1989

ECOLOGY OF THE VELVET SWIMMING CRAB LIOCARCINUS PUBER (L.) (BRACHYURA: PORTUNIDAE)

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ECOLOGY OF THE VELVET SWIMMING CRAB *LIOCARCINUS PUBER* (L.) (BRACHYURA: PORTUNIDAE)

-by-

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This thesis was submitted to the Council for National Academic Awards, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Polytechnic South West

July 1989

This work has not been accepted and is not concurrently being submitted for any other degree, and is a record of work carried out by the candidate himself.

Signed C.P. Norman

Candidate

Date 14/7/89

This is to certify that the work submitted here was carried out by the candidate himself. Due acknowledgement has been given to any assistance received.

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Ecology of the Velvet Swimming Crab Liocarcinus puber (L.) (Brachyura: Portunidae)

C.P. Norman

ABSTRACT

Currently, Liocarcinus puber is commercially fished in the United Kingdom and exported to Southern Europe. The species appears particularly vulnerable to overfishing and has a history of overexploitation. The present study has examined the reproduction, growth and diet of L. puber, to provide information on which to manage this fishery.

Immature Liocarcinus puber were first observed in late summer at a size of 6-10mm long carapace width (LCW). Growth in immature crabs was rapid, and sexual maturity occurred after approx. one year at 45mm (for males) and 40mm (for females) LCW. To differentiate the modal growth of mature crabs, probit analysis and computer models (ELEFAN) have been used. The life expectancy for L. puber is estimated at between 4-6 years, with males attaining a maximum size of approx. 95-100mm LCW and females 85-90mm LCW. From the end of the second year, moulting in both sexes was annual, and occurred in early summer for males and later in the year (generally late summer to autumn) for females. The reproductive cycle was also strongly seasonal with mating occurring at the time of the female moult. Evidence from the seasonal occurrence of different egg stages, and the ovarian development of ovigerous females, suggests that older females (II+ years) produce more than one brood each breeding season (January to July).

Stomach analyses established that the diet of Liocarcinus puber contained quantities of both animal and algal material, however, algae were the most abundant prey item. Animal prey items included a broad range of benthic organisms and variation in the diet was noted between depth zones, with the availability of prey items appearing to largely determine the diet. Analysis of laminarinase activity in the hepatopancreatic tissue showed levels comparable to the highest recorded for any crustacean. L. puber can survive for extended periods, and moult on an algal diet, however, it appears that an algal diet contains insufficient protein to fully support long-term growth in this crab.

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CHAPTER 1

GENERAL INTRODUCTION

1. General Introduction

Crabs of the genus Liocarcinus (Portunidae) are endemic to the north east Atlantic seaboard. Seven species of Liocarcinus are recorded from British waters and these occur over a broad range of latitude, typically from Scotland to the Moroccan coast. The northernmost record being Liocarcinus holsatus (Fabricius) at 71° N on the Norwegian coast (Christiansen 1969), and the most southerly record is for Liocarcinus corrugatus (Pennant) at 16° S off the Angolan coast (Clark 1986). The genus appears to predominate on the Atlantic coastal fringes with records of distribution in the Baltic and Mediterranean Seas being more sporadic. Liocarcinus puber (Linnaeus) is found from the Norwegian coast at 61° N (Chistiansen 1969) to the Spanish Sahara at 24° N (Capart 1951), and is limited in the Mediterranean Sea to the Spanish, French and Adriatic coastal areas (Clark 1986). Records of bathymetrical range indicate that Liocarcinus spp. are found predominantly in shallow, continental waters ranging from the lower shore down to 150m (Ingle 1983). The prefered substrata for most species are mud, sand or gravel, however, Liocarcinus puber is the exception and is found most commonly on rocky substrata. This latter species achieves the largest size of the genus (Ingle 1983).

The Plymouth Marine Fauna (1957) records all seven 'British' species of Liocarcinus within a radius of approximately 30km from Plymouth. Three species are described as 'common', and these are Liocarcinus depurator (Linnaeus), L. holsatus and L. puber; the remaining species are described as 'not uncommon' or 'occasional', and the only other portunid recorded as 'common' is Carcinus maenas (Linnaeus) (Plymouth Marine Fauna 1957).

Portunid crabs account for 5.4% of the total world crustacean

fishery catch and 19.7% of the world crab catch, and landings of 170,000 metric tonnes (MT) were recorded for 1985 (Table 1.1). Important species included in the world crab catch data include the blue crab Callinectes sapidus (Rathbun) (90,000 MT) on the east coast of the United States, Portunus trituberculatus (Miers) (29,000 MT) centered in the north west Pacific (with Korea and Japan being the main consumers), Scylla serrata (Forskal) (10,000 MT), and Portunus spp. (27,000 MT) in the western central Pacific (with the major consumers being Thailand) (FAO 1985). These fisheries predominate in shallow water and estuarine regions, and fishing techniques vary between differing locations and fisheries. Portunid fisheries, as with many other fisheries, suffer from marked variability of abundance of harvestable crabs (Dassow 1969). Such fluctuations are most apparent with species with complex life-history patterns such as the blue crab C. sapidus. Sulkin (1977) suggested that the size of the year class is particularly dependant on the survivorship of zoeae, but this is only one explanation of fluctuations of crab catches.

Fisheries for Liocarcinus spp. in the north east Atlantic have shown a similar variability in landings, with the fishery having a recent history of failure due to over-exploitation (Gonzalez Gurriaran 1981a, 1981b, P.H. MacMullen pers. comm.). Exploitation of portunid crabs in this area has centred largely in Spain and France, with smaller fisheries in Portugal and the Channel Islands. In Spain, commercially taken species are L. puber and L. depurator (as well as Carcinus maenas), although L. puber is the most important species (Gonzalez Gurriaran 1981b, FAO Yearbook 1985). Overfishing of L. puber in Spain has led to a marked decline in catch rates from 550 MT in 1975 to 136 MT by 1980 (Table 1.2). Statistics for the French fishery show a constant catch at approximately 1000 MT

between 1978 (when data first became available) to 1984, but a decline to 750 MT in 1985 (Table 1.2). The fisheries in both France and Spain have a large 'sport' fishing component, making enforcement of size limits difficult to impose (Gonzalez Gurriaran 1981a). The continued demand, and high price paid, for L. puber (eg. £10.00kg^{-1}, at Barcelona, Spain 1987) has enabled an export fishery to develop from the UK to Spain and France. Previously, L. puber was regarded as a pest species in the UK, taking bait intended for lobster (Homarus gammarus Linnaeus) and edible crab (Cancer pagurus Linnaeus). Fisheries, specifically for export of L. puber, now occur in north west Scotland, Cornwall and Wales (MacMullen 1983). Typically, the fishery uses baited pots in shallow water (<10m), often in intertidal regions, and export is via vivier lorry or boat (Whyman et al. 1985). Fishery data for Scottish stocks of L. puber first became available in 1984, and show a doubling in tonnage from 330 MT in 1984 to 694 MT in 1986 (FAO Yearbook 1985, Kinnear and Mason 1987). Records for the English and Welsh stocks are not available, and compared with Scotland, the southern fisheries are still in the early stages of exploitation (MacMullen pers. comm.).

The recent rapid expansion of the Liocarcinus puber fishery in the UK has highlighted the lack of biological data on which to manage stocks. Also, L. puber has been assumed to be an important predator on the nearshore sub-littoral community (Ebling et al. 1964), and the removal of this large, and rather active crab, especially in areas where other crustaceans have been heavily exploited, may have considerable consequences for the marine community as a whole. The aims of this study were to provide detailed data to help manage the fishery and to examine the role of L. puber in the nearshore sub-littoral.

Table 1.1. FAO statistics on worldwide crab and crustacean landings for 1979, 1982 and 1985. (MT - metric tonnes; sources of information = FAO Yearbook Vol. 50, 1980 and Vol. 60, 1985)

Landings (MT)	1979	1982	1985
Total marine crustacean catch	2,920,000	3,246,700	3,234,100
Total crab catch	784,493	797,440	886,837
Portunid crab catch - all areas	139,785	169,993	172,630
Liocarcinus spp. catch N.E. Atlantic	1,236	1,159	1,465

Table 1.2. FAO data on landings of Liocarcinus spp. from the principal European fisheries. (Units - metric tonnes; sources of information = FAO Yearbook Vol. 50, 1980; Vol. 60, 1985; Kinnear and Mason 1987)

Country	1975	1980	1981	1982	1983	1984	1985	1986
Spain	550	136	143	151	163	210	250	*
France	*	1006	1006	994	982	983	749	*
Portugal	*	17	15	14	19	25	36	*
Scotland	*	*	*	*	*	330	430	694
Total	-	1159	1164	1159	1164	1548	1465	-

(* indicates data not available)

CHAPTER 2

DESCRIPTION OF THE STUDY AREA

2.1. Introduction

Preliminary investigations established that Liocarcinus puber was relatively common in the nearshore sublittoral zone throughout Plymouth Sound and in the more sheltered areas along the adjacent coast. These early dives also indicated that the weather would be the most important factor determining sampling frequency at exposed sites. Therefore, all SCUBA sampling sites were located within The Sound to gain maximum protection from adverse weather, however, there is no reef within The Sound which is exposed at low water. To allow a littoral survey of L. puber, the site chosen was at Blackstone Rocks, Wembury (Fig. 2.1C).

2.1.1. Physical description of Plymouth Sound

The Sound is a natural harbour and, after the building of the Breakwater in the early 1800's, has provided refuge to shipping from all directions of storm. The flora and fauna of The Sound form a marine rather than an estuarine assemblage (Russel 1957). The estuarine regions of the Rivers Plym and Tamar, which empty into The Sound, are found above Laira Bridge and up-river from The Homoaze (Fig. 2.1B) (Russel 1957). The salinity of the surface water within The Sound, however, does vary slightly during a tidal cycle, particularly after periods of prolonged precipitation. Minimum salinity measured over a three month period in autumn 1974 at a depth of 4m was 32.8ppt (unpublished data collected for the Marine Biological Association aquarium).

Extensive seawater temperature data have been collected for the sea area around Plymouth for many years (Cooper 1958, Southward and

Butler 1972). Measurements of seawater temperature taken during the course of the present study showed a regular seasonal pattern consistent with the long-term records (Southward and Butler 1972). In summary, minimum temperatures occur in February/March and maxima during August/September (Fig. 2.2). The tidal range for the Plymouth area is relatively large with equinoxal spring tides reaching 6m above Chart Datum. The very extensive intertidal areas of the River Tamar, together with the rather constricted entrance to The Sound, cause currents in excess of 2.5 knots, particularly at The Narrows (Fig.2.1B).

Underwater visibility was estimated in the present study using the maximum number of chain links observable at F-Buoy whilst approximately one metre above the chain. Visibility varied markedly over the 24 month sampling programme with no obvious annual pattern (Fig. 2.3). Optimal visibility generally occurred at high water, particularly during periods of more settled weather. The sublittoral topography within The Sound varies greatly and there are numerous natural and artificial outcrops and reef systems. Sandy/muddy beds cover a large area of The Sound and undredged areas occur generally at a depth of 10-12m. The shipping channel, and naval mooring areas, are maintained to give a draft of 16m. The Narrows, an area with steep-sided limestone cliffs, extends to a depth of 30m and is kept free from fluvial deposits by the rapid tidal currents (Fig. 2.1B).

2.2. Zonation used in study

The preliminary survey for Liocarcinus puber established that relatively high numbers of crabs occurred in nearshore habitats, most noticably in areas of heavy kelp growth. To examine possible migratory

or feeding movements, three different areas occupied by L. puber were sampled. The criteria used to define the three areas (zones) were based on Hiscock (1985).

1) The eulittoral zone - defined as the zone above Chart Datum (C.D.).

2) The infralittoral zone - the boundary for this zone was dictated by the lowest level of abundant growth of brown algae. As this depth is dependant on water clarity (Hiscock 1985), its maximum depth will vary between geographical areas. In the present study, this zone ranged from C.D. down to a depth of 10m.

3) The circalittoral zone - extends below the depth of abundant brown algal growth and, for the present study, was generally below 10m.

2.2.1. Eulittoral zone

The eulittoral zone sampled was the reef at Blackstone Rocks, Wembury (Fig. 2.1C). The reef is composed of lower Devonian slate and forms a substantial marine platform which was cut during a period of higher sea level. Approximately 5000m^2 of shore are exposed at Chart Datum. Blackstone Rocks has a rich flora dominated by fucoid algae, with a dense growth of Laminaria digitata (Hudson) at extreme low water springs. The fauna of Wembury Bay is well documented (Kitching et al. 1934, Colman 1940, Morton 1954, The Plymouth Marine Fauna 1957). The high abundance and diversity of the flora and fauna at this site is caused partly by the presence of an uneven series of ridges running at right angles to the south easterly dip of the rock strata (Kitching et al. 1934). The gullies between these ridges are particularly well protected from wave action and provide sheltered niches on this relativly exposed shoreline (Potts 1985). Favourable aspect, and the

friable nature of the slate, allows cover for a markedly diverse fauna. Liocarcinus puber is a common member of this community and is found within crevices and under boulders in the protected gullies near the extreme low water mark. The physical conditions at Blackstone Rocks do not differ markedly from those experienced within The Sound. There is a maximum tidal range of 6m which, at high water spring tides, completely covers the reef. The reef is partially protected from south westerly gales by the Mewstone (Fig. 2.1B) but, due to the exposed aspect of Wembury, waves are common.

2.2.2. Infralittoral zone

Initial dives within this zone showed that large numbers of Liocarcinus puber were present at night, when they emerged to feed, yet few crabs were observed during the day, when they were generally well hidden in holes and crevices. These preliminary surveys indicated that one site did not have sufficient numbers of L. puber to sustain a long-term sampling programme, due to either inaccessability of crabs or limited area in which to sample. To overcome this potential problem of limited crab numbers, several sites within this zone were sampled (Fig. 2.1B).

Site 1) Bovisand - The shale bedrock around Bovisand has a dense growth of algae, with the laminarians Laminaria hyperborea (Gunnerus), Saccorhiza polyschides (Lightfoot) and Laminaria saccharina (Linnaeus) being particularly abundant; smaller algae and encrusting organisms occur beneath the kelp canopy. Approximatly 150m from the shore, the bedrock fines out into sand at 10m depth. Due to the deeply crevicular nature of the rock and the thick algal cover, few crabs were collected

during the day, although many were observed at night. The area is exposed to south westerly gales although it is also readily accessible in fine weather.

Site 2) The Hoe - This area is well protected from storm action by the Breakwater (Fig 2.1B) and was therefore accessible throughout the year. The area is current-dominated, and has a predominantly muddy-silt substratum with limestone outcrops. The latter have a covering of Laminaria hyperborea. Individuals of Liocarcinus puber were found associated with the limestone outcrops during the day and foraging over extensive areas of the substratum at night. Although L. puber was relatively common in this habitat, the number of available refuges in the limestone crevices was limited and this feature probably partially limited the total number of crabs present.

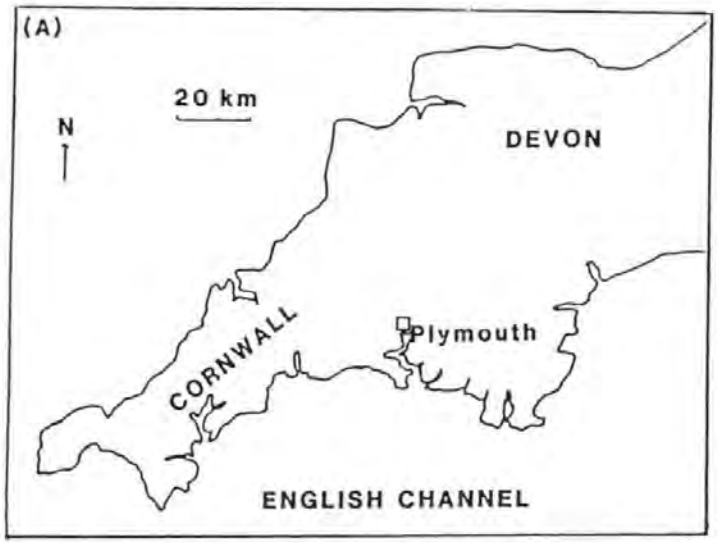
Site 3) The Breakwater Centre Fort - The northern, sheltered side of the Breakwater including the Centre Fort is accessible in most weather conditions, allowing year-round sampling. The substratum consists of large blocks of concrete with a comparatively thin algal cover.

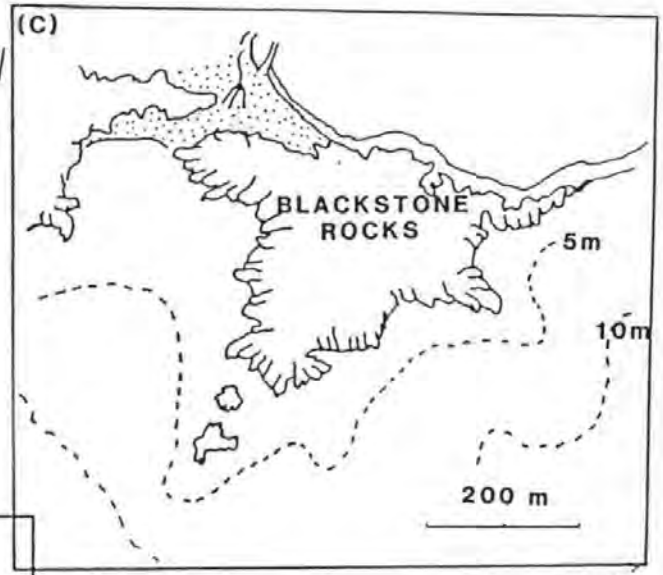
2.2.3. Circalittoral zone

The circalittoral site was at F Bouy, a large Admiralty Buoy situated 500m from Jennycliff Bay within Plymouth Sound (Fig. 2.1B). F Buoy is well protected from storms by the Breakwater and is anchored on a muddy-silt bottom. The buoy system consisted of three ground chains, each 250m long, connected to a riser chain and large buoy. The chain links were 0.8m long by 0.4m high, and formed a cave-like habitat in which crab and fish species sheltered. The area has moderate currents, up to 0.9 knots, and a fauna dominated by encrusting filter feeders

such as ascidians, crinoids and anemones, as well as other opportunists such as Gobius paganellus (Linnaeus), Gobius niger (Linnaeus), Cancer pagurus and L. puber. No growth of brown algae occurred on the chain links, however, large quantities of broken stipe and fronds were frequently observed banked by the tidal currents against the ground chains.

Figure 2.1. Map of the study area showing (A) the location of Plymouth in South-West England, (B) details of Plymouth Sound and environs, and (C) position of Blackstone Rocks, Wembury. For (C), dotted lines are depth contours.


(A)
20 km
N
DEVON
CORNWALL
Plymouth
ENGLISH CHANNEL


(C)
BLACKSTONE
ROCKS
5m
10m
200 m

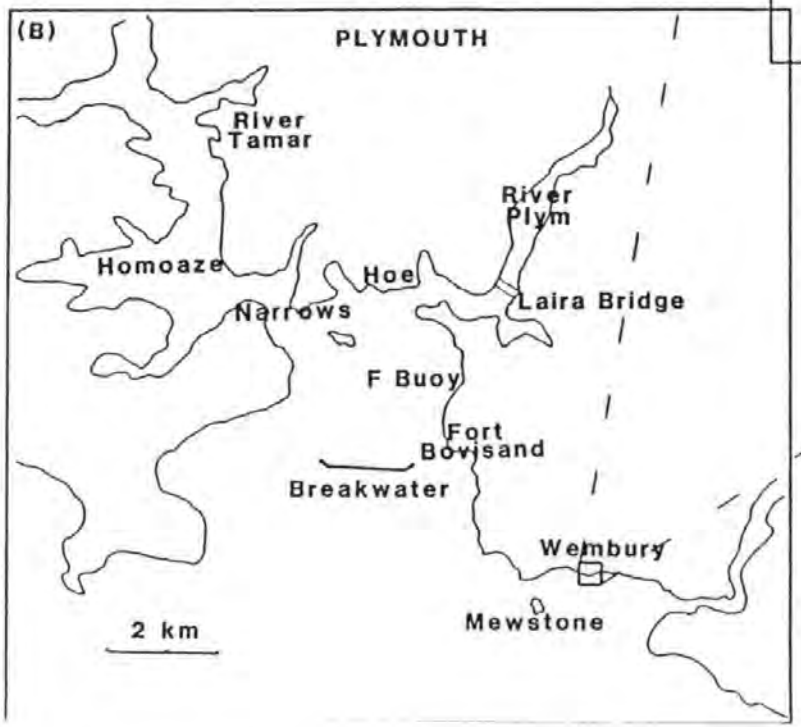

(B)
PLYMOUTH
River
Tamar
River
Plym
Homoaze
Hoe
Laira Bridge
Narrows
F Buoy
Fort
Bovisand
Breakwater
Wembury
Mewstone
2 km

Figure 2.2. Monthly seawater temperatures (±1 standard deviation) taken during SCUBA sampling at approximately 1 m above the sea floor from the infralittoral and circalittoral sampling sites during (A) January to December 1986; and (B) January to December 1987.

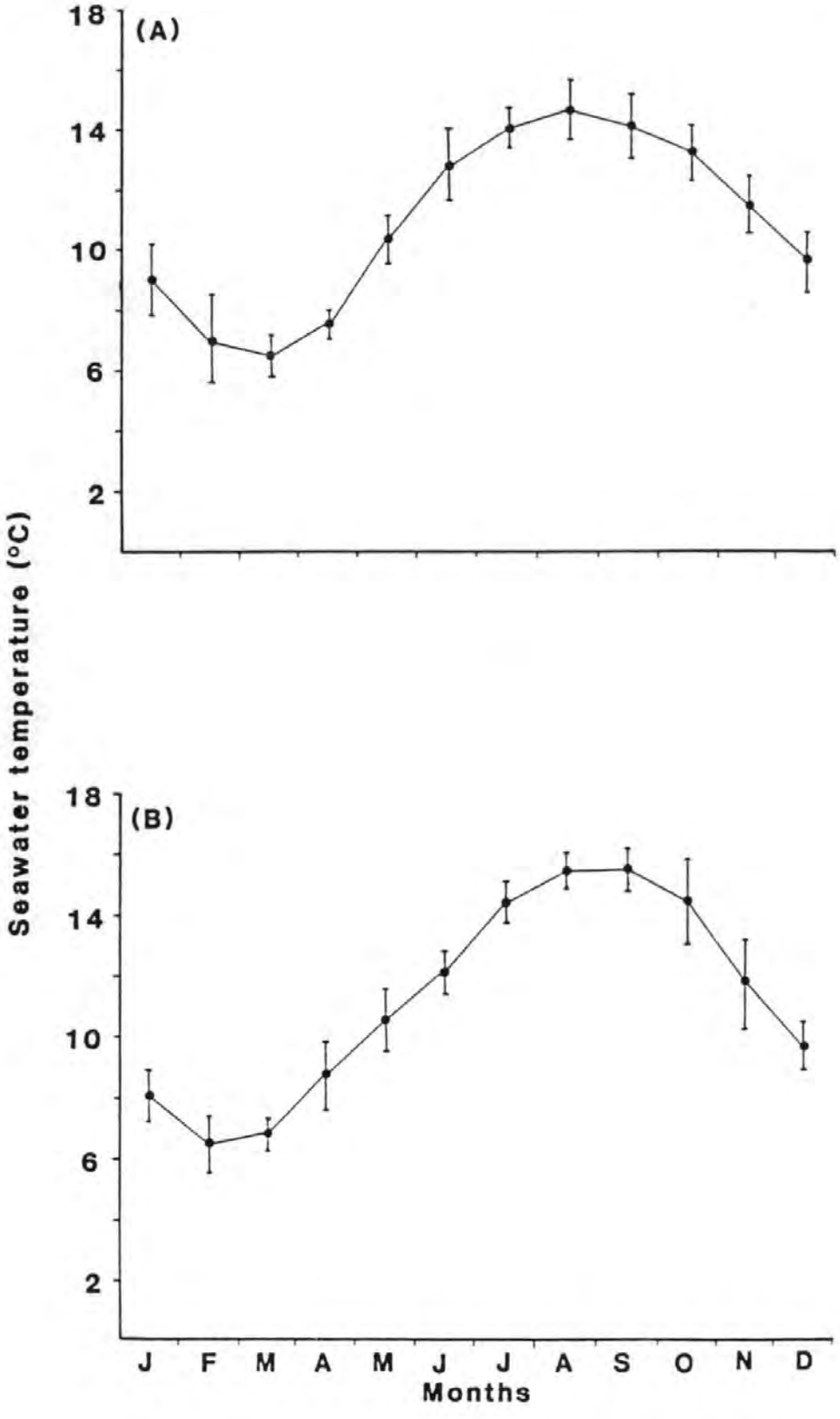
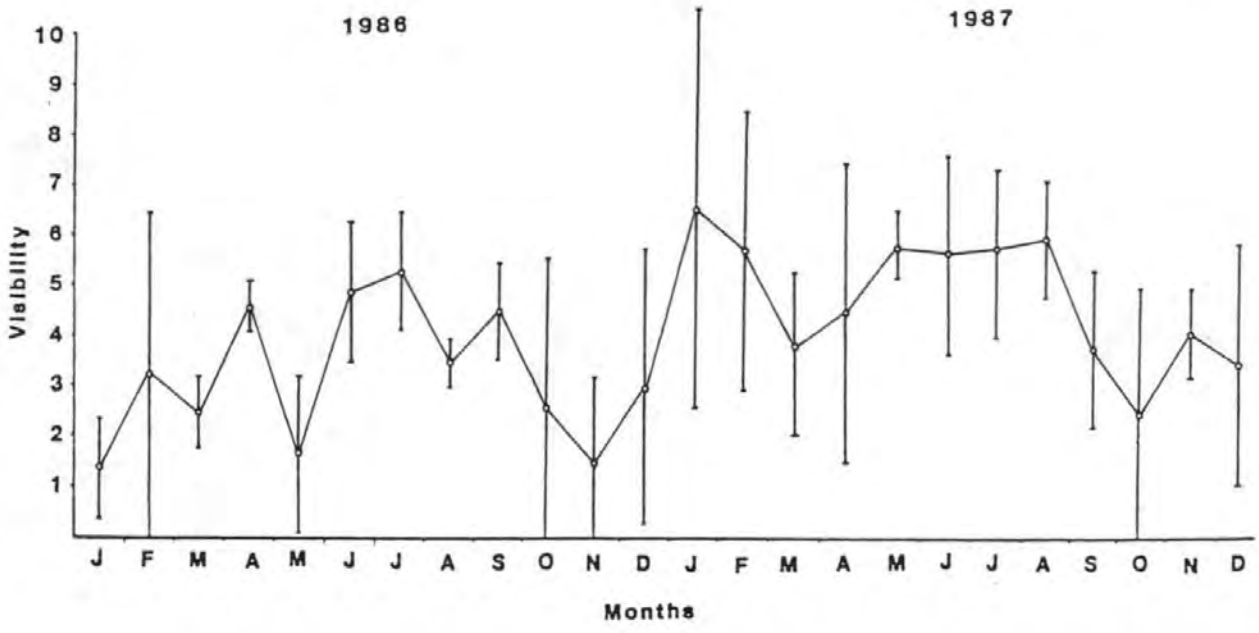

(A)
(B)
18
14
10
6
2
Seawater temperature (°C)
J F M A M J J A S O N D
Months

Figure 2.3. Mean monthly visibilities (± 1 standard deviation) measured at F Buoy estimated by a count of the number of links in the ground chain visible at a distance of one metre above the chain.


1986
1987
Visibility
10
9
8
7
6
5
4
3
2
1
J F M A M J J A S O N D J F M A M J J A S O N D
Months

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Sampling procedure

To allow a comparative seasonal study of Liocarcinus puber from its full vertical range, the three zones outlined in Chapter 2 were sampled with approximately the same amount of effort (measured in man hours per month) over a twenty four month period (January 1986 to December 1987). Techniques of capture varied between littoral and sub-littoral zones, but measurements taken from individual crabs were standardised for the three zones (see Section 3.2). To minimise, as much as possible, population and environmental disturbances, after the first six months, all measurements were taken in the field.

3.1.1. Eulittoral zone

At Blackstone Rocks, Wembury, sampling for Liocarcinus puber was carried out at times of low water spring tides when the crabs were found under boulders and in crevices on the low shore. Sampling was carried out only on tides which went lower than 1.0m above Chart Datum. The shore was visited 2-3 times each month and the sampling period was limited by the return of the tide to a maximum of 90 mins. Crabs, captured by hand by turning over boulders and inspecting crevices, were measured as outlined below (Section 3.2). Due to the historical biological importance of this unique site (Colman 1940), disturbance to the environment was kept to a minimum and all crabs, where possible, were returned to their original position on the shore.

3.1.2. Sub-littoral zones

SCUBA sampling was carried out by paired divers catching crabs by hand. At F Buoy, divers were positioned one each side of the ground

chain and, while one scared crabs through the chain links onto the open substrata, the other caught them by hand. The crabs were then either placed in a nylon meshed collecting bag for examination on board the diving vessel, or, for the migration study (Chapter 10), were measured underwater using purpose built underwater callipers. After measurements were recorded, crabs brought to the surface were returned to the sea floor and released adjacent to the chain system. The same procedure, using paired divers to catch crabs, was used in the infralittoral zone and crabs found in rock crevices were taken on board the diving vessel where measurements were taken.

3.2. Field data - parameters examined

For the first six months, all crabs collected, irrespective of zone, were taken to the laboratory and maintained in seawater aquaria prior to examination. For each crab, the sex, carapace width, moult stage, degree of limb loss, reproductive stage, wet weight, cheliped length and height, abdomen width and presence of any epifauna was recorded. After this initial period, all parameters were recorded in the field and measurements were taken to the nearest 0.1mm using hand-held vernier callipers. Field measurements included the sex, carapace width, moult stage, limb loss, reproductive state of the females and presence of epifauna. Details of the parameters examined are given below.

3.2.1. Sex - This is readily apparent in adults (40-100mm carapace width) from the shape of the abdomen. The male abdomen is acutely trianglar and white, whereas that of the female is broad and

hemispherical and is covered by dense setae or 'velvet', making the abdomen appear brown. Sexually immature crabs (<40mm carapace width) have white (non-setose) abdomens, but still may be separated into males and females by abdomen shape. Juvenile crabs (<8mm carapace width) were very difficult to sex accurately in the field and were returned to the laboratory. Using a binocular microscope, they were sexed by raising the abdomen and examining the number of pleopods present; males have a well developed pair of copulatory spines, whilst females have four pairs of finely haired pleopods.

3.2.2. Carapace width (CW) - During initial examinations, both the carapace width (as the total width to the ends of the anteriolateral spines) (Leavastu 1965) and the distance between the indentations of the fourth and fifth anteriolateral spines (Choy 1986a) were measured. The former measurement was subsequently taken as the better indicator of crab size, since it proved easier to record accurately in the field. To separate the two mesurements, long carapace width (LCW) is used here for the total width including spines, and short carapace width (SCW) for the distance measured between the indentations of the fourth and fifth anteriolateral spines. The relationship between these two parameters is given in Figure 3.1. Damage to the outer spines, found in approximately 1% of the population, occurred predominantly in large individuals suffering from shell erosion caused by chitiniverous bacteria (Johnson 1983). For individuals with damaged spines, the distance between the fourth and fifth spines was measured and the LCW estimated using the relationships shown in Figure 3.1. Juvenile crabs

(<8mm) were always measured in the laboratory using a micrometer eyepiece in a stereoscopic microscope.

3.2.3. Moult stages - The moult stage classification first devised by Drach (1939) and modified by Warner (1977) was adapted for use in the field as follows :

a) Soft - no calcification of the new exoskeleton.

b) Early papershell - a thin, flexible exoskeleton that is easily depressed when touched. The chelae are first to harden and are light brown.

c) Late papershell - the exoskeleton is hard with the exception of the branchiostegite region which is still compressible. The colour of the chela is still light brown.

d) Intermoult - the exoskeleton is completely hard and the chela colouration is dark.

e) Late intermoult - the exoskeleton is completely hard and chelar teeth may be well worn and have a less rounded appearance than for crabs in earlier moult stages; epifaunal growth is common.

3.2.4. Limb loss - Initial surveys showed a high percentage of crabs missing one or more limbs. The number and position of limb loss was recorded.

3.2.5. Reproductive state - Mature females were examined for the presence of eggs and sperm plugs. A sample of approximately twenty eggs was removed from each ovigerous female for egg developmental staging in the laboratory (Chapter 4). Eggs, removed using foreceps, were placed

in sea water and, where possible, were staged and measured whilst unpreserved. The presence of sperm plugs was noted by slightly raising the abdomen and examining the openings of the oviducts.

The following measurements were also recorded during the first six months of the sampling programme and subsequently on any crab returned to the laboratory.

3.2.6. Wet weight - Crabs were blotted dry and held out of water for 10 mins to remove excess water from the branchial chambers. They were weighed on a top-pan balance to an accuracy of $\pm$ 0.1g.

3.2.7. Cheliped length and height - The lengths of the major and minor chela were taken from the tip of the dactylus to the centre of the proximal end of the propodus. The height was measured from the highest point on the proximal end of the dactylus to the lower edge of the propodus .

3.2.8. Abdomen width - Measurements were taken at the fourth abdominal somite for both males and females.

3.3. Holding facility

To study growth, reproduction and diet, a holding tank 9.6m^3 situated at Fort Bovisand (Fig. 2.1B) was used. The tank was constructed in a Second World War pill box, sited on a reinforced concrete sea defence platform approximately 10m above the high water mark and adjacent to the sea. The tank was 5m long by 1.6m wide and the lip of the tank was 1.2m high (Fig. 3.2). The concrete was finished in cement render and ICI pondseal to prevent leaching of harmful materials

and seawater leakage. A continuous water flow was maintained via a Beresford PV41 pump which circulated approximately 40lmin^{-1} Water purity was maintained by a biological filter at the water intake and a sand/gravel filterbed at the outflow (Fig. 3.2).

The tank effectively experienced natural seasonal fluctations of light and temperature via the mesh grilled windows. The temperature and salinity of the sea water in the tank were measured twice weekly at each feeding visit. Salinity rose at a rate of approximately 1ppt per month and, to avoid any deleterious effects, half the tank's water was replaced by fresh sea water every two weeks. The general seasonal pattern for temperature was similar to that found from SCUBA data, however, tank seawater temperatures were slightly higher in summer and slightly lower in winter due to the smaller volume of water in the holding tank (Fig. 3.3). Fresh sea water was obtained at times of high water via a trench pump and pumped directly from the sea. The water sampling area has little freshwater input and water clarity was generally good. The area of sea water extraction, however, is prone to south westerly gales and high waves, and these conditions prevented pumping. A maximum salinity of 35.5ppt was recorded after a period when weather conditions prevented regular water changes.

Within the aquarium tank, crabs were kept individually in marked plastic boxes (0.5 x 0.3 x 0.3m). Each box was sub-divided into four compartments by perspex sections and covered with a perspex lid, which was held in place by sections of brick. The boxes were slatted both on the sides and the floor to enable adequate water flow. Prior to feeding, the crabs were inspected for moulting, mortality, torpid state, and spawning and hatching of any eggs.

Figure 3.1. Plot of short carapace width (SCW) against long carapace width (LCW) for (A) male, and (B) female Liocarcinus puber. Fitted regression lines have the following equations: males, LCW = -0.914+1.07SCW (n=32, r=1.00, P<0.001); and females, LCW = -0.317+1.06SCW (n=30, r=0.99, P<0.001).

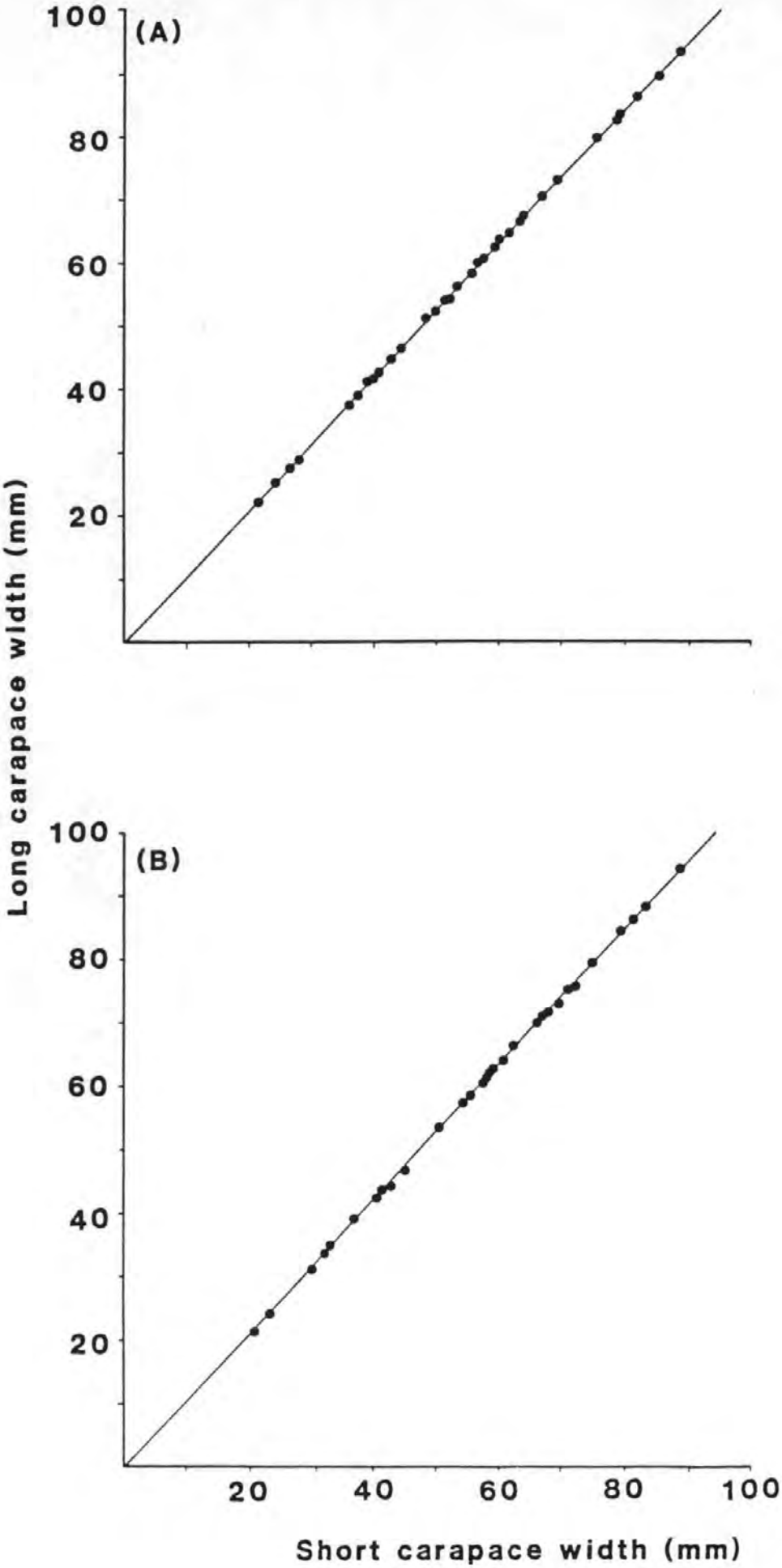
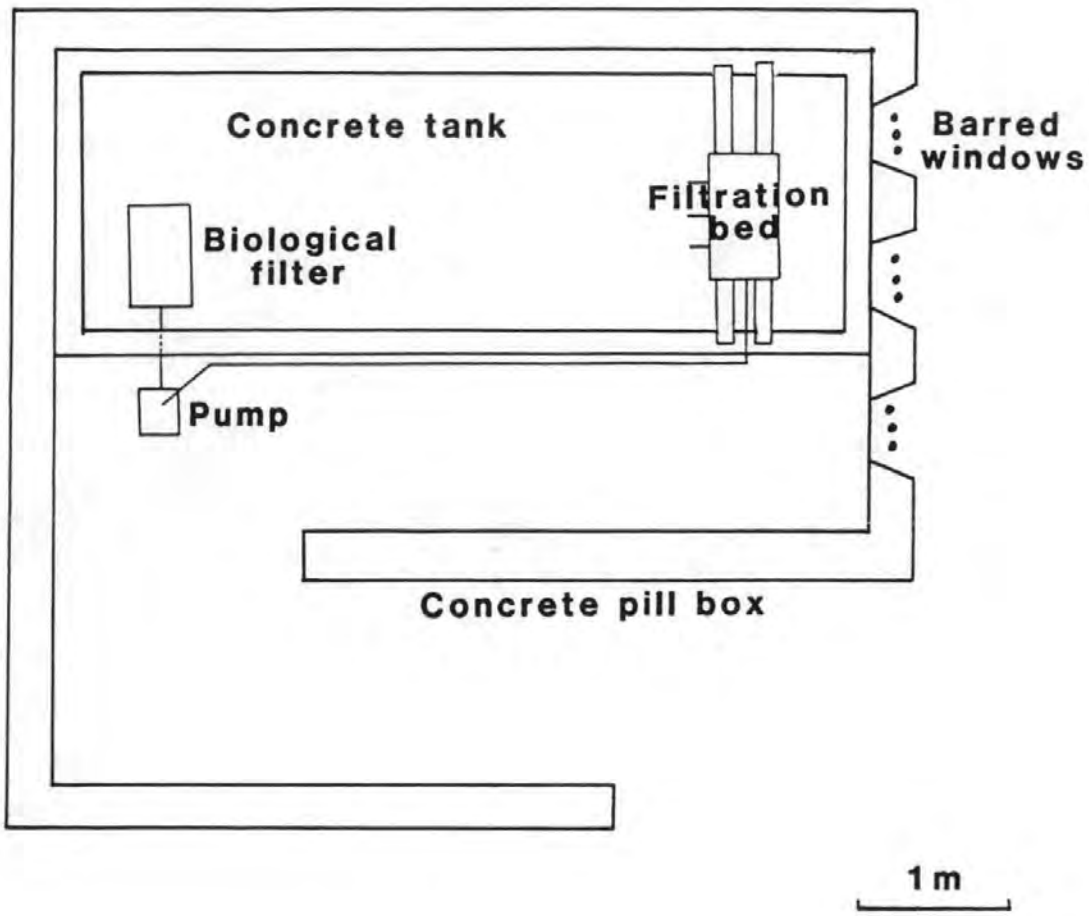

(A)
(B)
100
80
60
40
20
Long carapace width (mm)
20
40
60
80
100
Short carapace width (mm)

Figure 3.2. Schematic diagram of the holding facility at Fort Bovisand showing (A) horizontal plan and (B) vertical plan of holding tank, and position of pump and filtration bed.

(A)


Concrete tank
Barred windows
Filtration bed
Biological filter
Pump
Concrete pill box
1 m

(B)

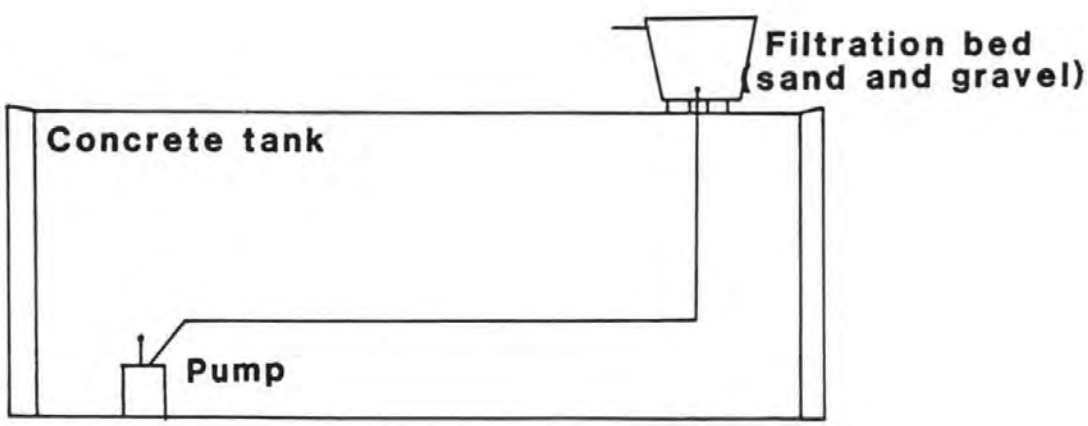
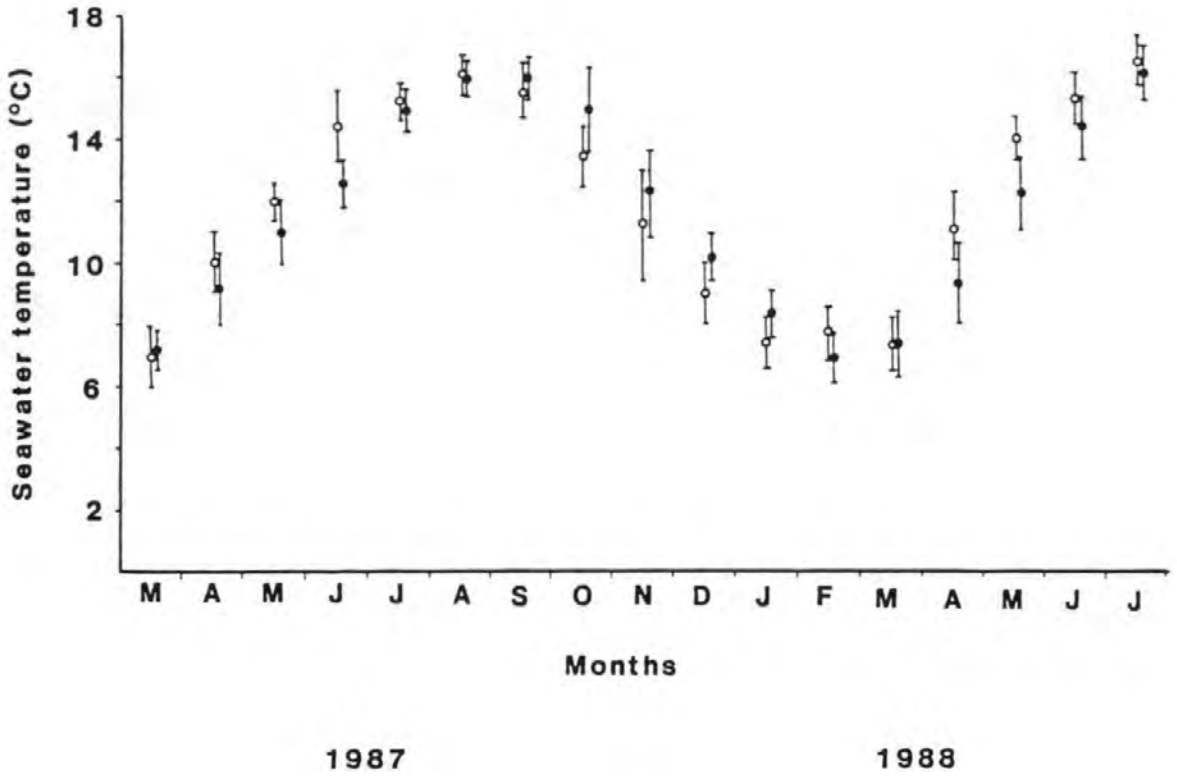

Filtration bed (sand and gravel)
Concrete tank
Pump

Figure 3.3. Mean temperatures (±1 S.D.) experienced in the holding facility at Bovisand (open circles) compared with the temperatures measured using SCUBA (closed circles).


Seawater temperature (°C)
18
14
10
6
2
M A M J J A S O N D J F M A M J J
Months
1987
1988

CHAPTER 4

REPRODUCTION

4.1. Introduction

Brachyuran reproduction has recieved much attention and recent reviews include those by Sastry (1983), Adiyodi and Adiyodi (1983, 1984) and Hartnoll (1985). This extensive literature base contains many reports of various aspects of reproduction of portunid crabs, although the species which have been investigated are generally the larger, commercially important species [eg. Callinectes sapidus (Churchill 1921, Hard 1942, Van Engel 1958, Tagatz 1968), Carcinus maenas (Broekhuysen 1936, Crothers 1968, Laulier and Demeusy 1974, Berill 1982), Portunus trituberculatus (Miers) (Oshima 1938), Portunus sanguinolentus (Herbst) (Ryan 1967a, 1967b), Portunus pelagicus (Linnaeus) (Pillay and Nair 1971, Fielder and Eales 1972), Scylla serrata (Forskal) (Hill 1975), Liocarcinus puber (Gonzalez Gurriaran 1981b, 1985a, Choy 1986a), Callinectes arcuatus (Ordway) and Callinectes toxotes (Ordway) (Paul 1982, Dittel et al. 1985), Ovalipes punctatus (De Haan) (Du Preez and McLachlan 1984b), Ovalipes stephensoni Williams (Haefner 1985a) and Ovalipes catharus (White) (Davidson and Marsden 1987)]. Many of these commercial species are from tropical and sub-tropical latitudes, and have less seasonal reproductive cycles than temperate species (Gonzalez Gurriaran 1981b, 1985a, Dittel et al. 1985, Choy 1986a). Indeed, Hartnoll (1985) found no clear reproductive pattern for portunids, and reported that some species showed multiple broods, others a singular brood in each post-puberty instar, whilst others, showed ovulation(s) in some but not necessarily all instars. The growth pattern also greatly influences the reproductive strategy of portunids, with species such as Callinectes sapidus undergoing terminal anecdysis at the puberty moult (Van Engel 1958). The reproductive ecology is complicated

further as species of the genus Callinectes, and species such as Scylla serrata and Portunus pelagicus, are found predominantly in estuarine and mangrove areas, and females migrate to fully marine areas prior to zoeal release (Van Engel 1958, Hill 1975, Paul 1982, Potter et al. 1983). Such marked differences in climate, growth pattern and female migratory behaviour make direct comparison of reproductive pattern between portunids complex.

For the purposes of description, reproduction can be divided into several distinct events, such as size of maturation, mating, and subsequent laying and incubation of eggs. Size of sexual maturity can be estimated by analysis of allometric growth rates (Teissier 1960, Hartnoll 1978b, 1982), and/or by examination of the percentage of crabs showing mature or maturing reproductive organs (Wenner et al. 1974, Somerton 1980). Allometric growth of brachyuran appendages has been extensively reported in the literature and has contributed greatly to the understanding of the interrelation between growth and reproduction (Huxley 1927, Przibram 1931, Drach 1936, Warner 1977, Hartnoll 1974, 1978b, 1982). The allometric growth method utilises changes in growth rate after puberty of various organs directly or indirectly involved with reproduction, and has been used to identify the size of maturity for several portunid species (Haefner 1985a, Choy 1986a, Davidson and Marsden 1987). This technique has been critised, however, as it identifies the 'minimum size of maturity' and may not represent effectively the size at which a significant reproductive output is achieved (Somerton 1980). The alternative technique of identifying the 'size class' with 50% of the sample showing mature reproductive organs, and therefore contributing significantly to reproductive output, may also lead to inaccuracies due to differences in

the criteria used to identify the developmental stages of reproductive organs.

Mating in portunids has been reviewed by Hartnoll (1969). In general, mating occurs at the time of female moult, with males showing elaborate pre- and post-moult care of the female. The male holds the female against his sternum using the second pair of walking legs and releases and stands guard immediately prior to the female moult. Ryan (1966) found that a pheromone released via the female urine near the time of moult elicits the initial recognition by the male. Once the old integument is shed, the female is inverted by the male and copulation occurs. Spermatophores are deposited into the oviducts where they, together with associated secretions, form a 'sperm plug' which may protude from the oviducal opening (Hartnoll 1969). The length of time, both pre- and post-moult, the male carries the female varies between species and may be influenced by external factors (Hartnoll 1969).

Brood size (number of eggs carried by a female at any one time) of tropical and sub-tropical portunid species, such as Callinectes sapidus, Portunus sanguinolentus and Ovalipes punctatus, vary from one to two million eggs in a single brood (Truitt 1939, Ryan 1967b, Du Preez and McLachlan 1984). Temperate species, such as Liocarcinus puber and Liocarcinus holsatus, have smaller broods and the maximum number incubated in a single brood approximates half a million (Gonzalez Gurriaran 1981b, 1985a, Choy 1986a). The egg size of temperate species is, however, larger than that reported for tropical portunids, and this difference has been interpreted as an adaptation to higher latitudes (providing larger reserves for the longer embryonic development and producing larger first stage planktonic zoea) (Sastry 1983). Measures of total fecundity (estimates of

egg production over the total life history) of portunids are generally lacking, due to difficulties in substantiating the number of broods per female per year and difficulties of analysis of growth rates. Peak periods of reproduction of tropical species, where reproduction may extend over a protracted period of the year, are particularly difficult to identify. Estimation of the number of broods in temperate species is also complicated by the effect of changes in temperature on rate of development of eggs (Wear 1974). For example, eggs of Liocarcinus depurator reared at a sea water temperature typical for early spring required three times as long to develop than those reared at temperatures equivalent for summer (Wear 1974).

The aims of this investigation of the reproduction of Liocarcinus puber were to examine the mating behaviour, and to examine the number of broods, brood size and the extent of mortality of eggs.

4.2. Materials and methods

4.2.1. Field sampling

All females collected during the regular monthly sampling programme (Section 3.2) were examined for the presence of sperm plugs, eggs and evidence of recent hatching of eggs. Recent hatching was inferred from the presence of empty egg cases still attached to the pleopods. A sample of approximately 20 eggs was removed from each ovigerous female and the stage of embryonic development determined (Section 4.2.2). The occurrence and sizes of the male and female in each mating pair were also noted.

To assess the size at which male and female Liocarcinus puber become sexually mature, measurements of the major (crusher) and minor (cutter) chelar height and length, abdomen width and length, and maximum 'depth' of body (thickness) were measured to the nearest 0.1mm for each crab collected during the first 6 months of the study (Section 3.2.3). For all allometric examinations, the independant variable (reference dimension) was the long carapace width (LCW) as carapace width has been generally used in similar studies on decapods including members of the family Portunidae (Du Preez and McLachlan 1984b, Haefner 1985a). The data were analysed using logarithmic transformations, as a log power function of the slope (b) allows an assessment of the degree of allometry shown by the dependant variable (Hartnoll 1982). Values of b significantly <1 indicate negative allometry and slopes significantly >1 indicate positive allometry (Hartnoll 1982). Significance of the degree of allometry was measured by comparing values of b against the

isometric slope of 1 using the Student's *t*-test (Draper and Smith 1966); lines not significantly different from 1 were taken as being isometric with the reference dimension. Discontinuities in the growth rate of appendages, and size range over which the puberty moult occurs, were detected using bivariate scatter plots and examination of residuals from regression analysis (Gore and Scotto 1983).

4.2.2. Gonadal and embryonic development

Tissue squash preparations, together with morphological development of the ovaries, were used to examine ovarian development. Ovaries were staged arbitrarily using criteria adapted from similar studies (eg. Laulier and Demeusy 1974, Simons and Jones 1981, Du Preez and McLachlan 1984b) (Table 4.1). Tissue squash preparations were also used to assess the abundance of spermatazoa in the vas deferens and testes. Immature testes and vas deferens were small and opaque and had no or relatively few spermatazoa. In contrast, the vas deferens and testes of mature males were large, white and rather fibrous, with very large numbers of spermatazoa. The size of sexual maturity was estimated based on the size at which 50% of the sample had developing ovaries or mature vas deferens (Wenner *et al.* 1974).

Eggs were examined under a binocular microscope (Kwoya Optical SDZ-PL) and assigned to a developmental stage (Table 4.2). Eggs were elliptical in early stages and became more spherical in later development. Egg volumes were calculated using the formula $4/3\ \pi\ xyz$ where x, y and z are the radii measured on three perpendicular axes (Wear 1974). The radii were measured to the nearest 0.01mm using a

micrometer eyepiece in a stereoscopic microscope. Three eggs from each brood were examined to obtain a mean radius and volume.

4.2.3. Numbers of eggs per brood

All females used to estimate brood size were handled with care during sampling to minimise any egg loss due to abrasion, stress or aggression from other crabs. It was noted that stressed females removed eggs from their pleopods. Hence, those collected by SCUBA were placed in individual containers in situ, and only females without shed eggs in the container were used for analysis of brood size. Ovigerous females with eggs in Stage 1 and 2a (Table 4.2) of development were preserved in 5% seawater formaldehyde solution for later examination of brood size. Each egg-laiden pleopod was subsequently cut at its base, and washed in distilled water to remove all traces of preservative and salt crystals. The eggs were freeze-dried to constant weight, transferred to a desiccator, and a sub-sample of between 50 to 100 eggs was weighed accurately to the nearest μg using a CAHN 29 Automatic Electrobalance. The number of eggs in the sub-sample was counted using a binocular microscope and the weight of an individual egg was recorded. The weight of the egg mass was recorded and the weight of the pleopods deducted after removal by dissection. Using the mean weight of one egg and total weight of the egg mass, an estimate of total number of eggs was achieved. The sub-sampling process of weighing 50 to 100 eggs was repeated three times to give three estimates of brood size. If the variation in the estimates of brood size was greater than 10%, the process was repeated.

To estimate brood mortality in Liocarcinus puber, females with

stage 3 and 4 eggs were examined using the same experimental procedure as above. The effects of limb loss on the brood size was also examined using females missing one or multiple limbs.

4.2.4. Egg development and hatch model

To estimate the periods of spawning (release of eggs onto the pleopods) and of egg hatching (into zoea), a model, using the relationship between temperature and the rate of egg development, was devised to simulate the reproductive strategy of Liocarcinus puber. The aim of the model was to identify the peak periods of egg hatch.

The egg hatch time was described by Belehradek (1935, 1957), who related the development of invertebrate eggs to ambient temperature by the following equation :

$$D = a\,(T - c)^{b} \qquad \text{(Equation 1)}$$

where: D is the egg developmental time from spawning to hatching in days, a and b are species parameters reflecting the response of the species to changes in temperature, and c is the temperature at which development time becomes infinite.

Belehradek's equation can be rearranged and expressed as the proportion of the total development (T.D.) achieved in one day at a temperature T°C :

$$Dr = \frac{1}{a\,(T - c)^{b}} \qquad \text{(Equation 2)}$$

where Dr is the daily development rate.

Values of a, b and c have been estimated previously for Liocarcinus puber (Choy 1986b) and have been used in this study (a= 24400, b= -2.23, and c= -3.09° C). The temperature data used in the

model were those collected during the sub-littoral sampling (Section 2.1.1) and they, together with the known parameters a, b and c, allowed an estimate of Dr for each day over the spawning period (Equation 2). For example, for each day with an ambient temperature of 7°C, 0.71% of total egg development occurs, whilst on days at 14°C, 2.2% of total development occurs. Therefore, knowing the approximate temperature for each day, the cumulative 'potential' egg development could be calculated (Fig. 4.12).

To gain an accurate estimate of the degree of advancement of the eggs sampled from the ovigerous females collected in the field (eg. whether the eggs were 10% or 70% through their development), ovigerous females were reared in the laboratory and the time required to develop through each egg stage was recorded (Table 4.3).

Although approximately the same sampling effort was put into each monthly collection (Chapter 3), monthly variations in female numbers occurred. To allow comparison of the fluctuations in number of ovigerous females, the number of females sampled per month was standardised using :

$$\text{No. of ovigerous females in monthly sample} \times \frac{\text{Mean number of females in monthly samples}}{\text{Number of females in monthly sample}}$$

Due to the extended time taken for winter eggs to develop compared to those spawned in the summer, winter-spawned eggs are more likely to be sampled than the summer spawned eggs (as the sampling effort was constant for each month). To gain a more representative measure of the total number of eggs spawned, the monthly estimates were standardised to those for the longest developmental time ie. over the

coldest period. The number of females spawning in a particular month was therefore multiplied by a factor of:

$$\text{No. of females spawning in any one month} \cdot \frac{\text{Maximum number of weeks required to complete development}}{\text{Number of weeks the sample requires to complete development}}$$

For example, using data from Figure 4.12 (Example II) of a female sampled on the 15th July, 1987 with eggs in Stage 2b (ie. 40% through their development), the date of spawning can be estimated using the cumulative curve by extrapolating back through 40% of 'potential' development to give a date of spawning of 1st July, 1987. Similarly, hatching can be estimated by extrapolating forward through 60% of 'potential' development to give an estimated date of egg hatching of 7th August, 1987, that is, a total period from spawning to hatching of approx. 5 weeks. Assuming that the longest period to complete development was as for Example I (17 weeks) and that sampling effort was constant, the number of females spawning in the month of January would be more likely to be sampled by a factor of 17/5 (3.4) than the females spawning in July. Therefore to allow a quantitative comparison between months the number of females spawning in July are modified by this factor. Spawn and subsequent hatch profile were estimated for the two years examined.

4.3. Results

4.3.1. Size of sexual maturity

Positive allometric growth occurred for both major and minor chelae in immature and mature male Liocarcinus puber (Figs 4.1 and 4.2; Tables 4.4 and 4.5). The level of allometry was greatly enhanced in mature males, particularly for chelar height (Table 4.4). Chelar length was also significantly positively allometric, but at a lower rate than chelar height, leading to a more pronounced height with size (Figs 4.1 and 4.2; Table 4.4). Immature females showed an isometric increase in all chelar measurements. Immature males showed a low, but signifcant positive allometric growth of both length and height for both chela (Figs 4.1 and 4.2; Table 4.4). Adult females showed a positive allometric growth in chelar height, whilst the length showed isometric and slight negative allometry for the minor and major chela respectively (Figs 4.1 and 4.2).

Examination of allometric growth via regression analysis allows an estimate of the point of intercept between the two growth phases ie. estimate of pubertal moult (Section 4.2.1). Male and female Liocarcinus puber show a clear increase in growth rate of the height of the major chela from 46mm and 41.5mm LCW repectively (Fig. 4.1). Other chela measurements for males, such as the height of the minor chela (Fig. 4.1) and major and minor propodus length (Fig. 4.2), also gave similar intercept values of 47.5, 47.0 and 46.0mm LCW respectively. Relative growth of the length of the major and minor propodus for females were isometric with no discontinuity in growth

(Fig. 4.2), whilst minor chelar height showed a clear increase in growth rate from 42.0mm LCW (Fig. 4.1).

Immature and mature females show a similar positive allometric growth rate of the sixth abdominal segment, however, a marked discontinuity occurred over a range from 35.8 to 41.4mm LCW (Fig. 4.3; Table 4.4). This growth discontinuity at the puberty moult indicates where the growth rate is greatly accelerated. Discontinuities also occurred for female abdomen length and body depth with mean values for both variables at 40.5mm LCW (Fig. 4.3), although only marginal allometric growth was shown for both parameters (Figs 4.3 and 4.4, Table 4.4). Males showed an isometric growth for abdominal width, abdomen length and body depth, suggesting that these parameters are of no significance in their sexual development (Figs. 4.3 and 4.4, Table 4.4).

The size of sexual maturity can also be estimated from the size at which 50% of the sample have been inseminated, or are ovigerous. Over spring (April-June), 50% of females in an ovigerous state occurred at 46.0mm LCW (Fig.4.5). For recently moulted females observed from July to November, 50% with sperm plugs (ie, inseminated) occurred at 44.0mm LCW (Fig. 4.5). In addition, 50% of males had large quantities of spermatozoa in the vas deferens at 48.5mm LCW (Fig. 4.5).

The difference between the size at maturity estimated from the two methods is small, indicating that individuals may play an active reproductive role after the pubertal moult without any further growth. This suggestion is supported further by the good agreement of size of the smallest ovigerous female (38.6mm LCW) and inseminated female (37.3mm LCW), compared with the size range estimated from allometric

data (35.8 to 41.4mm LCW based on abdominal width discontinuity) (Fig.4.6).

4.3.2. Mating

Mating pairs were observed regularly in the field from July until November each year (Table 4.6). In each mating pair, males were significantly larger than non-paired, mature males sampled from the same site at the same time (t= 4.77, df=64, P<0.001). This finding suggests that large males are more successful at pair forming and defending females than are smaller individuals. In general, males in the mating pair were larger than females (mean difference in size between mates was 16.8mm) (Table 4.6).. Only one pre-moult female was larger than the male and that was by a small amount (2.7mm) (Table 4.6). In several of the pairs, using data from moult increment (Chapter 5), females would grow to a larger post-moult size than the males (Table 4.6). One male, marked as part of the littoral tagging programme (Chapter 10), was recaptured on two occasions and, in each case, formed part of a mating pair with two different females (male 73.5mm recaptured on 10/9/87 and 29/9/87) (Table 4.6). These observations suggest that large males may be capable of numerous successful courtships over one season as females moulted over an extended period (late July to mid November).

Only 4 (out of 43) mating pairs comprised males carrying post-moult females, indicating that post-moult care by the male is relatively short in this species (Table 4.6).

The field survey of inseminated post-moult (soft and papershell moult stages) mature females (Fig. 4.6) found that 95% had mated successfully and had two sperm plugs intact, and 3 individuals (5%) had

only one duct plugged. Three post-moult mature females sampled had no sperm plugs visible and, on dissection in the laboratory, no sperm plugs were observed in the oviducts or spermathecae.

4.3.3. Ovarian development

The cycle of ovarian development was very closely related to the moult cycle. Females in late intermoult and soft and papershell post-moult stages had ovaries in either a spent or early recovering condition (Stage II ovaries) (Table 4.1). Ovigerous females with recently oviposited eggs also showed predominantly spent or early recovering ovaries. With increasing development of the eggs, however, some females were observed with maturing ovaries (Stage III). A high percentage of females with Stage 4 eggs and recently hatched eggs had maturing (Stage III) or ripe ovaries (Stage IV), particularly between March and July (Fig. 4.7). The development of the ovaries of these ovigerous or early post-ovigerous females indicates that a further brood occurs in a high percentage of these females, particularly from March to May (Fig. 4.7).

