ASPECTS OF THE BIOLOGY OF THE SQUAT LOBSTER, *MUNIDA RUGOSA* (FABRICIUS, 1775).

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To Ahmed, Tarneem and Vinous

DECLARATION.

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

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ABSTRACT.

The thesis presents a comparative study of respiratory physiology of the galatheids *Munida rugosa* (Fabricius, 1775) and *M. sarsi* Huus, 1935. Both species were obtained from the Firth of Clyde, *M. rugosa* from a depth range of 8-115m and *M. sarsi* from 95-115m depth.

To provide background information for this study, some aspects of the general biology of both species were studied, with greatest emphasis given to *M. rugosa*. Both species were collected mainly from sandy muds using creels and trawls. Studies of relative growth were carried out and indicated that sexual differences were particularly apparent in cheliped lengths (positive allometry in males) and abdomen widths (positive allometry in females). Heterochely was observed in both species and, although there was much variation, it was more commonly seen in large males. Extrapolation of relative growth regression relationships for males and females suggested that, in *M. rugosa*, sexual maturity occurs at approximately 17mm carapace length and at approximately 10mm carapace length in *M. sarsi*.

Preliminary studies on the reproductive cycle of *M. rugosa* indicated that ovary development occurred between spring and autumn. Ovigerous females were observed between November and May, and egg hatching occurred from March. There was a significant trend for larger females to carry a larger number of eggs than smaller females. Mean egg diameter was 0.89 ± 0.08mm.

Dietary analysis and observations of feeding behaviour indicated that *M. rugosa* is an omnivore with a preference for animal material. As well as feeding on macroscopic material, this species was also observed to deposit feed. The feeding behaviour of *M. sarsi* was similar. The morphology of the mouthparts

and stomach of *M. rugosa* are described and related to feeding. Some comparative information on *M. sarsi* is also provided.

A comparative study was made of the respiratory physiology of *M. rugosa* and *M. sarsi*. The gill formulae for the two species are presented. Gill areas were very similar and both species were characterized by having very low gill area values compared with other decapod crustaceans. This has been interpreted as reflecting their relatively inactive lifestyle.

Studies of cardiac and ventilatory activity were made for both species. It was observed that there was usually a high degree of bilateral coordination of scaphognathite activity with periods of reversed beating and cessation of scaphognathite activity occurring synchronously between both scaphognathites. In general, cardiac and scaphognathite activity were closely correlated. This was particularly evident in quiescent animals when periods of cardiac arrest were accompanied by cessation of scaphognathite activity.

The effects of temperature and oxygen availability on rates of oxygen consumption and on heart and scaphognathite rates were studied. The rates of oxygen consumption of both species were low compared with those of most other decapod crustaceans. Both *M. rugosa* and *M. sarsi* were found to be quite tolerant of hypoxia (Pc range 39 - 56 Torr). Survival under complete anoxia, however, was limited to 8 hours in *M. rugosa* and to 4 hours in *M. sarsi*.

The oxygen and carbon dioxide transporting properties of the blood of both species were also studied. No significant differences were found between the concentrations of the major ions in the blood of *M. rugosa* and *M. sarsi*. It was noted that the concentration of Mg^{2+} ions in the blood of both species was higher than in many other decapods. Values for the oxygen carrying capacity of the blood of *M. rugosa* and *M. sarsi* were similar (1.7 - 1.9 ml.100 ml^{-1}) and were within the range of values previously recorded for other decapod

crustaceans.

Oxygen dissociation curves were constructed spectrophotometrically using a diffusion chamber. The haemocyanin of both *Munida* species was found to have a low oxygen affinity (P_{50} = 20 - 39 Torr and 49 Torr in *M. rugosa* and in *M. sarsi* respectively at 10°C). These values are amongst the lowest reported for decapods. Values for the cooperativity ranged between 3.3 and 3.7. In both species, the haemocyanin exhibited a moderate, normal Bohr effect (θ = -0.4).

Measurements of the *in vivo* pH and Po_2 of the blood were also carried out. The mean values for the Po_2 of the pre- and post-branchial blood were 38 and 86 Torr respectively (pH = 7.7 and 7.9 respectively). There were no significant differences in these values between *M. rugosa* and *M. sarsi*. Interpolation of these Po_2 values on the dissociation curves constructed *in vitro* indicated that the post-branchial blood is fully saturated on leaving the gills. The values for the Po_2 of the pre-branchial blood were higher than in many other decapods and indicate that, in quiescent animals under normoxic conditions, only a small amount of the oxygen supplied to the tissues comes from the oxygen transported bound to the haemocyanin; most is derived from the oxygen carried in solution in the blood.

Carbon dioxide equilibrium curves for the blood were also constructed. The blood of both species showed a moderate capacitance for CO_2 transport (β = 3.45 and 3.37 $\mu mol.l^{-1}.Torr^{-1}$ for deoxygenated and oxygenated blood respectively). The Haldane coefficient calculated at Pco_2 = 6 Torr was between 0.56 and 0.69 in both species. In *M. rugosa*, the buffering capacity of the deoxygenated blood varied between -5.51 and -6.29 $mmol.l^{-1}pH\ unit^{-1}$ at temperatures between 10 and 20°C. The buffering capacities of the blood of *M. rugosa* and *M. sarsi* were very similar.

CHAPTER 1. INTRODUCTION

1.1. General context

In recent years there has been an increase of interest in the respiratory physiology of decapod crustaceans. This can clearly be seen when the reviews of Redmond (1955) and Wolvekamp & Waterman (1960) are compared with those of McMahon & Wilkens (1983), Mangum (1983) and Burggren & McMahon (1988). Many authors have pointed out the dangers of considering an animal's physiology in isolation, without a knowledge of its field biology. The philosophy of the ecophysiologist or physiological ecologist seeks to remedy this (Jorgensen, 1983). Thus, in the present work, although the emphasis is physiological, an attempt has been made to understand some aspects of the ecology and general biology of the species under investigation.

The progress made in the study of the respiratory physiology of decapods has been aided by considerable improvements in experimental techniques. Electron microscopy has aided the study of the morphology and anatomy of respiratory structures, while small, sensitive polarographic electrodes and electronic instrumentation have enabled oxygen consumption, heart and scaphognathite rates, and blood chemistry to be investigated with precision. It is the case, however, that detailed studies are confined to a relatively small number of species. Brachyuran and astacidean decapods have received the greatest attention (see McMahon & Wilkens, 1983). Comparatively little attention has been given to anomuran decapods.

Thus, published information on the respiratory physiology of anomurans is limited to only a few species, including the pagurid hermit crabs *Pagurus hirsutiusculus* (Young, 1963; Burggren & McMahon, 1981) and *Pagurus bernhardus* (Shumway, 1978; Davenport *et al.*, 1980; Bridges & Brand, 1980); the diogenid hermit crabs *Diogenes bicristimanus* (Sarojini & Nagabhushanam,

1968) and *Clibanarius vittatus* (Wernick & Penteado, 1983); the coenobitids *Birgus latro* (Cameron & Mecklenberg, 1973; Greenaway *et al.*, 1988; Morris *et al.*, 1988; Morris & Greenaway, 1989) and various *Coenobita* spp. (McMahon & Burggren, 1979; Burggren & McMahon, 1981; Achituv & Ziskind, 1985); and the galatheids *Galathea strigosa* (Bridges & Brand, 1980; Scammell & Hughes, 1982), *Pleuroncodes planipes* (Quetin & Childress, 1976), and *Munida quadrispina* (Burd, 1983, 1985, 1987). Most of the hermit crabs and the coenobitids listed above are semi-terrestrial, so there is little information on aquatic Anomura. There is a body of literature on the respiratory physiology of thalassinids (see Atkinson & Taylor, 1988). The Thalassinidae (burrow-dwelling 'mud-shrimps') are, however, no longer included in the Anomura, but have independent Infraorder status (see Bowman & Abele, 1982).

1.2. Galatheid biology

Galatheid 'crabs', are a cosmopolitan group (Family Galatheidae) of marine decapod crustaceans commonly known as 'squat lobsters' and are members of the Infraorder Anomura. Most galatheids are benthic in life style (Benedict, 1903). There are, however, species that swarm in pelagic waters, e.g. *Pleuroncodes planipes* and *Munida gregaria* (Boyd, 1967; Williams, 1980).

The benthic forms occur on a variety of substrata from rocks to soft mud. They occur widely from tropical seas to high latitudes and from the littoral zone down to abyssal depths (Abele, 1982). Recently, galatheids have been seen to be a characteristic component of hydrothermal vent communities, e.g. *Munidopsis lentigo* (Williams & Dover, 1983). The family Galatheidae Samouelle, 1819 contains several genera (7 according to Abele & Felgenhauer, 1982) and according to Henderson (1888), only the genera *Galathea* and *Munida* are found in shallow water. In general, species of *Munida* live at greater depths than *Galathea* spp., and *Munidopsis* species live deeper still

(Makarov, 1938; Wenner, 1982; Gore, 1983).

Species of *Galathea*, *Munida* and *Munidopsis* are recorded from around British and Irish coasts (Selbie, 1914; Allen, 1967; O'Riordan, 1968, 1984; Rice & de Saint Laurent, 1986; Attrill 1988). Including species occurring in the Porcupine Sea-bight, these are *Galathea dispersa, G. intermedia, G. machadoi, G. nexa, G. squamifera, G. strigosa, M. intermedia, M. micropthalama, Munida rugosa, M. sarsi, M. tenuimana, Munidopsis antonii, M. aries, M. bairdii, M. bermudezi, M. crassa, M. curvirostra, M. parfaiti, M. rosorata, M. serricornis* and *M. tridentata.* The common inshore species are the *Galathea* species (except *G. machadoi*) and *Munida rugosa.*

Most of the literature on the Galatheidea is concerned with taxonomic descriptions, habitat types and geographical distributions (e.g. Milne Edwards, 1880; Henderson, 1888; Benedict, 1903; Calman, 1911; Zimmermann, 1913; Laurie, 1926; Rayner, 1935; Brinkmann, 1936; Bull, 1937, Makarov, 1938; Mathews, 1932; Terslin, 1938; Eales, 1961; O'Riordan, 1968, 1984; Dennis, 1968; Pequegnat & Pequegnat, 1970; Samuelsen, 1970; Laird *et al.*, 1976; Campbell, 1980; Baba, 1977; 1979; Miyake, 1978; Fenwick, 1979; Ambler, 1980; Barnes, 1980; Williams, 1980; Tirmizi, 1966, 1980; Baba, 1981; Howard, 1981; Lewinsohn, 1981; Khodkina, 1981; Wenner, 1982; Adema, 1982; Baba, 1982; Zeldis & Jillett, 1982; Gore, 1983; Williams & Dover, 1983; Burd, 1983; Zeldis, 1985; Williams, 1984; Rice & Saint Laurent, 1986; Baba, 1986; 1987). Records of new species and their descriptions are continuing (e.g. Baba, 1988; Williams, 1988).

There is comparatively little information in the literature on the general biology of galatheids. Larval description and aspects of reproduction have received attention (e.g. Lebour, 1930 a,b; Rayner, 1935; Huus, 1935; Boyd, 1967; Williams & Brown, 1972; Gore, 1979; Wenner & Windsor, 1979; Williams, 1980, 1982; Wenner, 1982; Vera & Bacardit, 1986, 1987; Attrill,

1988), and so has parasitization (mainly by bopyrid isopods and rhizocephalan barnacles) (e.g. Benedict, 1903; Rayner, 1935; Brinkmann, 1936; Samuelson, 1970; Bursy, 1978; Wenner, 1982; Gore, 1983; Attrill, 1989). There are also few studies on feeding behaviour, and diet (e.g. Nicol, 1932; Gore, 1983; Bahamonde *et al.*, 1986). Other aspects of their biology are poorly known.

There are very few studies which have considered the functional morphology of the respiratory structures and which have investigated the respiratory physiology of members of the Galatheidae. Pike (1947) provided a brief description of the branchial morphology of *Galathea squamifera*, and Bridges (1979) investigated the respiratory physiology of *G. strigosa*. There are some members of this family which are known to experience low oxygen concentrations in their natural environment. For example, *Munida quadrispina* in Canadian fjords which become oxygen depleted at certain times of year (Burd, 1985) and *M. gregaria*, *Cervimunida johni*, *Pleuroncodes monodon* and *Pleuroncodes planipes* which occur in benthic concentration on the continental shelf, often in water of very low oxygen concentration (0.5-1 $ml.l^{-1}$) (Longhurst, 1967; Boyd, 1967; Quetin & Childress, 1976). Burd (1985) has also discovered that only the larger sized *M. quadrispina* can tolerate living at the low oxygen levels in fjords. In addition, it has been found that the gill development in *Munida quadrispina* is influenced by long-term exposure to the oxygen conditions in the habitat (Burd, 1987).

