</td>
<td>58</td>
<td>4.326</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>1.482</td>
<td>-2.740</td>
<td>0.044</td>
<td>0.909</td>
<td>65</td>
<td>8.310</td>
<td>+ve</td>
</tr>
<tr>
<td>PROPODUS DEPTH.</td>
<td>1.362</td>
<td>-2.188</td>
<td>0.018</td>
<td>0.976</td>
<td>136</td>
<td>20.111</td>
<td>+ve</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>1.275</td>
<td>-1.999</td>
<td>0.048</td>
<td>0.925</td>
<td>58</td>
<td>5.729</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>1.467</td>
<td>-2.513</td>
<td>0.056</td>
<td>0.915</td>
<td>65</td>
<td>8.33</td>
<td>+ve</td>
</tr>
<tr>
<td>ABDOMEN WIDTH.</td>
<td>0.899</td>
<td>-1.308</td>
<td>0.014</td>
<td>0.968</td>
<td>136</td>
<td>7.214</td>
<td>-ve</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>0.954</td>
<td>-1.433</td>
<td>0.049</td>
<td>0.868</td>
<td>58</td>
<td>0.938</td>
<td>0</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>0.780</td>
<td>-0.940</td>
<td>0.024</td>
<td>0.943</td>
<td>65</td>
<td>9.166</td>
<td>-ve</td>
</tr>
<tr>
<td>PLEOPOD LENGTH.</td>
<td>0.961</td>
<td>-1.056</td>
<td>0.017</td>
<td>0.950</td>
<td>153</td>
<td>2.294</td>
<td>-ve</td>
</tr>
<tr>
<td>&lt;13.0mm</td>
<td>1.104</td>
<td>-1.372</td>
<td>0.067</td>
<td>0.825</td>
<td>59</td>
<td>1.552</td>
<td>0</td>
</tr>
<tr>
<td>&gt;15.0mm</td>
<td>0.758</td>
<td>-0.445</td>
<td>0.019</td>
<td>0.950</td>
<td>80</td>
<td>12.736</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Based on testing the slope against a standard of 1.0, \( +ve \) = Positive allometry, \( 0 \) = Isometry, \( -ve \) = Negative allometry, P value for all regression lines < 0.05.
Table 1.4. Summary of allometric statistics for female *H. crenulatus*

\[ Y = ax^b \]

(Se(b)=std. error of b, r²= correlation coefficient, N=sample size, t statistic, A.S=Allometric status).

<table>
<thead>
<tr>
<th>LINE</th>
<th>b</th>
<th>a</th>
<th>Se(b)</th>
<th>r²</th>
<th>N</th>
<th>t</th>
<th>A.S stat</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARAPACE LENGTH</td>
<td>0.975</td>
<td>-0.035</td>
<td>0.004</td>
<td>0.997</td>
<td>128</td>
<td>6.25</td>
<td>-ve</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>0.969</td>
<td>-0.021</td>
<td>0.013</td>
<td>0.991</td>
<td>45</td>
<td>2.38</td>
<td>-ve</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>0.978</td>
<td>-0.043</td>
<td>0.011</td>
<td>0.990</td>
<td>71</td>
<td>1.88</td>
<td>0</td>
</tr>
<tr>
<td>DACTYL LENGTH</td>
<td>1.010</td>
<td>-1.279</td>
<td>0.011</td>
<td>0.983</td>
<td>128</td>
<td>0.90</td>
<td>0</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>1.053</td>
<td>-1.364</td>
<td>0.026</td>
<td>0.973</td>
<td>45</td>
<td>2.03</td>
<td>0</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>0.961</td>
<td>-1.137</td>
<td>0.044</td>
<td>0.870</td>
<td>71</td>
<td>0.87</td>
<td>0</td>
</tr>
<tr>
<td>PROPODUS LENGTH</td>
<td>1.133</td>
<td>-2.121</td>
<td>0.027</td>
<td>0.929</td>
<td>128</td>
<td>4.92</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>1.183</td>
<td>-2.218</td>
<td>0.098</td>
<td>0.769</td>
<td>45</td>
<td>1.85</td>
<td>0</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>0.974</td>
<td>-1.650</td>
<td>0.077</td>
<td>0.697</td>
<td>71</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>PROPODUS DEPTH</td>
<td>1.034</td>
<td>-1.590</td>
<td>0.017</td>
<td>0.965</td>
<td>128</td>
<td>2.00</td>
<td>0</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>0.998</td>
<td>-1.522</td>
<td>0.066</td>
<td>0.838</td>
<td>45</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>1.048</td>
<td>-1.631</td>
<td>0.048</td>
<td>0.871</td>
<td>71</td>
<td>0.99</td>
<td>0</td>
</tr>
<tr>
<td>ABDOMEN WIDTH</td>
<td>1.697</td>
<td>-2.594</td>
<td>0.026</td>
<td>0.961</td>
<td>172</td>
<td>26.80</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>1.643</td>
<td>-2.522</td>
<td>0.065</td>
<td>0.897</td>
<td>73</td>
<td>9.77</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>1.177</td>
<td>-1.084</td>
<td>0.058</td>
<td>0.846</td>
<td>76</td>
<td>3.05</td>
<td>+ve</td>
</tr>
<tr>
<td>PLEOPOD LENGTH</td>
<td>1.423</td>
<td>-2.166</td>
<td>0.034</td>
<td>0.928</td>
<td>132</td>
<td>12.44</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;11.0mm</td>
<td>2.202</td>
<td>-3.826</td>
<td>0.205</td>
<td>0.898</td>
<td>15</td>
<td>5.80</td>
<td>+ve</td>
</tr>
<tr>
<td>&gt;13.0mm</td>
<td>1.010</td>
<td>-0.994</td>
<td>0.027</td>
<td>0.926</td>
<td>109</td>
<td>0.54</td>
<td>0</td>
</tr>
</tbody>
</table>

Based on testing the slopes against a standard of 1, +ve = positive allometry, 0 = isometry, -ve = negative allometry, P value for all regression lines < 0.05.
Table 1.5.
Analysis of Covariance statistics for sexual dimorphism using CW as the covariate.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>DF</th>
<th>F value</th>
<th>Significance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carapace length</td>
<td>2,260</td>
<td>6.076</td>
<td>p=0.0026</td>
<td>Females wider</td>
</tr>
<tr>
<td>Dactylus length</td>
<td>2,260</td>
<td>311.00</td>
<td>p=0.0000</td>
<td>Males longer</td>
</tr>
<tr>
<td>Propodus length</td>
<td>2,260</td>
<td>157.2</td>
<td>p=0.0000</td>
<td>Males longer</td>
</tr>
<tr>
<td>Propodus depth</td>
<td>2,260</td>
<td>270.96</td>
<td>p=0.0000</td>
<td>Males deeper</td>
</tr>
<tr>
<td>Abdomen width</td>
<td>2,304</td>
<td>1665.29</td>
<td>p=0.0000</td>
<td>Females wider</td>
</tr>
</tbody>
</table>
Figure 1.2. Relationship between chela dactyl length and carapace width for male and female *H. cremulatus*. Regression equations are given in Tables 1.1 and 1.2.

Figure 1.3. Relationship between abdomen width and carapace width for both sexes. Regression equations are given in Tables 1.1 and 1.2.
Discussion.

At the outset, it is necessary to clarify the relationship between physical maturity and sexual maturity. Most authors refer to the pubertal moult as the attainment of sexual maturity. Inferences from such a statement can imply one or more of the following:

1) the crab has only just attained ripe gonads, yet gonad development may take place before the crab undertakes its pubertal moult.

2) the female attains an increase in the width of the abdomen which facilitates successful attachment of the eggs, or that their vulvae increase in aperture width to allow entry of the male pleopods, or that the male pleopods increase in size to maximise mating opportunities.

3) that the pubertal moult is marked by changes in allometry of a specific variable.

Thus the term sexual maturity is ambiguous and misleading when used in relative growth analysis as it's meaning encompasses gonadal development and morphological changes. Sexual maturity, in this thesis implies physiological maturity and refers to females which possess ripe gonads and males with fully developed spermatophores in their vas deferens.

It is more useful to apply the term physical maturity when discussing relative growth analysis. A true moult to puberty must result in a dimorphism of the specimens over a certain size range represented by a clear discontinuity in the growth curve and that the differences are not merely the result of allometric growth. In this concept, the pubertal moult refers to the size at which the secondary sexual characters have developed to ensure successful copulation. First copulation usually occurs shortly after the moult to puberty but in some graspsids, e.g. *Pachygrapsus transversus* (Hartnoll 1965) some of the larger pre-pubertal
females have already copulated. This is possible because in grapsids, the
genital apertures are fully developed before puberty. Grapsids which show
that pre-pubertal copulation and ovulation are theoretically possible at
any time include *Cyclograpsus integer*, *Pachygrapsus transversus*,
*Metapaulius depressus*, *Percnon gibbesi* and *Plagusia depressa* (Hartnoll
1965). In *H.crenulatus*, there is not one distinct moult to physical
maturity as is often the case for brachyurans which have a
pubertal/terminal moult e.g *Callinectes sapidus* (Churchill 1919),
*Corystes cassivelauenses* (Hartnoll 1972), *Ebalia tuberosa* (Schembri 1982)
*Maja sinuata* (Carlisle 1957) *Halicarcinus innominatus* (H.Menzies pers
comm) and various other species of spider crabs (Hartnoll 1963, 1965a).
*H.crenulatus* exhibits a change in allometry which may actually take place
over two successive molts but is used as an estimate of physical
maturity.

Hartnoll (1974) stressed that the differences in growth patterns
between the various organs can be correlated with the extent to which
they interact with the other structures in order to function effectively.
The growth sequence of a particular organ is thus determined by its
functional commitments to the reproductive phase of the animal’s life
history. Thus, the change in the growth pattern of the male chelae,
particularly the dactyl length dimension, the male pleopods and of the
female abdomen are a consequence of their reproductive importance. The
four major areas of growth discussed in this section are the carapace,
chelae, abdomen width and pleopods.

The relationship between carapace length and carapace width in females
was negatively allometric before physical maturity which implied that the
carapace grew wider rather than longer. This lateral expansion of the
carapace was associated with the dramatic increase in ovary volume during
oogenesis (Chapter 5). Following puberty, growth was isometric with mature females possessing near circular carapaces.

The relationship between male carapace length and width was isometric before physical maturity and then exhibited strong negative allometry. Male gonads do not require a large internal carapace volume during development, therefore lateral expansion of the carapace would be an excess expenditure of resources.

The chelae of male *H. crenulatus* reached a significantly larger size than that of similarly sized females. The growth of all chelae dimensions was positively allometric relative to carapace width and showed a tendency to become increasingly positive between 13.0mmCW and 15.0mmCW. This was taken to be the size of physical maturity in males. This did not merely involve a simple change whereby the growth of the chelae was increased relative to that of the body for there were simultaneous alterations to the growth rates within the chelae. A distinct moult to maturity was absent as there were no differences great enough to stand out above the normal variations.

The rapid rate of growth of the male chelae parameters is a consequence of the necessity of a larger chelae size to subdue a female before copulation and during agonistic displays among conspecifics (Hiatt 1948, Beer 1959). Although *H. crenulatus* was not observed participating in extensive courtship rituals, threat displays were common. These involved extending both chelae to their greatest width in order to exaggerate their overall appearance. Warner (1970) found that status and dominance hierarchy were determined to a large extent by the size of the chelae. Since growth of the chelae was positively allometric before physical maturity, it is possible that intra-specific combat occurred between males throughout their life.
Laboratory reared male *H.crenulatus* attained physical maturity at the 11th instar after approximately 392 days (Chapter 3).

Females showed a different pattern in chelae growth. The relative growth of the dactyl length and propodus depth was isometric with no significant puberty related changes. This was because the females do not require enlarged chelae for embracing during copulation and may not be involved in intra-specific combat to the extent of the males. The exception was propodus length which was positively allometric.

The growth of the female abdomen showed the characteristic positive allometry of the Brachyura (Lewis 1977, Finney and Abele 1981, Pohle and Telford 1982, Campbell and Eagles 1983, Davidson and Marsden 1987). Females in both the juvenile and adult phases exhibit positive allometry, although the second phase (> 11.0 mm CW) is at a lower level. This decrease in allometry following physical maturity is typical of the Brachyura (Hartnoll 1974). This change was quite pronounced and may occur during one moult to puberty instead of a continuous change in allometry as for the male chelae. This compared well with the size at which egg deposition occurs as the smallest ovigerous female measured 9.0 mm CW.

The high positive allometry of the abdomen width over the pubertal moult served to bring the organ to a functional size and shape to efficiently carry and protect the developing eggs. It also effectively increased the capacity for egg bearing as fecundity was positively correlated to carapace width (Chapter 5) which enhanced the overall reproductive fitness.

Laboratory reared female *H.crenulatus* attained physical maturity at the 9th instar within approximately 292 days (Chapter 3).

Growth of the male abdomen was isometric before physical maturity and then became negatively allometric in relation to carapace width. The
function of the male abdomen is to provide a protective cover for the pleopods (MacKay 1943) consequently it conformed to a narrower shape than in the females.

The male chelae and female abdomen resembled each other in that both exhibited high positive allometry in the pre-puberty phase and a change in allometry in the range of the 'pubertal moult'. These processes served to bring the organ to a functional size at maturity, whilst minimising the waste of resources which would have resulted had they been produced at this large size in the immature instars (Hartnoll 1974).

Following puberty, the relative growth of the male chelae increased to a still higher positive allometry, whereas that of the female decreased to a small positive allometry. Hartnoll (1974) proposed that the reason was because the chelae were independent effectors whose activities were not closely integrated with other organs. Consequently their relative size may advantageously increase further after puberty as the limit is set only by mechanical considerations and utilisation of available resources.

The female abdomen however, must work in conjunction with the sternum to provide an incubatory chamber; and having once attained a sufficient relative size at puberty to accomplish this, any disproportionate increase thereafter would diminish the efficiency of the mechanism, hence the reduction in the level of allometry following puberty.

The male pleopods illustrated a change from pre-puberty isometric growth to significant negative allometric growth in the post-puberty phase. This growth format is typical of many brachyurans (Finney and Abele 1981, Simons 1981). Hartnoll (1974) suggested that negative male pleopod allometric growth was an adaptive feature which reduced the variation in the size of the copulatory organs between mature males of
different carapace widths which ultimately facilitated greater overall sexual compatibility between males and females. An ability to mate with as wide a size range of females as possible would allow each male to maximise his reproductive potential.

Since the male pleopod had a functional relationship with the abdominal cover, it was not surprising that both variables showed the same changes in allometry before and after physical maturity.

Similarly, the functional relationship between the female pleopods and abdomen width in pre-pubertal crabs was clear with the occurrence of positive allometry. Following puberty, the pleopods grew isometrically in relation to carapace width. Reliance on the validity of this result was slightly dubious however, as the exopodites became increasingly curved and setose which reduced the accuracy of measurement.

It was more useful to observe the development of the pleopods through physical maturity. During oviposition, eggs are attached to the endopodite setae and were held in place and protected by densely setose exopodites creating an open but protective brood chamber. The endopodites had begun to kink and had long setae on them by 10.0mm CW which is within the range estimated for female physical maturity. Increasing the number of setae directly increases the number of egg attachment sites and maximises the reproductive potential of each female.

A summary of the size at puberty and maximum sizes attained for some graspid species of both sexes is shown in Table 1.6.

Males usually attain a larger maximum size than females. The resources expended in egg production reduce growth increments, while at the same time, the act of incubation lengthens the intermoult period and these together account for the female growing to a smaller size.
The minimum size at physical maturity of *H. crenulatus* differed between sexes with males entering the post-puberty phase 4mm larger than the females. This could be a consequence of the need for larger chelae to subdue the female during copulation.

In contrast, the other graspsids do not grow as large and the males moult to puberty before the females. *H. crenulatus* male reproductive fitness would be enhanced as they would be able to mate with a greater size range of females.

Table 1.6. Summary of graspid information.

<table>
<thead>
<tr>
<th>Species</th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puberty</td>
<td>Max.size</td>
</tr>
<tr>
<td><em>Aratus pisonii</em></td>
<td>9 to 13</td>
<td>24</td>
</tr>
<tr>
<td><em>Sesarma ricardi</em></td>
<td>8 to 9</td>
<td>16</td>
</tr>
<tr>
<td><em>Pachygrapsus transversus</em></td>
<td>6 to 7</td>
<td>15</td>
</tr>
<tr>
<td><em>Pachygrapsus gracilis</em></td>
<td>5 to 6</td>
<td>19</td>
</tr>
<tr>
<td><em>Cyclograpsus integer</em></td>
<td>5 to 6</td>
<td>11</td>
</tr>
<tr>
<td><em>Sesarma cinereum</em></td>
<td>6.8</td>
<td>19</td>
</tr>
<tr>
<td><em>Sesarma reticulatum</em></td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td><em>Hemigrapsus crenulatus</em></td>
<td>13 to 15</td>
<td>37.5</td>
</tr>
</tbody>
</table>
Chapter 2.