4.3.4. Embryonic development

The diameters of eggs of all egg stages sampled from the field were measured and mean diameters calculated (Table 4.9). The mean egg diameter increased by 97µm during development (Table 4.9). The mean volume of newly spawned eggs was $0.034mm^3$ ($\underline{n}$=23) and of eggs very close to hatching was $0.0695mm^3$ ($\underline{n}$=4), giving a percentage increase in volume of 103%.

4.3.5. Seasonal variation in percentage of ovigerous females

The monthly percentage of ovigerous females showed no significant difference between the two years sampled (Paired *t*-test, $t=0.44$; $df=10$; $P>0.7$) (Fig. 4.8). In the first year (1986-1987), ovigerous females were found over an 11 month period and over a 10 month period in the second year (Fig. 4.8). Ovigerous females were first observed in December of both years, and the peak period, with an excess of 68% of ovigerous females, occurred in March and April (Fig. 4.8). Post-ovigerous females were observed in April, May and June of both years indicating that this period is the peak period of zoeal release; April and May showed particularly high numbers of post-ovigerous females. Slight differences were, however, apparent between the two year's data. The percentage of ovigerous females in March and April 1987 peaked at 68% compared with 80% and 76% respectively in 1986 (Fig. 4.8). The data for May 1986 showed a marked decline in ovigerous females followed by a slight increase to approximately 30% ovigerous in June, July and August (Fig. 4.8A). In comparison, May 1987 showed a continued high level (65%) of ovigerous females and subsequent lower values in July and August (Fig. 4.8B).

Similarly, the egg stage data showed a consistent pattern between years (Fig. 4.9). In December, only Stage 1 eggs (recently spawned) were sampled, however, from January onwards there was an increase in later stage eggs and a decline in the percentage of Stage 1 eggs. Stage 4 (nearing hatching) eggs were present, albeit in low numbers, over the winter period, and were first observed in January 1986 and February 1987. The percentage of ovigerous females with Stage 4 eggs remained low until April when it started to increase and reached a

peak in May; this peak was particularly marked for 1987 (Fig. 4.9B). In June and July, there was an increase in Stage 1 and 2 eggs, indicating a second period of spawning. Stage 1 eggs, however, were observed in each month from December to August 1985-86, and from December to July 1986-87, suggesting that there is a continued production of eggs, and subsequently protracted release of zoeae over an extended period of the year.

When the percentages of females in an ovigerous state were separated into size groups (40-54, 54-68 and 68+ mm LCW) which approximated the estimated sizes of the first, second and third year female cohorts (Chapter 5), some interesting differences were observed (Fig. 4.10). The 40-54mm grouping (I year cohort) showed a lower percentage of ovigerous females over the winter period (December-March) than the other cohorts (Fig. 4.10). This I year cohort also had a delayed start in spawning compared with older individuals (Fig. 4.10). In addition, the 40-54mm grouping had a significantly lower percentage of ovigerous females during the sampling period compared with both the 68+ mm females (t=5.29; df=8; P<0.05) and the 54-68mm females (t=5.05; df=8; P<0.05).

4.3.6. Number of eggs per brood

The number of eggs (Stage 1 and 2a) per brood carried per female ranged from 48,000 to 366,000 (43.2-81.7mm LCW) and was directly related to carapace width (Fig. 4.11). Numbers of eggs per brood for the later egg stages (Stages 3b and 4) ranged from 74,000 to 307,000 (48.6-76.4mm LCW). Regression lines fitted to the data in Figure 4.11

are given in Table 4.7. The number of Stage 3 and 4 eggs were reduced compared with Stage 1 and 2a, and ANCOVA showed that this difference was significant ($P<0.05$) (Table 4.8). Examination of the regression lines predicted for early and late stage eggs indicates that brood mortality may be as high as 20% over the period of egg development. Females missing limbs had abnormally low and very variable brood sizes (Fig. 4.11) (Table 4.7). Egg numbers carried by females missing limbs were significantly lower than Stage 1 and 2a eggs ($P<0.01$) and Stage 3 and 4 eggs ($P<0.05$) (Table 4.8).

4.3.7. Egg development and hatch model

The cumulative plot of potential egg development shows a slow developmental rate over the period January to March (winter), and a subsequent increase in rate from April onwards (Fig. 4.12). The date of release of eggs onto the pleopods (spawning) and the hatching time of eggs for each ovigerous female has been estimated (to the nearest week) using Figure 4.12 and the data given in Table 4.3. The spawning extrapolations (Fig. 4.13A) for 1985-86 show two main peaks, one from December to mid-January, and another in early July. The hatching extrapolations also show a main peak of zoeal release in May (which correlates with the January spawn) and a further peak in August, which is consistent with the spawn peak of July (Fig. 4.13B).

The 1986-87 spawn data showed a more complex situation than the previous year, with peak periods of spawning occurring in January and May, and a smaller peak in early March (Fig. 4.14A). The hatch data show a peak of release of zoeae over a three month period (mid-April to mid-July), with three minor peaks apparent within this period of zoeal

release (Fig. 4.14B). The first, and largest, peak in the hatching of eggs occurred in May, which correlates with the spawning peak in January. The second peak, in early June, was consistent with the spawning peak in March, and the third peak, occurring in July, correlated with the spawn peak of May. (Fig. 4.14)

Small ovigerous females (40-54mm LCW) spawned later in the season over the spring period compared with the larger females (Fig. 4.10). In addition, ovigerous females of this small size class were relatively scarce, suggesting a lower reproductive effort by this size group. In view of these two differences for small ovigerous females compared with the other ovigerous groupings, the data for the small ovigerous females were plotted separately (Fig. 4.15). The model suggests that small ovigerous females have one brood, and spawning takes place predominantly in April and hatching in June (Fig. 4.15).

The predictions from the Hatch Model agree with the results obtained from examinations of the ovary and egg developmental stages (Section 4.3.4). The total data, indicate that there are two broods for the majority of the II and III year females, and a single brood, which commences later in the year, for the I year females.

4.4. Discussion

The size at which sexual maturity occurs for Liocarcinus puber can be readily ascertained by examination of allometric growth data. Males showed a marked positive allometric growth of all the chelar measurements recorded in this study, and increasingly so, following the puberty moult, allowing puberty moult to be estimated as the point of intercept. Females showed a marked positive allometric growth of the width of the abdomen and discontinuity at puberty. The increase in size of the male chela, and resulting sexual dimorphism of chelae, has been related to their use as a secondary sexual organ, being important in display, defense, territory, courtship, copulation and combat (Hartnoll 1974, 1985). Similarly, the sudden and substantial broadening of the female abdomen at puberty is generally related to the need for a suitable base for an incubatory chamber for the brooding of eggs (Hartnoll 1974, Warner 1977). Estimates, using allometric data, for the size at puberty for female L. puber range from 38.6 to 42.0mm LCW (mean 40.5mm LCW) (Table 4.10). This size range correlates well with the size of the smallest ovigerous female collected in the field and with the size of the smallest female sampled with sperm plugs (38.6mm and 37.3mm LCW respectively), and confirms that females are able to produce a brood without a further moult following puberty. Based on the allometric growth of the abdominal width, Choy (1986a) reported an estimated size of maturity of 40mm LCW for female L. puber from South Wales (Table 4.10). Similarly Drach (1933) found the discontinuity for abdomen growth to range from 40 to 46.3mm LCW for samples of L. puber from France

(exact location of sample site undisclosed). Allometric data from the present study, gave an estimate of the size of sexual maturity for males at approximately 46.5mm LCW (range of values estimated 45.8 to 47.5mm LCW). Similar estimates for male sexual maturity were given by Choy (1986a) (44mm LCW) (Table 4.11). Indeed, examination of all the allometric data for both sexes shows close correlation between samples from South Wales and Plymouth, with male maturation occurring at approximately 46mm LCW and females at approximately 40mm LCW.

The estimated size of sexual maturity for Spanish Liocarcinus puber, is noticably larger than that estimated from other localities (Tables 4.10 and 4.11). Unfortunately, the estimates for Spanish crabs are not based on allometric growth rates, but are extrapolated from the size at which 50% of a size class exhibited maturing or mature gonads (Gonzalez Gurriaran 1981b, 1985a). This method of estimating size of sexual maturity is dependant on the criteria chosen to demark 'maturity' and, as such, may yield variations between differing workers. Gonzalez Gurriaran (1981b, 1985a) reported females to be mature at 53mm LCW compared with estimates from other locations, based on the same technique, of 48mm LCW (Choy 1986a) and 46mm LCW (present study). Similarly, Gonzalez Gurriaran (1981b, 1985a) reported the size of sexual maturity for males to be 59mm LCW, compared with 52mm (Choy 1986a) and 48.5mm LCW (present study) for more northern populations. Gonzalez Gurriaran (1981b, 1985a) also found that the size of the smallest ovigerous female, and the smallest inseminated female (presence of sperm plugs), was markedly larger (49.5 and 47.4mm LCW respectively) than the equivalent sizes noted in the present study (38.6 and 37.3mm LCW respectively). Gonzalez Gurriaran (1981b) used a comprehensive data set for his work (over a thousand females) and

therefore, it would appear that Spanish crabs are maturing at a larger size than those from more northern latitudes.

Drach (1936) found two discontinuities in his study of the relative growth of the female abdomen, the first at sexual maturity (Table 4.10), and the second at a size approximately 50-60mm LCW, which he interpreted as being a lowering of allometric rate at a terminal moult. The studies by Gonzalez Gurriaran (1981b, 1985a) and Choy (1986a), together with the present study, all found females larger than 60mm LCW, and therefore the deduction that this reduction occurs at terminal moult appears spurious. Choy (1986a) reported that a reduction in the level of allometry for large mature females does occur and this is supported by results of the present study. Studies on Ovalipes spp. (Haefner 1985a, Davidson and Marsden 1987) also noted several discontinuities in abdominal growth. Whether these discontinuities coincide with moults is not readily apparent, but findings suggest that post-pubertal allometric growth in the abdomen may be limited by the area available for development over the thoracic sternites. Comparison of the size of sexual maturity of Liocarcinus puber with other species of portunids is limited, as few species have been adequately examined, particularly using allometric techniques. Gray and Newcombe (1938) estimated that male Callinectes sapidus attained maturity at 89mm CW. Davidson and Marsden (1987) found differences in the size of maturity for female (35mm CW) and male (40mm CW) Ovalipes catharus, and also noted a difference in size between the estimate of maturity from allometric data and the size of the smallest ovigerous females, suggesting that there is a moult between puberty and oviposition. Haefner (1985a) also observed differences in size of maturity for Ovalipes stephensoni, with females maturing at 51mm CW and males at

61mm CW. From these rather limited data it would appear that there is a general trend for males to mature at a larger size than females, although it is not clear from the data if both males and females mature at the same age.

In the population of Liocarcinus puber studied at Plymouth, mating, egg production and subsequent zoeal release showed distinct seasonal patterns. In this species, mating occurs at the time of moult which, for mature females, occurred from late summer to autumn (August to November). The mating behaviour of L. puber is typical for portunids, with a pre- and post-copulatory pair formation ('embrace'), and copulation occurring immediately after the female moult. This study found a predominance of male L. puber carrying pre-moult females, suggesting that defence from conspecifics may be a significant element in mating behaviour. The low number of females carried post-moult after copulation also suggests that post-copulatory care by males is minimal and probably lasts only long enough for peripheral hardening in the female cuticle prior to release. In a laboratory study, Gonzalez Gurriaran (1985a) found that pre-copulatory attendance of L. puber was between 1 to 9 days (mean 5 days) and attendance post-copulation ranged from 4h to 3 days; the number of conspecifics in the tank with the mating pair influence the period of courtship. Laboratory observations of Choy (1986a) showed that the largest male, from a range of sizes, successfully carried and subsequently mated with an introduced female. Choy (1986a) concluded that male mating success was largely dependant on male size.

Field evidence from the present study suggests that males found in a mating pair generally tended to be the large II and III year group (>60mm LCW) (Chapter 5), and that they may copulate several times during

the time of the main female moult season (July to November). Mating pairs were, however, observed in which the male was only marginally larger than the female; in one case the female was slightly larger than the male. Choy (1986a) found that the pre-moult female was always smaller than the male in the mating pair, although post-moult the female could exceed the size of the mating male. Similarly, Gonzalez Gurriaran (1981b, 1985a) found the majority of females were smaller than males in mating pairs, although several females exceeded or equalled the size of the male post-moult. As males moult several months prior to the female moult, similar post-moult sizes indicate that males and females from the same year class are able to mate. The success of mating for equal sized post-moult pairs has not been investigated in this study, although field evidence indicates that copulation and formation of sperm plug is not always successful.

Analysis of the percentage of females carrying eggs showed that oviposition first occurred in December, and the percentage of ovigerous females increased over the winter to reach maximum levels by March and April. Stage 1 eggs (newly spawned) were, however, observed in each month from December to August, indicating that, at least, limited egg production occurred over an extended period of the year. In Spain, Gonzalez Gurriaran (1985a) found high levels (approximately 50% of the females sampled) of ovigerous female L. puber from January to March and a lower, but consistent percentage (>20%), from April to July. He observed ovigerous females in all months of the year, although in very low numbers from September to November (Gonzalez Gurriaran 1985a). At Swansea, Choy (1986a) observed a peak in numbers of ovigerous females in January and February (>60% of sample) and again a consistent level (of approximately 20%) of ovigerous females from April to June. These findings suggest a similar

reproductive periodicity for L. puber between the different geographical areas.

The use of monthly percentages of ovigerous females as a criterion of reproductive effort, however, has limitations. For example, no account is taken of the marked change in rate of development of eggs with the rise in seawater temperature over the spring period. For species such as Liocarcinus puber where development time extends over a long period (months), the length of time the eggs are retained on the pleopods will shorten with increasing temperature. Thus, examinations of reproductive output utilising monthly percentages will tend to underestimate egg production in warmer months. The predictive model incorporating Belehadrek's equation, takes into account this variation in development time of eggs. Results indicate that there was a distinct seasonal pattern of periods of spawning and subsequent hatch of eggs in both years. Two peak periods of spawning (oviposition) and hatching of eggs occurred. The initial spawning peak occurs in early winter (December and January) and this brood hatches in May. The high percentage of ovigerous females over March/April period prior to hatch in May for both years suggests that a large percentage of females have one brood over this winter period. A second peak of spawning occurs after this 'winter' brood is hatched, giving a further peak in zoeal production later in the spring/early summer. Evidence to support the hypothesis that the second peak in egg production is a second brood comes from several sources. Firstly, examination of the ovaries of females carrying Stage 4 eggs and females that had recently released zoeae (egg cases still attached to the pleopods), showed that a large proportion had well developed ovaries. Gonzalez Gurriaran (1985a) concluded from an examination of the gonosomic

index for L. puber that 85% of the females sampled can have two successive spawnings over one intermoult period. Secondly, it is well documented in the literature that Liocarcinus puber reared in the laboratory produce multiple broods within one intermoult. In this study, two broods were observed. Choy (1986a) similarly observed two broods and Gonzalez Gurriaran (1981b) observed up to three broods in laboratory-reared females. Thirdly, it was observed that non-ovigerous females and, more particularly, females which had recently hatched eggs, were more active than the rather cryptic ovigerous females. The more marked foraging behaviour noted by the non-ovigerous females will lead to sampling bias of this group and therefore reduce the estimates of the percentage of females ovigerous.

The model also indicated that recently matured (I year cohort) females produced only one brood during their first breeding year. This brood occurs in spring, with a peak of spawning for this year class in April and hatching in June. The timing of the second brood predicted by the model for the II and III year cohorts was different between the two years studied; the 1985/86 season showed a delay in spawning compared with 1986/87. Reasons for this difference between years are not clear.

Data from the model suggested that zoeae were produced from March through to September in 1986 and to August in 1987. This prediction agrees with the general findings of Lebour (1928) who noted that zoeae ofLiocarcinus puber occurred in the spring and summer. Lindley (1987) noted zoeae of L. puber in the English Channel appraoches from March to October, and the Plymouth Marine Fauna (1957) records zoeae and megalopae from April to August. Allen (1967) noted zoeae of L. puber from May to October in the Clyde and Argyll area. These data suggest that zoeae are produced over a relatively broad period of the year and this may be an

adaptation to ensure that at least some zoeae find suitable conditions for survival (Bakun et al. 1982).

The hatch model may, however, be rather over simplistic. Amsler and George (1984) investigated egg development of Callinectes sapidus and suggested that partial diapause may occur in eggs during the gastrula phase at low ambient temperatures. In addition, Sulkin et al. (1976) suggested that C. sapidus may have an intrinsic rate of egg development and that development is not totally temperature dependant. These findings, however, do not appear to be involved in the egg development of Liocarcinus puber (Choy 1986a). Wear (1974) and Choy (1986a) both found close correlation of rate of development and ambient temperature for several other portunids including Liocarcinus holsatus, Liocarcinus pusillus (Leach) and Liocarcinus depurator. Hartnoll and Paul (1982) noted that newly spawned eggs of Carcinus maenas sampled from the Isle of Man were smaller in volume (by up to one third) than those used by Wear (1974) for the same species at Plymouth, and required a longer period to develop at the same temperature than Plymouth C. maenas. The size difference in egg volume at spawning between L. puber in the present study and Swansea (Choy 1986a) is small by comparison. The use of the hatch model as a tool to broadly assess periods of maximal and minimal egg production therefore appears valid for L. puber.

The percentage increase in egg volume between spawning ($0.034mm^3$, n=23) and hatching ($0.0695mm^3$, n=4) was 103%, which was a similar egg volume increase to that shown by other species of Liocarcinus (Wear 1974). The increase in egg diameter during maturation was similar for each of the three localities examined (Galicia, Swansea and Plymouth) (Gonzalez Gurriaran 1981b, 1985a, Choy 1986a and the present study) (Table 4.9). The

initial, and final, egg diameters, however, were different and suggest that Spanish eggs are smaller at each developmental stage than those from Plymouth and Swansea (Choy 1986a) (Table 4.9). The larger egg size at higher latitudes is consistent with the general trend of longer developmental period at lower temperatures associated with more northern latitudes (Vernberg 1962, Efford 1969, Jones and Simons 1983). The general pattern of smaller eggs and higher brood size is well confirmed in the literature between species (Sastry 1983) and this trend may also be apparent within species from different geographical locations.

At Plymouth, Liocarcinus puber showed the trend typical of all brachyurans of increasing fecundity with increase in body size (carapace width). Similar relationships have been described previously for Liocarcinus puber (Choy 1986a, Gonzalez Gurriaran 1981b, 1985a). The present study, however, established that brood sizes at Plymouth were larger than those recorded previously (Choy 1986a, Gonzalez Gurriaran 1981b, 1985a) (Fig. 4.16). Reasons for this difference may be experimental and/or environmental. In this study, care was taken to minimise egg loss during sampling by placing ovigerous females in individual containers whilst in situ. In the laboratory, ovigerous females frequently lost eggs during handling and from interactions with other crabs. Therefore, ovigerous females used to establish egg numbers were preserved immediately on return to the laboratory. Thermal differences and slight variations in salinity between the capture site and the holding facilities may also cause stress to the ovigerous females and mortality of eggs (Choy 1986a). In the present study, ovigerous females maintained in tanks in the laboratory were periodically observed fanning and preening their eggs, removing eggs that were dead or slowly developing. Egg loss was noted

also, particularly with females with large broods, via abrasion. The lower brood size for Spanish L. puber (Gonzalez Gurriaran 1981b, 1985a) may be explained partly by experimental design as they were sampled using a trawl which would cause loss of peripheral eggs. Gonzalez Gurriaran (1985a) also used eggs of all developmental stages in his analysis and therefore egg numbers are likely to be reduced due to mortality associated with development (Jewett et al. 1985). The lower brood size reported by Choy (1986a) is less easy to explain, as experimental procedures were similar to the present study. Further analysis of brood size from different geographical localities, allied with details of growth, would be particularly interesting, as L. puber may display a degree of 'plasticity' in the response of reproductive output to environmental variables. Similar variability in reproductive output between localities has been noted for other decapod crustaceans including Nepthrops norvegicus (Thomas 1964) and Heterozius rotundifrons A. Milne Edwards (Jones 1978).

Table 4.1. Details of stages of ovarian development based on morphological and microscopical criteria. Cell diameters given as mean values of axes.

Stage	Description
I	Immature - Ovary small (<0.1g) and transluscent (cells <50μm, prominent nucleus)
II	Spent/Early Developing - Light yellow, small (<0.5g) (cells 50-100μm; cells undergoing vitellogenesis)
III	Maturing - Yellow to orange, conspicuous organ covering much of the hepatopancreas (>0.5g) (large >100μm post vitellogenic oocytes)
IV	Ripe - Dark brown to purple, conspicuous organ covering much or all of the hepatopancreas (>0.5g) (ova >200μm)

Table 4.2. Morphological description of the egg developmental stages based on criteria adapted from Broekhuysen (1936) and Amsler and George (1984).

Stage	Description
1	Newly spawned, full of yolk and lacking cleavage
2a	Cleavage,few large cells (early blastula)
b	Many small cells visible (early gastula)
3	Eyespots first visible as slight pigment
4a	Chromatophores first visible
b	Chromatophores and eyespots well developed and heartbeat evident

Table 4.3. Mean time (±1 S.D.) required for eggs reared in the laboratory to mature through the four egg stages described in Table 4.2. Data originate from 6 ovigerous females spawned in the laboratory. Temperature = 13.5 + 1.5°C; 5-10 eggs were examined and staged each day.

Egg Stage	Mean time (Days)	Percentage
1	7.0 ±0.7	15.1
2	15.6 ±1.5	33.7
3	8.8 ±0.8	19.1
4	14.8 ±1.1	32.1
Total developmental time	46.2 ±1.6	

Table 4.4. Regression analyses of morphometric data. All measurements are based on long carapace width (LCW) as the independent variable. For all cases, the correlation was significant ($P<0.01$). Allometric status of the slope (b) of the logarithmic transformed regressions was tested against the isometric slope of 1 using Student's t-test (Draper and Smith 1966). Equations: M = the slope of the line, C = intercept at X = 0, a = intercept at ln(x) = 1, b = slope, r = correlation coefficient, n = number of data points.

$$Y = MX+C \qquad \ln(y) = a + b \ln(x)$$

Parameter	Sex	Size	mmLCW	M	C	a	b	r	N	t	P	Allometry
Major Chela Height	M	Immat.	<45	0.240	-0.684	-1.91	1.11	0.99	138	+8.05	<0.001	+
	M	Adult	>45	0.358	-6.27	-3.04	1.40	0.97	263	+108.63	<0.001	+
	F	Immat.	<40	0.212	-0.180	-1.66	1.02	0.99	111	+1.47	>0.01	0
	F	Adult	>40	0.225	-0.780	-1.75	1.05	0.95	150	+11.86	<0.001	+
Minor Chela Height	M	Immat.	<45	0.200	-0.441	-2.02	1.10	0.99	134	+7.35	<0.001	+
	M	Adult	>45	0.314	-6.06	-3.44	1.46	0.98	227	+121.45	<0.001	+
	F	Immat	<40	0.185	-0.194	-1.81	1.02	0.99	96	+1.21	>0.01	0
	F	Adult	>40	0.196	-0.558	-1.91	1.06	0.94	183	+14.16	<0.001	+
Major Chela Length	M	Immat.	<45	0.641	-0.806	-0.67	1.05	0.99	138	+3.77	<0.001	+
	M	Adult	>45	0.827	-9.62	-1.30	1.22	0.98	258	+65.16	<0.001	+
	F	Immat.	<40	0.629	-0.605	-1.69	1.03	0.99	111	+1.47	>0.01	0
	F	Adult	>40	0.602	-1.18	-0.35	0.97	0.98	150	-7.41	<0.001	-
Minor Chela Length	M	Immat.	<45	0.608	-0.917	-0.77	1.05	0.99	134	+3.65	<0.001	+
	M	Adult	>45	0.794	-9.83	-1.40	1.23	0.98	224	+67.79	<0.001	+
	F	Immat.	<40	0.584	-0.383	-0.63	1.02	0.99	96	+1.21	>0.01	0
	F	Adult	>40	0.584	-0.194	-0.55	1.00	0.97	183	0.0	>0.01	0
Width of the Sixth Abdominal Segment	M	All Sizes		0.195	+0.013	-1.66	1.01	0.99	357	+1.16	>0.01	0
	F	Immat.	<40	0.368	-2.06	-2.14	1.26	0.97	100	+17.70	<0.001	+
	F	Adult	>40	0.576	-7.06	-1.98	1.29	0.98	239	+71.03	<0.001	+
Length of Abdomen	M	All Sizes		0.442	+0.594	-0.72	0.98	0.99	283	-2.36	>0.01	0
	F	Immat.	<40	0.457	+0.165	-0.67	0.97	0.99	100	-2.38	>0.01	0
	F	Adult	>40	0.525	-1.72	-0.94	1.06	0.99	200	+16.46	<0.001	+
Depth of Body	M	All Sizes		0.371	+0.414	-0.88	0.98	0.99	283	-2.36	>0.01	0
	F	Immat.	<40	0.370	+0.437	-0.76	0.94	0.99	101	-4.79	<0.001	-
	F	Adult	>40	0.400	+0.075	-0.86	0.99	0.95	112	-2.84	>0.01	0

Table 4.5. Regression analysis of morphometrical data using short carapace width (SCW) as independent variable. Equations as for Table 4.4.

			$Y = MX+C$		$\ln(y) = A + B \ln(x)$			
Parameter	Sex	Size (mmSCW)	M	C	A	B	r	N
Major Chela Height	M	Immat. <43	0.256	-0.878	-2.00	1.15	0.99	138
	M	Adult >43	0.379	-6.39	-2.99	1.41	0.97	263
	F	All Sizes	0.233	-0.520	-1.76	1.06	0.97	261
Minor Chela Height	M	Immat. <43	0.216	-0.662	-2.10	1.13	0.99	134
	M	Adult >43	0.338	-6.44	-3.32	1.45	0.98	227
	F	All Sizes	0.207	-0.543	-1.94	1.08	0.96	279
Major Chela Length	M	Immat. <43	0.685	-1.32	-0.747	1.09	0.99	138
	M	Adult >43	0.878	-9.90	-1.28	1.23	0.98	258
	F	All Sizes	0.669	-0.896	-0.636	1.05	0.99	261
Minor Chela Length	M	Immat. <43	0.650	-1.41	-0.845	1.10	0.99	134
	M	Adult >43	0.840	-9.67	-1.33	1.23	0.98	224
	F	All Sizes	0.637	-1.06	-0.704	1.05	0.98	279
Width of the Sixth Abdomen Segment	M	All Sizes	0.208	-0.144	-1.71	1.03	0.99	357
	F	Immat. <38	0.385	-2.41	-2.41	1.26	0.97	100
	F	Adult >38	0.577	-7.15	-2.01	1.30	0.98	239

Table 4.6. Size-frequency data of mating pairs observed in the field. All females in pre-copulatory, 'hard-shelled' condition unless otherwise stated. * indicates where female will be expected to be larger than male post-moult.

Date	Site	Male (mmLCW)	Female (mmLCW)	Difference (mm)	Notes
29/7/86	F Buoy	80.0	57.0	23.0	
5/8/86	Wembury	69.9	51.2	18.7	
7/8/86	Wembury	73.7	62.6	11.1	
		67.4	43.7	23.7	
8/9/86	Wembury	76.1	57.2	18.9	
9/9/86	Wembury	69.2	53.2	16.0	
18/9/86	Wembury	75.8	64.9	10.9	
		68.2	63.1	5.1	*
		63.0	50.4	12.6	
23/9/86	F Buoy	70.5	73.2	-2.7	*
		77.0	42.0	35.0	
6/10/86	Wembury	68.3	43.7	24.6	
14/10/86	F Buoy	63.0	42.5	20.5	
3/11/86	Wembury	67.4	44.7		Post-copulatory
		76.8	41.2	35.6	
17/11/86	The Hoe	60.0	34.0	39.9	
13/7/87	Wembury	74.4	52.9		Post-copulatory
14/7/87	F Buoy	71.5	61.5	10.0	
29/7/87	F Buoy	80.0	70.2	9.8	
4/8/87	Bovisand	76.6	64.8		Post-copulatory
12/8/87	Wembury	74.3	59.9	14.4	
		72.2	44.0	28.2	
13/8/87	Wembury	72.5	45.6	26.9	
		71.6	57.9	13.7	
		65.0	38.8	26.2	
14/8/87	Wembury	56.2	48.3	7.9	
		47.8	38.6	9.2	
2/9/87	F Buoy	69.1	58.5	10.6	
7/9/87	Wembury	72.2	60.3	11.9	
8/9/87	Wembury	79.7	58.3	21.4	
		73.6	50.1	23.5	
9/9/87	Wembury	67.7	48.1	19.6	
10/9/87	Wembury	73.5	57.7		Post-copulatory
		65.3	47.9	17.4	
29/9/88	Wembury	61.7	57.4	4.3	*
		75.4	54.8	17.2	
		62.9	54.8	8.1	
		70.7	57.4	13.3	
		64.1	48.5	15.6	
		73.5	61.5	12.0	
		75.3	58.7	16.6	
			mean difference	16.8	

Table 4.7. Regression equations for the relationship between number of eggs carried in a brood and ovigerous female carapace width, where $\ln(y)$ is the natural log of the number of eggs carried and X is the carapace width (n = number of data points, r = correlation coefficient).

Female description	Equation	n	r
Newly spawned eggs (Stage 1 and 2a)	$\ln(y) = 9.14 + 0.0470X$	27	0.94
Well developed eggs (Stage 3 and 4)	$\ln(y) = 9.08 + 0.0452X$	9	0.90
Females missing limbs (All egg stages)	$\ln(y) = 9.50 + 0.0334X$	9	0.47

Table 4.8. Comparison (ANCOVA) of number of eggs carried related to stage of egg development and the effect of limb loss (data shown in Figure 4.11). (F values are given for a (the slope of the line) and b (the intercept where X=1) (d.f.: degrees of freedom; Sign : ** = 1%, * = 5%, NS = not significant).

Pairs of line tested	a			b		
	F	d.f.	Sign.	F	d.f.	Sign.
Newly spawned/Well developed (Stage 1+2b/Stage 3+4)	1.05	1/34	NS	4.21	1/35	*
Newly spawned/Missing limb (Stage 1+2b/Limb loss)	2.11	1/34	NS	15.56	1/35	**
Well developed/Missing limb (Stage 3+4/Limb loss)	0.587	1/16	NS	4.16	1/17	*

Table 4.9. Mean diameters (μm) (±1 S.D.) of the egg stages of Liocarcinus puber from the present study (n = number of eggs examined) and comparative data from Swansea (S. Wales) (Choy 1986b) and Galicia (N.W. Spain) (Gonzalez Gurriaran 1981c). Descriptions of egg stages are given in Table 4.2.

Egg stage	Plymouth		Swansea	Galicia
	n	Mean diameter	Mean diameter	Mean diameter
1 (newly spawned)	69	404 +14.60	380	360
2 (gastrulation)	252	430 +18.43		
3 (eyespots)	108	464 +24.97		
4 (chromatophores)	135	501 +27.98	489	460
Increase in diameter		97	109	100

Table 4.10. Comparative size data of sexual maturity for female *Liocarcinus puber* from different geographical locations (all measurements are given in mm LCW).

Parameter	Plymouth (present study)	Swansea (Choy 1986b)	Spain (Gurriaran 1981c)	France (Drach 1933)
Sixth abdomen discontinuity	35.8 - 41.4	40	-	40 - 46.3
Abdomen length	39.5 - 41.5	-	-	-
Body height	40.0 - 41.0	-	-	-
Chela major height	41.5	-	-	-
Chela minor height	42.0	-	-	-
Mean value from allometric data	40.6	40	-	43.15
50% of females with mature ovaries	46.0	48.0	52.7	-
Smallest ovigerous female sampled	38.6	39.3	47.4	-
Smallest female with sperm plugs	37.3	41.8	49.5	-

Table 4.11. Comparative size data of sexual maturity for male Liocarcinus puber from different geographical locations (all measurements are in mm LCW).

Parameter	Plymouth Present Study	Swansea Choy (1986b)	Spain Gurriaran (1981c, 1985)
Major chela height	45.8	44.0	-
Minor chela height	47.5	-	-
Major chela length	47.0	-	-
Minor chela length	46.0	-	-
Mean value of allometric data	46.5	44.0	-
50% of males abundant sperm.	48.5	48.0	52.7
Smallest male abundant sperm.	43.0	41.8	49.5

Figure 4.1. Relationships between chelar height and carapace width for Liocarcinus puber: male major chela (closed circles and solid line), female major chela (closed circles and dashed line), male minor chela (open circles and solid line), and female minor chela (open circles and dashed line). Each point is the mean of a 5mm size class and vertical bars represent the 95% confidence interval. Equations for the lines, degree of allometry and further details are given in Table 4.4.

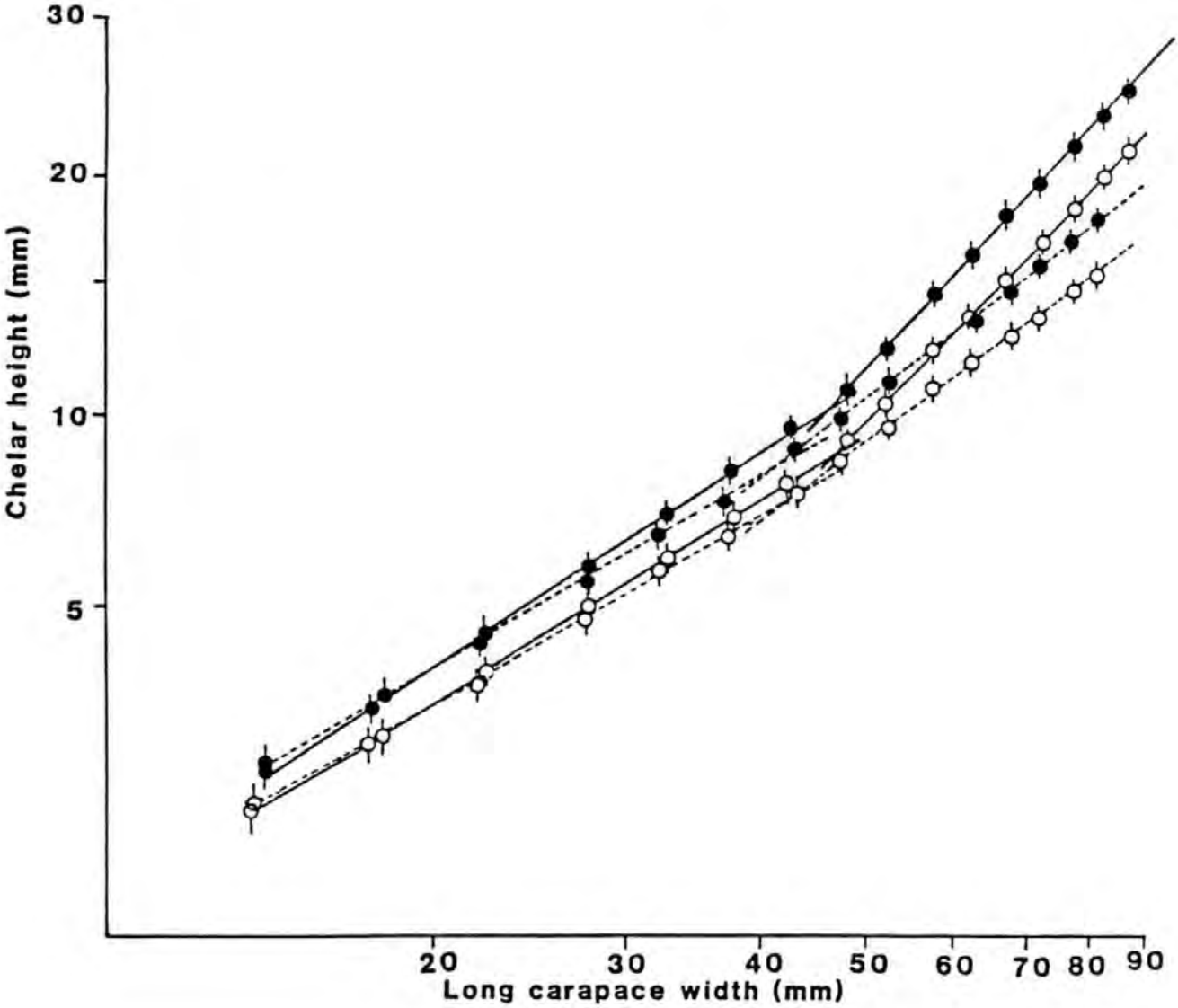

30
20
10
5
Chelar height (mm)
20
30
40
50
60
70
80
90
Long carapace width (mm)

Figure 4.2. Relationships between chelar length and carapace width for Liocarcinus puber: male major chela (closed circles and solid line), female major chela (closed circles and dashed line), male minor chela (open circles and solid line), and female minor chela (open circles and dashed line). Each point is the mean of a 5mm size class and vertical bars represent the 95% confidence interval. Equations for the lines, degree of allometry and further details are given in Table 4.4.

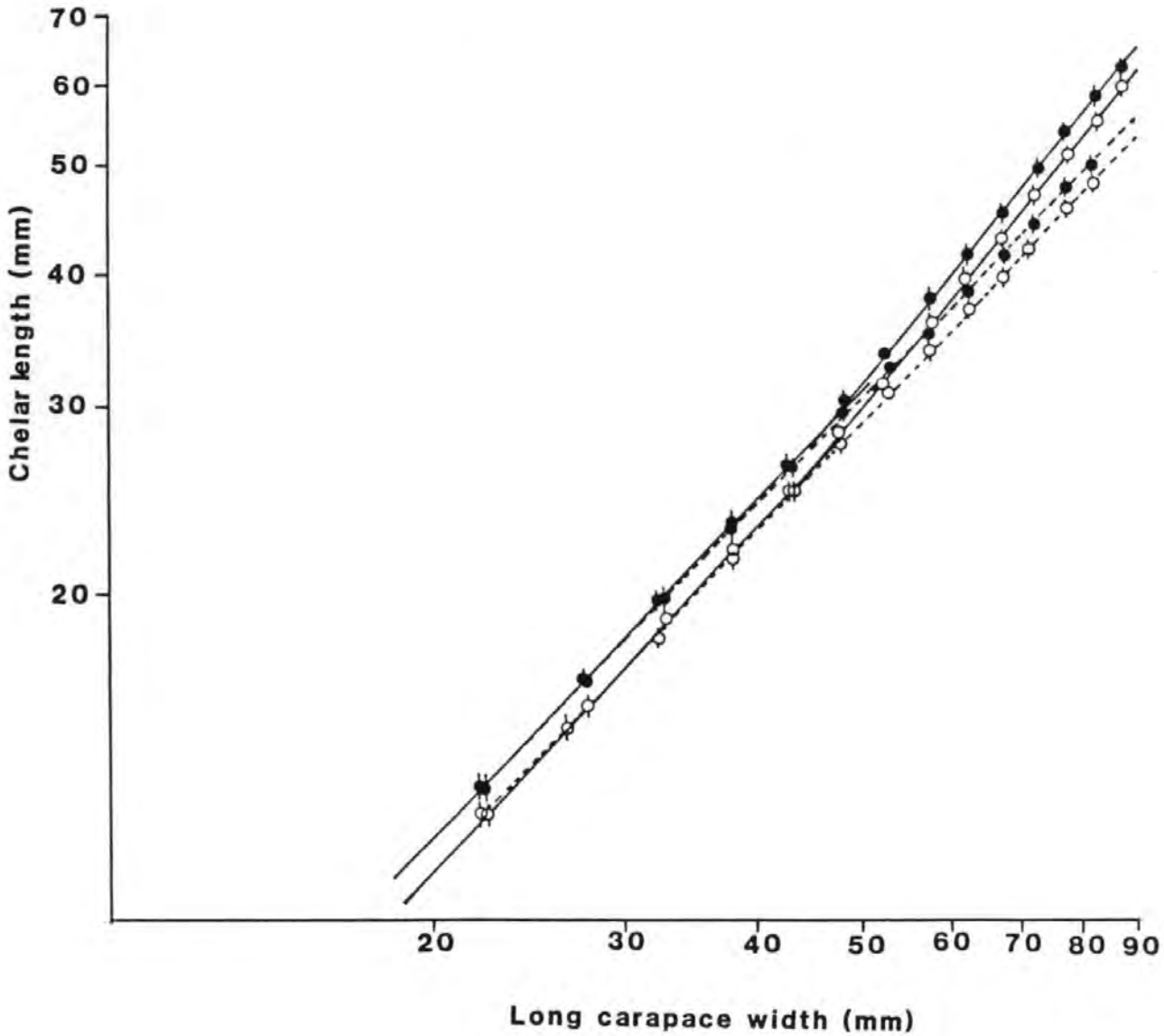

70
60
50
40
30
20
Chelar length (mm)
20
30
40
50
60
70
80
90
Long carapace width (mm)

Figure 4.3. Regression lines of the relationship between width of the sixth abdominal segment and carapace width for female (open triangles) and male (closed triangles) Liocarcinus puber. Equations, degree of allometry and further details are given in Table 4.4.

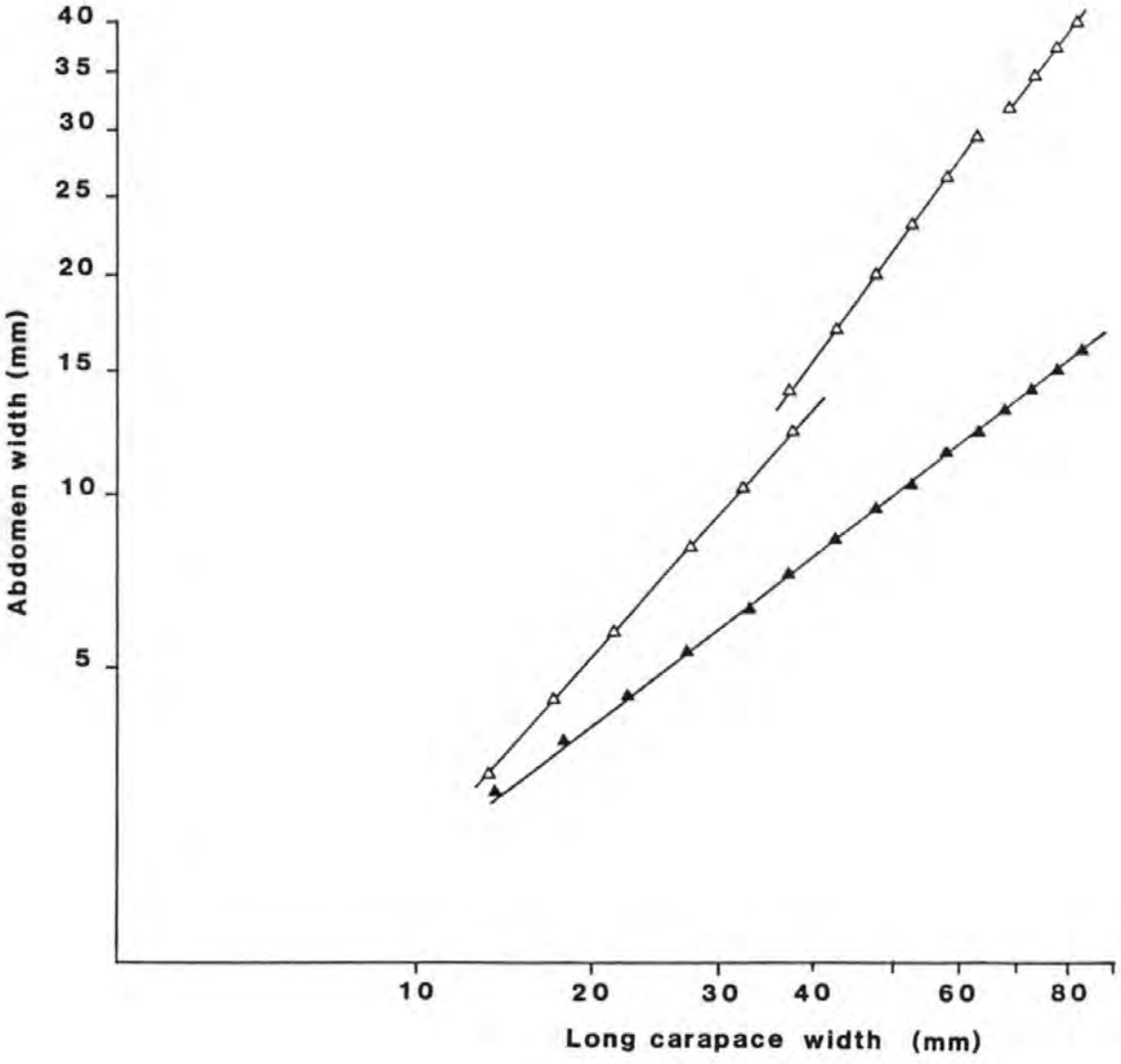

40
35
30
25
20
15
10
5
Abdomen width (mm)
10
20
30
40
60
80
Long carapace width (mm)

Figure 4.4. Relationships between carapace width and female abdomen length (closed squares and solid lines), male abdomen length (open squares and dashed lines), female body depth (closed circles and solid lines), and male body depth (open circles and dashed lines) for Liocarcinus puber. Equations for the lines, degree of allometry and further details are given in Table 4.4.

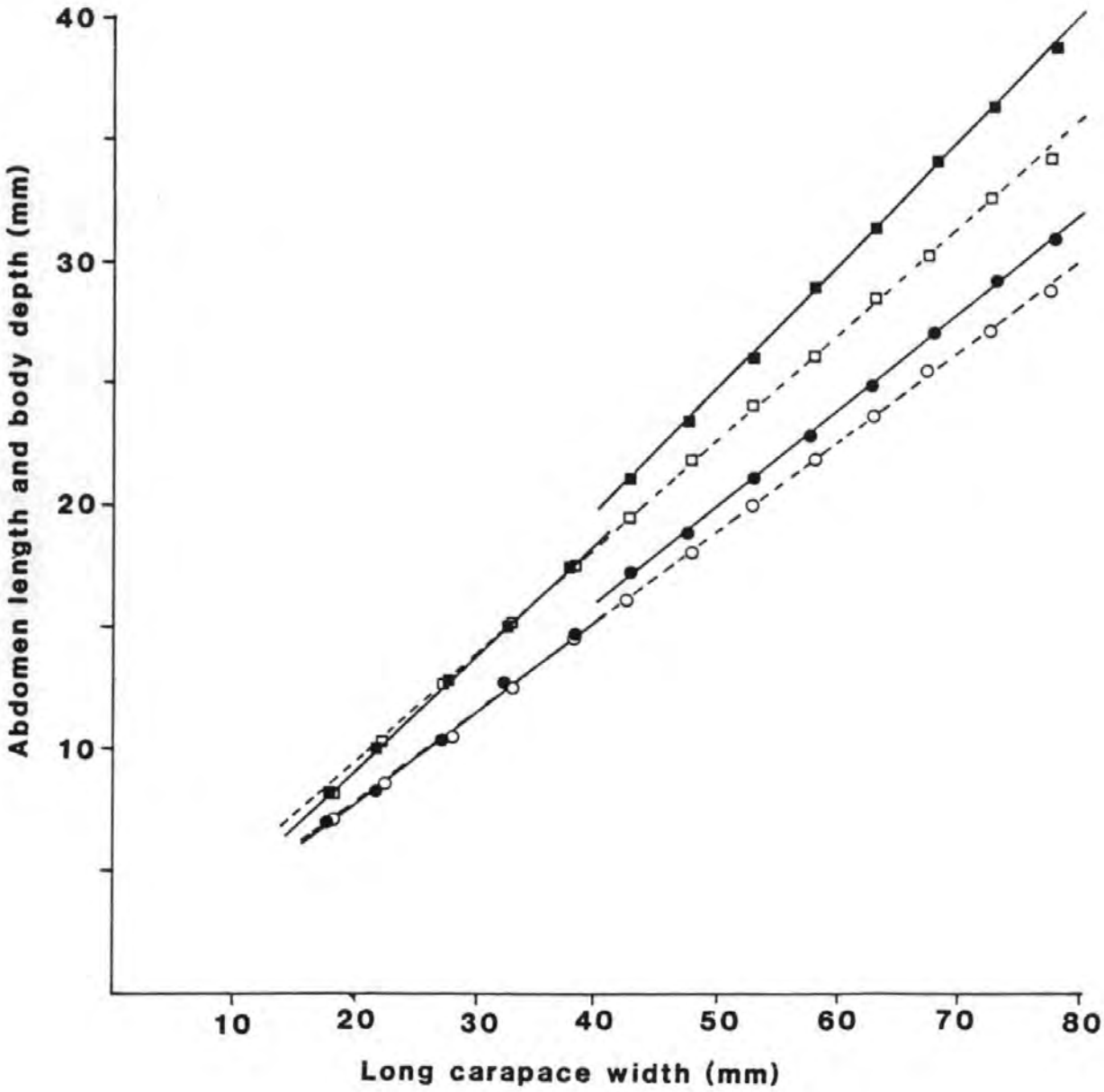

40
30
20
10
Abdomen length and body depth (mm)
10
20
30
40
50
60
70
80
Long carapace width (mm)

Figure 4.5. Proportion of Liocarcinus puber (grouped into 2mm size classes) showing various features of sexual maturity: females inseminated whilst in soft or papershell state (closed circles and dashed line; n=38, size range = 36-56mm LCW); females observed in an ovigerous state (open circles and solid line; n=46, size range = 38-56mm LCW); and males with an abundance of spermatazoa in the vas deferens (closed triangles and solid lines; n=62, size range = 42-54mm LCW).

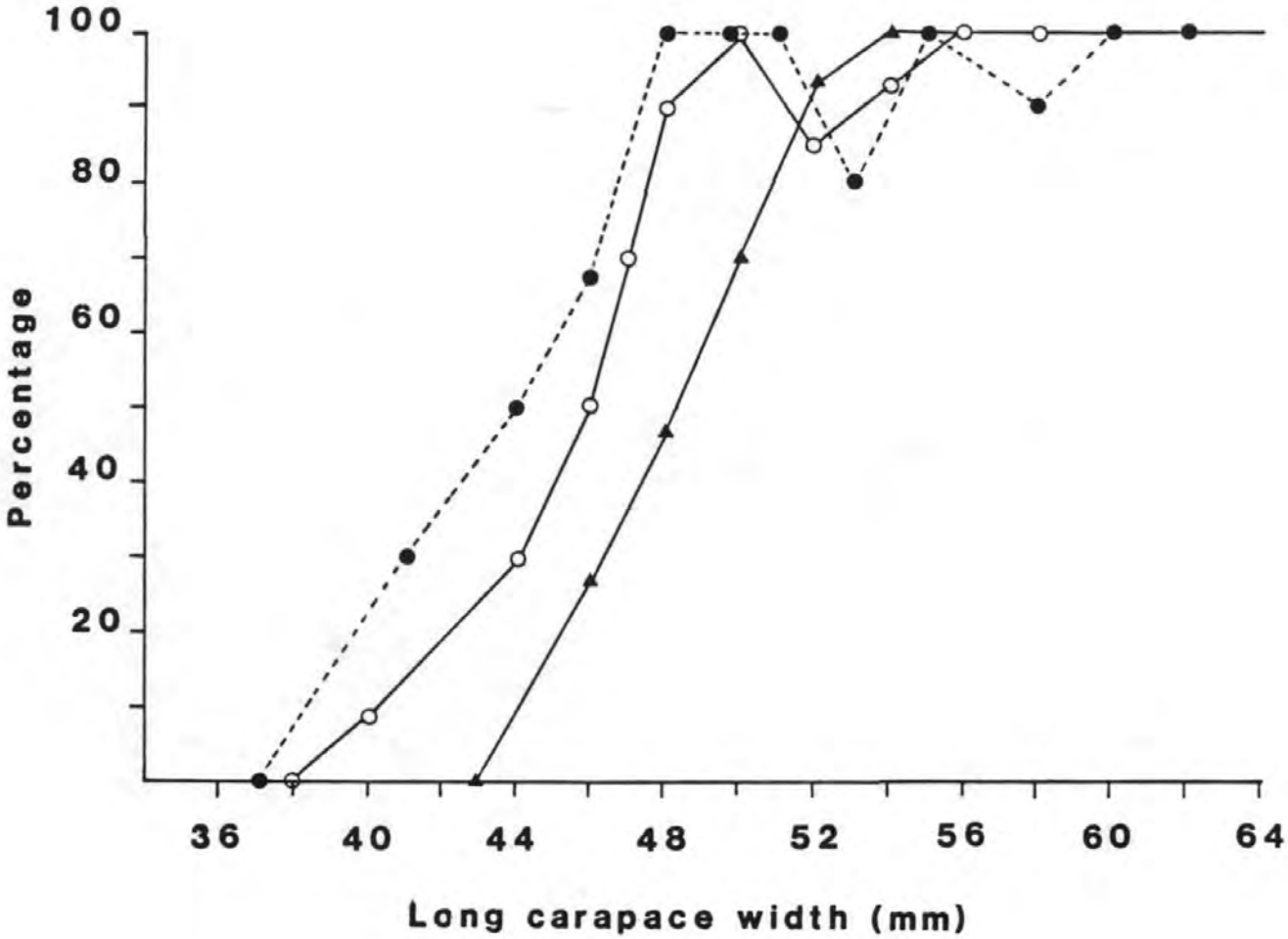

100
80
60
40
20
Percentage
36
40
44
48
52
56
60
64
Long carapace width (mm)

Figure 4.6. Size-frequency data (from November 1985 to December 1987) for female Liocarcinus puber showing external signs of sexual maturity : (A) extruding sperm plugs from the oviducts after insemination at moult (n=62), (B) carried in mating pairs (n=39) and (C) ovigerous (n=266).

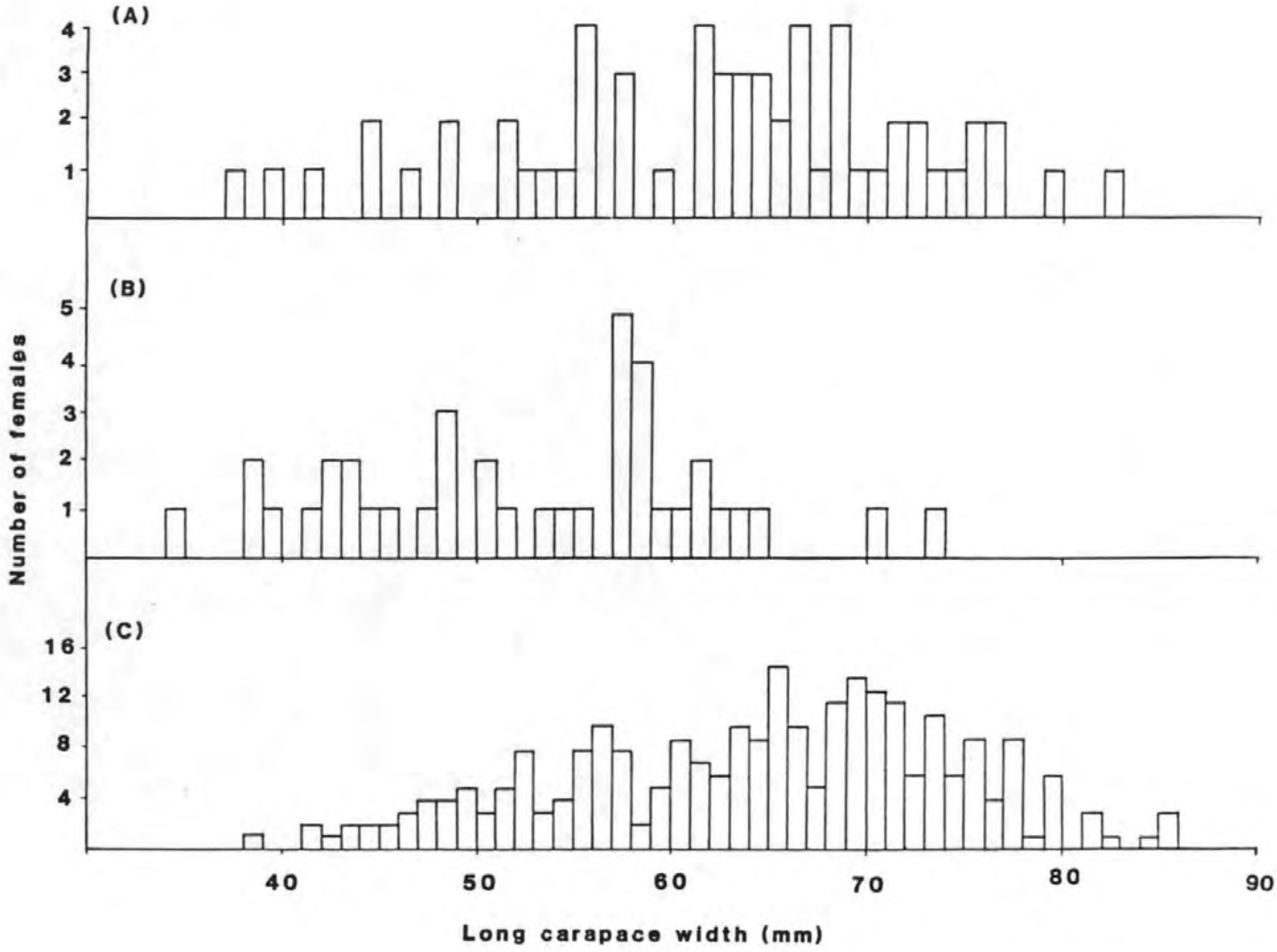

(A)
(B)
(C)
Number of females
Long carapace width (mm)
40
50
60
70
80
90

Figure 4.7. Percentage of female Liocarcinus puber with external signs of recent egg hatching or carrying late stage eggs (Stage 4, Table 4.2) with ovaries in the following different stages of development (see Table 4.1 for full description of stages): Stage II (clear), Stage III (wide hatching) and Stage IV (dots) (number of crabs examined is given above each histogram).

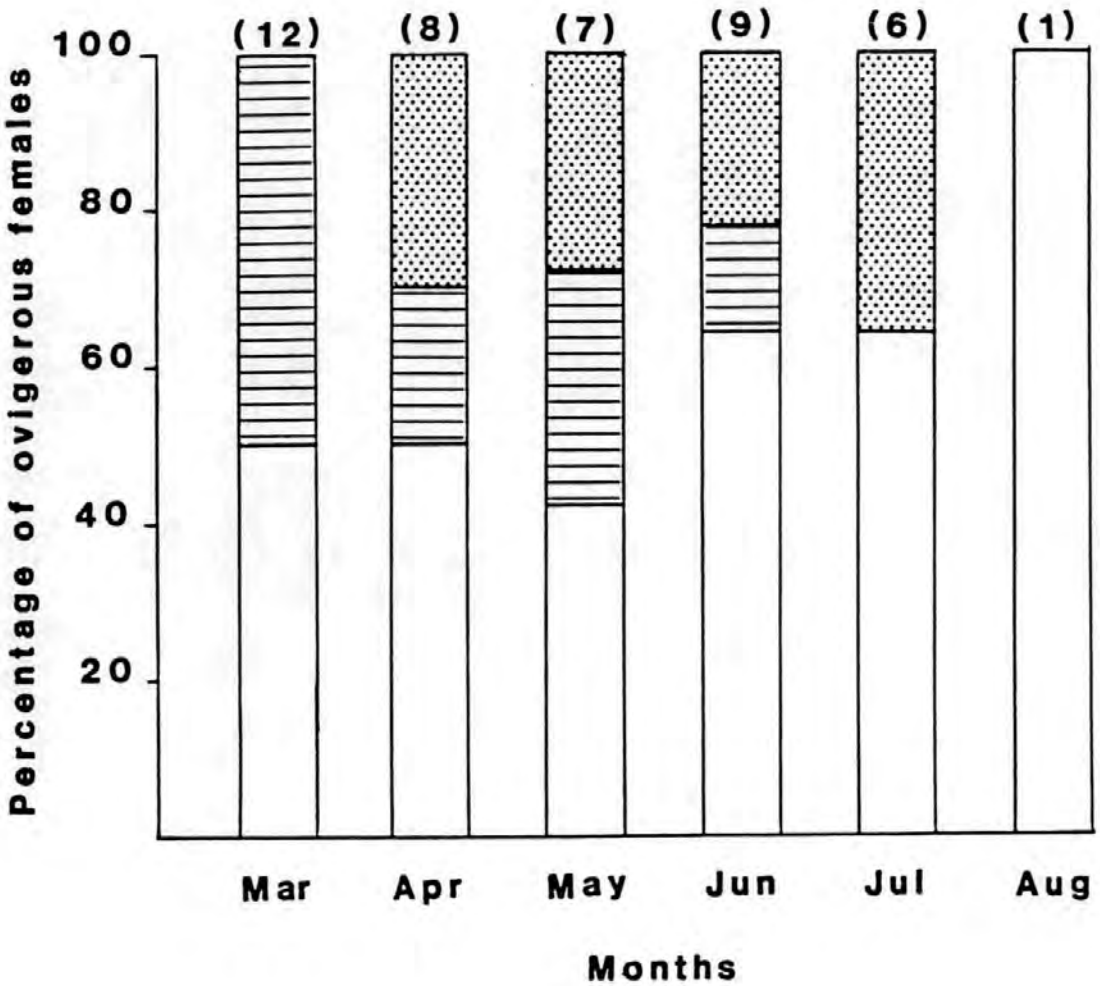

(12)
(8)
(7)
(9)
(6)
(1)
100
80
60
40
20
Percentage of ovigerous females
Mar
Apr
May
Jun
Jul
Aug
Months

Figure 4.8. Percentage of ovigerous (stippled), non-ovigerous (white) and recently spent (hatched) female Liocarcinus puber expressed as a proportion of total females (>40mm LCW) for (A) the period from November 1985 to October 1986 (n=471), and (B) November 1986 to 1987 (n=539).

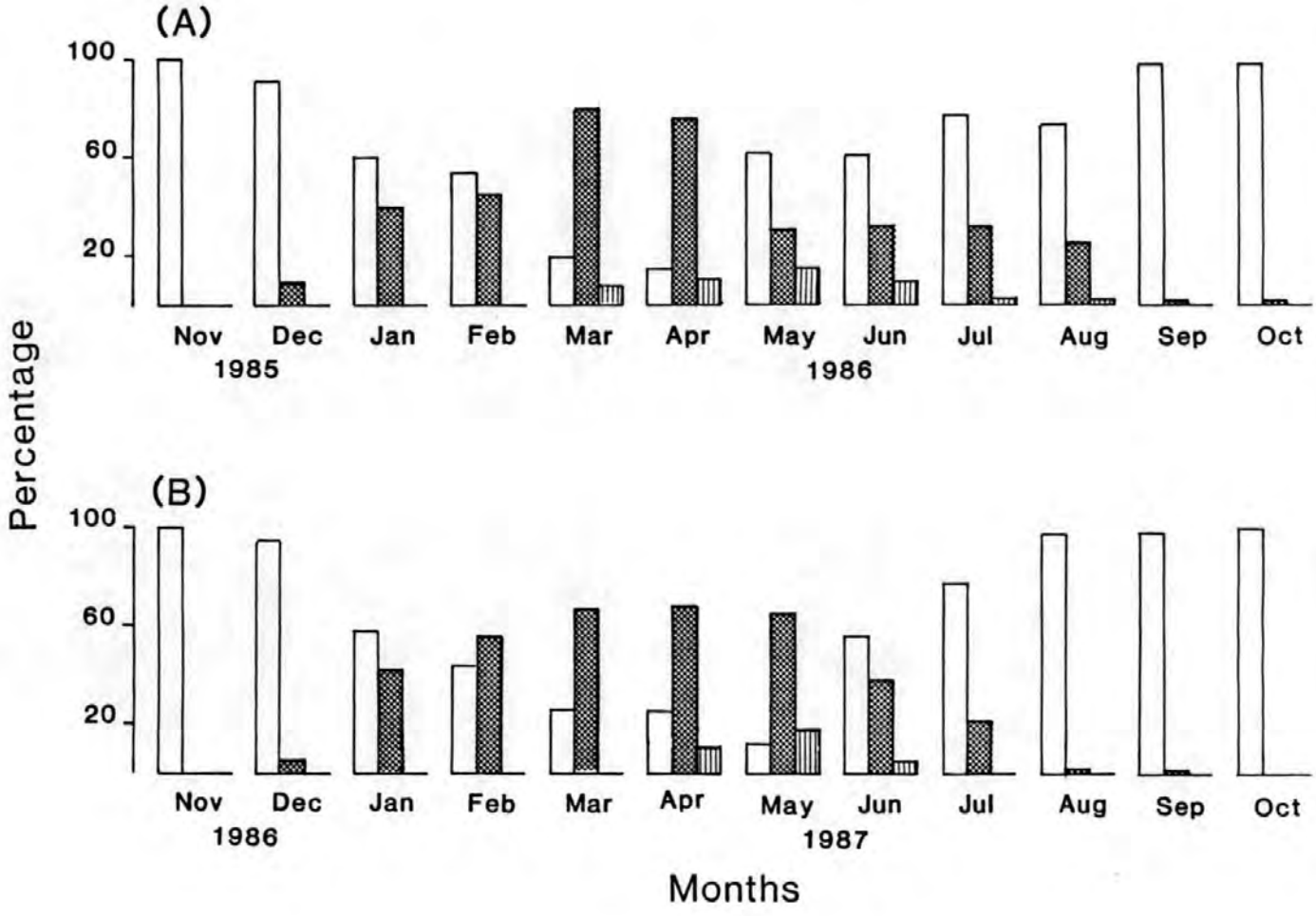
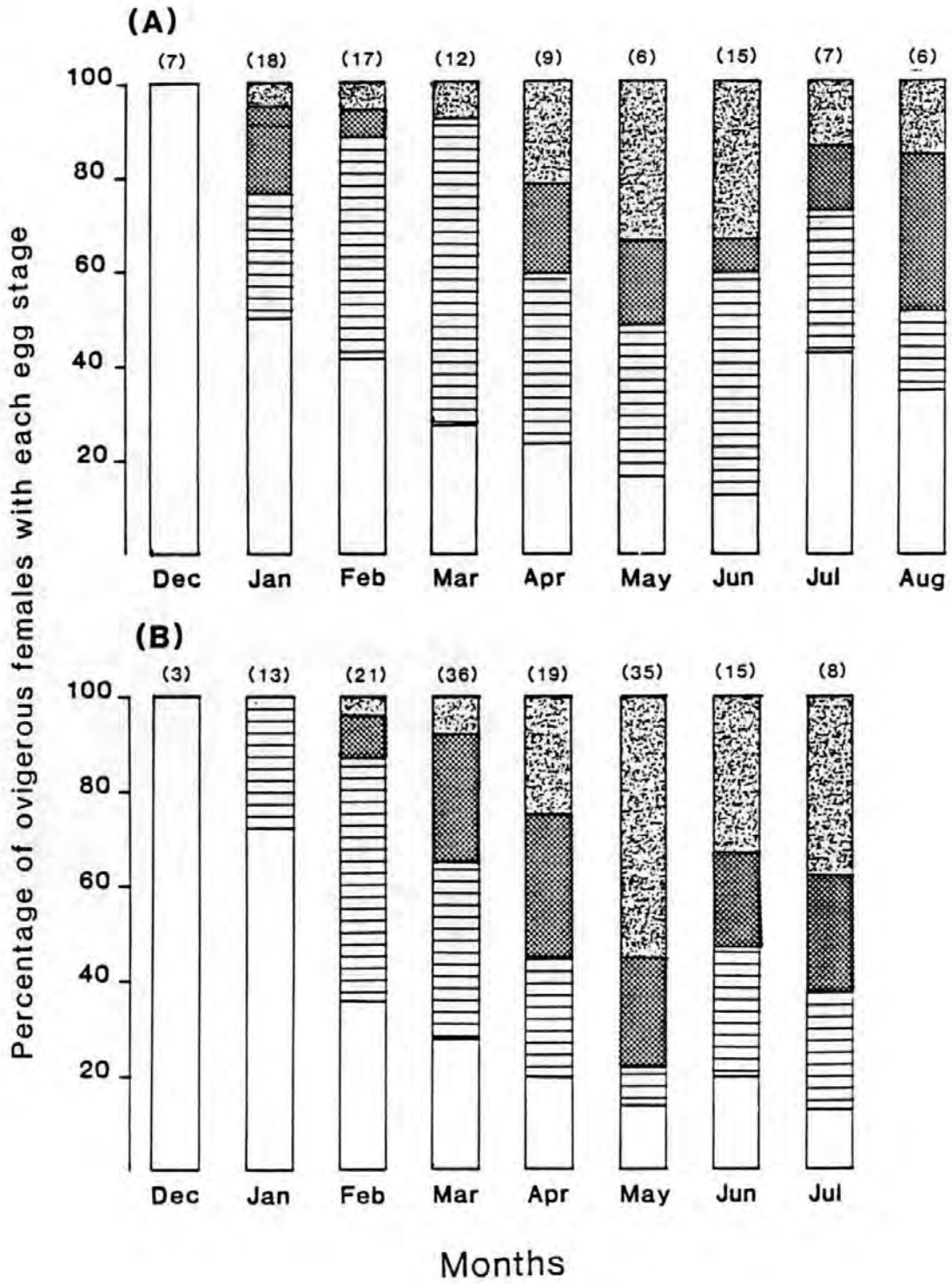

(A)
100
60
20
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
1985
1986
(B)
100
60
20
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
1986
1987
Percentage
Months

Figure 4.9. Percentage of ovigerous female Liocarcinus puber with egg Stage 1 (clear), Stage 2 (cross hatched), Stage 3 (stippled) and Stage 4 (grey) sampled for (A) December 1985 to August 1986 and (B) December 1986 to July 1987 (numbers of ovigerous females are given above each histogram and months with <3 ovigerous females are excluded).