1.3. Galatheid fisheries

It has been shown that galatheid 'crabs' are very important in the diet of sea lions (Hamilton, 1934), birds and whales (Mathews, 1932), humans (Rayner, 1935; Longhurst, 1967), and in the diet of several species of fish (Bahamonde, 1986). Recently, consideration has been given to fishery for pelagic *Munida gregaria* in New Zealand, primarily for use as food stock for cultured salmon

(Zeldis, 1985) and some galatheids are now marketed for human consumption.

The utilization of pelagic galatheids as a fishery resource has been considered by Longhurst (1967). Five known species of galatheids exhibit the phenomenon of mass occurrences mostly in highly eutrophic regions of the Atlantic ocean. *M. gregaria* and *Pleuroncodes planipes* occur in pelagic swarms as juveniles. At the time Longhurst wrote, a fishery off Chile for *Cervimunida johni* and *Pleuroncodes monodon* landed 10×10^3 metric tonnes yearly and it was estimated that the unexploited populations of *P. planipes* could produce similar or greater yields. It was estimated that the five species of galatheids may have a total annual potential of between 30×10^3 and 300×10^3 tonnes (Longhurst, 1967). Fisheries for Galatheidae in Chile began in 1953 and have become more-developed recently (Bahamonde *et al.*, 1986), particularly in relation to *Cervimunida johni* and *Pleuroncodes monodon. Cervimunida* and *Pleuroncodes* products have been imported to Britain and marketed in a similar manner to 'scampi' (Howard, 1981).

There is, however, an emergent fishery for the benthic *Munida rugosa* in Britain, particularly in Scotland (Howard, 1981; Scottish Sea Fisheries Statistical Tables, 1988). Here they are mainly caught by creel (usually *Nephrops* creel), but may also be landed as a trawled by-catch (Howard, 1981). Shellfish processing companies now handle the species and it may often be seen in fishmongers' shops and supermarkets (pers. obs.). The fishery is, however, opportunistic and small. Only 3.87 tonnes of 'squat lobsters' were landed in Scotland in 1988, valued at c.£4000. The tonnages in 1986 and 1987 were 1.86 and 0.66 tonnes, respectively (C.J. Chapman, pers. comm.). In the light of present information, some of the *Munida* taken from deeper water may have been *M. sarsi*. The landing statistics do not, in any case, separate species though it has been assumed that the catch consists of *M. rugosa*.

Howard (1981) noted that fishery research vessels off Scotland had caught up

to 52 kg per haul of *M. rugosa* west of the Outer Hebrides and that a 50 creel fleet of *Nephrops* creels shot on suitable ground might yield 25 kg. Howard indicated that the yield of usable meat is less than for *Nephrops norvegicus*. British *Munida* have been exported to Spain and France (C.J. Chapman, pers. comm.).

The type of biometric and biological information that is normally associated with a commercially exploited species is conspicuous by its absence in the case of *M. rugosa*.

1.4. The species studied

The two species investigated in this thesis are *Munida rugosa* (Fabricius, 1775) and *Munida sarsi* Huus, 1935. North-eastern Atlantic and Mediterranean species of the genus *Munida* Leach, 1820 have been subject to considerable taxonomic confusion in the past. Recently, Rice & de Saint Laurent (1986) have examined the literature and extensive museum collections and have resolved most of this confusion. They give long lists of the synonyms of *M. intermedia*, *M. rugosa*, *M. sarsi* and *M. tenuimana*, have interpreted the extensive confusion that exists in the literature and have shown how the confusion between the first three of these species has occurred. It is pointless to repeat this here, but suffice it to say that references to *Munida* spp. in the literature and particularly to *M. bamffica* (or *bamffia*) have to be examined with great care since any of the first three species may be the species referred to. One oversight by Rice & de Saint Laurent concerns the work of Ingrand (1937). Although on first sight the paper appears to deal with *M. rugosa* (as *M. bamffica*), the paper actually deals with *M. sarsi*. For some unaccountable reason the two species have been juxtaposed. Illustrations of anatomical detail labelled *M. sarsi* are of *M. rugosa* (as *M. bamffica*) and *vice versa*. The whole animal illustration of Ingrand (1937) clearly shows the specimen to be *M. sarsi*.

More is said of this in Chapter 2.

The most obvious specific characteristics of *M. rugosa* are its fairly uniform, dull red-brown colouration; its small eyes when compared with *M. sarsi*; the absence of spines on the fourth abdominal tergite; and, when examined closely, the presence of a distinctive spine on the distal external angle of the merus of the third maxilliped (see Chapter 2). The median rostral spine is usually uniformly coloured. These and other characteristics are examined in detail by Rice & de Saint Laurent (1986).

One of the problems associated with the taxonomic confusion surrounding these species is that their depth ranges, geographical variations and distributions are difficult to assess. Fortunately, Rice & de Saint Laurent (1986) have helped greatly here. Whereas much of the literature gives a wide depth distribution for *M. rugosa*, e.g. 0 (MLWS) - 1255m (Howard, 1981), it is likely that the deeper records represent confusion with other species. According to Rice & de Saint Laurent (1986), the species occurs from about 30 - 300m depth, though it has been observed in shallower water (Howard, 1981 and this study - see Chapter 2). In the Porcupine Sea-bight SW of Ireland, Attrill (1988) found that *M. rugosa* did not extend below 280m where it overlapped the distribution of *M. sarsi*. *M. rugosa,* as clarified by Rice & de Saint Laurent (1986), is an eastern Atlantic species extending from Shetland and Sognefjord (Norway) in the north to Madeira in the south. It extends into the Mediterranean at least as far east as the Adriatic.

Munida sarsi is normally a deeper water species than *M. rugosa*. Rice & de Saint Laurent (1986) state that it has been recorded most frequently from 200 - 800m depth and occasionally to about 1000m. They indicated that its greatest abundance appeared to be from 250 - 400m. Attrill (1988), sampling in the Porcupine Sea-bight, encountered a peak of density and biomass for this species at around 450m depth, though it extended from 205 - 815m depth. At

the bottom end of its range Attrill (1988) found that *M. sarsi* overlapped the distribution of *M. tenuimana* G.O. Sars, 1872. *M. sarsi* is described by Rice & de Saint Laurent (1986) as extending from North Cape (Norway) and Greenland to the southern Bay of Biscay and northern coast of Spain. It has not been recorded from off Portugal or from the Mediterranean.

The most obvious specific characteristics of *M. sarsi* are its mainly orange colouration with a very obvious white band across the anterior part of the carapace at the level of the bases of the rostral spines and including the orbital grooves; its large eyes; the presence of two spines on the fourth abdominal tergite; and the absence of a spine on the distal external angle of the merus of the third maxilliped (see Chapter 2). The median rostral spine is orange with a white patch near the tip. Further details are given by Rice & de Saint Laurent (1986).

For comparison, the depth and geographical distributions of the other species of eastern Atlantic *Munida* spp. are as follows. *M. intermedia* A. Milne Edwards and Bouvier, 1899, occurs between 120 - 800m depth in Mediterranean and the southern part of the eastern N Atlantic (S of the Goban Spur), with the most easterly Atlantic record being from 1360m in the Bay of Biscay (Rice & de Saint Laurent, 1986). *M. tenuimana* also occurs in the Mediterranean, and extends from the Atlantic waters Spain and Portugal to Iceland and Norway. Its depth distribution is 120 - 1775m, commonly 700 - 1400m (Rice & de Saint Laurent, 1986). Attrill (1988) recorded this species from 740 - 1410m depth in the Porcupine Sea-bight, with most occurring at 1296m. In this area, at 1630 - 1640m, Attrill encountered the little-known *M. micropthalama* A. Milne Edwards, 1880. This species has a wide N Atlantic distribution extending from Iceland to the Gulf of Mexico at depths of 120 - 2129m (Attrill, 1988).

1.5. Aim and structure of the thesis

The aim of the thesis was to provide information on the physiological ecology of two *Munida* species collected in the Firth of Clyde, Scotland. The species investigated were *Munida rugosa* and, to a lesser extent *Munida sarsi*. When the work commenced, it was directed at *M. rugosa* alone. A previously unknown Firth of Clyde population of *M. sarsi* was encountered during the study and the initial emphasis was changed to include it in the study. However, the relative rarity of this species meant that fewer data were collected than for *M. rugosa*. *M. sarsi* were only found in relatively deep water, so *M. rugosa* from equivalent depths, as well as from shallower water, were used in comparative physiological work. Chapter 2 provides information on aspects of ecology, including information on habitat, aspects of reproduction, relative growth, diet and feeding behaviour. In support of the work on feeding, information on the structure and function of the mouthparts and stomach is also provided. There was insufficient research vessel time available to undertake a comprehensive ecological programme so the above information was obtained from animals collected primarily for physiological experimentation. There is remarkably little published information on the biology of either species, so the acquisition of the information gathered in Chapter 2 was considered to be important in order to provide background and context to the physiological work which comprises most of the thesis. The biometric information may be useful in relation to the emergent fishery since very little such information appears to exist.

Chapter 3 presents information on the type, number and structure of the gills and data on gill areas are also provided. The basic pattern of scaphognathite-generated branchial ventilation is described under normoxic conditions and the effects of disturbance on heart and scaphognathite activity are described. In Chapter 4, heart and scaphognathite responses to declining oxygen tension are

described. Also, the effects of size, temperature and feeding on oxygen consumption are considered. Maximum survival time under anoxic conditions was also examined. In Chapter 5, comparative investigations were carried out on the oxygen and carbon dioxide transporting properties of the blood of both species. In addition, data on the major ions of the blood of both species are presented. Finally, Chapter 6 integrates the general conclusions reached during the study.

CHAPTER 2. ECOLOGY, RELATIVE GROWTH AND FEEDING BIOLOGY

2.1. INTRODUCTION

It has been noted in Chapter 1 that *Munida rugosa* has a wide geographical distribution. It is also reported to have a wide depth distribution, though as has been seen, nomenclature confusion has made it difficult to determine its geographical and bathymetric ranges with precision. Rice & de Saint Laurent (1986) indicate that the species occurs at depths of 30-300m in the NE Atlantic and Mediterranean and in the present work it was encountered as shallow as 8m. The species occurs in considerable numbers on both the east and the west coasts of Scotland, but is more abundant on the west coast (Howard, 1981).

The species occurs on a range of substrata from soft mud to bedrock (Fig. 2.1 A & B) and habitat preferences are noted in the present work. It has been noted that *M. rugosa* occupies the burrows of other species, for example, they have been reported from burrows of the red band-fish *Cepola rubescens* (Atkinson *et al.*, 1977) and those of the Norway lobster, *Nephrops norvegicus* (R.J.A. Atkinson pers. comm.). In each case the burrows had been vacated by the original occupants. The possibility of constructing their own burrows has also been suggested (Howard, 1981) and this was investigated in the present study.

Around the Scottish coasts, *M. rugosa* are commercially fished using creels. These catches are primarily by-catches from a fishery directed at crabs (*Cancer pagurus* and *Liocarcinus puber*) and particularly at Norway lobsters (*N. norvegicus*). Creeling was one of the collection methods used in the present work. Normally the creels were deployed at around 40m depth, but on one occasion a trawler fouled the creels and towed them into 95m depth before casting them adrift. On recovery, these creels were found to contain several specimens of *Munida sarsi*. This species was subsequently targetted and,

Fig. 2.1

Munida rugosa: (A) at the opening of a vacated burrow of a red band-fish (*Cepola rubescens*) in a muddy gravel substratum in the field, (B) on a coarse substratum in a laboratory aquarium tank.


A


B

although large numbers were never collected, sufficient were collected to make some morphological comparisons with *M. rugosa*. Comparisons which are mainly physiological also feature in later chapters. *M. sarsi* is a deep water, NE Atlantic species (see Chapter 1) and its occurrence in the Clyde Sea Area was unexpected. Some information on the local habitat of this species is given in the present chapter.

Limitations of research vessel time meant that a field-based research emphasis was impossible to achieve. Nevertheless, where possible, distributional, reproductive, dietary and morphological information was obtained. Such information is reported in this chapter.