Population Dynamics.

Introduction.

Little is known regarding the population biology of *Hemigrapsus crenulatus* despite its common appearance in estuarine and marine habitats throughout New Zealand. In an unpublished study, Walker (1970) noted an interesting pattern with a predominance of females at the lowest ebb and Jackson (1976) also noted an abundance of females subtidally. The present section documents the pattern of seasonal change with respect to population size and sex ratios and size structure of *H. crenulatus* in the Avon-Heathcote estuary.

Materials and Methods.

Monthly samples of *Hemigrapsus crenulatus* were taken from a study area in the Avon Heathcote estuary (Plate 3) from June 1985 to April 1987. For each sample, a search under ten rocks was made and all specimens collected. Males were recognised by the characteristic shape of their abdomen and in larger specimens by their enlarged chelae while females were identified by their wide abdomens and the presence of four pairs of pleopods (Plate 2). Each crab was assigned to one of the following classes:

1) Male
2) Female
3) Ovigerous female
4) Juvenile (crabs < 4.0mm CW).
Maximum carapace width was measured to the nearest 0.1mm with hand held vernier calipers for crabs > 4.0 mm CW and subsequently released so as not to deplete the population. Initially however, a small sample of crabs were fixed in 10% formalin and returned to the laboratory for relative growth analysis. Crabs < 4.0mm CW were also returned to the laboratory to be sexed and measured using a micrometer eyepiece in a stereomicroscope.

Analysis.

Monthly size frequency histograms were plotted and are presented in Figure 2.1. The size groupings used in the histograms were selected arbitrarily as a convenient method for data presentation.

Analysis of size frequency distributions by cumulative frequency analysis (Harding 1949, Cassie 1950, 1954) has frequently been used to determine the number of normally distributed components and their mean size in crustacean populations (Williams 1978, Simons 1980, Siegel and Wenner 1985). Unfortunately the use of this mathematical model was not possible due to small sample sizes, rapid growth of small individuals, and lack of synchrony in recruitment.

Monthly sample sizes are shown in Table 2.1 in addition to sex ratios (males : females). A chi-square test was used to determine whether these ratios differed significantly from 1:1.

Preliminary sampling revealed that H.crenulatus occupied a narrow muddy zone in the sampling area and predominantly occurred beneath large rocks which were scattered over the substrate. The patchy distribution and large size of some of the rocks made transect quadrat sampling an unsuitable sampling strategy.

The proportions of males, females, ovigerous females and juveniles occurring in each month from June 1985 to April 1987 were calculated as were the sex ratios in each size class.
Results.

Monthly Population Size and Sex Ratios.

Table 2.1 shows the number of male and female *Hemigrapsus crenulatus* caught in each month. During the summer months (November 1985 to March 1986 and December 1986 to March 1987) there was a decrease in the total number of crabs.

The results suggest that the sex ratios followed a definite pattern with males outnumbering the females in Spring and Summer (September to March) followed by a return to equal proportions of both sexes in June and August of both years. The overall sex ratio for the 21 months sampled was 1365 males : 921 females indicating a 1.48 : 1 ratio. The highest proportion of females was recorded in July 1985 (0.68 : 1).

Size Frequency Distribution.

Size frequency histograms (Figure Series 2.1) show that the population comprised crabs between 2.0mm CW and 38mm CW and was dominated by crabs in the post-puberty size classes (12-20mm CW). This intermediate size class range conveyed the typical bell shaped curve to the majority of the monthly histograms.

Growth of small crabs was rapid (Chapter 3), and they were quickly absorbed into the middle size classes (12-20mm) within a year. For example, sampling in November 1986 yielded only 4% males in the 8.0-9.9mm size range which subsequently increased to 10.5% in the 10 -11.9mm size class in the following month, December. In February 1987, the males probably moulted once more and were therefore common in the 12-13.9mm size class range.
Thus the size structure of the population varied monthly with continual growth of the smaller crabs which eventually became obscured by the larger crabs. This was counterbalanced by a continual loss of large males, especially in the post-breeding period from January to April/May in both years. The absence of any skewness in the population histograms confirmed the loss of the larger specimens. The females, however remained in the 12-20mm size class ranges especially in the breeding season when their ovigerous condition could have been responsible for preventing further growth. The largest male found in the Avon-Heathcote estuary was 37.5mm CW and the largest female measured 27.5mm CW.

The breeding season.
The first year sampling program revealed that ovigerous females were evident from June 1985 to February 1986 with a peak in December. Sampling was not undertaken in September and October 1985, however it is highly probable that ovigerous females were present.

Sampling in the second year revealed a similar breeding season which extended 8 months from June 1986 to January 1987 with a high percentage of ovigerous females in October 1986. Ovigerous females of intermediate size (14-20mm CW) formed the majority of the breeding females. The smallest ovigerous female measured 5.0mm CW and the largest measured 26.0mm CW.

Recruitment.
Recruitment into the population occurred asynchronously and varied from month to month. Newly recruited individuals were almost totally absent in the 1985-1986 Summer except for a small peak in February to April. The pattern of recruitment was more obvious the following year when juveniles
were sampled in increasing proportions from October 1986 to April 1987. Therefore recruitment followed the breeding season with the first obvious appearance of juveniles in October, 3 months after the first females became ovigerous.

Table 2.2 shows the total numbers of males and females in each size class over the entire sampling period. Samples are dominated by crabs in the mid size class range.
Table 2.1. Monthly totals and sex ratios of male and female *H. crenulatus* from June 1985 to April 1987 (NS = Not significant, * = significance at the .05 level, *** = significance at the .01 level.)

<table>
<thead>
<tr>
<th>Month</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>Sex Ratio</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 1985</td>
<td>47</td>
<td>56</td>
<td>103</td>
<td>0.84:1</td>
<td>NS</td>
</tr>
<tr>
<td>July</td>
<td>62</td>
<td>90</td>
<td>152</td>
<td>0.68:1</td>
<td>*</td>
</tr>
<tr>
<td>August</td>
<td>75</td>
<td>75</td>
<td>150</td>
<td>1:1</td>
<td>NS</td>
</tr>
<tr>
<td>November</td>
<td>50</td>
<td>28</td>
<td>78</td>
<td>1.78:1</td>
<td>***</td>
</tr>
<tr>
<td>December</td>
<td>61</td>
<td>26</td>
<td>87</td>
<td>2.30:1</td>
<td>***</td>
</tr>
<tr>
<td>January 1986</td>
<td>28</td>
<td>9</td>
<td>37</td>
<td>3.10:1</td>
<td>***</td>
</tr>
<tr>
<td>February</td>
<td>55</td>
<td>27</td>
<td>82</td>
<td>2.03:1</td>
<td>***</td>
</tr>
<tr>
<td>March</td>
<td>49</td>
<td>30</td>
<td>79</td>
<td>1.63:1</td>
<td>*</td>
</tr>
<tr>
<td>April</td>
<td>83</td>
<td>55</td>
<td>138</td>
<td>1.50:1</td>
<td>*</td>
</tr>
<tr>
<td>May</td>
<td>90</td>
<td>57</td>
<td>147</td>
<td>1.57:1</td>
<td>***</td>
</tr>
<tr>
<td>June</td>
<td>67</td>
<td>64</td>
<td>131</td>
<td>1.04:1</td>
<td>NS</td>
</tr>
<tr>
<td>July</td>
<td>74</td>
<td>50</td>
<td>124</td>
<td>1.48:1</td>
<td>*</td>
</tr>
<tr>
<td>August</td>
<td>66</td>
<td>72</td>
<td>138</td>
<td>0.91:1</td>
<td>NS</td>
</tr>
<tr>
<td>September</td>
<td>99</td>
<td>48</td>
<td>147</td>
<td>2.06:1</td>
<td>***</td>
</tr>
<tr>
<td>October</td>
<td>74</td>
<td>73</td>
<td>147</td>
<td>1.01:1</td>
<td>NS</td>
</tr>
<tr>
<td>November</td>
<td>85</td>
<td>54</td>
<td>139</td>
<td>1.57:1</td>
<td>***</td>
</tr>
<tr>
<td>December</td>
<td>61</td>
<td>17</td>
<td>78</td>
<td>3.50:1</td>
<td>***</td>
</tr>
<tr>
<td>January 1986</td>
<td>48</td>
<td>25</td>
<td>73</td>
<td>1.92:1</td>
<td>***</td>
</tr>
<tr>
<td>February</td>
<td>48</td>
<td>15</td>
<td>63</td>
<td>3.20:1</td>
<td>***</td>
</tr>
<tr>
<td>March</td>
<td>63</td>
<td>14</td>
<td>77</td>
<td>4.50:1</td>
<td>***</td>
</tr>
<tr>
<td>April</td>
<td>80</td>
<td>36</td>
<td>116</td>
<td>2.22:1</td>
<td>***</td>
</tr>
<tr>
<td>Totals</td>
<td>1365</td>
<td>921</td>
<td>2286</td>
<td>1.48:1</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 2.2. Total numbers of male and female *H. crenulatus* and sex ratios in each size class (NS Not significant, * at .05 level, *** at .01 level).

<table>
<thead>
<tr>
<th>Size class (mm CW)</th>
<th>Males</th>
<th>Females</th>
<th>Totals</th>
<th>Sex Ratio</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles</td>
<td>49</td>
<td>20</td>
<td>69</td>
<td>2.45:1</td>
<td>***</td>
</tr>
<tr>
<td>4-5.9</td>
<td>53</td>
<td>38</td>
<td>91</td>
<td>1.39:1</td>
<td>NS</td>
</tr>
<tr>
<td>6-7.9</td>
<td>59</td>
<td>45</td>
<td>104</td>
<td>1.31:1</td>
<td>NS</td>
</tr>
<tr>
<td>8-9.9</td>
<td>64</td>
<td>42</td>
<td>106</td>
<td>1.52:1</td>
<td>*</td>
</tr>
<tr>
<td>10-11.9</td>
<td>120</td>
<td>45</td>
<td>165</td>
<td>2.66:1</td>
<td>***</td>
</tr>
<tr>
<td>12-13.9</td>
<td>162</td>
<td>138</td>
<td>300</td>
<td>1.17:1</td>
<td>NS</td>
</tr>
<tr>
<td>14-15.9</td>
<td>182</td>
<td>174</td>
<td>356</td>
<td>1.04:1</td>
<td>NS</td>
</tr>
<tr>
<td>16-17.9</td>
<td>152</td>
<td>183</td>
<td>335</td>
<td>0.83:1</td>
<td>NS</td>
</tr>
<tr>
<td>18-19.9</td>
<td>130</td>
<td>132</td>
<td>262</td>
<td>0.99:1</td>
<td>NS</td>
</tr>
<tr>
<td>20-21.9</td>
<td>86</td>
<td>57</td>
<td>143</td>
<td>1.50:1</td>
<td>*</td>
</tr>
<tr>
<td>22-23.9</td>
<td>69</td>
<td>42</td>
<td>111</td>
<td>1.64:1</td>
<td>*</td>
</tr>
<tr>
<td>24-25.9</td>
<td>50</td>
<td>8</td>
<td>58</td>
<td>6.25:1</td>
<td>*</td>
</tr>
<tr>
<td>26-27.9</td>
<td>53</td>
<td>1</td>
<td>54</td>
<td>53:1</td>
<td>***</td>
</tr>
<tr>
<td>28-29.9</td>
<td>54</td>
<td>0</td>
<td>54</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>30-31.9</td>
<td>28</td>
<td>0</td>
<td>28</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>32-33.9</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>34-35.9</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>36-37.9</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Totals</td>
<td>1300</td>
<td>905</td>
<td>2205</td>
<td>1.43:1</td>
<td>***</td>
</tr>
</tbody>
</table>
June 1985 N=103.

July N=152

August N=180.
November N=139.

December N=78.

January 1987 N=73.
February N=63.

March N=77.

April N=116.
Figure 2.2

Monthly percentages of male, female, ovigerous female and juvenile *H.crenulatus* from June 1985 to April 1987.
Monthly Population Samples

Discussion.

*Hemigrapsus crenulatus* occurred predominantly in the lower reaches of the intertidal zone in shallow depressions beneath rocks where the mud was saturated with water. Often as many as forty specimens could be found beneath one rock (approx. 45cm x 40cm x 35) in addition to a smaller population of the anomuran *Petrolisthes elongatus*.

Thus *H.crenulatus* achieved high population densities, especially in the Winter months, however densities decreased during Summer with a significant loss of females from the population. In other species, this decrease in female numbers has been attributed to seasonal changes in temperature, daylength and availability of food resources which accelerates breeding activity and provides the cue for the females to migrate further down shore (Pillay and Ono 1978). Other suggestions for these short term migrations are to ensure that the developing brood is kept moist (Boolootian et al 1959), to ensure the zoeae are released into salinity concentrations within their tolerance range (Jones 1981) or to redistribute the crabs periodically in a manner that adjusts densities to food resources (Aspey 1978).

Transect sampling within the sampling area was not possible so definitive results regarding the distribution of ovigerous *H.crenulatus* could not be made. However during the Summer months, all ovigerous females occurred under rocks at the very lowest reaches of the sampling area and were always bathed in water. These results suggest that some short term downshore migrations were occurring either to ensure brood immersion in water or that zoeae were released into favourable conditions.
As a general rule, estuarine populations appear to have biased sex ratios (Wenner 1972) with both females (Wildish 1970, Jones and Naylor 1971, Fish and Fish 1978, Jones 1978) and males (Jones 1974, 1980, Begg 1980, Carroll 1982) being favoured. Nye (1977) proposed that there was a general tendency for female grapsids to outnumber males as in *Cyclograpsus punctatus* (Broekhuysen 1941), *Pachygrapsus crassipes* (Hiatt 1948), *Hemigrapsus edwardsi* (Wear 1970) and *Helice crassa* (Nye 1977) but that differences were only significant if a large sample was considered.

When *H.crenulatus* were sorted into 2mm size classes, it was found that males were significantly more numerous than females except in the middle size classes. Equality in these size ranges may have resulted from the tendency of reproductively active females to defer somatic growth thereby causing them to accumulate in the L2 to 18mm CW size intervals (Colby and Fonseca 1984).

Disparity of sex ratios in crustaceans may also be the result of sex reversal or sexual differences in life span, migration and mortality (Dillery and Knapp 1970, Leigh 1970, Moly 1970, Wenner 1972, Winget et al 1974, Swartz 1976). The effects of differential migration and differential mortality remain obscure in *H.crenulatus* however sex related differences in growth (Chapter 3), are likely to be responsible for males dominating the largest size classes.

Benthic invertebrates with large numbers of eggs usually have long larval lives with many larval instars and high mortality (Thorson 1950). Furthermore, semi-terrestrial crabs such as *Sesarma pictum* (Pillay and Ono 1978) have restricted breeding seasons whilst low intertidal crabs such as the grapsids *Hemigrapsus nudus, H.oregonensis* (Boolootian et al 1959), *H.penicillatus* (Pillay and Ono 1978) have extended breeding seasons and are capable of producing successive large batches of small
eggs. Such crabs are known as r-selectionists (Stearns 1976). *H. crenulatus* could be considered a typical example as it’s reproductive output is large (Chapter 5) and it also has an extended breeding period (8-9 months) which produces asynchronous recruitment patterns.

Recruitment into the population was minimal in the first year of sampling (February to April’86) which was probably a consequence of the previous lack of ovigerous females. Recruitment within the second sampling year was stronger with juveniles first appearing in October and in successive months until April 1987 when the sampling program was terminated.

Throughout the study, juveniles were never really common. Their scarcity in the collections may have been due to the combination of small size and cryptic coloration which made them difficult to collect in quantity. Alternatively these small stages may have a different habitat from larger crabs and may not be restricted to the intertidal regions as much as the adults (Dell 1968a). In fact, juveniles were only ever recovered from the dense algae (*Ulva* sp.) which sometimes surrounded the rocks at very low tide.