(A)
(7)
(18)
(17)
(12)
(9)
(6)
(15)
(7)
(6)
100
80
60
40
20
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
(B)
(3)
(13)
(21)
(36)
(19)
(35)
(15)
(8)
100
80
60
40
20
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Percentage of ovigerous females with each egg stage
Months

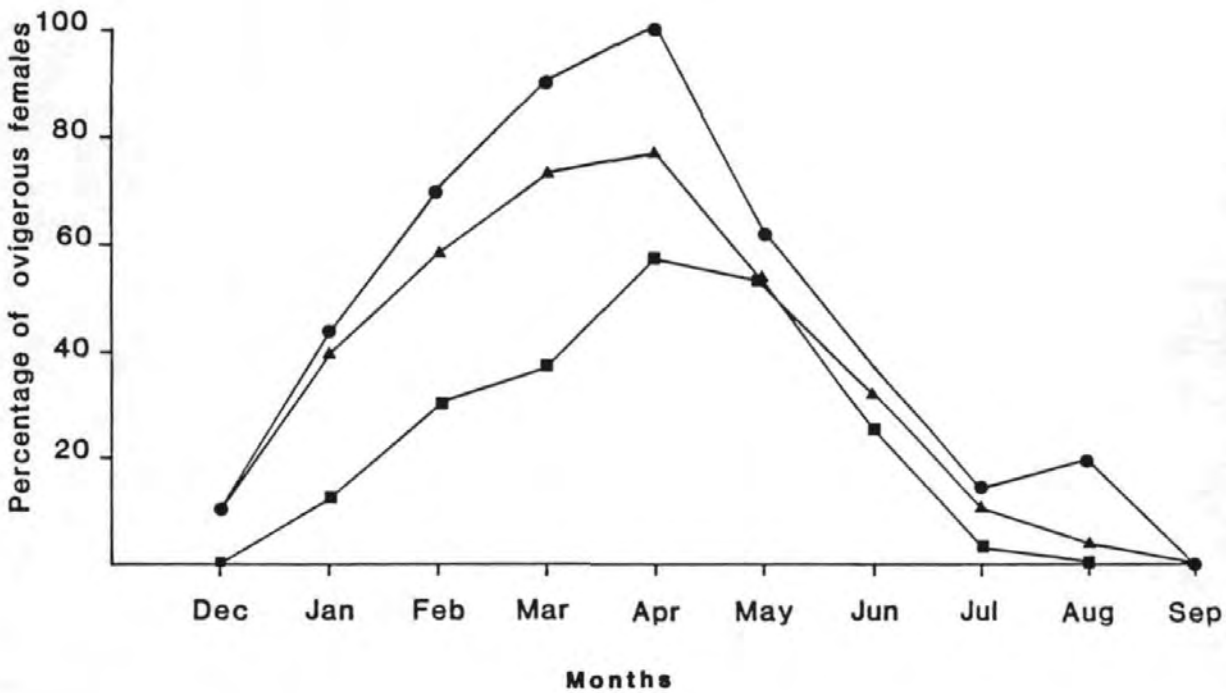

Percentage of ovigerous females
100
80
60
40
20
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Months

Figure 4.11. Relationship between brood size and carapace width for ovigerous Liocarcinus puber carrying Stage 1 and 2a eggs (closed circles and solid line), Stage 3 and 4 eggs (open circles and short hatched line), and females with Stage 1-4 eggs but suffering limb loss (open squares and intermitant dashed and dotted line). Details of regression equations are given in Table 4.7.

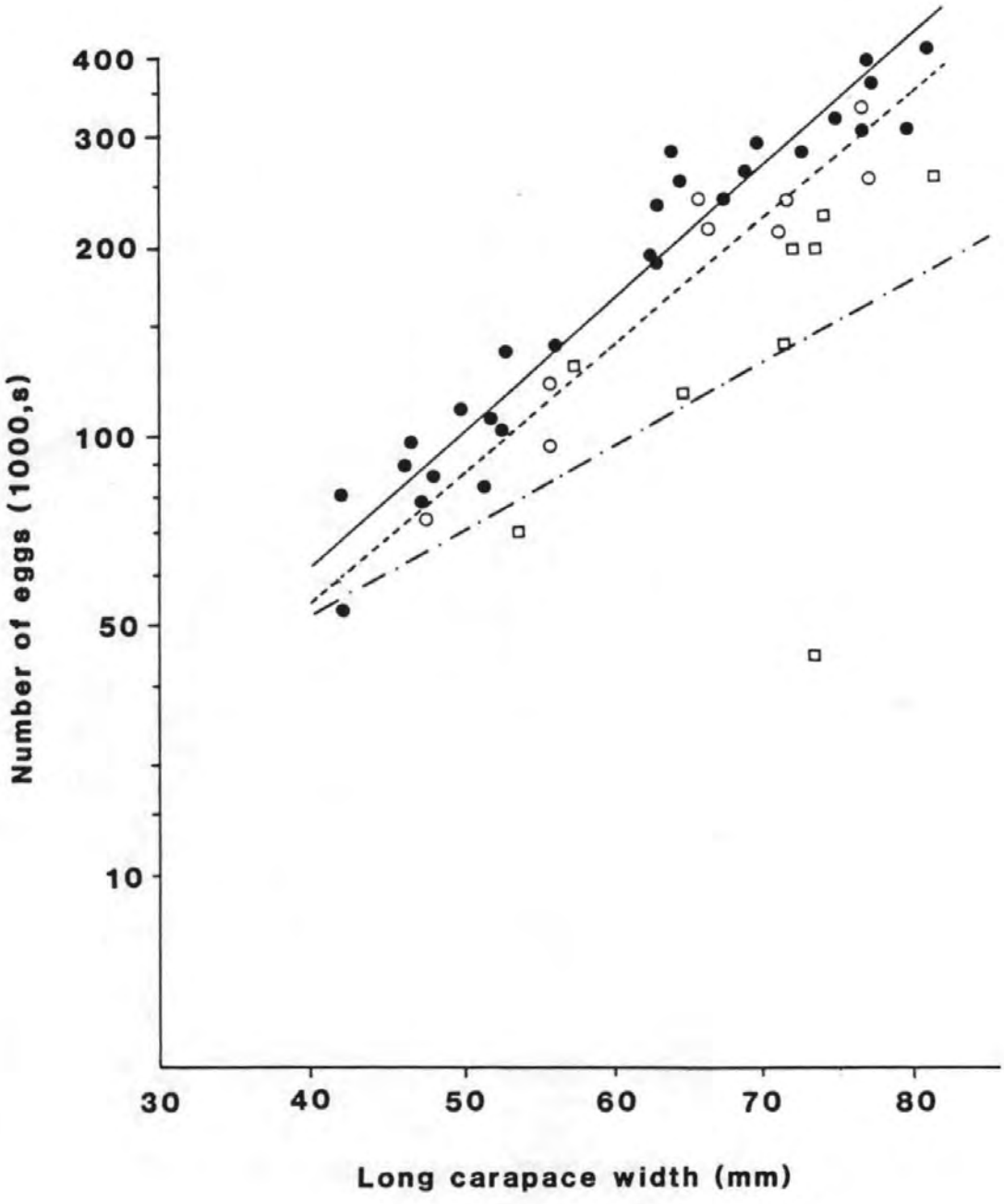

400
300
200
100
50
10
Number of eggs (1000,s)
30
40
50
60
70
80
Long carapace width (mm)

Figure 4.12. Cumulative plot of potential egg development over the period December to August 1985-86 (open circles, broken line) and 1986-87 (closed circles, solid line). Example I and II are of ovigerous females sampled on 21/3/87 and 15/7/87 with Stage 2b eggs (ie. 40% through their total development).

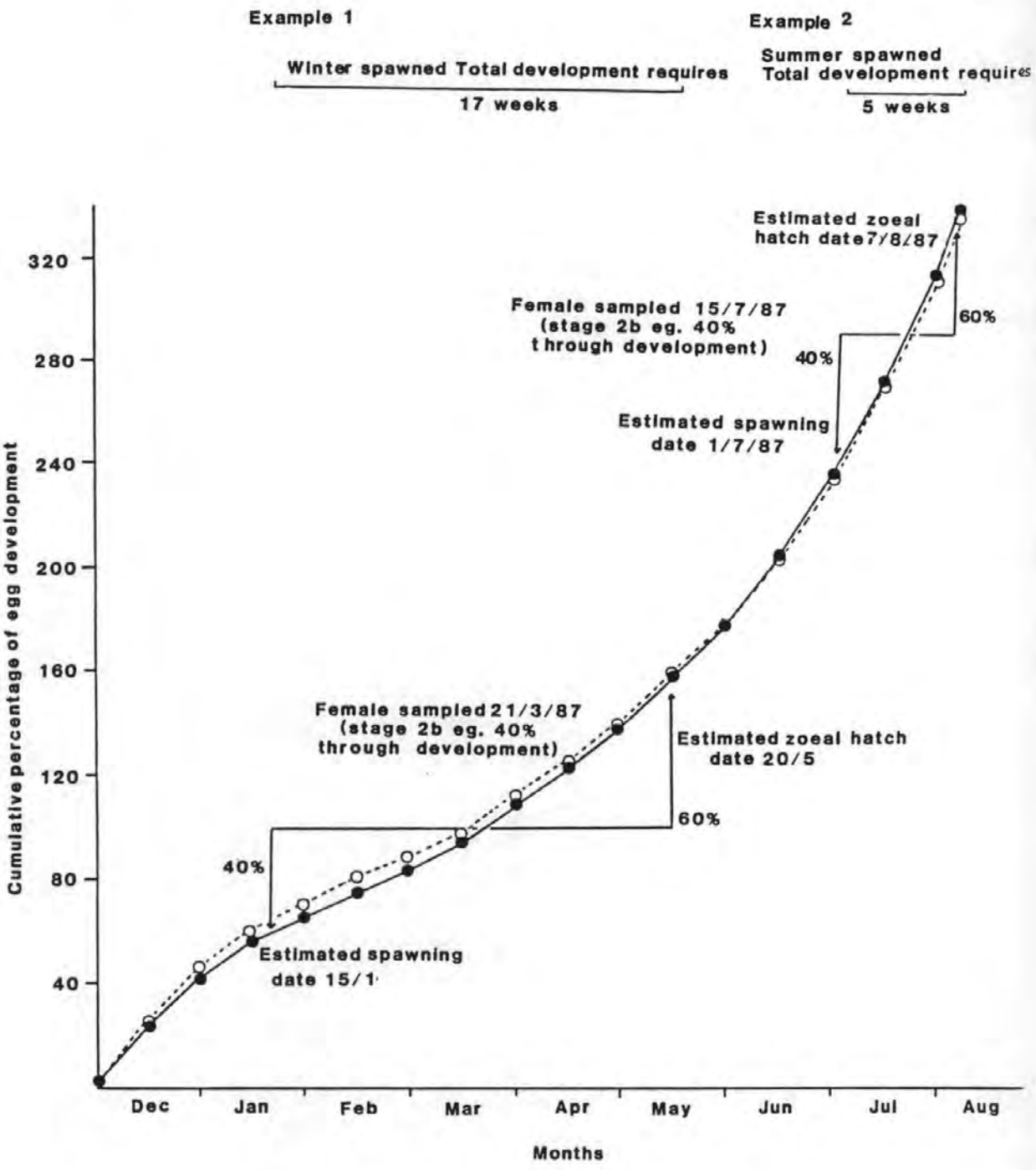

Example 1
Winter spawned Total development requires
17 weeks
Example 2
Summer spawned
Total development requires
5 weeks
Estimated zoeal
hatch date 7/8/87
Female sampled 15/7/87
(stage 2b eg. 40%
through development)
40%
60%
Estimated spawning
date 1/7/87
Female sampled 21/3/87
(stage 2b eg. 40%
through development)
Estimated zoeal hatch
date 20/5
60%
40%
Estimated spawning
date 15/1
Cumulative percentage of egg development
320
280
240
200
160
120
80
40
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Months

Figure 4.13. Estimates using the Hatch Model (Fig. 4.12) of levels of (A) spawning and (B) hatching for ovigerous female Liocarcinus puber sampled from December 1985 to September 1986.

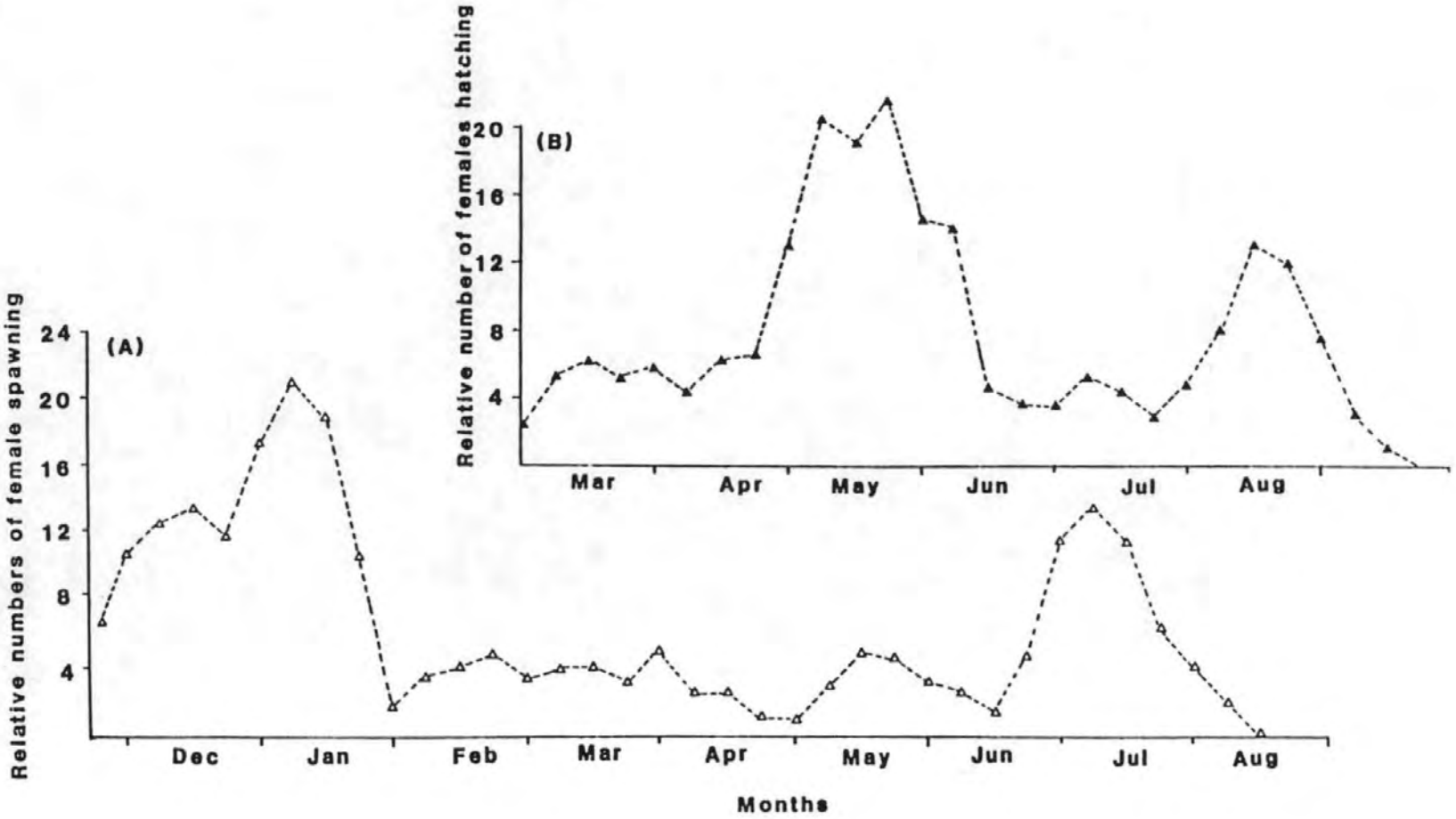

(A)
Relative numbers of female spawning
24
20
16
12
8
4
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Months
(B)
Relative number of females hatching
20
16
12
8
4
Mar
Apr
May
Jun
Jul
Aug

Figure 4.14. Estimates using the Hatch Model (Fig 4.12) of levels of (A) spawning and (B) hatching for ovigerous female Liocarcinus puber sampled from December 1986 to August 1987.

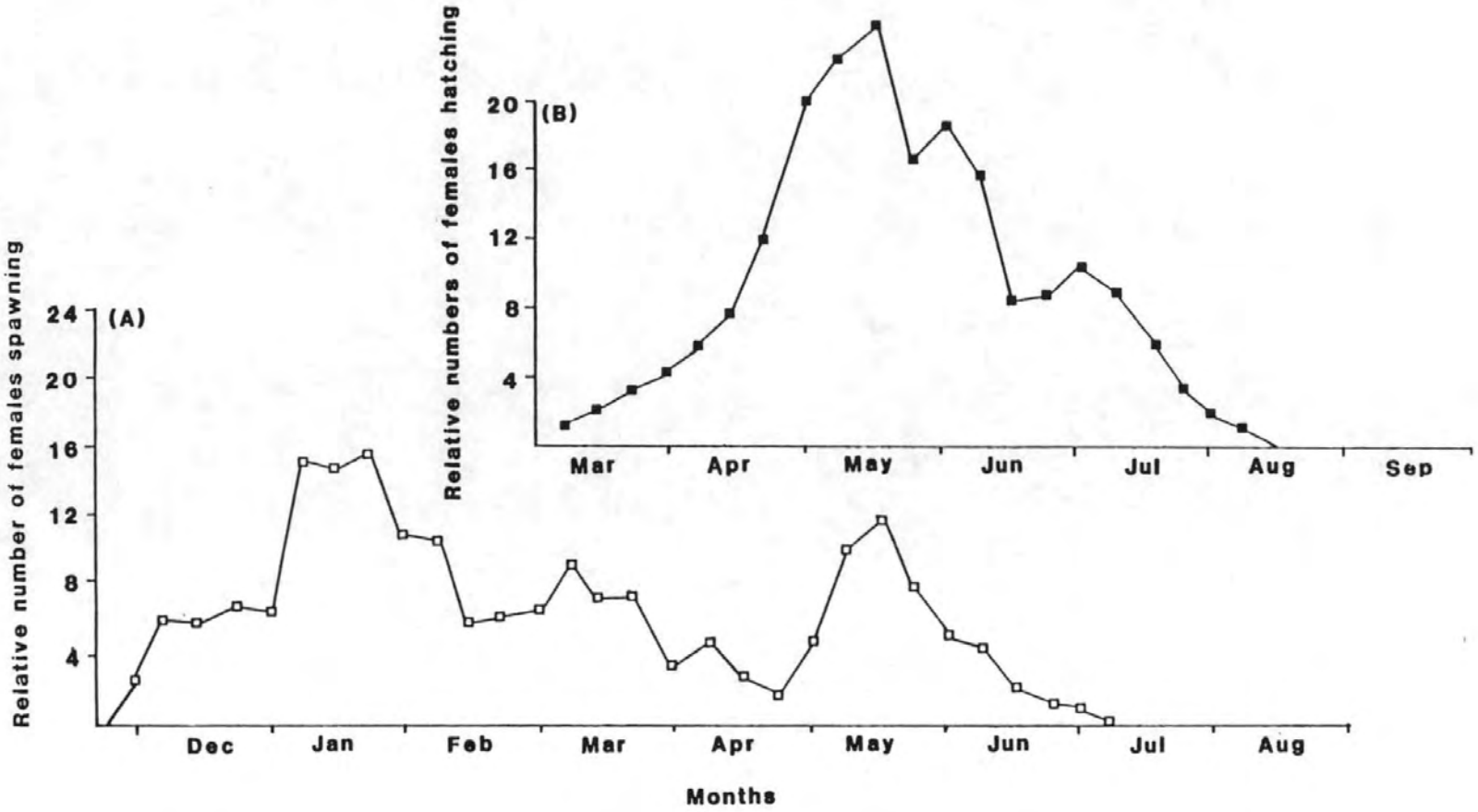

(A)
Relative number of females spawning
24
20
16
12
8
4
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Months
(B)
Relative numbers of females hatching
20
16
12
8
4
Mar
Apr
May
Jun
Jul
Aug
Sep

Figure 4.15. Estimates using the Hatch Model (Fig 4.12) of levels of (A) spawning and (B) hatching of ovigerous female Liocarcinus puber (40-54mm LCW) for December 1986 to July 1987.

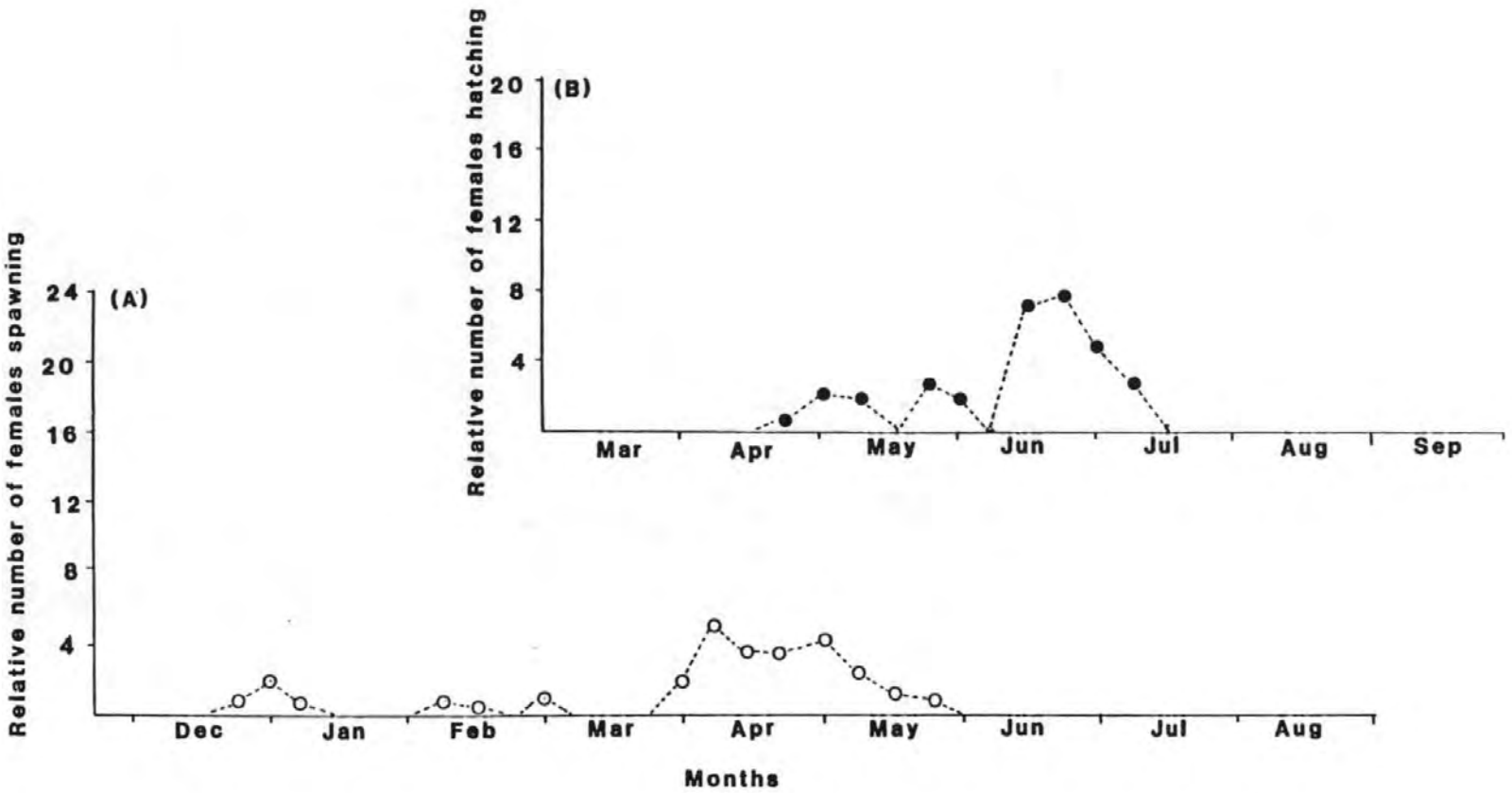
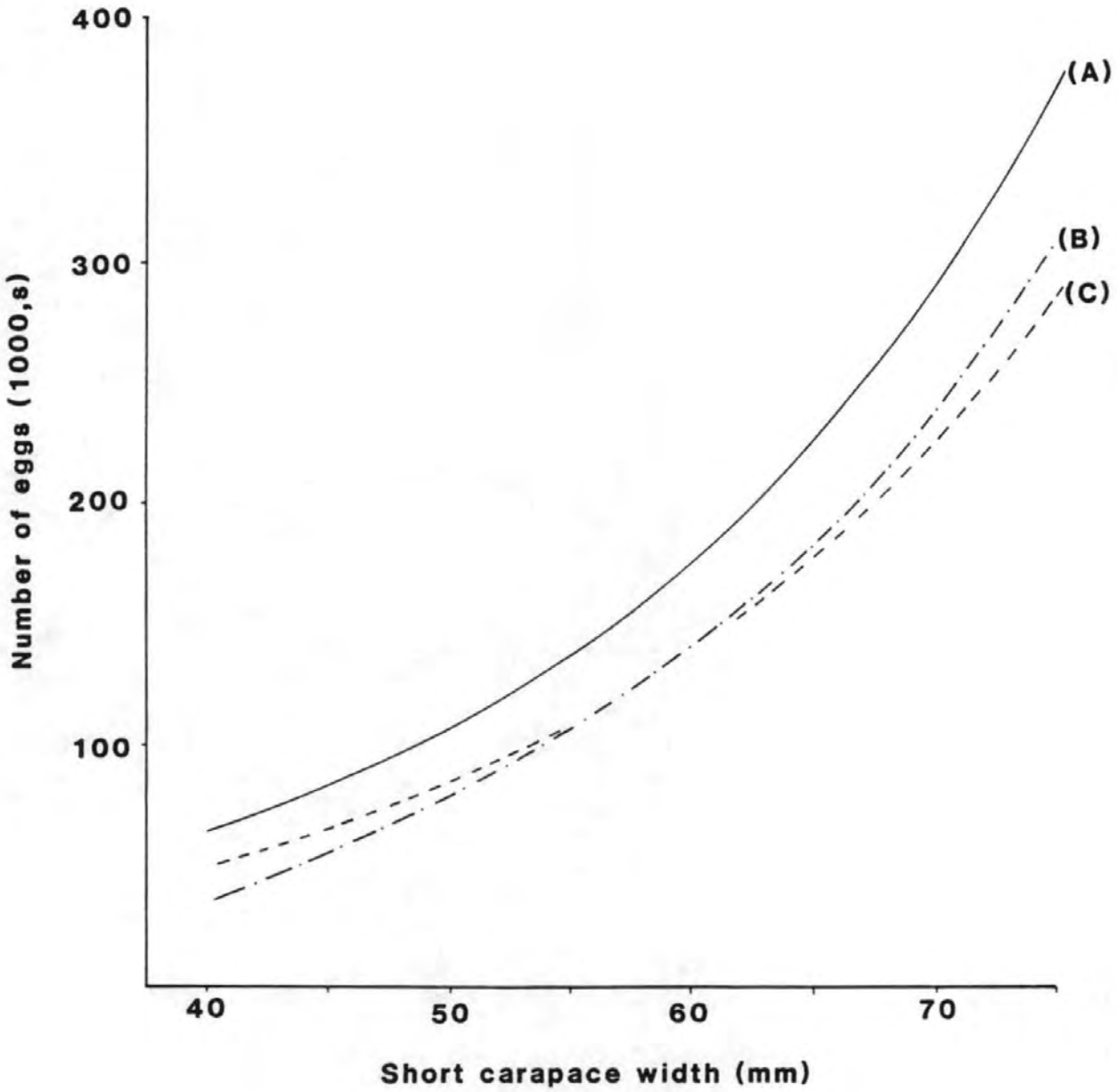

(A)
Relative number of females spawning
24
20
16
12
8
4
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Months
(B)
Relative number of females hatching
20
16
12
8
4
Mar
Apr
May
Jun
Jul
Aug
Sep

Figure 4.16. Brood size of Liocarcinus puber against short carapace width (SCW) for (A) the present study (Stages 1 and 2a eggs), (B) Gonzalez Gurriaran (1981b, 1985a) and (C) Choy (1986a). The equations are (A) $\ln Y = 9.10+0.0502X$, (B) $Y = 0.105\ X^{3.446}$ and (C) $\ln Y = 8.754+0.051X$, where Y = number of eggs and X = SCW.


400
300
200
100
Number of eggs (1000,s)
(A)
(B)
(C)
40
50
60
70
Short carapace width (mm)

CHAPTER 5

GROWTH

5.1 Introduction

In brachyurans, increase in size occurs solely at times of the moult when the hard outer exoskeleton is shed together with all other hard structures. Therefore, moulting leads to a step-wise growth pattern, and the time between moults is referred to as the intermoult period and the increase in size at moult as the moult increment. Periodic shedding of all hard structures, as occurs in brachyurans, precludes their use in direct age/size estimates, and attempts to find other anatomical structures on which to base age, such as the number of lamellae in the endocuticle (Yano and Kobayashi 1969) and number of segments in the exopodites of the antennules (Conan 1978), have so far proved unsuccessful. Consequently, crab growth studies have relied on indirect methods of estimating age at a particular size and have primarily used one, or a combination, of the following approaches: 1) examination of moult stages based on field observations, 2) rearing individuals in captivity, 3) mark-recapture experiments of wild individuals, and 4) analysis of size-frequency distributions from field data.

5.1.1. Examination of moult stages based on field observations

Observations of soft crabs with intact exuviae on the shore provide an exact measure of moult increment in the field as well as establishing the time of moult (ie. the date at which moulting occurs). This method does not, however, allow any assessment of the intermoult period, and therefore each observation can only be assessed as an isolated

event. Analysis of these data therefore yields strong supportive evidence of when and the extent of increment, but yields no information on the actual rate of growth. Similarly, examination of crabs in early post-moult stages (soft and papershell) only yields information on the time of moulting. Examination of moult stages have been used in many studies of crab growth and have provided information on increment and seasonality of moult (eg. Broekhuysen 1936, Naylor 1962, Edwards 1965, Tagatz 1969 and Gonzalez Gurriaran 1985b).

5.1.2. Rearing of individuals in captivity

Laboratory rearing, under varying conditions of temperature, food, salinity and light, has been used in many studies of crustacean growth and results have been compared with growth rates based on size-frequency and tagging experiments (Kurata 1962, Leffler 1972, Klein Breteler 1975a, 1975b, Chittleborough 1976, Hartnoll 1978a). The growth rate of reared crabs has been shown to be particularly dependant on both ambient temperature and food availability (Klein Breteler 1975b, Hartnoll 1982). For example, elevated temperature appears to reduce the intermoult period whilst largely unaffecting the increment at moult (Hughes et al. 1972, Botsford 1985). Reduction in the intermoult period at elevated temperatures is enhanced by an abundance of food, which may also affect the size increment at moult (Klein Breteler 1975b). Hartnoll (1982) concluded that as the effects of laboratory rearing on the percentage increment and intermoult period may be appreciable and variable, the use of rearing experiments to examine growth rates must be treated with caution.

5.1.3. Mark-recapture experiments of wild individuals

Mark-recapture experiments have been used extensively for commercial crustacean species to examine migratory movements, growth and mortality (Edwards 1964, Bennett 1970, Conan and Gundersen 1979). Estimation of growth using tagging techniques requires some mechanism by which the tag is maintained throughout the shedding of the exoskeleton. Tagging methods have been reviewed extensively (Chittleborough 1974, Bennett and Lovewell 1983), and the main criticism is that the placement of an externally protruding tag may damage the integument and alter the behaviour and feeding activity, and hence affect the growth, of individuals (Cooper 1970, Hill and Wassenburg 1985). In addition, elevated mortality has been noted in tagged individuals, and tagging may make the individual more vulnerable to predation and infection (Fujita and Takeshita 1979, Gonzalez Gurriaran 1981c, pers. obs.). A novel approach, which appears to reduce some of the deleterious effects of tagging, uses binary coded micro-wire inserted into the body musculature which is retained through moulting (Jefferts et al. 1963). The cost of both detecting (using an electro-magnetic device) and monitoring returns makes this latter approach economically viable only for large scale programmes.

5.1.4. Analysis of size-frequency distributions from field data

The use of size-frequency data to estimate growth was pioneered by Petersen (1891) for fin-fish, but was superceeded, in temperate waters, by the discovery of direct ageing methods, such as the use of annular growth rings of otoliths (Lea 1913). For groups such as crustaceans and, particularly, for tropical fin-fish (where direct ageing of fish using annular ring growth is difficult due to less marked seasonal fluctuations),

the use of size-frequency data in modal class progression analysis has continued. One method, utilising probability paper (Harding 1949, Cassie 1954), has been applied, with some success, to crustaceans to identify cohorts (Poole 1967, Powles 1968a, 1968b, Farmer 1973, Thomas 1973, Gonzalez Gurriaran 1985b). The technique may be criticised because it relies on a level of subjectivity in that the worker has to pick the points of inflexion between cohorts and assess a size to age relationship to elucidate one of the growth parameters (t_o). To overcome some of the problems caused by such subjectivity computer programmes, such as ELEFAN, have been developed primarily for use for tropic fin-fisheries (Pauly 1987). ELEFAN examines the best fit to a series of monthly poly-modal distribution frequencies and assesses the degree of 'closeness of fit' between the actual and estimated curves. Computer-aided size-frequency techniques have been used with some success for crustacean and molluscan growth studies (Pauly and Calumpong 1984, Pauly et al. 1984, Choy 1986a, Pauly 1987).

Use of size-frequency analysis to determine growth of crabs by both probability paper and computer-aided model relies on the species having identifiable cohorts, each with a normal distribution. Problems arise where distinct cohorts do not occur, eg., when larval release, and subsequent recruitment, occurs over an extended period of the year, with no clear peak period of recruitment (Du Preez and McLachlan 1984a, Dittel et al. 1985). Variations in the date of settlement and limb loss (Chapter 6) also result in the size-frequency distribution for any one cohort being made up of several 'moult classes'. Size-frequency analysis using modal progression techniques is complicated further by the step-wise nature of brachyuran growth, as ELEFAN and the probability paper technique, predicts

a curve (ie. an estimate of continuous growth) for what is essentually a discontinuous growth pattern.

All four of the above techniques were used in this study in order to achieve as accurate an estimate as possible of the growth rate of Liocarcinus puber. Particular attention has been paid to the analysis of the size-frequency data collected from the monthly field samples (Chapter 3).

5.2. Materials and Methods

5.2.1. Field measurements

The methods used in the regular monthly sampling programme have been outlined in Chapter 3.

Recently moulted soft crabs, together with their intact exuviae, were occasionally observed in the littoral zone. The exuviae were measured in the field and the soft crabs were placed in sea water, returned to the laboratory and measured after a period exceeding 5h. The latter time interval was based on a previous study of Liocarcinus puber which showed that soft crabs required between 4-5h post moult to reach full expansion (Gonzalez Gurriaran 1981b).

5.2.2. Laboratory-rearing procedure

Following the recommendations of Hartnoll (1982), the rearing environment approximated, as closely as possible, the physical parameters experienced in the natural habitat, with temperature and light being allowed to fluctuate seasonally. Details of the holding facilities at Bovisand have been described previously (Chapter 3). Crabs were reared at Bovisand and their growth was examined over a 19 month period (January 1986 - July 1987). Due to the aperture size in the holding crates, only crabs >30mm long carapace width (LCW) were reared. Each was fed twice weekly with approximately the same quantity of food. The diet consisted of one to two opened Mytilus edulis (L.) (3-4cm), or strips of Pollachius

pollachius (L.) (approximately 4 by 2cm), fronds of Fucus spp. and strips of Laminaria spp.. Algae were added for nutritional purposes and to allow the crabs some cover. Prior to feeding, crabs were examined for moulting and any uneaten material from the previous feeding was removed.

5.2.3. Analysis of the size-frequency data

The twenty four month size-frequency data set (January 1986 - December 1987) was analysed using poly-modal size-frequency analysis (Harding 1949, Cassie, 1954) to elucidate any modes in the distribution. For each month, the size-frequency data for the three zones were combined and grouped into 1mm size-frequency classes. The method requires the size-frequency distribution to be plotted cumulatively on probability paper; points of inflexion on the graph demark separate cohorts. Data for male and females were plotted separately and, where sample numbers were low (<100 individuals), monthly samples were combined to give bimonthly totals. Increases in modal size of cohorts between months were estimated and the growth parameters (L_∞ and K, see below) were determined using the Gulland-Holt method (Gulland 1983).

Growth was expressed using the mathematical formula of Von Bertalanffy (1938) which in its simple form is :

$$Lt = L_\infty \; 1 - e^{[-K(t-t_o)]} \qquad (1)$$

where Lt is the length of the animal at time t, L_∞ is the asymptotic length, K is the growth constant describing the rate at which the length of the animal approaches L_∞, and t_o is the function to descibe the time at which length would approximate zero.

Data from the rearing experiments and the size-frequency field sampling were analysed to estimate L_∞ and K using the Gulland Holt plot

(eg, see Fig. 5.13), where $K = -b$ and $L\infty = a/K$ [a is the intercept of the x-axis and b is the slope (Gulland and Holt 1959)]. The third parameter of the von Bertalanffy equation, to (time at which length approximates zero), was estimated by re-arranging equation (1):

$$to = t + (1/K)\ \ln[(L\infty - Lt)/L\infty] \qquad (2)$$

Therefore, if the length at a given age (t) is known, then to can be estimated. To estimate the length at a given age (t), a degree of subjectivity is required, as this relationship may not be readily apparent from the field data. The best estimates for to are from young, but fully recruited cohorts to the adult population (Pereiro 1982, Gulland 1983). To remove some of the more subjective elements (such as 'picking' the points of inflexion, and estimation of to) of the probability paper technique, and to allow comparison of results between size-frequency analysis techniques, the model ELEFAN (electronic length frequency analysis) was also used to analyse the size-frequency data (Pauly and David 1981). ELEFAN separates the normally distributed components of the distribution, identifies the growth parameters and generates the growth curve which minimises the sum of squared deviations from the means of the component distribution (Pauly 1987). The output of the model yields a ratio of the 'explained sum of peaks' over 'available sum of peaks' (ESP/ASP ratio) (ie, a measure of goodness of fit of the predicted size-frequency curve).

As a function of the ELEFAN programme, a seasonally oscillating growth equation was plotted using a modification from Gaschutz *et al.* (1980) :

$$Lt = L\infty\ [1-e(-K[-(t-to)+(C/2\pi)(\sin(2\pi(t-ts))])] \qquad (3)$$

where ts is the winter point - 0.5 (winter point is the coldest month of the year expressed as a fraction and is the start of the sinusoidal cycle)

and C is the intensity of the seasonal growth oscillation (C = 0 when there is no seasonal oscillation and C = 1 where seasonal growth is apparent). The ELEFAN programme therefore assumes that the data conform to the von Bertalanffy equation and, by varying the values for L∞, K, C and ts and optimising the ESP/ASP ratio, ELEFAN selects the best fit parameters to the data set. The programme requires no assumption of age at length to derive to but extrapolates from the size-frequency data.

5.3. Results

5.3.1. Size-frequency distributions

Immature crabs (<40mm LCW) were well represented in the littoral sampling at Wembury throughout the year (Figs 5.1 and 5.2). Recruitment of post-larval crabs (size range between 5-10mm LCW) was first noted in August 1987 and during October 1986 (in females only). During 1987, this post-larval size class formed a marked mode in the size-frequency distribution from September to December and reached a modal size of 15-20mm LCW by December. Moulting appears to be minimal for all sizes from January to June as there was no modal growth in the size-freqency data (Figs 5.1 and 5.2). Growth of immature male and female crabs can be inferred from June to December 1986 when crabs increased from 15-20mm to 30-45mm LCW. Larger females (post moult >37mm LCW) of this mode may mature and mate at this stage producing viable eggs over the following spring (Chapter 4). The growth of this cohort beyond the second winter is not readily discernable

sub-littoral occurred over most of the year, however, the most marked period of recruitment was in August, September and October for 1986 (Fig. 5.3). Similar trends were not observed for the same period for 1987, possibly due to poor recruitment (supporting the notion of poor recruitment based on littoral zone sampling for this year class). The size-frequency distribution of adult females >50mm LCW showed no clear separation into recognisable modal groups (Fig. 5.3). Male recruitment to the adult sub-littoral stock also occurred over much of the year, but was most obvious from June to December, when immature crabs were growing (Fig. 5.4). Large individuals, of the size range 60-80mm LCW, formed the main size group sampled during the sub-littoral survey. Within this latter size range, no clear modal size groups could be readily equated to any year classes.

5.3.2. Moult periodicity based on field samples

Throughout this section 'periodicity' refers to the time (ie. date) of moulting, and 'intermoult period' refers to the length of time between successive moults for an individual.

Immature Liocarcinus puber were observed in early post moult stages (ie. soft and papershell) from April to December (Fig. 5.5A). Very low levels of early post moult individuals were observed from January to April, indicating that growth was negligable over this period. Increased moulting rate, and hence growth, occurred from May and reached a peak from June to September when 15% of all immature crabs sampled were either in a soft or papershell condition (Fig.5.5A). The proportion of immature crabs in soft and papershell condition declined from September to a minimal level in December. Immature crabs complete the stages from soft crab to

intermoult stage within 7-17 days (Table 5.1). Therefore, the relatively high levels of crabs in early post moult stages found from May to November suggest that immature crabs moult several times over this period (Fig. 5.5A).

The seasonal variation of occurrence of juveniles with heavily worn exoskeletons, and with high settlement of epizooites, was consistent with the pattern just described for soft crabs (Fig. 5.5B). There was a clear peak of late intermoult immature crabs in April with few being found at other times of the year. These data suggest that there was a low degree of moulting over winter. The marked decline in percentage in late intermoult individuals in May and June suggests further that a high proportion moulted over this period (Fig. 5.5B).

Mature females (>40mm LCW) showed a very seasonal growth pattern based on field evidence (Fig. 5.6A). Early post moult females were observed from August to November, with a peak of occurrence in August and a smaller peak in November (Fig. 5.6A). There was a significant difference between the size of individuals moulting in the two peak periods, with smaller mature females (40-50 mm LCW) making up the November peak compared with August (>50mm LCW) (ANOVA, $F=8.15$, $df=1,32$, $P<0.05$). The percentage of mature females with heavily worn and fouled exoskeletons (ie. late intermoult) also showed a marked seasonal pattern (Fig. 5.6B). A peak in July was followed by a decline from August onwards. The steady increase of females in the late intermoult stage over the winter and spring suggests that moulting, particularly for larger individuals, is an annual occurrence, with the main moult period being from October to November.

Mature males (>45mm LCW) in early post-moult stages (soft and papershell) were more common in the sub-littoral zones compared with the

littoral zone (Fig. 5.7A). In the sub-littoral, mature males were observed in early post moult stages primarily from June to August, although a few large individuals moulted in March (Fig. 5.7A). In the littoral zone, moulting occurred later in the year and extended from June to October (Fig. 5.7A). The sizes of mature male crabs in early post moult were compared (ANOVA) to identify any trend in sizes of males moulting between months (Table 5.2). Significant difference ($P<0.05$) in the size of male crabs was observed between months for the sub-littoral zones, with large individuals (>65mm LCW) moulting early in the year (March, June and July) and smaller males (45-65 mm LCW) moulting later (from July to October) (Table 5.2). In contrast, there was no clear size trend observed for male Liocarcinus puber in the littoral zone, except that those moulting in September were smaller than in July, August and October (Table 5.2). The frequency of occurrence of male crabs in late stage intermoult showed a similar pattern for each of the three zones (Fig. 5.7B). A decline in percentage of late intermoult crabs occurred in June, suggesting that early summer was the main moulting period. The progressive increase in the level of mature males in late intermoult stages from December to May suggests that little moulting activity takes place over winter (Fig. 5.7B).

5.3.3. Moult periodicity based on observed moults

Observations of soft crabs with intact exuviae on the shore allowed an assessment of both moult periodicity and moult increment in the natural population (Fig. 5.8). Immature soft crabs (<20mm LCW) with intact exuviae were never collected in the field in this study. Soft individuals with exuviae of 20-60mm LCW were found, but predominantly in the littoral zone under large boulders. Mature males (>60mm LCW) appeared

to moult only in the sub-littoral and incremental data for this size class results from mark-recapture experiments for the circalittoral zone, where several crabs were repeatedly recaptured before and immediately after moult (Chapter 10). Mature females were rarely observed with an intact exuvia. The majority of mature females are protected by males whilst moulting and, as the males are fully mobile even whilst in copula, they transport the females away from their moult site. The two mature females observed with intact exuvia were found under stones in the littoral zone without the attendance of a male. The periodicity of these field observations, although based on small numbers, indicates that large males (>60mm LCW) moult in mid-summer (June/July) and smaller individuals of both sexes moult from June to November (Fig. 5.8).

In the laboratory, Liocarcinus puber maintained under 'natural' conditions of light and temperature (Section 3.3) showed a very marked seasonal moulting pattern (Figs 5.9 and 5.10). Small males (31.8-55mm LCW) moulted up to three times over the nineteen month period, whilst larger males (>55mm LCW) moulted twice (Fig. 5.9). The first peak period of moulting occurred in early summer (June and July), when all males moulted. The second period of moulting occurred in late autumn (late September to mid-December) and was made up entirely of small (<55mm LCW) individuals. A third peak occurred over a more extended spring and summer period, when the largest individual moulted in late March (increasing from 70.6 to 81.2mm LCW) and other males moulted from May through to the termination of the experiment at the end of July (Fig. 5.9). Large females (>50mm LCW) moulted once in late summer (July-September), and smaller individuals (37.2 - 43.0mm LCW) moulted in mid-summer and again between late autumn and early winter (Fig. 5.10).

5.3.4. Increment at moult - comparison of 'wild' and laboratory-reared data

To use data from laboratory-reared individuals to estimate growth parameters for the generation of growth curves, the reared individuals must conform to the growth pattern of their 'wild' counterparts. The moult periodicity, as examined above, showed close agreement between 'wild' and reared individuals. Comparison of the relationship of premoult size versus post moult size can be used to identify differences between the percentage increase at moult, and has been used in this study to compare 'wild' data (soft crabs found with intact exuviae) and reared crabs (Fig. 5.11). The premoult : postmoult relationship for Liocarcinus puber was linear for all sizes of males, whilst females showed a slight reduction in increment for larger individuals (>55mm LCW) (Fig. 5.11). No significant difference was observed between the ratio of premoult size to postmoult size for 'wild' crabs and 'laboratory reared' crabs for males or females ($P>0.05$) (Fig. 5.11; Table 5.3). Similarly, no significant differences were detected between growth factor (percentage increment at moult) and premoult carapace width for 'wild' and 'laboratory reared' for male or female crabs ($P>0.05$) (Fig. 5.12; Table 5.3). The semi-logarithmic plot of female growth factor against carapace width showed a marked discontinuity at 55mm LCW, whilst the male plot showed an increase in the variability of the data points for larger individuals (Fig. 5.12).

5.3.5. Growth equations

The monthly size-frequency data for the three zones were combined, grouped into 1mm size classes and modes were identified using the

probabality paper technique. An example of this graphical technique is illustrated in Figure 5.13. Modes in the size-frequency distribution for each monthly, or bimonthly, sample were identified by this technique (Fig. 5.14). The increment between the modes of the samples indicates growth and, using information from the analysis of the periodicity of moult from the wild (Section 5.3.3), modes have been joined (Fig. 5.14). The data for the Gulland-Holt plot (Fig. 5.15A) were obtained from Figure 5.14 using mid-Febuary as the point of minimal growth, and estimating the apparent modal growth for six monthly periods (starting mid-Febuary). The parameters for the Von Bertalanffy (1938) growth equation were estimated subsequently from the Gulland-Holt plot and are summarised in Table 5.4; the growth curves are shown in Figure 5.16. The growth curve shows a rapid initial growth and subsequent decline in rate of increment with age (Fig 5.16).

The very similar patterns of moult periodicity based on field observations and laboratory-reared individuals, together with no significant differences between moult increment of wild and laboratory-reared crabs (Section 5.3.4), has enabled the laboratory-reared data to be used to estimate growth parameters for the Von Bertalanffy equation. Six-monthly estimates of growth (starting mid-February as the point of minimal growth) were taken from Figures 5.9 and 5.10. and growth parameters were estimated using the Gulland-Holt plot technique (Fig. 5.15B). Values of the growth parameters are summarised in Table 5.4 and growth curves plotted in Figure 5.16. The growth curve for males was similar to the curve produced using the probability paper technique, however, the female curve showed an earlier and more marked reduction in growth rate (Fig. 5.16).

The results of the ELEFAN analysis on the modal growth of Liocarcinus puber gave growth parameter estimates that were very similar to

those estimated using the other methods (Table 5.4). The 'explained sum of peaks' to 'available sum of peaks' ratio (ESP/ASP) had, as anticipated, low values (0.116 for females and 0.057 for males). These, in part, result from ELEFAN predicting a continuous growth pattern to the data, and from the variability found within each year class due to factors such as varying time of recruitment and effects of limb-loss (Chapter 6). Parameters derived from ELEFAN are given in Table 5.4 and the Von Bertalanffy growth curve in Figure 5.16. The output of the ELEFAN programme incorporates a function to allow for the seasonal anecdysis over the winter period and Figure 5.17 shows the seasonal growth curve predicted by ELEFAN for L. puber. A growth line describing the discontinuous mode of growth for L. puber has also been superimposed onto the ELEFAN predicted data, using information on moult periodicity and increment (Sections 5.3.3 and 5.3.4) (Fig.5.17). The growth pattern derived from ELEFAN varied between males and females from a size of 55mm LCW, suggesting a change in rate of growth between sexes (Fig. 5.18). This difference between sexes was also noted for laboratory-reared individuals (Fig. 5.11). This decrease in increment at moult for female crabs >55mm LCW, and delayed moult in females, means that males of the same age will be larger than females and therefore well capable of fertilising similarly aged females (Chapter 4). The seasonalised growth data predicted from ELEFAN (Table 5.5) have been added to the original size-frequency data for the total survey (Figs 5.19 and 5.20). The histograms suggest that the recruitment period may vary markedly between years and that the time of peak recruitment may differ in any one year. The data also strongly indicate that crabs beyond their fourth winter become less abundant compared with younger cohorts. Based on the curve predicted from ELEFAN, the life expectancy for L. puber

end of the fourth year (Figs 5.19 and 5.20; Table 5.5).

DISCUSSION

The growth curves predicted in this study from the size-frequency data and from laboratory-rearing data showed similar values for the rate constant (K) and asymptotic length (L_∞) of the Von Bertalanffy equation (Fig. 5.16; Table 5.4). The growth curves for laboratory-reared females, however, showed a lower size to age estimate for large females (>55mm LCW) than the estimates from the probability paper and ELEFAN analysis of the size-frequency distributions. This difference may be explained partly by problems experienced by females attaching their eggs to the pleopods. The failure of eggs to attach to the pleopods of captive females has been noted in several other crab studies, and a soft substratum appears necessary for successful attachment for portunids and cancrid crabs (Crothers 1968, Edwards 1971). Even with a sandy substratum, however, laboratory-reared females were observed to have broods with very low egg numbers and, on several occassions, were observed to physically remove the few eggs that had successfully attached. The reared females subsequently produced more ovulations, with each one failing to adhere. This increased reproductive effort may, therefore, have reduced the growth for these individuals.

Growth of *Liocarcinus puber* has been examined previously (Gonzalez Gurriaran 1981a, 1981b, 1985b and Choy 1986a), and a summary of the predicted growth parameters is given in Table 5.4 and the growth curves are shown in Figure 5.16. Gonzalez Gurriaran (1985b) (using the probability paper technique and supportive field data) estimated a more rapid development for *L. puber* from Ria de Arousa, Spain than from Plymouth, England. Gonzalez Gurriaran (1985b) predicted that after one

year females achieved 48mm SCW (50.5mm LCW) and males 54mm SCW (57mm LCW), whereas Plymouth L. puber requires two years to reach comparative sizes (Fig. 5.16). The age of sexual maturity predicted by Gonzalez Gurriaran (1985b) is similar to that estimated in the present study (1 year) but, due to the differing rates of growth, Spanish crabs are larger at maturity than Plymouth (Chapter 4). Choy (1986a) gave a size of maturity and L_∞ for L. puber in South Wales which were similar to the present study, yet the rate constant (K) for Welsh L. puber was comparable with that of Spanish L. puber. Choy (1986a) also used ELEFAN to examine of growth of L. puber and Liocarcinus holsatus from South Wales. These species, particularly adults, showed a markedly discontinuous growth pattern, with an intermoult period of up to one year (Choy 1986a). ELEFAN has also been used in growth studies for several invertebrate species (some showing discontinuous growth, such as penaeids) and ESP/ASP values obtained range from 0.2-0.457 (Rodriguez 1977, Pauly and Calumpong 1984, Pauly et al. 1984, Mathews et al. 1987). The ESP/ASP ratios from Choy's (1986a) study for L. puber were extremely high (0.669 and 0.854 for males and females respectively) and were higher than literature values for animals with continuous growth! It would appear that Choy (1986a) mis-interpreted the output from the ELEFAN programme as he used an early edition of the computer programme (S.Choy, pers. comm.). The ESP/ASP ratios obtained for L. puber at Plymouth (0.057 and 0.116 for male and female respectively) suggest low agreement between model and data as would be expected from the mode of growth of the crab, yet the model gave a clear optimum ESP/ASP ratio, suggesting that the values for K and L_∞ were reliable.

The study of growth in other portunids has utilised several techniques and met with varying success. Broekhuysen (1936) and Naylor

(1962) examined the modal increment of Carcinus maenas through monthly samples. Van Engel (1958) traced the growth of immature Callinectes sapidus through to the terminal moult at an age of eighteen months. Paul (1982) examined the progression of modes in the growth of Callinectes arcuatus and C. toxotes and constructed growth curves for the juvenile and maturing cohorts. Similarly, Potter et al. (1983) traced the growth of juvenile and sub-adult Portunus pelagicus but found a strongly bimodal distribution, and were unable to distinguish cohort structure of the latter mode. Du Preez and McLachlan (1984a) examined Ovalipes punctatus but failed to distinguish modal progression due to the prolonged spawning and recruitment of this species. Dittel et al. (1985) also found difficulty in distinguishing modes in the size-frequency distribuion for Callinectes arcuatus due to prolonged spawning. In the majority of these investigations, the discrimination of modal growth of juvenile instars has been successfully identified, but clear definition of adult year classes has been limited to species with a determinate growth pattern and those with terminal anecdysis at maturity [eg. C. sapidus (Van Engel 1958, Tagatz 1969)]. Hartnoll (1982, 1985) could identify no clear growth pattern for portunids, although several species have a determinate growth pattern, with a terminal moult caused by a continual over-production of the moult inhibiting hormone (MIH) [eg. Carcinus maenas (Carlisle 1957)]. Choy (1986a) suggested that L. puber undergoes terminal anecdysis over a size range of 78-85mm SCW for males and 68-73mm SCW for females after a set number of moults. The present study found a mode in the size-frequency distribution that gives support to Choy's (1986a) prediction, but several Plymouth specimens were also observed >10mm larger than the terminal moult size suggested by Choy (1986a). This latter finding indictes that L. puber

shows either a determinant growth pattern with there being marked variation in size increments between individuals in the study area, or an indeterminant growth pattern with moulting continuing until mortality occurs. Without further information on the hormonal cycle controlling moulting in L. puber it is difficult to argue with conviction which growth pattern L. puber conforms to.

The percentage increment at moult (growth factor) has recieved much attention and early investigators believed that the percentage increment was fixed (generally at about 25%, ie. doubling in volume) (Przibram 1929). Values of percentage increment have, however, been shown to vary markedly, and values in excess of 400% have been reported [eg. Pachygrapsus crassipes Randall (Hiatt 1948)]. In this study, percentage increment for Liocarcinus puber decreased with size for both males and females. The plot of female growth factor based on laboratory-reared individuals showed a discontinuity for larger females indicating a reduction in percentage increment. This phenomenon may, as explained above, be partially due to unsuccessful adhesion of eggs to the pleopods and subsequent multiple ovulations. ELEFAN, however, also predicted a slower growth rate for females >55mm LCW compared with males (Fig. 5.18). Therefore, it appears that this discontinuity is real, with laboratory rearing accentuating the phenomenon. A reduction of growth factor in females at puberty has been noted for several species (eg. Cancer magister Dana) and this has been interpreted as a response to competition for resources between the processes of growth and reproduction (Hartnoll 1982, 1985). For L. puber this reduction in growth factor occurred at a size of approx. 55mm LCW, considerably larger than maturity (approx. 40mm LCW, Chapter 4), suggesting that for L. puber the 55mm LCW size group demarks

the point where reproduction rather than growth is dominant. This further supports the theory that the I cohort females (35-55mm LCW) may only produce one brood and that resources are channelled primarily into growth for these smaller females and that a large reproductive effort occurs for females >55mm LCW (II cohort+ females).

Variation in crustacean growth rates with differing environmental factors such as substratum, temperature, food availability, salinity, and density-dependant parameters has recently been well documented in the literature (Annala et al. 1980, Berrill 1982, Jones and Simons 1983, Conan 1985, Siegel and Wenner 1985, Chapman and Howard 1988). Variations in the size at sexual maturity, and the growth parameters of L_∞ and K have been shown for crustacean species from differing localities due to variations in the above mentioned factors. A clear example of this variation has been given by Chapman and Howard (1988), working with Nep.hrops norvegicus (L.). These authors found marked variation in growth parameters between 'stocklets' and suggested that density-dependant factors, which were correlated with substratum, were the prime cause of variation. Conan (1985) further suggested that growth parameters for N. norvegicus from the same locality may vary from year to year. Annala et al. (1980) suggested that the geographical variation in the size of sexual maturity in the spring crayfish Jasus edwardsii (Hutton) was due to the interaction of several factors, such as temperature, food availability, metabolic rate and population density. This apparent 'plasticity' of crustacean growth may, in part, explain the difference in growth rate for Liocarcinus puber between Spanish and Plymouth populations. Lower temperatures, and possibly greater degree of density-dependant factors, may play an important role in slowing and reducing growth in Plymouth waters.

For example, the variation in recruitment of juvenile L. puber reported in this study between the poor recruitment of 1986 compared with 1985 and 1987, will lead to marked differences in density-dependant factors between these cohorts, such as lower intraspecific competition and aggression leading to reduced limb-loss between siblings for the 1986 year class. The effects of these density-dependant factors, and the possibility of trophic variation between localities (Chapter 7), suggest that there is likely to be both temporal and spatial variation in the growth of L. puber.

Thus for Liocarcinus puber, further variability in life-history structure and growth rate may be expected between Plymouth and the current main fishery area on the West coast of Scotland, where L.puber is reported to to attain a larger size than in S.W. England (MacMullen 1983). The level of growth parameters such as K, L_∞ and size of sexual maturity may therefore vary significantly between populations and will require local examination prior to use in an overall fishery management context.

Table 5.1. Estimates of period between moulting and complete hardening of the cuticle for wild and laboratory-reared Liocarcinus puber. Data for wild individuals are from littoral and sub-littoral mark-recapture experiments (Chapter 9). Reared Liocarcinus puber are from Bovisand where temperature fluctuated seasonally (Chapter 3). (Male and female data combined; n= number of crabs).

Crab type	Size Range (mm LCW)	n	Estimated mean period to harden (days)	Actual mean (±1S.D.) to harden (days)
'Wild'	40-60	4	30	
Reared	30-40	5		17.4 ±5.5
	40-60	11		25.5 ±5.4
	> 60	4		36.2 ±10.9

Table 5.2. Mean postmoult size (mm LCW) of male Liocarcinus puber observed in early post moult stages (soft and papershell) in the three sampling zones (n = number of early post moult males sampled and numbers in parentheses are ±1S.D.). Data for 1986 and 1987 combined.

Zone	March	June	July	Aug.	Sept.	Oct.
Circalittoral	83.2	73.7	68.4	64.2	-	-
	(1.1)	(8.7)	(10.6)	(11.8)	-	-
	n=3	n=21	n=22	n=8	-	-
Infralittoral	-	74.2	70.3	54.2	-	-
	-	(7.7)	(7.1)	(4.8)	-	-
	-	n=20	n=22	n=4	-	-
Littoral	-	-	60.2	56.1	51.0	58.8
	-	-	(10.6)	(7.2)	(8.8)	(5.6)
	-	-	n=10	n=14	n=13	n=5

Table 5.3. Results of ANCOVA between (A) ratio of premoult to postmoult size and (B) growth factor and premoult size, between 'wild' and laboratory-reared male and female Liocarcinus puber (N.S. indicates not significant, P>0.05).

Pairs of lines tested	Intercept			Slope		
	F	df	Sign.	F	df	Sign.
(A)'wild'/ laboratory-reared						
male	1.43	1,35	N.S.	4.03	1,34	N.S.
female	0.05	1,24	N.S.	2.81	1,23	N.S.
(B)'wild'/ laboratory-reared						
male	0.26	1,33	N.S.	1.56	1,32	N.S.
female	0.04	1,21	N.S.	1.62	1,20	N.S.

Table 5.4. Von Bertalanffy growth parameters calculated for Liocarcinus puber for a) Plymouth (present work), b) Ria d'Arousa, Spain (Gonzalez Gurriaran 1985b) and c) Swansea (Choy 1986a). Parameters for Ria d'Arousa and Swansea are converted to long carapace width (LCW) (Chapter 3) for comparison. A value for t_o is not given for the ELEFAN programme as it is assessed internally in the programme and is not incorporated in the data output (Pauly 1987).