Thus, observations on gonad maturation in female *M. rugosa* proved possible, together with some egg counts from ovigerous females. The results were compared with other reproductive information from Scottish waters (Allen, 1967; Comely & Ansell, 1989) and elsewhere (Zariquey Alvarez, 1968; Attrill, 1988).

In many Crustacea, changes in the relative growth of various body proportions have been shown to occur at various stages of development and particularly at sexual maturity (Teissier, 1960; Hartnoll, 1982). Ingrand (1937), used size allometry to indicate secondary sexual differentiation in pereiopod lengths in *M. bamffica* and *Galathea squamifera* from French waters (Golfe de Gascogne). There is some confusion, however, as to the identity of the *M. bamffica* of Ingrand (1937). Rice & de Saint Laurent (1986) and Atrill (1988) assume it to be *M. rugosa*. However, an examination of the figures and corresponding text of Ingrand's paper indicates that the characteristics of *M. sarsi* have been applied to *M. rugosa* and *vice versa*. Therefore it is likely that the morphometric data in Ingrand's paper also relate to *M. sarsi* and not to *M. rugosa*. Without access to her original material, this is impossible to fully

resolve, but according to Dr A.L.Rice (pers. comm. to Dr R.J.A. Atkinson), it is almost certain that Ingrand's paper refers to *M. sarsi* throughout. With an awareness of this problem, results from Clyde Sea Area animals were compared with Ingrand's work. Attrill (1988) provides some biometric and ecological information for *M. sarsi* from the Porcupine Sea-bight off SW Britain and Rios (1979) presents some biometric data for *M. rugosa* from Dunstaffnage Bay, near Oban, on the Scottish west coast. Both workers include information on relative growth which is taken into account when discussing the present work.

All crustaceans have the same basic mouthparts (though particular mouthparts may be absent in some groups), with anterior thoracopods taking on a mouthpart role in higher Crustacea such as decapods. Nevertheless, the diversity of mouthpart form and function is enormous (Marshall & Orr, 1960; McLaughlin, 1980). Regarding diet, the majority are generalists and will take anything edible that comes their way; a number have more than one kind of feeding mechanism (Marshall & Orr, 1960). Diet and the morphology of the mouthparts and major chelipeds were investigated in the present study. Their morphology has been shown to reflect differences in diet in many decapods (e.g. Greenwood, 1972; Caine, 1975; Kunze & Anderson, 1979; Schembri, 1982a). For example, amongst fifteen species of hermit crabs, Schembri (1982a) described various methods of deposit-feeding, browsing, suspension-feeding, predation and scavenging. Each species adopted one or two primary feeding mechanisms, but also a number of secondary mechanisms. One example, *Pagurus rubricatus* was predatory, detrivorous, macrophagous, and to a small degree, a suspension feeder (Schembri, 1982a, b). Such diversity was reflected in mouthpart differences, particularly in relation to setation (Schembri, 1982a, b).

The morphology of the stomach of decapod crustaceans has also been related

to the type of food taken, though phylogenetic constraints cannot be overlooked (Patwardhan, 1935b; Schaefer, 1970; Caine 1975). Therefore, its structure was investigated in *Munida rugosa* and *M. sarsi*. Some background information on the stomach of *M. rugosa* was provided by Patwardhan (1935b).

2.2. MATERIALS AND METHODS

2.2.1. Ecology

The *Munida rugosa* obtained for the present study were caught by creel, trawling or by SCUBA divers in the Clyde Sea Area NW of Little Cumbrae, around Great Cumbrae and in Loch Fyne. The animals were obtained from 8-115m depth and from substrata ranging from bedrock to soft mud. Divers provided some information on the habitat preferences of the species; otherwise, general substratum information was deduced from charts, research vessel echo-soundings and local knowledge.

2.2.2. Relative growth; heterochely

During 1986, some morphometric measurements of different parts of *M. rugosa* were carried out using Vernier callipers. Both sexes were examined. The aim was to discover whether allometric growth occurred. Measurements included rostral length (median spine), 'total' body length (excluding rostrum, i.e. from the orbit to the posterior edge of the telson in a fully extended animal), carapace length (from posterior margin of the orbit to the mid-posterior region of the carapace), carapace width (maximum), abdominal width (maximum width of the second tergite), total length of chelipeds, cheliped dactylar and propodal length; cheliped propodal width, and the lengths of the meri of the pereiopods. Various combinations of these measurements were then plotted on logarithmic axes as indicated by Hartnoll (1982) and regression relationships indicated. The dimension chosen to be representative of the general size of the animal was normally carapace length

and was plotted as the independent variable on the x-axis. The dimensions of the body part whose relative growth was under investigation were plotted as the dependent variable on the y-axis. Relative growth is thus expressed by $Y = aX^b$ or $\log Y = \log a + b.\log X$ where a is the y-intercept and b is the allometric growth constant or relative growth rate. If b is greater than 1, this indicates positive allometry; if b = 1, growth is isometric; if b is less than 1, this indicates negative allometry (variable growing slower than body size reference dimension).

Data were derived from 105 individuals which were obtained from 40m depth, NW of Little Cumbrae, Firth of Clyde. Care was taken to ensure that animals used in body weight computations were intact (varying degrees of limb loss were often seen). The relationship between body wet weight (g) and carapace length was obtained for both males and females.

It is important to note that various workers have used differing measurements of carapace length in *Munida* spp. The measurement taken here (which follows the protocol for *Nephrops norvegicus*) may be regarded as equivalent to the measurements taken by Ingrand (1937) and by Attrill (1988). Although there are slight positional differences in each case, comparative measurements indicated that the differences between them are minimal. In the case of Brinkmann (1936), however, the measurement is taken over a greater distance and Attrill's conversion factor has been accepted so that where Brinkmann's data are referred to, Attrill's converted values of carapace length have been used. It should be noted that the carapace length measurement taken by Ingrand (1937) is, at first sight, similar to that of Brinkmann (1936); both are median measurements from the posterior edge of the carapace to the base of the rostrum. However, judging from the rather inaccurate drawing given by Ingrand (1937), her measurement does not extend as far anteriorly as that of Brinkmann (1936). Although Attrill (1988) does not comment on this, he must

have reached a similar conclusion since he accepts Ingrand's carapace length values as they stand.

Measurements for relative growth computations were taken soon after animals were collected, but a note of the form of propodal 'finger' of each chela ('arched' or 'straight') was not taken at this time. Material was stored for later examination. Half of the *Munida rugosa* material was, however, lost in the Zoology Department fire of 1988. An investigation of chela morphology was carried out using the remaining animals and some collected subsequently. Whether or not this 'arched chela' feature could be identified in the lost samples was assessed by an examination of their chela dimensions. The occurrence of heterochely is therefore assessed in Section 2.3.2, with further reference to it in Section 2.3.6.

2.2.3. Aspects of reproduction

Reproductive information was obtained incidentally and was not a primary objective. Collection sites are indicated in the results section. Egg measurements were confined to just 18 freshly collected females (see below). For these, the total number of eggs carried and the average egg diameter was obtained. It was found to be important to use freshly obtained animals and fresh eggs because freezing the eggs caused damage and formalin preservation changed egg size. Since the data obtained from frozen eggs were deemed unreliable, they were excluded from analysis.

The eggs were collected by firstly carefully removing the pleopods. Then, with the aid of a microscope, all eggs were separated and removed by forceps taking care not to damage or lose any egg. The eggs were placed on a slide and mean diameter for >50 eggs was obtained using an eye piece micrometer after multiplying by a correction factor at the required magnification. The mean value for egg size from each female was obtained as follows: 10 groups of 25

eggs were placed on pieces of pre-weighed aluminium foils on a Cahn microbalance. The remaining egg mass was weighed as a single batch of eggs. The egg groups were then dried in a freeze drier (Edwards Pirani 501) for 24h. The dry weights of the egg groups were calculated. The mean value for the 10 groups of 25 eggs was calculated. Finally, the total number of eggs was estimated by dividing the weight of the whole batch by the mean weight of the 10 groups. The actual total number of eggs carried by the female was this value plus 250 (i.e. 25 eggs x 10 groups). The estimated total number of eggs was correlated with the carapace length (a function of animal size). A regular sampling programme was not possible (ship-time constraints) and the animals that were obtained were restricted in size range because of gear selection effects.

An attempt was made to obtain some information on the reproductive cycle of *M. rugosa* by observing the occurrence of ovigerous females from January to December. Preserved ovaries of different individuals were examined and classified into immature, intermediate, advanced, and spent according to the general appearance, colour, size, and visibility of ova.

2.2.4. Diet

One series of animals (n = 11) used in the dietary analysis were obtained by creel during May, June and October 1986 at depths of 30-50m NW of Little Cumbrae in the Firth of Clyde. A few individuals (n = 5) were also obtained during this period at 18-20m depth in Loch Fyne (by divers). Both groups were injected with 10% formalin within 2h of capture and preserved in a freezer until examined. For those animals which had been collected in creels baited with fish, any fish remains found in the gut were ignored. The majority of individuals examined (n = 114) were those obtained during December and January 1988 by trawling. These were obtained from 32-115m (mostly 76-

115m) depth NW of Little Cumbrae in the Firth of Clyde. Here, data were also analysed according to the time of capture. Individual *M. rugosa* were killed immediately following removal from the trawl by dipping them first in fresh water which rapidly caused immobilization, followed by immersion in 90^{o}C water. They were then kept frozen for a short time before being dissected and their stomachs removed. Carapace length and sex were also recorded. Individual stomachs were placed in 10% formalin and examined 1-4 weeks later.

Each individual stomach was placed on its ventral side in a petri dish and its general appearance was noted. Then a longitudinal cut was made carefully, using a fine scissors, and the stomach was opened. The stomach fullness was assessed by eye (empty (<10%), 10-25%, 26-50%, and >50% full). Contents were removed by overturning the stomach and flushing it with water. This was to ensure that all the fine particles were separated from the tissues and that the stomach ossicles were clean. The stomach contents were examined using a light microscope at magnifications ranging from x50 to x250. A total number of 130 stomachs were examined. Identifications of the gut contents were restricted to the general level and specific identifications were not attempted. Much of gut material was highly digested and such contents were included under the term 'unidentified'. Because of the finely divided nature of much of the gut contents, it was not possible to analyse the relative abundance of the various dietary items.

2.2.5. Feeding behaviour

This information is based only on laboratory observations. Animals were placed individually in a small clear 'perspex' tank which was provided with shelter. To avoid disturbance, observations were carried out using a time-lapse video camera under dim illumination. Sediment was provided in some cases and not in others. The animals were examined first for their preference for

food items, then their foraging techniques were observed. The food items included in these observations were the kelp *Laminaria digitata*, a red alga *Rhodymenia palmata*, the errant polychaete *Nephtys hombergii*, the bivalve *Abra alba*, the sedentary polychaete *Sabella pavonina* and the brittle star *Ophiocomina nigra*. All of these species occurred in areas where *M. rugosa* were encountered (detached thalli in the case of the algae). Live and dead food was presented.

2.2.6. Functional morphology of the mouth parts, chelipeds and stomach

The morphology of the mouth parts and the stomach of *M. rugosa* were examined using both light and electron microscopy. The mouth parts comprised the mandibles, first and second maxillae, first, second and third maxillipeds. The antennules were also examined. Cheliped morphology and their role in feeding was also observed. The protocol of preparation for electron microscopy was the same as given in Chapter 3. In addition, the stomach was examined and its general internal organization observed by sectioning after decalcification in EDTA for a few days followed by the staining procedure mentioned in Chapter 3. The identification of different types of setae was made following Thomas (1970), Factor (1978) and Schembri (1982a, b, c). General setal terminology is adopted, e.g. pappose, plumose, plumodenticulate, serrate, serrulate, triserrate, triserrulate, cuspidate, simple, and subdivisions of each setal type are ignored (as in McLaughlin, 1980, 1982). General mouthpart nomenclature follows McLaughlin (1980, 1982).

For mouthparts, the following orientational terminology is adopted (see Schembri, 1982c), assuming mouthparts to be in their resting position. The inner surface of a mouthpart is that facing the mouth, the outer surface is that facing away from the mouth, the lateral edge is that which is directed away

from the midline of the body and the medial edge is that which is nearest to the midline of the body.

2.2.7. *Munida sarsi*

61 specimens (47 males, 14 females) of *Munida sarsi* were obtained from 95-115m depth in the Main Channel between the Cumbraes and Bute. It was therefore possible to provide a limited amount of comparative data for this species.