Recruitment may be restricted by larval mortality regulated by changes in environmental salinity and temperature such that larval settlement is successful only when physico-chemical conditions are optimal. Other factors such as predation and dispersal of the larvae will also modify recruitment patterns. Furthermore, the life cycle of an animal is usually timed by some environmental variables so that the offspring are produced at a period favorable for their survival (Giese 1959). Recruitment in *H. crenulatus* occurs in late Spring and Summer when temperatures increase and therefore growth is faster and also when phytoplankton blooms occur in the Avon-Heathcote estuary (Schmidt 1971).
Chapter 3

Absolute growth.

Introduction.

In the Crustacea, growth occurs by a series of moults which separate the instars. The rate of growth is determined by the increase in size at each moult (growth increment) and the time interval between successive moults (intermoult period). The terms used in the analysis of absolute growth are often ambiguous. In this thesis, growth increment refers to the absolute increase in size at each moult and percentage growth factor is synonymous with the percentage growth increment.

Early descriptions of growth increments in Brachyura are fairly common (Brooks 1886, Fowler 1909, Pearson 1908) and it was soon discovered that smaller individuals had larger percentage size increments than larger individuals. This relationship is true of grapsids (e.g. *Cyclograpsus punctatus* (Broekhuysen 1941) *Pachygrapsus crassipes* (Hiatt 1948), *Aratus pisonii* (Hartnoll 1965), *Pachygrapsus marmoratus* (Vernet-Cornubert 1958a), *Sesarma cinereum* and *Sesarma reticulatum* (Seiple and Salmon 1987) and of other crabs (*Ovalipes punctatus* (Du Preez and McLachlan 1984), *Cancer magister* (MacKay and Weymouth 1936)) but not of *Callinectes sapidus* (Churchill 1918, Gray and Newcombe 1938).

Descriptions of intermoult period are less common. The tagging method involves the assumption that intermoult period is unaffected and there also difficulty in ascertaining whether specimens have moulted more than once. The alternative and perhaps most reliable method is direct observation of captive crabs over a long time period. However the fact that intermoult period and also growth increment in some species are

Various approaches have been employed in the quantitative description and analysis of crustacean growth and have recently been reviewed by Botsford (1985). Most methods require the collection of both intermoult period and growth increment data in order to generate a description of absolute growth in terms of size and age.

The Hiatt growth diagram relates postmoult to premoult size and the regressions obtained are used to define growth constants (Hiatt 1948). However, this method assumes that the percentage growth factor at successive mouls remains constant. Mauchline (1976) suggested that it was more accurate to fit the non-linear moult data with a hyperbola. Somerton (1980) questioned the advisability of employing a hyperbola and instead fitted several straight lines to the data.

A plot of percentage growth factor and intermoult period versus premoult size provides a more realistic and informative description of growth (Mauchline 1977). The slope defines the rate at which the percentage increment declines with size and so has a major bearing on the maximum size a species can attain. The regression equations obtained are used to generate growth curves relating size and age.

The difficulty of obtaining intermoult period and growth increment data in the field makes laboratory studies essential although increments may be smaller than those in the wild (Hartnoll 1982). Direct observation allows accurate assessment of both parameters for successive mouls.
Therefore, growth predictions can be made beyond the size range of the original observations.

Within Crustacea, there is a great diversity in the patterns of growth. Four patterns have been proposed by Hartnoll (1982):

1) Indeterminate growth: Moulting continues indefinitely after puberty with no obvious terminal ecdysis.
2) Determinate growth: a) With variable instar number and maturity occurring before the final instar.
   b) With variable instar number and maturity delayed until the final instar.
   c) With constant instar number and maturity delayed until the final instar.

The classification of determinate growth requires some mechanism to inhibit further moulting and to maintain the state of terminal ecdysis. Although a fixed number of mature instars is frequently reported, it is often caused by seasonal mortality rather than a physiological incompetence for further moulting (Hartnoll 1982).

The aim of this section was to investigate whether Hemigrapsus crenulatus had determinate or indeterminate growth and whether it conformed to the prevalent brachyuran growth pattern of a reduction in percentage growth factor and an increased intermoult period with increasing size. Hypothetical growth curves generated from successive moult data of a few small individuals of both sexes will show the number of instars required reach the size of physical maturity and the age at maturity. It was also of interest to determine whether the observed intermoult periods of larger crabs did conform to one of the four growth formats proposed by Hartnoll (1982).
Materials and Methods.

Crabs (n=90) ranging in size from 3.0mm to 31.0mm carapace width (males) and 2.4mm to 25.0mm C.W (females) were collected from the Avon Heathcote estuary in February 1986. An attempt was made to separate the crabs into 2.0 mm size groupings with 3 to 4 crabs of each sex within each group. Crabs < 20.0mm CW were each placed in a small clear plastic container (6cm x 4cm x 3.5cm) which had gauze (mesh size 1.0mm) fitted to both ends to allow water movement. Crabs > 20.0 mm CW were maintained in larger plastic containers (12cm x 6cm x 5cm) with punched holes. Each container had a small stone for ballast and approximately thirty containers were placed in each of three large tanks (60cm x 40cm x 30cm) which had continual water circulation. Tanks were cleaned at monthly intervals.

These crabs were fed approximately three times a week for a year on mussel (Perna canaliculata) and each specimen was checked at least four times a week to record any moulting activity. Newly moulted crabs were measured two days after moulting to allow hardening of the exoskeleton. Carapace width was the reference measurement and secondary sexual characters of immature crabs were also measured for relative growth analysis (Chapter 1). When deaths occurred, replacement crabs were approximately the same size and sex.

Analysis.

Mauchline's method (1977) was chosen as the most convenient to describe the absolute growth of Hemigrapsus crenulatus. Percentage growth factor was defined as the percentage increase in carapace width at each moult. Least squares linear regressions were obtained from plots of growth
increment, percentage growth factor and log percentage growth factor against premoult carapace width. Similarly intermoult period (in days) and log intermoult period were plotted against premoult carapace width. Intermoult periods were recorded only after each crab had moulted once. This decreased the risk of recording a shortened intermoult period due to collection between successive moult periods or due to stress (Hiatt 1948, Sweat 1968, Childress and Price 1978).

The size of the crab and length of intermoult period at each instar were calculated from the regression equations thus allowing estimations of the number of moult periods to reach puberty and to reach maximum size.

The statistical packages 'Statistix' and BMDP 1r and 1v were employed to compute the linear regressions and the analysis of covariance for a total of 282 growth increment and 170 intermoult data points. The latter test was used to determine whether regression slopes were significantly different for males and females.
Results.

All captive crabs moulted at least once during the year (except for two males greater than 32.0mm CW) with the frequency of moulting decreasing with increasing crab size. Smaller crabs moulted more frequently and some individuals moulted through eight or nine instars to nearly reach the size of maturity within the year (see appendix).

Growth Factor.

Regression analysis for both sexes of growth increment, percentage growth factor and log percentage growth factor against premoult carapace width are shown in Table 3.1. Slopes of all regressions were significantly different from zero at 0.01 level (t-test).

Plots of male and female growth increment are shown in Figures 3.1 and 3.2 respectively. The positive slope of the regression line in both sexes indicates that actual growth increment increases with increasing size.

The negative slope of the percentage growth factor regressions (whether transformed or not) indicates an overall decrease in the percentage increment with size.

However, when the moult records for 118 males and 161 females were grouped into 2 mm CW intervals and the mean carapace width, mean absolute growth increment and mean percentage growth factors calculated, a more accurate picture of the growth sequence in males (Table 3.2) and females (Table 3.3) was obtained.

There is an increase in the percentage growth factors up to the size of maturity (13.5 mm CW males, 9.0mm CW females) then a decrease with the decline being more pronounced in the females.

Analysis of covariance revealed that males had significantly greater growth increments than females (Table 3.5).
Table 3.1. Absolute Growth Regression Analysis.

Growth Increment and Percentage Growth Factor.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Range CW (mm)</th>
<th>N</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Value/Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>2.8-32.7</td>
<td>119</td>
<td>G.Inc = 0.127 cw + 0.445</td>
<td>0.71</td>
<td>17.25 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G.Fac = -0.277 cw + 20.96</td>
<td>0.12</td>
<td>4.05 **</td>
</tr>
<tr>
<td></td>
<td>(males &lt; 13.5mm CW)</td>
<td></td>
<td>G.Fac = 0.385 cw + 17.81</td>
<td>0.11</td>
<td>5.4 **</td>
</tr>
<tr>
<td></td>
<td>(males &gt; 13.5mm CW)</td>
<td></td>
<td>G.Fac = -0.290 cw + 21.01</td>
<td>0.00</td>
<td>1.5 N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Log G.F = -0.006 cw + 1.30</td>
<td>0.12</td>
<td>4.00 **</td>
</tr>
<tr>
<td>F</td>
<td>2.4-27.6</td>
<td>163</td>
<td>G.Inc = 0.086 cw + 1.02</td>
<td>0.37</td>
<td>9.74 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G.Fac = -0.572 cw + 25.03</td>
<td>0.31</td>
<td>8.52 **</td>
</tr>
<tr>
<td></td>
<td>(females &lt; 9.0mm CW)</td>
<td></td>
<td>G.Fac = -0.505 cw + 23.29</td>
<td>0.01</td>
<td>0.75 N</td>
</tr>
<tr>
<td></td>
<td>(females &gt; 9.0mm CW)</td>
<td></td>
<td>G.Fac = -0.800 cw + 28.94</td>
<td>0.43</td>
<td>2.51 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Log G.F = -0.015 cw + 1.42</td>
<td>0.34</td>
<td>9.17 **</td>
</tr>
</tbody>
</table>

Intemoult Period.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Range CW (mm)</th>
<th>N</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Value/Signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>3.0-35.2</td>
<td>69</td>
<td>I.P = 6.78 cw - 10.05</td>
<td>0.84</td>
<td>19.04 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(males &lt; 13.5mm CW)</td>
<td>I.P = 4.38 cw + 10.29</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(males &gt; 13.5mm CW)</td>
<td>I.P = 12.14cw - 109.01</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Log I.P = 0.033 cw + 1.26</td>
<td>0.80</td>
<td>16.49 **</td>
</tr>
<tr>
<td>F</td>
<td>2.9-27.6</td>
<td>55</td>
<td>I.P = 6.324 cw - 9.70</td>
<td>0.66</td>
<td>10.32 **</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(females &lt; 9.0mm CW)</td>
<td>I.P = 0.375 cw - 36.52</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(females &gt; 9.0mm CW)</td>
<td>I.P = 10.04 cw - 64.34</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Log I.P = 0.036 cw + 1.31</td>
<td>0.63</td>
<td>9.89 **</td>
</tr>
</tbody>
</table>
Table 3.2. Summary of mean growth increments and mean percentage growth factor per size class in male *H. crenulatus*.

<table>
<thead>
<tr>
<th>Size (mmCW)</th>
<th>Crab No.</th>
<th>Size $(\bar{x})$</th>
<th>Growth Increment $(\bar{x})$</th>
<th>P. Growth Factor $(\bar{x})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3.9</td>
<td>13</td>
<td>3.37</td>
<td>0.54</td>
<td>16.39</td>
</tr>
<tr>
<td>4-5.9</td>
<td>18</td>
<td>4.47</td>
<td>0.89</td>
<td>18.70</td>
</tr>
<tr>
<td>6-7.9</td>
<td>12</td>
<td>7.0</td>
<td>1.31</td>
<td>18.98</td>
</tr>
<tr>
<td>8-9.9</td>
<td>10</td>
<td>8.95</td>
<td>1.79</td>
<td>20.09</td>
</tr>
<tr>
<td>10-11.9</td>
<td>9</td>
<td>10.75</td>
<td>2.06</td>
<td>19.97</td>
</tr>
<tr>
<td>12-13.9</td>
<td>11</td>
<td>12.67</td>
<td>2.23</td>
<td>20.30</td>
</tr>
<tr>
<td>14-15.9</td>
<td>11</td>
<td>14.81</td>
<td>2.70</td>
<td>18.27</td>
</tr>
<tr>
<td>16-17.9</td>
<td>8</td>
<td>16.80</td>
<td>2.70</td>
<td>16.06</td>
</tr>
<tr>
<td>18-19.9</td>
<td>9</td>
<td>18.91</td>
<td>2.48</td>
<td>13.15</td>
</tr>
<tr>
<td>20-21.9</td>
<td>6</td>
<td>20.95</td>
<td>2.81</td>
<td>13.37</td>
</tr>
<tr>
<td>22-23.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24-25.9</td>
<td>2</td>
<td>25.5</td>
<td>3.65</td>
<td>14.31</td>
</tr>
<tr>
<td>26-27.9</td>
<td>3</td>
<td>26.63</td>
<td>4.13</td>
<td>15.49</td>
</tr>
<tr>
<td>28-29.9</td>
<td>4</td>
<td>28.62</td>
<td>3.75</td>
<td>12.97</td>
</tr>
<tr>
<td>30-31.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>32-33.9</td>
<td>1</td>
<td>32.7</td>
<td>4.30</td>
<td>13.14</td>
</tr>
</tbody>
</table>
Table 3.3. Summary of mean growth increments and mean percentage growth factor per size class in female *H. crenulatus*.

<table>
<thead>
<tr>
<th>Size (mm)</th>
<th>Crab No.</th>
<th>Size (x)</th>
<th>Growth Increment (x)</th>
<th>Percentage Increment (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3.9</td>
<td>3</td>
<td>3.23</td>
<td>0.60</td>
<td>19.97</td>
</tr>
<tr>
<td>4-5.9</td>
<td>8</td>
<td>5.36</td>
<td>0.88</td>
<td>16.54</td>
</tr>
<tr>
<td>6-7.9</td>
<td>17</td>
<td>7.18</td>
<td>1.47</td>
<td>20.34</td>
</tr>
<tr>
<td>8-9.9</td>
<td>12</td>
<td>9.15</td>
<td>2.05</td>
<td>22.32</td>
</tr>
<tr>
<td>10-11.9</td>
<td>17</td>
<td>11.24</td>
<td>2.29</td>
<td>20.43</td>
</tr>
<tr>
<td>12-13.9</td>
<td>22</td>
<td>12.96</td>
<td>2.19</td>
<td>16.86</td>
</tr>
<tr>
<td>14-15.9</td>
<td>28</td>
<td>14.86</td>
<td>2.40</td>
<td>16.22</td>
</tr>
<tr>
<td>16-17.9</td>
<td>22</td>
<td>17.00</td>
<td>2.78</td>
<td>16.33</td>
</tr>
<tr>
<td>18-19.9</td>
<td>15</td>
<td>18.76</td>
<td>2.58</td>
<td>13.75</td>
</tr>
<tr>
<td>20-21.9</td>
<td>10</td>
<td>20.49</td>
<td>2.64</td>
<td>12.87</td>
</tr>
<tr>
<td>22-23.9</td>
<td>3</td>
<td>22.33</td>
<td>2.50</td>
<td>11.21</td>
</tr>
<tr>
<td>24-25.9</td>
<td>4</td>
<td>24.62</td>
<td>2.22</td>
<td>9.02</td>
</tr>
<tr>
<td>26-27.9</td>
<td>1</td>
<td>27.6</td>
<td>2.50</td>
<td>9.05</td>
</tr>
</tbody>
</table>

Estimation of numbers of instars to reach maturity and maximum size.

Separate regression lines were fitted to both pre-puberty and post-puberty data on percentage growth factor versus premoult size (Table 3.1) and the equations were used to calculate the number of instars to reach maturity and to reach the maximum size.

The smallest male found measured 3.0mm and it is assumed that this crab
would be in its 2nd crab instar on the basis of the length of the 5th zoeal instar. This size was substituted into the pre-puberty equation, the percentage growth factor calculated and the resulting value added to 3.0 mm to obtain the carapace width of the next instar. Calculation of the first mature carapace width was then substituted into the post-puberty regression equation.

The smallest female measured 2.4 mm however the same starting point of 3.0 mm CW was used to ensure comparability of growth curves. Table 3.4 shows the iterations involved to determine the number and size successive instars in the growth of male and female *H. crenulatus* from the percentage growth factor regressions.

It is estimated that males attain maturity in their 11th instar, and females in their 9th instar (assuming that a size of 3.0 mm is the 2nd cranial instar). Furthermore, males pass through 19 instars to reach their maximum size (largest male caught was 37.5 mm CW) while females moult a total of 1 times (largest female measured 27.1 mm CW).

**Intermoult Period.**

Regression analysis of intermoult period and log intermoult period against premoult size are also shown in Table 3.1. All regressions were significant at the 0.01 level.