	Male	Female
a) Plymouth		
1) Probability paper technique		
$L\infty$	107.25	97.8
K	0.337	0.362
t_o	-0.214	-0.268
2) ELEFAN analysis		
$L\infty$	110	94
K	0.33	0.448
ESP/ASP	0.057	0.116
3) Reared individuals		
$L\infty$	114.80	91.20
K	0.278	0.348
t_o	-0.354	-0.44
b) Ria d'Arousa, Spain.		
$L\infty$	109	96
K	0.65	0.67
t_o	-0.041	-0.048
c) Swansea, Wales		
$L\infty$	107	83
K	0.608	0.65
t_o	-0.03	-0.05

Table 5.5. Seasonal growth curve data predicted from ELEFAN for (a) male and (b) female Liocarcinus puber. Measurements predicted for the 15th of each month. Both curves used Febuary as the 'winter point', ie. the coldest month of the year and C=1 (where C is a measure of the intensity of seasonal growth oscillation). Parameters predicted by ELEFAN are the growth rate constant K, the asymptotic length L_∞, and the 'expected sum of peaks' to 'available sum of peaks' ratio (ESP/ASP). Numbers 1-12 indicate months of year (1=January etc.).

(a) Male - K=0.33, L_∞=110; ESP/ASP = 0.057

1	2	3	4	5	6	7	8	9	10	11	12
							1.4	7.3	12.3	16.4	18.9
20.2	20.6	20.6	20.9	22.1	24.4	27.8	31.9	36.1	39.8	42.7	44.5
45.4	45.7	45.7	45.9	46.8	48.5	50.9	53.9	56.9	59.5	61.6	62.9
63.6	63.8	63.8	64.0	64.5	65.8	67.5	69.6	71.8	73.7	75.2	76.1
76.6	76.8	76.8	76.9	77.3	78.2	79.4	81.0	82.6	83.9	85.0	85.7
86.0	86.1	86.1	86.2	86.5	87.1	88.0	89.1	90.3	91.2	92.0	92.5
92.8	92.8	92.8	92.9	93.1	93.6	94.2	95.0	95.8	96.5	97.1	97.4
101.1	101.1	101.1	101.2	101.3	101.5	101.8	102.2	102.7	103.0	103.3	103.5
103.6	103.6	103.6	103.6	103.7	103.9	104.1	104.4				

(b) Female - K=0.448; L_∞=94; ESP/ASP = 0.116

1	2	3	4	5	6	7	8	9	10	11	12
								2.0	8.1	12.9	15.9
17.4	17.8	17.8	18.2	19.5	22.2	26.0	30.6	35.2	39.1	42.2	44.1
45.1	45.3	45.3	45.6	46.4	48.2	50.6	53.5	56.5	59.0	60.9	62.1
62.7	62.9	62.9	63.1	63.6	64.7	66.3	68.1	70.0	71.6	72.9	73.6
74.0	74.1	74.1	74.2	74.6	75.3	76.3	77.5	78.7	79.7	80.5	81.0
81.2	81.3	81.3	81.4	81.6	82.0	82.7	83.4	84.2	84.9	85.4	85.7
85.8	85.9	85.9	85.9	86.1	86.4	86.8	87.3	87.7	88.2	88.5	88.7
88.8	88.8	88.8	88.8	88.9	89.1						

Figure 5.1. Size-frequency distributions for female Liocarcinus puber sampled in the littoral zone. Numbers in parentheses indicate numbers of crabs sampled.

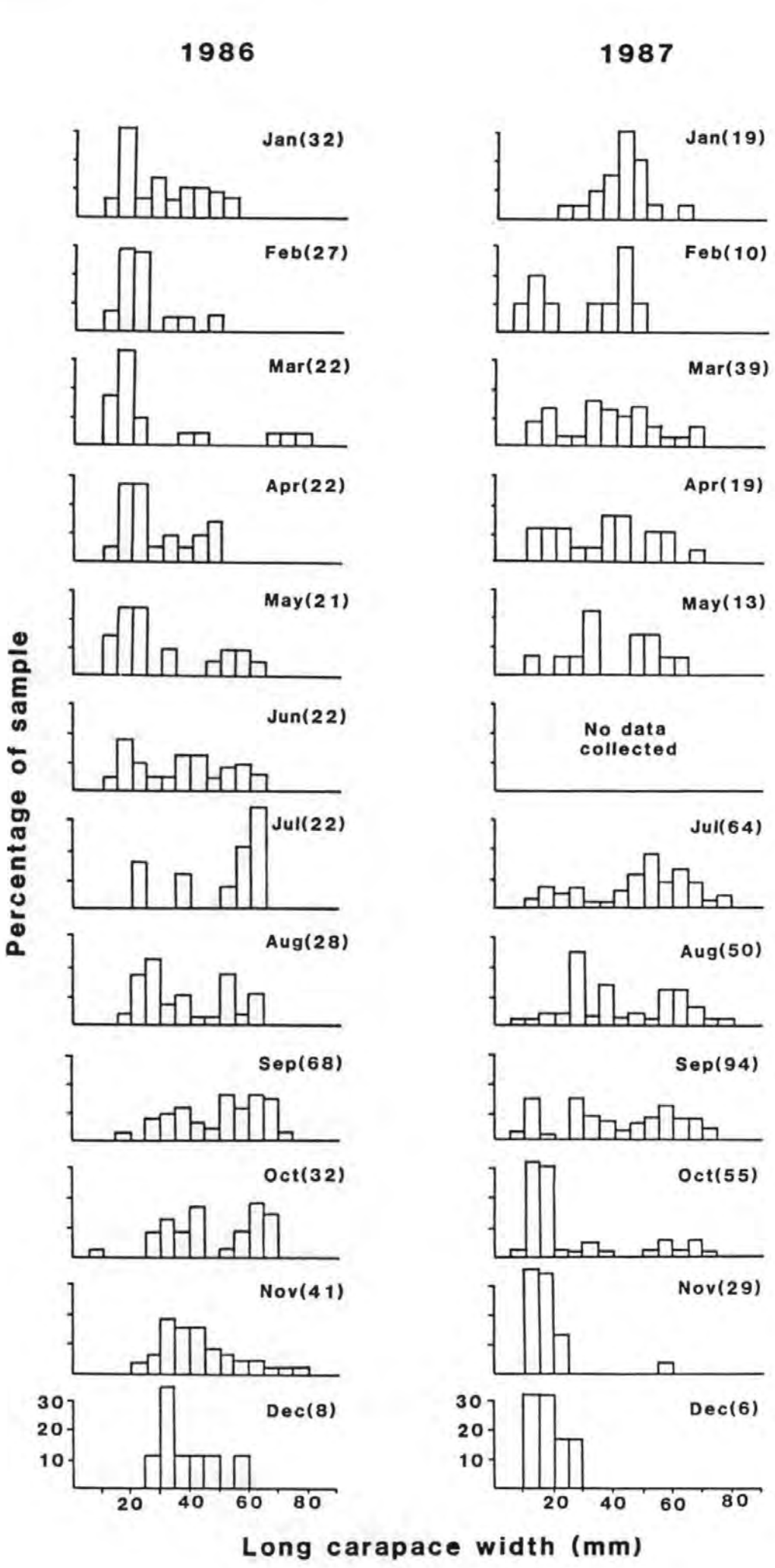

1986
1987
Jan(32)
Jan(19)
Feb(27)
Feb(10)
Mar(22)
Mar(39)
Apr(22)
Apr(19)
May(21)
May(13)
Jun(22)
No data collected
Jul(22)
Jul(64)
Aug(28)
Aug(50)
Sep(68)
Sep(94)
Oct(32)
Oct(55)
Nov(41)
Nov(29)
Dec(8)
Dec(6)
Percentage of sample
30
20
10
20
40
60
80
Long carapace width (mm)

Figure 5.2. Size-frequency distribution for male *liocarcinus puber* sampled in the littoral zone. Numbers in parentheses indicate numbers of crabs sampled.

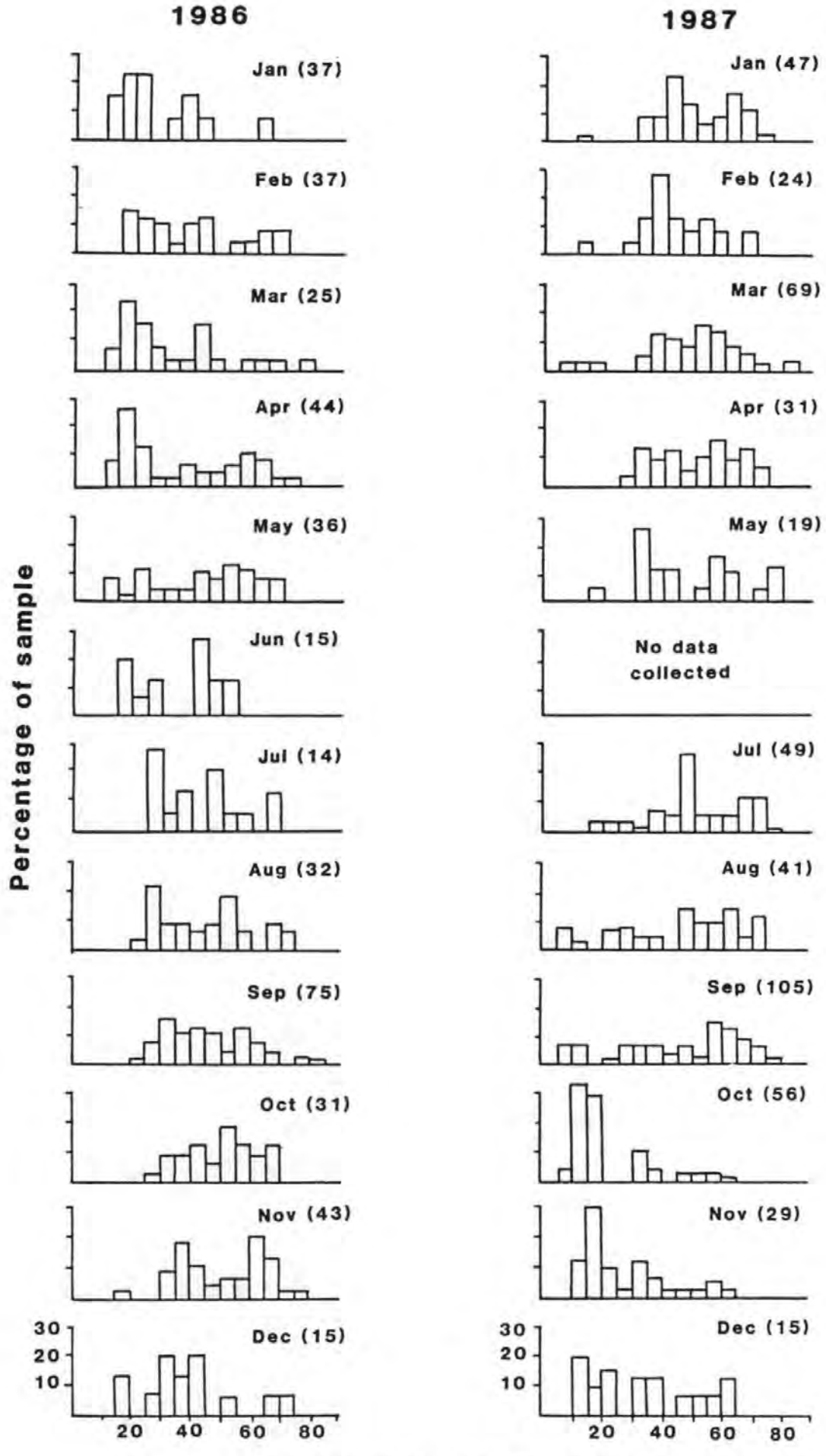

1986
1987
Jan (37)
Feb (37)
Mar (25)
Apr (44)
May (36)
Jun (15)
Jul (14)
Aug (32)
Sep (75)
Oct (31)
Nov (43)
Dec (15)
Jan (47)
Feb (24)
Mar (69)
Apr (31)
May (19)
No data collected
Jul (49)
Aug (41)
Sep (105)
Oct (56)
Nov (29)
Dec (15)
Percentage of sample
30
20
10
20
40
60
80
Long carapace width (mm)

Figure 5.3. Size-frequency distribution for female Liocarcinus puber sampled in the infralittoral and circalittoral zones. Numbers in parentheses indicate numbers of crabs sampled.

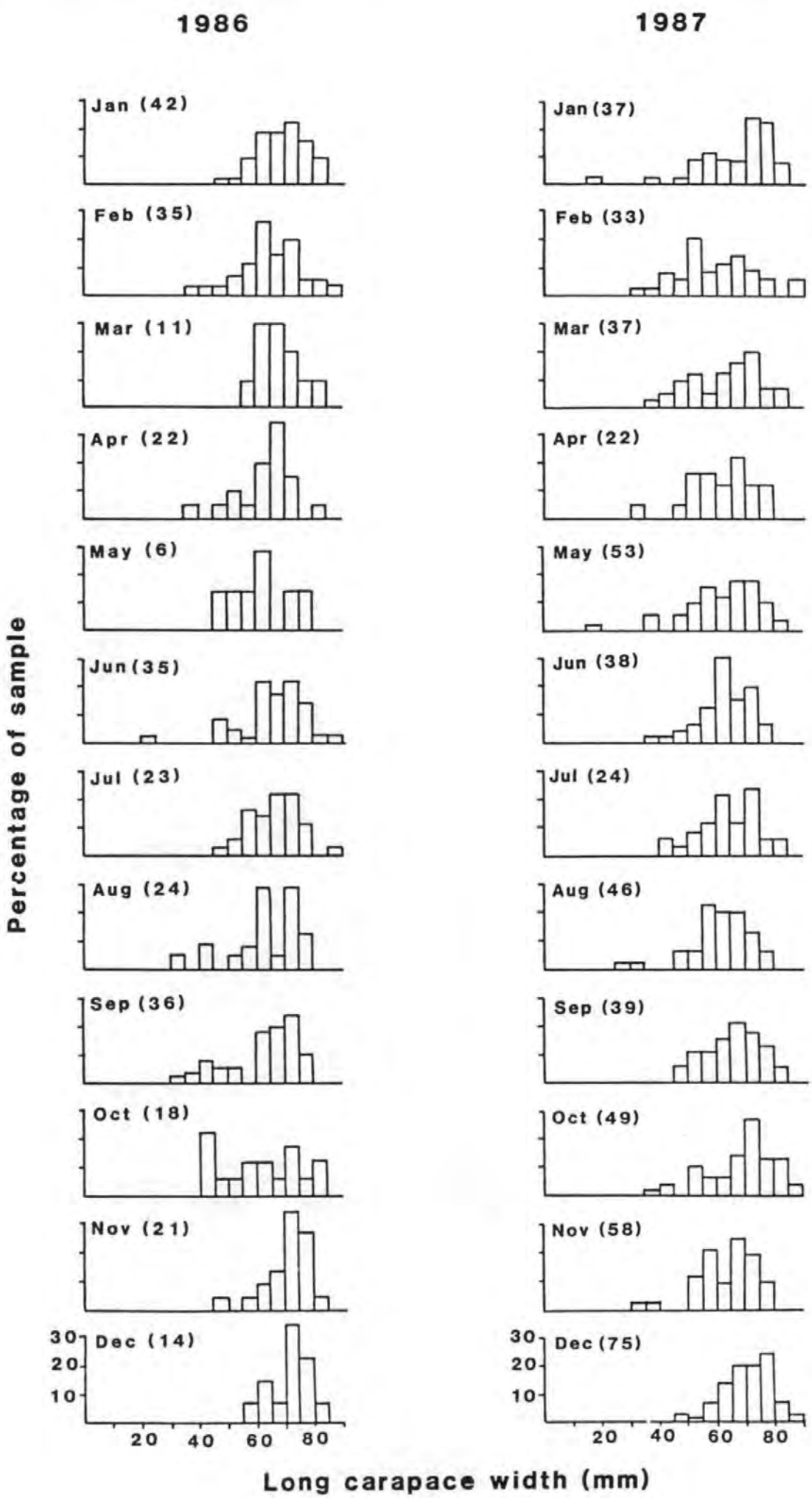

1986
1987
Jan (42)
Feb (35)
Mar (11)
Apr (22)
May (6)
Jun (35)
Jul (23)
Aug (24)
Sep (36)
Oct (18)
Nov (21)
Dec (14)
Jan (37)
Feb (33)
Mar (37)
Apr (22)
May (53)
Jun (38)
Jul (24)
Aug (46)
Sep (39)
Oct (49)
Nov (58)
Dec (75)
Percentage of sample
30
20
10
20
40
60
80
Long carapace width (mm)

Figure 5.4. Size-frequency distribution for male Liocarcinus puber sampled in the infralittoral and circalittoral zones. Numbers in parentheses indicate numbers of crabs sampled.

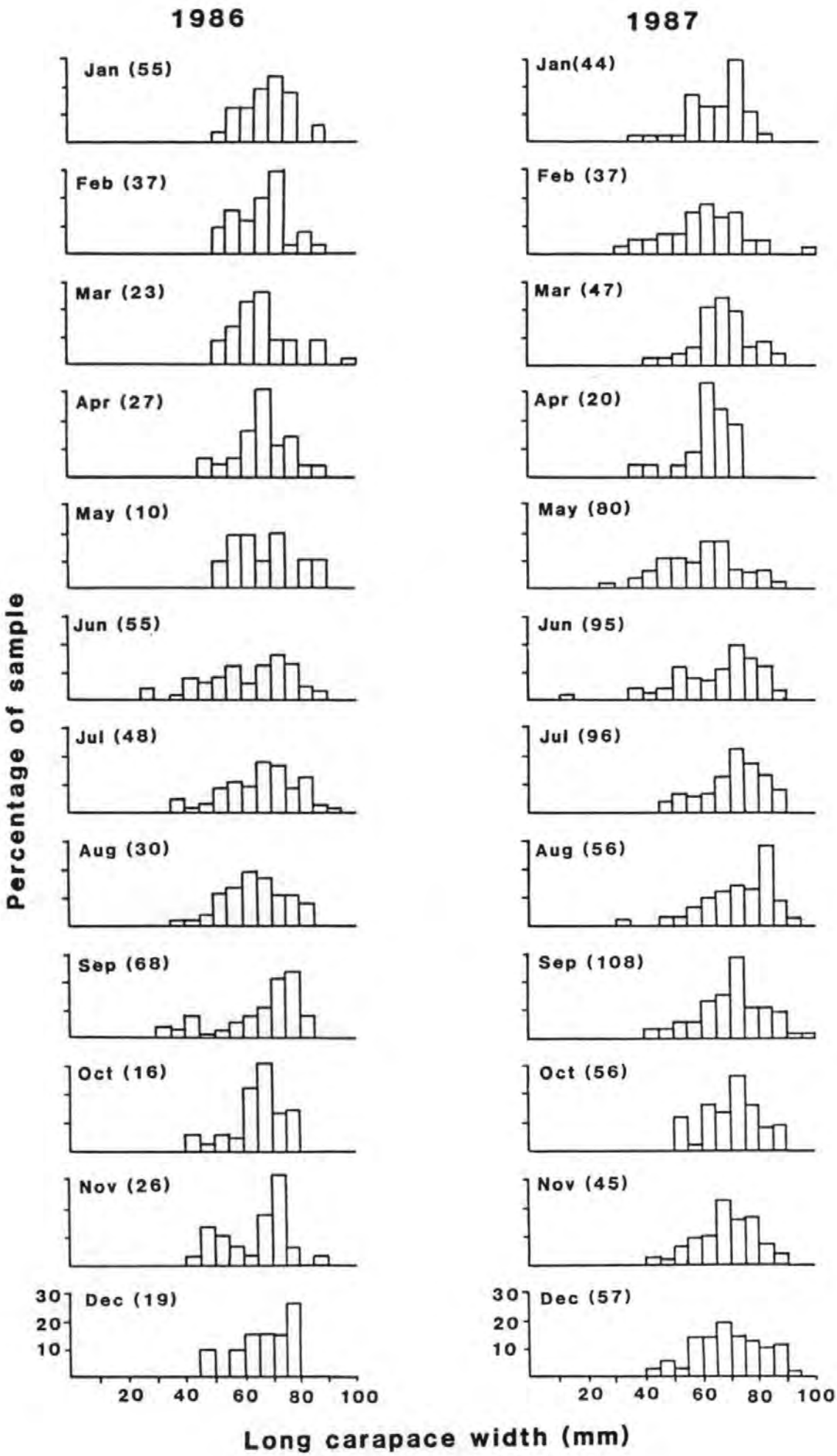

1986
1987
Jan (55)
Jan(44)
Feb (37)
Feb (37)
Mar (23)
Mar (47)
Apr (27)
Apr (20)
May (10)
May (80)
Jun (55)
Jun (95)
Jul (48)
Jul (96)
Aug (30)
Aug (56)
Sep (68)
Sep (108)
Oct (16)
Oct (56)
Nov (26)
Nov (45)
Dec (19)
Dec (57)
30
20
10
20
40
60
80
100
Percentage of sample
Long carapace width (mm)

Figure 5.5. Percentage of juveniles (females<40mm LCW, males <45mm LCW) sampled in (A) soft and papershell moult stages and (B) in late intermoult condition. Data from both years combined and percentages are expressed as the number of juveniles sampled in the appropriate moult stage over the total number of juveniles sampled each month. For (A), closed triangles indicate soft and papershell crabs combined, and open triangles represent soft crabs. Number of crabs sampled were soft, 19; papershell, 39; early intermoult, 688; and late intermoult, 89.

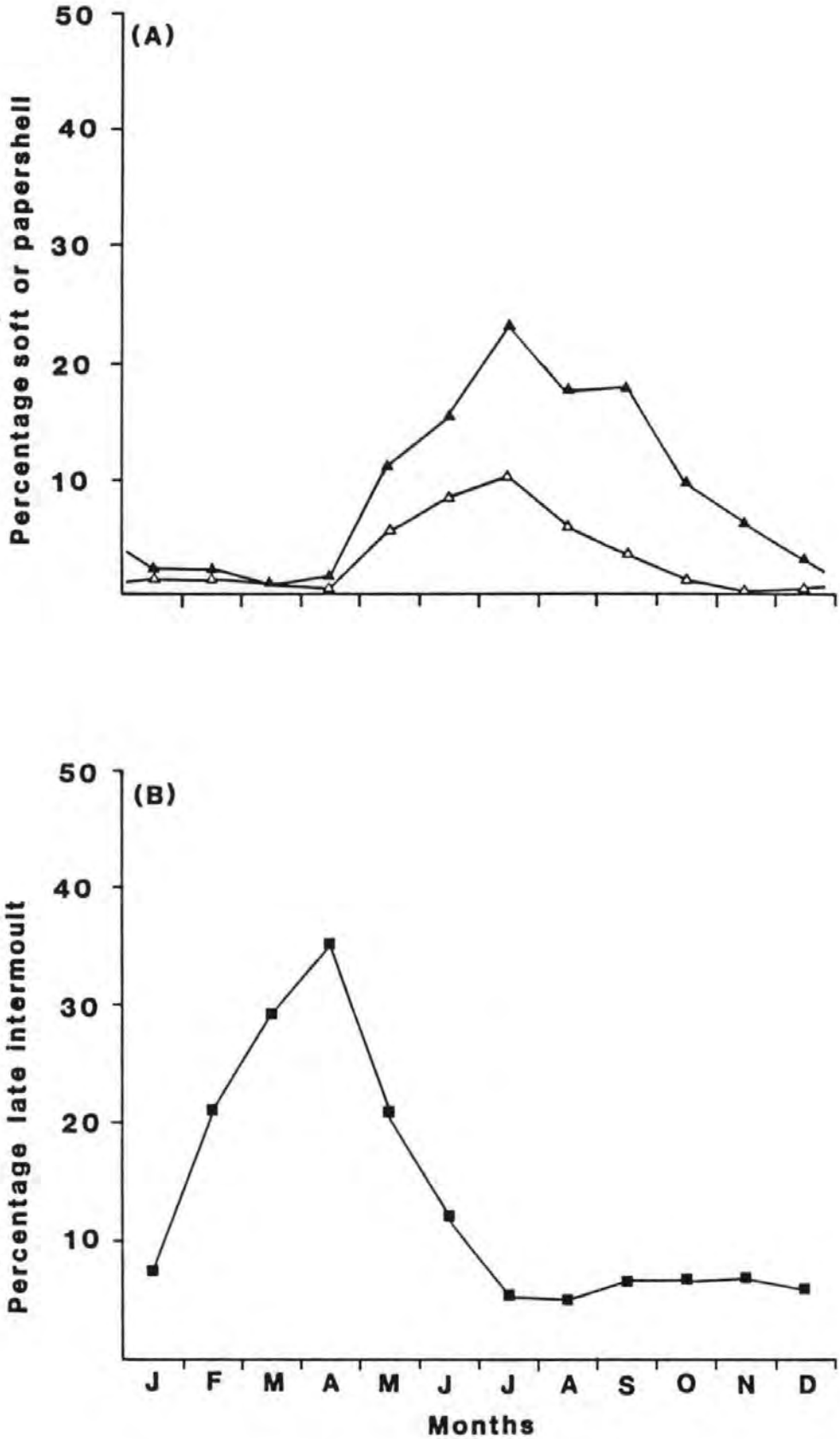

(A)
Percentage soft or papershell
50
40
30
20
10
(B)
Percentage late intermoult
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Months

Figure 5.6. Percentage of mature female Liocarcinus puber (>40mm LCW) sampled in (A) early post moult stages (soft and papershell) and (B) in late intermoult condition. Data for two years combined and percentages are expressed as the number of females sampled in the appropriate moult stage over the total monthly number of females sampled for each zone. For (A), closed triangles indicate percentage for the circalittoral zone, open squares for the infralittoral zone and closed square for the littoral zone. Note the change in vertical scaling between (A) and (B). Dashed lines indicate data not available. Numbers of crabs sampled were :

Zone	Moult Stage		
	soft+papershell	early intermoult	late intermoult
Circalittoral	18	319	244
Infralittoral	15	75	90
Littoral	41	168	126

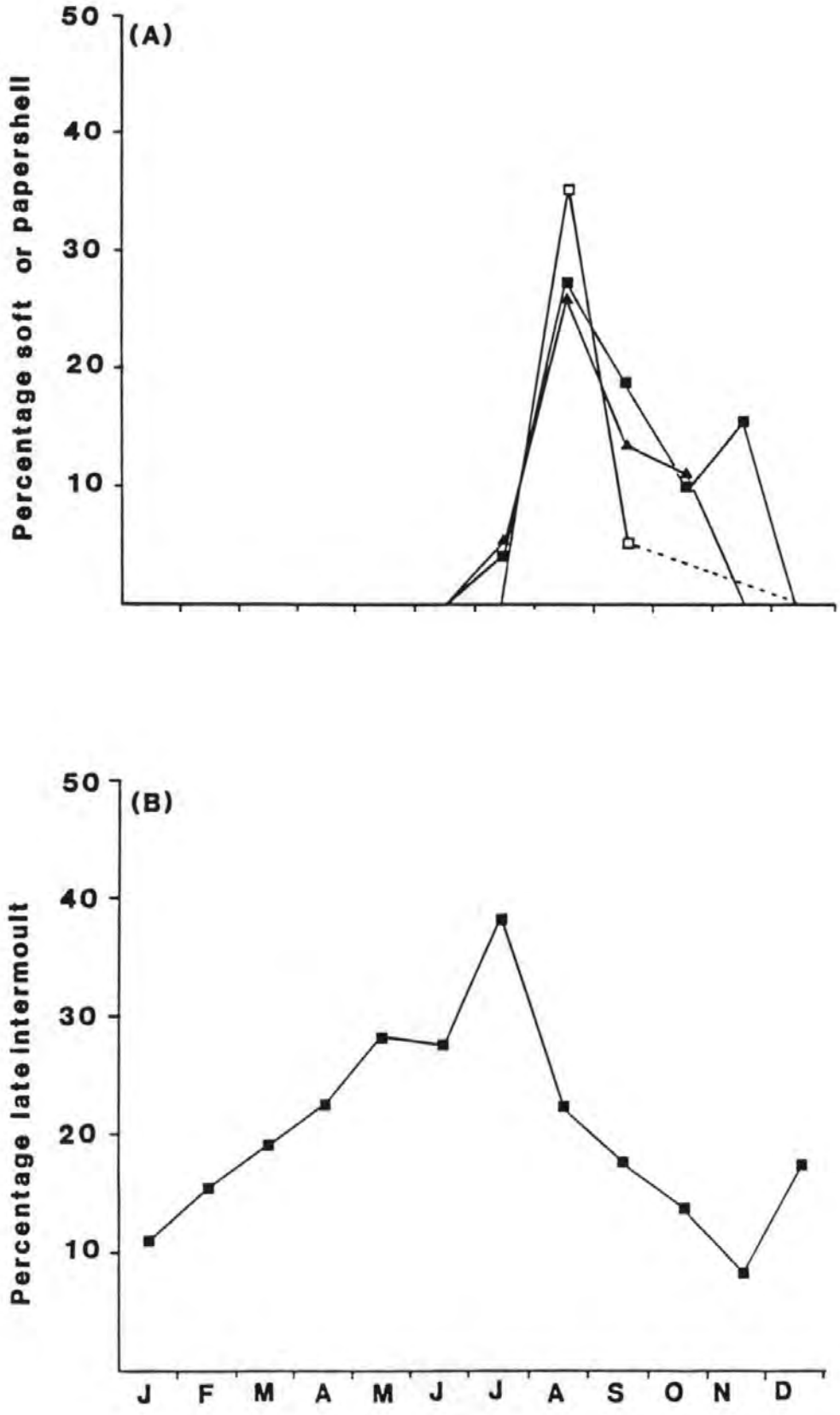

(A)
Percentage soft or papershell
50
40
30
20
10
(B)
Percentage late intermoult
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D

Figure 5.7. Percentage of mature male Liocarcinus puber (>45mm LCW) sampled in (A) early post moult stages (soft and papershell) and (B) in late intermoult condition (B). Data for two years combined and percentages are expressed as the number of males sampled in the appropriate moult stage over the total monthly number of males sampled for each zone. For (A) and (B), closed triangles indicate the percentage of soft and papershell crabs in the circalittoral zone, open squares for the infralittoral zone, and closed squares for the littoral zone. Note the change in vertical scaling between (A) and (B). Dashed line indicates data not available. Numbers of crabs sampled were :

Zone	Moult Stage		
	soft+papershell	early intermoult	late intermoult
Circalittoral	65	529	243
Infralittoral	40	146	100
Littoral	44	314	126

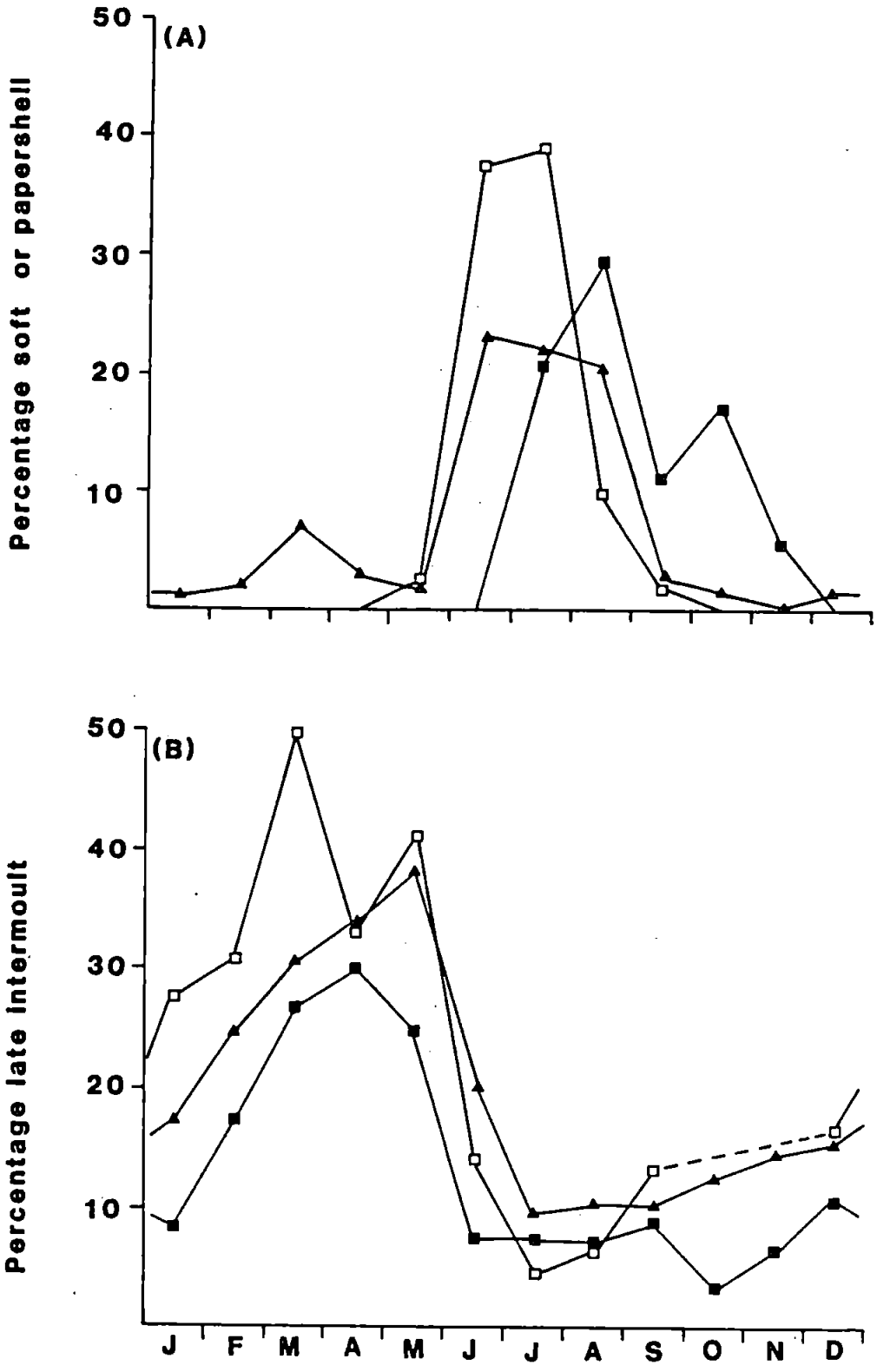

(A)
Percentage soft or papershell
50
40
30
20
10
(B)
Percentage late intermoult
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D

Figure 5.8. The periodicity of individuals observed in the field in a soft moult stage with intact exuviae. Data for large males (>60mm LCW premoult) come from mark-recapture experiments in the circalittoral zone (Chapter 10). Closed circles indicate males ($\underline{n}$=9) and open circles females ($\underline{n}$=6). Numbers in parentheses are the post moult size achieved by the soft crab measured in the laboratory 6h after sampling.

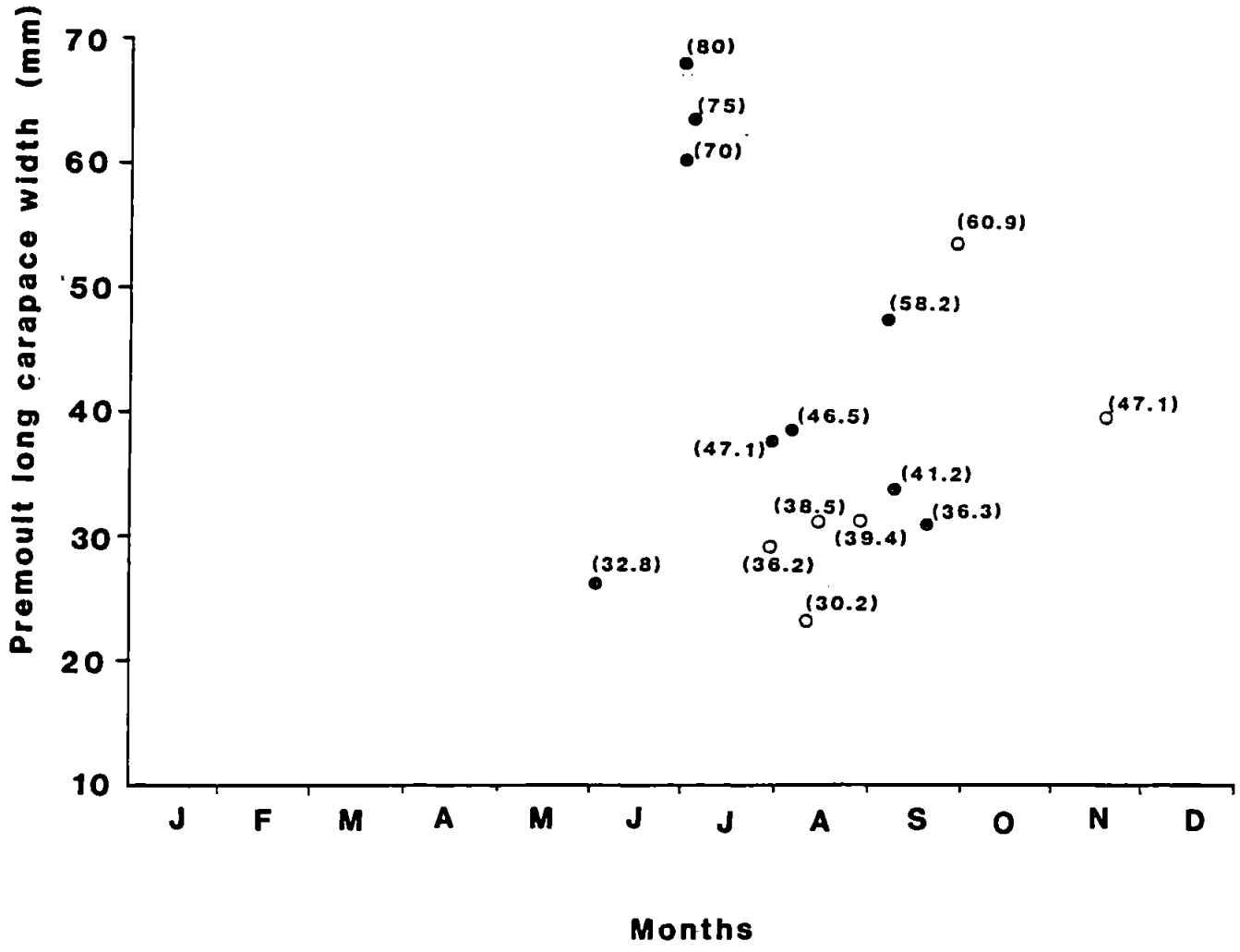

Premoult long carapace width (mm)
70
60
50
40
30
20
10
J
F
M
A
M
J
J
A
S
O
N
D
Months
(80)
(75)
(70)
(60.9)
(58.2)
(47.1)
(46.5)
(47.1)
(41.2)
(38.5)
(36.3)
(39.4)
(32.8)
(36.2)
(30.2)

Figure 5.9. Moult periodicity and increment of laboratory-reared male Liocarcinus puber. Data graphically presented to illustrate the periods of high moulting incidence. Closed circles indicate the premoult size and date of moult. Dashed lines to open triangles represent size increment and period of time for the carapace to fully harden. Closed circles with dashed lines which terminate prior to full hardening indicate that the crab died during moulting (n=10).

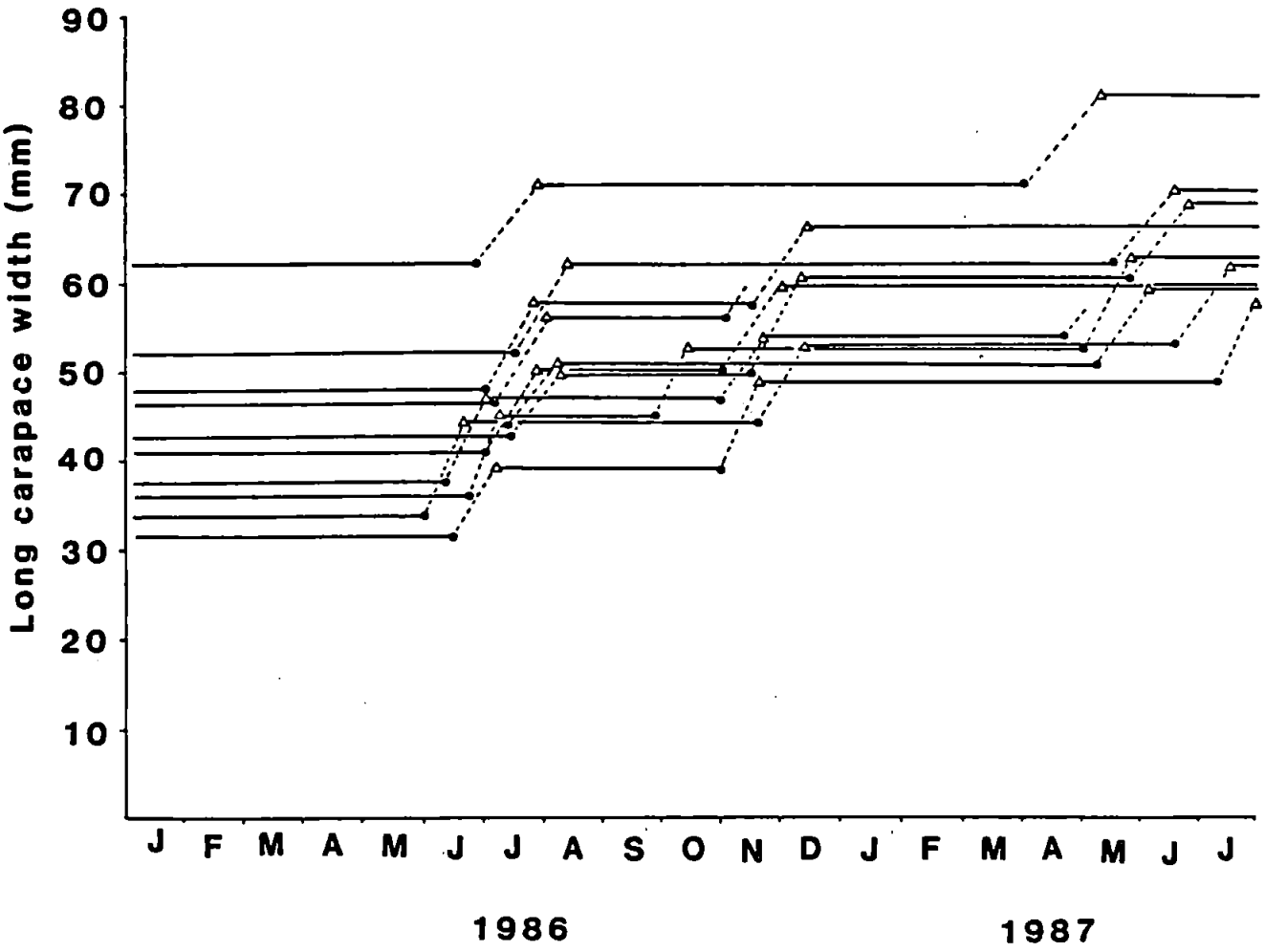

Long carapace width (mm)
90
80
70
60
50
40
30
20
10
J F M A M J J A S O N D J F M A M J J
1986
1987

Figure 5.10. Moult periodicity and increment of laboratory-reared female Liocarcinus puber. Data graphically presented to illustrate the periods of high moulting incidence. Closed circles indicate the premoult size and date of moult. Dashed lines to open triangles represent size increment and period of time for the carapace to fully harden. Closed circles with dashed lines which terminate prior to full hardening indicate that the crab died during moulting (n=10).

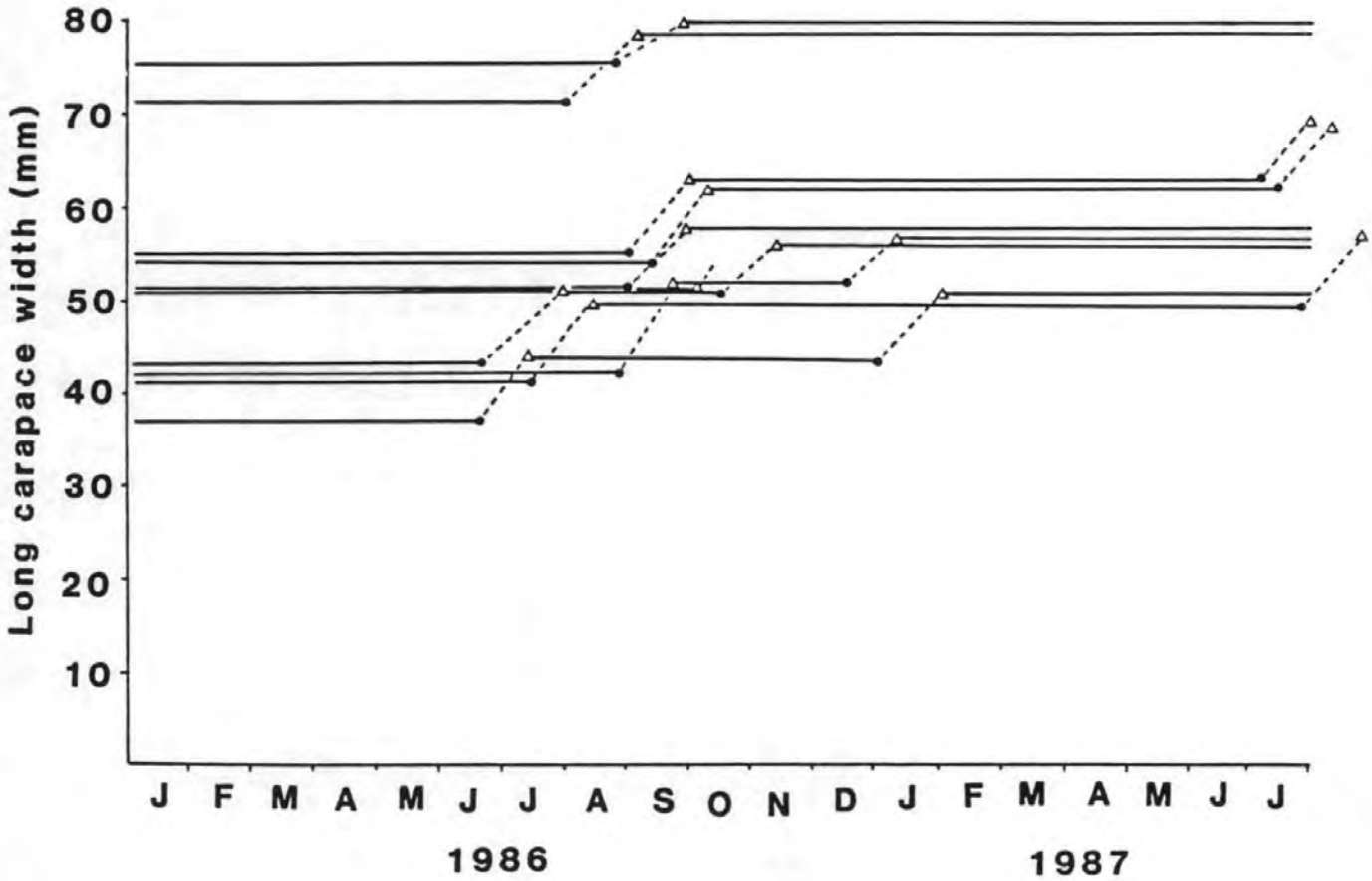

Long carapace width (mm)
80
70
60
50
40
30
20
10
J F M A M J J A S O N D J F M A M J J
1986
1987

Figure 5.11. Premoult versus postmoult plots for (A) male and (B) female Liocarcinus puber. Open circles are for 'wild data' ie. sampled in a soft condition with intact exuviae or from mark-recapture experiments. Closed circles are from laboratory-reared experiments. Regression equations are: male 'wild', $\underline{Y} = 4.486 + 1.109\underline{X}$ ($\underline{n} = 8$, $\underline{r} = 0.999$); male laboratory reared, $\underline{Y} = 7.082 + 1.041\underline{X}$ ($\underline{n} = 31$, $\underline{r} = 0.994$); female 'wild', $\underline{Y} = 6.796 + 1.015\underline{X}$ ($\underline{n} = 7$, $\underline{r} = 0.999$); and female laboratory reared $\underline{Y} = 10.57 + 0.921\underline{X}$ ($\underline{n} = 18$, $\underline{r} = 0.995$).

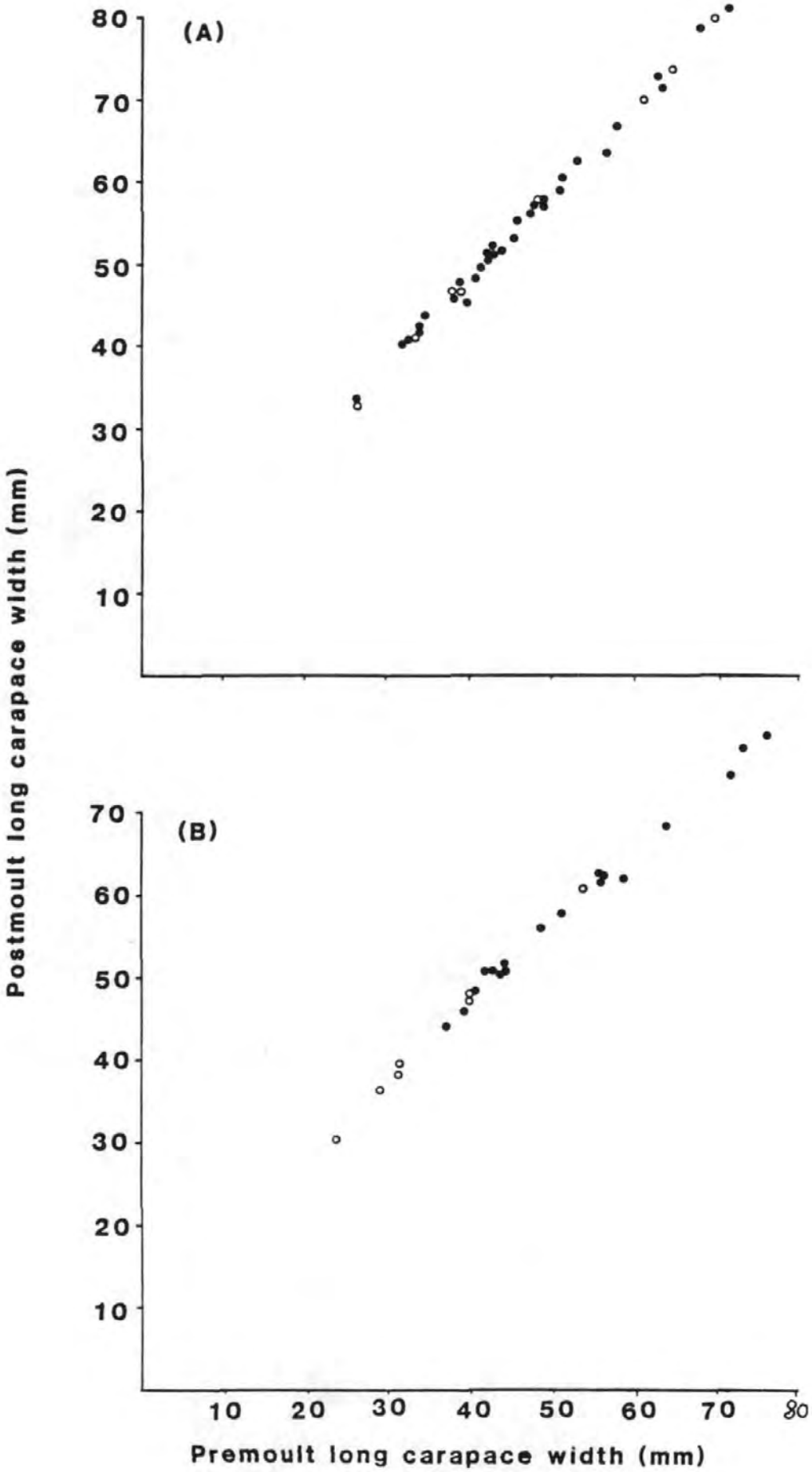

(A)
(B)
Postmoult long carapace width (mm)
Premoult long carapace width (mm)
10
20
30
40
50
60
70
80

Figure 5.12. Semi-log plot of percentage moult increment (growth factor) versus premoult plots for (A) male and (B) female Liocarcinus puber. Open circles are for 'wild data' (ie. sampled in a soft condition with intact exuviae or from mark and recapture experiments) and closed circles are from laboratory-reared crabs (experiments carried out at Bovisand under natural light and seasonally fluctuating temperatures). Regression equations: male $\underline{Y} = e^{(3.669 - 0.014\underline{X})}$ ($\underline{n}$=38, $\underline{r}$=0.739); and female less than 55mm LCW $\underline{Y} = e^{(4.032 - 0.028\underline{X})}$ ($\underline{n}$=18, $\underline{r}$=0.792); and for total females (regression line not shown) $\underline{Y} = e^{(4.372 - 0.035\underline{X})}$ ($\underline{n}$=25, $\underline{r}$=0.622)

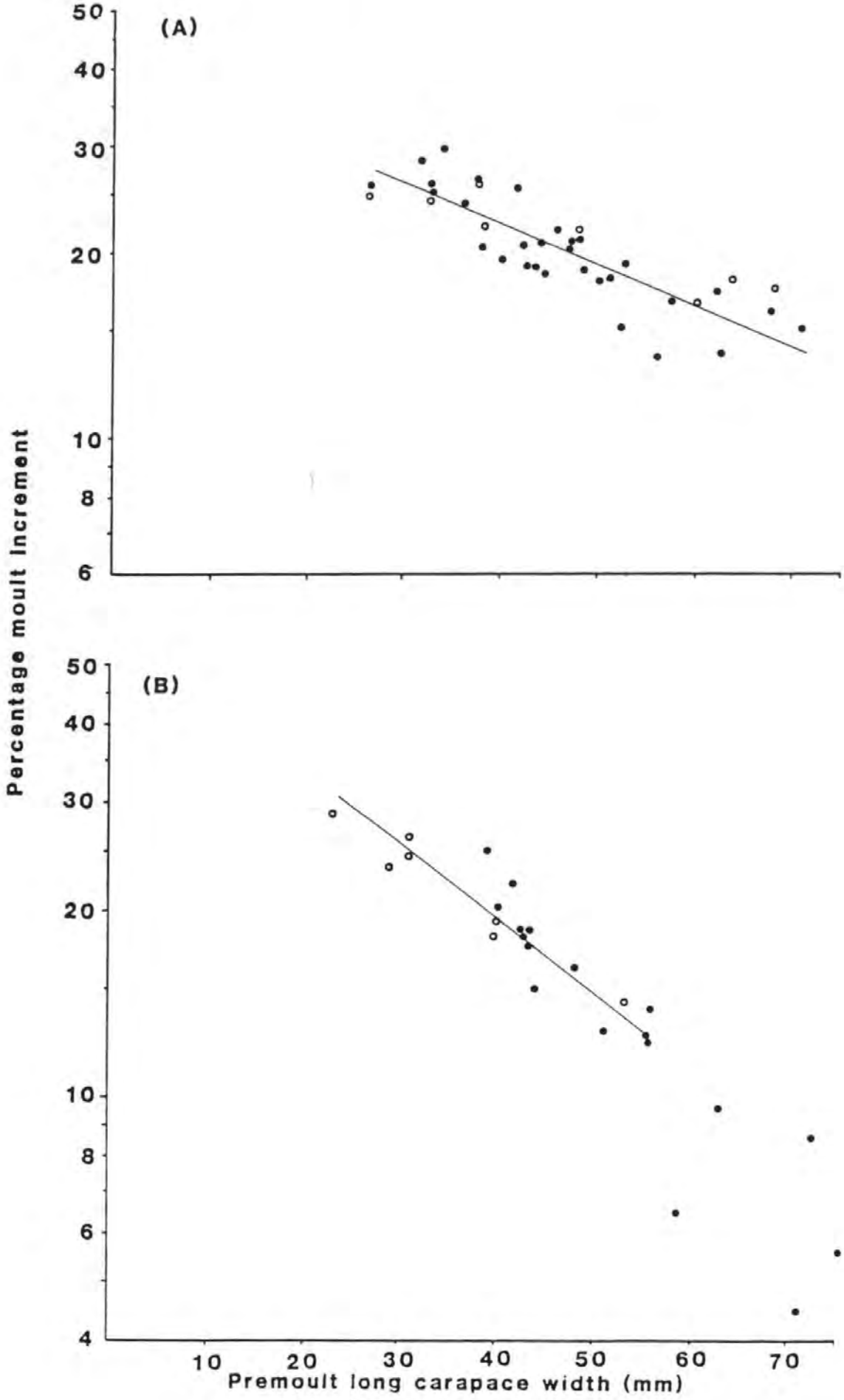

(A)
(B)
50
40
30
20
10
8
6
4
Percentage moult increment
10
20
30
40
50
60
70
Premoult long carapace width (mm)

Figure 5.13. An example of the probability paper technique using female data for October 1987. Closed circles indicate the cumulative plot of the size-frequency data. Points of inflexion are illustrated by arrows. Size-frequency data plotted in 2mm intervals on the vertical axis.

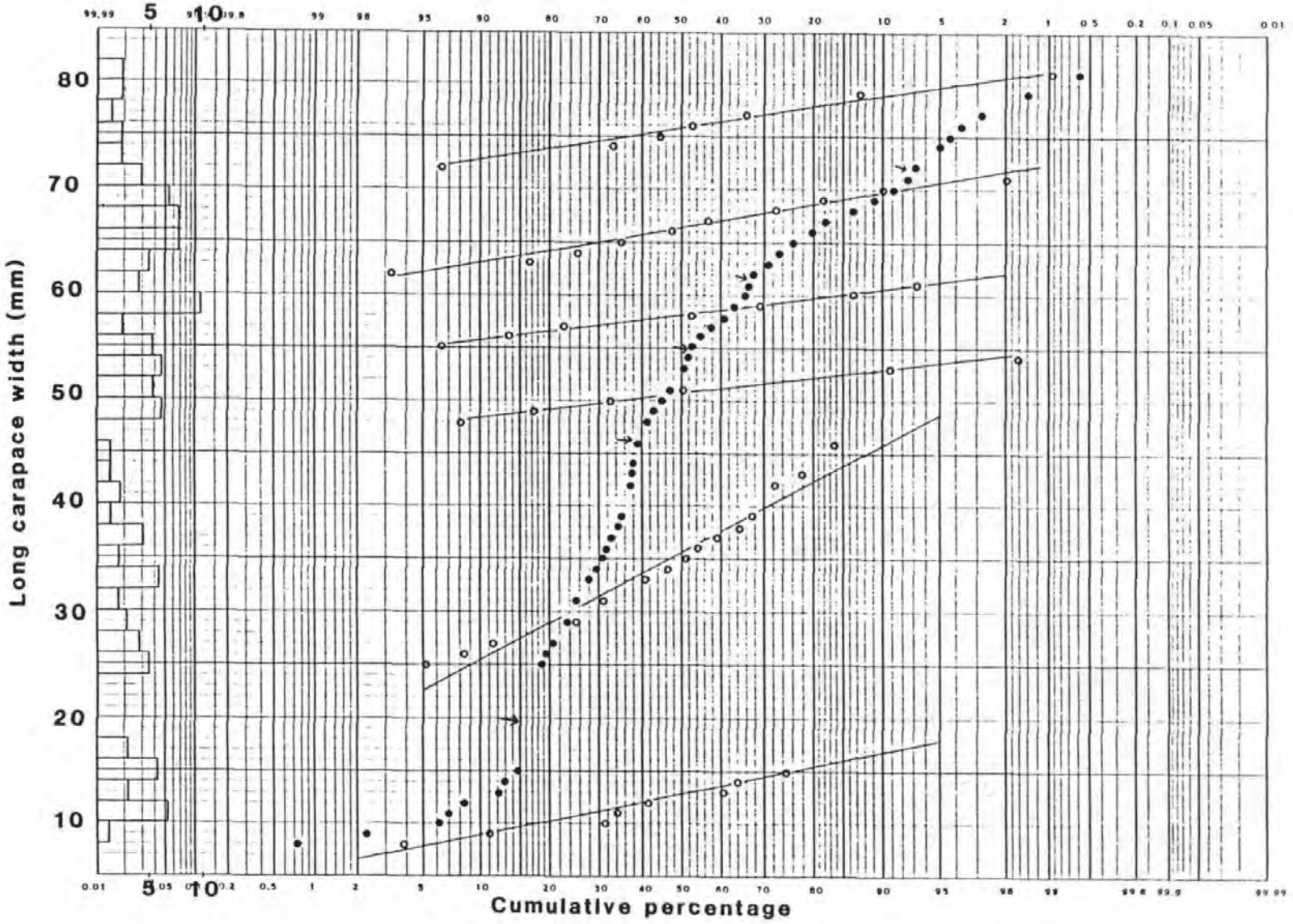

Long carapace width (mm)
80
70
60
50
40
30
20
10
Cumulative percentage

Figure 5.14. Plot of the mean modal sizes estimated using the probability paper technique for (A) males and (B) females. Dotted lines join probable cohorts between the months sampled.

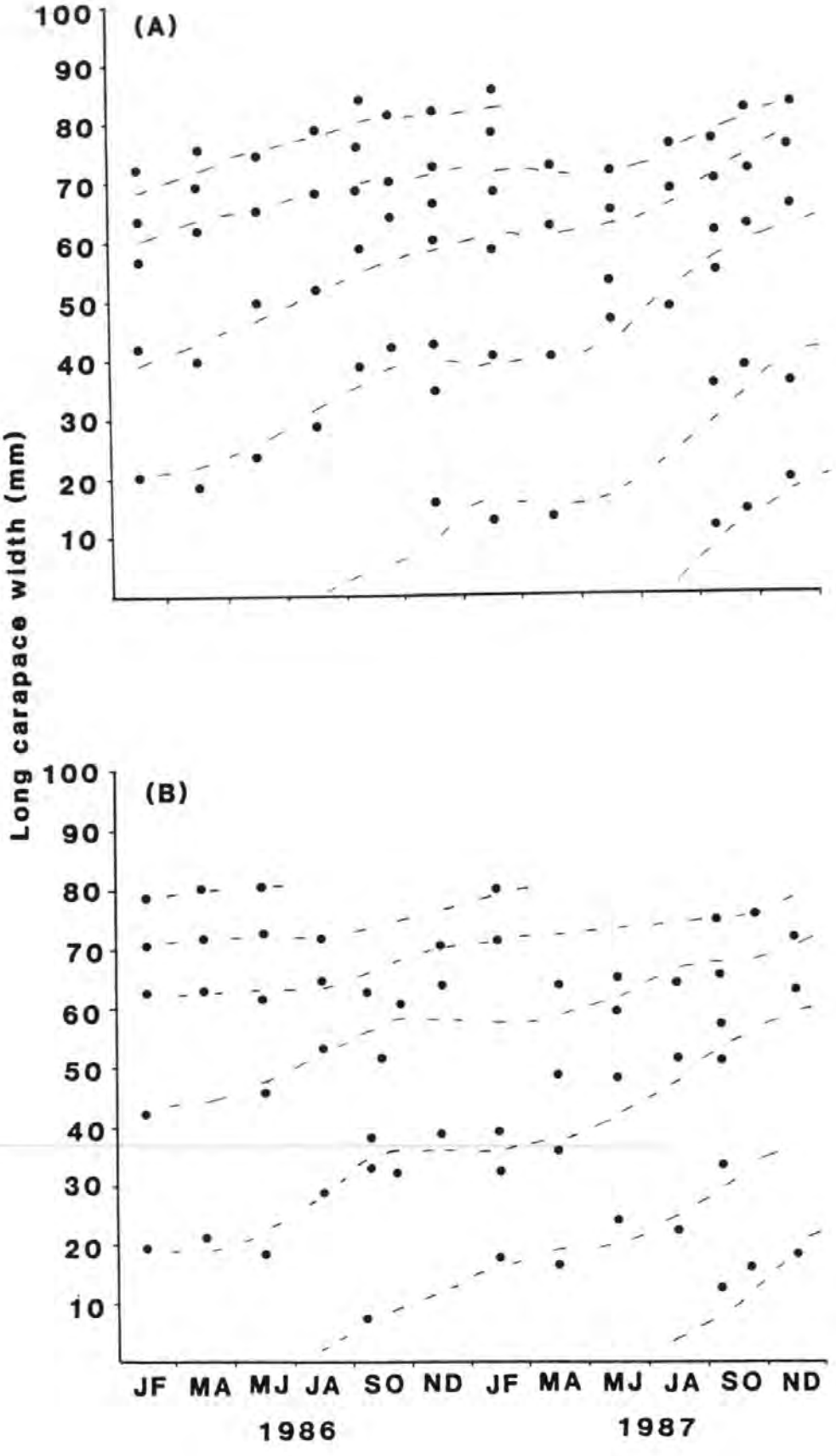

(A)
(B)
Long carapace width (mm)
100
90
80
70
60
50
40
30
20
10
JF MA MJ JA SO ND JF MA MJ JA SO ND
1986
1987

Figure 5.15. Gulland-Holt plots for (A) 'wild' data and (B) laboratory-reared data: closed circles are for male and open circles female Liocarcinus puber. For (A) and (B), points taken from examination of size-frequency data using probability plots to elucidate cohort growth. Points are taken over two six monthly periods commencing February and August for cohorts that could readily be indentified. Regression equations are : 'wild' males $\underline{Y} = 0.695 - 0.00648\underline{X}$ ($\underline{n}=9$, $\underline{r}=0.992$); 'wild' female $\underline{Y} = 0.682 - 0.00697\underline{X}$ ($\underline{n}=9$, $\underline{r}=0.964$); laboratory-reared males $\underline{Y} = 0.613 - 0.00534\underline{X}$ ($\underline{n}=23$, $\underline{r}=0.65$); and laboratory-reared females $\underline{Y} = 0.611 - 0.0067\underline{X}$ ($\underline{n}=14$, $\underline{r}=0.843$).