2.3. RESULTS

2.3.1. Ecology

Munida rugosa were collected from several types of substratum. The grounds traversed by trawls were of gravelly muddy sand, sandy mud and mud (BGS, 1985), but were mostly of sandy mud. The creels were deployed on sandy mud and muddy sand grounds close to submarine basalt outcrops with associated boulder screes. It was found that more animals were caught in creels when they were deployed near to rocks than when they were further out on the sediment plain. Divers (S.J. Anderson, R.J.A. Atkinson, M. Davies) reported observing *M. rugosa* on open bedrock, beneath stones and rock overhangs (particularly at the bedrock/sediment interface), in rock crevices, amongst hydroid and bryozoan 'turf' and on the sediment plain. Here they were often adjacent to stones and other cover, but also occurred in burrows. These burrows were reportedly those of other species. The shallowest depth from which divers reported *M. rugosa* was 8m in Loch Fyne. The greatest depth from which they were trawled in the present study was 115m in the Cumbrae Main Channel: deeper areas were not investigated.

In the laboratory, the species was often observed occupying crevices and hollows beneath rocks, but many specimens clung to vertical rock faces or the

walls of aquaria. Aquarium animals were generally very inactive and remained virtually motionless for many hours at a time. Animals were observed to occupy hollows in the muddy substrata of tanks, but none were observed to burrow independently.

Few small animals were collected by creel and trawl. Gear selection probably accounts for much of this, though divers also reported seeing few small animals which suggests more secretive behaviour or different habitat utilization than large specimens. Size-frequency distributions of the animals used in studies of relative growth are given in Fig. 2.2 The peak carapace length for males was between 25 - 30mm and at approximately 25mm for females.

2.3.2. Relative growth; heterochely

When weight \ length relationships in *M. rugosa* are considered, no significant difference between the sexes is apparent (Table 2.1., Fig. 2.3). The relationships between total length and carapace length are given in Table 2.1 and Fig. 2.4. In this case a sexual difference is apparent ($P < 0.05$).

When the relative growth of various body parts is examined, various sexual differences become apparent. The abdomen width in the females is always greater ($P < 0.05$) than that of males of the same carapace length (Table 2.1., Fig. 2.5). In the case of females, positive allometry is indicated (b significantly greater than 1, see Section 2.2.2.). In males, however, negative allometry is apparent with the abdominal growth rate being less than that of the reference dimension (carapace length).

There was also a significant difference in the rate of the growth of the chelipeds (total length) of males and females (Table 2.1., Fig. 2.6). Because heterochely occurred in some individuals (see below) and left or right handedness could occur (see Section 2.3.6.), it was considered wise to pool data from both chelipeds (Table 2.1). Although regression relationships for left and

Fig. 2.2

Size frequency histograms for the male and female *M. rugosa* used in the investigation of relative growth.

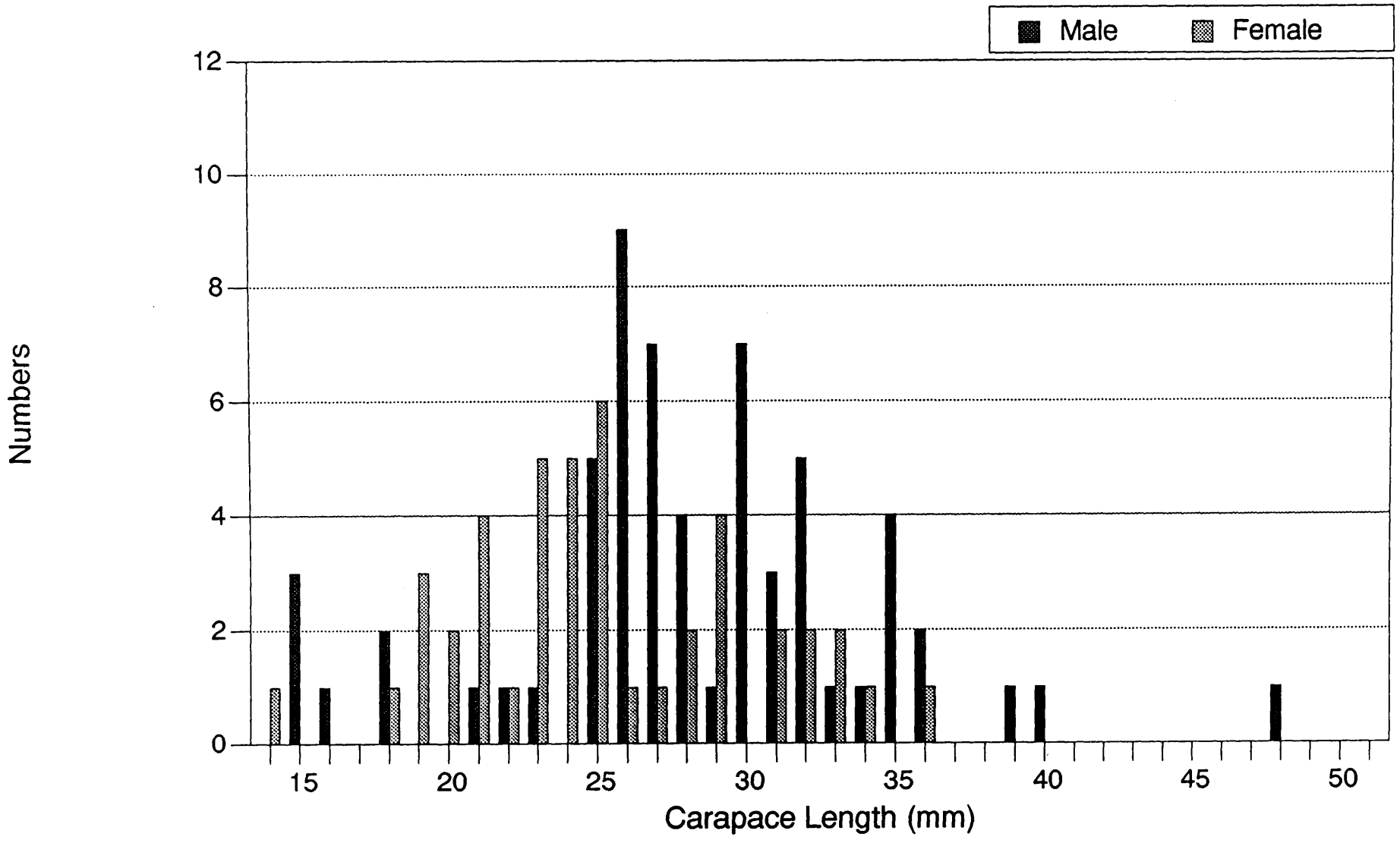

Male
Female
Numbers
0
2
4
6
8
10
12
15
20
25
30
35
40
45
50
Carapace Length (mm)

Table 2.1. Regression coefficients for the relationships between different body measurements and the carapace length of male (M) and female (F) *Munida rugosa*. The regression lines calculated for the data for male and female animals have been compared using covariance analysis. The F values for the slopes (F_s) and elevations (F_e) of these lines are given and any significant difference between the slopes (P) is also indicated. A modified t-test was used to compare the slopes of the regression lines (b) with a value of 1. A value of 'b' which did not differ significantly from 1 indicates isometric growth; a value greater than 1 indicates positive allometry and a value of less than 1 indicates negative allometry. For further details see text.

cl = carapace length (mm); wt = fresh body weight (g); cw = carapace width; tlr = total length from tip of rostrum; tlo = total length from orbit; abdw = abdominal width; Lchl & Rchl = left and right chela length; Lpro & Rpro = left and right propodus length; Ldac & Rdac = left and right dactylus length; Lchw and Rchw = left and right chela width; a = elevation and b slope of the regression equations; r = correlation coefficient; n = number of animals; t = t value, allo. = allometry.

(Note: the t value for the weight/carapace length was calculated but compared with a slope of 3).

Table 2.1.

	sex	a	b	r	n	F_s	F_e	P_s	t	allo.
wt/cl	F	-2.76	2.84	0.97	44	00.07	00.37	>0.05	-1.47	iso
	M	-2.66	2.78	0.91	61				-1.33	iso
cw/cl	F	-0.06	1.01	0.99	44	04.33	01.97	<0.05	0.70	iso
	M	0.11	0.89	0.94	61				-2.50	-ve
tlr/cl	F	0.55	0.97	0.98	44	25.38	17.91	<0.05	-1.20	iso
	M	0.69	0.85	0.95	61				-3.50	-ve
tlo/cl	F	0.40	1.02	0.98	44	11.28	15.36	<0.05	0.50	iso
	M	0.67	0.81	0.93	61				-4.60	-ve
abdw/cl	F	-0.19	1.12	0.98	44	11.66	55.49	<0.05	3.40	+ve
	M	0.07	0.90	0.94	61				-2.30	-ve
Lchl/cl	F	0.63	0.94	0.96	38	04.98	21.8	<0.05	-1.40	iso
	M	0.31	1.20	0.89	52				2.30	+ve
Rchl/cl	F	0.60	0.95	0.94	35	03.45	34.44	>0.05	-0.80	iso
	M	0.36	1.17	0.92	53				2.40	+ve
Lpro/cl	F	0.33	0.92	0.93	38	02.25	18.07	>0.05	-1.40	iso
	M	0.06	1.15	0.82	52				1.30	iso
Rpro/cl	F	0.32	0.92	0.88	35	02.56	30.30	>0.05	-0.90	iso
	M	0.06	1.15	0.88	53				1.80	+ve
Ldac/cl	F	0.16	0.87	0.90	38	06.50	19.52	<0.05	-1.90	-ve
	M	-0.25	1.20	0.88	51				2.20	+ve
Rdac/cl	F	0.07	0.92	0.85	34	02.38	34.31	>0.05	-0.80	iso
	M	-0.16	1.14	0.90	53				1.80	+ve
Lchw/cl	F	-0.92	1.23	0.83	38	03.81	16.27	>0.05	1.60	iso
	M	-1.46	1.68	0.84	51				4.40	+ve
Rchw/cl	F	-0.67	1.04	0.90	35	04.96	27.70	<0.05	0.40	iso
	M	-1.25	1.53	0.84	53				3.80	+ve

Pooled data of left and right for males and females *M. rugosa*.

chl./cl	F	0.60	0.90	0.90	073	8.42	55.80	<0.05	-01.50	iso
	M	0.30	1.20	0.90	105				03.30	+ve
chw./cl	F	-0.81	1.15	0.84	073	8.12	42.76	<0.05	-10.40	+ve
	M	-1.35	1.60	0.84	104				05.81	+ve
wid./cl	F	-0.67	1.05	0.92	031	9.04	33.98	<0.05	00.59	iso
	M	-1.44	1.68	0.87	048				04.86	+ve
nar./cl	F	-0.66	1.03	0.91	031	3.24	14.46	>0.05	00.35	iso
	M	-1.17	1.45	0.81	048				02.88	+ve
lon./cl	F	0.64	0.93	0.95	031	6.59	35.90	<0.05	-01.22	iso
	M	0.29	1.22	0.91	048				03.03	+ve
sho./cl	F	0.60	0.95	0.91	031	3.52	17.30	<0.05	-00.73	iso
	M	0.30	1.20	0.90	048				02.36	+ve

Fig. 2.3

The relationship between body fresh weight (g) and carapace length (mm) for male (□) and female (⊠) *M. rugosa*.