Plots of intermoult period against premoult carapace width are shown in Figures 3.3 and 3.4 for males and females respectively. The positive slope of the lines indicate increasing time between moults as size increased. Prepuberty regression equations revealed an initial zero slope with no significant relationship between intermoult period and carapace width in female crabs (t-tests). There was however a positive correlation between the variables in post-puberty females. An increasing slope from pre-puber
to post-puberty growth was also observed in the males although the relationship was significantly positive throughout.

Analysis of covariance revealed no significant differences in the intervals between moults of males and females (Table 3.5). Nearly all the females were not ovigerous in the laboratory and therefore their intermoult intervals were probably decreased. However, in the field this would not be the case.

Estimation of age at sexual maturity, life span and intermoult period at each instar.

A similar procedure was used to calculate age at each moult by substituting a starting point of 3.0mmCW into pre-puberty and post-puberty regression lines (Table 3.4).

This method indicated that male *H.crenulatus* reach maturity after approximately 392 days, a little over a year and live for nearly five years if they reach the maximum size.

Females reach maturity after 292 days and take approximately 3.5 years to reach their maximum size (Table 3.4). However, this laboratory data may well be an underestimate since ovigerous females were not included.

The size and number of instars, together with the age at each instar were used to plot a hypothetical growth curve for both male and female *H.crenulatus* (see Figure 3.5).

The curves are sigmoid, with the slope of the curve increasing initially, then declining following puberty. The point at which the sexes diverge is 13.0mm.
Table 3.4. Summary of information used to formulate growth curves in *H. crenulatus*. (P.G.F, percentage growth factor; I.P, intermoult period.)

<table>
<thead>
<tr>
<th></th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.W</td>
<td>P.G.F</td>
<td>I.P</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>18.06</td>
<td>22</td>
</tr>
<tr>
<td>3.54</td>
<td>18.11</td>
<td>24</td>
</tr>
<tr>
<td>4.18</td>
<td>18.16</td>
<td>27</td>
</tr>
<tr>
<td>4.93</td>
<td>18.23</td>
<td>30</td>
</tr>
<tr>
<td>5.82</td>
<td>18.30</td>
<td>34</td>
</tr>
<tr>
<td>6.88</td>
<td>18.39</td>
<td>38</td>
</tr>
<tr>
<td>8.14</td>
<td>18.50</td>
<td>43</td>
</tr>
<tr>
<td>9.64</td>
<td>18.63</td>
<td>49</td>
</tr>
<tr>
<td>11.43</td>
<td>18.78</td>
<td>56</td>
</tr>
<tr>
<td>13.57</td>
<td>17.06</td>
<td>65</td>
</tr>
<tr>
<td>15.88</td>
<td>16.40</td>
<td>55</td>
</tr>
<tr>
<td>18.48</td>
<td>15.66</td>
<td>83</td>
</tr>
<tr>
<td>21.37</td>
<td>14.82</td>
<td>115</td>
</tr>
<tr>
<td>24.53</td>
<td>13.90</td>
<td>150</td>
</tr>
<tr>
<td>27.93</td>
<td>12.90</td>
<td>188</td>
</tr>
<tr>
<td>31.53</td>
<td>11.86</td>
<td>230</td>
</tr>
<tr>
<td>35.52</td>
<td>10.78</td>
<td>273</td>
</tr>
<tr>
<td>39.34</td>
<td>-</td>
<td>322</td>
</tr>
</tbody>
</table>

18 instars  4.9 yrs.     15 instars  3.5 yr
Table 3.5. Analysis of Covariance statistics for growth increment and intermoult period using premoult size as the covariate.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>DF</th>
<th>F value</th>
<th>Significance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermoult period</td>
<td>2, 120</td>
<td>1.034</td>
<td>NS</td>
<td>No dif. in sex</td>
</tr>
<tr>
<td>Growth Increment</td>
<td>2, 278</td>
<td>6.424</td>
<td>p=.001</td>
<td>males larger</td>
</tr>
<tr>
<td>Growth Factor</td>
<td>2, 278</td>
<td>4.778</td>
<td>p=.009</td>
<td>males larger</td>
</tr>
<tr>
<td>Log Growth Factor</td>
<td>2, 278</td>
<td>8.149</td>
<td>p=.000</td>
<td>males larger</td>
</tr>
</tbody>
</table>
Figure 3.1. Relationship between absolute growth increment and premoult carapace width in male *H. crenulatus*. Regression equations given in Table 3.1.

Figure 3.2. Relationship between absolute growth increment and premoult carapace width in female *H. crenulatus*. Regression equations given in Table 3.1.
Figure 3.3. Relationship between intermoult period and premoult carapace width for male *H. crenulatus*. Regression equations given in Table 3.1.

Figure 3.4. Relationship between intermoult period and premoult carapace width for female *H. crenulatus*. Regression equations given in Table 3.1.
Intermoult Period vs Premoult Size.

Moies N=71

Intermoult Period (days)

Premoult Carapace Width (mm)

260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
4

Intermoult Period vs Premoult Size.

Females N=53

Intermoult Period (days)

Premoult Carapace Width (mm)

260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
4
Figure 3.5. Hypothetical growth curves for male and female *H. crenulatus*. 
Hypothetical growth curves

Females    Males

Carapace Width (mm)

0 250 500 750 1000 1250 1500 1750 2000
Discussion.

Methods of studying absolute growth include collecting recently moulted individuals together with their cast integuments (e.g. as for *Carcinus maenas* (Hogarth 1975), *Hemigrapsus sanguineus* (Kurata 1962) and *Pachygrapsus crassipes* (Hiatt 1948)), tagging and recovering crabs in the field, or obtaining increments and intermoult period from captive specimens in the laboratory.

The first two methods were neither feasible nor possible so the results obtained for intermoult period and increments of captive *H. crenulatus* must be accepted. It is appreciated however that the effects of captivity on the growth increment can be both appreciable and variable. For example, captive specimens generally have smaller increments than wild specimens (MacKay and Weymouth 1935, Drach 1939, Hiatt 1948, Kurata 1962, Sweat 1968) and the period of confinement can also play a major role (Vernet-Cornubert 1958b). Clearly the effect varies and depends on the conditions of maintenance and the adaptability of the species.

Despite this, analysis of absolute growth in *Hemigrapsus crenulatus* has indicated that both male and female adults conform to the prevalent condition in Crustacea, that is, a general decrease in the percentage growth factor with an increase in size. Other grapsids which undergo a similar growth format include *Cyclograpsus punctatus* (Broekhuysen 1941), *Pachygrapsus crassipes* (Hiatt 1948), *P. marmoratus* (Vernet-Cornubert 1958b), *Hemigrapsus sanguineus* (Kurata 1962), *Aratus pisonii* (Hartnoll 1965, Warner 1967), *Sesarma cinereum* and *S. reticulatum* (Seiple and Salmon 1987). Generally, intermoult period lengthens with increasing size and *Hemigrapsus crenulatus* was no exception although it must be accepted that intermoult periods in the laboratory are likely to be longer than in the
wild (Hartnoll 1982).

The maximum size of a crustacean is determined by both percentage growth factor and intermoult period and the slopes of the log percentage growth factor regressions have a major bearing on the size that a species can attain. In general, species with a shallow slope reach a larger maximum size. Furthermore, a steep slope in the log intermoult regressions indicates that the mouls become infrequent while the crustacean is still small and thus will tend to limit the maximum size attainable.

The slope of log percentage growth factor in male *H. crenulatus* (-0.006) is steeper compared to other crab records of regression slopes (e.g. *Carcinus maenas* slope - 0.0034 Maximum CW 50mm Crothers 1967; *Pisa tetraodon*, slope -0.002, max. CW 45mm Vernet-Cornubert 1960, *Carcinus mediterraneus*, slope -0.002, max CW 50mm Veillet 1945). The steeper slope means that *H. crenulatus* does not grow as large, maximum recorded size was 37.5mm CW.

Absolute growth analysis revealed that the effect of sex and maturity on growth increment was significant. In both male and female *Hemigrapsus crenulatus*, the percentage growth factor increases with size up to the onset of sexual maturity and then declines much more rapidly. The point at which the growth curves diverge between sexes has previously been used to determine the size of maturity (Grey and Newcombe 1938). Using this criteria, *Hemigrapsus crenulatus* should be mature at approximately 13.0mm CW. Relative growth analysis does validate this conclusion with regard to males however females become mature between 9.0 and 11.0mm CW. A decrease in percentage increment also occurred at sexual maturity in *Pachygrapsus crassipes* (Hiatt 1948), *Cancer magister* (Butler 1961, Wilder 1953), *Hemigrapsus sanguineus* (Kurata 1962) and for the lobster *Homarus*
americanus (Templeman 1936, Ennis 1972).

The decline in percentage growth factor in *H. crenulatus* is much more pronounced in females. It has been suggested that with the onset of maturity, a large amount of energy must be used to nourish the developing oocytes. Consequently, the rest of the body of the female will be undernourished as compared with the male due to the energy allocated to reproduction rather than growth (Kurata 1962). However, exceptions do exist, for example, if the development of the ovary does not occur, (possibly due to parasitism) the adult female can sustain moulting and growth comparable to the adult male e.g. *Cambaroides japonicus* and *Hemigrapsus sanguineus* (Kurata 1962). Furthermore, Kurata (1962) found that a change in the growth pattern of *Hemigrapsus sanguineus* occurred independently of actual maturation of the ovary.

Unfortunately, the sample size was insufficient to allow sacrifice of any of the captive *H. crenulatus* females to determine whether ovarian development had occurred. Therefore it is not known whether the decline in percentage growth factor can be directly attributed to reproduction in the females. However the fact that females did not grow as large as the males and the production of a brood by a few of the captive females does suggest that energy allocation for reproductive purposes was the most likely cause.

The effect of sex and maturity also affects the intermoult period. After maturity is attained in both sexes, the intermoult periods become increasingly longer. Larger animals find moulting more expensive in terms of energy resources than smaller ones. In female brachyurans such as *Cyclograpsus punctatus* (Broekhuysen 1941), *Rhithropanopeus harrisi* (Turoboyski 1973) and *Cancer pagurus* (Bennett 1974), the intermoult periods are further prolonged due to the egg-bearing periods when
moulting is inhibited. The overall result is that post-puberty females usually moult less frequently and consequently grow more slowly and hence reach a smaller size than males. Such a pattern was observed in female *H. crenulatus* despite the absence of an incubation period. This suggests that egg-bearing may not be the sole reason for decreased female growth rate but this point requires clarification and was not within the scope of this thesis.

Both growth increment and intermoult period are affected not only by size, sex and maturity but also by various external factors such as temperature, salinity, light and food supply and internal factors such as endogenous rhythms, parasitism and loss of limbs. The extent to which these factors exert an influence is not identical for the two aspects of the crustacean growth. Unfortunately, it was not possible to discern the effects of temperature, salinity and light on the growth of *H. crenulatus* as these variables had to be maintained at a constant level throughout the experiment.

Deficiency in diet, either in terms of quantity and quality of food tends to decrease growth increment and increase intermoult period as shown in *Panulirus longipes* (Chittleborough 1975) and *Carcinus maenas* (Klein-Breteler 1975b). It is unlikely that food supply affected the growth of *H. crenulatus* in this study since they were fed liberally every two days and often some food remained at the next feeding period, so it assumed that they were satiated.

A loss of appendages results in the reduction in increment (Travis 1954, Kurata 1962, Fielder 1964) since part of the energy resources must be diverted to the regenerating limb and also since the animal may be handicapped by it's loss. A few *H. crenulatus* self-autonomized their chelae which may have resulted in a decrease in food uptake and
ultimately reduce growth increments. The effect of loss of appendages can involve either a lengthening or shortening of the intermoult period but depends on the number of appendages lost and whether the loss occurs early or late in the intermoult period (Hartnoll 1982).

During the course of the study, it was noted that some crabs collected in the population samples were parasitised by epicarid isopods which are thought to extend the moulting frequency in Brachyura (Veillet 1945). They were found in a large sack which covered the hepatopancreas in dorsal view. Unless *H. crenulatus* specimens were killed and dissected, it was impossible to know, a priori, which crabs were infected.

This analysis has permitted reasonable estimates of maximum size and maximum life span of both male and female *H. crenulatus*. Males pass through a total of 19 instars after 4.9 years whilst females pass through 16 instars after 3.5 years. These estimates include one more instar to account for growth from the megalopa stage.

Unfortunately there are very few estimates of life span in grapsids. Male *Pachygrapsus crassipes* pass through 25 instars to their maximum size of 47mm CW after approximately 2.8 years while females of the same species pass through 28 instars to reach a maximum carapace width of 44mm CW in 2.8 years; the prolonged ovigerous intermoults slow the growth of females relative to that of males (Hiatt 1948).

There is a general trend for grapsids to have smaller growth increments than sub-tidal crabs (Hartnoll 1965) which is thought to be due to their semi-terrestrial existence. Consequently they are subjected to increased risk from desiccation and probably predation. Thus the advantage of a large size increase at each moult is sacrificed for greater need of rapid hardening of the new integument. Males become sexually mature at a slightly larger size than females, but then growth continues after
puberty and the largest males are always slightly larger than females of corresponding age, resulting from more frequent moult and larger increments. *Hemigrapsus crenulatus* however has a larger range of percentage increments, females reach maturity before males and males attain a much greater size (see Table 3.6 for summary of grapsid growth information).

The growth format of *H. crenulatus* probably follows an indeterminate pattern due to the shape of the growth curves for both sexes. The curves are sigmoid, with the slope of the curve increasing initially then beginning to decline. However, the growth curve still has a significant positive slope even at maximum size. With growth curves of this type, some species reach a much larger size than others (Table 3.6) because of the different rate of change of increment and intermoult with size.

Within *H. crenulatus*, the form of the growth curve differs between the sexes. This is a familiar pattern with similar growth between the sexes up to maturity but subsequently slower growth in females due to smaller increments and possibly longer inter-moult associated with egg production and incubation or at least preparation for the latter. Hartnoll (1982) suggested that the maximum size is not determined by the most obvious mechanism of a cessation of moult at a particular size but by the continuous operation of growth regulating mechanisms active at a specified level from early post-larval life. In this way, species may still extend their period of moult, yet maintain a body size appropriate to their ecological niche. Such an adaptive feature is likely to be advantageous because moult does perform other functions besides growth, such as damage repair by regeneration, the removal of metabolites and the preparation for incubation.
There is no indication that a terminal anec dysis occurs in Hemigrapsus crenulatus, nor has it been reported in any other grapsid crabs (Hartnoll 1965). In the largest specimens which had self-autonomised appendages, new limb buds were developing instead of the formation of a hard calcareous layer at the breakage plane which is common in crabs which undergo a terminal anec dysis. All the evidence gained from this study of absolute growth in H.crenulatus conforms to the indeterminate growth format with mortality from various causes tending to set an upper limit to the size reached.
<table>
<thead>
<tr>
<th>Grapsid species</th>
<th>Author</th>
<th>Percentage increment</th>
<th>Size at maturity</th>
<th>Maximum Size</th>
<th>Percentage increment</th>
<th>Size at maturity</th>
<th>Maximum Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aratus pisoni</td>
<td>Hartnoll 1965</td>
<td>3-10</td>
<td>9-13</td>
<td>24</td>
<td>3-10</td>
<td>15-17</td>
<td>23</td>
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<tr>
<td>Cyclograpsus punctatus</td>
<td>Broekhuysen 1941</td>
<td>4-21</td>
<td></td>
<td></td>
<td></td>
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<td>9-20</td>
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<tr>
<td>Cyclograpsus ineger</td>
<td>Hartnoll 1965</td>
<td>5-6</td>
<td>11</td>
<td>7</td>
<td></td>
<td></td>
<td>10</td>
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<tr>
<td>Pachygrapsus crassipes</td>
<td>Hiatt 1948</td>
<td>8-15</td>
<td>12</td>
<td>47</td>
<td>15</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Pachygrapsus verrucosus</td>
<td>Vernet-</td>
<td>5-15</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pachygrapsus transversus</td>
<td>Cornubert 1958a</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pachygrapsus gracilis</td>
<td>Hartnoll 1965</td>
<td>6-7</td>
<td>15</td>
<td>7-8</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Sesarma ricordi</td>
<td>Hartnoll 1965</td>
<td>5-6</td>
<td>19</td>
<td>6-8</td>
<td></td>
<td></td>
<td>17</td>
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<tr>
<td>Hemigrapsus crenulatus</td>
<td>Present</td>
<td>13-20</td>
<td>13-15</td>
<td>37</td>
<td>9-22</td>
<td>9-11</td>
<td>26</td>
</tr>
<tr>
<td>Sesarma reticulatum</td>
<td>Seiple, Salmon '87</td>
<td>8.8</td>
<td>11</td>
<td>20*</td>
<td>8.1</td>
<td>10</td>
<td>24*</td>
</tr>
<tr>
<td>Sesarma cinereum</td>
<td>&quot;</td>
<td>8.2</td>
<td>6.8</td>
<td>20*</td>
<td>10.5</td>
<td>8.5</td>
<td>20*</td>
</tr>
</tbody>
</table>

* represent estimated maximum sizes shown on reference graphs.
Chapter 4.