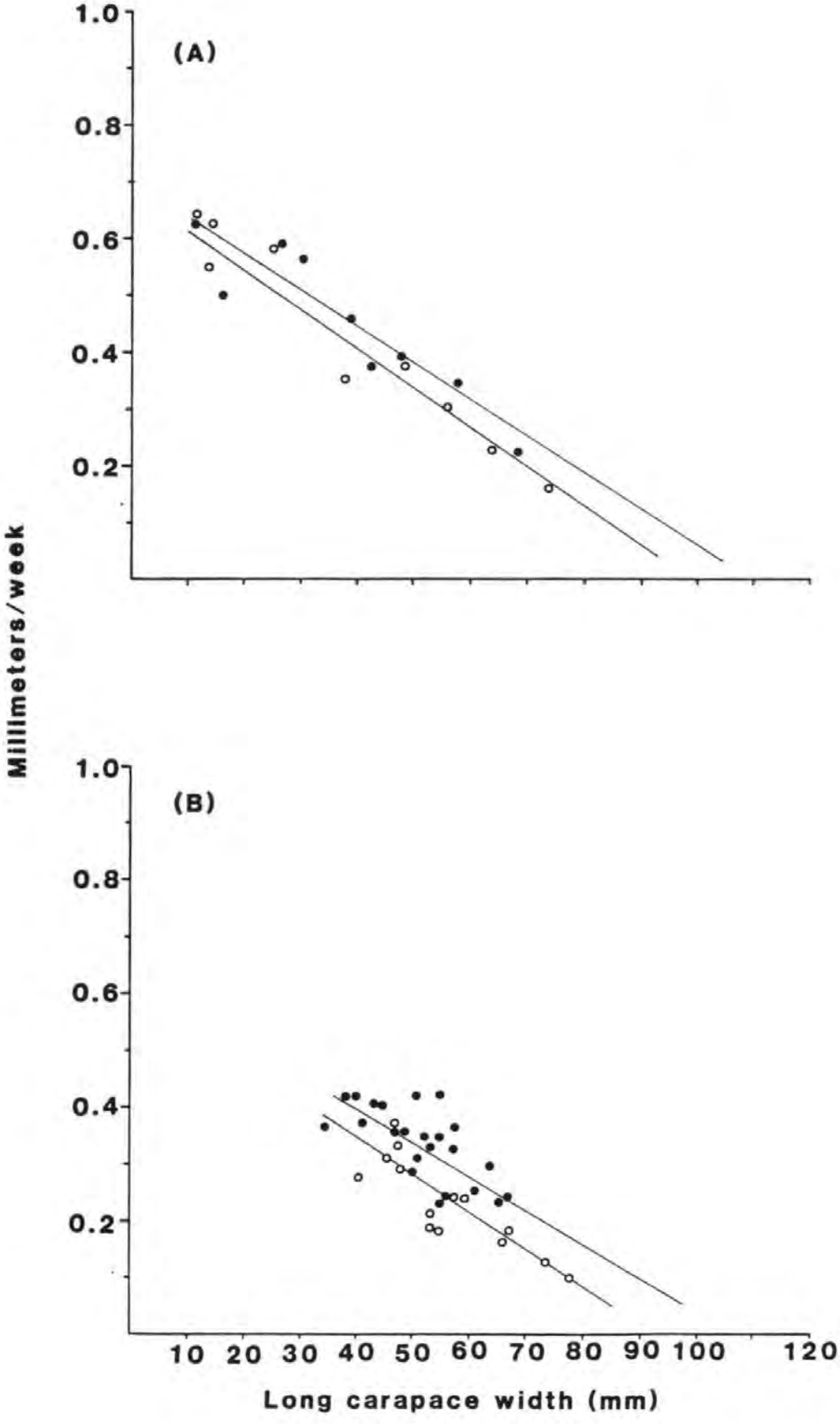

(A)
(B)
1.0
0.8
0.6
0.4
0.2
Millimeters/week
10 20 30 40 50 60 70 80 90 100 120
Long carapace width (mm)

Figure 5.16. Growth curves for (A) male and (B) female Liocarcinus puber. Data originate from (1) Gonzalez Gurriaran (1985b), (2) Choy (1986a), (3) ELEFAN (this study), (4) probability paper technique (this study) and (5)laboratory-reared data (this study).

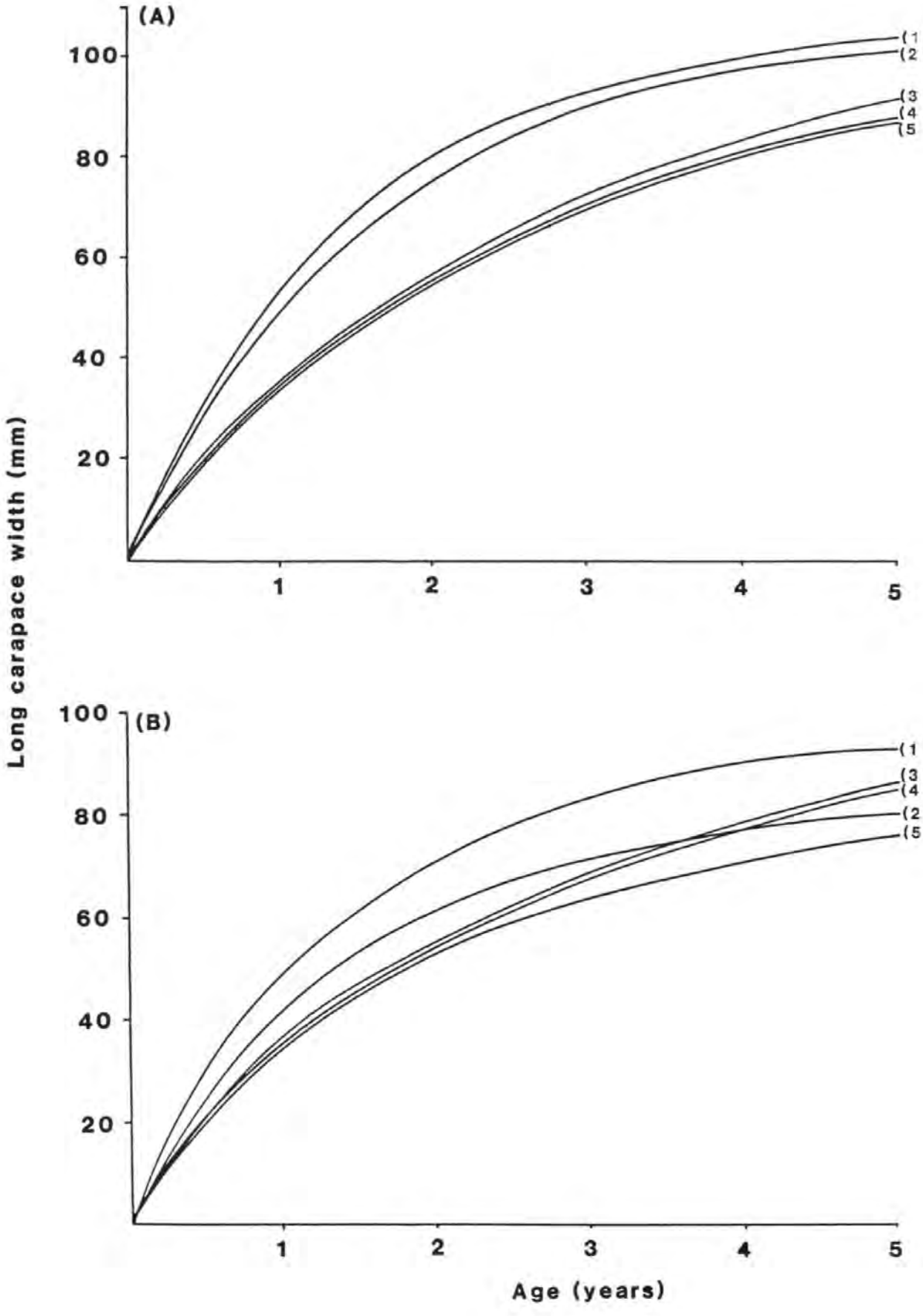

(A)
100
80
60
40
20
1
2
3
4
5
(1
(2
(3
(4
(5
Long carapace width (mm)
(B)
100
80
60
40
20
1
2
3
4
5
(1
(3
(4
(2
(5
Age (years)

Figure 5.17. Seasonal growth curve of Liocarcinus puber showing anecdysis over the winter period for (A) males and (B) females. Seasonalised growth curve from ELEFAN. The discontinuous growth curve (step-like curve) is based on the ELEFAN predicted curve using estimates of growth increments and intermoult periods from field evidence collected from this study.

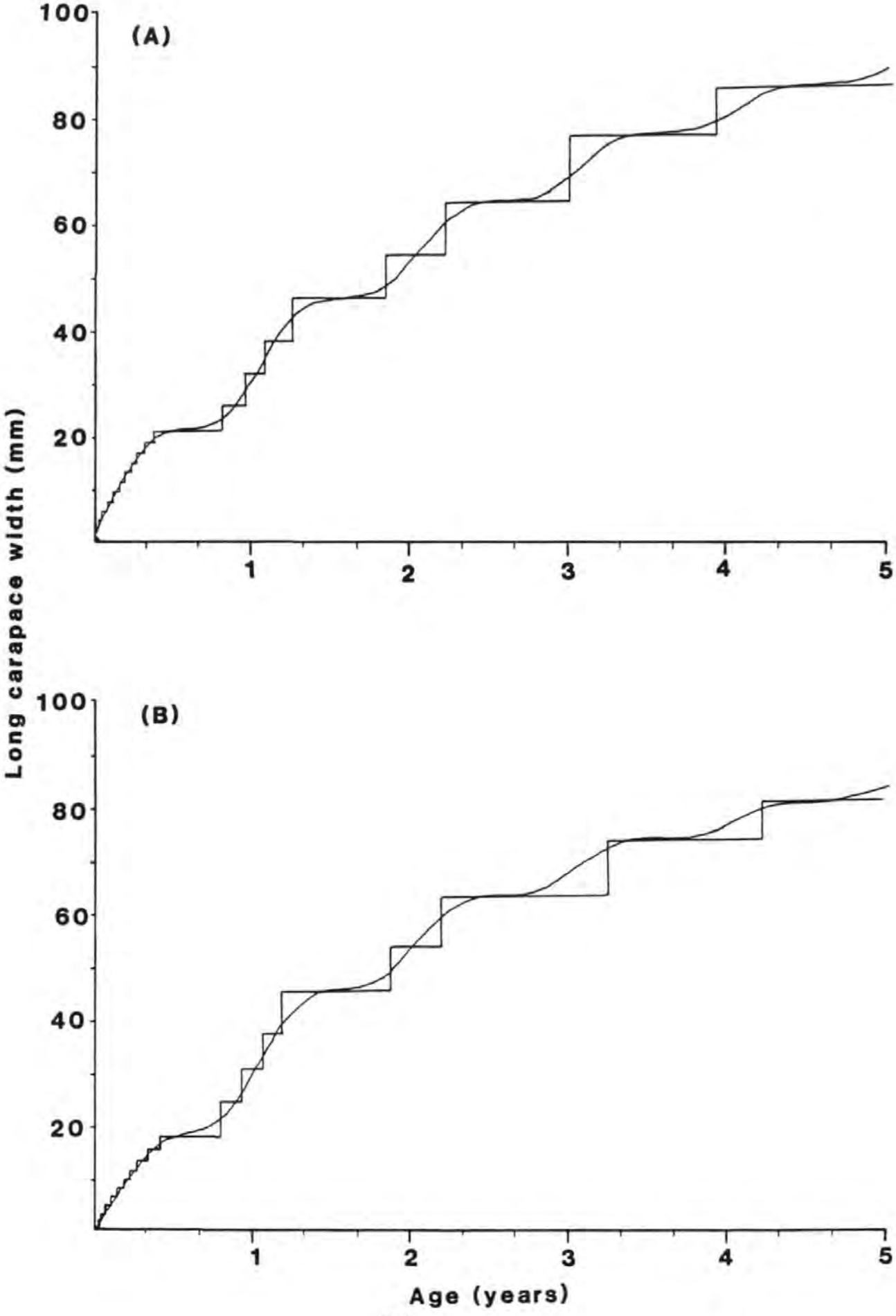

(A)
(B)
100
80
60
40
20
1
2
3
4
5
Long carapace width (mm)
Age (years)

Figure 5.18. Seasonal growth curves allowing comparison of male and female data for (A) continuous curve predicted by ELEFAN and (B) discontinuous curve (sizes based on ELEFAN curve, and increment and intermoult periods based on field evidence from this study).

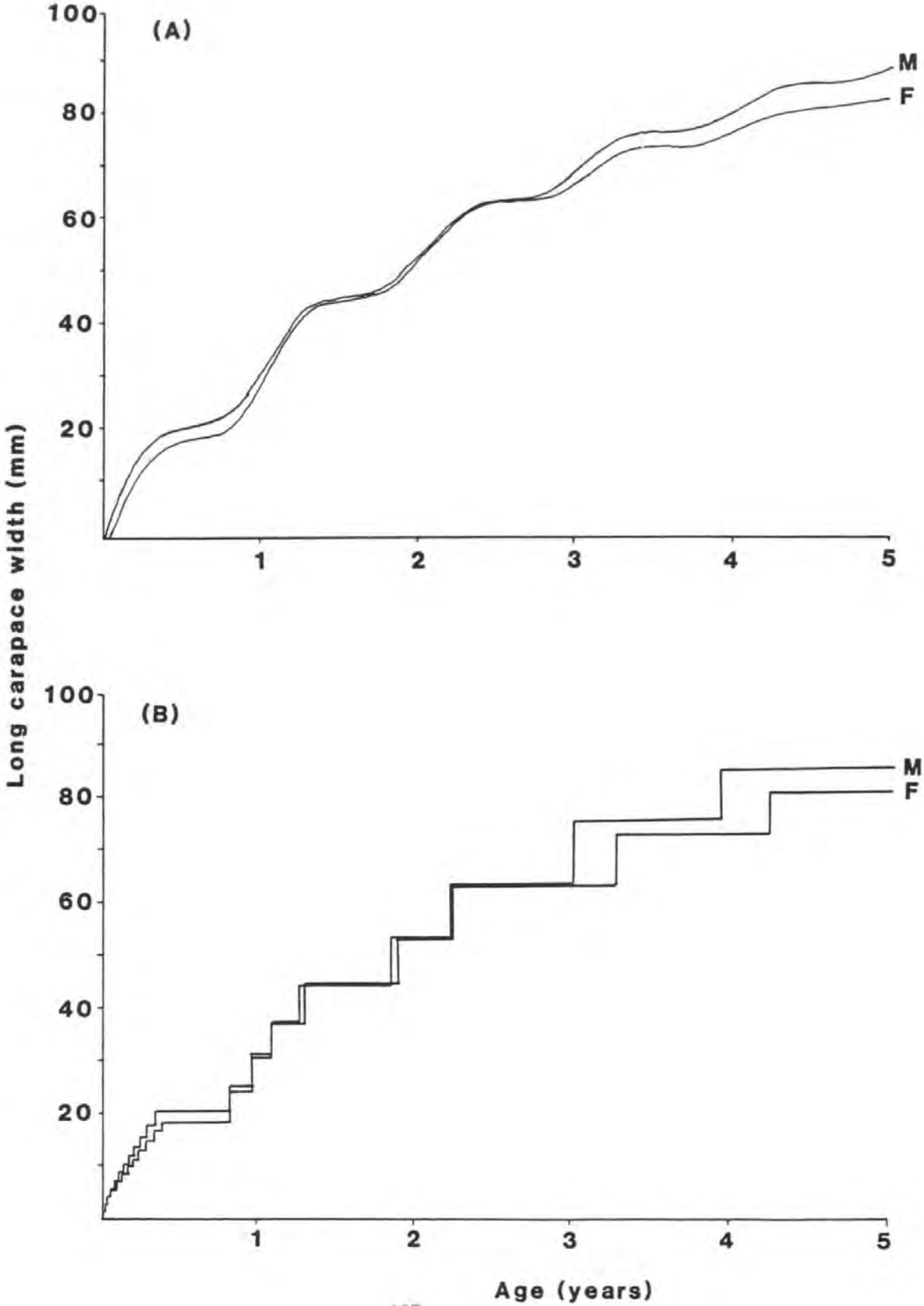

(A)
100
80
60
40
20
M
F
1
2
3
4
5
(B)
100
80
60
40
20
M
F
1
2
3
4
5
Long carapace width (mm)
Age (years)

Figure 5.19. Size-frequency data for male Liocarcinus puber, all zones combined, with growth curves predicted from ELEFAN.

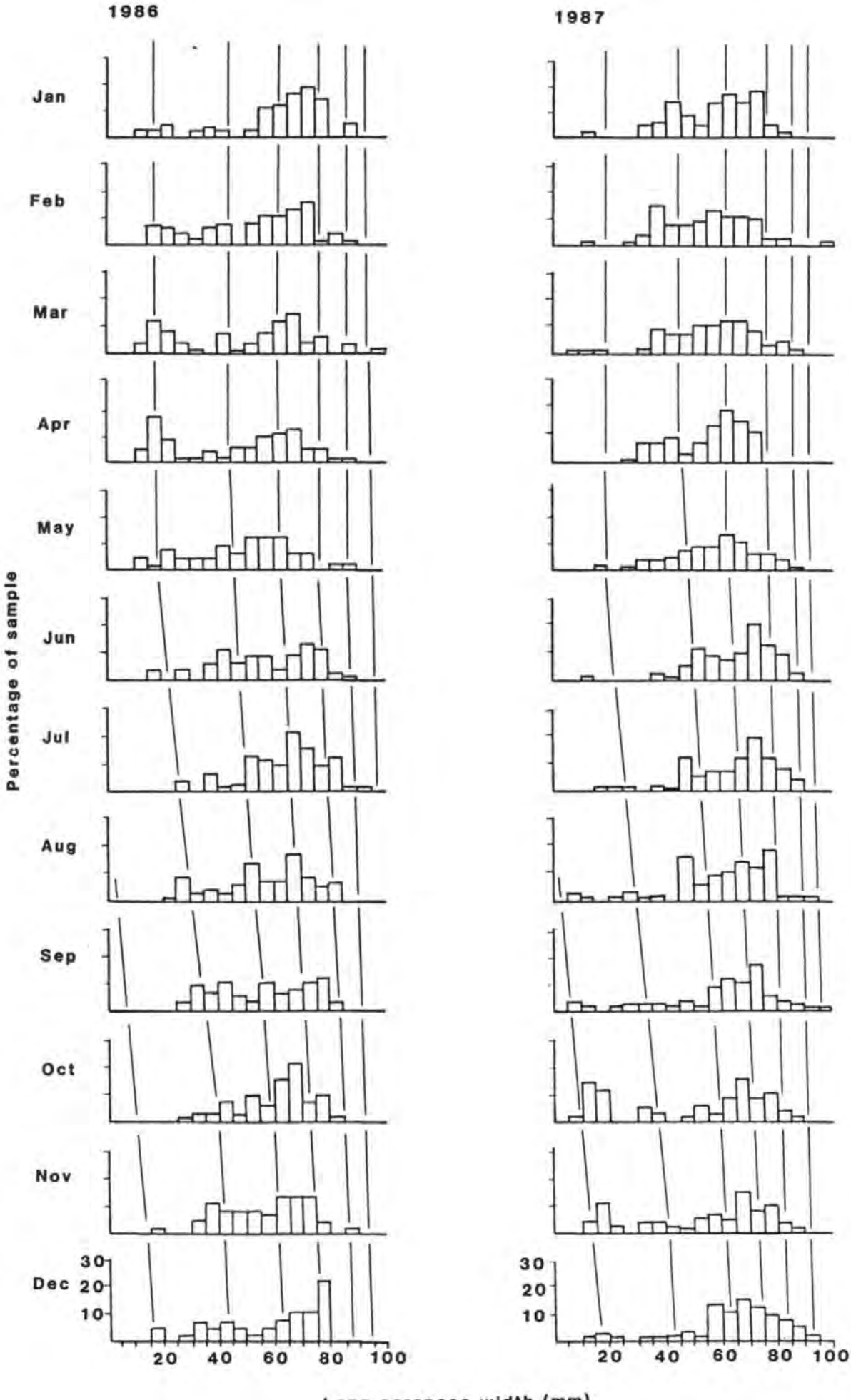
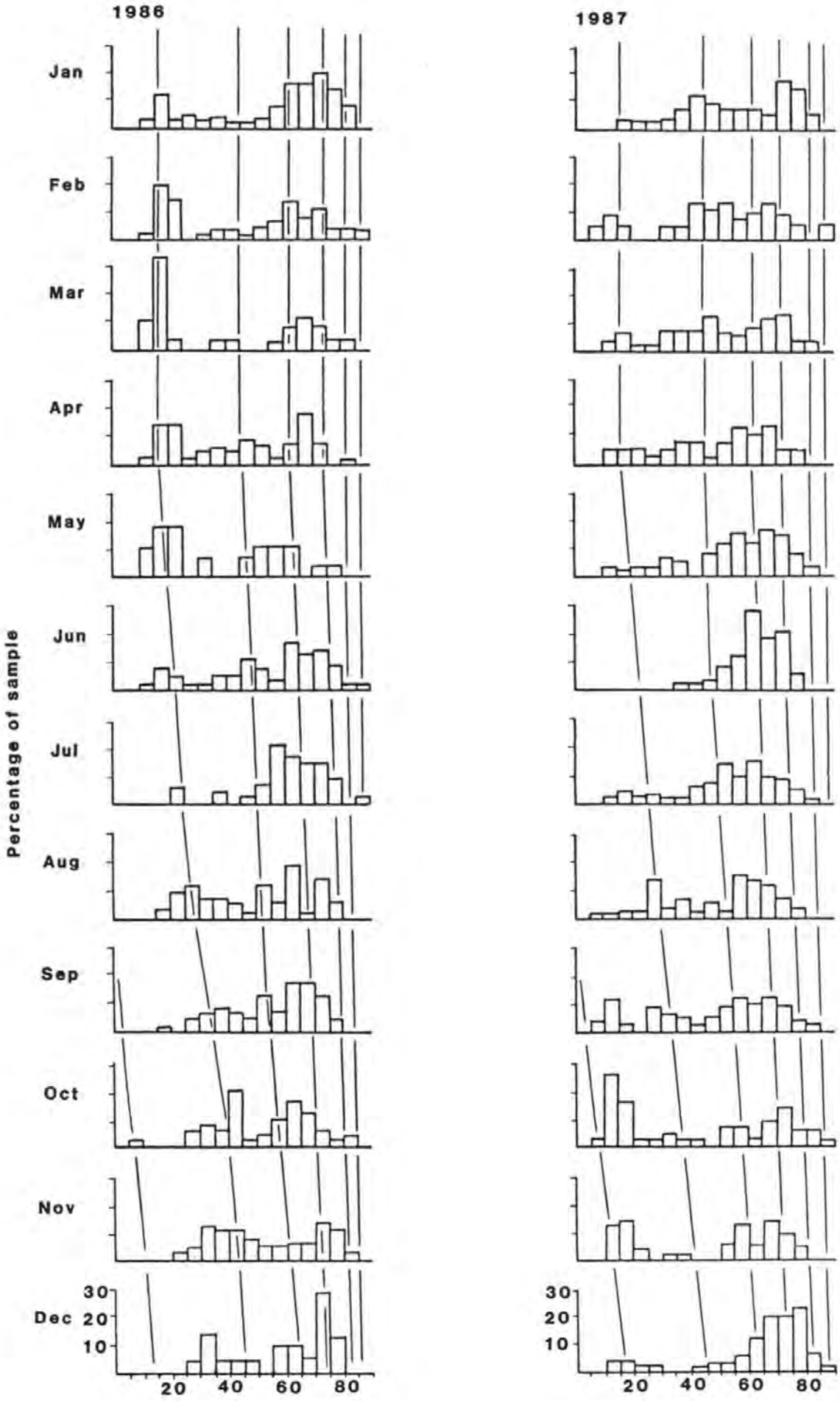

1986
1987
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Percentage of sample
30
20
10
20
40
60
80
100
Long carapace width (mm)

Figure 5.20. Size-frequency data for female Liocarcinus puber, all zones combined, with growth curves predicted from ELEFAN.


1986
1987
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Percentage of sample
30
20
10
20
40
60
80
Long carapace width (mm)

CHAPTER 6

LIMB LOSS

6.1 Introduction

Brachyurans have the ability to autotomise limbs between the basi-ischium and the coxa in response to potential predators or as a result of physical damage (Bliss 1960). This behaviour of limb autotomy may deter, or delay, aggression whilst the victim is able to escape (Warner 1977). Limb autotomy and the process of regeneration of limbs has been reviewed by Bliss (1960), McVean (1982) and Skinner (1985). Several early studies examined regeneration with particular reference to its effects on species which show heterochely (Wilson 1903, Przibram 1931, Huxley 1932). In species of hermit crabs and the lobster (Hommarus gammarus), once the position of the major (crusher) and the minor (cutter) chela was established, the same chelal type was regenerated after autotomy (Przibram 1931). This, however, was not the case for several species of heterochelous Brachyura, and Przibram (1931) noted that in Carcinus maenas, Liocarcinus corrugatus and Liocarcinus depurator reversal could occur. After autotomy of the major chela, the minor chela, in subsequent moults, developed into the major chela, and the regenerating chela into a minor chela (Przibram 1931). This phenomenon of reversal of site of the major chela has been noted also in other brachyurans such as Calappa spp. (Oxystomata) (Lewis 1969), Menippe mercenaria (Savage and Sullivan 1978), and the pistol shrimp Alpheus spp. (Przibram 1901, Wilson 1903).

Przibram (1931) noted that small specimens (<10mm CW) of the shore crab (C. maenas) showed complete reversal of the chela dentition at the subsequent moult, whilst larger crabs took from one to three moults to complete reversal of dentition. The dentition of both chela in the

intervening moults showed minor (or cutter) style dentiton. Savage and Sullivan (1978) similarly found that complete reversal of the chela in adult Menippe mercenaria took two to three moults. Kiortsis and Trampusch (1965) suggested that "the pincer chela is phylogenetically more primitive than the partner crusher, so that the latter is liable to regenerate through the pincer stage".

Limb loss in crustaceans has also been examined with regard to its effect on growth (Bennett 1973, Savage and Sullivan 1978). Singular, or multiple, limb loss has been shown to lower the size increase in subsequent moults by up to 25% for Mennippe mercenaria and Cancer pagurus (Savage and Sullivan 1978, and Bennett 1973 respectively). The intermoult period is also affected by limb loss, and precocious moulting has been reported for Carcinus maenas after multiple limb loss (Skinner and Graham 1972).

During field sampling of Liocarcinus puber it was noted that singular and multiple limb loss was a common occurrence within the population. The degree of limb loss and the level of reversal of chela was examined to assess the possible effect on growth and as a possible measure of predatation pressure on this species.

6.2 Materials and Methods

6.2.1. Field examination

All crabs examined in the field study (Chapter 3) were examined for missing limbs, limb bud formation and development, and the reversal of chelae. In Liocarcinus puber, the major chela (crusher) is predominantly on the right one. A small percentage of left-handed crabs were observed and some crabs had both claws showing the general morphology of the cutter (minor) chela. To examine the level of chelar reversal and to determine whether the percentage of left handed crabs increases through ontogeny, the chelar formation of all crabs examined was recorded.

6.2.2. Chelotomy experiments - effect on growth

To extend Przibram's (1931) observations of chelae reversal in various European portunids, and examine the effect of the removal chelae on moult increment, a sample of juvenile crabs (<20mm LCW) with normal right hand major chelar dentition and full limb complement was maintained in the laboratory. Crabs were kept either as a control (no autotomy) or the right (major) chela was autotomised by pressure being applied to the carpus of the limb using forceps. Eight specimens of both chelotomised and control animals were held individually in circular containers (the latter consisted of the base and lid of a Petri dish, separated by stout plastic to make a enclosure of a height of 70mm). The containers were maintained in seawater aquaria at a temperature of 13.5 $\pm$ 1.5°C. The juveniles were fed twice weekly on opened Mytilus edulis, pieces of Pollachius pollachius and

brown seaweed (normally Fucus spp. or strips of Laminaria spp.). Each crab was inspected prior to feeding for evidence of moult.

6.3. Results

6.3.1. Chela reversal

All the chelotomised crabs underwent reversal of dominance of the chelae in the ensuing moults, whereas the right hand major chela of the control crabs continued to develop as the major chela. For the former group, no immediate reversal of chela was noted and all specimens required at least two moults for full reversal. In the intermediate stages, both chelae resembled minor or cutter chelae with no enlarged proximal peg, however the left chela showed an enlarged double tooth at the proximal end of the dactylus.

6.3.2. Field analysis of left-handed crabs

The percentage of left-handed crabs, and those with both chelae in the intermediate stages increased with crab size (Fig. 6.1A). Both sexes within the 0-19mm LCW group showed a low level of 'left-handedness' (mean percentage for this size group was approx. 9%). Size groups larger than 20mm LCW showed higher levels of left-handedness (mean percentage level of approx. 20%), with no difference between sexes. (Fig.6.1A).

6.3.3. Field analysis of limb loss

The percentage of Liocarcinus puber showing limb loss also increased with crab size (Fig. 6.1B). This increase in the percentage of crabs missing limbs was linear for both males and females (Fig. 6.1B). The level of limb loss (ie. singular or multiple) showed little difference between sexes, although larger crabs (>40mm LCW) showed a slight increase

in the percentage with multiple limb loss (Fig. 6.2). In over 70% of cases for both sexes showing multiple limb loss, the limbs were sequentual (eg. removal of left hand leg 1, 2 and 3). The site of limb loss was also examined, using the combined male and female data, to examine any change of site of loss with increasing size (Fig.6.3). Juvenile stages show a higher percentage of chelae loss than the walking legs, and the rear paddle was infrequently lost. In larger individuals, the level of loss of each limb more closely approximates that which would be expected if limbs loss was not site orientated (Fig. 6.3). In all size groups, however, chelae losses were high.

6.3.4. Effect of limb loss on growth

As part of the chelae reversal experiment, the growth increment of control and chelotomised crabs was examined. Due to escape and subsequent cannibalism of some of the original control crabs, the control experiment was repeated one month later, therefore fewer increments were noted for these crabs due to insufficient time. The size increment of chelotomised individuals was traced over an ensu ing three to five moults (Fig. 6.4). All specimens showed a reduced initial moult increment and, generally, a lower increment in the ensu ing moult (Fig. 6.4). Two to three moults after autotomy, the growth factor showed levels similar to those of the control (Fig. 6.4). Comparison of the intermoult period was not possible due to the loss of the initial control animals.

handed (Hamilton et al. 1976). The relative importance of the two causes of autotomy in Liocarcinus puber as proposed by Bliss (1960) as an escape response to either predation or to damage by, for example, the movement of rock by wave action, is difficult to assess from field evidence alone. The effects of damage to crabs by adverse climatic conditions may be significant, particularly in the littoral zone. Predation, however, is likely to be a major cause of limb loss, and, as such, may allow some indirect measure of predator pressure. Examination of the percentage of adult L. puber which are left-handed between geographical localities may allow some indication of predator pressure exerted on the population. A similar study examining C. maenas from the Isle of Cumbrae, Scotland, however, found comparative levels of chelal reversal [eg. 21% left-handed for adult (>55mm CW)] (Abby-Kalio and Warner, in press). The marked similarity of the percentage left-handed between these studies suggests that there may be some behavioural feed-back mechanism between predator pressure and 'risk' that an individual may take.

Figure 6.1. Percentage of males (closed circles) and females (open circles) showing (A) left handedness and (B) limb loss. Data from May 1986 to April 1987, all sampling sites combined; number of crabs examined, male 868, female 659. Regression equations for (B) percentage showing limb loss are : male $\underline{Y} = 11.0 + 0.392\underline{X}$, ($\underline{r}$=0.981), and female $\underline{Y} = 12.2 + 0.301\underline{X}$ ($\underline{r}$=0.784).

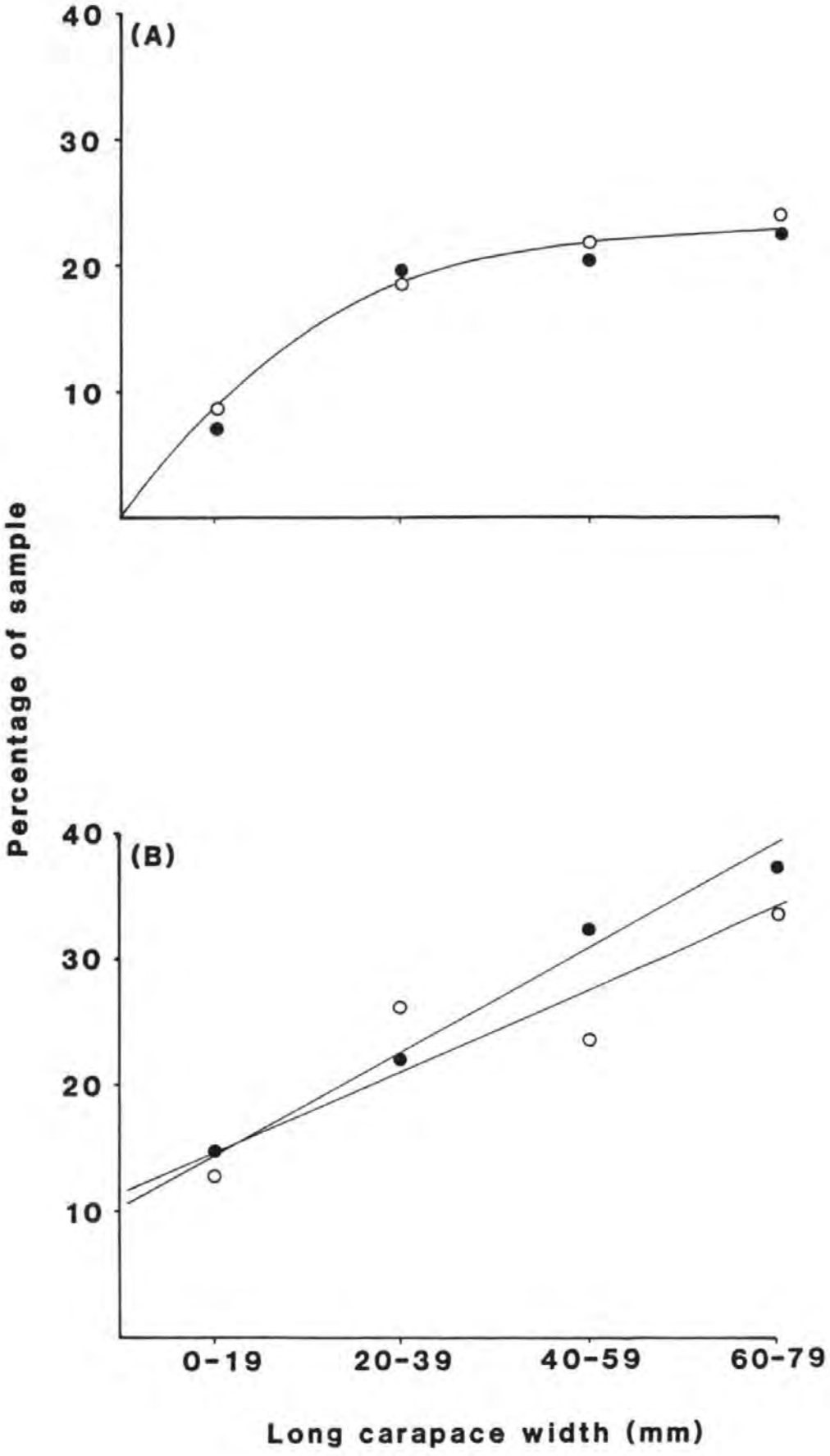
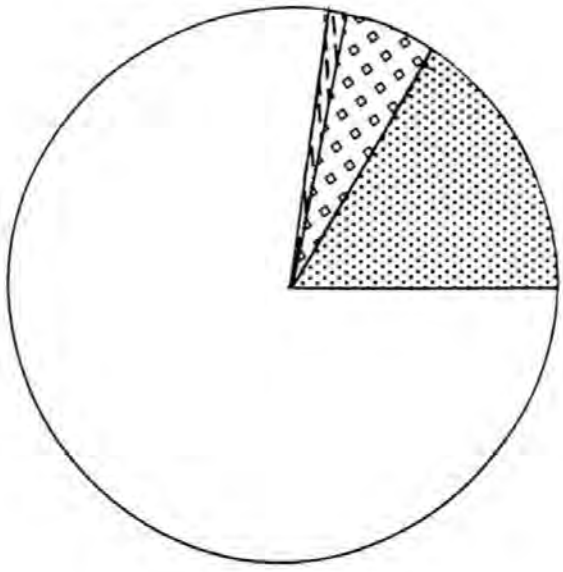
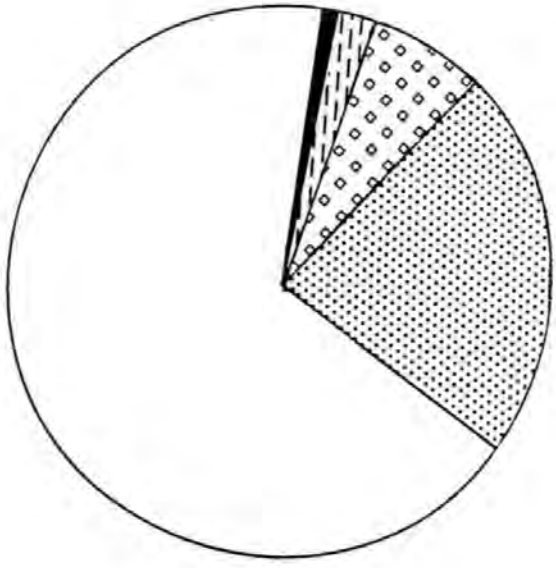

40
(A)
30
20
10
Percentage of sample
40
(B)
30
20
10
0-19
20-39
40-59
60-79
Long carapace width (mm)

Figure 6.2. Percentage of crabs showing one or multiple limb loss for (A) immature males (<45mm LCW) (n=221); (B) adult males (>45mm LCWO (n=647); (C) immature females (<40mm LCW) (n=227); (D) adult females (>40mm LCW) (n=432). Unshaded represents no limb loss; stippled - 1 limb missing; squares - 2 limbs missing; vertical dashes - 3 limbs missing; black - 4 or more limbs missing.

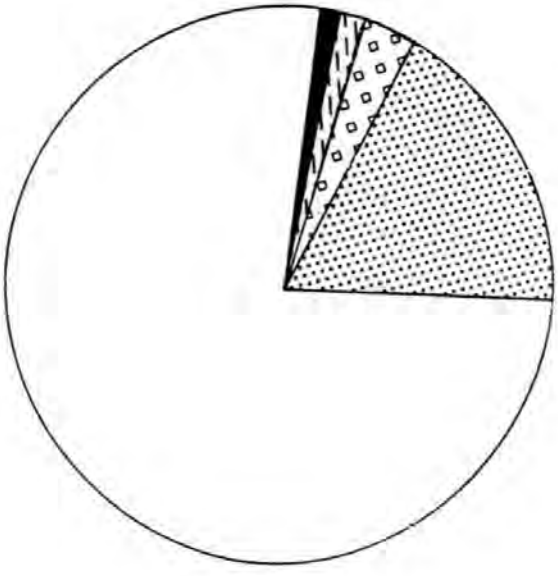
(A)



(B)



(C)



(D)

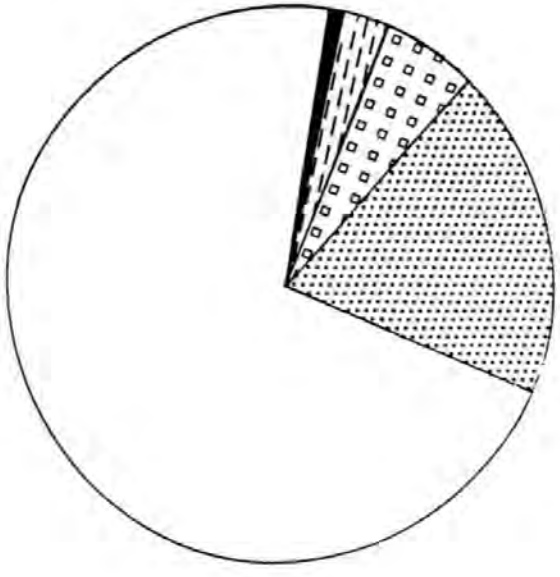


Figure 6.3. Diagram to represent the most frequently shed limb by Liocarcinus puber for four size classes (A) <20mm LCW; (B) 20-39mm LCW; (C) 40-59mm LCW; and (D) 60-79mm LCW (males and females combined, n indicates the total number of limbs lost of sample, using the same sample sizes as for Fig. 6.2). Numbers adjacent to limbs indicate the relative frequency of lose for each size class. Bar graph shows the same data for ease of comparison. The 10% level ie. the percentage expected if each limb was equally autotomised is marked on each bar graph. LC to L4 indicate left-hand chela to left-hand leg 4 (rear paddle); RC to R4 indicate right-hand chela to right-hand leg 4 (rear paddle).

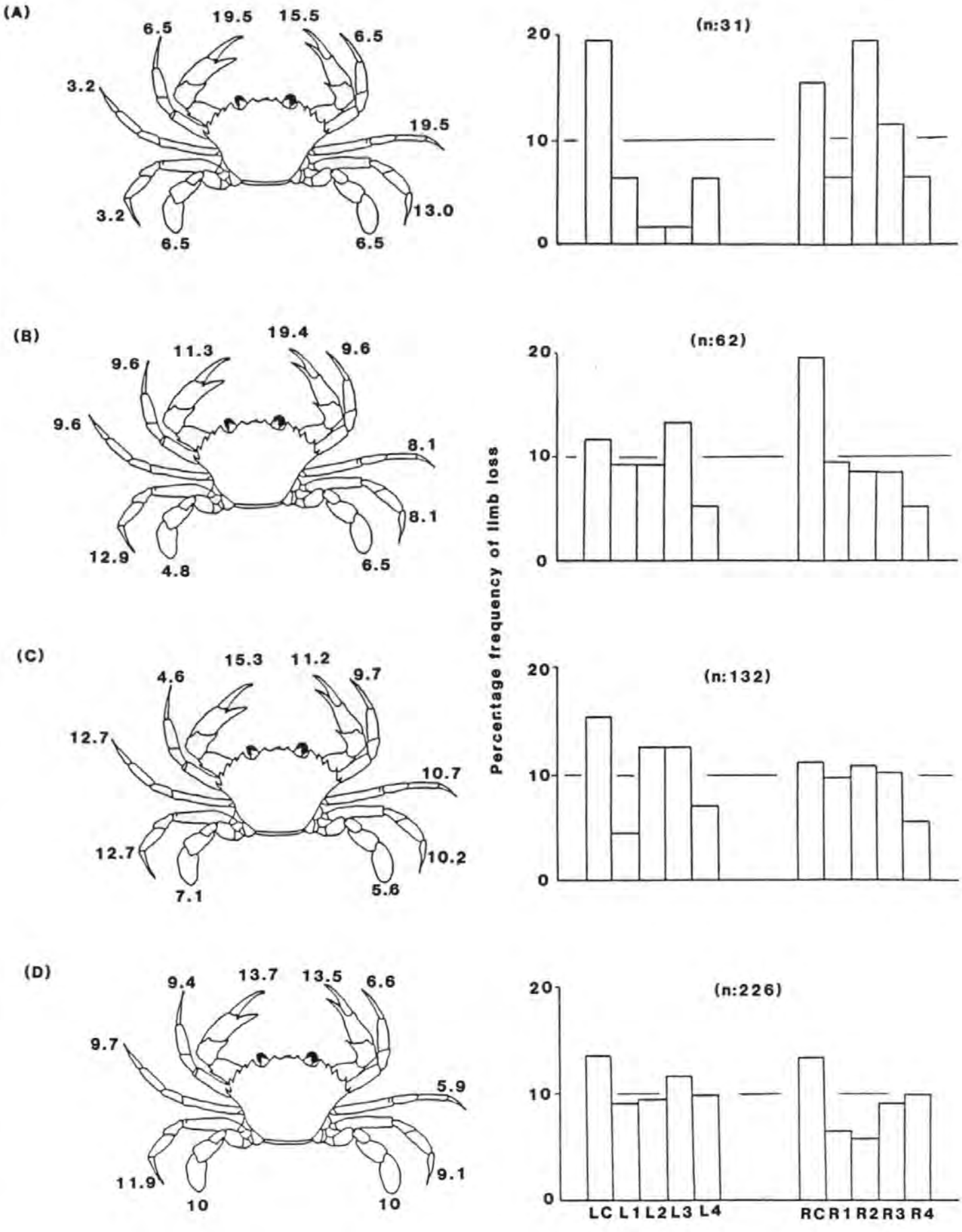
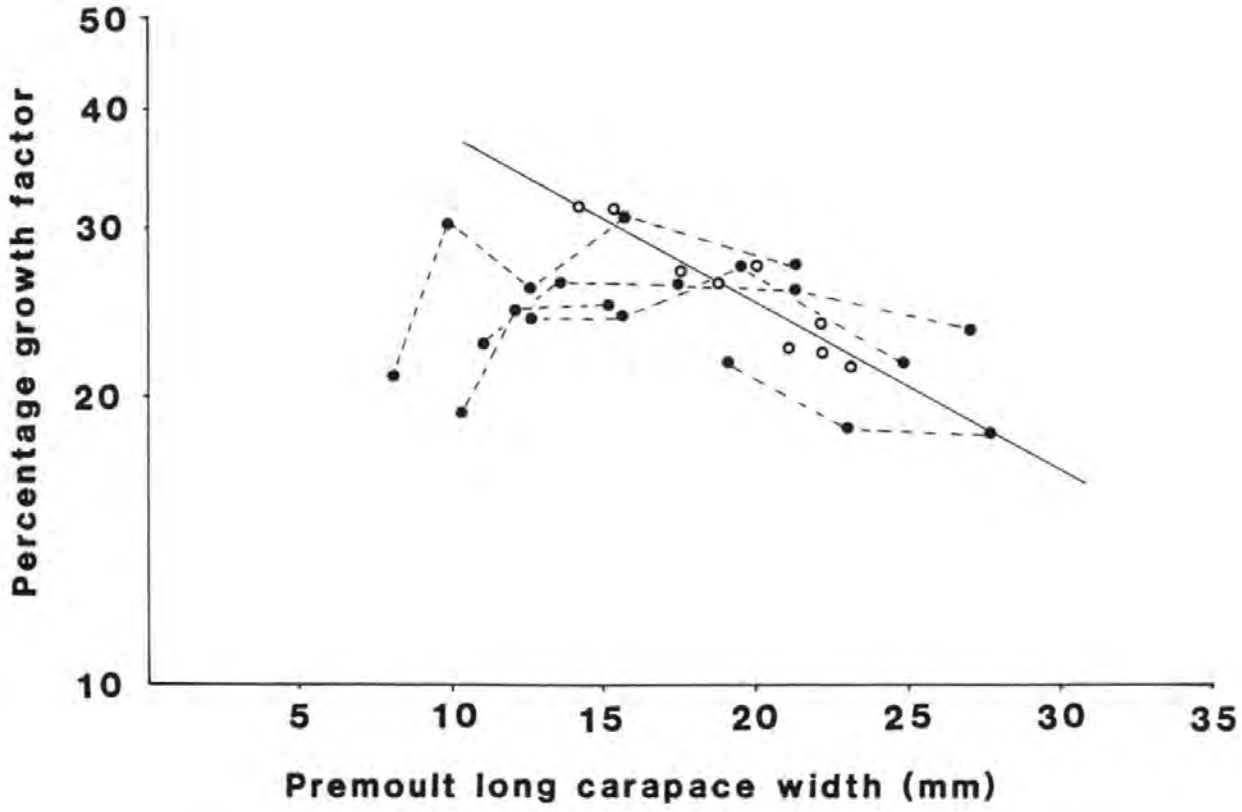

(A)
6.5
19.5
15.5
6.5
3.2
19.5
3.2
13.0
6.5
6.5
(n:31)
20
10
0
(B)
19.4
11.3
9.6
9.6
9.6
8.1
8.1
12.9
4.8
6.5
(n:62)
20
10
0
(C)
15.3
11.2
4.6
9.7
12.7
10.7
12.7
10.2
7.1
5.6
(n:132)
20
10
0
(D)
9.4
13.7
13.5
6.6
9.7
5.9
11.9
9.1
10
10
(n:226)
20
10
0
LC L1 L2 L3 L4
RC R1 R2 R3 R4
Percentage frequency of limb loss

Figure 6.4. Plot of growth factor for crabs reared with full compliment of limbs (open circles) and with the right hand chela autotomised (closed circles). Dashed lines join successive moults of the same individual for autotomised crabs. Regression line for crabs with full compliment of limbs is $\underline{Y} = e^{(4.05 - 0.0408\underline{X})}$, ($\underline{n}$=9, $\underline{r}$=0.952) and for autotomised crabs (not shown) $\underline{Y} = e^{(3.27 - 0.00595\underline{X})}$, ($\underline{n}$=24, $\underline{r}$=0.05).


50
40
30
20
10
Percentage growth factor
5
10
15
20
25
30
35
Premoult long carapace width (mm)

CHAPTER 7

FEEDING AND NATURAL DIET

7.1 Introduction

Brachyurans may be described generally as opportunistic omnivores, as few species have tended towards specialisation in feeding techniques or prey choice (Warner 1977). Studies of the diet of portunids, however, have shown that this group of crabs is an exception and has specialised, showing a preference for a carnivorous diet with cannibalism also being commonly reported (Laughlin 1982, Abello and Cartes 1987, Wear and Haddon 1987). This conclusion is based mainly on work carried out on species of commercial importance (Tagatz 1968, Hill 1976, Matsui et al. 1986), or those that predate on commercially exploited molluscs (Van Engel 1958, Wear and Haddon 1987). In addition, the dietary preference of portunids comes predominantly from studies based in the tropics and sub-tropics, where species have been shown to prey heavily on molluscs and crustaceans, with fish, detritus and polychaetes occasionally being found in stomach contents (Patel et al. 1979, Paul 1981, Williams 1982). Typically, the diet of these tropical and sub-tropical portunids is closely related to the relative abundance of prey species available, suggesting a markedly opportunistic feeding strategy (Du Preez 1984, Wear and Haddon 1987). Slight differences in diet do occur, however, between species of portunids inhabiting estuaries and mangrove swamps (Hill 1976, Paul 1981, Laughlin 1982), and those of open sandy shores (Williams 1982, Du Preez 1984, Wear and Haddon 1987). Although portunids examined from estuarine and mangrove areas predate heavily on crustaceans and molluscs, they show more omnivorous tendencies than those from sandy shores. For example, species

of the genus Callinectes have been reported in the literature to utilise plant material; C. sapidus feeds on the angiosperm Spartina alterniflora and algae (Ulva spp.) (Van Engel 1958, Tagatz 1968), and C. arcuatus, C. toxotes Ordway, C. danae Smith and C. ornatus consume various plant materials (Moncada and Gomez 1980, Paul 1981). Algae and plant material have also been found in the stomachs of estuarine specimens of Scylla serrata, but were assumed to result from accidental ingestion during consumption of other prey items (Hill 1976).

Temperate species of portunids appear to have a more diverse diet than those of the tropics and sub-tropics, and this increased prey diversity may be related to the more marked changes in the seasonal abundance of prey items in temperate compared with low latitudinal regions. Temperate species examined for dietry contents include Carcinus maenas (Ropes 1968, Elner 1977, 1981, Rangeley and Thomas 1987), Liocarcinus depurator (Abello and Cartes 1987), L. puber (Gonzalez Gurriaran 1978, Choy 1986a, 1986b) and L. holsatus (Choy 1986a, 1986b). The diet of L. holsatus and L. depurator is broadly similar to that reported for other portunids, and consists mainly of crustaceans, with bivalves and fish for L. holsatus, and fish, cephalopods, foraminifera and polychaetes for L. depurator also being important (Choy 1986b, Abello and Cartes 1987 respectively). Both these species of Liocarcinus are found in the circalittoral zone (10-100m depth) and on substrata ranging from sand to mud (Ingle 1980). The temperate portunids, C. maenas and L. puber, are found in the infralittoral and littoral zones of rocky shorelines, although C. maenas occupies a broad spectrum of intertidal and infralittoral habitats ranging from estuarine to moderately exposed open shores (Ingle 1980, Elner 1981). Elner (1981) showed that C. maenas was able to predate on a wide variety of

prey items with differences in diet between sampling sites reflecting the availability of food types in each habitat. The diet of C. maenas at one site (Menai Bridge, N.Wales) showed high utilisation of crustaceans and algae, in contrast to a bivalve-dominated diet reported for C. maenas in North America (Ropes 1968, Elner 1981). Choy (1986b) examined L. puber from the rocky intertidal zone in South Wales, and found extremely high quantities of algae in the stomachs of all sizes of crab, and suggested that L. puber fed selectively on, and was able to digest, brown algae. Other important prey items for adult L. puber included brachyurans and Mytilus edulis (Choy 1986b). Gonzalez Gurriaran (1978) examined a population of L. puber from the circalittoral zone under rafts for the cultivation of mussels, and found a markedly lower dependance on algae and a higher relative importance for both crustaceans and molluscs compared with rocky intertidal individuals (Choy 1986b).

To gain a clearer understanding of the trophic role played by this large and highly aggressive crab in the nearshore community, the stomach contents of Liocarcinus puber were examined to establish the diet, and to study the effects of habitat, season and moult stage on the diet, and hence the predatory behaviour, of L. puber.

7.2. Materials and Methods

7.2.1. Field sampling - analysis of stomach contents

A sample of approximately 15 Liocarcinus puber was examined each month (January 1986-December 1987) to estimate the percentage occurrence and percentage volume of the main prey items in the stomach. Crabs used for stomach analyses were taken predominantly from samples captured in the morning, as portunids have been noted as nocturnal predators (Kitching et al. 1959, Hill 1976). Field sites for sampling for stomach content analysis were: Wembury for the littoral sampling site, The Hoe for the infralittoral site and F Bouy for the circalittoral (Chapter 2). Crabs were preserved within one hour of capture in 10% saline formaldehyde. For large individuals, the carapace was lifted to ensure penetration of the preservative. All crabs were processed in the laboratory within one week of preservation. The volume of the cardiac stomach was measured using a displacement technique (Section 7.2.2), and then dissected and the contents identified to the lowest possible taxon. Each prey item was separated and its percentage volume estimated from the original stomach volume (Section 7.2.2). Differences in diet between sexes, immature and mature crabs and sampling sites were based on the occurrence of prey species observed in gut contents using G-tests (Sokal and Rohlf 1969).

7.2.2. Stomach volume

To estimate the volume of prey items in the stomach, an objective method to analysise gut volume was devised, which was a modification of the technique used by Hill (1976). The stomach was ligatured at the oesophagus

and the volume measured by the displacement of water using a side-arm test-tube (which was purpose-blown to give a sharp lip at the water level at the side-arm). The level of water was topped up prior to each measurement and the effects of surface tension were reduced by adding detergent. The weight of displaced water was measured using a top pan balance to give the volume of the stomach. To enable an objective assessment of the percentage stomach fullness, the volumes of both completely full and empty stomachs, over a range of crab sizes, were measured. Twenty-four crabs were starved for 72h and then fed to satiation on fish flesh. When no more food was accepted, each crab was killed, dissected, the oesophagus ligatured and the stomach volume measured by the displacement technique. A second group of crabs was starved for 72h and then dissected to enable an estimate of the volume displaced by empty stomachs. A range of crab sizes was used for both experiments. The relationship of stomach size to carapace width enabled the percentage fullness of crabs examined in the monthly samples to be estimated by comparing their stomach volumes to the stomach volume relationships shown in Figure 7.1.

7.2.3. Stomach clearance rate

To examine the rates at which soft and hard material were digested by Liocarcinus puber, serial slaughter experiments were carried out. Initially, crabs were starved for 72h and maintained individually in cleaned seawater tanks (30x20x20cm). Twelve crabs were fed a known weight of soft fish flesh and, after 30 mins, any left-over tissue was recovered, wet weighed and dry weighed (by drying for a period of 24h at 100°C). After 1, 2, 3 and 6h, the crabs were serially slaughtered. At each time interval, the stomach contents were examined, and weighed as above to

ascertain the percentage of fish flesh digested. The same process was tried using brown algae as the prey item.

Defaectation rates were also examined. Twelve Liocarcinus puber were starved for a period of 72h and maintained in cleaned seawater tanks as above. Crabs were fed to satiation on fish tissue and all remaining food removed after 0.5h. The period prior to the production of faeces, and the length of time over which faecal material was produced, was noted. Using the same experimental protocol, defaecation rates of crabs fed on brown algae (Fucus vesiculosis and Laminaria digitata) were examined.

7.3. Results

7.3.1. Stomach volume

The volume of full and empty stomachs of Liocarcinus puber was related to crab carapace width (Fig. 7.1). The fitted regression lines showed an exponential increase in stomach volume to carapace width for both full and empty stomachs. The difference between these two lines gives the maximum volume of material that can be contained by the stomach for a given size crab. The 'percentage fullness' of L. puber sampled from the wild was therefore estimated using the calculated regressions from Figure 7.1.

7.3.2. Variation in stomach contents between zones

The percentage volumes and percentage occurrence of each of the major prey items for crabs sampled in the littoral, infralittoral and circalittoral zones are shown in Table 7.1. and Figure 7.2. In all zones and for each sex, algae, particularly brown algae, were the most significant prey item by volume. The second most important prey item by volume varied between zones (Fig. 7.2). For the infralittoral zone, molluscs were the second most abundant prey, whilst crustaceans were the second most abundant prey in the circalittoral zone. There was a significant difference in prey species between the three zones for both males (littoral vs. infralittoral, $G=29.55$, $df=2$, $P<0.01$; littoral vs. circalittoral, $G=95.9$, $df=2$, $P<0.01$; infralittoral vs. circalittoral, $G=32.5$, $df=2$, $P<0.01$), and females (littoral vs. infralittoral, $G=42.7$,

df=2, P<0.01; littoral vs. circalittoral, G=134.3, df=2, P<0.01; infralittoral vs. circalittoral, G=35.6, df=2, P<0.01). There was a close similarity between the diets of male and female Liocarcinus puber for each zone, although females consistantly consumed more algae than males (Table 7.1). No significant difference was observed in the occurrence of prey items between males and females from the littoral (G = 1.03, df = 2, P = >0.01) and circalittoral zones (G = 8.1, df = 2, P = >0.01). A significant difference, however, occurred between the sexes in the infralittoral zone (G = 21.0, df = 2, P = <0.01), with females having a higher volume of algae than males (Fig. 7.2).

Liocarcinus puber from the littoral zone showed a particularly high level of algae consumption. For example, approx. 80% of females with material in their stomachs had algae present (Table 7.1 and Fig. 7.2). For both sexes in the littoral zone, molluscs and unidentified material/sand each comprised approx. 10% of the percentage volume (Table 7.1 and Fig. 7.2). For the littoral crabs, crustacean prey was observed only in males, although the low number of females sampled may partially explain this difference (Table 7.1). The diet of immature crabs from the littoral zone was also examined (Table 7.2). G-tests showed a significant difference between immature and adult diets (G = 12.64, df = 2, P = <0.01), with the occurrence of molluscs and crustaceans being higher for immature than adult crabs sampled from the same zone (Table 7.2). Examination of prey items consumed by volume, however, suggests that immature and adult crabs consumed similar volumes of molluscan material (Table 7.2). These observations indicate that immature crabs feed on small molluscs more frequently than mature individuals. Specific prey items observed from the stomach of immature crabs included barnacle cirri, whole specimens and

parts of the anomurans Pilumnus hir tellus (L.) and Porcellana platycheles (Pennant), and shell fragments of Littorina spp.. Specific prey items identified from adults from the littoral zone included whole specimens of P. hir tellus and Carcinus maenas.

Specimens of Liocarcinus puber from the infralittoral zone showed high levels of both algae and molluscs, with bivalves being a common prey item (Table 7.1 and Fig. 7.2). The majority of the bivalves appeared to be small specimens (<1cm) and those identified from shell fragments include Spisula spp., Anomia ephippium (Jeffreys) and Tellina crassa (Pennant). Other prey items identified from gut contents included masticated fragments of hermit crabs (Pagurus spp.), the squat lobster (Galathea squamifera Leach) and also fragments of juvenile swimming crab (L. puber).

At the circalittoral sampling site at F Buoy, the ground chains of the buoy (Section 2.1.1) acted as a detrital trap. This site had the greatest diversity of animal prey species with crustaceans being a particularly important group (Table 7.1 and Fig. 7.2). Specific prey items identified from gut contents included limbs and whole specimens of Liocarcinus puber and Carcinus maenas, and well-masticated pieces of Pagurus spp., Liocarcinus spp. and fragments of the test of Echinocardium cordatum (Pennant).

7.3.3. Seasonal variation of stomach contents

The percentage of crabs with empty stomachs varied seasonally (Fig. 7.3). In the littoral zone, female crabs showed a rather constant percentage with approx. 50% having empty stomachs throughout the year, with a slight decrease over the late autumn and early winter (Fig. 7.3). Males in this zone showed a more marked variation with all those collected

in January/February (n=8) having empty stomachs; a further peak in percentage of empty stomachs occurred in May/June (Fig. 7.3). Infralittoral zone females showed a peak with empty stomachs in July/August, although there was a rather constant level at approx. 40% for the rest of the year. Males from the infralittoral also showed a relatively constant annual level except for March/April, when food items were present in all specimens (n=12). The circalittoral zone showed evelated levels with empty stomachs for females in March/April and July/August; and for males from March to June (Fig. 7.3). In both the infralittoral and circalittoral zones, females had a high proportion of algae in their stomachs throughout the year, and particularly during winter (Fig. 7.4). For females, peak predatory periods occurred in May/June ie. post zoeal release and prior to female moulting (Chapters 4 & 5) (Fig. 7.4). Males showed high levels of algal consumption throughout the year, and although predation on molluscs and crustaceans also occurred over the entire year, the percentage volume occupied by these prey items increased in late autumn/early winter for the infralittoral and circalittoral respectively (Fig. 7.5).

7.3.4. Effect of moult stage on stomach contents

The diet was also analysed in relation to the moult stage to determine whether any prey item was prefered during pre- or post- moult (Fig. 7.6). Premoult and soft crabs showed predominantly empty stomachs, suggesting that feeding ceases immediately before and after moult for Liocarcinus puber (Fig. 7.6). The moult stages of early papershell, late papershell and intermoult crabs all showed similar profiles of percentage fullness. Each stage had approx. 40% with empty stomachs and approx. the

same percentage with gut fullness (5-20%) (Fig. 7.6). Intermoult crabs, however, showed a slightly higher percentage of stomachs with 50-100% fullnes than the papershell stages. The similarity of proportions of crabs in each gut fullness stage between the three moult stages suggests that early papershell Liocarcinus puber are fully capable of feeding by this moult stage (Fig. 7.6).

The percentage composition of the diet of the three moult stages (early papershell, late papershell and intermoult) indicated a similar dietry composition, with brown algae making up a large proportion of the diet (Fig. 7.7). Bivalve shell was observed in early papershell individuals, although it is not known whether this is detrital shell or whether crabs of this moult stage can successfully predate on bivalves (Fig. 7.7). Crabs in late papershell also relied heavily on brown algae as the main prey item and had a similar dietry composition to those in early papershell. Intermoult crabs showed a higher diversity of prey groups and lower reliance on brown algae, although brown algae still contributed very significantly to the diet (Fig. 7.7).

7.3.5. Stomach clearance rate

Figure 7.8 shows that Liocarcinus puber is capable of a relatively rapid rate of digestion of fish flesh, with approx. 50% of the food being digested by 4.5h after ingestion. After 1 and 2h, pieces of gill and the harder sections of the fish mouthparts were still clearly recognizable. After 3h, identification of the original meal was difficult and after 6h few large pieces of solid material were evident.

The same experiment was attempted using brown algae (Fucus vesiculosis and Laminaria digitata), but met with limited success as no

individual readily consumed the algae on demand. Indeed, Liocarcinus puber was somewhat reluctant to eat brown algae in the laboratory, and only consumed it after long periods of starvation (>5 days) and then infrequently at night. Therefore, to examine the rate of digestion of brown algae, freshly caught crabs from the littoral zone were used (as gut content analysis showed a high percentage occurrence of brown algae in these individuals). The lack of detailed information on quantity and time of feeding precluded a quantitative examination, but allowed an estimate of approx. time required prior to complete evacuation of algal material from the stomach. Sixteen freshly caught L. puber were kept in clean aquaria in the laboratory without food, and stomach contents were examined after 6, 12, 24 and 48h of captivity. Algae were not observed in any of the four stomachs examined after 6h, but large quantities of bivalve shell were observed in one stomach. After 12h, algae were observed in two of the four stomachs examined in a well digested, but still recognisable, state. After 24h, two crabs were observed with brown algal fragments in their stomachs and their stomachs contained quantities of brown fluid. After 48h, one stomach was observed with pieces of shell as well as some algal fragments, indicating that brown algae and shelly material may be retained for a considerable longer period in the stomach compared with softer prey items.

To further examine the rate of digestion, the defaecation rates were examined. Liocarcinus puber fed on fish tissue, first started defaecating 15h after feeding and faeces were produced up to 60h after feeding. A further twelve crabs were maintained as above and fed overnight on a known weight of algae. Five of the specimens consumed algae and commenced defaecating approx. 20h after feeding, and continued to produce faeces up to six days after feeding.

7.3.6. SCUBA observations of natural diet

Routine sampling of crabs using SCUBA was generally carried out during the day (Chapter 3) and during these dives Liocarcinus puber were occasionally observed feeding. Direct observations of feeding were mainly of L. puber preying on large prey items such as polychaetes, crustaceans and fish. Ragworms, Nereis spp., were observed to be taken, particularly in areas of silty sediment and rocky outcrops. Crustaceans being consumed included the squat lobster Galathea squamifera, and appendages of both Carcinus maenas and Liocarcinus puber. A large specimen of L. puber (65mm LCW), on the upper reaches of Bovisand Harbour wall within half a metre of the water surface, was observed using the chelae and walking legs to crush and feed on barnacles. On several occasions, L. puber were observed feeding on the remnants of fish fillets which were thrown from trawlers passing over F Buoy. L. puber was observed feeding on this fish and protecting it by outmanouvering and running away from the slower Cancer pagurus (Bell). On another occasion, a large L. puber was observed feeding on fish flesh whilst sitting on the top of a crab pot! It had presumably escaped with its prize of fish whilst two small C. pagurus were observed still in the pot. Suprisingly, few observations of L. puber eating algae were noted. One large female was observed consuming Ulva lactata during a night dive in the littoral zone at Blackstone Rocks, Wembury. Another observation was of a large male consumming a piece of detrital Fucus vesiculosis whilst 'in copula'.