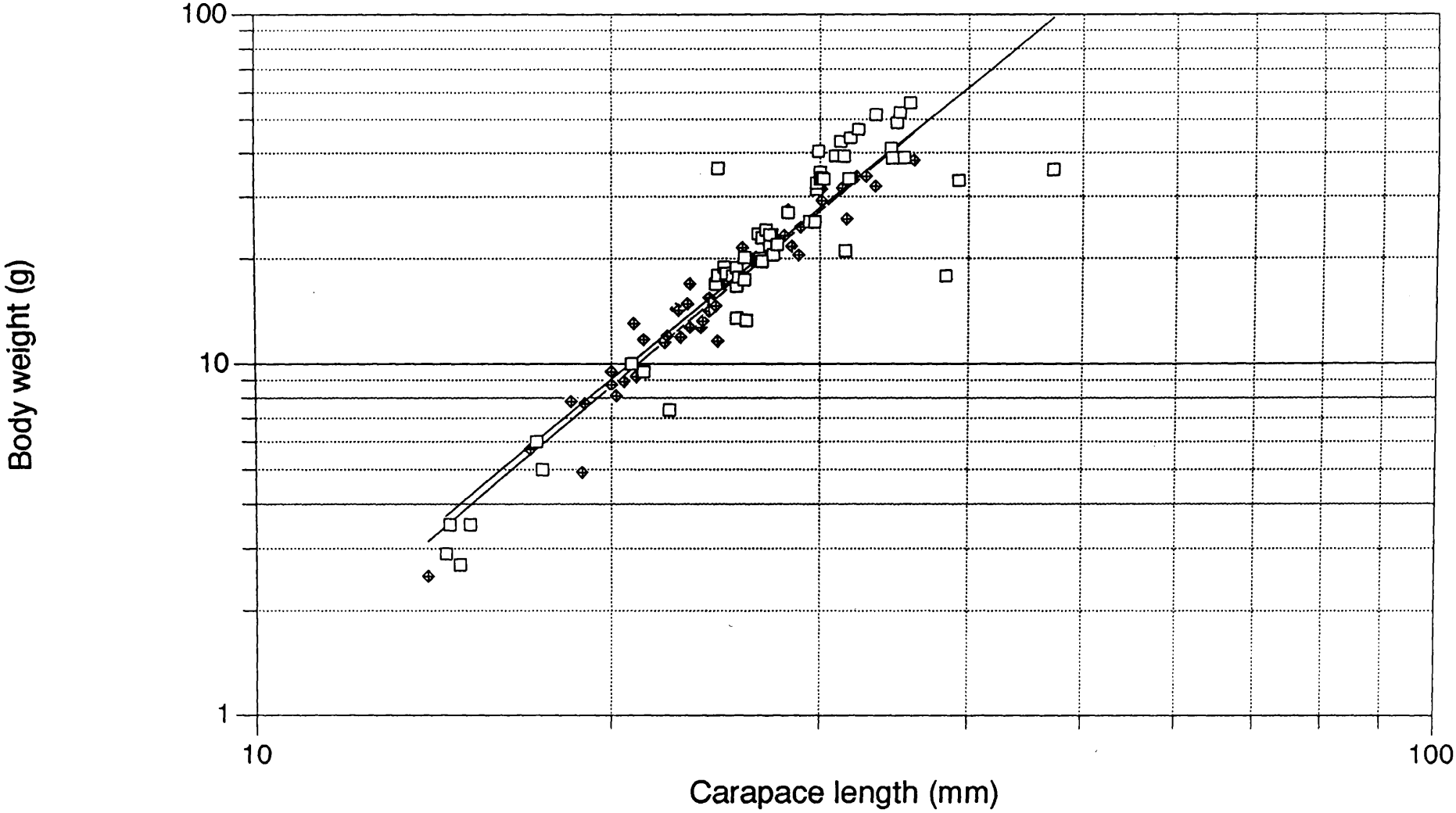

100
10
1
Body weight (g)
10
100
Carapace length (mm)

Fig. 2.4

The relationship between total length excluding rostrum and carapace length for male (□) and female (⊠) *M. rugosa*.

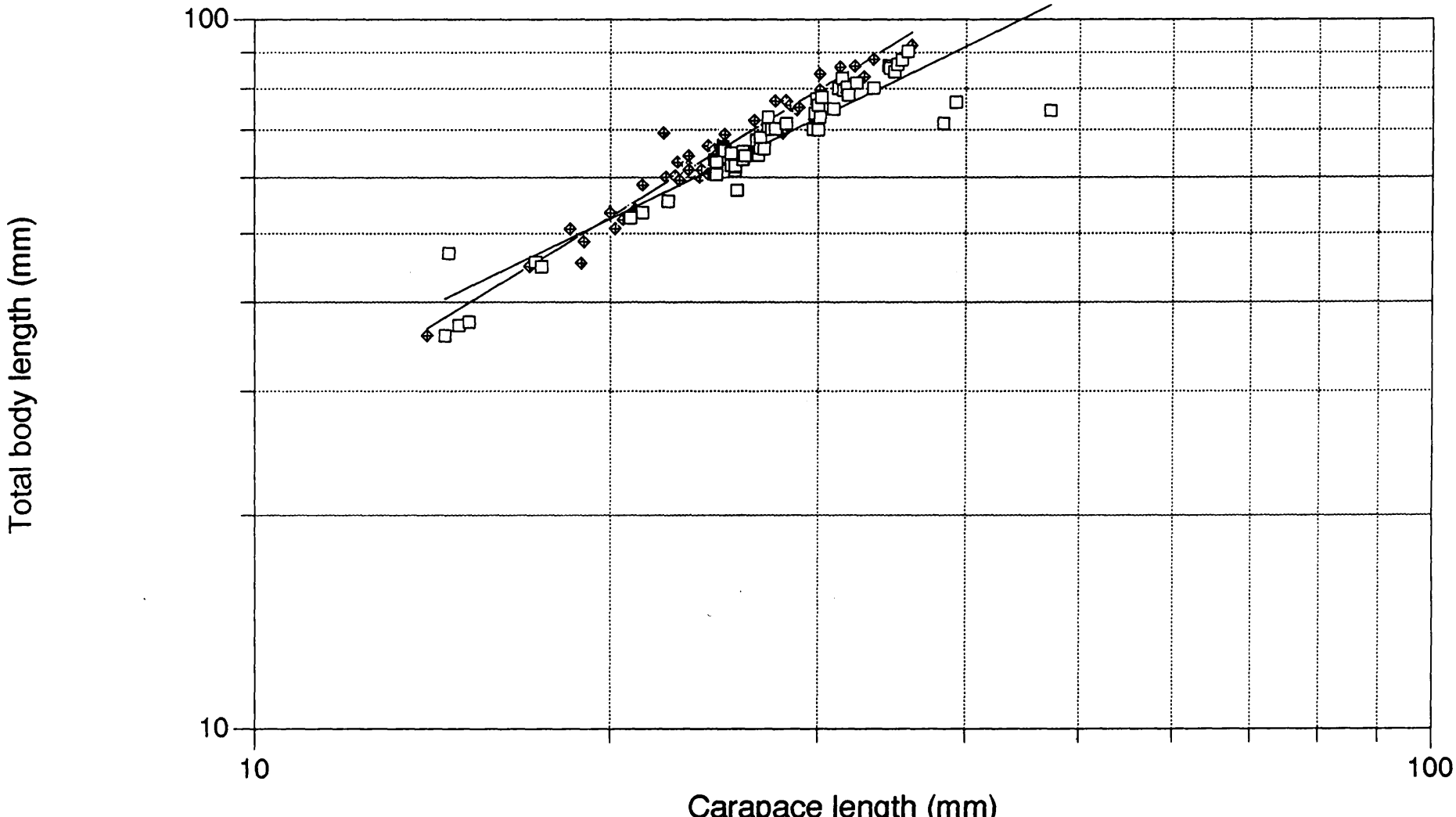

100
10
10
100
Total body length (mm)
Carapace length (mm)

Fig. 2.5

The relationship between abdomen width and carapace length for male (□) and female (⊠) *Munida rugosa*.

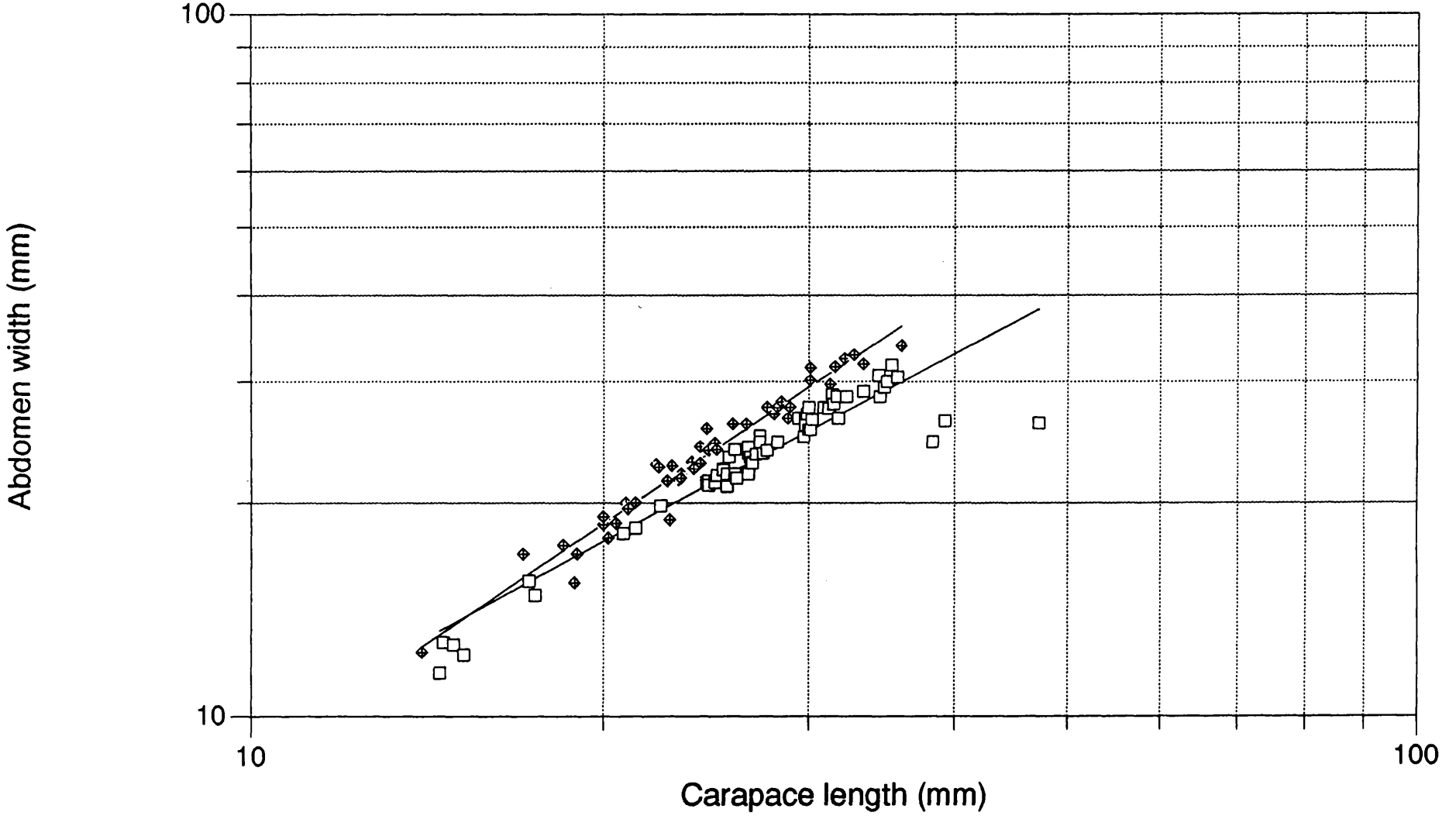
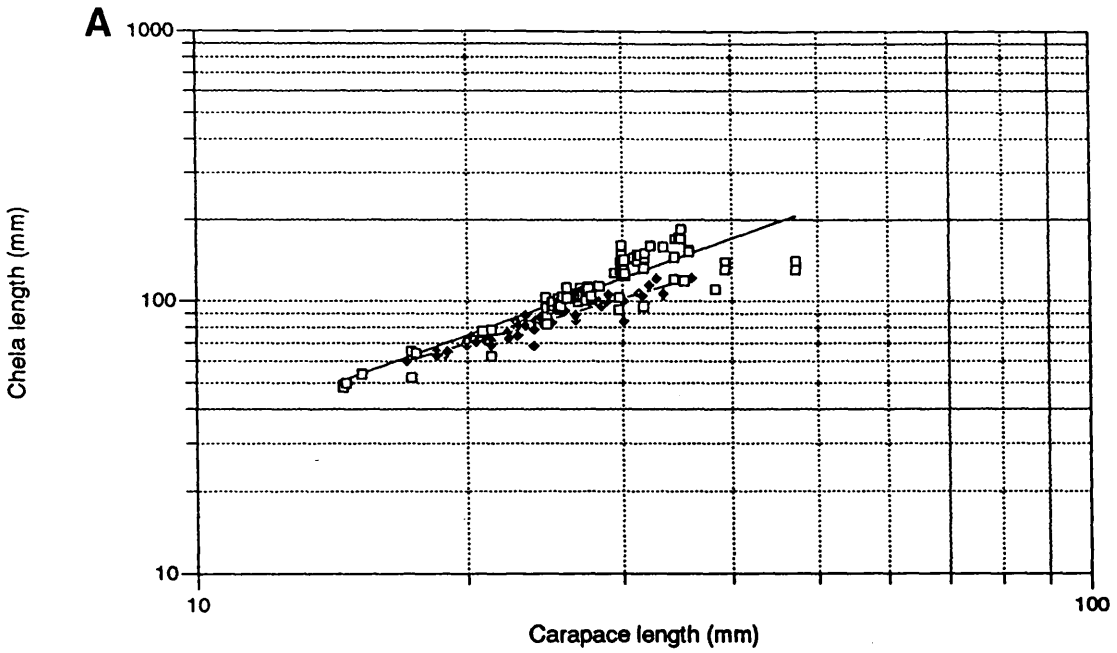
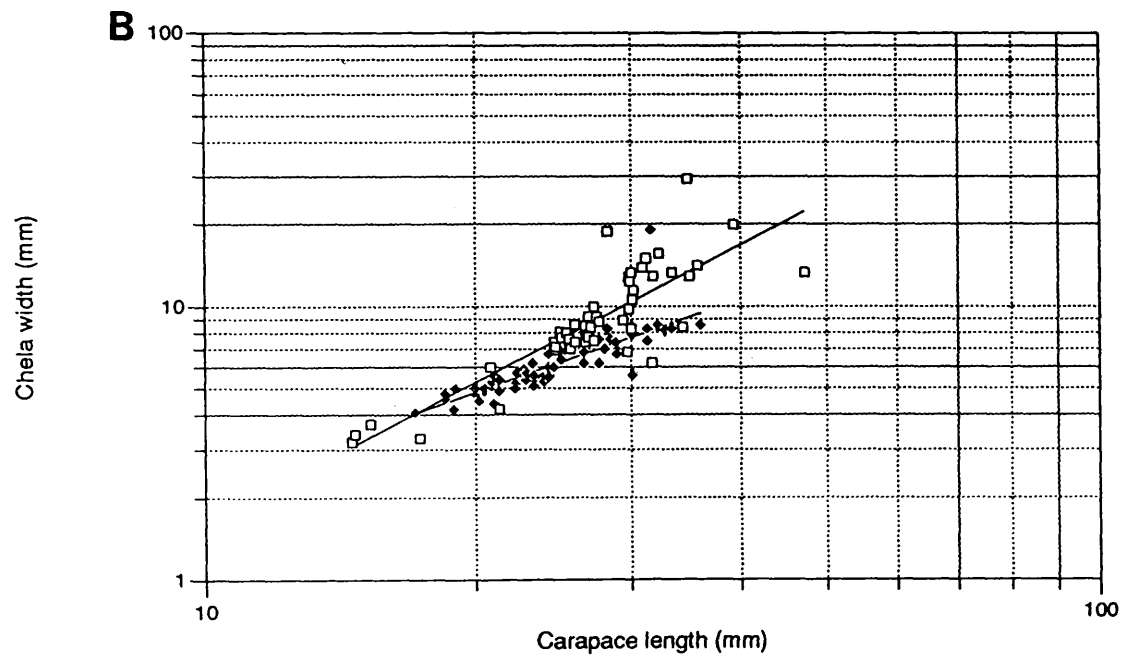