Moulting.

Introduction.

The act of moulting is a critical stage in the life history of crustaceans. If external physical factors such as temperature and salinity are not adequate, the individual may be unable to complete its moult and will die (Conan 1985). Synchrony of moulting with favorable environmental conditions would seem to be a beneficial mechanism enhancing survival.

A seasonal pattern of moulting has emerged within the Crustacea, especially in those species which also exhibit a seasonal incidence of mating, spawning and egg incubation.

Growth and reproduction are antagonistic processes. Growth requires energy resources before ecdysis, and to complete tissue growth afterwards, and these resources will not be available for egg production. In addition, the acquisition if these resources may incur mortality and the act of ecdysis itself also involves a major risk; both prevent further reproductive participation. Nevertheless, larger females generally produce more eggs (Hines 1982) so that further growth can be advantageous in terms of overall reproductive success.

However, reproduction can never enhance growth, but must always restrict it. Resources are required for ovary maturation, the female intermoult may be prolonged by egg incubation and incubatory behaviour may restrict foraging.

Since the reproductive cycle must be phased with seasons in order that young may hatch when food and environmental conditions are adequate, the
moult cycle should be phased with seasonal fluctuations of the natural environment (Conan 1985).

A seasonal pattern of moulting does not imply that all individuals will moult during each moult period. Instead, each individual can prepare to moult according to its age and state of maturity and when a moult is possible, it will tend to take place during a moult season (Conan 1985). Seasonality of moulting has been recorded for a number of grapsids with a peak moulting incidence usually occurring before and after a breeding season.

Variables such as temperature (Dall 1965, Jefferies 1964) and photoperiod (Bishop and Herrnkind 1976, Benayoun and Fowler 1980) have been proposed as factors affecting the seasonality of moulting. Crab size is another factor as larger crabs moult less frequently. In some crabs, post puberty moulting is limited by a definite terminal anec dysis which is mediated by distinct changes in the endocrine system (Carlisle 1957, Hartnoll 1972). The terminal anec dysis may not immediately follow the pubertal moult e.g Carcinus maenas (Broekhuysen 1936, Carlisle 1957), Portunus sanquinolentus (Ryan 1967). However in many crabs, especially the hymenosomatids (Hartnoll 1963; 1965a), the pubertal moult is the same as the terminal moult so there is only one mature instar, though one in which a number of ovulations may occur.

The aim of this section was to determine whether a moulting season existed for Hemigrapsus crenulatus as this might provide clues as to when copulation occurred and whether moulting occurred within or after the breeding season.
Materials and Methods.

The crabs collected in the population samples (Chapter 2) and those used in the absolute growth experiment (Chapter 3) were used to establish a field and laboratory moulting season for *Hemigrapsus crenulatus*.

Field Study.

Crabs can be assigned to one of three stages of the moult cycle. If a membranous layer is present under the exoskeleton, the crab is in the intermoult/early premoult stage of the moult cycle (Hiatt 1948, Passano 1960). If the crab is soft or has no membranous layer present, the crab is termed postmoult. An obvious new carapace beneath the old one places the crab into the premoult category. The premoult and postmoult categories can be grouped together as crabs exhibiting moulting activity.

However it was not deemed necessary to excise a section of carapace for every crab collected in the field as such a procedure would soon deplete the population. Intermoult ('hard') and postmoult ('soft') crabs were easily recognisable without dissection and premoult crabs could be identified by the formation of a split at the base of the abdomen. Crabs used in the gonad index calculation (N=35 each month) were examined in greater detail by excision of the carapace.

Laboratory study.

Monthly incidence of moulting of crabs kept separately and maintained in large tanks was noted. Monthly mortality was also recorded. The temperature of the water was relatively constant at approximately 15-17 celcius.
Results.

The incidence of male and female *Hemigrapsus crenulatus* undergoing moulting in the field and in the laboratory is shown in Figures 4.1 and 4.2 respectively.

Field Study.

There appears to be an annual moulting cycle for female *H. crenulatus*, with a peak in the percentage of moulting females occurring each year in April. Thus moulting occurs approximately two months after the last ovigerous female was recorded and two months before the beginning of the breeding season in June. During the breeding season, frequency of moulting and consequently growth in females is considerably retarded.

The moulting season of the males is less distinct than that of the females but a similar smaller peak in percentage moulting occurred in March and May 1986 followed by a period of low moulting activity.

Laboratory Study.

Both male and female *H. crenulatus* showed a higher moulting frequency in the laboratory and exhibited two peaks in moulting activity during the year at approximately the same time.

The first increase in moulting activity for both sexes was observed in March, three months after the appearance of the last ovigerous female in the laboratory, although moulting in male *H. crenulatus* was at a higher intensity than in females.

In September, nearly 50% of the captive females moulted and the following month, a similar peak in the percentage of males undergoing ecdysis was also observed.
Moulting.

Moulting commenced with rupture along the carapace/abdominal hinge (N=5 individuals). During the next five minutes, the crab would pitch slowly forwards as more of the new soft carapace appeared at the slit. The new abdomen was then withdrawn and finally the legs, and chelipeds. Actual active emergence from the carapace took approximately one minute but this period increased with increasing size of the individuals (e.g 30.0 mm CW, 2.5 minutes).

The first sections to harden were those portions of the walking legs which were subject to autonomy, followed by the anterior areas of the carapace. Knudsen (1959) suggests that these parts of the crab’s body are those which are frequently attacked by predators; unless fed, captive hard-shelled *H.crenulatus* would often incapacitate their soft shelled counterparts by removing their walking legs or chelae.
Figure 4.1. Monthly moulting activity in the field in male and female \textit{H.crenulatus}.

Figure 4.2. Monthly moulting incidence in the laboratory for male and female \textit{H.crenulatus}.
Seasonal Moulting Incidence.

Field Study.

Seasonal Moulting Incidence.

Laboratory Study.

Percentage molting.
Discussion.

Differences were obtained in the moulting season of *Hemigrapsus crenulatus* in that under natural conditions an annual moulting period just prior to the breeding season (April) was observed in the females, but in the laboratory both sexes showed a high incidence of moulting in April. Laboratory crabs moulted again just after the breeding season in late September.

The possible explanations for the absence of a second moulting peak in the field are numerous. Decapods are known to be vulnerable to predation and cannibalism during moulting (Reaka 1976, Botsford and Wickham 1978, Laughlin 1982) and it is generally assumed that they seek spatial and temporal refugia during ecdysis until their carapace hardens, they regain motility, and they can defend themselves adequately (Lipcius and Herrnkind 1982). Furthermore, crabs may remain in premoult condition for a number of days yet only those actually in the process of shedding their carapace were detected within the sampling time period. Thus the data on moulting frequencies must be interpreted with caution as the field samples might not actually reflect the prevalence with which newly moulted and premoult crabs *H. crenulatus* actually exist.

Fewer crabs were found in the population samples in Summer (Chapter 2) and it could be that during the higher temperatures, *H. crenulatus* (especially females) move downshore into the cooler water which might explain the apparent absence of a second moult.

An example of habitat partitioning for moulting purposes is exemplified by *Callinectes sapidus* (Hines, Lipcius and Haddon 1987). The males utilized the tidally influenced proportion of the main tributary as a moulting habitat and it is thought that this selection confers three
advantages for the crab. Firstly there may be an osmotic advantage to moulting in reduced salinities because of the need to take up large quantities of water to expand the exoskeleton immediately after ecdysis. Secondly, moulting in the creek habitat may minimise mortality due to predation and cannibalism during the vulnerable period and finally, by aggregating to moulт, crabs may gain an advantage in acquiring minerals for recalcification by consuming cast exoskeletons (Vigh and Dendinger 1982).

Temperature has been proposed as one factor that affects the seasonality of moulting. Cold temperatures slow down crustacean metabolism (Jefferies 1963, Dall 1965) and moulting is depressed below certain thresholds (Aiken and Waddy 1976). Moulting may even be inhibited by rising temperatures e.g in *Cancer irroratus* (Haefner and Van Engle 1975) however it is doubtful that temperatures were sufficiently high in August and September to inhibit moulting in the field but perhaps the winter temperatures (August x=7.5°C, September x=11.0°C) were sufficiently low to reduce moulting activity in *H. crenulatus*.

In the present study, *H. crenulatus* were maintained in the laboratory at a relatively high constant temperature (16-18°C) throughout the year, consequently metabolism proceeded at a faster rate. A shift in time of the peak ovigerous period was evident due to the higher temperatures with the majority of laboratory females carrying eggs in August (cf October in the field).

Most female Brachyura remain in intermoult while vitellogenesis proceeds in the ovaries. It is not until oviposition and release of the young that the crab enters the early premoult stage which is characterised by quick withdrawal of stored calcium from the hepatopancreas (Adiyodi 1968). Following release of the young,
H. crenulatus moulted between 5 to 54 days later (Chapter 5). The high incidence of moultng in September signifies the termination of the breeding season for some females. Others however were successful in producing another brood after a moult or two broods within a moult cycle (see Chapter 5). This latter phenomenon which requires high energy reserves is similarly present in other graspsids, Hemigrapsus penicillatus (Pillay and Ono 1973), Helice crassa (Nye 1977) and Sesarma cinereum (Salmon and Seiple 1987), Cyclograpsus integer (Hartnoll 1985).

Seasonality of moultng has been recorded for a number of New Zealand graspsids with a peak moultng incidence usually occurring after a breeding season. Cyclograpsus lavauxi shows an increase in moultng activity from January to April approximately 2 months after the last ovigerous female was recorded (Begg 1980). Female Leptograpsus variegatus moult in November and December before becoming gravid and again during February after egg hatching (Trenery 1984). Little conclusive information was obtained by Begg (1980) about moultng in Hemigrapsus edwardsi except that there is an absence of moultng crabs during the ovigerous season (April to August) when berried fema'es are unable to moult. In December, the greatest number of soft female's was recorded although it was uncertain whether a moultng peak occurred before this time. Finally moultng was observed in Plagusia chabrus from May to September which is the period just before the mating season (Almarzah 1985).

A similar pattern of seasonal moultng occurs in graspsids overseas. Cyclograpsus granulosus adults moult at the beginning and end of the summer breeding season (August, then again between January and April) (Griffin 1969). In Brachynotus spinosus, moultng occurs only at the end of a breeding season with a peak around February for females and around April for males (Griffin 1969). Broekhuysen (1941) found that wild and
captive *Cyclograpsus puctatus* moults frequently but most often during spring (September and November) and summer (January and April). Another grapsid *Pachygrapsus crassipes* also moults several times during the year but with a post-breeding summer peak between June and October (Hiatt 1948).

It can be concluded that grapsids generally confine their moulting activity to the periods just before and after the breeding season which results in seasonality of moulting. The laboratory moulting pattern of *H. crenulatus* is similar to that of *Helice crassa* in that *H. crassa* females moulted in late summer between February and April 1971 but more than half of these females also moulted between November and December 1970. Nye (1977) hypothesized that the difference may be due to sex but as the females were mostly smaller than the males, the difference could have been due to size as smaller crabs moult more frequently than large crabs (Hartnoll 1982). Seasonality of moulting in relation to size could not be determined in the present study. This was because immature crabs were not replaced every month, therefore even though they moulted several times throughout the year (Chapter 3 and appendix), they eventually grew into the "mature" category so their continual moulting became obscured.

Often mating seasons are established in relation to peak moulting periods. In all species, the males copulate only while in the fully hardened condition but in some species the female must be soft shelled (Hartnoll 1969). Consequently the mating season is obvious as moulting and mating occurs more or less simultaneously. All grapsid crabs are thought to mate in the hard shelled condition (Hartnoll 1969, Warner 1977) with the exception of *Pachygrapsus crassipes* (Hiatt 1948). However even in this case the female is hard enough to actively respond to a male (Bvobjerg 1960). Female *H. crenulatus* moults prior to the males which
suggests that *H. crenulatus* conforms to the typical grapsid mating pattern with males copulating with females in the hard shelled condition (Yaldwyn 1966). Therefore establishing a moulting season does not necessarily indicate the mating season in *Hemigrapsus crenulatus* as copulation is possible at any time with both sexes mating during the intermoult cycle.

Most brachyurans are capable of storing sperm for long periods of time. However it is more feasible that mating would occur when the females possess ripe ovaries, just prior to the breeding season, in preference to mating well beforehand with risks to sperm viability and possible energy expended by the female in maintaining a ripe ovary. Therefore even though *H. crenulatus* was never observed mating in the field or in the laboratory, it seems reasonable to suggest that male *H. crenulatus* would mate in June when the ovarian index is high (Chapter 5) and throughout the breeding season with non-ovigerous females. This would account for the multiple ovipositions by some females later in September and October which ultimately extended the breeding season.

Events in the moulting cycle are sometimes obvious. Prior to shedding the old carapace, calcium is withdrawn from it, causing it to become brittle. Another sign of approaching ecdysis is the change in carapace colour from dark to light brown (Turoboyski 1973). These features were not readily observed in *H. crenulatus*, however imminent moulting was characterised by the absence of feeding as is often observed in other Brachyura.
Chapter 5.

Reproductive Biology.

Introduction.


Of the nine grapsid crabs occurring in New Zealand, only one, the estuarine mud crab *Helice crassa*, has been the focus of much research including both the North and South Island populations (Wear 1970a, Nye 1977, Jones 1980 and Jones and Simons 1983). The breeding biology of other New Zealand Grapsids has received less attention with only records of ovigerous females, predictions concerning brood incubation intervals and a mention of the likelihood of multiple ovipositions (Dell and Marshall 1967, Wear 1970 and Bacon 1971a, 1971b, Begg 1980, Trecery 1984, Almarzah 1985).

Published information on the reproductive biology of *Hemigrapsus crenulatus* is limited. Thomson and Anderton (1921) noted ovigerous females in January and February in Otago Harbour. Wear (1970) collected ovigerous females in Auckland in October 1964 and again in Wellington in October and November 1967 and recorded size of freshly deposited eggs. The five zoeal stages have been described (Wear and Fielder 1985).
The reproductive cycles of Crustacea have been studied by various authors making use of different methods. These include the percentage ovigerous females present, staging brood development, gonad index, appearance of ripe gametes and the histology of the gonad (Boolootian et al 1959, Heydorn 1968, 1969, Pillay and Nair 1971, Haley 1975, Pillay and Ono 1978, Subramoniam 1979, MacFarlane and Moore 1981, Schlagmann et al 1986). This section involves experimental and natural habitat observations of mating systems, multiple ovipositions, gonad development, brood development, incubation intervals and fecundity to achieve understanding of the reproductive cycle of *Hemigrapsus crenulatus* in the Avon-Heathcote estuary.
Materials and Methods.

Breeding Cycle

Information regarding the breeding cycle of *H. crenulatus* was obtained by collecting crabs of both sexes at monthly intervals and recording sex, size, and whether females were ovigerous. Brood development was staged according to Table 5.1. Each month, a sample of approximately fifteen post-puberty males and females (as defined in Chapter 2) were killed in 10% formalin and returned to the laboratory.

All ovigerous females collected were brought back live to the laboratory for observations on egg incubation and embryological development. Both ovigerous and some non-ovigerous females were maintained in a re-cycled sea water aquarium system under a 12:12 hour photoperiod at a salinity of approximately 36.0ppt and temperature of 16° celsius. A soft (mud and sand mixture) substrate was provided in each aquarium to facilitate the attachment of the eggs to the pleopods (Edwards 1966). Crabs were fed chopped mussel (*Mytilus edulis* or *Perna canaliculus*) at least twice a week.

Gonad Index and Development.

A gonad index was determined monthly for at least ten post-puberty individuals of each sex. Specimens which were not in a healthy condition or lacked a full complement of limbs or had recently moulted, were not included in the sample. Each crab *CW* was measured to 0.01mm and wet body weight recorded to the nearest 0.01g.