7.4. Discussion

In this study, brown alga was the food item most important by volume and most frequently observed in the stomachs of Liocarcinus puber for all three depth zones examined. This finding was somewhat suprising due, not only to the reported preference of portunids for a carnivorous diet, but also to the fact that brown algae do not grow in the circalittoral zone (although detrital algae were commonly observed at F Buoy). The next most important prey items varied between zones, with both molluscs and crustaceans being major dietry constituents, and echinoderms, polychaetes and fish also preyed on. These findings support Choy (1986a, 1986b) who examined L. puber in the littoral zone at two sites on the Gower Peninsula (S.Wales) and found key prey items, in descending order of importance, to be: algae (Laminaria spp. and Fucus spp.), crustaceans (Porcellana platycheles) and molluscs (Mytilus edulis). Gonzalez Gurriaran (1978) sampled the circalittoral zone below a large area of mussel raft culture and identified the following prey items, in descending order of importance : crustaceans (Pisidia longicornis), molluscs (Mytilus edulis), algae (Saccorhiza spp. and Laminaria spp.) and echinoderms [Paracentrotus lividus (Lamarck) and Psammechinus spp.]. These differences in relative importance of prey species between L. puber from different locations are likely to be due to differences in habitat and depth from which the crabs were sampled (Choy 1986a, 1986b, Gonzalez Gurriaran 1978). In this study, marked differences in the relative importance of prey groups were noted between the three depth zones occupied by L. puber, with the relative importance of prey items being dependant on their availability. An example of this being the decrease in the relative importance of algae in the diet with increasing depth.

The findings of manipulative field studies implicate Liocarcinus puber as an important predatory species on the nearshore benthic community (Kitching et al. 1959, Ebling et al. 1963, Muntz et al. 1965). For example, studies at Loch Inne strongly suggest an inverse relationship between density of crabs of the species L. puber, Cancer pagurus and Carcinus maenas and the distribution of the edible mussel Mytilus edulis and the dogwhelk Nucella lapillus (Kitching et al. 1959, Ebling et al. 1963). In addition, Ebling et al. (1963) showed L. puber capable of preying on all sizes of M. edulis and, in areas dominated by M. edulis, L. puber were unable to exploit this potential food source due to either lack of crevice cover, wave exposure or inaccessability. The ability of L. puber to predate on molluscs has been examined in laboratory trials, and the results show L. puber capable of preying on large bivalves and gastropods by various handling, and shell cracking methods (Warner 1984, ap Rheinhalt and Hughes 1985, ap Rheinalt 1986, Choy 1986a, 1986b, Lake et al. 1987). Study of chelar morphology, however, suggests that L. puber is best suited to preying on relatively soft-bodied, benthic animals such as crustaceans (ap Rheinallt and Hughes 1985, ap Rheinallt 1986). This intepretation was supported indirectly by Choy (1986a, 1986b) who found that crustacean prey was preferred to molluscan prey, which in turn was preferred to algae in prey choice experiments.

The diet of crabs from the littoral zone in this study, was rather restricted, with algal material making up approx. 75% of the diet by volume. This finding, however, may be a result of the timing of sampling, rather than reflecting a biological difference. At Wembury, low water spring tides [the period of littoral sampling (Chapter 3)] occur predominantly at mid-day or early afternoon. The timing of high water (6am

and 6pm), and the possible effects of wave action, may make this a poor period of feeding for L. puber due to the limit period for night-time foraging at depths of water unaffected by wave action. L. puber has also been shown to be poorly adapted to aerial exposure (Johnson and Uglow 1985, Taylor 1988) and the physiological stress caused by emersion may reduce the crab's ability to successfully forage for active prey. The diet of immature crabs taken from the same location showed a lower dependance on algal material and a higher proportion of animal tissue compared with adult L. puber. Similar findings of higher predation levels on animal tissue for immature than adult L. puber were also noted by Choy (1986a, 1986b) for littoral samples. Immature L. puber are found higher on the shore than are adults (Choy 1986b) and may, due to their smaller size, be able to utilise surface water and crevices to better advantage than adults. Therefore, immature L. puber may be more suited to utilise the upper fringe area of their distribution than adults. As a consequence, analysis of the dietary composition for adult L. puber solely from shore collections may give an inaccurate assessment of the diet as a whole. The two examinations of diet of littoral L. puber currently available have shown particularly high levels of algae (Choy 1986b, present study).

In general, there was little sexual difference between the major dietary constituents in each of the three zones, although female crabs from the infralittoral zone showed a significantly higher occurrence of algae than males. This difference may, in part, be explained by the inclusion of ovigerous females, which were frequently observed in the infralittoral zone (Chapter 10). Ovigerous females appear to utilise less mobile prey, such as brown algae, whilst males are more mobile and are therefore capable of a more diverse diet. Seasonal variations in the percentage fullness of

the stomachs of Liocarcinus puber were noted in this study and may, in part, be related to the moult cycle (Chapter 5). The effects of the moult cycle on the diet have also been examined previously (Ropes 1968, Gonzalez Gurriaran 1978 and Abello and Cartes 1987). High percentages of empty stomachs have been reported for premoult and soft-shelled crabs, while early and late papershell stages showed a percentage with empty stomachs similar to that of hard shelled crabs (Ropes 1968, Abello and Cartes 1987). This study found similar trends, and stomach contents of L. puber in the early papershell stage indicated active feeding. Gonzalez Gurriaran (1978) reported the highest percentage of occurrence of algae in the foregut of recently moulted L. puber.

Findings from the current study indicate algae to be utilised throughout the year. Seasonal variations in composition of diet were noted, with animal tissue being more frequently observed over the autumn and early winter than at other times. Choy (1986b) noted that the relative importance of brown algae to Liocarcinus puber increased over the spring and summer, and suggested that this period was trophically optimimal for algal feeding. Choy (1986b) also reported an increase in the proportion of animal prey over the autumn and early winter.

Several common benthic animals observed at the sampling sites were not recorded as prey items in this investigation, particularly highly mobile crustaceans such as prawns and mysids. Hill (1976) and Paul (1981) noted that prawns did not appear in the stomachs of 'wild' portunids, although immobile prawns were readily consummed in the laboratory. Fish rarely appeared in the gut contents of Liocarcinus puber, apart from those sampled at F Buoy where detrital fish was occasionally observed. Therefore, these fast moving groups appear not to

be markedly exploited by L. puber. Other groups frequently observed in the field at the sampling sites, yet not observed in the stomach contents nor being consumed in the field, included anthozoans, brittlestars, starfish, crinoids, ascidians, nudibranchs and sponges.

The rate of gut clearance by swimming crabs has been investigated for Scylla serrata (Hill 1976), Ovalipes catharus (Haddon and Wear 1987) and Liocarcinus puber (Choy 1986a, 1986b, present study). The rate of clearance of O. catharus was markedly affected by external temperature and an increase in temperature from 9.5 to 19.5°C reduced the time for complete clearance of the stomach from 14.5h to 5.33h (Haddon and Wear 1987). The length of time for 50% of the food to be digested at 20°C was 1h for S. serrata and O. catharus, although complete digestion of stomach contents took longer for S. serrata than O. cathurus (Haddon and Wear 1987). The rate of digestion of soft material for L. puber in this study was in accordance with that found by Choy (1986b). The digestion of 50% of the food at 13.5°C took between 4.5 and 5h (present study and Choy 1986b respectively). Choy (1986b) estimated that complete evacuation of soft animal tissue occurred after 20h at 13.5°C. Direct comparisons of the three species are difficult due to different experimental temperatures, although it would appear that L. puber takes longer to digest food than O. catharus (Haddon and Wear 1987, present study). As lower temperatures are experienced at higher compared with low latitudes, longer periods are required to digest material for temperate compared with tropical species. For portunids in temperate regions, such as L. puber, digestion of material may take a considerable period prior to more food being able to be ingested. Therefore, the volume of the stomach, that is the amount of food that can be ingested at any one time, is of obvious importance.

Stomach size of portunids is related to carapace width (Fig. 7.9). For the same sized individual, O. catharus has a markedly larger foregut than either S. serrata or L. puber. If L. puber, as may be anticipated from the slow digestion rate, digests much of it's food during the day, the size of stomach would be expected to be larger than the stomach size of the sub-tropical species O. catharus, which has a rapid enough rate of digestion to enable the crab to feed repetitively through the night. The smallness of stomach size of L. puber compared to that of O. catharus suggests that the intake per night of L. puber is lower than that of O. cathurus, and this may be related to differences in the metabolic rate and subsequently the rate of growth.

Evidence from this study suggests that the rate of digestion of brown algae is slower than for soft animal tissue. Therefore, the more recalsitrant items, such as mollusc shell and algae, will be found in the stomach for longer periods, and examination of diet by stomach content analysis may over emphasise the role of these materials. The high quantities of brown algae reported here, however, suggest that they do form a major food source for Liocarcinus puber, particularly for post-moult, ovigerous female and littoral crabs. In this study, L. puber showed a preference for detrital brown algae which, presumably, has a high bacterial content. Choy (1986b) also noted a preference for fronds with epiphytic growth for L. puber in laboratory feeding trials. The relative importance of the nutrient gain for L. puber from the bacteria, the epiphytes and from the algae itself is difficult to ascertain directly from the present data. To gain a clearer understanding of the importance of brown algae in the diet of L. puber, its ability to digest laminarin (the main carbohydrate

storage material in brown algae) has been investigated and results are given in Chapter 8.

Table 7.1. Summary of the diet of Liocarcinus puber examined for the littoral, infralittoral and sub-littoral. A is the percentage volume occupied by each prey item, and B is the percentage occurrence.

	Littoral				Infralittoral				Circalittoral			
Sex	Male		Female		Male		Female		Male		Female	
No. of specimens:	63		30		110		67		64		62	
	A	B	A	B	A	B	A	B	A	B	A	B
Algae												
Brown	65.8	63.5	67.7	70.0	43.4	36.4	54.5	44.7	25.6	22.0	30.9	24.2
Red	3.7	7.9	7.8	10.0	1.0	3.6	2.6	3.0	-	-	5.4	6.4
Green	1.0	4.8	4.1	3.3	-	-	2.1	3.0	-	-	-	-
Calcareous	2.5	1.6	-	-	-	-	-	-	-	-	-	-
Total Vol. Algae	73.0		79.6		44.4		59.2		25.6		36.3	
Mollusca												
Bivalves	6.1	6.7	5.1	6.4	27.0	21.8	21.2	20.8	14.1	25.0	10.2	24.2
Gastropods	3.3	4.8	4.4	3.3	6.5	5.5	1.6	3.0	-	-	-	-
Total Vol. Mollusca	9.4		9.5		33.5		22.8		14.1		10.2	
Crustacea	5.0	4.8	-	-	12.2	5.5	10.6	9.0	24.9	7.8	28.0	6.4
Echinodermata	1.0	1.6	2.1	3.3	1.0	1.8	0.5	3.0	4.7	14.1	4.1	12.9
Pisces	-	-	0.5	3.3	-	-	-	-	9.8	10.9	2.8	6.5
Polychaeta	2.1	3.2	-	-	1.7	1.8	-	-	1.5	3.1	-	-
Unidentified material mud, sand and gravel	9.5	36.5	8.3	40.0	7.2	53.6	6.9	32.8	19.4	67.2	18.6	51.6

Table 7.2 The diet of immature (male <45mm, female <40mm LCW) and mature (male >45mm, female >40mm LCW) Liocarcinus puber sampled from the littoral zone. Data are combined for the two sexes. A is percentage volume occupied by each prey item and B is percentage occurrence.

Number of specimens:	Immature 96		Mature 93	
	A	B	A	B
Algae				
Brown	62.7	45.8	66.4	65.6
Red	2.4	4.2	5.0	8.6
Green	0.4	1.0	2.0	4.3
Calcareous	-	-	1.7	1.1
Total Vol. Algae	65.5		75.1	
Mollusca				
Bivalves	5.2	16.6	5.8	6.5
Gastropods	4.8	6.3	3.7	4.3
Total Vol. Mollusca	10.0		9.5	
Crustacea	10.0	8.3	3.4	3.2
Polychaeta	4.8	2.1	1.4	2.2
Echinodermata	-	-	1.3	2.2
Pisces	-	-	0.2	1.1
Unidentified material, mud, sand and gravel	9.7	16.6	9.1	37.6

Figure 7.1. The volumes of empty (open circles) and full (closed circles) stomachs of Liocarcinus puber in relation to crab long carapace width. Crab sexes are combined and fitted regression equations are : full stomachs, $\ln y = -10.8 + 2.71 \ln x$ ($r = 0.91$, $n = 24$); empty stomachs, $\ln y = -12.5 + 2.83 \ln x$ ($r = 0.95$, $n = 15$).

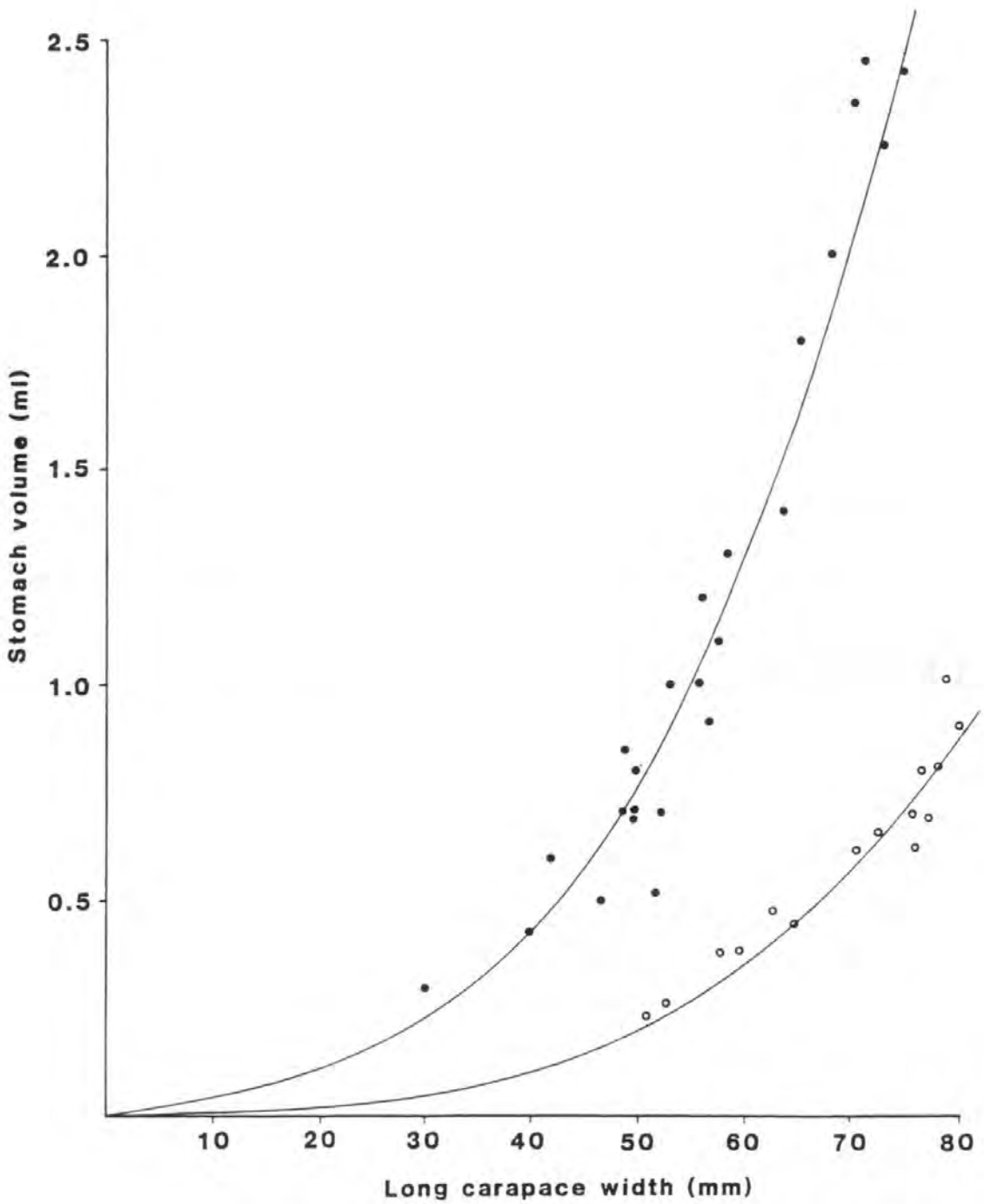

2.5
2.0
1.5
1.0
0.5
Stomach volume (ml)
10
20
30
40
50
60
70
80
Long carapace width (mm)

Figure 7.2. Variation in the percentage of the total volume of each prey item between the littoral, infralittoral and circalittoral zones for (A) male, and (B) female Liocarcinus puber: algae (closed circles), molluscs (open triangles), crustaceans (closed triangles), unidentified material, sand and mud (open circles with dashed line), fish (open squares), echinoderms (open circles with solid line), and polychaetes (closed squares).

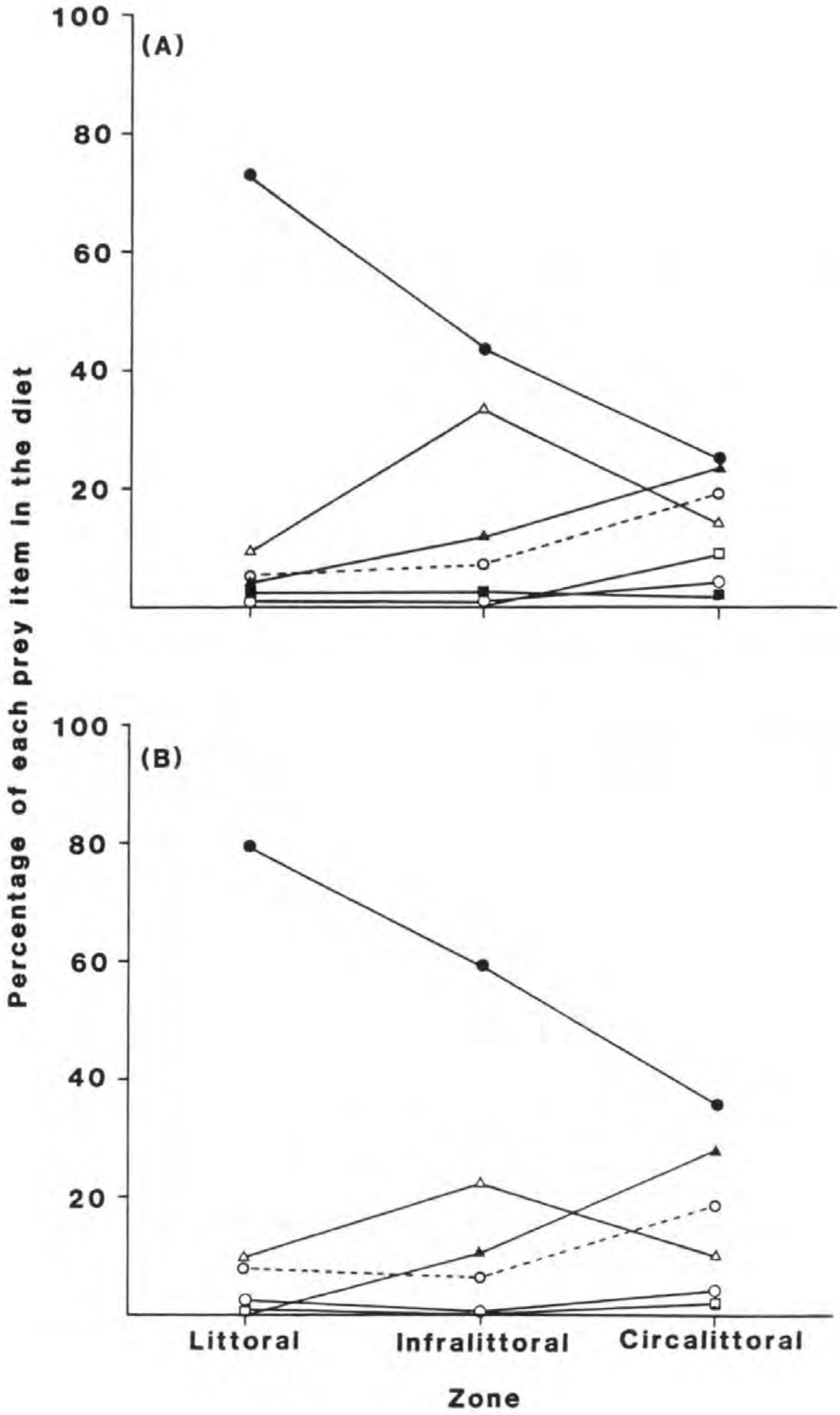

100
(A)
80
60
40
20
Percentage of each prey item in the diet
100
(B)
80
60
40
20
Littoral
Infralittoral
Circalittoral
Zone

Figure 7.3. Bimonthly variation in the percentage of adult Liocarcinus puber with empty stomachs for (A) the littoral zone, (B) the infralittoral zone, and (C) the circalittoral zone (males, closed circles and dashed line; females open circles and solid line).

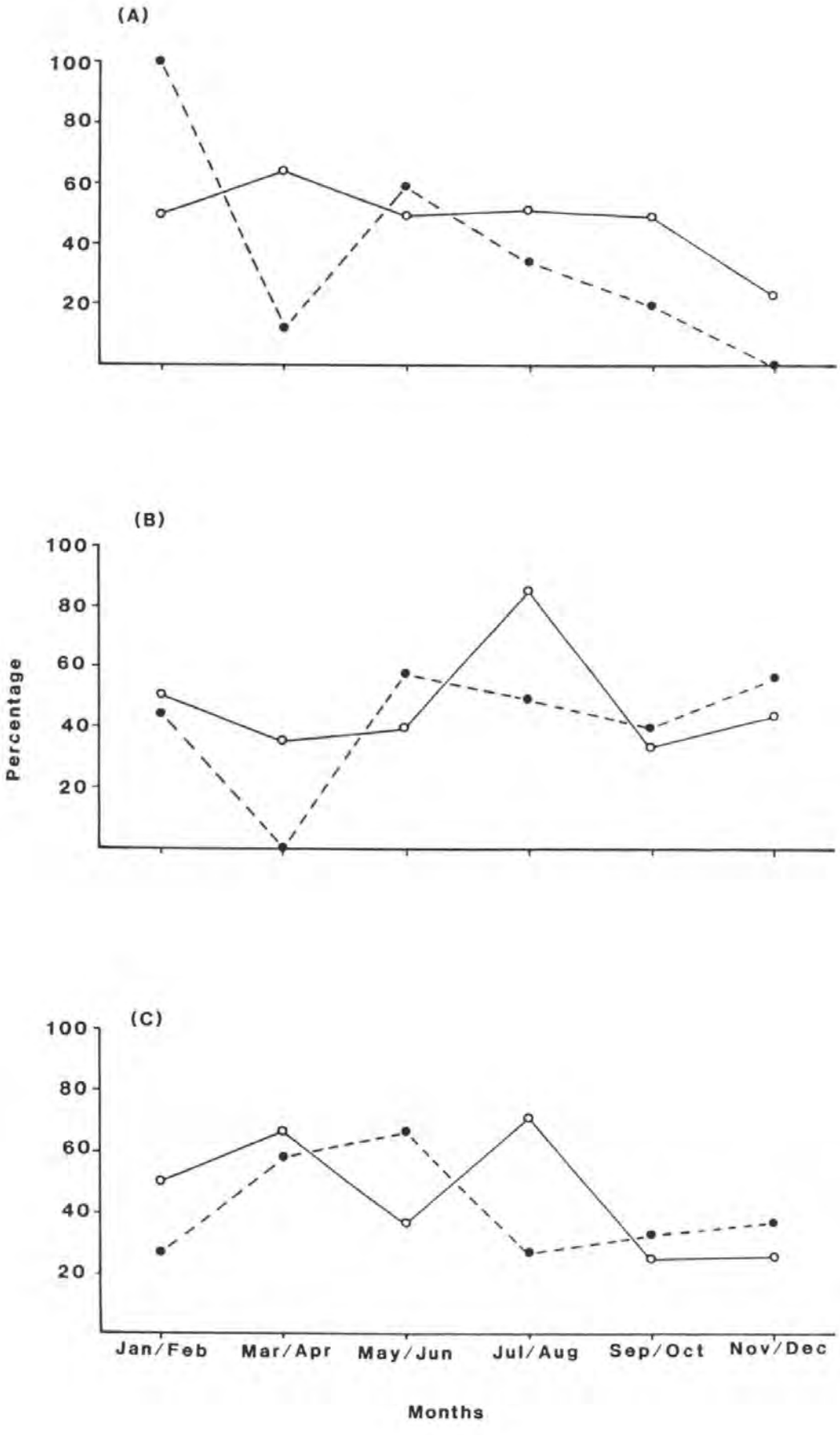

(A)
100
80
60
40
20
(B)
100
80
60
40
20
Percentage
(C)
100
80
60
40
20
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Months

Figure 7.4. Bimonthly variation of the dietary composition for female Liocarcinus puber expressed as the percentage volume of the four major prey groups in the diet of (A) infralittoral, and (B) circalittoral crabs. The littoral zone is not represented due to lack of sufficient samples.

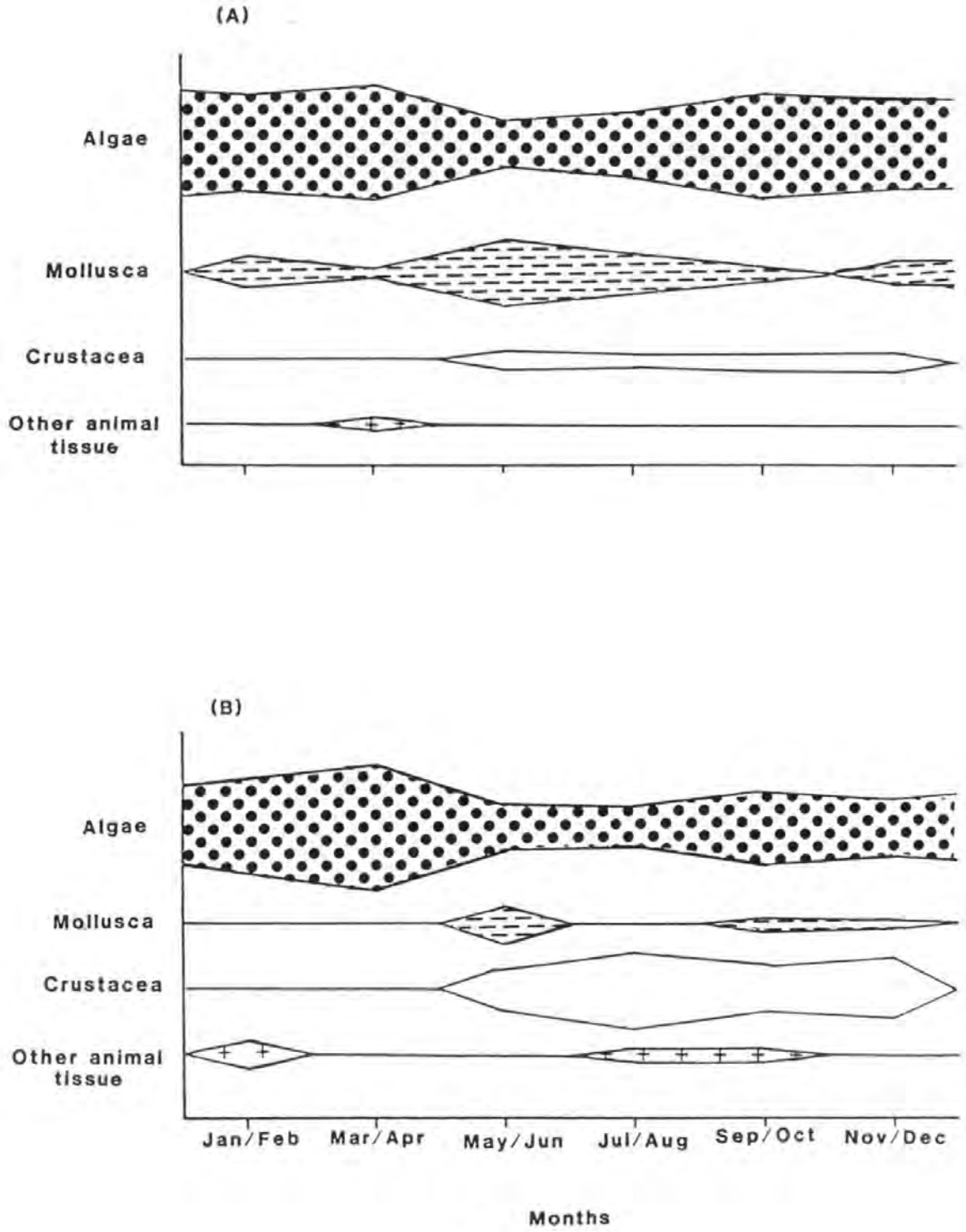

(A)
Algae
Mollusca
Crustacea
Other animal tissue
(B)
Algae
Mollusca
Crustacea
Other animal tissue
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Months

Figure 7.5. Bimonthly variation of the dietary composition of male Liocarcinus puber expressed as the percentage volume of the four major prey groups in the diet of (A) littoral, (B) infralittoral, and (C) circalittoral crabs.

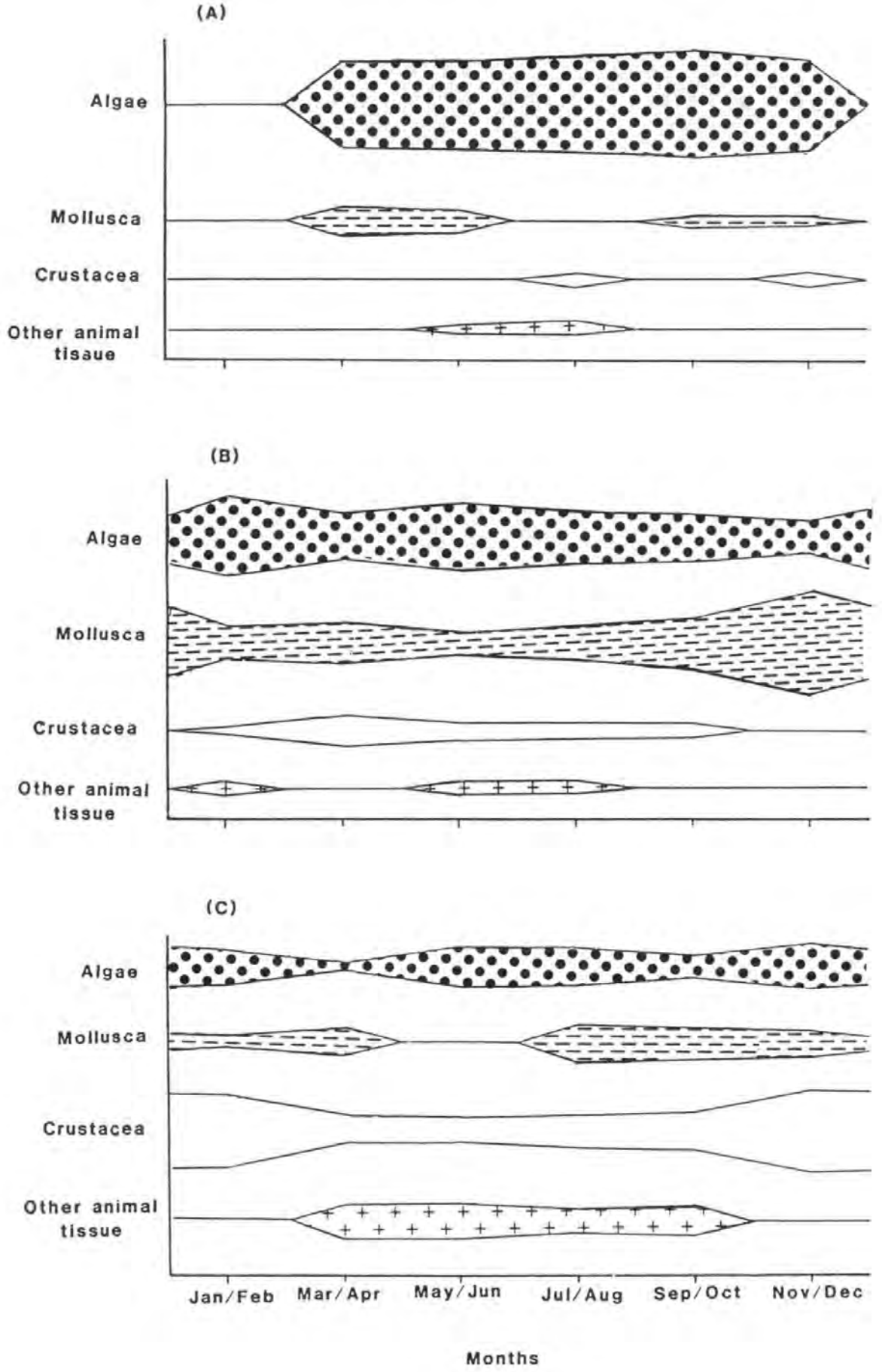

(A)
Algae
Mollusca
Crustacea
Other animal tisaue
(B)
Algae
Mollusca
Crustacea
Other animal tissue
(C)
Algae
Mollusca
Crustacea
Other animal tissue
Jan/Feb
Mar/Apr
May/Jun
Jul/Aug
Sep/Oct
Nov/Dec
Months

Figure 7.6. The percentage fullness of stomachs of Liocarcinus puber in crabs of different moult stages. Gut fullness is divided into Stage 1 (0-5% gut fullness), Stage 2 (5-20% fullness), Stage 3 (20-50% fullness), Stage 4 (50-100% fullness). Number of crabs examined is shown in parentheses.

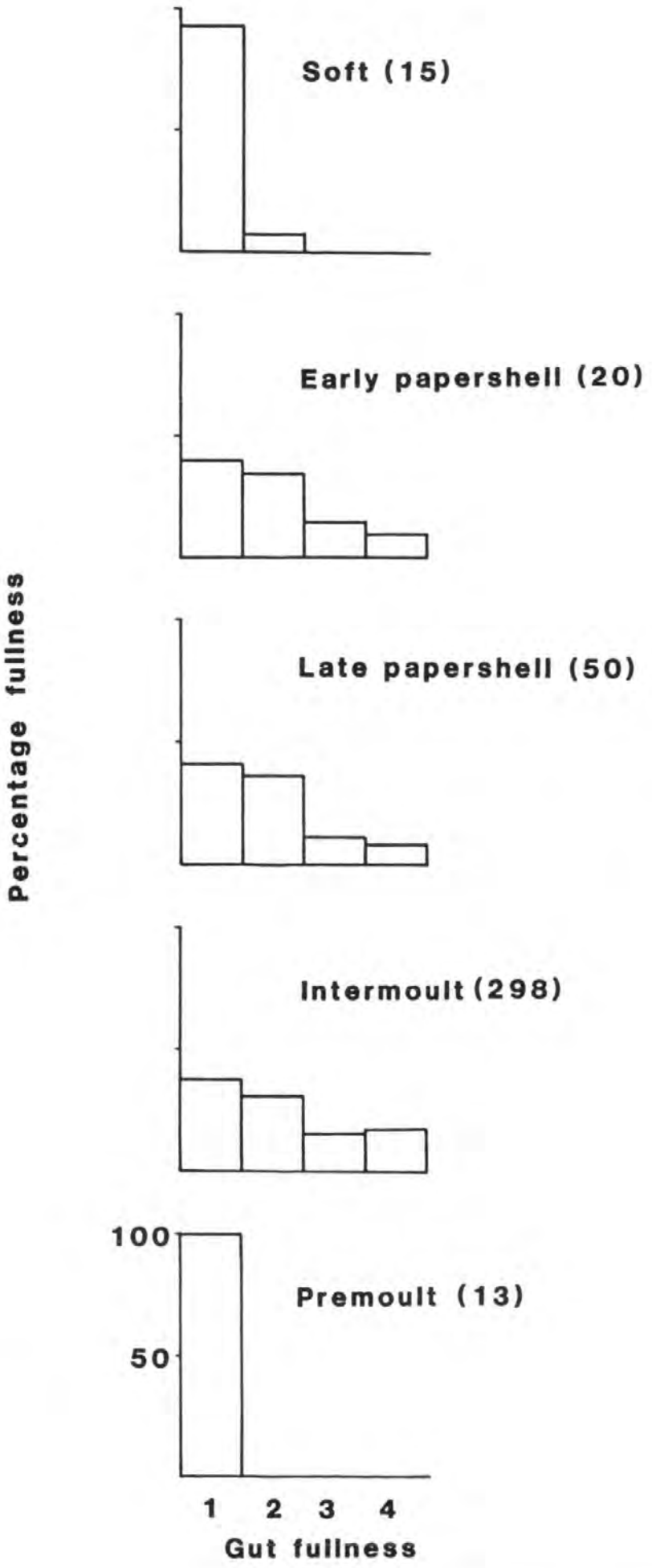
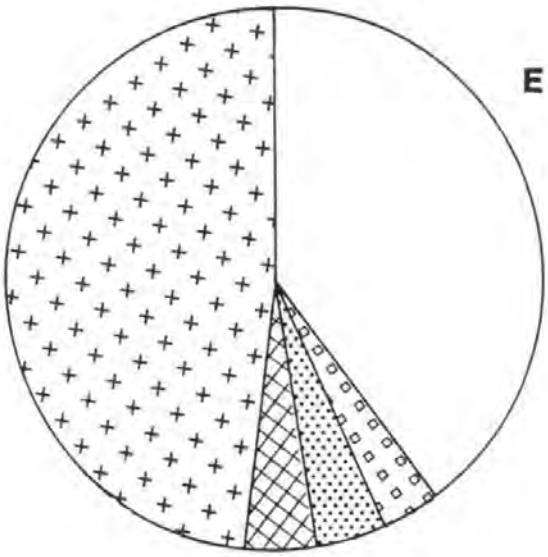
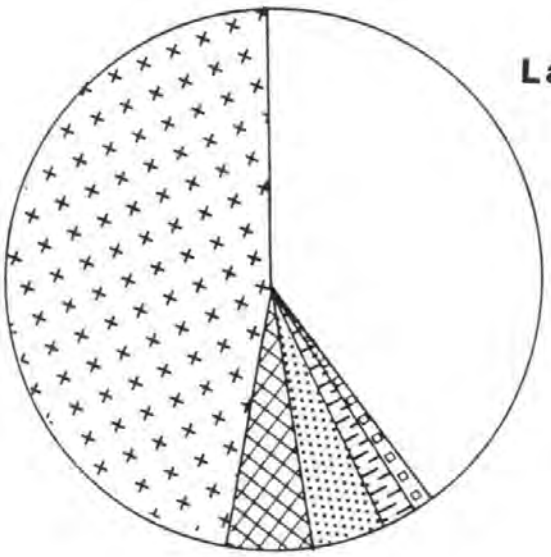

Soft (15)
Early papershell (20)
Late papershell (50)
Intermoult (298)
Premoult (13)
Percentage fullness
100
50
1
2
3
4
Gut fullness

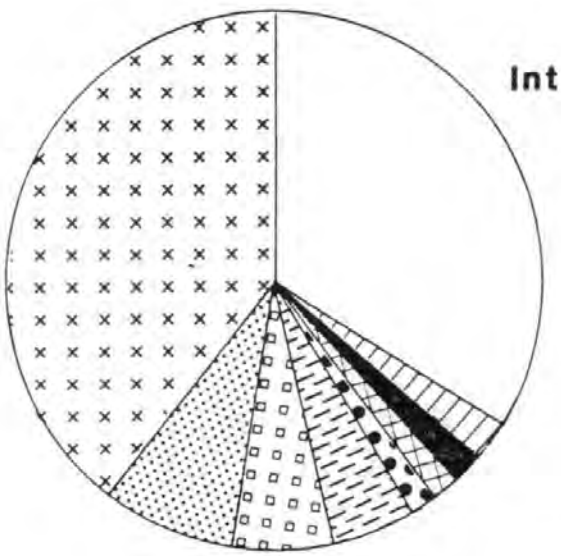
Figure 7.7. The percentage composition of the diet of crabs in early papershell, late papershell and intermoult. For each moult stage, the percentage of the sample with empty stomachs is estimated as for Figure 7.6 and the dietary composition is estimated by volume. Soft and premoult crabs are not shown as too few were observed with any stomach contents. Numbers examined are shown in parenthesis. Key to stomach contents: empty (clear), brown algae (crosses), bivalve (open squares), gastropods (large dots), crustacea (dashes), unidentified material (stippled), red algae (cross hatched), green algae (black), and fish, echinoderms and polychaetes (striped).



Early papershell (20)



Late papershell (50)



Intermoult (298)

Figure 7.8. Amount of food remaining in the stomach after a single meal of soft fish. Mean points (±1 S.D.) represent three estimates of the percentage of weighed samples remaining in the stomach of *Liocarcinus puber* after 1, 2, 3 and 6h. Fitted regression line has the formula; $\ln y = -0.212 - 0.392 \ln x$ ($r = 0.724$, $n = 12$).

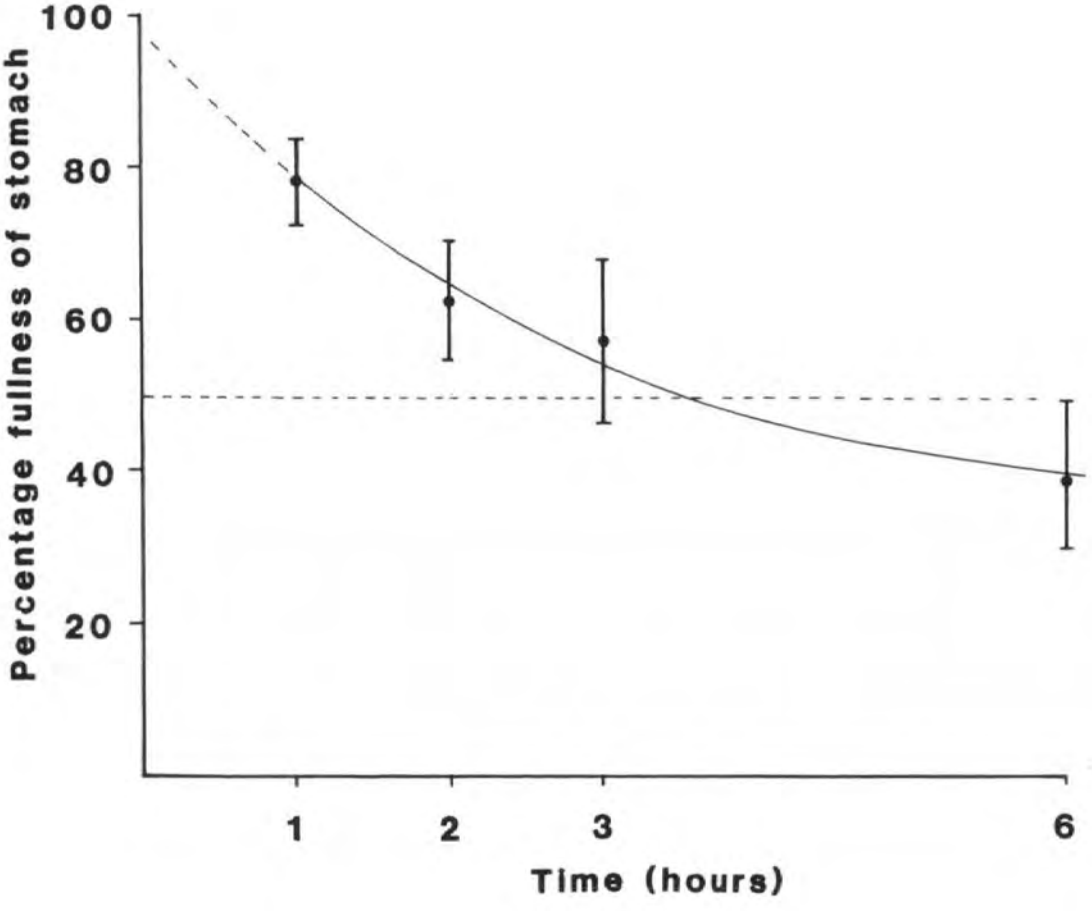
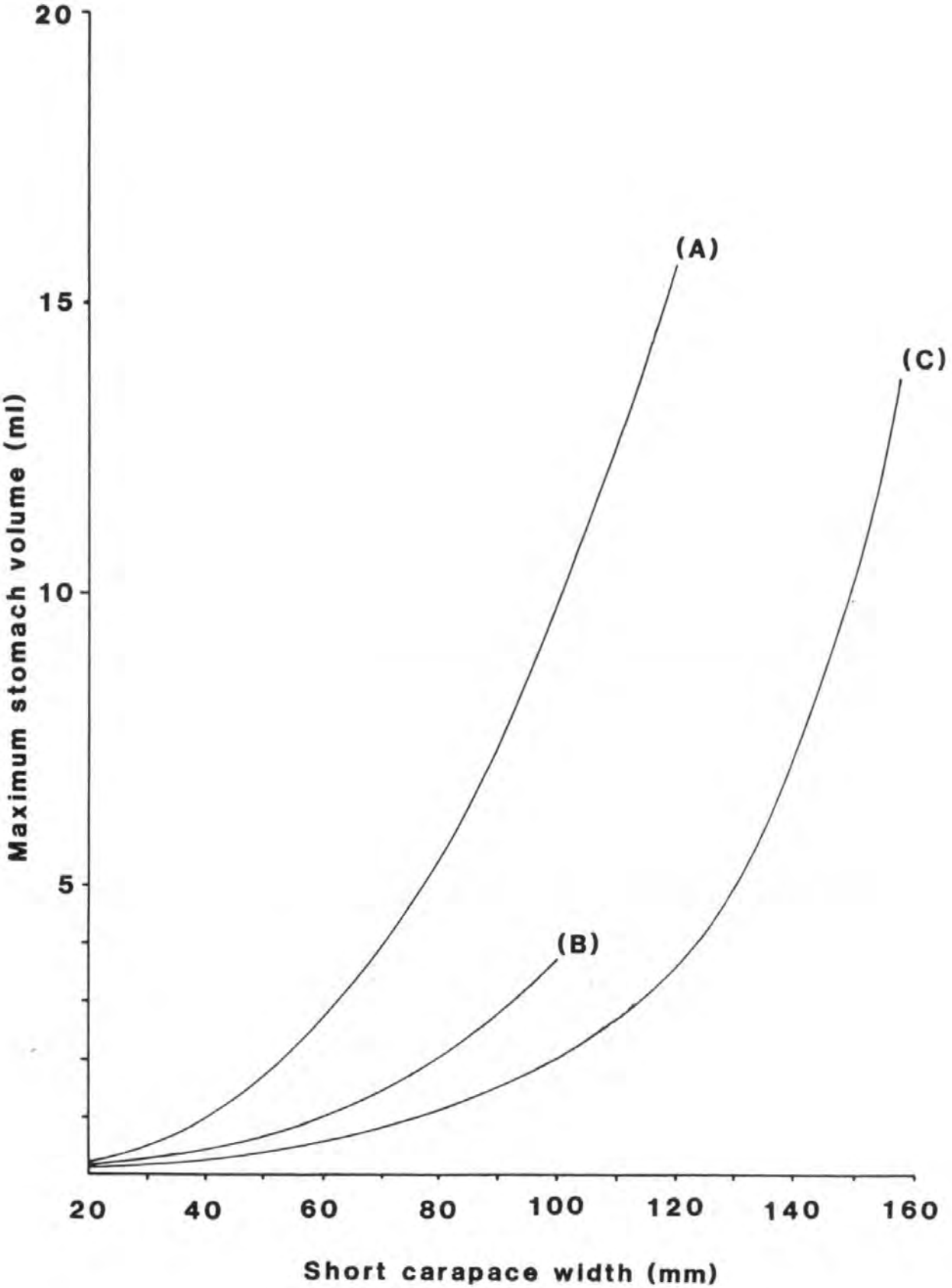

Percentage fullness of stomach
100
80
60
40
20
1
2
3
6
Time (hours)

Figure 7.9. The maximum volume of material that can be contained by the stomach for (A) Ovalipes catharus (Haddon and Wear 1987), (B) Liocarcinus puber (present study), and (C) Scylla serrata (Hill 1976).


20
15
10
5
Maximum stomach volume (ml)
(A)
(B)
(C)
20
40
60
80
100
120
140
160
Short carapace width (mm)

CHAPTER 8

LAMINARINASE ACTIVITY

8.1. Introduction

Gut content analysis of Liocarcinus puber established that the most common food item, both by frequency of occurrence and volume, was brown algae (Chapter 7). As already mentioned (Chapter 7), there are several sources of nutrition associated with brown algae, including the alga itself, the bacteria associated with both growing and detrital algae, and the often rich epiphytic fauna growing on the alga. It was not possible to identify which sources were utilised by L. puber from gut content analysis, however, preliminary laboratory observations indicated that L. puber consumed brown algae which lacked visible epiphytes and also fed upon frond sections at the growing margin of the stipe where bacterial activity is likely to be low. These observations suggest, that the alga itself may be a direct nutritional source, and any epiphytic and bacterial fauna form an additional source of nutrition.

In general, alga is regarded as a poor source of protein for animals due to the low organic nitrogen levels it contains and, as a consequence, is unsuitable as a sole food source, without additional protein (Mann 1972). For example, Russel-Hunter (1970) calculated that animals require a C:N ratio lower than 17:1, and Mann (1972) found C:N ratios in Laminaria spp. ranging from 13.8:1 to 27.2:1. Alga is, however, a rich source of carbohydrate. The principle storage product of brown algae is laminarin which constitutes up to 10-30% of the dry weight of Laminaria spp. (Chapman and Chapman 1980). The levels of laminarin within individual plants, however, is variable. For example, in Laminaria saccharina, the laminarin content for the active growing sections adjacent to the stipe is low, and increases up the frond (Black 1954). The

structure of the polysaccharide sugar, laminarin, is beta -1,3- linked glucans $(C_5 H_{10} O_5)_n$, which hydrolysis to glucose. Liocarcinus puber therefore, may be utilising this rich carbohydrate source and supplementing the paucity of protein found in algae, by the animal protein sources in its rather varied diet (Chapter 7).

To test the theory that Liocarcinus puber is able to degrade laminarin to its constituent glucose molecules, an enzyme assay technique developed by Sova et al. (1970) has been used. To further assess the comparative ability of L. puber to digest laminarin, sympatric crab species were also examined to establish whether the use of brown algae in the diet was specific to L. puber or of a more universal nature.

8.2. Materials and Methods

8.2.1. Laminarinase determination

The method used in this assay is modified from Sova et al. (1969) and summarised in Figure 8.1. All crabs examined were obtained via SCUBA from either the infralittoral zone (for 'shallow water' Liocarcinus puber) or the circalittoral zone (for all other species and for 'deep water' L. puber). Only crabs in intermoult stages were examined. The crabs were starved in the laboratory for 72h to standardise glucose levels within the hepatopancreas. Crabs were maintained in the laboratory in a recirculating seawater aquarium at 13.5 ± 1.5°C. To minimise any contamination of the results due to consumption of algal material growing on the tank walls, the tank was cleaned thoroughly prior to the arrival of fresh specimens. The crabs were sexed and carapace width measured prior to the removal of hepatopancreatic tissue. They were killed immediately prior to dissection by driving a sharp object through the cerebral ganglion. The crab carapace was then dissected away to reveal the cardiac, gill and hepatopancreatic region. Hepatopancreas tissue was excised and 0.1g weighed accurately. The tissue sample was placed in a micro-homogeniser and homogenised for 2 min in 1ml of acetate buffer (pH 5.6). The resultant homogenate was centrifuged for 10 min at 9,000 revs. to remove any suspended solid material. 0.1ml of the supernatant fluid ('enzyme solution') was added to the 'blank' (0.9ml of sodium acetate buffer), and to the 'sample' (0.5ml of laminarin solution and 0.4ml of sodium acetate buffer). The laminarin solution was made up from commercial powder (B.D.H.) to a strength of 0.4g/100ml in distilled water and the acetate buffer (pH 5.6) from 0.1M

sodium acetate buffer solution. Two sets of 'blanks' and 'samples' were tested for each crab examined. A further blank of 0.5ml of laminarin solution and of 0.5ml of sodium acetate buffer was used for each analysis to ensure that there was no contamination of the stock solution or reduction of the laminarin prior to experimentation. Fresh standard solutions and laminarin solution were made up daily, and buffer solution weekly, to reduce the effects of bacterial or chemical contamination or degradation.

The 'blanks' and 'samples' were then incubated at 37°C for 1h, and the assay was terminated by denaturing any protein by boiling for 7 min. The solution was cooled under running water and the amount of glucose in the test solutions was measured spectrophotometrically using a commercial glucose test kit (Sigma). The test is based on the peroxidase and glucose oxidase (PGO) enzymes which, in combination with a colour reagent, allow the photometric determination of glucose. The combined colour reagent and solution from the assay were combined in a ratio of 10:1 and incubated for 30 min at 37° C. The resultant colour change was then measured at 450nm using a Cecil CE 303 spectrophotometer. The glucose standard solution was linear over the range 0-20mg/100ml (Fig. 8.2). Comparison of the glucose levels in the 'blanks' and 'sample' solutions gave an estimate of the quantity of glucose formed by the reduction of the laminarin whilst incubating with the laminarinase from the hepatopancreas. The level of laminarinase activity/wet weight of protein was then estimated. Activity was measured in units which were the amount of enzyme which produced 100µg of reducing sugar per hour under experimental conditions.

The assay, the levels of laminarinase in the hepatopancreas of

four sympatric crabs: Liocarcinus puber, L. depurator, Carcinus maenas and Cancer pagurus. In addition specimens of Liocarcinus puber were examined from a depth of >20m, from a site several kilometres offshore, where there is no algal growth and little evidence of detrital algae being available as a prey item.

8.2.2. Protein determination

As levels of enzyme in animal tissue are usually presented as units of enzyme per milligram wet weight of protein, the protein content of the hepatopancreas of was measured using the Folin-Lowry technique (Lowry et al. 1951). The technique involves the formation of a copper/protein complex which is subsequently measured spectrophotometrically (Lowry et al. 1951). The percentage protein composition was established for each crab species examined for laminarinase activity.

Hepatopancreas tissue (0.1g) was digested overnight in 100ml of 1M sodium hydroxide. The solution was centifuged at 1750 revs for 10 min, and 0.5ml of supernatant was removed and added to 2.5ml of a freshly prepared mix of solutions (1% copper sulphate, 2% sodium potassium tartrate and a 2% solution of anhydrous sodium carbonate in 0.1M sodium hydroxide - in a ratio of 1:1:100 ml). Folin-Ciocaeteau reagent (BDH) 0.25ml was then added and the reaction was allowed to stand for 25 min. Bovine serum albumin was used as a standard protein solution and, with the sample reactions, was read at 750nm using a Cecil CE 303 spectrophotometer. Standards gave a linear curve between 0-85µg/ml (Fig. 8.3). The test curves for the hepatopancreas fell outside this range and therefore a 50% dilution of the original hepatopancreas/sodium hydroxide solution was used. As the solution of hepatopancreas/sodium hydroxide was rather opaque, the

original technique used centrifugation to remove some of this heavier cellular debris. This procedure may, however, bias the protein estimation by solidifying out some of the heavier protein material. To examine any bias, the experiment was repeated using both centrifuged and non-centrifuged samples.

8.3. Results

8.3.1. Laminarinase determination

The levels of laminarinase activity are given in Table 8.1. Liocarcinus puber from the infralittoral (shallow water <10m) had significantly higher levels of laminarinase activity than both Carcinus maenas and Cancer pagurus (ANOVA $F=34.32$, $df=4,30$, $P<0.05$). The laminarinase levels for the specimens of L. puber taken by SCUBA from deep water (>20m) also showed high levels of laminarinase activity. Values for C. maenas and C. pagurus were both low, suggesting that active digestion of laminarin in these species is negligable.

8.3.2. Protein determination

The estimates of percentage protein content of the hepatopancreas samples are given in Table 8.2. The limited data suggest that there is little difference in the percentage of protein both within and between species. Liocarcinus puber, L. depurator and Carcinus maenas showed relatively small variation in the values, suggesting that the amount of protein in the hepatopancreas of these species is constant at a level of approximately 11.4 - 12.6% protein. Centrifugation also had little effect on the estimated level of protein. Analysis of variance (ANOVA) showed no significant difference between the means values of percentage protein of the hepatopancreas of Liocarcinus depurator, Carcinus maenas and Liocarcinus puber (centrifuged and non-centrifuged) ($F=0.047$, $df=3,10$, $P>0.1$).

8.4 Discussion

There are few published reports on the levels of carbohydrases and, in particular, laminarinases in marine invertebrates. Studies that are available have shown high levels of laminarinase activity in certain bivalve molluscs, particularly associated with the crystalline style, and also in some crustaceans (Sova et al. 1970, Hylleberg Kritensen 1972). Sova et al. (1970) found comparable levels to those measured in the present study; for example, 3.5 units/mg protein were recorded for Cancer pygmaeus compared with the value of 5.1 units/mg protein for Cancer pagurus found in this study. Other crustacean species investigated by Sova et al. (1970) included Hapalogaster dentata, Pachycheles stevensii, Pagurus ochotensis and Hemigrapsus sanguineus which had laminarinase levels of 52, 47, 34 and 28 units/mg protein respectively. The levels of laminarinase recorded in this study for Liocarcinus puber are amongst the highest levels for any crustacean so far studied (Sova et al. 1970). These findings suggest that L. puber is capable of reducing laminarin and that laminarin forms a potentially significant source of carbohydrate.

Hylleberg Kristensen (1972) found negligible levels of laminarinase in Carcinus maenas and suggested that laminarin was of no significance as a food source for this species. These findings, although, in agreement with the present study which found a mean value of 8.3 units/mg protein for C. maenas, are somewhat suprising as some studies have found C. maenas to regularly consume algal material (Ropes 1968, Elner 1977). This apparent contradiction suggests that C. maenas may consume algae primarily to gain access to the epiphytes and bacteria, or

may show increased levels of laminarinase in certain localities where algae is preyed upon. Evidence from this study between 'shallow' and 'deep' water L. puber suggests that there may be a limited degree of enzyme enhancement related to food availabilty of a food item. The high levels of laminarinase found for deep water L. puber, however, indicate that the level of enzyme production is primarily genetically controlled. Crabs from the 'deep water' sampling site will rarely (if ever) be exposed to quantities of algal material, and no evidence was found of any detrital algae at this site on over 10 sampling visits, over one complete year.

Paine and Vadas (1969) extensively analysed the calometric value of algal material and found that green algae were calorifically higher than red algae which in turn were higher than brown algae (4.9, 4.75 and 4.45 kcal/ash-free g dry weight, respectively). The same authors also found that, within each algal group, ephemeral species had a higher calorific value than annual species, which in turn were higher than perennial species. Therefore, the large browns may be considered to be the least attractive group from a calorific stand-point. Some herbivores, such as species of urchins and abalone, do prey on large browns, and it is suggested that long-term availability may be more important than calorific content in their selection (Fuji 1962, Leighton and Boolootian 1963, Paine 1977). Little variation occurs in the calorific content of brown algae with season (Paine and Vadas 1969, Jensen et al. 1985), suggesting that this source of carbohydrate is available year-round.

Night-time SCUBA observations of feeding of Liocarcinus puber found little evidence of active herbivory (Chapter 7). On several occasions, individuals were seen to 'sit on' brown algae, yet there was no evidence of any grazing on the algal frond. In addition, slight movement

of Laminaria, through wave action, appeared to effectively deter L. puber from gaining access to the lamina blades. These observations suggest that L. puber does not directly graze, to an appreciable extent, on live laminarians but feeds on detrital algae. Detrital algae is defined here as any algal material which has broken off from the fixed stipe. The local abundance of this material will depend on factors such as storm frequency, aspect to prevailing wave and current direction, and whether this material is entrained sub-littorally or cast onto the shore. At sheltered sites such as F Buoy, detrital algae were common and often extremely abundant, being pushed onto the chains by tidal currents. In infralittoral areas, detrital algae were also observed frequently, particularly in gulleys and areas where material settles out. The utilisation of detrital algae, rather than living algal material, for L. puber may be advantageous as it is may be more easily accessible than living plants, and may be in a state of decay and therefore bacteria rich. Detrital algae was often observed in sections or strips, broken from lamina blades, and these pieces may be more easily manipulated L. puber whilst feeding.

The apparent year-round consumption of brown algae by Liocarcinus puber (Chapter 7) together with the demonstrated ability to reduce laminarin, confirms that brown algae is a major source of carbohydrate for L. puber. The protein content of brown algae is low, and herbivores, such as the sea-urchin (Stronglylocentrotus droebachiensis), require gut bacteria to enhance their protein intake (Fong and Mann 1980). To examine whether L. puber is capable of long-term survival on a diet of brown algae, a rearing experiment using various combinations of animal or algal food sources, was carried out to determine the survival and growth of L. puber on these diets (Chapter 9).

Table 8.1. Laminarinase levels found in inshore crabs.

Species	Enzyme activity (units activity mg protein^{-1})		
	n	Mean	S.D.
Liocarcinus puber (shallow water <10m)	14	40.28	±19.0
Liocarcinus puber (deep water >20m)	5	32.34	±23.0
Liocarcinus depurator	5	19.53	±15.6
Carcinus maenas	6	8.31	±6.3
Cancer pagurus	5	5.10	±4.2

Table 8.2. Comparison of percentage protein content of the hepatopancreas.

Species	Protein content of hepatopancreas (%)		
	n	Mean	S.D.
Liocarcinus puber			
Centrifuged	4	11.4	0.86
Non-centrifuged	3	12.5	2.10
Liocarcinus depurator	3	11.4	1.07
Carcinus maenas	3	12.6	0.95

Figure 8.1. Flow diagram to summarize the laminarinase assay technique.

ASSAY TECHNIQUE

(MODIFIED FROM SOVA ET AL. 1970)

STARVE CRABS FOR 72 HOURS PRIOR TO EXPERIMENTATION

EXCISE HEPATOPANCREAS OF KNOWN WEIGHT (0.1g)

HOMOGENISE (SOLUTION MADE UP TO 1cm^3 IN ACETATE BUFFER)

CENTRIFUGE FOR 10 MINUTES AT13000 revs.

SAMPLE	BLANK
0.5ml LAMINARIN SOLUTION	0.9ml SODIUM ACETATE BUFFER
0.4ml SODIUM ACETATE BUFFER	0.1ml ENZYME SOLUTION
0.1ml ENZYME SOLUTION	

INCUBATE 1 HOUR AT 37°C

REACTION TERMINATED BY BOILING FOR 7 MINUTES

GLUCOSE PRODUCED IS MEASURED SPECTROPHOTOMETRICALLY BY THE GLUCOSE OXIDASE METHOD

LEVEL OF GLUCOSE ORIGINALLY IN HEPATOPANCREAS PLUS QUANTITY PRODUCED BY ENZYME IN ASSAY	LEVEL OF GLUCOSE ORIGINALLY IN HEPATOPANCREAS

DIFFERERENCE YIELDS A MEASURE OF THE ACTIVITY OF ENZYME IN THE HEPATOPANCREAS

Figure 8.2. Standard curve to show range of linearity for concentrations of glucose. Reagents used are from SIGMA test kit and wavelength 750nm.