100
10
10
100
Abdomen width (mm)
Carapace length (mm)

Fig. 2.6

The relationships between cheliped lengths (A) and cheliped widths (B) and carapace length for male (□) and female (⊠) *M. rugosa*. Both chelipeds from each animal are used in these analyses.


A
1000
100
10
10
100
Chela length (mm)
Carapace length (mm)


B
100
10
1
10
100
Chela width (mm)
Carapace length (mm)

right chelae against carapace length were calculated (Table 2.1.), the occurrence of heterochely complicates the interpretation of these data. Therefore, distinctions between 'longer' and 'shorter' and 'wider' and 'narrower' chelipeds were also made, without reference to right and left (Table 2.1.).

It was found, however, that there was no pronounced handedness bias for either cheliped length or width (see below) so that heterochely-induced variation in the data for right and left chelipeds will equally influence each data set. The clearest sexual differences are seen when chela widths for males and females are compared (Fig. 2.7, Table 2.1).

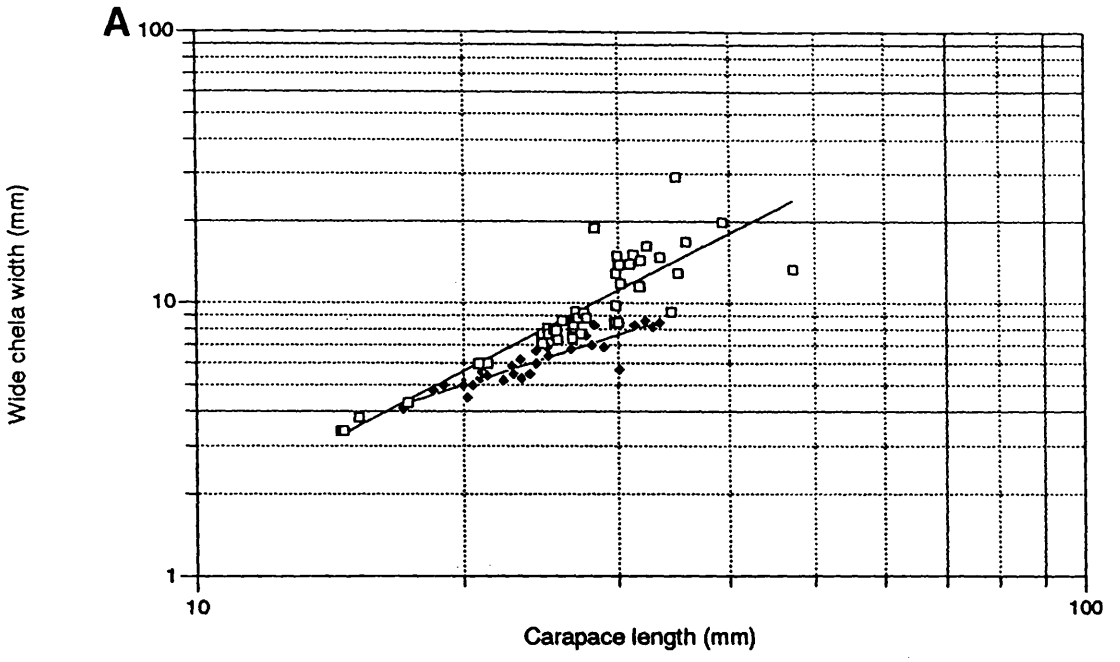
The relationship between the lengths of the meri of the walking legs and carapace length for male and female *M. rugosa* is shown in Table 2.2. and Fig. 2.8. Covariance analyses of these relationships for both males and females indicates that there is no significant difference in the rates of growth of the meri of the pereiopods within one sex. There is no evidence of sexual differences in the growth rates of pereiopods 1 - 4 over the size range of animals available. There is, however, a slight difference between the sexes in the case of pereiopod 5 (a significant difference in slopes). It should be noted that there are differences in the size of the meri of males and females (Table 2.2). The meri of pereiopods 1 - 4 show no evidence of allometry, though negative allometry is evident in the case of pereiopod 5 of both sexes (Table 2.2.).

Heterochely

Considering all the measurement data for *M. rugosa* with both chelipeds, the following results are obtained:

Fig. 2.7

The relationship between the widths of the wider of the two chelae (A) and the narrower of the two chelae (B) and carapace length for male (□) and female (◆) *M. rugosa*.


A
100
10
1
10
100
Wide chela width (mm)
Carapace length (mm)

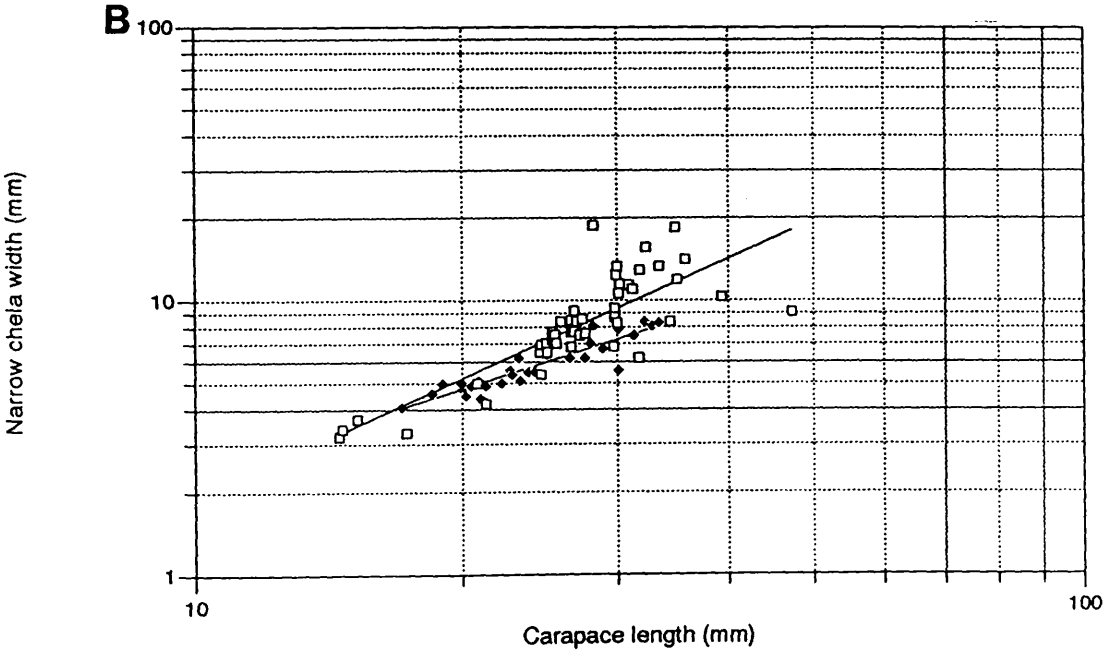

B
100
10
1
10
100
Narrow chela width (mm)
Carapace length (mm)

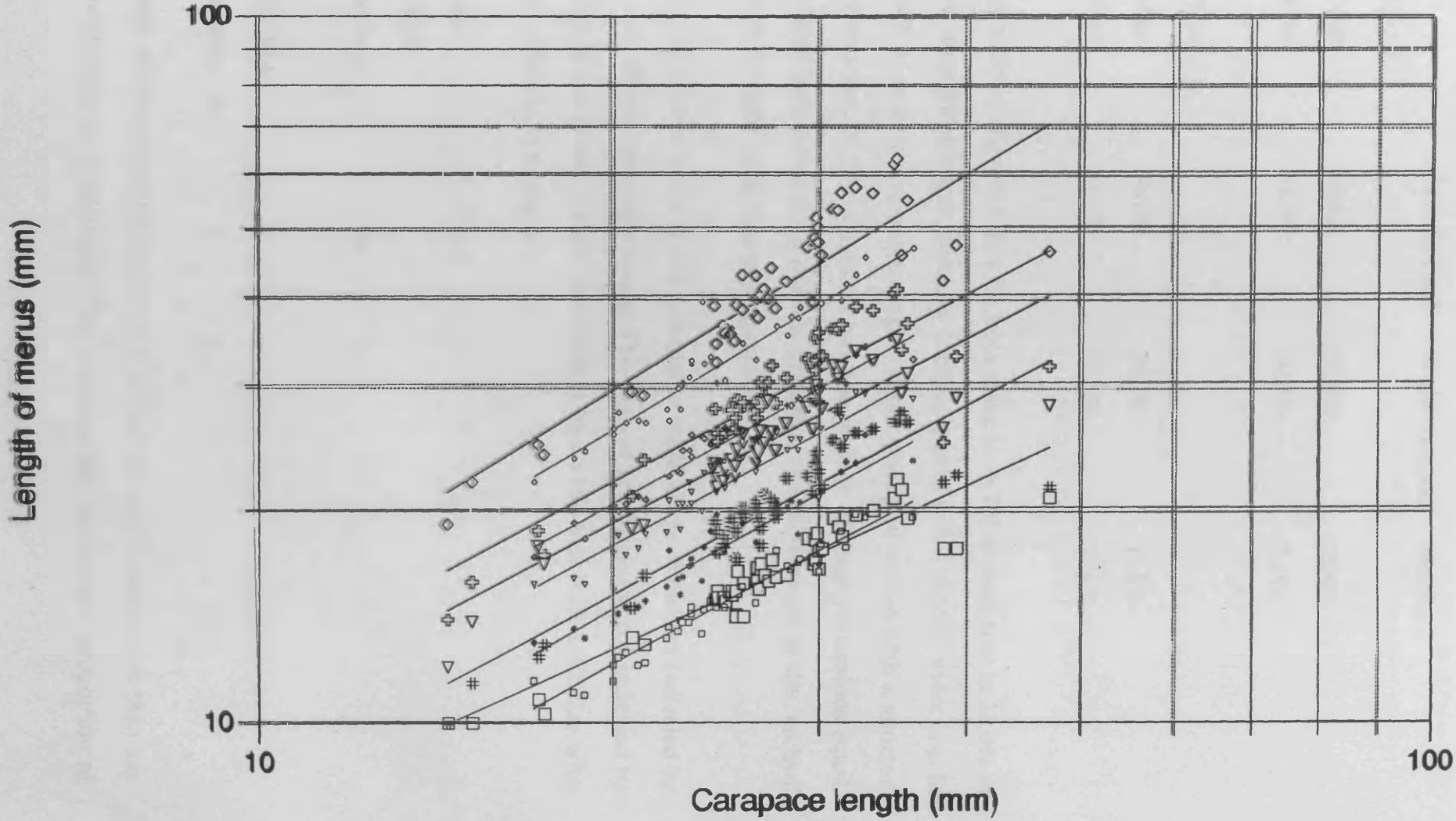
Table 2.2. Regression coefficients for the relationships between the merus length of pereiopods 1-5 and the carapace length of male (M) and female (F) *Munida rugosa*. The regression lines calculated for the data for male and female animals have been compared using covariance analysis. The F values for the slopes (F_s) and elevations (F_e) of these lines given and significant difference between the slopes (P) is also indicated. A modified t-test was used to compare the slopes of the regression lines (b) with a value of 1. A value 'b' which did not differ significantly from 1 indicates isometric growth; a value of greater than 1 indicates positive allometry and a value of less than 1 indicates negative allometry. For further details see text.