The gonads were carefully dissected out and weighed to the nearest 0.01g. Ovarian developmental stage was recorded (Table 5.2) and presence
of spermatophores was noted. The wet weight of the gonad was then divided by the wet weight of the entire body and this factor was multiplied by 100 to give a gonad index. The mean values for both sexes in each month were calculated to obtain the average reproductive condition of the population. Low gonad index values denoted inactivity of the gonad, high values indicated the onset of the breeding season and a subsequent precipitous fall in the values suggested a spawn out condition. This method was also useful for indicating the possibility of production of successive broods during the same breeding season.

Histology

In species possessing considerable nutritive tissue in the gonads, both an increase and a decrease in gonad index may actually be a consequence of changes in the number or mass of nutritive cells without a corresponding change in gametogenic tissue (Moore 1937, Pearse 1969).

Samples of gonad tissue used in the gonad index calculation were also used for histological purposes. Gonads were fixed in 10% formalin for at least 24 hours and then dehydrated through a series of 50%, 70%, 90% and 100% absolute alcohol at fixed time intervals of 30, 90, 15 and 15 minutes respectively. After remaining in a terpineal solution for up to six hours, samples were infiltrated with melted wax over at least three changes and imbedded. Sections of gonad material were cut at seven microns and stained with Ehrlich’s haematoxylin and eosin. The five developmental stages of female gonad tissue are shown on Plates 5a to 5e in addition to mature spermatozoa in the male (Plates 5f, 5g).
Brood Biology.

Incubation intervals

Captive crabs which were known to have extruded a brood recently and ovigerous females collected from the field with stage 1 eggs (Table 5.1) were used to determine incubation intervals in a range of salinities.

Forty four ovigerous females were separated into three groups and transferred to one of the following salinities: 11, 18 and 36ppt. Salinities were prepared by adding re-reated distilled water to pasteurised sea water with 0.2cc penicillin. Each female was isolated in a glass jar containing 350mls of filtered sea water under a 12/12 hour light cycle at a constant temperature of 15° celcius. Survival was monitored daily, the water was changed at two day intervals and the crabs were fed at three day intervals.

A sample of eggs (n=30 approx.) was plucked at random every three to four days until the eggs either hatched or were aborted. Between three and six eggs were removed at random and the brood was assigned to one of four categories according to stage of egg development. Egg size was measured using a micrometer scale in a stereo microscope to an accuracy of 0.001mm along two perpendicular axes. Although slightly oval, egg volume was calculated as for a sphere from the formula \( \frac{1}{6}\pi l^3 \) (where \( l \) is the mean of the short and long diameters).

The mean volume of those eggs classified in the same developmental stage was recorded every third day to determine the overall mean egg volume. Multiple regression analysis and analysis of variance were used to determine whether salinity affected egg volume.

Student’s t-test was used to determine whether there were differences in incubation intervals and egg volumes in the various salinities. At the end of the incubation period, egg hatching was recorded, zoeae removed
and measured and some larvae were used in larval rearing trials (Chapter 6).

**Multiple Ovipositions**

Spent females (n=20), used in the incubation experiments were maintained in communal tanks without males to determine whether a second brood would be produced prior or subsequent to moulting. Intervals between releasing the first brood to extruding the second brood or moulting were recorded.

Spent females (n=20) were also maintained in a tank with males to investigate their outcome of multiple oviposition success when there was an opportunity for mating. Observations on copulatory activity were made.

**Fecundity**

Decapod fecundity estimates are made by either counting mature ova (pre-spawning estimate) or number of eggs released (post-spawning estimate). For the latter, volumetric (Warner 1967, Diaz et al 1983) or gravimetric (Somerton and Meyers 1983, Somerton and MacIntosh 1985, Bycroft 1986) methods may be used. However, the accuracy of the results is greatly dependant on how well the eggs are freed from the setae and adhering material (Choy 1985).

Twenty five ovigerous females with a full complement of limbs, ranging from to 9.0mm to 26.0mm CW carapace width were used. The carapace width and wet weight of each female was recorded before snipping off pleopods with the attached eggs and placing them in a sieve of sufficient mesh size to retain the eggs. The sieve was then immersed in household sodium hypochlorite bleach solution (4% available chlorine) at concentrations between 75% and 85%. At random intervals, the pleopods were shaken until
all the eggs were freed. The eggs were subsequently rinsed in distilled water. A subsample of 500 eggs was counted from each brood under a stereomicroscope to be used as a reference weight and then the total egg sample was dried to a constant weight at 60° celcius. An estimate of fecundity was obtained by dividing the weight of the entire egg mass by the weight of the subsample and multiplying by 500. Regression equations of fecundity on carapace width were obtained using Statistix package.
Table 5.1  Stages of egg development.

Based on field collections and laboratory observations of incubated eggs.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Egg mass ruddy orange to brick red or yellow in colour. Newly deposited eggs completely filled with yellow or brown yolk globules, a white polar body may be seen. No cellular cleavage.</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Cellular cleavage apparent at the animal pole, clear tissue cap visible but no eye pigment. Sponge is yellow, brick red in colour.</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Bright red or purple eye pigment visible, yolk reduced, some abdomen and limb development, no chromatophores.</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Egg sponge grey or mauve in colour, colourless embryo with large purple or black eye pigment. Yolk is small and bilobed, abdomen and limbs are well developed and moving. Heartbeat is regular, red and black chromatophores are obvious. Hatching is imminent at this stage.</td>
</tr>
<tr>
<td>Stage</td>
<td>Colour of ovary</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>1 Undeveloped</td>
<td>Colourless, white or ivory</td>
</tr>
<tr>
<td>2 Slight</td>
<td>White, ivory.</td>
</tr>
<tr>
<td>3 Moderate</td>
<td>Pale yellow.</td>
</tr>
<tr>
<td>4 Developed</td>
<td>Yellow, orange or brick red.</td>
</tr>
<tr>
<td>5 Well developed. Bright orange or brick red.</td>
<td>Fully mature ovary nearly obscures the hepatopancreas in dorsal view. Only a small portion of the hepatopancreas and the coiled midgut caeca are visible between the ovary and branchial chamber. Individual oocytes large (up to 1mm in diameter), yolky. Subsequent to this stage, the eggs are spawned onto the pleopods.</td>
</tr>
</tbody>
</table>
Results.

Breeding Period.

Ovigerous *H. crenulatus* females were sampled from June 1985 to February 1986 and again in June 1986 to January 1987. The breeding period spanned 8-9 months through late winter, spring and early summer. Over 50% of the female population were ovigerous by October 1986 (Chapter 2).

The long length of the breeding season suggested that:
1) there could be multiple broods or
2) single broods but asynchronous breeding.

Gonad Index and Development.

Figure (5.1) shows the monthly mean gonad index values for male and female *H. crenulatus* during the 1986/1987 season.

Females.

Fig (5.2) shows the monthly ovarian developmental stages which contributed to the calculation of the gonad index.

Ovaries were either undeveloped or spent in February but a proportion of the females had well developed ovaries by May indicating an increase in the gonad index. This proportion increased over successive months until September when a large majority of females had mature ovaries; they contributed to the high gonad index value in September (x=6.4).

Following September, females with undeveloped or spent ovaries reappeared in the population (causing the gonad index to decrease) indicating the onset of oogenesis but other females still had maturing ovaries. By February 1987, however the ovarian cycle was complete and
females possessed either undeveloped or slightly developed ovaries. They contributed to the low gonad index value of 0.12.

The results indicate that oogenesis was completed within 16 weeks and that there was a possibility of multiple ovipositions by some females during one breeding season.

Males.

The male gonad index remained relatively constant as there was no marked increase in size of the testes and vas deferens relative to body weight. There were however changes in the macroscopic appearance of the male gonads. From January to April the testis and vas deferens were small and translucent. A very slight increase in size and a change in colour (from translucent to white) of the testis was correlated with the onset of sperm production. Sperm release into the vas deferens was observed macroscopically by the appearance of a white substance in the proximal vas deferens which became distended. Spermatophores could be seen in the distal vas deferens surrounded by seminal fluid. Spermatophores were present throughout the year.
Figure 5.1. Monthly mean gonad index for male and female *H. crenulatus*. Vertical bars represent standard deviations.

Figure 5.2. Monthly ovarian stages.

U - Undeveloped.
S - Slightly developed.
M - Moderate development.
D - Developed.
W.D - Well developed.
Histology.

Gametogenesis: Primordial germ cells appear early in development and either migrate to or form the locus of the gonads. The germ cells produce gonial cells; oogonia in females and spermatogonia in males.

Oogenesis.

The five developmental stages of the ovary are represented histologically in Photomicrographs 5a to 5e.

Stage 1 (Undeveloped ovary).
Plate 5a shows the ovary in an undeveloped stage. The germinative zone, where the oocytes develop is only just visible.

Stage 2 (Slight development).
Plate 5b shows the germinative zone with oocytes in early stages of development. The nucleus contains partially separated chromosomes which are dispersed in the nuclear sap called the germinal vesicle. At this stage the nucleus contains a conspicuous nucleolus and the oocytes are called previtellogenic. Oocytes are surrounded by a layer of follicle cells which are spindle shaped. It is thought that they are capable of transporting protein into the oocyte.

Stage 3 (Moderate development).
Plate 5c shows an ovary in moderate development. The oocytes increase in size. Follicle cells are still visible.

Stage 4 (Developed).
The uptake and accumulation of nutrients (vitellogenesis) causes rapid growth of the oocytes. Nucleoli are no longer visible and the cytoplasm shows a granular consistency.
(Plate 5d).

Stage 5 (Well developed).
Yolk distends the oocytes as vitellogenesis continues and the cytoplasm
becomes increasingly granular with the formation of yolk and fat globules making sectioning difficult. Follicle cells are often just visible and form a tight layer around the large oocyte.

Young oocytes are evident within germinative strands in conjunction with well developed ooctes (Plate 5e) or in conjunction with fibrous connective tissue which is characteristic of very early oogenesis. It is possible that these oocytes will develop to become the next brood.

**Spermatogenesis.**

Mitotic divisions of spermatogonia in the testes result in the formation of primary spermatocytes. Meiotic divisions of the primary spermatocytes produce secondary spermatocytes. The second meiotic division produces haploid spermatids (four spermatids for each primary spermatocyte). Once differentiation of the spermatids into mature spermatozoa occurs, spermatogenesis is complete.

The testes was composed of relatively long and complexly folded seminiferous tubules. Some of the stages in the transformation of the spermatocyte into a spermatozoan could be found in passing from one end of the tubule to another. Mature sperm and spermatids were seen in the seminiferous tubules of over 30 samples of testes (Plate 5f).

The mature sperm, upon entrance into the proximal vas deferens became surrounded by epithelial secretions which consolidated the sperm into a compact mass and formed the acellular layers of the spermatophore (Plate 5g), Binford (1913), Johnson (1960), Greenwood (1972), Dudenhauen and Talbot(1982). Once the spermatophores have been formed in the proximal vas deferens, they are moved distally where they are stored and surrounded by seminal fluid until they are released at the time of copulation.

Regenerating or spent testes were never found in any of the males.
Plate 5a. Undeveloped ovary.

Plate 5b. Slightly developed.

Abbreviations.

GZ. Germinative Zone.
PO. Previtellogenic oocytes.
FC. Follicle cells (surrounding oocyte).
Plate 5f. Anterior testes.

Plate 5g. Spermatophores in vas deferens.

Abbreviations.

ST. Seminiferous tubules.
SP. Spermatophore.
S. Sperm.
Brood Biology.

Field Study.

Fig (5.3) shows the number of ovigerous females present each month and the developmental stage of the broods.

Females with recently extruded broods (Stage 1) were present throughout the breeding season indicating that either some females were ovipositing more than once or that females become ovigerous throughout the 1986/1987 breeding season.

Females with "imminent hatching" broods (Stage 4) first appeared in early September suggesting that incubation period extended between 8 and 12 weeks. The majority of the female population were ovigerous by October 1986. The large proportion of females in October with stage 1 broods (n=20) followed the high gonad index recorded in the preceding month.

Effect of salinity on incubation time.

The development of the broods in 36ppt, 18ppt and 11ppt salinity are shown in Figures 5.4, 5.5 and 5.6 respectively.

Successful development through to hatching stage was completed within a minimum 45 days of extrusion of the brood in 36ppt and within a minimum 58 days in 18ppt. However, in 18ppt the broods often deteriorated due to death of some of the eggs and were aborted thus reducing the overall clutch size by the end of the experiment.

Brood development in 11ppt was unsuccessful with eggs only proceeding to stage 3 (N=1 female) and the majority of females aborting their eggs between 20 and 40 days.

The greater egg volume attained within 1 or 2 days of the beginning of each experiment was due to swelling by initial uptake of the water by osmosis. This dramatic increase was best illustrated in the most dilute salinity, 11ppt (Fig. 5.6).
Table 5.3 shows the mean number of days and mean egg volume for each development stage in addition to percentage occurrence of hatching, adult mortality and aborted broods.

The days spent in each stage generally decreased as development proceeded. The longest time duration was spent in stage 1 or 2 when the eggs showed little increase in size and were filled with yolk which accounts for the brick red or yellow colour of a freshly spawned brood.

Cellular cleavage and appearance of a transparent tissue cap signified development to the stage 2 category. The cap continued to increase in size to form a crescent shape on top of the metabolised yolk. As the eggs enlarged, the yolk was further metabolised. Embryonic eye pigments were seen between 30 and 40 days in 36 ppt and development of the limbs was also noticeable during the ‘eyed’ stages. Development proceeded at a rapid rate after formation of the heart and hatching occurred approximately 9 days later in 36ppt.

Multiple regression analysis indicated that egg volume was significantly affected by salinity:

\[
\text{Egg vol.} = 0.0157 + 0.00016 \text{ Days} - 0.0019 \text{ Salinity}, \text{ DF}=399, F \text{ value} = 113.5, p=0.0000.
\]

A summary of regression equations, slopes, and correlation coefficients is shown in Table 5.4.

Egg volumes were significantly larger in 11ppt than in 18ppt (\(t=2.943, p=.005\), DF=259) and were also significantly greater in 18ppt than 36ppt (\(t=3.391, p=.005\), DF=309).
Figure 5.3. Monthly totals of females carrying
S.1 - Stage 1 broods.
S.2 - Stage 2
S.3 - Stage 3
S.4 - Stage 4

Figure 5.4 Mean daily egg volume in 36ppt salinity.
Horizontal bar represents hatching time.
Monthly Brood Stages.


No. of broods

<table>
<thead>
<tr>
<th>Month</th>
<th>S.1</th>
<th>S.2</th>
<th>S.3</th>
<th>S.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
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<td></td>
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<td>Aug</td>
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<td>Sept</td>
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<td>Oct</td>
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<td>Nov</td>
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<td>Dec</td>
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<tr>
<td>Jan</td>
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<td></td>
</tr>
<tr>
<td>Feb</td>
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</tr>
</tbody>
</table>

Mean egg volume vs incubation time.

Salinity (36ppt).

Egg Volume (cu)

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.5. Mean daily egg volume in 18ppt salinity. Horizontal bar represents hatching time.

Figure 5.6. Mean daily egg volume in 11ppt salinity.
Table 5.3. Mean egg volume and number of days spent in each developmental stage in 36ppt, 18ppt, 11ppt salinity. (% A aborted, % H hatched, % M moulted).

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Developmental Stage.</th>
<th>% A</th>
<th>% H</th>
<th>% M</th>
</tr>
</thead>
<tbody>
<tr>
<td>36ppt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Days</td>
<td>16.2</td>
<td>17.7</td>
<td>11.3</td>
<td>8.7</td>
</tr>
<tr>
<td>X egg vol.</td>
<td>0.010</td>
<td>0.012</td>
<td>0.014</td>
<td>0.016</td>
</tr>
<tr>
<td>Std.dev</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>N</td>
<td>47</td>
<td>56</td>
<td>26</td>
<td>14</td>
</tr>
<tr>
<td>18ppt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Days</td>
<td>22.0</td>
<td>17.3 + 3.5</td>
<td>15 + 7.9</td>
<td>8.2 + 2.6</td>
</tr>
<tr>
<td>X egg vol.</td>
<td>0.013</td>
<td>0.017</td>
<td>0.018</td>
<td>0.021</td>
</tr>
<tr>
<td>Std.dev</td>
<td>0.001</td>
<td>0.003</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>34</td>
<td>38</td>
<td>15</td>
</tr>
<tr>
<td>11ppt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Days</td>
<td>26.6</td>
<td>25</td>
<td>19</td>
<td>62.5</td>
</tr>
<tr>
<td>X egg vol.</td>
<td>0.016</td>
<td>0.017</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Std.dev</td>
<td>0.002</td>
<td>0.001</td>
<td>0.00005</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>68</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Table 5.4. Effect of salinity on egg volume.

\[ Y = bx + a. \]

<table>
<thead>
<tr>
<th>Line</th>
<th>b</th>
<th>a</th>
<th>( r^2 )</th>
<th>Std.error</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>11ppt</td>
<td>5.84x10^{-5}</td>
<td>1.59x10^{-2}</td>
<td>0.01</td>
<td>5.19x10^{-5}</td>
<td>89</td>
</tr>
<tr>
<td>18ppt</td>
<td>2.14x10^{-4}</td>
<td>1.07x10^{-2}</td>
<td>0.68</td>
<td>1.09x10^{-5}</td>
<td>174</td>
</tr>
<tr>
<td>36ppt</td>
<td>1.56x10^{-4}</td>
<td>9.05x10^{-3}</td>
<td>0.58</td>
<td>1.13x10^{-5}</td>
<td>139</td>
</tr>
</tbody>
</table>

Egg Hatching.