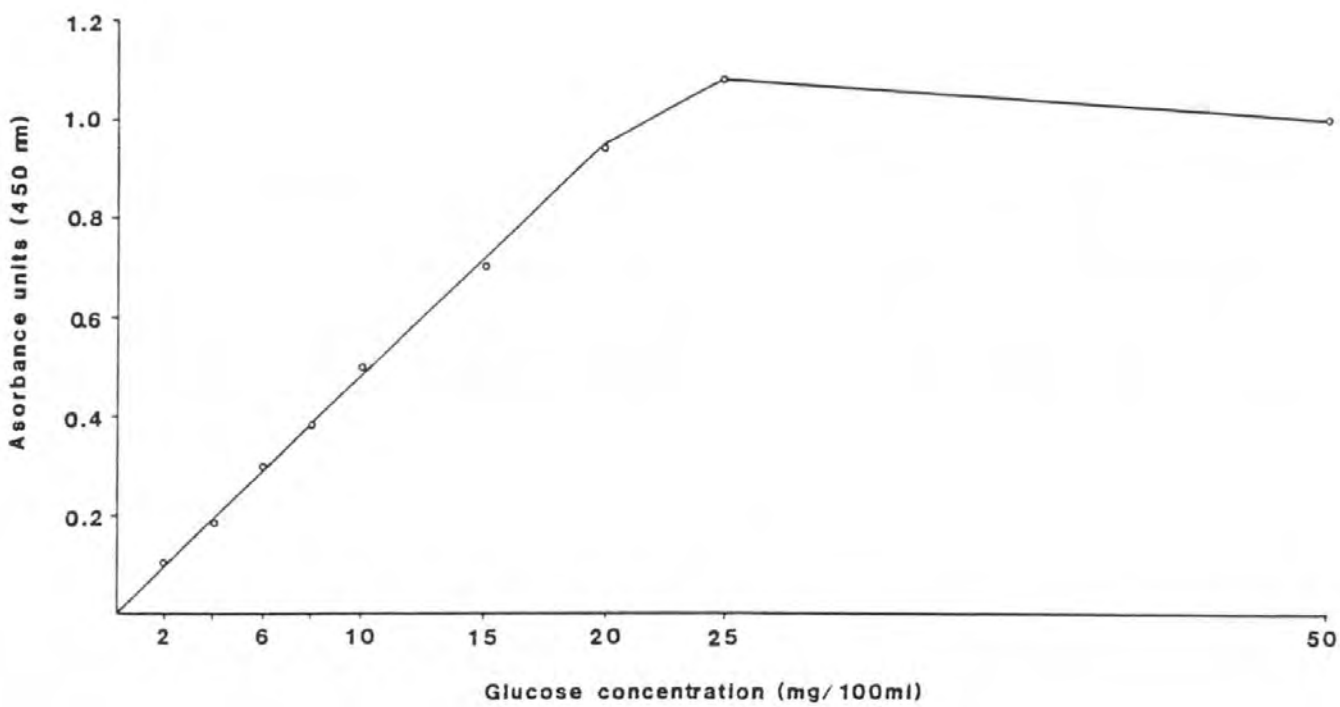
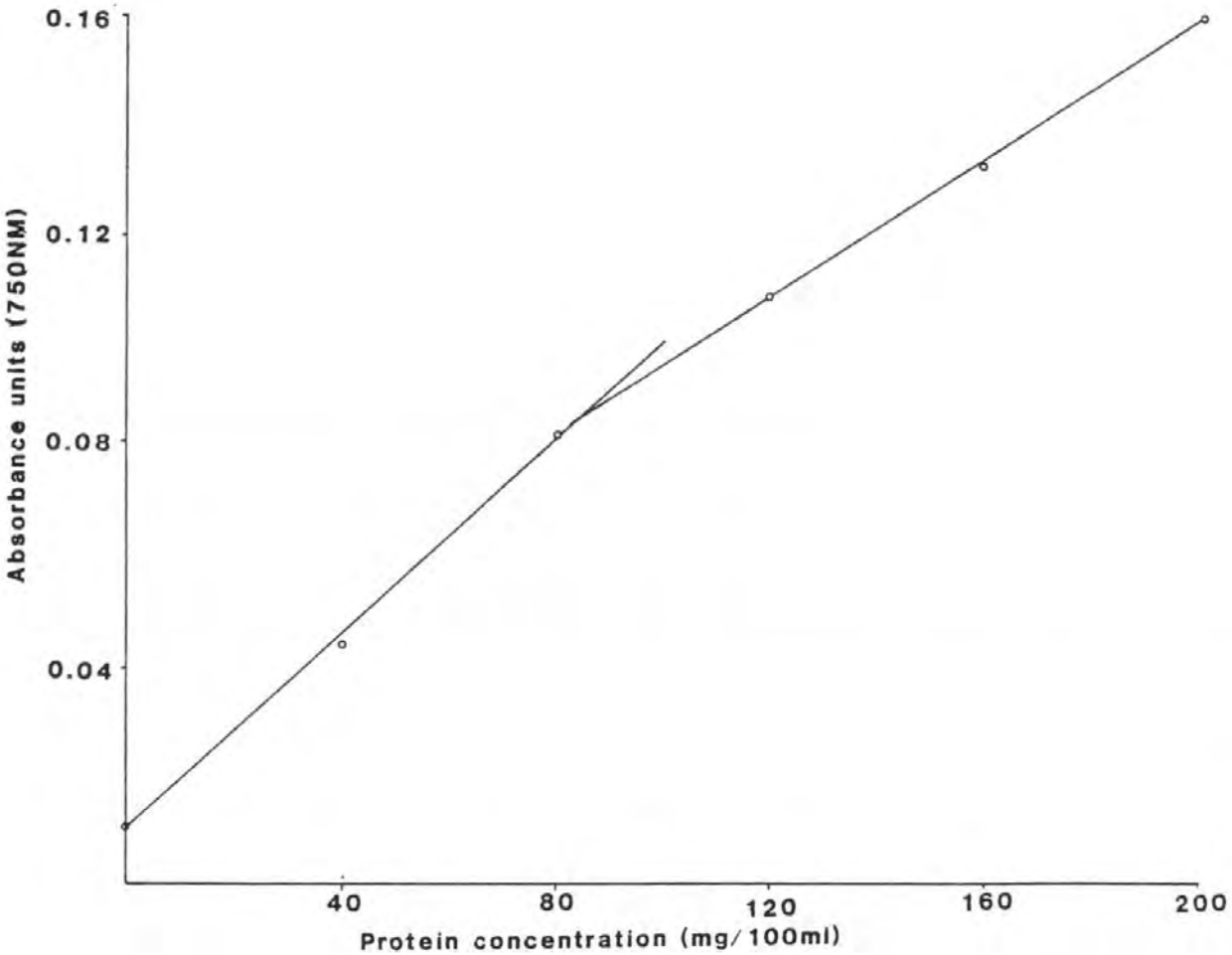

1.2
1.0
0.8
0.6
0.4
0.2
Asorbance units (450 m)
2
6
10
15
20
25
50
Glucose concentration (mg/100ml)

Figure 8.3. Standard curve of Bovine Serum Albumin (BSA) for the determination of protein levels (Lowry et al. 1951).


0.16
0.12
0.08
0.04
Absorbance units (750NM)
40
80
120
160
200
Protein concentration (mg/100ml)

CHAPTER 9

LABORATORY FEEDING TRIAL

9.1 Introduction

Results from Chapter 8 established that Liocarcinus puber was capable of reducing laminarin to glucose at a significantly higher rate than other sympatric crabs and the levels of laminarinase measured for L. puber were similar to the highest levels previosly recorded for Crustacea (Sova et al. 1970).

The nutritional requirements of Crustacea are similar to other higher metazoans (New 1976, 1980, Conklin 1980). For example, a range of essential amino acids, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, trypotophan and valine is required for growth in Cancer (Lasser and Allen 1976) and Homarus (Gallagher and Brown 1975). Other essential dietry requirements are lipids, carbohydrates, vitamins and minerals (Dall and Moriarty 1983). Laminarales have high levels of carbohydrate, vitamins and minerals (Chapman and Chapman 1980), although the quantity of protein has been reported as limiting for animal nutrition (Russel-Hunter 1970). This shortfall in levels of nitrogen in brown algae has been overcome by other herbivores, such as echinoids, by utilising gut bacteria (Fong and Mann 1980) and by juvenile gastropods which selectively feed on detrital algae, and thereby utilise the additional protein source of bacteria (Smith et al. 1985). Whether Liocarcinus puber is able survive on a diet based solely on algal material is examined in this chapter.

9.2. Materials and Methods

9.2.1. Rearing protocol

To examine the survival and growth increment of Liocarcinus puber fed on different diets, crabs were maintained in the holding facility at Bovisand (Chapter 3). Fifty-six crabs were allocated randomly to one of four dietry groups : 1) starved (12 crabs), 2) fed on mussel only (15 crabs), 3) fed on algae only (16 crabs) and 4) fed on a mixed diet of fish, mussel and algae (13 crabs). Uneven crab numbers were due to difficulties in collecting sufficient numbers as well as initial escapment from holding boxes. The crabs were collected in a single sampling session from Wembury beach on the 27th of March 1988. Size of crab ranged from 35-65mm LCW. The sample was biased in favour of males (39:17) and no ovigerous females were sampled. The crabs were fed twice weekly and any uneaten food from the previous feeding session was removed prior to new food being given. Food items consisted of opened Mytilus edulis (3-4cm long), strips of Pollachius pollachius (approximately 4 by 2cm), and strips of brown algae. The brown algae used was taken from young Laminaria digitata collected at low water spring tides or via SCUBA. The fronds were cut into stips each of approx. 10 by 4cm. Any epiphytes observed on the strips, such as Patina pellucida (L.), were removed prior to feeding. Where heavy infestation by epifauna was apparent the frond was disgarded. Prior to each feeding, crabs were inspected for moulting and survival.

9.2.2. Weight measurements

After 4 months, surviving crabs were culled and processed as follows. For each crab, the stomach was removed to avoid any contamination of its contents on the weighings. The hepatopancreas was then removed, taking care to remove all sections including the posterior midgut lobes, and dried to constant weight at 100° C. The remainder of the crab was similarly dried to constant weight. Once constant weight was achieved, the carapace and hepatopancreas were ash-free dry weighted in a muffle furnace at 600° C for 48h. The weight of the inorganic residue was subsequently used to estimate the weight of the dry organic matter via subtraction from the total dry weight of the crab. To compare crabs reared on various diets at Bovisand with their 'wild' counterparts, a further sample of 7 male crabs was collected from Wembury in early August 1988, and dry weighted as above.

9.2.3. Laminarinase levels

The technique to examine the levels of laminarinase in the hepatopancreas has been outlined in Chapter 8. When the crabs were freshly killed for the dry weight examination, a sub-sample of hepatopancreas was used to compare the laminarinase levels between the feeding trials.

9.3. Results

9.3.1. Mortality

Figure 9.1 shows the percentage survival of Liocarcinus puber fed over a four month period (April-July inclusive) on the four diets. The starved animals showed the highest mortality, but survived for prolonged periods (50% mortality after 2.5 months), with death being largely associated with unsuccessful moults (see below). The longest period of survival was 15 weeks (Fig. 9.1). Prior to death, control crabs were noted to undergo an extensive period of 'lethergy' in which no aggressive response to disturbance occurred. The mortality of crabs fed on the other diets, was generally low (Fig. 9.1). Crabs fed solely on algae, however, showed relatively higher mortality towards the end of the trial, and also showed a 'lethargic' response on inspection.

9.3.2. Increment at moult

Crabs moulted in all four dietry treatments. Four of the starved individuals died whilst moulting and only one individual of this group moulted successfully (male, premoult 43.7mm LCW, percentage increment 13.2%). Eight successful moults were recorded from each of the crab groupings fed on the mixed diet and the mussel only diet, and nine moults occurred for crabs fed on the algae only diet. The percentage increment data for males from these moults was compared with the laboratory-reared crabs fed on a_{Λ} (Figure 9.2). Analysis of covariance (ANCOVA) of the fitted lines shown in Figure 9.2 showed that the percentage increment of individuals fed on algae was significantly lower than that of laboratory-

reared crabs fed on mixed diet [F(elevation)=33.8, df=1,43, P<0.01; F(slope)=1.74, df=1,42, P>0.05]. Crabs fed on mussels showed no significant difference in slope or elevation to those fed on a mixed diet ((P>0.05). Too few females moulted during this experiment to enable a statistical comparison. This was due largely to the later moulting of females compared with males (Chapter 5) and the lower number of females in the experiments. Of the females used, 66% and 75% fed on mussel and a mixed diet respectively moulted, and no females fed on algae successfully moulted.

9.3.3. Inorganic composition

At the termination of the experiment, the weight of the inorganic residue of crabs was not significantly different (ANCOVA, P>0.05) between the three diets, nor from the sample of crabs taken from the wild at the same time as the experimental crabs were culled (Fig. 9.3). These results indicate that diet largely does not affect the quantity of inorganic material present in the exoskeleton and other 'hard' components of the crabs.

9.3.4. Organic composition

The organic content of the algal-fed crabs did, however, differ significantly from that of 'wild' crabs [ANCOVA, F(elevation)=90.8, df=1,11, P<0.01; F(slope)=1.0, df=1,10, P>0.05]; and of crabs fed on a mixed diet [ANCOVA, F(elevation)=5.6, df=1,11, P<0.05; F(slope)=1.5, df=1,10, P>0.05] (Fig. 9.4). These data suggest that a diet of algae, is insufficient to maintain an organic weight comparable to crabs fed on a diet containing animal tissue.

9.3.5. Hepatopancreas weight

Figure 9.5 shows the dry weight of the hepatopancreas in relation to crab carapace width. Males fed on algae had a significantly lower hepatopancreas weight compared with both 'wild' [ANCOVA, F(elevation)=13.6, df=1,12, P<0.01; F(slope)=2.14, df=1,11, P>0.05] and mussel-only fed crabs [F(elevation)=6.4, df=1,11, P<0.05: F(slope)=0.52, df=1,10, P>0.05) (Fig. 9.5).

9.3.6. Laminarinase levels

Two-way ANOVA using Scheffes multiple comparison method established that crabs fed on algae only had significantly higher laminarinase content per milligarm wet weight of protein in the hepatopancreas compared with crabs fed on the other diets and the 'wild' crabs (data taken from Chapter 8) (ANOVA F=10.58, df=3,45, P<0.01) (Table 9.1). No significant differences were observed between the 'wild', mussel only and mixed diet groups. The levels of laminarinase found for individuals fed solely on algae were markedly variable, and ranged from 13.6 to 241.8 units laminarinase/mg wet weight protein, with a high standard deviation (63.1 units).

9.4. Discussion

This experiment was designed to examine the longevity of crabs fed solely on algae compared with starved crabs and crabs fed on other diets. The ability of Liocarcinus puber to survive for extended periods on a diet of algal material was somewhat surprising as was their ability to moult under this dietry regime, considering that brown algae has been reported as having insufficient protein to support animal growth (Russel-Hunter 1970). At the termination of the experiment, crabs fed on a diet of algae were showing signs of starvation (lethargy) and showed a rise in mortality in the last month of the experiment. The low organic weights observed in algal-fed individuals suggest that reserves of lipid and carbohydrate had been utilised, and also that catabolism of certain proteins (eg. in reproductive tissues) may also have occurred to fuel essential metabolic functions. It would therefore appear likely that protein deficiency was the prime cause of the poor condition, mortality and lower percentage increase at moult for crabs fed on algae. The longevity of algal-fed compared to the starved crabs, however, strongly supports the theory, outlined in the previous chapter, that L. puber is able to gain a significant quantity of its carbohydrate requirements from brown algae.

Crabs fed on algae showed significant increases in laminarinase levels compared with crabs fed on the other diets. Two hypotheses may be raised to explain this result. Firstly, crabs maintained on a diet of algae were able to raise enzyme levels to achieve the maximal return for food ingested. Secondly, the highly variable levels of enzyme and poor condition of the hepatopancreas of the crabs fed on algae indicates that these crabs were in late stages of starvation and that laminarinase

production was maintained whilst other less useful protein systems were catabolised to fuel basal metabolism; hence the relative quantities of laminarinase were enhanced. The more consistant levels of laminarinase observed for all the other feeding trials including the animal only diet, however, supports the notion that the laminarinase levels are constant and that lack of usage of the laminarinase enzyme system does not necessarily lead to the lowering of the enzyme concentration. This conclusion is endorsed further by the observations that deep water Liocarcinus puber (Chapter 8) also had laminarinase levels only slightly reduced compared with the infralittoral sample. This suggests that laminarinase concentrations are relatively 'fixed' and the marked rise in laminarinase levels for the algae only trial is an artefact of the poor condition of the animals.

Ability to utilise brown algae as a source of carbohydrate may give Liocarcinus puber a major advantage over sympatric species, particularly when other more beneficial food material is unavailable. The high utilisation of brown algae reported for L. puber (Chapter 7), suggests that this carbohydrate source is used throughout the year by all size classes, by both sexes and in each moult stage where feeding was observed. Brown algae must therefore play a major role in the life-history of L. puber and this may partly explain the limited depth distribution of this crab. Liocarcinus puber has been reported from between 0-10m depth (Plymouth Marine Fauna, 1957), ie. the depth range at which brown algal typically grows in the Plymouth region.

Whether Liocarcinus puber is able to directly graze brown algae or whether only detrital algae is fed on, has not been fully determined in this study. The lack of field observations of L. puber grazing, or even

regularly observed on kelp blades, suggests that detrital algae is more probably used. The use of detrital algae as the principle source of algal material in the diet, is further supported by the observations from this study and Choy (1986b) that detrital and heavily colonised pieces of brown algae were preferentially consumed.

Table 9.1. Laminarinase concentrations found for Liocarcinus puber under the dietry regimes of algae only, mixed diet, mussel only and from 'wild' data (Chapter 8).

Dietry regime	Enzyme activity (units activity mg protein^{-1})		
	n	Mean	S.D.
Shallow water 'wild' L. puber	14	40.3	19.0
Algae only feeding trial	12	110.8	63.1
Mussel only feeding trial	13	42.2	32.6
Mixed diet trial (algae, mussel & fish)	12	37.3	20.1

Figure 9.1. Percentage survival of crabs fed on the four different diets (n is number of crabs at commencement of each feeding trial).

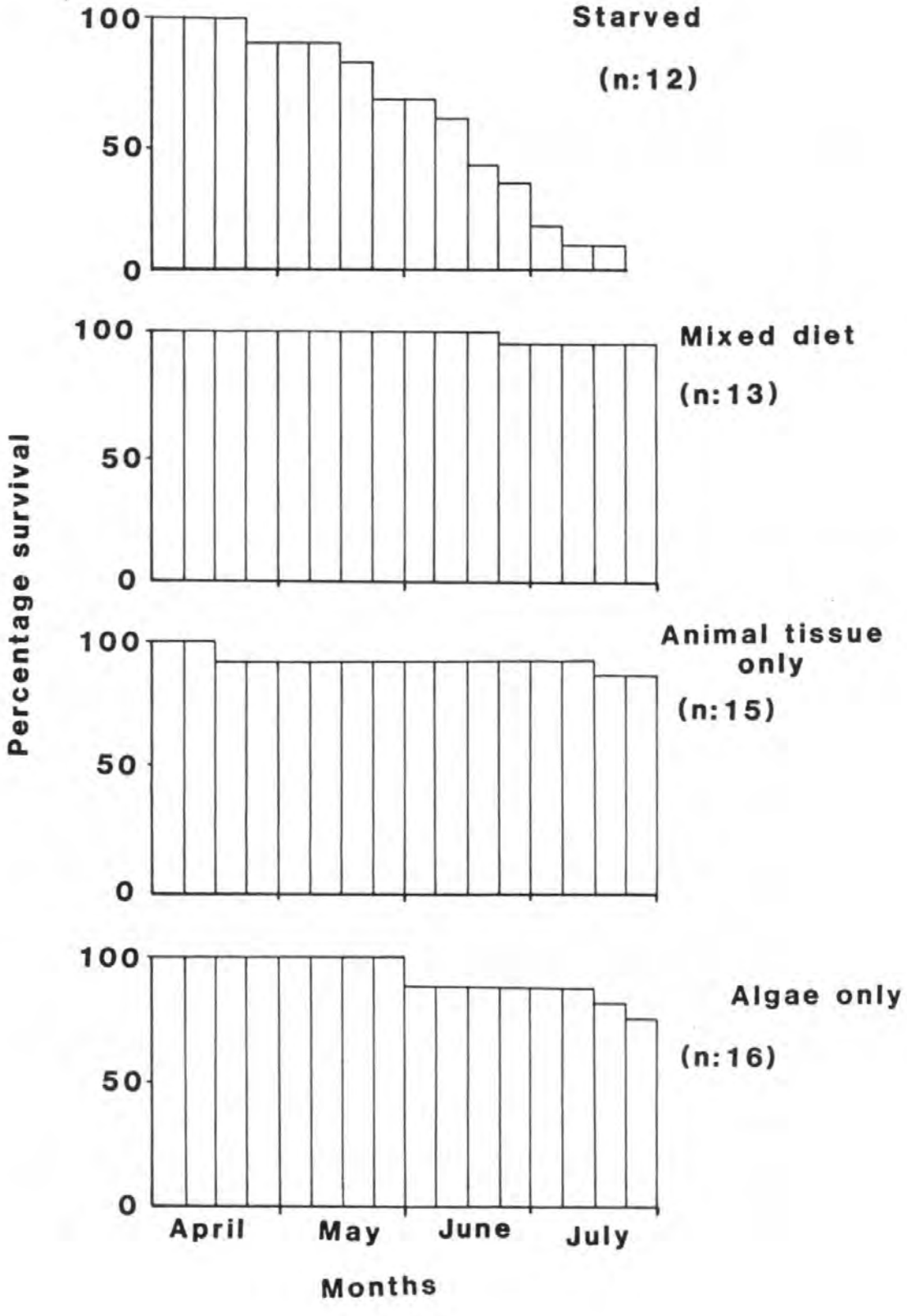

Starved
(n:12)
Mixed diet
(n:13)
Animal tissue only
(n:15)
Algae only
(n:16)
100
50
0
Percentage survival
April
May
June
July
Months

Figure 9.2. Male growth factor (percentage increment at moult) versus premoult carapace width (LCW) for crabs fed on algae [open circles; fitted regression line (B) $\ln Y = -0.504 \ln X + 4.628$, $r=-0.43$, $n= 10$], mussel only [open triangles; fitted regression line (not shown for clarity of presentation) $\ln Y = -1.161 \ln X + 7.388$, $r=-0.83$, $n= 6$], and a mixed diet [closed circles; fitted regression line (A) $\ln Y = -0.624 \ln X + 5.407$, $r=-0.74$, $n= 37$]. Mixed diet values include results from the growth experiment (Chapter 5).

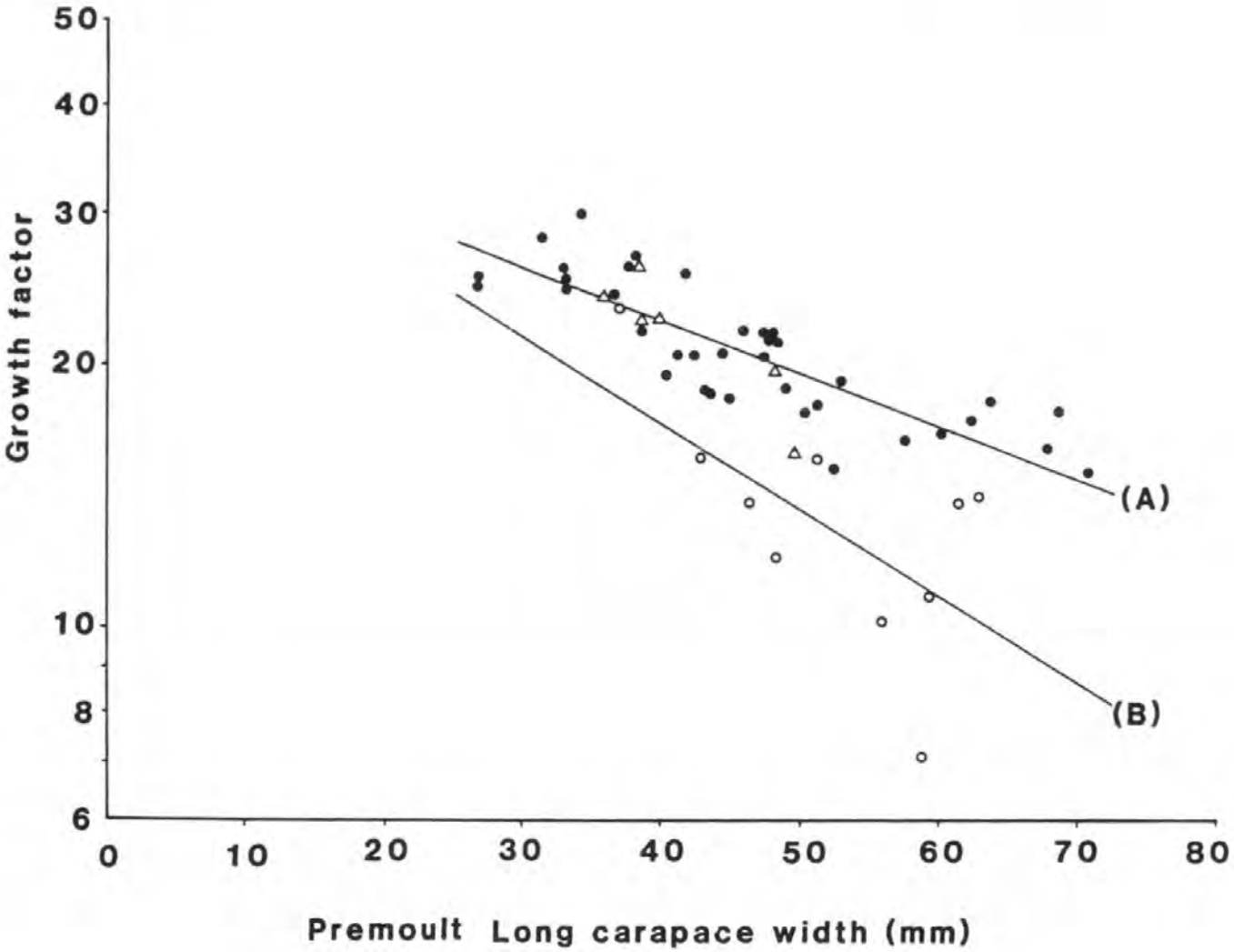

50
40
30
20
10
8
6
Growth factor
(A)
(B)
0
10
20
30
40
50
60
70
80
Premoult Long carapace width (mm)

Figure 9.3. Weight of inorganic material (AFDW) for male crabs fed on algae [open circles; $\ln Y = 3.020 \ln X - 10.77$, $r = 0.984$, $n = 7$], mussels [open triangles; $\ln Y = 2.873 \ln X - 10.08$, $r = 0.966$, $n = 6$], mixed diet [closed squares; $\ln Y = 2.781 \ln X - 9.614$, $r = 0.810$, $n = 7$], compared with crabs sampled from Wembury [closed circles $\ln Y = 3.637 \ln X - 13.26$, $r = 0.987$, $n = 7$]. Regression lines were found not to significantly differ and therefore not shown.

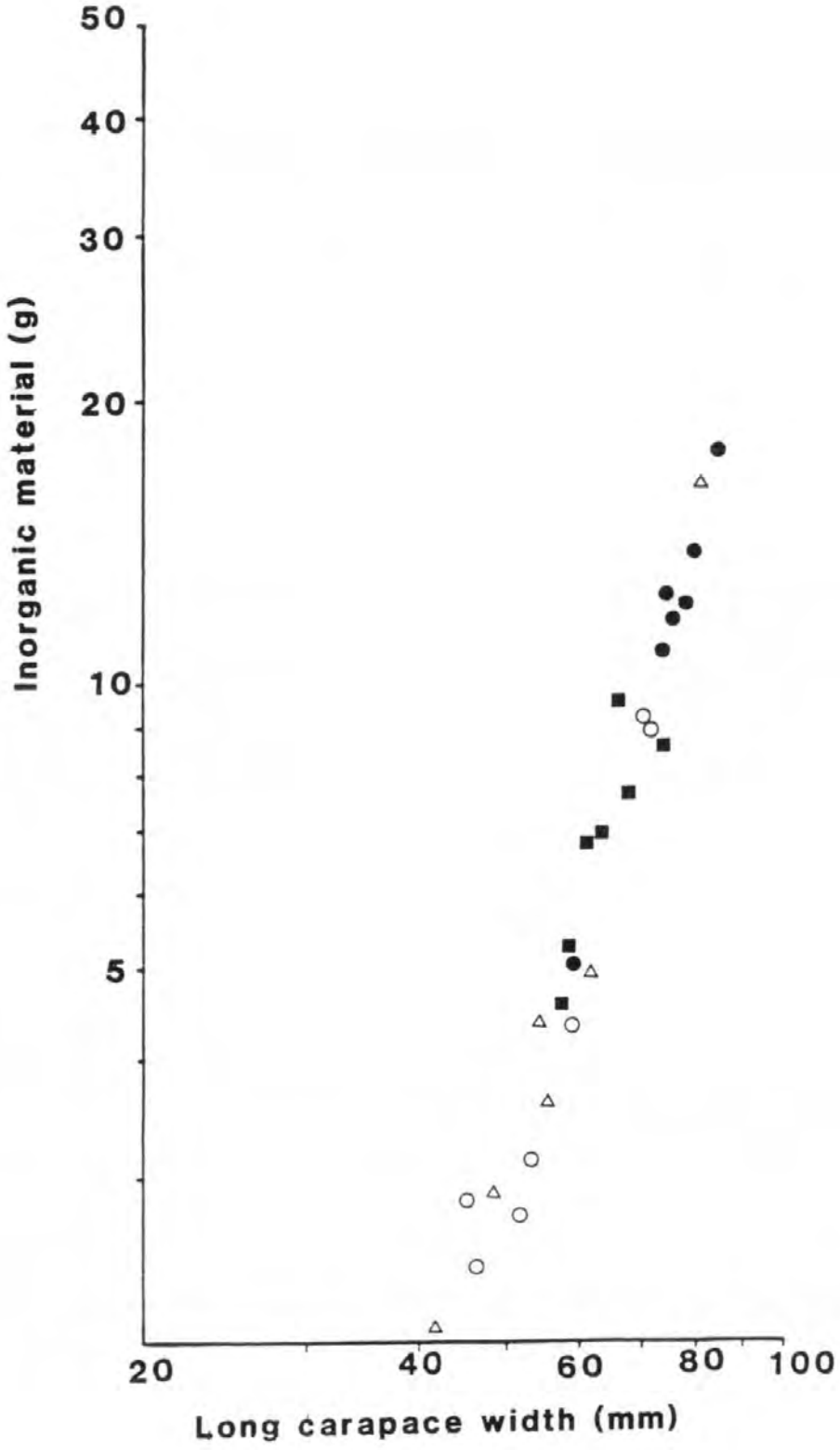

50
40
30
20
10
5
Inorganic material (g)
20
40
60
80
100
Long carapace width (mm)

Figure 9.4. Weight of organic material in body for males fed on algae [open circles; fitted regression line (B) $\ln Y = 3.006 \ln X - 10.369$, $r = 0.990$, $n=7$], mixed diet [closed squares; fitted regression line (C) $\ln Y = 2.384 \ln X - 7.4180$, $r = 0.780$, $n=7$], mussels [open triangles; $\ln Y = 2.365 \ln X - 7.5150$, $r = 0.833$, $n=6$], compared with crabs sampled from Wembury [closed circles; fitted regression line (A) $\ln Y = 3.356 \ln X - 11.287$, $r = 0.984$, $n=7$] . Only significantly different regression lines are shown on figure, details of analysis of covariance are given in text.

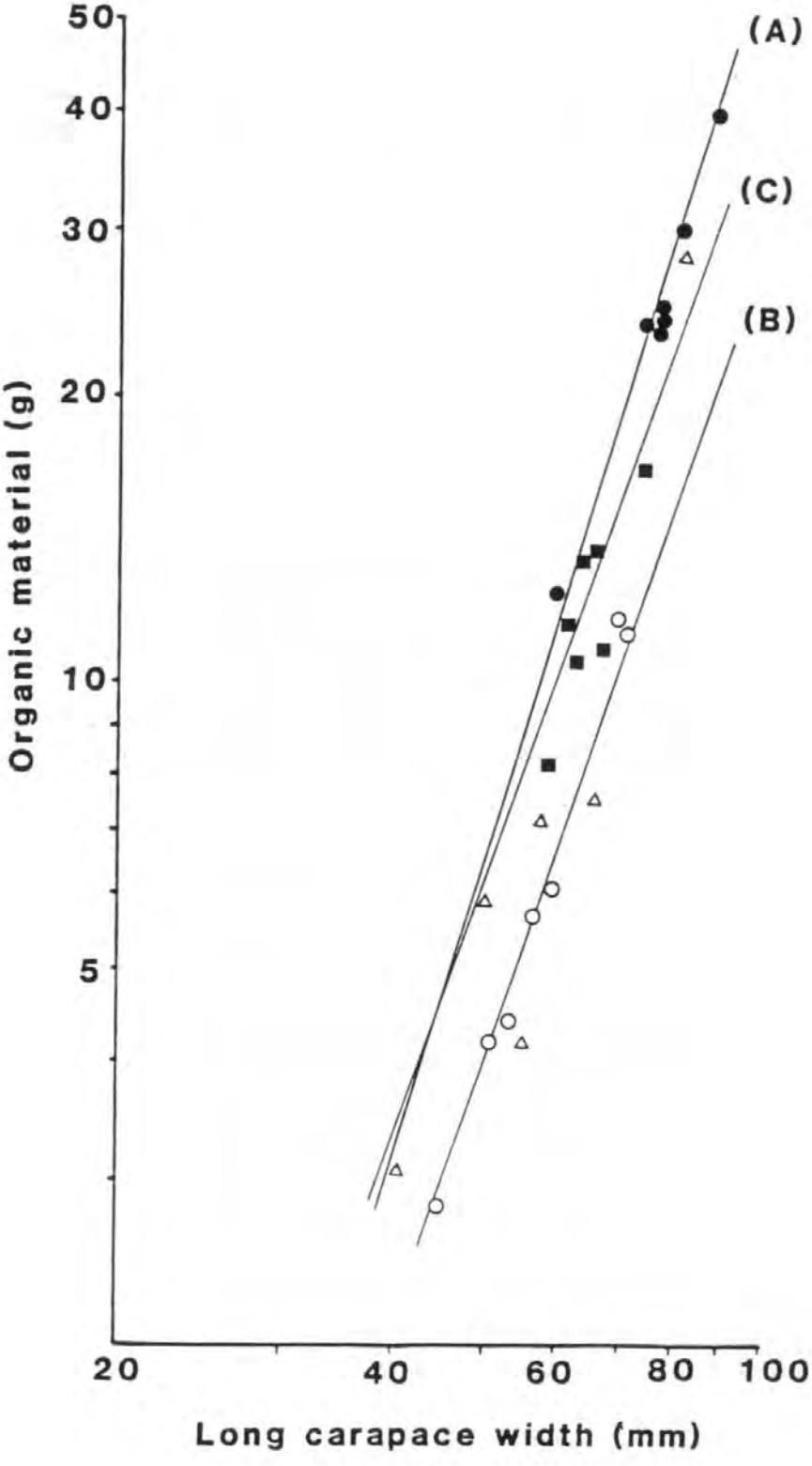
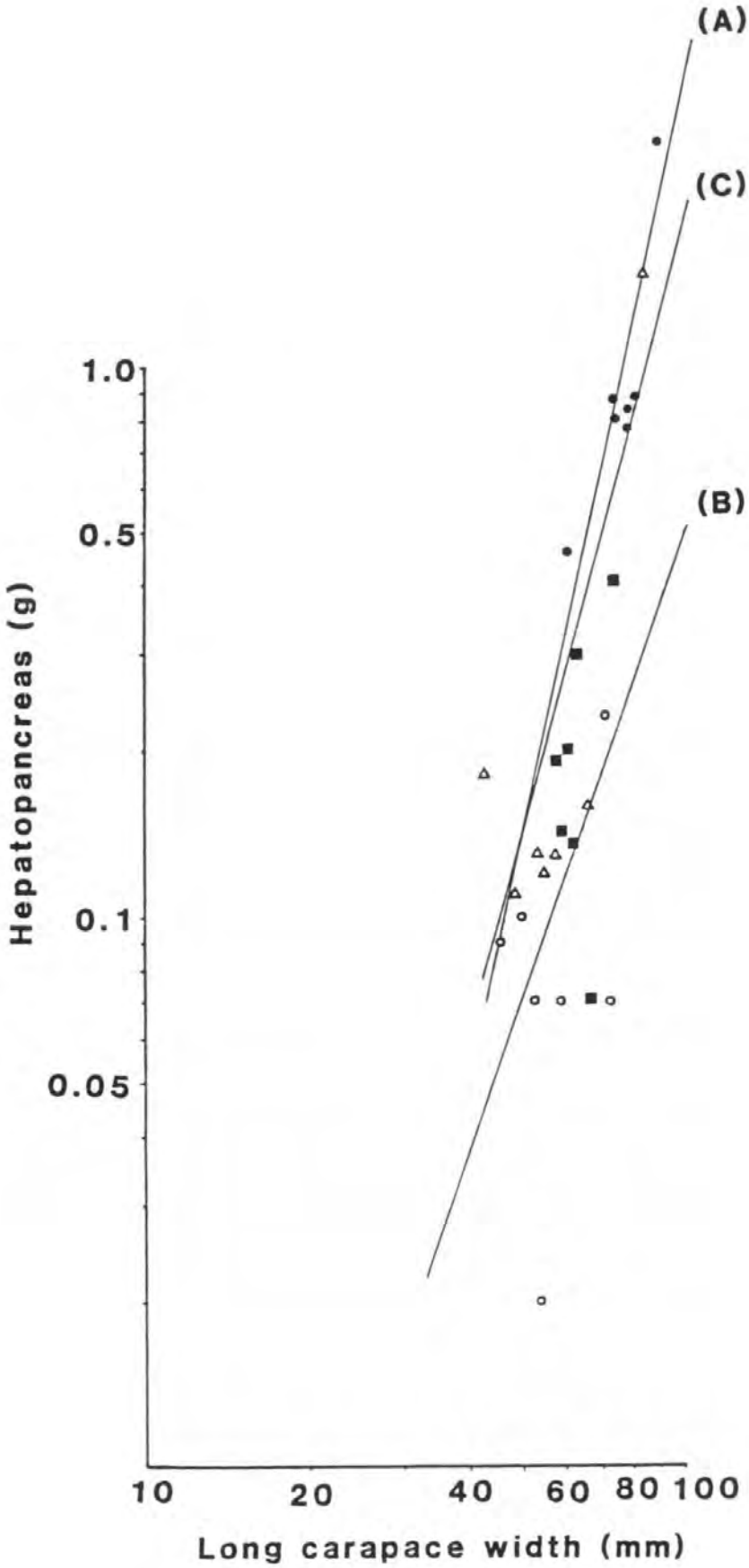

50
40
30
20
10
5
Organic material (g)
(A)
(C)
(B)
20
40
60
80
100
Long carapace width (mm)

Figure 9.5. Weight of hepatopancreas for male crabs fed on algae [open cirles; fitted regression line (B) $\ln Y = 1.143 \ln X - 7.197$, $r = 0.28$, $n=7$], mussels [open triangles, fitted regression line (C) $\ln Y = 2.625 \ln X - 12.271$, $r = 0.72$, $n=6$], mixed diet [closed squares, $\ln Y = 1.695 \ln X - 8.772$, $r = 0.23$, $n=7$], and comparitive data from crabs sampled at Wembury [closed circles, fitted regression line (A) $\ln Y = 4.665 \ln X - 20.420$, $r = 0.82$, $n=7$]. Only significantly different regression lines are shown on the figure, details given in text.


(A)
(C)
(B)
1.0
0.5
0.1
0.05
Hepatopancreas (g)
10
20
40
60
80
100
Long carapace width (mm)

CHAPTER 10

FIELD OBSERVATIONS OF MOVEMENT

10.1 Introduction

A preliminary survey of the distribution of Liocarcinus puber within Plymouth Sound suggested that the main habitats occupied by this crab were the rocky nearshore sub-littoral and the littoral fringe region at low water spring tides. These observations were consistent with the Plymouth Marine Fauna (1957) which reported L. puber as a common species amongst stones on all rocky shores from 'between tide-marks' down to approximately 10m. In a review of crab distribution around the U.K., L. puber was identified as being found in fully marine and rocky coastal areas (Clark 1986). Allen (1967) recorded L. puber from 80m and it has also been noted in the present study that occasional 'pockets' of L. puber occurred in deeper water (>25m) associated with either wrecks or rocky outcrops. The density of L. puber at these deeper water enclaves, however, appears to be lower than that of the infralittoral zone. It would appear, therefore that L. puber is a characteristic species of the nearshore sub-littoral and littoral fringe.

Many brachyurans are very mobile with some species showing highly orientated movement patterns (Herrnkind 1983), including short-term foraging patterns, which are often tidally initiated, and long-term migrations which are primarily associated with reproductive behaviour and moulting cycles. Long-term migrations have been shown in several commercially important crabs such as Cancer pagurus (Edwards 1964, Bennett and Brown 1983) and Callinectes sapidus (van Engel 1958). Short-term migratory patterns occur in several nearshore species such as Carcinus maenas and Callinectes sapidus (Naylor 1962, Atkinson and Parsons 1973, Nishimoto 1980). These are tidally initiated with active foraging movement up shore with the rising tide and subsequent movement down the

shore prior to low water. The portunid Carcinus maenas shows both types of movement patterns with a seasonal long-term movement of adults offshore in winter and very marked short-term foraging patterns. The distance covered by short-term foraging movements of temperate portunids, however, has recieved relatively little attention. Naylor (1962), using baited traps, showed that C. maenas was capable of covering considerable distances up and down the shore over one tidal cycle. Mark-recapture data for C. maenas also indicate high mobility with generally little retension of crabs in one area (Edwards 1958). Hill (1978) tracked Scylla serrata using ultrasonic transmitters and found that individuals covered large distances in a single night, with a mean distance of 461m (n=12) and range between 219 and 910m. Hill (1978) concluded that individuals did not occupy a distinct territory, but did tend to remain in the same general area.

Choy (1986a) examined the rhythmical activity patterns of Liocarcinus puber and L. holsatus, and found increased locomotor activity during periods of darkness, and evidence to suggest that L. puber showed a circatidal rhythm as found for Carcinus maenas (Naylor et al. 1971). This chapter examines field evidence to identify any short- and long-term migratory behaviour found in L. puber.

10.2. Materials and Methods

10.2.1. Catch rate per unit effort

During the littoral sampling programme at Blackstone Rocks, Wembury (Chapter 3), careful note was taken of the collection time to enable an estimate of the relative abundance of adult and juvenile Liocarcinus puber on the shore. The initial survey of L. puber in the littoral zone found the most productive sampling area to be a band from Extreme Low Water Spring Tides (ELWST) to approximately 1m above Chart Datum. Therefore, sampling took place in this area on the Low Water Spring Tides each month with approximately the same effort of 4-6 man hours sampling. Similarly, the catch rate for the sub-littoral zones was recorded, however, this was so influenced by water visibility that little information on the subtidal abundance of L. puber could be gained.

10.2.2. Littoral tagging

A tagging programme was carried out in order to examine any short-term movements of adults between the littoral and sub-littoral. An area of approximately $75m^2$ on the western fringe of Blackstone Rocks was used as the study site (Fig. 10.1). The site, approximately rectangular, consisted of loose boulders and surrounding bedrock. Tidal height ranged from 0.2-1.1m above Chart Datum and was therefore only fully emersed at ELWST.

To investigate the daily movements and residence periods of Liocarcinus puber in one area, crabs were marked and released back into their original position on the shore. The marking programme was carried

out over three spring tidal periods in three consecutive months (July, August and September, 1987). All crabs >40mm LCW were numbered on their abdominal surface using waterproof nail varnish. By marking the abdominal surface, this also ensured that on re-examination of the area, coded individuals were not preferentially sampled, as each crab had to be caught, to fully inspect the abdominal surface. Individuals similarly marked in the laboratory had recognizable numbers after five months.

The size of the population, and rate of migration away from the parent population within the sampling area, was estimated using the Fisher-Ford method (Fisher and Ford 1947). The size of the parent population was estimated from the formula:

$$\underline{Ni} = \frac{\underline{ni}+1}{\underline{mi}+1} \times \underline{Mi}$$

where $\underline{Ni}$ is the population size on day $\underline{i}$, $\underline{ni}$ is the number caught on day $\underline{i}$, $\underline{mi}$ is the number of marked individuals caught on day $\underline{i}$, and $\underline{Mi}$ is the number of marks at risk on day $\underline{i}$. The rate of migration from the sampling area was estimated using the 'survival time' method described in the Fisher-Ford (1947) model. The mortality rate was assumed to approximate zero over the sampling periods eg. from the seventh to the eleventh of September, and therefore any reduction in the estimate of total numbers of crabs inhabiting the reef will be caused by emigration.

10.2.3. Sub-littoral tagging

Tagging experiments were also instigated for the infralittoral and circalittoral zones. Two short-term sub-littoral tagging programmes were carried out at F Buoy over the period 16th September, 1986 to 24th

November, 1986, and again from 1st June, 1987 to 15th September, 1987. Approximately weekly visits were made to the north east chain of F Buoy to code and recapture marked individuals. In order to minimise disturbance to individuals, all tagging and subsequent measurements were carried out *in situ*, and crabs were returned to their original location on the chain. The crabs were marked using the technique devised for *C. maenas* (Edwards 1958) by cutting one or more of the prominent anterio-lateral spines using nail clippers. This procedure was tested initially in the laboratory on six individuals of *Liocarcinus puber*; the spine did not regrow and the lesions soon sealed. The clipped spines were clearly distinguishable in the field from naturally damaged spines by their straight edges. The coded tag was also recognisable post moult and crabs showed no ability to regenerate spines over a single moult cycle. The laboratory-coded individuals showed no mortalities over the four month period for which they were held.

In the field, each crab was individually coded by spine clipping one or more of the ten available spines. To aid re-identification, the spines were numbered from one to ten from the left hand side of the dorsal surface. The outer spines (spine one and ten) were not used in the coding as these spines were kept intact to enable the accurate measurement of the carapace width of the individual. To further assist the re-identification of coded individuals, the sex and moult stage were recorded and carapace width was measured using purpose built underwater calipers (Fig. 10.2). The calipers gave an accuracy of $\pm$0.5mm when used underwater. The technique to measure crabs involved both divers, one to hold the crab in position whilst the other moved the sliding arm of the caliper and recorded the measurement. In practice the procedure was time-consuming but effective.

Initially, crabs of the infralittoral zone were also marked, however, problems in relocating the precise area sampled, particularly in low visibility, and the complex structure of inshore reef systems allowing crabs to 'hide' within the reef system, precluded representative resampling of this area and therefore infralittoral tagging experiments were aborted.

10.3. Results

10.3.1. Catch Rates

Catch rates for littoral crabs showed marked seasonal variations, indicating that immigration/emigration may occur at certain times of the year (Fig. 10.3). Immature crabs (<40mm LCW) showed peaks of catch rates in February/March 1986, October 1986 and, most noticeably, in October 1987 and April 1988 (Fig. 10.3A). Mature crabs (>40mm for females and >45mm for males) showed marked sexual differences in capture rates, particularly over the winter period when few females were captured (Fig. 10.3B). In general, peaks in the catch rate for adult females occurred in late summer (October 1986, August 1987) (Fig. 10.3B), whereas males showed a more constant presence in the littoral zone with peaks of abundance occurring intermitently (October 1985, May 1986, October 1986, April 1987 and September 1987) (Fig. 10.3B).

Mean monthly catch rates gives a clearer pattern (Fig. 10.4). Immature crabs had a marked peak in October and a smaller, less clear, peak in February (Fig. 10.4B). Both peaks coincided with the extreme low spring tides over the equinox periods (Fig. 10.4A). The October peak was made up largely of '0' group individuals of a size range of between 8-15 mm LCW. This group first appeared on the shore in large numbers from September onwards (Chapter 5). The decline in catch rate of immature crabs between the period of the autumn and spring equinox tides in September and February may indicate the high mortality rate of this size group. In July, there was a reduction in the numbers of immature crabs caught, with

the modal size of the '0' group reaching 30+ mm LCW by July/August (Chapter 5). Decline in numbers of Liocarcinus puber of this size range may reflect recruitment to the sub-littoral population at this size (Chapter 5). Apart from the peaks of numbers in October and February and decline in July, the numbers caught per man hour remained constant between 4.2 and 6.2 crabs person hour throughout the rest of the year. These data infer that immature crabs remained in the littoral zone throughout the year.

The catch rate data for mature females (>40mm LCW) in the littoral zone showed marked seasonal variability (Fig. 10.4C). Mature females were relatively common over the summer (June to September), but more scarce at other times (October and May) (Fig. 10.4C). Very few females were sampled between January and April, when the majority of mature females were ovigerous (Chapter 4). The few females sampled between January and April were predominantly non-ovigerous, although a few ovigerous females were collected from low on the shore on exceptionally low tides (<0.5m above C.D.).

Males showed a relatively stable presence in the littoral zone, throughout the year, and numbers were only slightly elevated in summer and lowered in winter (Fig. 10.4D). There was, however, strong correlation of high numbers caught and equinox spring tides.

10.3.2. Sex ratio

The sex ratio for immature Liocarcinus puber (all sites combined) did not deviate significantly from 1:1 (χ^2 = 14.31, df = 11, P >0.1) (Fig. 10.5A; Table 10.1). The sex ratio for adults, however, was significantly different from 1:1 (χ^2 = 299.8, df = 11, P<0.001), with males prodominating giving a 59.4 : 40.6 % ratio male to female (Table

10.1). The reason for the deviation of sex ratio for adults compared to the 1:1 ratio of immature crabs is not clear. It may result from a sampling bias of males which, due to their pronounced aggressive displays when disturbed, which as a consequence, makes males more conspicuous than females. The similarity of the adult mean sex ratio for the three zones (40.5%, 38.5% and 41.4% for the littoral, infralittoral and circalittoral respectively, Table 10.1), however, suggests that this bias was consistant between zones. The monthly changes in sex ratio, show a distinct pattern between the three zones, suggesting seasonal movement of one section of the population (Fig. 10.5). Over winter, the sex ratio for the littoral shows a marked decline (ie. relative loss of females), particularly from February to May, which is matched by a corresponding increase in the infralittoral zone (Fig. 10.5). During, winter, many females were ovigerous (Chapter 4). From June until September, there was an increase in sex ratio in the littoral zone with high numbers of mature females observed on the shore (Fig 10.5B). There was an increase in sex ratio in both sub-littoral zones in August when many females were observed in pre-moult coupling prior to mating (Chapter 5). From October onwards, there was a steady decline in the sex ratio in the littoral zone and a concurrent increase in the circalittoral (no data obtained for the infralittoral) indicating an offshore movement of females over winter.

10.3.3. Littoral tagging

The mark-recapture data for littoral crabs showed few returns (Table 10.2). As the time between sampling was short (8 weeks), mortality is unlikely to have any significant influence, suggesting that the low returns were due to emigration. As there appeared to be no significant

loss of individuals (Section 10.3.1) over the three month period, there must also be a high level of immigration into the area. Certain male individuals were recaptured in each of the three months sampled. The most noticeable individual was a male of 56.2mm LCW originally marked on 12th July, 1987 and subsequently recaptured on the 15th July, 1987 and then on the 14th August, 1987 and was again sampled on 7th, 8th, 9th and 10th of September, 1987. Three other male crabs were also captured repeatedly, and these four individuals were recaptured a total of sixteen times. The total number of recaptures of the eighty two crabs marked was thirty three (Table 10.2). It would appear, therefore, that a small number of male crabs are permanently resident on this portion of the reef, whilst the majority of individuals migrate in and out of the area possibly as a result of feeding excursions. Estimates for the size of the male population that effectively utilises the sampling area for the sampling period in September varied from twenty-three to ninety-seven males. The 'survival rate' was estimated at 85% indicating that one sixth of the males move away from the sampled area over each 24h period.

Females showed very low returns compared with males and no individual was recaptured more than once (Table 10.2). The numbers recaptured for the September period were too low to analyse using the Fisher-Ford model. The data, however, indicate that females are highly mobile, as reported for males.

On return to the littoral sampling site (Fig. 10.1) after eight months from the termination of the tagging programme, out of eight male crabs captured, three were clearly marked. All three crabs were only previously observed once in the tagging programme, at the time of marking. The recapture of these crabs at the same site after such a protracted

period suggests that, although there is a high degree of short-term emigration from the sampling area, the crabs may show limited long-term lateral movement.

10.3.4. Littoral movement - observations *in situ*

Onshore movements by *Liocarcinus puber* were assessed qualitatively by night-time snorkelling at Blackstone Rocks on two occasions during August 1987. *L. puber* were observed at all levels within the intertidal. The lack of large numbers of adult *L. puber* at high shore levels at low water during the day, suggests that *L. puber* migrates up the shore with the tide and back down, to near, or below the low water mark with the receding tide.

10.3.5. Sub-littoral tagging

Tagging returns at F Buoy showed several multiple recaptures (Table 10.3), however, the low number of individuals marked, and low numbers recaptured, prevented analysis using the Fisher-Ford model. The recapture data generally indicate a mobile population with the majority of individuals remaining at F Buoy for a limited period.

10.4 Discussion

From the rather limited data presented here, Liocarcinus puber appears to display similar long- and short-term movement patterns, to those described for Carcinus maenas (Edwards 1958, Naylor 1962, Crothers 1968, Atkinson and Parsons 1973). The lower catch rates in the littoral zone for adult L. puber over the winter (Choy 1986a, present study) suggest a general seaward movement during the coldest months. Naylor (1962), however, found that mature C. maenas actively foraged over the intertidal at night during winter, and concluded that the 'seaward' movement in winter was to a depth at low water where the majority of individuals were not emersed. The same interpretation appears valid for L. puber which remained comparatively abundant in the infralittoral zone. The stimulus for this movement is not known, however, Crisp et al. (1964) noted high mortalities of L. puber during the extreme winter of 1963, and suggested that low temperatures and aerial exposure may be critical for this species.

Mark-recapture experiments of adult Liocarcinus puber in the littoral zone indicated a highly mobile population, with low residence times. Based on the extremely high daily loss rates of marked individuals and the consistantly high number of crabs observed Edwards (1958) concluded for C. maenas also showed high emigration to and immigration from the sub-littoral on a nightly basis. The littoral mark-recapture experiment for L. puber established that few crabs remained in the study area, and those that did appeared to remain permanently at this position on the shore. The majority of marked crabs moved away from this area overnight, substantiating the in situ observations which showed a short-term, on-shore

migration at night-time high water.

Evidence from catch rate data for immature L. puber and C. maenas indicate that immature crabs remain in the littoral zone and show little migratory movement (Naylor 1962, Choy 1986a, present study). Naylor (1962) showed marked differences in the littoral distribution of immature (<30mm CW) and mature C. maenas. In general, immature C. maenas remain in the littoral zone throughout the year whereas their larger counterparts migrate from the lower shore at night to forage over the shore as a whole (Naylor 1962).

Male Liocarcinus puber showed a more consistant presence in the littoral than females, with females only exceeding male numbers in June and July (present study). This increase in the relative abundance of females on the shore coincides with a large percentage of females finishing brooding eggs (Chapter 4) and the commencement of moulting of mature males (Chapter 5). Few mature males were observed moulting, or in a soft moult stage, in the littoral zone, whilst large numbers of fresh exuviae were observed in the sub-littoral suggesting that prior to moulting male L. puber move into the sub-littoral. Mature female L. puber were found on the shore over winter but in low numbers; the same was reported for C. maenas (Naylor 1962). The high numbers of ovigerous females in the sub-littoral over winter, infers that reproductive females move to the sub-littoral to ensure optimal conditions for egg development (ie. more constant salinity, temperature and oxygen levels) (present study). This movement to the sub-littoral by ovigerous females has also been noted for C. maenas (Naylor 1962).

The similarity between Liocarcinus puber and Carcinus maenas in movement pattern and their common dietry preference (Ropes 1968, Elner

1977, Choy 1986a, 1986b, present study) suggests that competition may occur between these two species. L. puber and C. maenas, however, seldom co-exist on the same shore level (pers. obs.). C. maenas is physiologically adapted to fluctuating environmental conditions, including salinity (Broekhuysen 1936), oxygen (Taylor 1988), aerial exposure (Johnson and Uglow 1985) and siltation (Crothers 1968), and may be considered as an estuarine and littoral crab coping well with long periods of aerial exposure. Conversely, L. puber shows little physiological adaptation to fluctuating conditions (Johnson and Uglow 1985, Taylor 1988), but prodominates in fully marine, rocky sub-tidal areas where its larger size, agility and broad diet (Chapter 7) may partially explain its dominance. Any competitive interaction due to similarity in foraging behaviour of the adults, therefore, probably only occurs at the interface of these two habitats.

The similarity in short-term and long-term migratory behaviour and diet of adults of the two species suggests that they both play a similar predatory role in the intertidal in their respective habitats. Where the two species are found on the same shore, such as Wembury, where conditions are fully marine, they generally occupy different shore levels (Lippett 1987). Liocarcinus puber occupies the lower shore and Carcinus maenas, is restricted to the upper reaches of the shore (Lippett 1987). This mutual exclusion of the two crab species may, in part, be explained by predatory pressure of L. puber on the smaller C. maenas (ap Rheinalt 1986, Chapter 7). Lippett (1987), in a study of the seasonal vertical distribution of these crab species at Wembury, found the distribution of C. maenas increased down the shore over the winter period coinciding with the offshore movement of L. puber, and receded back up the shore in early

summer with the onshore movement of L. puber. These observations suggest that C. maenas utilises its greater ability to withstand aerial exposure compared with L. puber to obtain a refuge and to exploit the upper reaches of the shore.

The movement of Liocarcinus puber over the littoral, and the low retension of marked crabs at F Buoy, implies a free-ranging lifestyle with little apparent long-term (ie. greater than one tidal cycle) territoriality of a particular area or crevice. The highly mobile behaviour of L. puber noted from the littoral zone may be extreme as crabs may move away from the area after being stressed at periods of spring tides by aerial exposure. The recapture of marked crabs at the same site after a period of eight months, however, suggests that crabs may remain in approximately the same area over extended periods of time.

Table 10.1. Sex ratio data for immature (male <45mm, female <40mm LCW) (all sites combined) and for adults in the littoral, infralittoral and circalittoral zones (two years data combined). ($N♀$ = number of females sampled; $N♀+♂$ = total number of crabs sampled; % = sex ratio ie. $N♀/N♀+♂$)

	IMMATURE			ADULTS								
	All Sites			Littoral			Infralittoral			Circalittoral		
Month	$N♀$	$N♀+♂$	%	$N♀$	$N♀+♂$	%	$N♀$	$N♀+♂$	%	$N♀$	$N♀+♂$	%
January	28	58	48.3	17	57	29.8	5	17	29.4	72	158	45.6
February	39	76	51.3	6	34	17.6	14	34	41.2	51	102	50.0
March	39	72	54.1	18	69	26.1	6	11	54.5	41	106	38.7
April	27	57	47.4	11	41	26.8	18	31	58.1	24	57	42.1
May	22	48	45.8	14	42	33.3	27	70	38.6	28	72	38.8
June	14	29	48.3	13	26	50.0	31	80	38.7	40	131	30.5
July	21	38	55.3	60	108	55.5	15	56	26.8	37	134	27.6
August	40	65	61.5	38	84	45.2	40	85	47.1	27	66	40.9
September	65	141	46.1	97	203	47.8	20	72	27.7	52	170	30.6
October	64	131	48.8	27	73	37.0	-	-	-	56	128	43.7
November	54	98	55.1	24	63	38.1	-	-	-	77	148	52.0
December	9	22	40.9	5	14	35.7	3	9	33.3	86	156	57.1
Total	422	835		330	814		179	465		591	1428	
Site Sex Ratio	422/835 = 50.5			330/814 = 40.5			179/465 = 38.5			591/1428 = 41.4		
Mean of Sex Ratio							1100/2707 = 40.6%					

Table 10.2 Littoral mark-recapture data for (A) males and (B) females. Numbers in parentheses indicate individuals that were repeatedly recaptured.

(A)

Date 1987	Number Captured	Number Marked	Date of recapture 12/7	13/7	15/7	12/8	13/8	14/8	7/9	8/9	9/9	10/9	11/9
12/7/	9	9	-										
13/7/	14	14	1	-									
15/7/	7	7	2(1)	2	-								
12/8/	5	5	0	1	0	-							
13/8/	8	8	0	1	0	1	-						
14/8/	5	5	1(1)	0	0	0	1	-					
07/9/	9	9	1(1)	0	1	1(1)	1	1	-				
08/9/	10	10	1(1)	0	0	1(1)	0	1(1)	1	-			
09/9/	12	12	1(1)	0	1(1)	1(1)	0	0	1	0	-		
10/9/	3	3	1(1)	0	0	0	1	1(1)	1	0	2	-	
11/9/	3	0	0	0	0	0	0	1(1)	0	1	1	0	

Marks at risk = 82 Recaptures = 33
Multiple recapture =16

(B)

Date 1987	Number Captured	Number Marked	Date of recapture 12/7	13/7	15/7	12/8	13/8	14/8	7/9	8/9	9/9	10/9
12/7/	19	19	-									
13/7/	15	15	2	-								
15/7/	1	1	1	0	-							
12/8/	9	9	0	0	0	-						
13/8/	9	5	0	0	0	0	-					
14/8/	8	1	1	0	0	0	0	-				
07/9/	9	9	0	0	0	0	0	0	-			
08/9/	7	3	0	0	0	1	1	0	1	-		
09/9/	14	14	0	0	0	0	0	0	2	0	-	
10/9/	13	3	0	0	0	0	0	0	0	0	0	-
11/9/	3	0	0	0	0	0	0	0	0	0	0	0

Marks at risk = 79 Recaptures = 9

Table 10.3. Sub-littoral mark-recapture data for (A) males sampled from the 16/9/86 to the 19/1/87, and (B) from 1/6/87 to the 11/8/87. Numbers in parentheses indicate individuals that were repeatedly recaptured.

(A)

Date	Number Captured	Number Marked	Date of recapture 16/9	23/9	10/10	14/10	4/11	24/11	19/1
16/9/86	14	14	-						
23/9/86	17	0	3	-					
10/10/86	21	21	2(1)	-	-				
14/10/86	14	5	2(2)	-	0	-			
04/11/86	18	0	0	-	4	0	-		
24/11/86	6	0	0	-	2	0	-	-	
19/1/87	9	0	0	-	1(1)	0	-	-	-

Marks at risk = 40

Recapture total = 14
Multiple recaptures = 4

(B)

Date	Number Captured	Number Marked	Date of recapture 1/6	8/6	22/6	29/6	14/7	21/7	29/7	11/7
01/6/87	8	8	-							
08/6/87	5	5	1	-						
22/6/87	14	2	2	0	-					
29/6/87	8	8	2(2)	0	0	-				
14/7/87	7	6	1(1)	0	0	0	-			
21/7/87	13	7	0	1	0	0	0	-		
29/7/87	6	3	0	0	0	0	0	0	-	
11/8/87	9	0	0	0	0	0	0	0	0	

Marks at risk = 39

Recapture total = 7
Multiple recaptures = 3

Figure 10.1. Area sampled for the littoral tagging experiment at Blackstone Rocks, Wembury. The area sampled was of broken rock and stones substrata between Chart Datum and 1.0m above C.D.. The approximate area of the sampling zone was $75m^2$.

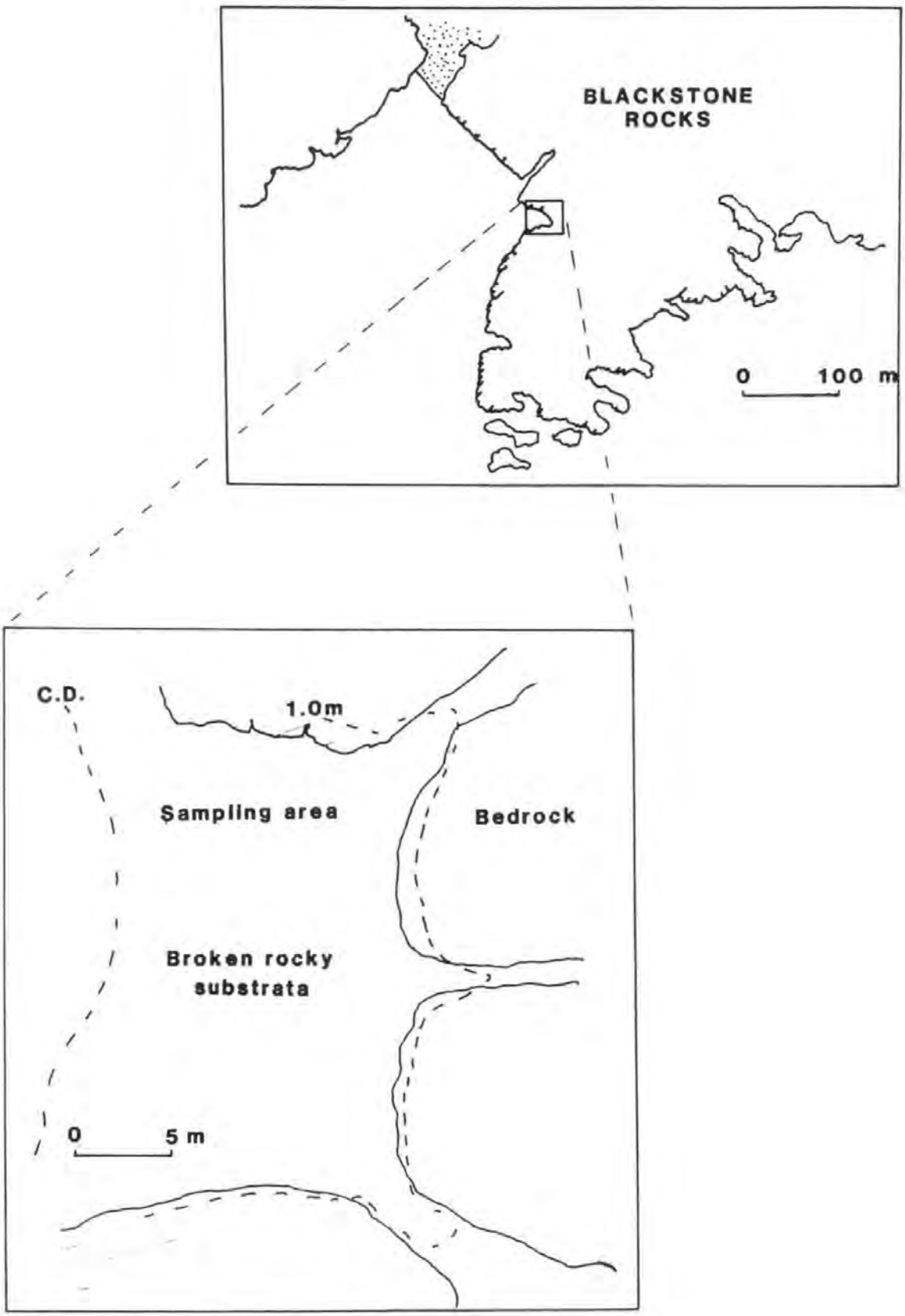
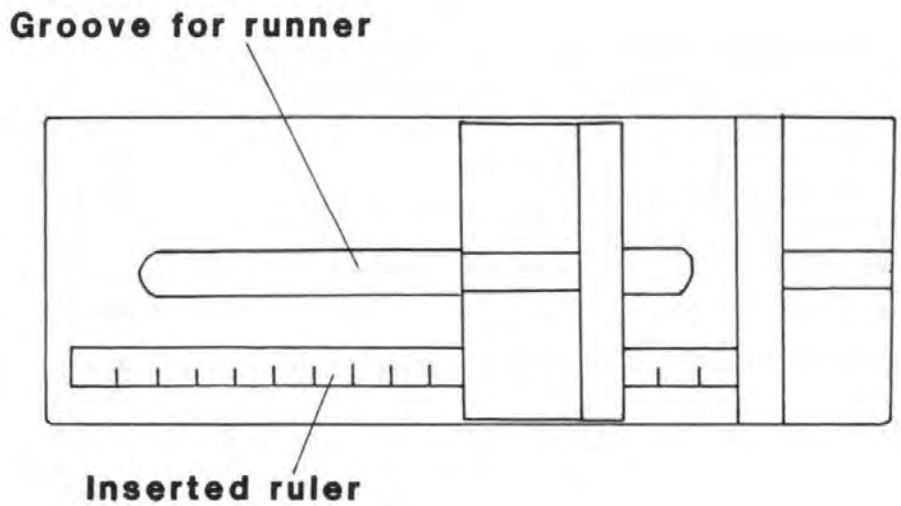

BLACKSTONE
ROCKS
0 100 m
C.D.
1.0m
Sampling area
Bedrock
Broken rocky
substrata
0 5 m

Figure 10.2. Design of the underwater calipers used to measure crab carapace width, (A) is vertical view and (B) horizontal profile.