	sex	a	b	r	n	F_s	F_e	P_s	t	allo.
P_1/cl	F	0.1	1.0	0.9	37	0.02	23.8	>0.05	0.06	iso
	M	0.1	1.0	0.9	50				0.20	iso
P_2/cl	F	0.1	0.9	1.0	37	0.33	04.4	>0.05	-1.6	iso
	M	0.2	0.9	0.9	50				-1.6	iso
P_3/cl	F	0.4	0.9	0.9	37	0.50	07.1	>0.05	-1.3	iso
	M	0.2	0.9	0.9	50				-2.2*	-ve
P_4/cl	F	-0.1	1.0	0.9	37	0.58	02.4	>0.05	-0.8	iso
	M	0.0	0.9	0.9	50				-1.9*	-ve
P_5/cl	F	-0.1	0.9	1.0	37	4.44	00.4	<0.05	-2.2*	-ve
	M	0.1	0.8	0.9	50				-5.0*	-ve

cl = carapace length (mm), P_1-P_5 = merus length of pereiopods 1-5. allo. = allometry, * = just significant.

Fig. 2.8

The relationship between the length of the meri of the pereiopods and carapace length for male (A) and female (B) *M. rugosa*. Pereiopods 1 - 5 = (◇), (✣), (▽), (#) and (□), respectively.


100
10
10
100
Length of merus (mm)
Carapace length (mm)

	Left cheliped	Right cheliped	Neither
MALE			
longer	39.6%	50.0%	10.4%
wider	47.9%	50.0%	2.1%
FEMALE			
longer	46.7%	36.7%	16.6%
wider	43.3%	33.3%	23.4%

An analysis of pooled data for both sexes (n = 79) showed that in 38.0% of cases there was a longer, narrower cheliped paired with a shorter, wider one. In 40.5% of cases there was a longer, wider cheliped paired with a shorter, narrower one. 21.5% of animals had one or both of these dimensions equal. Cheliped length was not a reliable indicator of the presence of the 'arched' chela v. 'straight' chela type of heterochely.

The numbers of animals with unequal or equal chela widths are indicated by the ratio of left:right chela widths. This form of left handedness is indicated by a ratio greater than 1, right handedness by a ratio less than 1. Chelae with equal widths have a ratio of 1.

Ratio	>1	<1	1
MALE			
Numbers	21	26	1
FEMALE			
Numbers	14	9	8

These differences are more likely to reflect the small sample size than any real difference in handedness. The observation that a larger proportion of

females than males had chelae of equal width may reflect a predominance for heterochely in males, but larger samples are needed in order to confirm this.

In the case of chela length, the corresponding ratios indicated that 19 males and 15 females had longer left chelipeds and 24 males and 11 females had longer right chelipeds. 5 males and 1 female had chelipeds of equal length.

The bilateral differences in length and width do not necessarily indicate the presence or absence of the 'arched' chela type (see Section 2.3.6., and Fig. 18 A-C) It was noticed however, that the propodus of the 'arched' type of chela was always wider than in the 'straight' form, so that marked bilateral differences in chela width are likely to indicate the presence of an 'arched' and a 'straight' chela (see Fig. 2.19C). There was, however, much bilateral variation in chela lengths and widths in animals in which both chelae were of the same type.

Only the samples that survived the Zoology Department fire and those collected subsequently could be directly examined for the presence of the 'arched' type of chela. Using this material, the analysis of those animals which had both chelipeds (left = L, right = R) (n = 43) indicates:

	L. arched R. straight	R. arched L. straight	Both arched	Both straight
MALE n = 25	7%	7%	2%	42%
FEMALE n = 18	0%	0%	0%	42%

The samples also contained a few animals with only one cheliped (n = 8), but none of these was of the 'arched' type. It was noticed that the arching of the

propodal 'finger' was more pronounced in larger specimens. The smallest animal with an 'arched' chela was of 26.8mm carapace length.

This small sample suggests that the development of the 'arched' chela form is a male phenomenon (though on this sample size its expression in females cannot be excluded, see discussion). This is also suggested by the relative growth data. When the regression relationships for chela widths in males and females are compared, it is evident that a bilateral difference between the chela widths is only apparent in males, with this difference in widths becoming more pronounced with increasing size.

2.3.3. Aspects of reproduction

The smallest ovigerous female included in the fecundity estimation had a carapace length of 20.6mm. It is not known, however, whether this is actual minimum size for the ovigerous females. This animal carried 2244 eggs and one of 20.9mm carapace length carried 2432 eggs. The lowest number of eggs was carried by an animal of 21mm carapace length (see Table 2.3). The largest ovigerous females (cl = 30.1 and 31.4mm) carried 4064 eggs and 10004 eggs respectively.

There was no significant difference between the diameters of fresh eggs (Table 2.3). In addition, there was no correlation between the average egg diameter and carapace length of the females (Fig. 2.9 A). The total number of eggs plotted against carapace length is shown in (Fig. 2.9 B). There was, however, a significant trend for larger females to carry a larger number of eggs than smaller females.

Ovigerous females were observed in November, December, January, February, March, April, and May. Only non-ovigerous females were found in June, July, August, September and October. During early December, and in

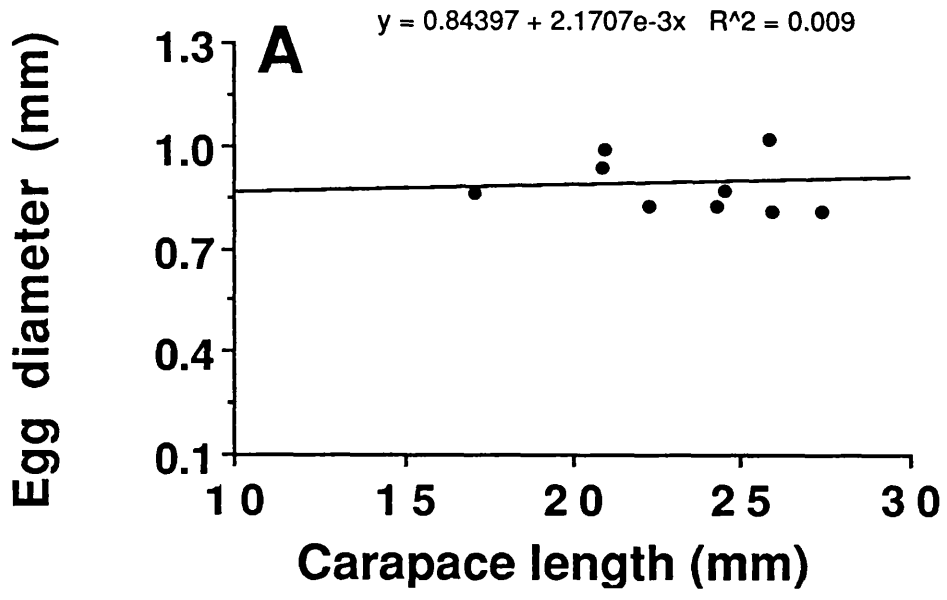
Table 2.3. Total number and the mean diameter of eggs carried by female *M. rugosa*.

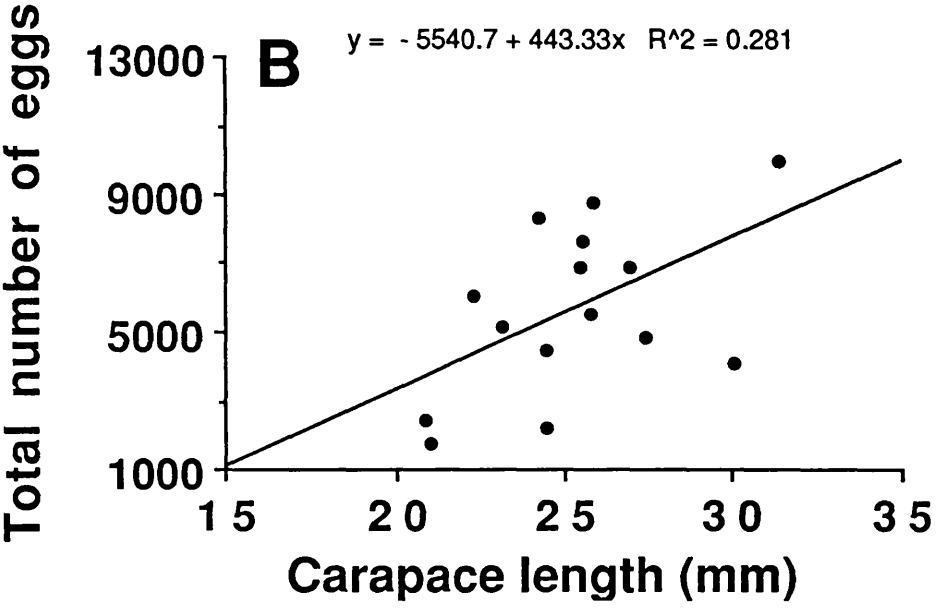
No.	Date	Site	Depth (m)	CL (mm)	No. eggs	Diam. (mm ± s.d.)
1	Feb.	Loch Fyne	20	17.1	-	0.865 ± 0.03
2	Feb.	Loch Fyne	20	21.0	1746	0.993 ± 0.05
3	Mar.	F.Channel	30	25.8	5545	0.023 ± 0.09
4	May	L.Cumbrae	30	30.1	4064	1.001 ± 0.15
5	Mar.	F.Channel	40	20.9	2432	0.941 ± 0.09
6	Mar.	L.Cumbrae	40	24.5	2238	0.870 ± 0.08
7	Dec	M.Channel	115	23.2	5152	FE
8	"	"	"	24.5	4491	FE
9	"	"	"	25.5	6852	FE
10	"	"	"	25.6	7670	FE
11	"	"	"	27.0	6903	FE
12	"	"	"	31.4	10004	FE
13	Jan.	"	"	22.3	6031	0.825 ± 0.09
14	Feb.	"	"	20.6	2244	(2.25 ± 0.2)
15	"	"	"	24.0	6347	(2.25 ± 0.2)
16	"	"	"	24.3	8286	0.823 ± 0.09
17	"	"	"	25.9	8784	0.813 ± 0.07
18	"	"	"	27.4	4845	0.806 ± 0.07

FE = frozen eggs; in parentheses = preserved in 10% formalin solution for 2.5 days; the rest are fresh eggs used within 2-6 days of animal capture. L = Little Cumbrae, F = Fairlie, M = Main, CL = carapace length. The data are presented in order of increasing depth. Within the same month and at the same depth, animals are listed in order of increasing size.

Fig. 2.9

For female *M. rugosa*, the relationship between egg diameter (A) and the total number of eggs carried (B) and carapace length. The correlation for (A) was significant ($P < 0.05$), that for (B) was not ($P > 0.05$).