Many publications concerning sexual and reproductive behaviour in crabs give considerable detail about courtship, mating and egg attachment. However, larval release as a separate event from egg attachment has been overlooked in most studies.

Davis (1964, 1965, 1968) has documented the hatching process in a number of aquatic invertebrates and suggested that bursting of the inner membrane of the egg and the final emergence of the larva were either brought about through the struggles of the larvae or through action of the mother. Only a few authors make a special reference to larval release e.g Warner (1967), Saigusa (1982) and Mac Diarmid (1985). The reason for this probably lies in the difficulty of observing such phenomena in the field or in the aquaria.

Ovigerous females (N=20) which had stage 4 broods were maintained in glass jars and closely monitored to observe hatching behaviour. The sequence of events during hatching were as follows:

Shortly before hatching, the female raised herself on the tips of the peraeopods and flexed her body up and down without the abdomen touching the ground. Simultaneously, the female made jerking body movements of
varying intensity which elevated her body to different heights. The female then arched her body which partially opened the abdomen. Subsequently, the female fully opened the abdomen which beat in a rhythmical fashion. Often the chelipeds were alternately dug into the brood which served to propel the newly hatched larvae anteriorly into the surrounding water. If a rock was present in the jar, the female often clung to it and vigorously vibrated her abdomen while releasing the larvae. This behaviour could allow more efficient dispersal of the larvae.

The time duration of releasing the entire brood differed among females. Those females maintained in 36ppt released their broods within 0.5 to 6 hours. The liberated zoeae were highly active and demonstrated a strong positive phototactic response. They swam with their heads uppermost and were propelled by spasmodic rapid flicks of their abdomens. Unless removed, the zoeae would sink to the bottom of the jar and were dead within 10 hours of release.

Those females maintained in 18ppt had a reduced brood size due to abortion or death of some of the eggs and brood release spanned 2 to 24 hours. Often the zoeae could not sustain swimming for more than 2 hours and would sink to the bottom of the jar.

A preliminary microscopic examination of these cultured zoeae showed that their morphology was the same as stage 1 zoeae described by Wear (1970) and that the larvae did emerge as prezoae.
Fecundity.

The number of eggs was positively correlated with female size (Fig 5.7). This was undoubtedly due to their increased gonad size and larger abdomen and pleopods for egg attachment. The smallest ovigerous female found was 9.0mm C.W, which was within the range estimated for the female pubertal moult (9-11mm CW Chapter 1).

The relationship between fecundity and female size proved to be an exponential function. The regression equation, fitted by the method of least squares was:

\[
\ln Y = 6.06 + 0.171 \text{CW.} \quad (r^2=0.70, \text{N}=26).
\]

Transmoult sperm retention.

Table 5.5 shows the outcome of events during isolation of females which had previously produced one brood. Moulting always followed release of the first brood and the incidence of multiple ovipositions was low when there was no opportunity for remating. Transmoult sperm retention was evident in \textit{H.crenulatus} as fertile broods were still produced.

Table 5.6 shows the outcome of events when females had the opportunity to mate following deposition of one brood. There was a higher success rate of multiple ovipositions when an opportunity for mating was available. This experiment also proved that \textit{H.crenulatus} females could oviposit without an intervening moult. Individual oviposition and moulting sequences from which these tables were formed, are outlined in the appendix.
Figure 5.7  Relationship between fecundity and carapace width in female *Hemigrapsus crenulatus.*
Table 5.5. Multiple oviposition outcomes of isolated females (N=20).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moulted without producing a second brood.</td>
<td>80%</td>
</tr>
<tr>
<td>Moulted, produced an infertile brood.</td>
<td>10%</td>
</tr>
<tr>
<td>Moulted, produced a fertile brood which aborted.</td>
<td>5%</td>
</tr>
<tr>
<td>Moulted, produced a fertile brood which hatched.</td>
<td>5%</td>
</tr>
</tbody>
</table>

Table 5.6. Multiple oviposition outcomes of females with an opportunity for mating (N=20).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No moulting or production of a second brood.</td>
<td>10%</td>
</tr>
<tr>
<td>Moulted without producing a second brood.</td>
<td>30%</td>
</tr>
<tr>
<td>Moulted, produced an infertile brood.</td>
<td>15%</td>
</tr>
<tr>
<td>Moulted, produced a fertile brood which aborted.</td>
<td>10%</td>
</tr>
<tr>
<td>Moulted, produced a fertile brood which hatched.</td>
<td>5%</td>
</tr>
<tr>
<td>No moulting, produced an infertile brood.</td>
<td>10%</td>
</tr>
<tr>
<td>No moulting, produced a fertile brood.</td>
<td>20%</td>
</tr>
</tbody>
</table>
Discussion.

While many authors have used the presence of ovigerous female crabs to indicate the level of reproductive activity (e.g. Reese 1968, Nye 1977, Jones 1977, 1978), I have used this criterion only to define the duration of the breeding period in *Hemigrapsus crenulatus*. The breeding period is an extended one, over 8 or 9 months of the year from June to either January or February of the following year with pronounced activity in October 1986 when most of the females were ovigerous. Wear (1970) collected ovigerous females from the Manganui estuary in Auckland in October 1964, and noted the presence of ovigerous females in the Hutt river estuary in Wellington harbour in late November. Thomson and Anderton (1921) noted ovigerous females in January and February in Otago Harbour.

General trends of reproductive response with change in latitude have been investigated previously (Giese 1959, Vernberg 1962, Jones and Simons 1983) and it is factors such as temperature (Fusaro 1980) and photoperiod which probably influence life history characteristics. At higher temperatures (and lower latitudes), more broods are produced and more females become ovigerous within a reproductive season due to stimulation and initiation of earlier ovarian development (Kinne 1970, Annala et al 1980, Armitage and Landau 1982). *H. crenulatus* is widespread throughout New Zealand so it is quite feasible that observed changes in the timing of the peak breeding season from the present study and those reported by Wear (1970) and Thomson and Anderton (1921) are a result of temperature changes with latitude.

An extended breeding season may mean that the individual females are producing several successive broods during the year or that they are
breeding asynchronously i.e some females are in earlier stages of ovary maturation, some are spawning and some are already spent (Giese 1959). *H. crenulatus* shares a very similar breeding period to the Tasmanian grapsids *Brachynotus spinosus* and *Cyclograpsus granulosus* (Griffin 1969) but it is much longer than in other New Zealand grapsids. It’s closest relative *Hemigrapsus edwardsi* has a Winter breeding season extending from March to August (Mear 1974). Using this criterion, *H. crenulatus* is a late Winter, Spring and early Summer breeder.

A more reliable and precise picture of the reproductive cycle in this species has been obtained by using the gonad index method in conjunction with descriptions of the ovarian development and brood biology. The results furnish supplementary evidence to that obtained from a study of the incidence of ovigerous females.

It is evident that breeding in *H. crenulatus* extends for several months of the year with pronounced activity during October. It is clear however that during certain months, individuals in different ovarian stages are present, representing a heterogenous population as seen from the range of variation in the gonad index and the ovarian stages.

Gonad maturation was disrupted by a temporary but complete resting period in January and February 1987 when the ovaries were either undeveloped or only slightly developed. Although the ovaries of mature females were inactive during the resting period, a few ovigerous females were still to be found but once their eggs hatched, the breeding season was terminated.

Ovigerous females were absent from March to May of both years but ovaries were recuperating with a proportion of females having well developed ovaries by May. The presence of females with well developed ovaries in the population was usually followed by peaks in oviposition
(presence of stage 1 eggs) in the following month. The present data suggest a peak in the gonad index in September due to the majority of females possessing fully ripe ovaries, followed by a peak in oviposition in October.

The presence of females with slightly developed ovaries during the peak breeding season in conjunction with the occurrence of females with stage 1 broods throughout the season further suggests the possibility of production of successive broods within one breeding season.

The Brachyura differ from all other Crustacea in that following copulation, the spermatozoa are stored in internal non-integumental spermathecae which are not shed when the female moults (Hartnoll 1983). Thus the sperm are stored until the eggs become mature and move down the oviducts where they are fertilised before being spawned onto the pleopods. Ryan (1965) suggested that spermatozoa may remain through ecdysis in Portunus sanguinolentus and Broekhuysen (1941, 1955) noted that normal development of extruded eggs took place in the grapsid Cyclograpsus punctatus and the hymenosomatid Hymenosoma obiculaire which had moulted but had not copulated again. Transmoult sperm retention was also evident in the stone crab Menippe mercenaria (Cheung 1966) which spawned ten successful broods after one ecdysis without copulation.

Evidence obtained from the present study indicated that although transmoult sperm retention without further copulation was possible in Hemigrapsus crenulatus, the incidence was very low. A higher success rate in producing a second brood was achieved when the females moulted following release of the first brood and had the opportunity for mating or alternately foregoing the intervening moult.

Broekhuysen (1941) found that Cyclograpsus punctatus can fertilise several successive egg batches after one copulation without an
intervening moult. Such an occurrence is not unusual in the grapsids e.g. Hemigrapsus oregonensis, H.nudus (Knudsen 1964), H.penicillatus Sesarma pictum (Pillay and Ono 1978), S.reticulatum and S.cinereum (Seiple and Salmon 1987), S.intermedia (Kyomo 1986) and Pisa tettradon (Vernet-Cornubert 1958). Whilst H.crenulatus did not produce a second brood without further copulation and an intervening moult, it does not preclude the possibility that one fertilization is sufficient for more than one batch of eggs. When an opportunity for remating occurred, H.crenulatus was successful in producing another brood without an intervening ecdysis.

Females capable of spawning again without an intervening moult may increase production by minimising the time required to produce more young, or may assure continued reproduction under favourable conditions (Morgan et al 1983). Furthermore, the ability to spawn more than once through a breeding season permits H.crenulatus to disperse offspring temporally. Spatial and temporal dispersal decrease the variance in number of surviving offspring in heterogenous environments and raise the mean frequencies of the genotype in the next generation (Strathmann 1974, Gillespie 1977, Wilbur 1977).

Since environmental factors such as temperature and photoperiod generally affect neurosecretory activity in arthropods (Highnam and Hill 1969), a sharp rise in temperature would undoubtedly influence the eyestalk neurosecretory system controlling ovarian development causing the onset of oogenesis. Lower temperature may also induce regression or reabsorption of the ovaries in ovigerous and non-ovigerous females prior to the resting period (Highman and Hill 1969, Haefner 1977).

Successive broods in H.crenulatus were characterised by a reduction in the number of eggs produced. This feature was also apparent in Scylla
serrata (Ong 1966) and *Macropipus depurator* (Wear 1974). During ovarian development, substantial quantities of protein and more especially lipid are required (Babu 1987). These organic substrates are normally synthesised from food intake but during the peak of breeding activity or when successive broods are produced, the stored lipid may be transferred from the hepatopancreas to the ovaries (Pillay and Nair 1973, Wear 1974, Paulus and Laufer 1987). Hence the smaller number of eggs occurring in successive batches produced by *H.crenulatus* may reflect the females' inability to mobilize sufficient protein and lipid reserves to keep pace with antagonistic requirements of metabolic activity and growth. Under these circumstances, a compromise may occur when the ovaries regress or are absorbed to allow nutrient replenishment; this phase marks the resting or quiescent period in January and February of *H.crenulatus*. Thus in the reproductive cycle of this species, temperature and perhaps nutrition play the vital roles in timing gametogenesis.

The reproductive cycle of female *H.crenulatus* is relatively easy to discern due to the changes in size, shape and colour of the ovary and the ovigerous condition. However the male testes do not reflect such a clear picture. It is evident that the testis is small in size compared to the female ovary due to the smaller unit cost of male gametes. Furthermore the fluctuations in gonad index are not so pronounced and do not show a definite cycle indicative of growth, maturity and collapse through spawning as is evident in the females.

The present study indicated that *H.crenulatus* exhibited continuous testicular activity throughout the year due to the presence of sperm and spermatophores. The male gonad index failed to show an upward trend before the breeding season as was incited in *Portunus pelagicus*, *Uca annulipes* and *Metapenaeus affinis* (Pillay and Nair 1971) although there
was a macroscopic change in colour and size of the testis at the onset of the breeding season. There is a paucity of information concerning spermatogenic cycles in male brachyurans although the structure and formation of crustacean spermatophores has recently been the subject of much investigation (Hinsch and Walker 1974, Uma and Subramoniam 1979, Kooda-ciso and Talbot 1982, Dudenhauen and Talbot 1983). The spermatophores of male H.crenulatus conform to the simplest type within the Crustacea. They are small and ellipsoid and are composed of a sperm mass surrounded by a thin acellular wall (Hinsch and Walker 1974) although time did not permit further detailed analysis of their structure.

The consequence of continual spermatogenesis in H.crenulatus means that males are capable of mating at any time. However it has been suggested that searching behaviour by the males is only elicited upon stimulation by a pheromone released in the females' urine (Ryan 1966) or by tactile or chemical stimuli upon contact (Veillet 1945). It is the females which restrict mating opportunities, either because they are ovigerous or because they may be in a 'soft' condition whereas continual spermatogenesis allows male H.crenulatus to mate at any time.

In contrast to the males, histological examination of the ovaries of female H.crenulatus revealed that the ovarian cycle could be divided into different stages according to oocyte size and whether they were previtellogenic or vitellogenic. No stage corresponding to a spent or degenerating condition was found as in other brachyurans (Haefner 1977, Du Preez and McLachlan 1984) nor were any ovigerous females dissected to determine either histological or macroscopic ovarian stage. However, some females were capable of spawning another brood within a minimum of 10 days of releasing the first brood. This indicates that oogenesis
continues during incubation and further supports the hypothesis that successive broods may be produced in the field. A similar time interval (approx. 10 days) between liberation of one batch of eggs and production of another was observed in the grapsids Hemigrapsus penicillatus (Pillay and Ono 1973), Sesarma intermedia (Kyomo 1986), S. cinereum (Seiple and Salmon 1987) and 30 days in Helice crassa (Nye 1977) and Sesarma reticulatum (Seiple and Salmon 1987).

The mud crab Rhithropanopeus harrisii was induced to breed and spawn during the non-breeding season by maintaining a 12:12 day-night photoperiod and a water temperature that would be encountered in the field during the normal breeding season (Goy 1985). Little (1968) also induced spawning in Palaemonetes pugio by increasing temperature and photoperiod whilst Sulkin et al (1976) reported that proper temperature and diet alone were sufficient to induce spawning in Callinectes sapidus. The factors responsible for coordinating cycles of both gonad activity and egg deposition in H. crenulatus remain obscure however the regularity of seasonal spawning do suggest that temperature is an influential factor in addition to perhaps the nutritional state.

Increase of brood size with female size is very extensively documented in the Crustacea (Hines 1982, Hartnoll 1985, Wenner et al 1987) and is in fact an almost universal feature of crustacean reproduction (Diaz 1980, Somerton and Meyers 1983). In H. crenulatus the smallest female (9.0mmCW) carried approximately 1000 eggs whilst the largest female (26.0mmCW) carried approximately 35,000 eggs. This estimate was within the range of the grapsids Sesarma cinereum and S. reticulatum (Seiple and Salmon 1987), Hemigrapsus nudus and H. oregonensis (Knudsen 1964) and H. edwardsi (J. Pringle pers. comm) on the basis of their specific size ranges.
Grapsids usually conform to a basic pattern of indefinite post-puberty moulting (Hartnoll 1983). This allows egg production to commence at a small size, thus offsetting the risk of reproductive failure due to early mortality. Also in temperate regions, breeding is usually seasonal so that resources can be channelled into egg production and the larvae released at a period favorable for their survival (Giese 1959) but diverted into growth at other times of the year.