(A)


Groove for runner
Inserted ruler
0 2cm

(B)

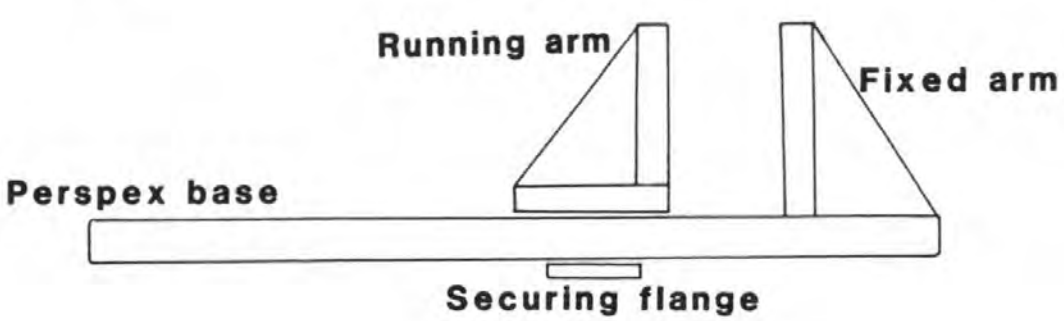

Running arm
Fixed arm
Perspex base
Securing flange

Figure 10.3. Catch per unit effort for (A) juveniles and (B) adults (open circles male; open triangles female). Data from the littoral sampling site at Blackstone Rocks; dashed lines indicate insufficient data due to low (<1 man hour) sampling effort.

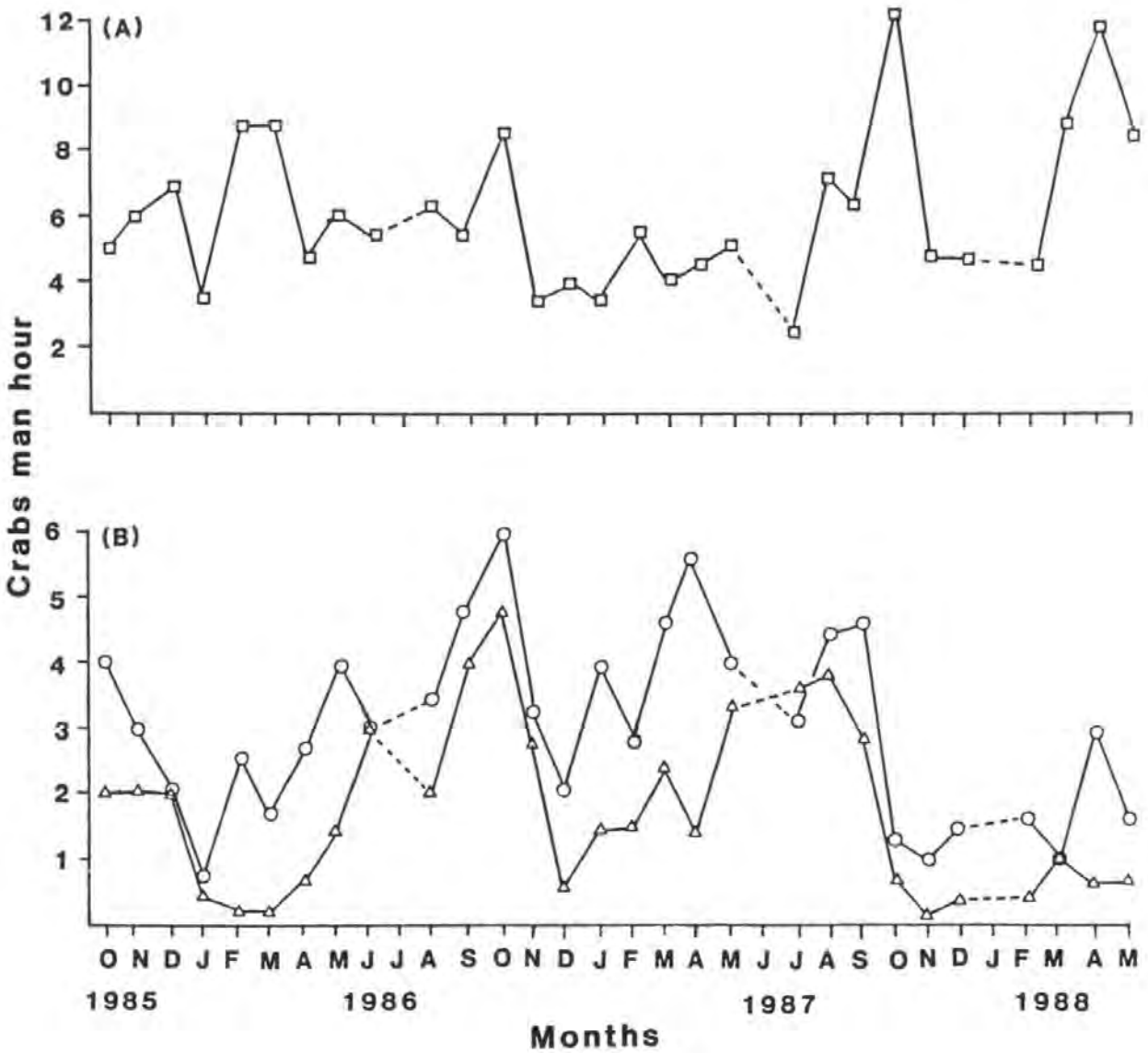

(A)
12
10
8
6
4
2
(B)
6
5
4
3
2
1
Crabs man hour
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M
1985
1986
1987
1988
Months

Figure 10.4. (A) Mean monthly seawater temperature (closed circles) taken via SCUBA from approximately one meter above the substratum at F Buoy (1986 and 1987 data combined), together with the mean of the two year's lowest astronomical tide for each month for Plymouth (closed triangles). (B) Mean monthly catch per man hour for juvenile Liocarcinus puber at Blackstone Rocks, Wembury. (C) Mean monthly catch per man hour for adult female Liocarcinus puber at Blackstone Rocks, Wembury. (D) Mean monthly catch per man hour for adult males Liocarcinus puber at Blackstone Rocks, Wembury. In each case, vertical line represents ± 1 S.E.

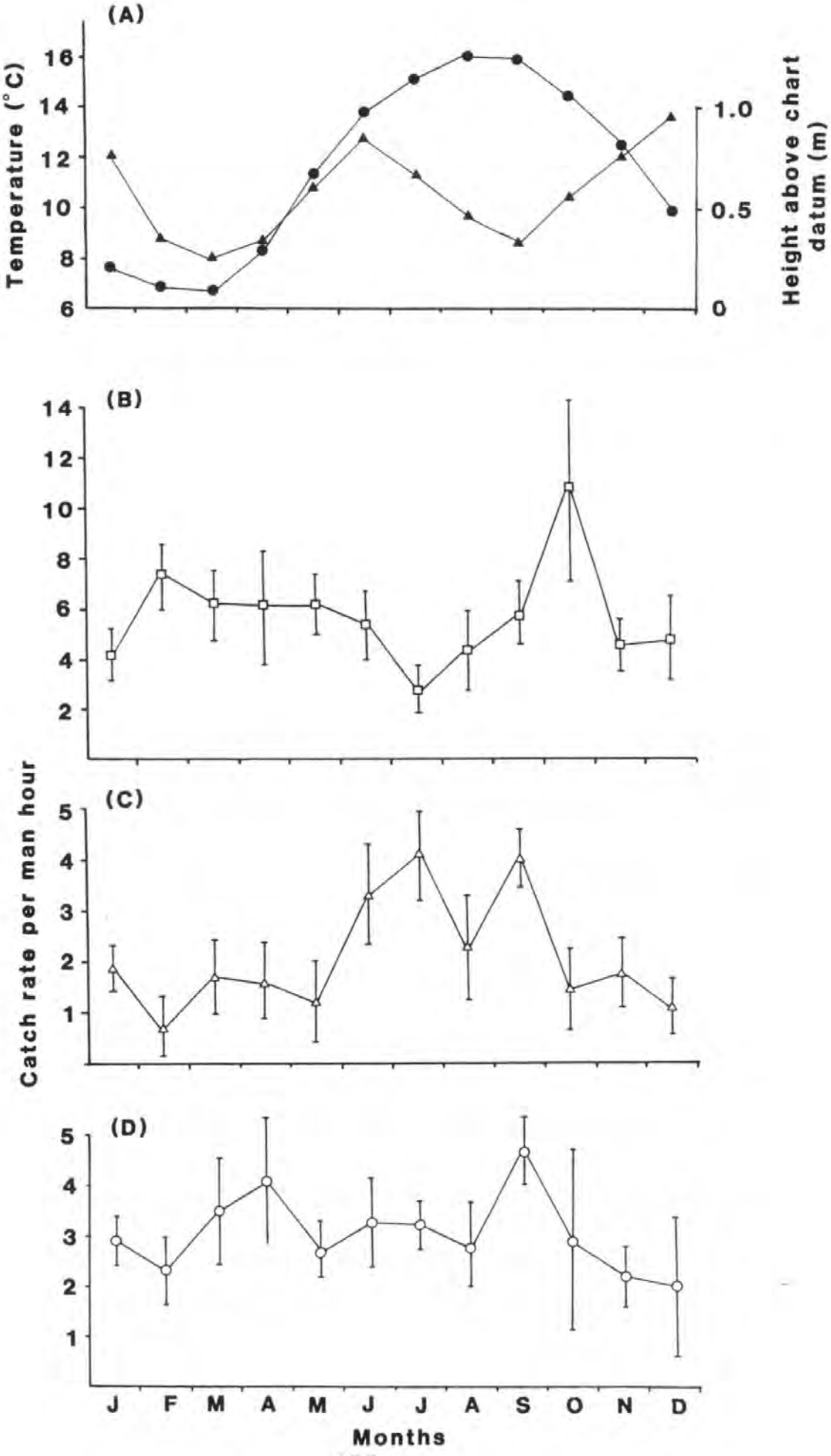
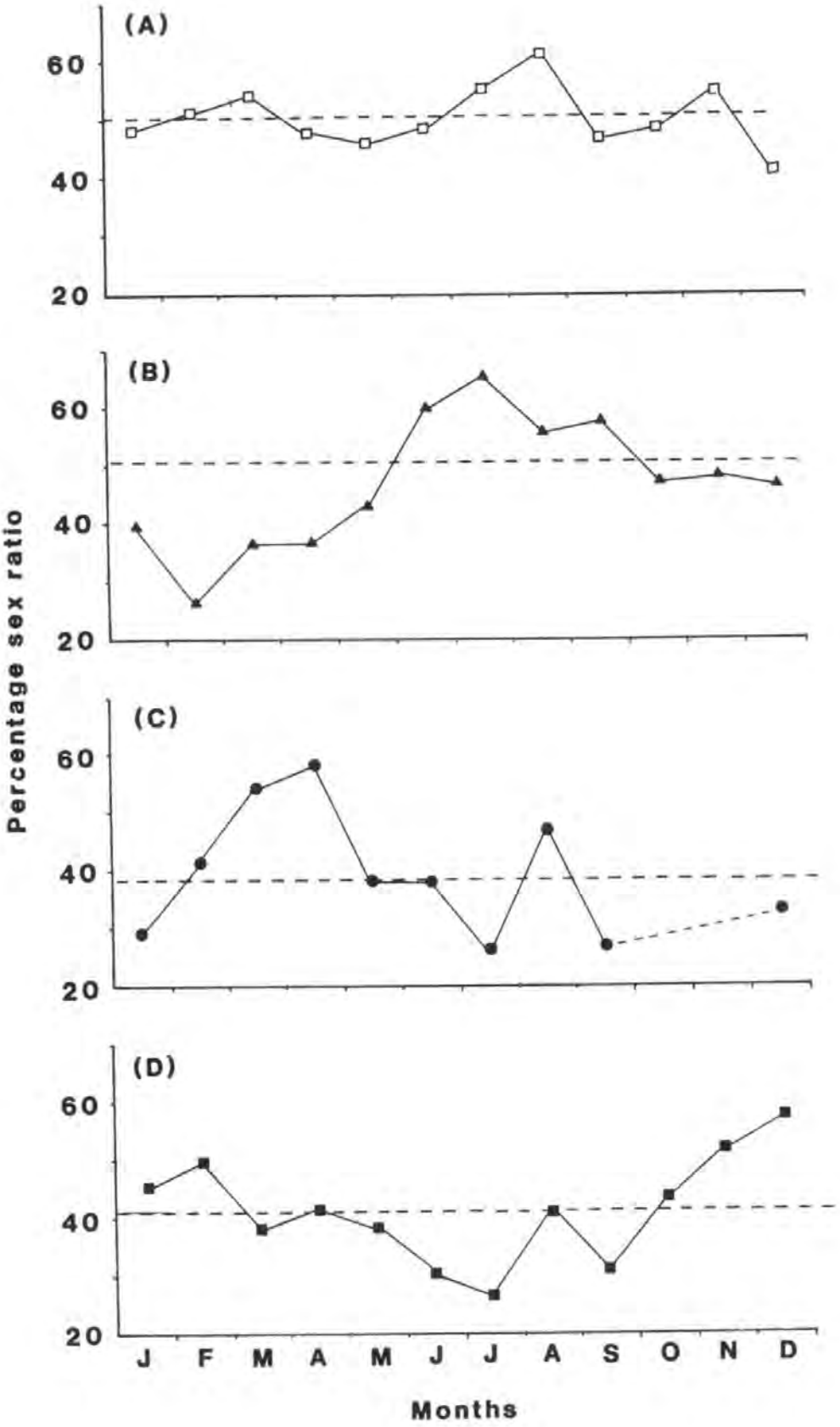

(A)
Temperature (°C)
16
14
12
10
8
6
1.0
0.5
0
Height above chart datum (m)
(B)
14
12
10
8
6
4
2
(C)
5
4
3
2
1
Catch rate per man hour
(D)
5
4
3
2
1
J F M A M J J A S O N D
Months

Figure 10.5. Sex ratios (expressed as the percentage of females over the combined total of males plus females). (A) sex ratio for juveniles (male <45mm and female <40mm LCW), data from all sites combined; (B) sex ratio for adult Liocarcinus puber in the littoral zone; (C) sex ratio for adult L. puber in the infralittoral zone; and (D) sex ratio for adult L. puber in the circalittoral zone.


(A)
(B)
(C)
(D)
60
40
20
Percentage sex ratio
J F M A M J J A S O N D
Months

CHAPTER 11

GENERAL DISCUSSION

11.1 General Discussion

World-wide, certain species of portunid crabs are very heavily exploited, and fisheries such as that for the blue crab Callinectes sapidus on the east coast of the USA is of major economic importance to the region. Alverson (1971), however, suggested that, taken as a group, portunids may be under-fished and that scope is available for further expansion particularly for tropical species. Reasons for the low exploitation of certain species may be related to their small maximum size and to difficulties in maintaining caught crabs in optimal condition for market. The portunid species of the N.E. Atlantic region have, until recently, been exploited predominantly only in Southern Europe. In Southern Europe, the species exploited are Liocarcinus puber, L. depurator and Carcinus maenas. However, compared with other economically exploited portunids, the European species are small, with L. puber, the largest of these species, reaching a maximum size of 125mm carapace width (MacMullen 1983). L. puber is also difficult to maintain, and high mortality occurs during the live export of this species (Whyman et al. 1985).

In general, portunid crabs, predominate in shallow water and often in estuarine or lagoon-type systems, where they may be exposed to fluctuating salinities (Tagatz 1968, Hill 1976, Paul 1982, Potter 1983). These habitats are characterised by soft sediments and most portunids show some degree of burrowing into the substrata for concealment during the day (Hill 1978, Glass 1985). Liocarcinus puber, however, appears to be the exception, as it shows strong preference for rocky and fully marine environments (Ingle 1980, 1983, Clark 1986, pers. obs.). Members of the genus Liocarcinus also show little

tolerance to fluctuating salinities, and only L. holsatus occupies a range of salinities down to approx. 24ppt. (Broekhuysen 1936). The extent, and dependance, L. puber has for rocky substrates has not been fully evaluated in this study, although L. puber was found almost exclusively in a 'defendable' rocky crevice during the day. No evidence was observed of any ability of L. puber to 'dig' or excavate a suitable site, a practice commonly observed in sympatric species such as Cancer pagurus which excavates shallow holes (pers. obs.), and Goneplax rhomboides which builds complex burrow systems (Atkinson 1974). The reliance of L. puber on crevices may partly limit population size in the present study area, as aggressive interactions between conspecifics over suitable 'hiding' sites were observed, particularly at dawn (pers. obs.).

Liocarcinus puber shows a very varied diet, typifying an opportunistic feeding style, with a broad range of prey species being consumed. The use of large quantities of brown algae in the diet appears to be unique for portunids, as they are generally regarded as carnivores (Warner 1977). The distribution of L. puber in the present study, was found to extend from approx. 1m above Chart Datum to a few infrequent specimens observed during SCUBA at depths >25m. L. puber, although, were only observed in large numbers in the infralittoral zone and shallow circalittoral zone (to approx. 12m). This rather limited distribution over which L. puber is abundant is also the depth range over which brown algae grows within the Plymouth area. It is therefore tempting to suggest a direct association between brown algae and L. puber, however, it should be noted that within the study area rocky substrata is only found to approx. 12m, with areas deeper than this generally having a soft sediment. On hard substrata at depths greater than 12m, such as on wrecks and rocky outcrops, L. puber are

observed but at low numbers compared to shallower depths. The effect of predation by L. puber on the nearshore sub-littoral community is difficult to assess directly from the data presented in this study. Further analysis of the rate of digestion of different food items, as well as examination of diet by stomach contents over a nightly cycle, may assist in further interpreting the effect of the removal of this species from this area.

Evidence from Gonzalez Gurriaran (1985a), Choy (1986b) and the present study strongly suggests that the larger crabs (>54mm LCW) (II+ year cohort, present study) have multiple broods over the period from December to August, whereas the I cohort produces a single brood over the spring period (March to June). The production of several broods of eggs leads to zoeae hatching over a broad period of the year (March to September) and subsequently recruitment may also occur over an extended period. Recruitment of juveniles in the littoral zone showed very marked variation between years, in this study, with the recruitment of the 1986 year class being extremely weak, with only a few individuals of the size range 5-10mm LCW being observed. The successful recruitment of first stage crabs depends on adequate survival of the zoeal and megalopa stages (Bakun and Parrish 1981). Survival of brachyuran zoeae is dependant on physical, predatory and trophic parameters of which the requirements for each zoeal stage are generally poorly understood (Haefner 1985b, McConaugha 1985). Bukan et al. (1982), suggest that appropriate survival environments occur as 'windows' in time and space. In environmental conditions where these 'windows' may be open for a short period or infrequently for the requirements of a species, there is a long and continous spawning, whilst short and localised spawning are indicative of stable conditions (Bakun et al. 1982).

The failure of the 1986 year class and the rather extended period over which zoeae are produced (Chapter 4), suggests that survival of the zoeae of Liocarcinus puber may vary significantly both temporally and spatially.

One of the most marked differences found between this study and that of Gonzalez Gurriaran (1981a, 1985b) is the rate of growth achieved in the first year. Gonzalez Gurriaran (1981a, 1985b) estimated that the Spanish 0 cohort of Liocarcinus puber reached a mean size of 50-55mm LCW, whilst results of this study suggests that a size range of 35-45mm LCW is typical. The variation in growth rate and maximum size shown by certain crustacean species suggests that within the growth format of various species there is a degree of 'plasticity' with growth dependant on local factors. Key parameters that may affect growth are physical factors such as temperature, substratum and salinity; and biological factors such as density dependant parameters and predation. The possible compound effect of variations of these factors on growth of L. puber, may be reflected by the differences observed in maximum size and size at maturity between studies (Gonzalez Gurriaran 1985b, Choy 1986a, present study). This variation in growth rates and size of maturity have been noted in several commercial species [eg. Nephrops norvegicus (Chapman and Howard 1988)]. Consequently to further the management of crustaceans, a greater understanding of the effects of these environmental and biological factors on growth are required. For L. puber the examination of parameters that are readily obtainable, such as size of sexual maturity (via relative growth studies), between geographical areas, may allow a clearer identification of the extent of, and possible factors influencing, the differences in growth format noted above.

A decline of landings of Liocarcinus puber has been noted after

periods of high fishing effort in certain sites in north west Scotland (P.H. MacMullen, pers. comm.). The concern about the state of the fishery has led to the introduction of a minimum landing size of 65mm carapace width, though to effectively conserve stocks of L. puber, protection of both ovigerous females, and some degree of protection for larger individuals may be required. The protection of the I year class, as introduced, allows some degree of reproductive effort, although it is rather low compared to the II year and older females (65+mm LCW) (Table 11.1). Large males (II and III year) are also required within the population to ensure successful mating of the larger females. Therefore a level of protection, either by lowering effort in certain areas or by leaving some areas unfished may allow the maintainance of a high reproductive effort. To further the management of Liocarcinus puber greater detail on the variation in growth between geographical areas is required. For other portunid fisheries world-wide there is a general paucity of detailed information on which to base management, due largely to problems faced in indentifying adult growth. Species that have been well documented, such as Callinectes sapidus, are those with a clearly determinate growth pattern allowing cohorts to be readily indentified. Therefore, some degree of assessing discontinuous growth is clearly required, and it is the author's opinion that the most appropriate approach is via a computer method, such as ELEFAN, but allowing the integration of discontinuous growth parameters. For further examination of growth in a commercial crustacean species in the UK, L. puber may prove to be a good species on which to base studies, due to its relatively short life-span and ease of monitoring juveniles.

Table 11.1. Size/age estimates of the fecundity of Liocarcinus puber from Plymouth U.K., for a female with a 4 year life expectancy.

Age	Mean size mm LCW	Reproductive state, number of broods	Fecundity (eggs)
'I' grp.	41	Female may mature, mate; produce single brood.	70,000
'II' grp.	65	Female copulate and produce two broods.	400,000
'III' grp.	74	Female copulate and produce two broods	740,000
		Total fecundity	1,210,000

ACKNOWLEDGEMENTS

I would like to express my sincerest thanks to Dr. Malcolm Jones for his guidance throughout this study and for his perception and inspiration in developing a SCUBA orientated project. I hope that the results of this study justify his dedication and drive in obtaining funding. I also wish to express my sincere thanks to Dr. M. Uttley who has been of great assistance in advice on statistics, and Dr. G. Warner for his helpful advice on sampling procedure. My thanks also goes to Drs. Ian Johnson and Peter Campbell who have sustained my enthusiasm in this field of work, and have been a wealth of assistance and advice. Thanks especially to Anne Torr for her uncomplaining stamina and her mastery of the difficult task of catching 'crabs'.

I would also like to thank the members of the Polytechnic Diving Unit and particularly Mr. John Vaudin and Mr. Frank Knott, who ensured that diving facilities were always available and for their understanding of the requirements of scientific diving. I would like to thank the 200+ divers who have assisted in sampling during their stay at the Polytechnic, inparticular I would like to thank Mr. Roger Haslem, Miss Joan Edwards, Dr. P. Campbell, Mr. Mark Burdass, Dr. Mark Costello aand Mr. Jerry Taylor.

I would also like to thank Joan for her keen sense of humour in the face of adversity, her immense powers of common sense and her unstinting support, both above and below water, over the past three and a half years. Without her, this project would still be in its early stages of infancy.

I would also like to take this opportunity to thank Devon County Council for their financial support of this study.

REFERENCES

Abby-Kalio, N.J. and G.F. Warner, in press. Heterochely and handedness in the shore crab Carcinus maenas (L.) (Crustacea: Brachyura). Zool. J. Linn. Soc..

Abello, P. and J. Cartes, 1987. Observaciones sobre la alimentacion de Liocarcinus depurator (L.) (Brachyura: Portunidae) en el Mar Catalan. Inv. Pesq. 51(1)): 413-419.

Adiyodi, K.G. and R.G. Adiyodi, 1983. Reproductive biology of invertebrates. Vol 1. Oogenesis, oviposition and oosorption. John Wiley & Sons, New York, 770pp.

Adiyodi, K.G. and R.G. Adiyodi, 1984. Reproductive biology of invertebrates. Vol.2. Spermatogenesis and sperm function. John Wiley & Sons, New York, 720pp.

Allen, J.A., 1967. The Fauna of the Clyde Sea Area - Crustacea: Euphausiacea and Decapoda; with an illustrated key to British species. Scot. Mar. Biol. Assoc., 116pp.

Alverson, F.G., 1971. Internation Trade - Crab. FAO Development Program, Rome, 16: 1-19.

Amsler, M.O. and R.Y. George, 1984. The effect of temperature on the oxygen consumption and development rate of the embryos of Callinectes sapidus Rathbun. J. Exp. Mar. Biol. Ecol., 82: 221-229.

Annala, J.H., J.L. McKoy, J.D. Booth and R.B. Pike, 1980. Size at the onset of sexual maturity in female Jasus edwardsii (Decapoda: Palinuridae) in New Zealand. N.Z. J. Mar. Freshwat. Res., 14(3): 217-227.

ap Rheinallt, T., 1986. Size selection by the crab Liocarcinus puber feeding on mussels Mytilus edulis and the shore crab Carcinus maenas: the importance of mechanical factors. Mar. Ecol. Prog. Ser., 29(1): 45-53.

ap Rheinallt, T. and R.N. Hughes, 1985. Handling methods used by the velvet swimming crab Liocarcinus puber when feeding on molluscs and shore crabs. Mar. Ecol. Prog. Ser., 25: 63-70.

Ary, R.D., C.K. Bartell, R.M. Grisoli and M.A. Poirrer, 1985. Morphological changes in regenerating chelipeds of the blue crab, Callinectes sapidus, as indicators of the progression of the moult cycle. J. Shellfish Res., 5(1): 1-8.

Ary, R.D., C.K. Bartell and M.A. Poirrier, 1987. The effects of chelotomy on moulting in the blue crab Callinectes sapidus. J. Shellfish Res., 6(2): 103-108.

Atkinson, R.J.A., 1974. Behavioural ecology of the mud-burrowing crab Goneplax rhomboides (L.). Mar. Biol. (Berlin), 25: 239-252.

Atkinson, R.J.A. and A.J. Parsons, 1973. Seasonal patterns of migration and locomotor rhythmicity in populatios of Carcinus maenas. Neth. J. Sea Res., 7: 81-93.

Bakun, A. and R.H. Parrish, 1981. Environmental inputs to fishery population models for eastern boundary current regions. In, Sharp, G.D., (Ed.): I.O.C. Workshop Report No. 28, UNESCO, Paris: 67-104.

Bakun, A., J. Bayer, D. Pauly, J.G. Pope and G.D. Sharp, 1982. Ocean Science in relation to living resources. Can. J. Fish. Aquat. Sci., 39(7): 1059-1070.

Belehradek, J., 1935. Temperature and living matter. Protoplasma Monogr., 8: 1-277.

Belehradek, J., 1957. Physiological aspects of heat and cold. Ann. Rev. Physiol., 19: 59-82.

Bennett, D.B., 1970. Crab Tagging in S.W. England. ICES Shellfish and Benthos Comm., K13: 5pp.

Bennett, D.B., 1973. The effect of limb loss and regeneration on the growth of the edible crab Cancer pagurus L.. J. Exp. Mar. Biol. Ecol., 13: 45-53.

Bennett, D.B. and C.G. Brown, 1983. Crab (Cancer pagurus) migrations in the English Channel. J. Mar. Biol. Ass. U.K., 63: 371-391.

Bennett, D.B. and S.R.J. Lovewell, 1983. Lobster (Homarus gammarus (L.)) tagging trials in England. MAFF Fish. Res. Tech. Rep., 71: pp.1-7.

Berrill, M., 1982. The life cycle of the green crab Carcinus maenas at the northern end of its range. J. Crust. Biol., 2(1): 31-39.

Black, W.A.P., 1954. Concentration gradients and their significance in Laminaria saccharina (L.) Lamour. J. Mar. Biol. Ass. U.K., 33: 49-60.

Black, W.A.P. and W.J. Cornhill, 1951. A method for the estimation of fucosterol. J. Sci. Fd. Agric., 2: 387-390.

Bliss, D.E., 1960. Autotomy and Regeneration. In, Waterman, T.H., (Ed.): Physiology of Crustacea, Vol. 1. New York, London: Chap. 17, 561-589.

Botsford, L.W., 1985. Models of growth. In, Wenner, A.M., (Ed.): Crustacean Issues 3, Factors in Adult Growth. A.A.Balkema, Rotterdam/Boston: 171-191.

Broekhuysen, G.J., 1936. On development, growth and distribution of Carcinides maenas (L.). Arch. Neerl. Zool., 2: 257-399.

Cassie, R.M., 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshwat. Res., 5: 513-522.

Capart, A., 1951. Crustaces Decapodes Brachyures. In: Expeditions Oceanographique Belge dans les Eaux Cotieres Africaines de l'Atlantique Sud (1948-1949). Resultats Scientifiques. 3(1): 11-205.

Chapman, C.J. and F.G. Howard, 1988. Environmental influences on Norway lobster (Nephrops norvegicus) populations and their implications for fishery management. In, Fincham, A.A. and R.S. Rainbow, (Eds.): Aspects of Decapod Crustacean Biology. Symp. zool. Soc. Lond., 59: 343-353.

Chapman, V.J. and D.J. Chapman, 1980. Seaweeds and their uses. Chapman and Hall, London, 225pp.

Christiansen, M.E., 1969. Marine Invertebrates of Scandinavia. No.2 Crustacea, Decapoda, Brachyura. Universitetsforlaget, Oslo. pp.1-143

Chittleborough, R.G., 1974. Development of a tag for the western rock lobster. Rep. CSIRO Australia Div. Fish. Oceanogr. Mar. Lab. Cronulla, Sydney, (58): 19pp.

Chittleborough, R.G., 1976. Growth of juvenile Panulirus longipes cygnus George on coastal reefs compared with those reared under optimal environmental conditions. Aust. J. Mar. Freshwat. Res., 27: 279-295.

Choy, S.C., 1986a. Ecological studies on Liocarcinus puber(L.) and L.holsatus (Fabricius) (Crustacea, Brachyura, Portunidae) around the Gower Peninsula, South Wales. Ph.D. Thesis. University of Wales. 341pp.

Choy, S.C., 1986b. Natural diet and feeding habits of the crabs Liocarcinus puber and L. holsatus (Decapoda, Brachyura, Portunidae). Mar. Ecol. Prog. Ser., 31: 87-99.

Churchill, E.P., 1921. Life history of the blue crab. Bull. U.S. Bur. Fish., 36: 95-128.

Clark, P.F., 1986. North-East Atlantic Crabs; an atlas of distribution. Marine Conservation Society, Ross-on-Wye. 252 pp.

Colman, J.S., 1940. On the faunas inhabiting intertidal seaweeds. J. Mar. Biol. Ass. U.K., 24: 129-83.

Conan, G.Y., 1978. Average growth curves and life history in a Nephrops norvegicus population from Northern Bay of Biscay. ICES, Shellfish Comm., K, 21: pp 58.

Conan, G.Y., 1985. Periodicity and phasing of molting. In, Wenner, A.M., (ed.): Crustacean Issues 3, Factors in Adult Growth. Balkema, Rotterdam, Boston: 73-101.

Conan, G.Y. and K.R. Gundersen, 1979. Growth curve of tagged lobsters (Homarus gammarus) in Norwegian waters as inferred from the relative increase in size at moulting and frequency of moult. Rapp. P-v. Reun. Cons. Int. Explor. Mer., 175: 155-166.

Conklin, D.E., 1980. Nutrition. In, J.S. Cobb and B.F. Philips, (Eds.): The Biology and management of Lobsters. Academic Press, New York: 277-300.

Cooper, L.H.N., 1958. Sea temperatures in Plymouth Sound. J. Mar. Biol. Ass. U.K., 37: 1-3.

Cooper, R.A., 1970. Retention of marks and their effects on growth, behaviour and migration of the American lobster (Homarus americanus). Trans. Am. Fish. Soc., 99: 409-417.

Crisp, D.J., J. Moyse and A. Nelson-Smith, 1964. The effects of the severe winter of 1962-63 on marine life in Britain. J. Anim. Ecol., 33: 165-210.

Crothers, J.H., 1967. The biology of the shore crab, Carcinus maenas (L.); 1. The background - anatomy, growth and life history. Fld. Stud., 2: 4407-434.

Crothers, J.H., 1968. The biology of the shore crab Carcinus maenas (L.); 2. The life of the adult crab. Fld. Stud., 2: 579-614.

Dall, W. and D.J.W. Moriarty, 1983. Nutrition and Digestion. In, Bliss, D,E., (Ed. in chief): The Biology of Crustacea. Vol. 5. Academic Press, New York, London: 215-262.

Dassow, J.A., 1969. Crab industry. In, Firth, E.D., (Ed.): The encyclopedia of marine resources. Van Nostrand Reinhold Co., New York: 105-154.

Davidson, R.J. and I.D. Marsden, 1987. Size relationships and relative growth of the New Zealand swimming crab Ovalipes catharus (White, 1843). J. Crust. Biol., 7(2): 308-317.

Dittel, A.I., Epifano, C.E. and J.B. Chavarria, 1985. Population biology of the portunid crab Callinectes arcuatus (Ordway) in the Gulf of Nicoya, Costa Rica, Central America. Est. Coastal and Shelf Science, 20: 593-602.

Drach, P., 1933. Sur la croissance de l'abdomen chez les Brachyoures. C. R. Acad. Sci., Paris, 197: 93-95.

Drach, P., 1936. Croissance allometrique et dimorphisme sexual chez les Brachyoures. C.R. Acad. Sci., Paris, 203: 820-822.

Drach, P., 1939. Mue et cycle d'intemue chez les crustaces decapodes. Ann. Inst. Oceanogr., Monaco, 19: 103-391.

Draper, N.R. and H. Smith, 1966. Applied regression analysis. John Wiley and Sons, Inc., New York, 407pp.

Du Preez, H.H., 1984. Molluscan predation by Ovalipes punctatus (De Haan). J. Exp. Mar. Biol. Ecol., 84: 55-71.

Du Preez, H.H. and A. McLachlan, 1984a. Biology of the three-spot swimming crab Ovalipes punctatus (De Haan). II. Growth and moulting. Crustaceana, 47(2): 113-120.

Du Preez, H.H. and A. McLachlan, 1984b. Biology of the three spotted swimming crab Ovalipes punctatus (De Haan). III. Reproduction, fecundity and development. Crustaceana, 47(3): 285-297.

Ebling, F.J., J.A. Kitching, L. Muntz and C.M. Taylor, 1964. The ecology of Lough Inne. XIII. Experimental observations of the destruction of Mytilus edulis and Nucella lapillus by crabs. J. Anim. Ecol., 33: 73-82.

Edwards, R.L., 1958. Movements of individual members of a population of the shore crab, Carcinus maenas L., in the littoral zone. J. Anim. Ecol., 27: 37-45.

Edwards, E., 1964. The use of the suture tag for the determination of growth increments and migrations of the edible crab (Cancer pagurus). ICES CM. Shellfish Comm. 42, pp8.

Edwards, E., 1965. Observations on the growth of the edible crab (Cancer pagurus). Rapp. P-v. Reun. Cons. per. int. Explor. Mer., 156: 62-70.

Edwards, E., 1971. A contribution to the bionomics of the edible crab Cancer pagurus (L.) in English and Irish waters. PhD. thesis, Nat. Univ. Ireland, Galway, 123pp.

Efford, I.E., 1969. Egg size in the sand crab Emerita analoga (Decapoda, Hippidae). Crustaceana, 16: 15-26.

Elner, R.W., 1977. The predatory behaviour of Carcinus maenas (L.). Ph.D. thesis. University College of North Wales, Bangor, 91pp.

Elner, R.W., 1981. Diet of green crab Carcinus maenas (L.) from Port Herbert, Southwestern Nova Scotia. J. Shellfish Res., 1(1): 89-94.

Farmer, A.S., 1973. Age and growth in Nephrops norvegicus(L.) (Decapoda: Nephropidae). Mar. Biol., 23: 315-325.

FAO 1980. Yearbook of fishery statistics 1980. Vol.50. Food and Agriculture Organization of the United Nations - Rome, X: 385pp.

FAO 1985. Yearbook of fishery statistics 1985. Vol.60. Food and Agriculture Organization of the United Nations - Rome, viii: 461pp.

Fielder, D.R., and A.J. Eales, 1972. Observations on courtship mating and sexual maturity in Portunus pelagicus (L. 1766) (Crustacea: Portunidae). J. Nat. Hist., 6: 273-277.

Fong W.C. and K.H. Mann, 1980. Role of gut flora in the transfer of amino acids through a marine food chain. Can. J. Fish. Aquat. Sci., 37: 88-96.

Fisher, R.A. and E.B. Ford, 1947. The spread of a gene in natural conditions in a colony of the moth Panaxia dominula (L.). Heredity, 1: 143-147.

Fuji, A., 1962. Studies on the biology of the sea urchin. V. Food consumption of Strongylocentrotus intermedius. Jap. J. Ecol., 12: 181-186.

Fujita, H. and K. Takeshita, 1979. Tagging technique for tanner crab long-term tag. Bull. Far Seas Fish. Res. Lab., 17: 223-226.

Gallagher, M. and W.D. Brown, 1975. Composition of San Francisco Bay brine shrimp (Artemia salina). Agric. Food Chem., 23: 630-633.

Gaschutz, G., D. Pauly, and N. David, 1980. A versatile BASIC program for fitting weight and seasonally oscillating length growth data. ICES. Statistics Cttee. D. 6: 23pp.

Glass, C.W., 1985. Observations on the ecology and behaviour of swimming crabs in a Scottish loch. Prog. in Underwater Sci., 11: 125-129

Gonzalez Gurriaran, E., 1978. Introduccion al estudio de la alimentacion en la necora, Macropipus puber (L.) (Decapoda - Brachyura) en la Ria de Arosa (Galicia- Espana). Bol. Inst. Esp. Oceanog., 4(241): 71-80.

Gonzalez Gurriaran, E., 1981a. Preliminary data on the population dynamics of the velvet swimming crab (Macropipus puber L.) in the Ria de Arousa (Galicia, N.W. Spain). ICES Shellfish and Benthos Comm., K16: 1-21.

Gonzalez Gurriaran, E., 1981b. La necora Macropipus puber (L.) (Decapoda, Portunidae) en los poliginos de bateas de la Ria de Arousa: Contribucion al estudio de su biologia. Resumen de la tesis presentada para aspivar al grado de Doctor en Biologia. Dept. Zool., Facul. Biol., Univ. Santiago de Compostela, Espania.

Gonzalez Gurriaran, E., 1981c. Primeros datos sobre el crecimiento en la ecdisiis y pruebas de sistemas de marcado de la necora, Macropipus puber (L.) (Decapoda, Brachyura) en el laboratotio. Boln. Inst. Espan. Oceanogr., 6(300): 115-136.

Gonzalez Gurriaran, E., 1985a. Reproduccion de la necora Macropipus puber (L.) (Decapoda, Brachyura), y ciclo reproductivo en la Ria de Arousa (Galicia, N.W. Espana). Boln. Inst. Espan. Oceanogr., 2(1): 10-32.

Gonzalez Gurriaran, E., 1985b. Crecimento de la necora Macropipus puber (L.) (Decapoda, Brachyura) en la Ria de Arousa (Galicia, N.W. Espana): y primeros datos sobre la dinamica de la pobalacion. Boln. Inst. Espan. Oceanogr., 2(1): 33-51.

Gray, E.H. and C.L. Newcombe, 1938. The relative growth of parts in the blue crab Callinectes sapidus Rathbun. Growth, 2: 235-246.

Gore, R.H. and L.E. Scotto, 1983. Studies on decapod Crustacea from the Indian River region of Florida. XXV. Carapacial and abdominal allometry of five species of subtropical parthenopid crabs (Brachyura, Parthenopidae). Crustaceana, 44: 1-22.

Gulland, J.A., 1983. Vol. 1. Fish stock assessment. A manual of basic methods. FAO/Wiley Series on Food and Agricult. John Wiley and Sons, New York, 223pp.

Gulland, J.A. and S.J. Holt, 1959. Estimation of growth parameters for data at unequal time intervals. J. Cons. Perm. Int. Explor. Mer., 25(1): 47-49.

Haddon, M. and R.G. Wear, 1987. Biology of feeding in the New Zealand paddle crab Ovalipes catharus (Crustacea, Portunidae). N.Z. J. Mar. Freshwat. Res., 21: 55-64.

Haefner, P.A. Jr., 1985a. Morphometry, reproduction, diet and epizoites of Ovalipes stephensoni Williams, 1976 (Decapoda, Brachyura). J. Crust. Biol., 5(4): 658-672.

Haefner, P.A. Jr., 1985b. The biology and exploitation of crabs. In, Bliss, D.E., (Ed. in chief): The Biology of Crustacea, Vol. 10, Academic Press, New York: 111-166.

Hamilton, P.V., R.T. Nishimoto and G.J. Halusky, 1976. Cheliped laterality in Callinectes sapidus (Rathbun). Biol. Bull. (Woods Hole), 150: 393-401.

Hard, W.L., 1942. Ovarian growth and ovulation in mature blue crab Callinectes sapidus Rathbun. Chesapeake Biol. Lab. Contrib., 46: 17pp.

Harding, J.P., 1949. The use of propability paper for the graphical analysis of polymodal frequency distributions. J. Mar. Biol. Assoc. U.K., 28: 141-153.

Hartnoll, R.G., 1969. Mating in the Brachyura. Crustaceana, 16: 161-181.

Hartnoll, R.G., 1974. Variations in growth pattern of some secondary sex characteristics in crabs (Decapoda, Brachyura). Crustaceana, 27: 131-136.

Hartnoll, R.G., 1978a. The effect of salinity and temperature on the post-larval growth of the crab Rithropanopeus harrisii. In, D.S. Mclusky and A.J. Berry, (Eds.): Physiology and Behaviour of marine organisms. Pergamon Press, Oxford: 349-358.

Hartnoll, R.G., 1978b. The determination of relative growth in Crustacea. Crustaceana, 34: 281-293.

Hartnoll, R.G., 1982. Growth. In: In, Abele, L.G., (Ed.): The Biology of Crustacea, Vol 2. Embrology, morphology and genetics. Academic Press, New York, London: 111-196.

Hartnoll, R.G., 1985. Growth, sexual maturity and reproductive output. Crustacean Issues, A.A. Balkema, Rotterdam, 3: 101-128.

Hartnoll, R.G. and R.G.K. Paul, 1982. The embryonic development of attached and isolated eggs of Carcinus maenas. Int. J. Invert. Reprod., 5: 247-252.

Hartnoll, R.G. and P. Gould, 1988. Brachyuran life history strategies and the optimization of egg production. Symp. Zool. Soc. Lond., 59: 1-9.

Herrnkind, W.F., 1983. Movement patterns and orientation. In, Bliss, D .E., (Ed.): Biology of Crustacea, Vol. 7. Academic Press, New York: 41-106.

Hiatt, R.W., 1948. The biology of the lined shore crab (Pachygrapsus crassipes Randall). Pac. Sci., 2: 135-213.

Hill, B.J., 1975. Abundance, breeding and growth of the crab Scylla serrata in the South African estuaries. Mar. Biol. (Berlin), 32: 119-126.

Hill, B.J., 1976. Natural food, foregut clearance rate and activity of the crab Scylla serrata. Mar. Biol. (Berlin), 34: 109-116.

Hill, B.J., 1978. Activity, track and speed of movement of the crab Scylla serrata in an estuary. Mar. Biol. (Berlin), 47:135-141.

Hill, B.J. and T.T. Wassenburg, 1985. A laboratory study of the effect of streamer tags on mortality, growth, moulting and duration of nocturnal emergence of the tiger prawn Penaeus esculentus (Hanswell). Fisheries Res., 3(3): 223-235.

Hiscock, K., 1985. Aspects of the Ecology of Rocky Sublittoral Areas. In, Moore, P.G. and R. Seed, (Eds.): The Ecology of Rocky Coasts. Hooder and Stoughton, Lond: 290-329.

Hughes, J.T., J.J. Sullivan and R. Shleser, 1972. Enhancement of lobster growth. Science, 177: 1110-1111.

Huxley, J.S., 1927. Further work on heterogonic growth. Biol. Zentralbl., 47: 151-163.

Huxley, J.S., 1932. Problems of relative growth. Second edition (Methuen, London), pp312.

Hylleberg Kristensen, J., 1972. Carbohydases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol., 14: 130-142.

Ingle, R.W., 1980. British Crabs. Oxford Univ. Press & Br. Mus. nat Hist., 222pp.

Ingle, R.W., 1983. Shallow water crabs. Synopses of the British fauna; a new series. Vol. 25, 205pp.

Jefferts, K.B., P.K. Bergman and H.F. Fiscus, 1963. A coded wire identification system for macro-organisms. Nature, 98: 460-462.

Jensen, A., M. Indergaard and T.J. Holt, 1985. Seasonal variations in the chemical composition of Saccorhiza polyschides (Laminariales, Phaeophyceae). Botanica Marina, 28: 375-381.

Jewett, S.C., N.A. Sloan and D.A. Somerton, 1985. Size at sexual maturity and fecundity of the fjord dwelling golden king crab Lithodes aequinispina Benedict from Northern British Colombia. J. Crust. Biol., 5(3): 377-385.

Johnson, I. and R.F. Uglow, 1985. Some effects of aerial exposure on the respiratory physiology and blood chemistry of Carcinus maenas (L.) and Liocarcinus puber (L.). J. Exp. Mar. Biol. Ecol., 194: 151-165.

Johnson, P.T., 1983. Diseases caused by Viruses, Rickettsiae, Bacteria and Fungi. In, Bliss, E.D., (Ed.): Biology of Crustacea. Vol.6. Academic Press, New York: 1-78.

Jones, M.B., 1978. Aspects of the biology of the big-handed crab, Heterozius rotundifrons (Decapoda, Brachyura), from Kaikoura, New Zealand. J. R. Soc. N.Z., 7: 259-272.

Jones, M.B. and M.J. Simons, 1983. Latitudinal variation in reproductive characteristics of a mud crab, Helice crassa (Grapsidae). Bull. Mar. Sc., 33(3): 656-670.

Kinnear, J.A.M., and J. Mason, 1987. A preliminary study of an unfished population of the velvet swimming crab Liocarcinus puber (L.). ICES Shellfish Comm., 6: 1-4.

Kitching, J.A., I.T. Macan and C.H. Gilson, 1934. Studies in Sublittoral Ecology. (1) A Submarine Gulley in Wembury Bay, South Devon. J. Mar. Biol. Ass. U.K., 19: 677-707.

Kitching, J.A., J.F. Sloane and F.J. Ebling, 1959. The Ecology of Lough Inne. VIII. Mussels and their predators. J. Anim. Ecol., 28: 331-341.

Kiortsis, V., and H.A.L. Trampusch, 1965. Regeneration in animals and related problems. North Holland Publ. Co., Amsterdam, 568pp.

Klein Breteler, W.C.M., 1975a. Growth and moulting of juvenile shore crabs, Carcinus maenas, in a natural population. Neth. J. Sea Res., 9: 86-99.

Klein Breteler, W.C.M., 1975b. Laboratory experiments on the influence of environmental factors on the frequency of moulting and the increase in size at moulting of juvenile shore crabs, Carcinus maenas. Neth. J. Sea Res., 9(1): 100-120.

Kurata, H., 1962. Studies on the age and growth of Crustacea. Bull. Hokkaido Reg. Fish. Res. Lab., 24: 1-115.

Laevastu, R., 1965. Manual of methods in fisheries biology. FAO manuals in fishery science. No. 1. 46pp.

Lasser, G.W. and W.V. Allen, 1976. The essential amino acid requirements of the Dungeness Crab (Cancer magister. Aquaculture, 7: 235-244.

Laughlin, R.A., 1982. Feeding habits af the Blue Crab Callinectes sapidus Rathbun, in the Apalachicola Estuary, Florida. Bull. Mar. Sci., 32(4): 807-822.

Laulier, M. and N. Demeusy, 1974. Etude histologique du fonctionement ovarien au cours d'une maturation de ponte chez le crabe Carcinus maenas L. (Crustace, Decapode). Cah. Biol. Mar., 15: 343-350.

Lebour, M.V., 1928. The larval stages of the Plymouth Brachyura. Proc. Zool. Soc. Lond., 2: 473-560.

Lea, E., 1913. Further studies concerning the methods of calculating the growth of herrings. Publ. Circonst. Cons. Int. Explor. Mer., 66: pp36.

Leffler, C.W., 1972. Some effects of temperature on the growth of juvenile blue crabs, Callinectes sapidus, in the laboratory. Mar. Biol., 14: 104-111.

Leighton, D.L. and R.A. Boolootian, 1963. Diet and growth in the black abalone Haliotis cracherodii. Ecol., 44: 227-238.

Lewis, J.E., 1969. Reversal of asymetry of chelae in Calappa Weber 1795 (Decapoda: Oxystomata). Proc. Biol. Soc. Washington, 82: 63-80.

Lindley, J.A., 1987 Continuous plankton records: the geographical distribution and seasonal cycles of decapod crustacean larvae and pelagic post-larvae in the nort-eastern Atlantic Ocean and the North Sea, 1981-3. J. Mar. Biol. Ass. U.K., 67(1): 145-169.

Lippett, T., 1987. Feeding ecology of intertidal crabs. BSc. Hons. Project, Plymouth Polytechnic, 85pp.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 19: 265-275.

MacMullen, P.H., 1983. The Fishery of the Velvet Swimming Crab Macropipus puber . Sea Fish Indust. Auth. Tech. Rep., No. 218, 15pp.

McConaugha, J.R., 1985. Nutrition and larval growth. In, Wenner, A.M., (Ed.): Larval Growth, Crustacean Issues 2, Rotterdam, Boston: 127-155.

McVean, A.R., 1982. Autotomy. In: The Biology of Crustacea. Vol. 4. Neural Integration and Behavior. (Bliss D.E, ed.-in-chief), Academic Press, New York: Chap. 4, 107-132.

Mann, K.H., 1972. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. I. Zonation and biomass of seaweeds. Mar. Biol., 12: 1-10.

Mann, K.H., 1982. Ecology of coastal waters - a systems approach. Studies in Ecology. Vol. 8. Blackwell Scientific Publications. 322pp.

Mathews, C.P., M. Al-Hossaini, A.R. Abdul Ghaffar and M. Al-Shoushani. 1987. Assessment of short-lived stocks with special reference to Kuwait's shrimp fisheries: a contrast of the results obtained from traditional and recent size-based techniques pp.147-166. In, Pauly, D. and G.R. Morgan, (Eds.): Length-based methods in fisheries research. ICLARM Conference Proceedings, 13: 486pp.

Matsui S., Y. Hagiwara, H. Tou and H. Tsukahara, 1986. Study on the feeding habit of the Japanese blue crab Portunus trituberculatus (Miers). Sci. Bull. Fac. Agric. Kyushu. Univ., 40(2-3): 175-181.

Moncada F.G. and O. Gomez, 1980. Algunos aspectos biologicos de tres especies del genero Callinectes (Crustacea, Decapoda). Rev. Cub. Invest. Pesq., 5(4): 1-35.

Montfrans, J.V., J.Capelli, R.J.Orth and C.H.Ryer, 1986. Use of microwire tags for tagging juvenile Blue Crabs (Callinectes sapidus Rathbun). J. Crust. Biol., 6(3): 370-376.

Morton, J.E., 1954. The crevice faunas of the upper intertidal zone of Wembury. J. Mar. Biol. Ass. U.K., 33: 187-224.

Muntz, L., F.J. Ebling and J.A. Kitching, 1965. The Ecology of Lough Inne. XIV. Predatory activity of large crabs. J. Anim. Ecol., 34: 315-329.

Naylor, E., 1962. Seasonal changes in a population of Carcinus maenas (L.) in the littoral zone. J. Anim. Ecol., 31: 601-609.

Naylor, E., R.J.A. Atkinson and B.G. Williams, 1971. External factors influencing the tidal rhythms of shore crabs. J. Interdisipl. Cycle Res., 2(2): 173-180.

New, M.B., 1976. A review of dietry studies with shrimp and prawns. Aquaculture, 9: 101-144.

New, M.B., 1980. A bibliography of shrimp and prawn nutrition. Aquaculture 21: 101-128.

Nishimoto, R., 1980. Orientation, movement patterns and behaviour of Callinectes sapidus Rathbun (Crustacea: Portunidae) in the intertidal. Ph.D. Diss. Florida State Univ., Tallahassee, Florida.

Oshima, S., 1938. Biological and fishery research in Japanese blue crab Portunus trituberculatus (Miers). J. Imp. Fish. Exp. Sta., 9: 208-212.

Paine R.T., 1977. Controlled manipulations in the marine intertidal zone, and their contributions to ecological theory. In, Goulden, C.E., (Ed.): The changing Scenes in Natural Sciences, 1776-1976, Acad. of Nat Sci. Spec. Publ.12, Philadelphia: 245-270.

Paine, R.T. and R.L. Vadas, 1969. The effects of grazing by sea-urchins Strongylocentrotus spp. on benthic algal populations. Limnol. Oceanogr., 14: 710-719.

Patel, N.M., N.D. Chhaya and M. Bhaskaran, 1979. Stomach contents of Portunus pelagicus (Linn.) from AD net catches. Indian J. Mar. Sci., 8: 48-49.

Paul, R.K.G., 1981. Natural diet, feeding and predatory activity of the crabs Callinectes arcuatus and C. toxotes (Decapoda, Brachyura, Portunidae). Mar. Ecol. Prog. Ser., 6: 91-99.

Paul, R.K.G., 1982. Abundance, breeding and growth of Callinectes arcuatus (Ordway) and Callinectes toxotes (Ordway) (Decapoda, Brachyura, Portunidae) in a lagoon system on the Mexican Pacific Coast. Est. Coast. Shelf Sci., 14: 13-26.

Pauly, D., 1987. A review of the ELEFAN system for the analysis of length-frequency methods of study of the growth, mortality and recruitment in fish and aquatic invertebrates, pp. 7-34. In, Pauly, D. and G.R. Morgan, (Ed.): Length- based methods in fisheries research. ICLARM Conference Proceedings 13: 468pp.

Pauly, D. and N. David, 1981. A BASIC program for the objective extraction of growth parameters from length-frequency data. Meeresforsch., 28: 205-211.

Pauly, D. and H. Calumpong, 1984. Growth, reproduction and mortality of the sea hare Dolabella auricularia (Gastropoda: Aplysiidae) in the Central Visayas, Philippines. Mar. Biol. (Berlin), 79: 289-293.

Pauly, D., J. Ingles and R. Neal, 1984. Application to shrimp stocks of objective methods for the estimation of growth, mortality and recruitment related parameters from length-frequency data (ELEFAN I and II). In, Gulland, J.A. and B.J. Rothschild, (Ed.): Paneid shrimps: Their biology and management. Fishing News Books, Farnham, Surrey, England: 220-234.

Pereiro, J.A., 1982. Modelos al uso en dinamica de poblaciones marinas sometidas a explotacion. Inf. Tech. Instit. Espan. de Oceanografia, 1: 1-255.

Petersen, C.G.J., 1891. Eine Methode zur Bestimmung des Alters und des Wuchses der Fische. Mitt. Dtsch. See-fischerei Ver., II: 226-235.

Piavaux, A., 1977. Distribution and localisation of the digestive laminarinases in animals. Biochem. Sys. Ecol., 5: 231-239.

Plymouth Marine Fauna. Third Edition, 1957. Latimer, Trend & Co. Ltd., Plymouth, 457 pp.

Powell, G.C., 1964. Fishing mortality and movements of adult male king crabs Paralithodes camtschatica (Tilesius), released seaward from Kodiak Island, Alaska. Trans. Am. Fish. Soc., 93(3): 295-300.

Potter, I.C., P.J. Chrystal and N.R. Loneragan, 1983. The biology of the blue manna crab Portunus pelagicus in an Australian estuary. Mar. Biol., 78(1): 75-85.

Potts, G.W., 1985. The nest structure of the Corkwing Wrasse. Crenilabrus melops (Labridae : Teleostii). J. Mar. Biol. Ass. U.K., 65: 531-546.

Poole, R.G., 1967. Preliminary results of the age and growth study of the market crab (Cancer magister) in California: The age and growth of Cancer magister in Bodega Bay. Mar. Biol. Assoc. India Symp. Ser., 2(2): 553-567.

Powles, H.W., 1968a. Distribution and biology of the spider crab Chionoecetes opilio and Hyas araneus, in the Gulf of St. Lawrence. Fish. Res. Board Can. Manus. Rep. Ser., No. 884, 15pp.

Powles, H.W., 1968b. Observations on the distribution and biology of the spider crab, Chionoecetes opilio. Fish. Res. Board Can. Manus. Rep. Ser., No. 997, 106pp.

Przibram, H., 1901. Expermentelle studien uber regeneration. Arch. Entwicklungsmech Org., Vol. XI.

Przibram, H., 1929. Quanta in biology. Proc. R. Soc. Edinburgh, 49: 224-231.

Przibram, H., 1931. Connecting laws in Animal Morphology. (University Press, London), 62pp.

Rangeley, R.W. and M.L.H. Thomas, 1987. Predatory behaviour of juvenile shore crab Carcinus maenas (L.). J. Exp. Mar. Biol. Ecol., 108: 191-197.

Rodriguez, A., 1977. Contribution to the knowledge of the biology and fishing of the prawn Panaeus kerathurus (Forskal 1895) from the Cadiz Gulf (Spanish South Atlantic Region). Invest. Pesq., 41(3): 603-637.

Ropes, J.W., 1968. The feeding habits of the green crab Carcinus maenas (L.). Fishery Bull. Fish. Wildl. Serv. U.S., 67: 183-203.

Russel, F.S., 1957. Descriptions of the Main Collecting Grounds. In, Plymouth Marine Fauna. Latimer, Trend & Co. Ltd., Plymouth: xiii-xxxix.

Russel-Hunter W.D., 1970. Aquatic productivity. Macmillan, New York. 285pp.

Ryan, E.P., 1966. Pheromone: evidence in a decapod crustacean. Science, 151: 340-341.

Ryan, E.P., 1967a. Structure and function of the reproductive system of the crab Portunus sanguinolentus (Herbst) (Brachyura: Portunidae). I. The male system. Proc. Symp. Crustacea, Part II. Mar. Biol. Ass. India, 506-521.

Ryan, E.P., 1967b. Structure and function of the reproductive system of the crab Portunus sanguinolentus (Herbst) (Brachyura: Portunidae). I. The female system. Proc. Symp. Crustacea, Part II. Mar. Biol. Ass. India, 522-544.

Sastry, A.N., 1983. Ecological aspects of reproduction. In, Vernberg, F.J., and W.B. Vernberg, (Eds.): The Biology of Crustacea. Vol 8. Environmental adaptations. Academic Press, New York: 179-270.

Savage, T. and J.R. Sullivan, 1978. Growth and claw regeneration of the stone crab Menippe mercenaria. Florida Mar. Res. Pubs., 32: 1-22.

Siegel, P.R. and A.M. Wenner, 1985. Field and laboratory studies of sand crab growth. In, Wenner, A.M., (Ed.): Factors in adult growth, Crustacean Issues 3. A.A. Balkema, Rotterdam, Boston: 165-171.

Simons, M.J. and M.B. Jones, 1981. Population and reproductive biology of the mud crab, Macrophthalmus hirtipes (Jacquinot, 1853) (Ocypodidae), from marine and estuarine habitats. J. Nat. Hist., 15: 981-994.

Skinner, D.M., 1985. Molting and regeneration. In, Bliss, E.D., (Ed.): The biology of Crustacea. Vol. 9, Integument, pigment and hormonal processes. Academic Press, New York: 43-146.

Skinner, D.M. and D.E. Graham, 1972. Loss of limbs as a stimulus to ecdysis in Brachyura (true crabs). Biol. Bull. (Woods Hole), 143: 222-233.

Smith, B.D., E.L. Cabot and R.E. Foreman, 1985. Seaweed detritus versus benthic diatoms as important food resources for two dominant subtidal gastropods. J. Exp. Mar. Biol. Ecol., 92(2,3): 143-157.

Sokal, R.R. and F.J. Rohlf, 1969. Biometry - The principle and practice of statistics in biological research. W.H. Freeman and Co., New York, 859pp.

Somerton, D.A., 1980. A computer technique for estimating the size of sexual maturity in crabs. Can. J. Fish. Aquatic Sc., 37(9): 1488-1494.

Southward, A.J. and E.I. Butler, 1972. A note on further changes of sea temperature in the Plymouth area. J. Mar. Biol. Ass. U.K., 52: 931-937.

Sova, V.V., L.A. Elyakova and V.E. Vaskovsky, 1970. The distribution of laminarinases in marine invertebrates. Comp. Biochem. Physiol., 32: 459-464.

Sulkin, S.D., 1977. Blue crab study in Chesapeake Bay. Chesapeake Biol. Lab. Contrib. 77, 86pp.

Sulkin, S.D., E.S. Branscomb and R.E Miller, 1976. Induced winter spawning and culture of larvae of the blue crab, Callinectes sapidus. Aquaculture, 8: 103-113.

Tagatz, M.E., 1968. Biology of the blue crab, Callinectes sapidus Rathbun, in the St. John's River, Florida. Fish. Bull. Fish. Wildl. Serv. U.S., 67: 17-33.

Tagatz, M.E., 1969. Growth of juvenile blue crabs, Callinectes sapidus Rathbun, in the St. Johns River, Florida. Fish. Bull. Fish. Wildl. Serv. U.S., 67: 281-288.

Taylor, A.C., 1988. The ecophysiology of decapods in the rock pool environment. In, Fincham, A.A., and P.S. Rainbow, (Eds.): Aspects of Decapod Crustacean Biology. Proc. Sym. Zool. Soc. (Lond)., 59: 227-261.

Teisser, G., 1960. Relative growth. In, Waterman, T.H., (Ed.): The physiology of crustacea. Vol. 1. Academic Press, New York: 537-560

Thomas, H.J., 1964. The spawning and fecundity of the Norway lobster (Nephrops norvegicus) around the Scottish coast. J. Cons. Int. Explor. Mer., 29: 221-229.

Thomas, J.C., 1973. An analysis of the commercial lobster (Homarus americanus) fishery along the coast of Maine, August 1966 through December 1970. Nat. Mar. Fish. Serv. Spec. Sci. Rep. - Fish, No. 667, 57pp.

Thorson, G., 1950. Reproduction and larval ecology of marine bottom invertebrates. Biol. Rev., 25: 1-45.

Truitt, R.V., 1939. The blue crab. In: Our water resources and their conservation. Chesapeake Biol Lab. Contrib., 27: 10-38.

Van Engel, W.A., 1958. The blue crab and its fishery in Chesapeake Bay. Part I. Reproduction, early development, growth and migration. Comm. Fish. Rev., 20(6): 6-17.

Vernberg, F.J., 1962. Latitudinal effects on physiological properties of animal populations. A. Rev. Physiol., 24: 517-546.

Von Bertalanffy, L., 1938. A quantitative theory of organic growth. Hum. Biol., 10: 181-213.

Warner, G.F., 1977. The Biology of Crabs. Elek. Science, London, 202pp.

Warner, G.F., 1984. The cracking of winkles by crabs: contrasting techniques in Carcinus and Liocarcinus. J. Zool. Soc. Lond., 196: 431-438.

Wear, R.G., 1974. Incubation in British decapod Crustacea, and the effects of temperature on the rate and success of embryonic development. J. Mar. Biol. Ass. U.K., 54: 745-762.

Wear, R.G. and M. Haddon, 1987. The natural diet of the crab Ovalipes catharus (Crustacea, Portunidae) around Central and Northern New Zealand. Mar. Ecol. Prog. Ser., 35: 39-49.

Wenner, A.M., C. Fusaro and A. Oaten, 1974. Size at onset of sexual maturity and growth rate in crustacean populations. Can. J. Zool., 52: 1095-1106.

Whyman, H., R.F. Uglow and P.H. MacMullen, 1985. A study of mortality rates of the velvet crab during holding and transport. Sea Fish Indust. Auth. Tech. Rep., No. 259, 22pp.

Williams, M.J., 1981. Methods for analysis of natural diet in portunid crabs (Crustacea : Decapoda : Portunidae). J. Exp. Mar. Biol. Ecol., 52: 103-113.

Williams, M.J., 1982. Natural food and feeding in the commercial sand crab Portunus pelagicus Linnaeus, 1766 (Crustacea : Decapoda : Portunidae) in Moreton Bay, Queensland. J. Exp. Mar. Biol. Ecol., 59: 165-176.

Wilson, E.B., 1903. Notes on the reversal of asymetry in the regeneration of the chelae in Alpheus heterochelis. Biol. Bull. (Woods Hole), 4: 197-210.

Yano, I. and S. Kobayashi, 1969. Calcification and age determination in Crustacea. I. Possibility of age determination in crabs on the basis of number of lamellae in cuticles. Bull. J. Soc. Sci. Fish, 35: 34-42.

Yong, M. and R.A. Silkman, 1975. A computer program for analysis of polymodal frequency distributions (ENORMSEP), FORTRAN IV. Fishery Bull. Fish Wildl. Serv. U.S., 73(3): 681.

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