Exploitation of highly nutritional resources in an estuary is thought to be responsible for increased fecundity (Fish and Fish 1978, Haynes et al 1976). Pillay and Nair (1971) suggested that high fecundity of estuarine decapods is a compensation for high larval mortalities due to the harsh environmental conditions and losses due to dispersal to unfavorable settlement sites. Furthermore, individuals of a similar size are said to produce more eggs per brood at low compared with high latitudes (Vernberg 1962, Reaka 1979, Campbell and Eagles 1983, Jones and Simons 1983). This response implicates temperature and it's relationship with metabolic adaption as the major determinant of fecundity. For example, at high temperatures, rapid growth and high metabolism result in less energy going into egg production than at low temperatures (Diaz 1980) although high temperatures may increase the likelihood of multiple ovipositions. Alternatively, at lower temperatures, females with smaller growth and reduced metabolism may channel more energy into producing eggs each with high nutritional reserves (Woodward and White 1981, Babu 1987) which require a longer incubation time. Unfortunately, there are no records of fecundity estimates in *H. crenulatus* from other parts of New Zealand to use as a comparison.

It has been suggested that the energy required for larval life determines the size of the egg (Pillay and Ono 1973, Crisp 1974) thus
larger eggs with their greater energy reserves also tend to result in fewer larval instars (Rice 1980). Within the Grapsidae, there are also correlations between number of zoeal stages and adult habitat. In freshwater species, there are two zoeal stages, in strictly marine forms five and estuarine species most commonly have four zoeal instars (Rabalais and Gore 1985).

Wear (1970a) has recorded the size of freshly laid and mature eggs from New Zealand graspsids. Results indicate that freshly laid eggs of *H.crenulatus* are similar to the predicted size (0.28 x 0.26mm) as are the mature eggs (0.34 x 0.33mm). The eggs of all other graspsids are larger with the exception of *Helice crassa* (Wear 1970a, Jones 1980).

Incubation time is also related to egg size. For example, the eggs of *Sesarma cinereum* receive a lesser degree of parental investment (per offspring) being smaller and are carried for only 28 days while eggs of *S.reticulatum* are larger and carried for approximately 45 days (Seiple and Salmon 1987). The planktonic development of *S.cinereum* through 4 zoeal stages takes from 25 to 30 days (Costlow et al 1960) while larvae of *S.reticulatum* pass through only 3 zoeal stages after 15 to 30 days. Ultimately this decreased time must be due to the increased investment by the adult females.

The small size of female *H.crenulatus* maturity (9.0mmCW), the positive correlation between brood number and female size, the fact that there are five zoeal stages, the ability to produce successive broods and a minimum incubation time of 45 days in the laboratory all suggest that this species shows little parental investment per offspring but great parental investment per female. Furthermore, Eliss (1968) has pointed out that swimming crabs and lower intertidal crabs such as *Hemigrapsus penicillatus* (Pillay and Ono 1973) produce smaller and greater numbers of
eggs than crabs adapted to the upper intertidal region or semi-terrestrial species. *H. crenulatus* is basically a low intertidal dweller. Also, longer planktonic life (approximately 8 weeks) favours dispersal and *H. crenulatus* is found throughout the North and South Islands (Dell 1968).

I was not able to precisely determine the incubation period of *H. crenulatus* in the field as there was considerable overlap in brood stages over the breeding season. However, in the laboratory, the minimum incubation interval was 45 days although it is appreciated that temperature and salinity are all important in influencing incubation time (Kinne 1971, Wear 1974, Steele and Steele 1975). A minimum incubation time of 45 days indicates that this species is capable of spawning at least twice during the breeding season assuming that the ovary is regenerating while the female is ovigerous.

In studies on the hatching process in aquatic decapods Davis (1964a, 1964b, 1965a, 1965b, 1966) found that egg size during incubation was due to either a slow but steady osmotic swelling of the inner egg membrane or to swelling of the membrane itself. In both cases, the size increase is brought about by uptake of the water which increases the internal pressure of the egg up to the size of hatching.

Results from the present study undertaken in the laboratory suggest that there is an initial uptake in water (especially at 11ppt) followed by a gradual increase in egg size. Incubation in 36ppt (Range 45 to 60 days) accelerated the rate of embryonic development and also resulted in a higher development success to hatching stage. Eggs of *H. crenulatus* can tolerate marked dilution of the external medium as illustrated by their normal development in 18ppt although incubation period was significantly longer. A dilute salinity may be responsible for physiological adaptation
of the embryonic larvae allowing for maximum salinity tolerance on hatching. Prolonged immersion in 11ppt could not be tolerated and resulted in disruption of embryonic development, cytolysis and abortion although it would appear that eggs may be able to tolerate short term drastic dilutions in salinity without detrimental effects. Similar responses to low salinity media have been reported for other estuarine crabs (Sandoz and Rogers 1944, Roberts 1971, Jones and Simons 1981).

Courtship behaviour and copulation were never observed in *H. crenulatus* throughout their 2 year laboratory confinement, nor in the field. *Hemigrapsus* species in general copulate with virtually no courtship (Hartnoll 1969) and are thought to mate with the female in the hard condition with the female above the male. Thus mating behaviour in *H. crenulatus* remains obscure except for one observation by Yaldwyn (1966) who noted that it did conform to the grapsid pattern.
Chapter 6.

Larval development.

Introduction.

The notion that certain life history stages are subject to limitation by abiotic environmental factors was first enunciated by Shelford (1915). Since then, considerable evidence has been amassed which demonstrates that brachyuran egg and larval stages tend to be less tolerant than the adults to abiotic changes (Sandoz and Rogers 1944, Costlow and Bookhout 1959, Costlow 1967, Young and Hazlett 1978). Estuarine organisms, in particular, must often tolerate the wide variation in temperature and salinity, particularly during low tide when estuary margins become exposed and residual rain pools are formed. Salinity and temperature, as well as the combination of these two physical factors have been shown to effect the larval development of crabs in several ways (Costlow and Bookhout 1962, Costlow et al 1962, 1966, Chamberlain 1962, Vinuesa et al 1985).

Many authors have demonstrated that temperature produces significant differences in the developmental rates of crustacean larvae. Increased temperature usually reduces the time required to complete each larval stage (Sandifer 1973, Dawirs 1979, Nakanishi 1981, Anger 1984, Blaskowski and Moreira 1986 Hartnoll and Mohamedeen 1987), although there is only often a small difference between the highest temperature tolerated and that producing deleterious effects on biological function (Boyd and Johnson 1963, Chamberlain 1961, 1962, Costlow et al 1960, 1962, 1966).

A great deal of information is also available on the effects of salinity on many aspects of the biology of larval stages (Kinne 1971,
Christiansen and Costlow 1975, Roberts 1971, McNamara et al 1982, 1983). In general, salinity strongly affects growth and survival with tolerance ranges unique for each developmental stage and species (Roberts 1971). Salinity may also influence the vertical distribution of planktonic crustacean larvae (Roberts 1971, Sulkin and Van Heuvelen 1982). This in turn can determine the extent of larval displacement into and out of estuaries and ultimately the dispersal of the species (O'Connor and Epifanio 1985).

Wear and Fielder (1985) have described the larval development of the New Zealand grapsids, Hemigrapsus edwardsi, H. crenulatus, Helice crassa and Cyclograpsus lavauxi through each of the five zoeal stages. However little published information is available on the effects of salinity or temperature on any of these zoeae (Jones and Simons 1983).

Since large numbers of zoeae incubated under known salinity and temperature became available (Chapter 5 Incubation experiments) it was decided to attempt to rear them to the megalopa stage. The major aim of this experiment was to determine the length of time spent in each of the five zoeal stages at each temperature to determine their approximate life span in the plankton and how this relates to prepubertal growth of subsequent instars (Chapter 3). This experiment would also provide information on the optimum temperature for survival and enable a description of the megalopa which was not included by Wear and Fielder (1985).

If the zoea could be reared successfully through to the megalopa, then an investigation into the effects of salinity (100%, 50% and 30% in combination with the optimum temperature) on the larval development could also be undertaken.
Materials and Methods.

Larvae were hatched from ovigerous females maintained in jars containing 300mls water of 36ppt and 18ppt salinity at 20°Celsius and from ovigerous females captive in the laboratory.

_H. crenulatus_ larvae are initially released as prezoaeae and normally shed the prezoal cuticle within 30 minutes. Only zoaeae collected within five hours of hatching and which responded photopositively by active swimming were used.

The zoea were removed and placed in plastic rearing pottles (4.0cm x 3.0cm x 3.0cm) each containing 20mls of filtered sea water (36ppt salinity) which had previously been aerated and brought to the required temperature. Larval density was held at 10 stage 1 zoea per pottle and between n=3 and n=10 pottles were used at each temperature. For example, the abbreviation, T2 n=8 means trial 2 consisted of 8 pottles each containing 10 zoaeae. Mass rearing in jars containing 500mls sea water was also attempted.

Optimum temperature for rearing zoea is approximately 20°Celsius (Wear pers comm) so three temperatures were chosen: 20°C, 15°C, (using the constant temperature rooms) and room temperature (17-18°C). All pottles in the constant temperature rooms received a 12:12:L:D regime and were placed on a gentle shaking machine to aerate the water. Vigorous aeration can cause the larvae to be stranded on the sides of the pottles (Hartnoll and Mohamadeen 1987).

Every day the zoea were fed with freshly hatched _Artemia_ nauplii, fresh plankton, when available or homogenised baby food. These foods were offered in various combinations. The water was also changed every day and 0.2cc penicillin added to prevent fouling (T. Osborne pers comm).
The zoea were carefully removed, every day, and placed in a shallow dish under a stereomicroscope to record deaths or metamorphosis. Death was obvious from lack of movement of appendages or internal organs and an opaque appearance. Mean intermoult period and percentage survival was recorded at each temperature.
Results.

Figures 6.1, 6.2 and 6.3 show the daily percentage survival of *H. crenulatus* stage 1 zoeae at 15°Celsius, 17-18°Celsius (room temperature) and 20°Celsius respectively.

In all temperatures, all larvae (N=555) failed to moult and remained in zoeal stage 1 until death.

The mortality rate was highest at the highest temperature (20°C) with less than 30% survival in both trials by the third day of incubation. All zoeae had died by the 5th day (Fig. 6.3).

A similar pattern was evident at room temperature although the zoeae survived for a slightly longer time but 100% mortality occurred by the 7th day (Fig. 6.2).

Survival of the zoeae was the highest in 15°Celsius with over 80% of the larvae alive by the 3rd day. However, a steady decrease in numbers followed with the last death occurring in 3 trials by the 8th day. One exception was trial 4 in which 50% of the zoeae were still alive by the 15th day but without mouling to the second zoeal instar.
Figure 6.1. Temperature tolerance of Stage 1 zoea larvae of *H. crenulatus* (15°C, 4 trials).

Figure 6.2. Temperature tolerance of Stage 1 zoea larvae (Room temp., 2 trials).
Temperature tolerance of zoea.

15°C temp.

Temperature tolerance of zoea

Room Temperature.
Figure 6.3. Temperature tolerance of Stage 1 zoea larvae (20°C, 2 trials).

Temperature tolerance of zoea.
Discussion.

The method described for rearing zoeae (Costlow and Bookhout 1962, Costlow, Bookhout and Monroe 1966, Costlow and Fagetti 1967, Wear and Fielder 1985, T. Osborne pers comm) has previously been successful for rearing zoeae. All trials with H.crenulatus resulted in the death of the stage 1 zoeae which failed to moult successfully to the next stage. This was despite rearing attempts in different combinations of temperature and salinity, mass and individual rearing and varying larval densities within pottles and with different foods. Factors which may have been responsible for the inhibition of moulting include salinity, temperature, starvation and genetic variability.

Salinity is the most widely varying parameter within estuaries and could therefore be a major determinant of larval survival and development. The larvae would be subjected to a variety of salinities during transportation from spawning areas to the lower reaches of the estuary (Sandifer 1973, Goy 1976, Christy 1982, Dittel and Epifanio 1982).

The majority of H.crenulatus stage 1 zoeae were reared in 36ppt salinity although a few attempts were also made in 18ppt salinity.

The reason for choosing 36ppt as a test salinity was due to successful embryonic development of the entire brood compared to lower salinities where losses occurred (see Incubation experiments, Chapter 5).

Most often, crabs have a semi-lunar rhythm (Christy 1978, 1982, Saigusa and Hidaka 1978, Saigusa 1981, 1982) in which the number of females releasing larvae varies with the phase of the moon. Saigusa (1982) suggested that the actual time of hatching is regulated by the combination of solar day and local tide cycles. By entering the water
column near the time of high tide, the larvae are potentially exposed to high salinity and that release at high tide avoids stressful salinity conditions (Forward et al. 1982) and aids larval dispersal (Christy 1978, 1982, Bergnin 1981, Saigusa 1981).

Although a larval release rhythm was not observed in H. crenulatus in the laboratory, ovigerous females occur further down the shore in the field. The reason for this distribution could be that incubating broods need to be immersed in water to prevent dehydration or the ovigerous females anticipate the rising tide and move downshore to release their broods (Jones 1981). Thus, stage 1 H. crenulatus zoeae would initially be subjected to high salinities before transportation to areas of lower salinities due to mixing water currents. One would expect that laboratory reared zoeae would be preadapted to high salinities especially if the embryos developed in the same salinity.

Temperature alone can affect the overall rate of development. Hartnoll and Mohamadeen (1987) found that larvae of Piliumnus hirtellus show better survival at 15°Celsius but at 20°Celsius, a larger proportion reach the megalopa and first crab instar. Improved survival at higher temperatures may be an artifact of captivity, in that the more accelerated development reduces exposure to any adverse factors of the laboratory environment such as bacterial or fungal infection. A daily water change and the addition of penicillin prevented the occurrence of any infection during rearing attempts of H. crenulatus larvae.

When combined with extremes in salinity, temperature may also affect the survival and duration of specific zoeal stages and the rate of their development. This has been investigated in many brachyuran zoeae, Callinectes sapidus (Sandoz and Rogers 1944), Sesarma cinereum (Costlow Bookhout and Monroe 1960), Panopeus herbstii (Costlow, Bookhout and
Monroe 1962), *Rhithropanopeus harrisii* (Costlow, Bookhout and Monroe 1966). Optimal conditions of temperature and salinity for survival, duration and metamorphosis of the instar differ between the species. Temperatures and salinities used in the laboratory were within the range that the newly hatched *H. crenulatus* zoeae would experience in the field yet they still failed to moult to the second zoeal instar. All the evidence pointed to the fact that some other factor was preventing metamorphosis.


The main reason for the failure of *H. crenulatus* to moult to the second zoeal instar was probably due to starvation. The freshly hatched *Artemia* larvae were larger than the stage 1 zoeae and so it appeared mechanically impossible for the zoeae to grasp the prey. Recent digestion of *Artemia* results in a pinkish colour appearing in the transparent gut (T. Osborne pers comm) but this distinctive feature was never observed.

A feature apparent in this study and from others (Hartnoll and Mohamedeen 1987, Anger 1984) is that different batches of zoeae of the same species, even though reared as far as possible under experimental conditions, showed marked differences in daily survival, especially in 15°C celcius. There is always variation from hatch to hatch which may be genetically determined or caused by seasonal differences in the
nutrition of the females during oogenesis affecting the storage of yolk reserves in the oocytes (Kunisch and Anger 1984). There may also be differences in oxygen availability in different layers of the egg mass which affects the overall development and hatching of the brood. Finally, differences in food supply have also been used to explain marked differences in survival (Hartroll and Mohamedeen 1987).
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Appendix.

Absolute growth increments and intermoult periods of four individuals in captivity for the duration of a year.

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<th>Crab Sex</th>
<th>Date of moult</th>
<th>Interval (Days)</th>
<th>Carapace width after moult (mm)</th>
<th>Size increase</th>
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<td>-</td>
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Individual data from isolated females showing the date of release of the first brood, date of moulting, growth increment (G.I), total number of days to moul since hatching, date of extruding the second brood, total number of days to produce 2nd brood after moulting and it's incubation time under the abdomen until abortion (A) or hatching (H) occurred, *=fertile brood.

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Individual data from females which had the opportunity to mate showing date of release of the first brood, date of moulting, growth increment (G.I), total number of days to moulting since hatching, date of extruding the second brood, total number of days to produce second brood after moulting and it's incubation period until hatching (H) or abortion (A) occurred, * fertile brood.

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