A
y = 0.84397 + 2.1707e-3x R^2 = 0.009
Egg diameter (mm)
1.3
1.0
0.7
0.4
0.1
10
15
20
25
30
Carapace length (mm)


B
y = - 5540.7 + 443.33x R^2 = 0.281
Total number of eggs
13000
9000
5000
1000
15
20
25
30
35
Carapace length (mm)

March, April and May the samples contained both ovigerous and non-ovigerous females. In addition, in April and May samples, some eggs had hatched and other eggs were observed to hatch when animals caught at this time were placed in aquaria. This suggests that eggs are incubated on the pleopods for at least 5 months with hatching occurring in the spring.

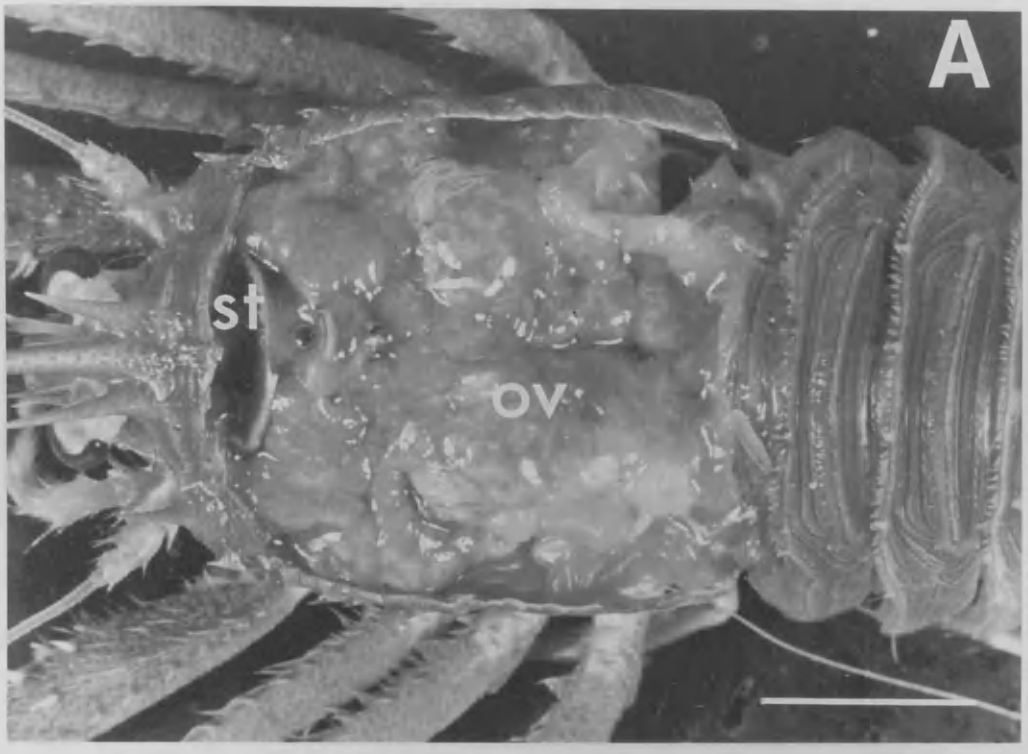
The examination of the preserved ovaries obtained over the cycle revealed some agreement between the state of the ovary development and the occurrence of the ovigerous females. Advanced ovaries (i.e. enlarged orange in colour, full of developed but unreleased eggs were found during July, August and September, intermediate ovaries (yellowish-orange, large, with different sized ova visible and with some of them with the yolk separated from the wall) during March, April, May, June and July. Developing ovaries at an early stage in the developmental cycle were white with very small ova present. The only immature ovary observed occurred in a small female which was obtained from Loch Fyne (weight = 2.47g). This ovary was transparent, empty and thread-like. Ovaries in an intermediate and advanced condition are shown in Fig. 2.10.

2.3.4. Diet

The diet of *M. rugosa* appears to be very variable (Table 2.4) and indicates an ability to adopt different modes of feeding (see 2.3.5). During this investigation, no 100% empty stomachs were obtained. Although some stomachs could be regarded as virtually empty (< 10% full), microorganisms, sand particles, setae and small amounts of undigested tissue were found entrapped by the stomach ossicles. The % stomach fullness was divided to four categories as follows: category 1, >50%; category 2, 26-50%; category 3, 10-25%; category 4, <10%. Out of 114 stomachs obtained from the trawl catch of animals in 1988, 32.5% (9.6% Female + 22.9% Male) were in category 1, 36.8% (10.5% F. + 26.3% M.) in category 2, 11.4% were in category 3 (3.5% F.

Fig. 2.10

Photographs of ovaries of *M. rugosa* in an intermediate (A) and advanced (B) stage of development. Scale bars = 10mm.


A
st
ov

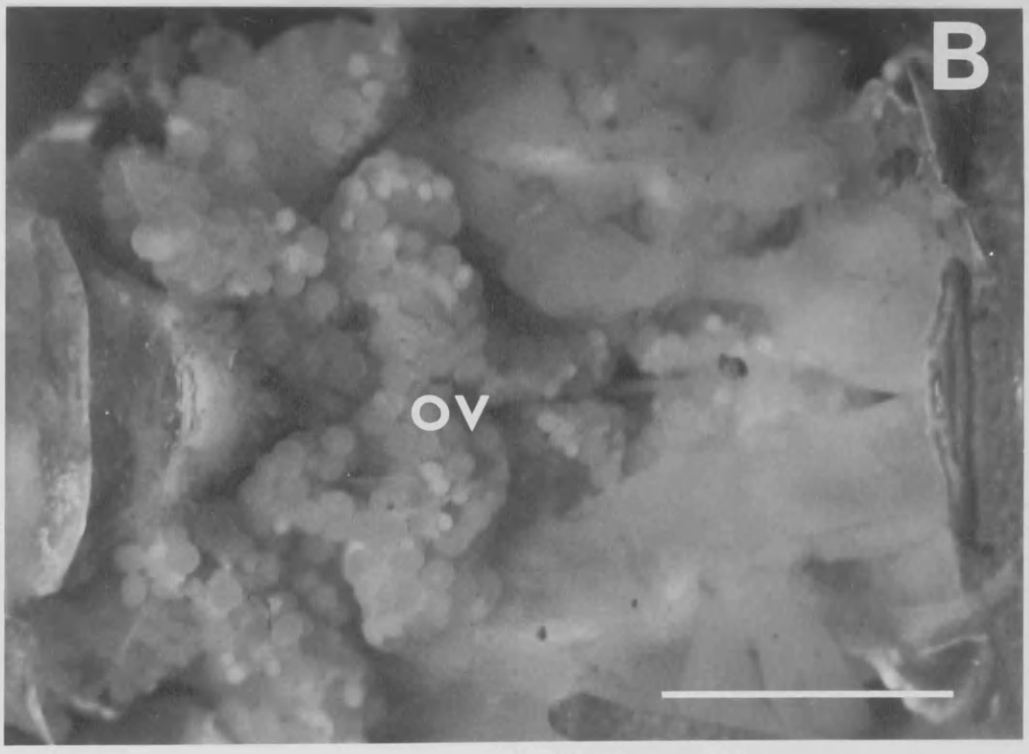

B
ov

Table 2.4. Stomach contents analysis of *M. rugosa* obtained by trawls (n = 114) and from creels (n = 16) i.e. 130 stomachs.

Dietary items.	No.	% of number containing an item.
sediment particles	130	100%
polychaete setae & jaws	83	64%
crustacean parts	79	61%
foraminifera	43	33%
bivalve shell & tissues	35	27%
unidentified cf.	35	27%
algae	34	26%
gastropod remains	24	18%
eggs	18	14%
sponge fragments	1	<1%
unidentified tissue	*	*

* = all stomachs contained some soft, finely divided, highly digested, unidentified organic matter. cf = calcareous fragments. Foraminiferan % occurrence may have been underestimated due to their small size and the difficulty in separating them from sediment grains in the stomach.

+ 7.9% M.) and 19.3% (11.4% F. + 7.8% M.) were in category 4.

In addition, many animals had strands of synthetic fibre in their stomachs from nets, creels, etc. This has also been found in *Nephrops norvegicus* (Bailey *et al.*, 1986) in the same general area.

The diet appears to be mainly of animal material, with polychaetes and crustaceans appearing to dominate. Variation in polychaete setae and jaws indicated that several species were taken. The crustacean component of the diet included euphausids, (*Thysanoessa raschi*) mysids, and small carideans.

Algal material contributed to the diet, though in laboratory observations the algal species used were less attractive than animal tissue. The occurrence of sediment in almost all the stomachs is indicative of periods of deposit feeding (note the foraminifera in the gut).

Detailed analysis of stomach fullness should be based on periodic sampling over 24 hours so that the daily variation in the fullness and the type of diet can be established. This was not possible, but preliminary observations showed that more full stomachs (category 1) were found in the morning catches than in afternoon catches, suggesting a nocturnal feeding peak (see below).

To summarize these data, animals were divided into those with a stomach fullness category of 1 (>50% full) and those in categories 2-4 combined (<50% full) as follows:

	MORNING			AFTERNOON		
	n	nF	nM	n	nF	nM
>50% full	29	10	19	8	1	7
<50% full	42	15	27	35	14	21

In percentage terms, 40.9% (14.1% F & 26.8% M) of animals from morning catches had stomachs which were >50% full, while the corresponding number from afternoon catches was 18.6% (2.3% F & 16.3% M). The trend for greater stomach fullness in morning catches was particularly apparent in males.

The catches contained more males than females (see Table 2.5). A total of 74 males were examined and 35.1% of these had full stomachs (i.e. >50% full = fullness category 1) while 27.5% of the 40 females examined had stomachs in this fullness category.

The examination of the effect of the body size on the diet is not assessed since the majority of the individuals included in the analysis were roughly of similar sizes i.e. females cl = 26.7 ± 4.5mm, n = 40 and males cl = 27.9 ± 5.0mm, n = 74. (see Table 2.3).

2.3.5. Feeding behaviour

The normal resting orientation of *M. rugosa* was for pereiopods 2-4 and the reflexed abdomen to be in contact with the substratum (in the case of a vertical or inclined solid surface, pereiopods 2-4 were effective in enabling the animal to cling on). The chelipeds were extended, resting on the substratum and with the 'fingers' of each chela apposed. The third maxillipeds were orientated downwards (towards the substratum) and slightly inwards. The antennae were spread apart, and the antennules moved almost continuously with only very brief pauses. During such pauses, the antennules were often seen to be lowered to touch the raised third maxillipeds in a brushing sort of action. The third maxillipeds then moved towards the mouth.

When food was presented to individual *M. rugosa*, each moved very slowly towards it and seemed to preferentially select the larger pieces. Food capture and initial manipulation was achieved using the chelate first pereiopods and/or the third maxillipeds. Small pieces of food, in particular algal fragments, were

Table 2.5. Percentage stomach fullness of *M. rugosa* in trawl catches in December, 1988 (n = 114). The creel and (SCUBA) catches are not included because the time of the catch was not precisely known.

Date	Time (GMT)	Dur. (min)	Depth (m)	Site	n	nF	nM	%
05/12/88	10.30-11.30	60	32-42	NC	29	11	18	69.0%
05/12/88	13.00-14.00	60	33-36	NC	28	10	18	35.7%
06/12/88	09.40-10.40	60	83-100	WB	9	2	7	66.7%
06/12/88	11.05-12.00	55	83-100	WB	4	1	3	50.0%
06/12/88	13.35-14.35	60	76-108	MCh	15	5	10	33.3%
19/12/88	10.46-11.53	67	95-114	SMCh	29	11	18	48.3%

Dur = duration; nF = number of females; nM = number of males; n = total number in catch; % full = percentage of stomachs in fullness category 1; NC = north Cumbrae; WB = west Bute; MCh = Main Channel; SMCh = south Main Cumbrae Channel.

often difficult to grasp and were sometimes swept away in the exhalant respiratory currents. Larger pieces of algae were grasped and held near the mouth using both chelae. After manipulation, an edge was eventually placed between the cutting mandibles. These cut through the algae thallus a tiny piece at a time. The outline of each cut edge was curved. The algal thallus was moved upwards by the pushing action of the chelipeds and the third maxillipeds. The second maxillipeds also were used in the manipulation of the food by pulling it towards the mandibles. The three-segmented mandibular palps moved up and down behind the cutting edges of mandibles. They moved downwards when the mandibles were apart and upwards during closure. When feeding, the labrum was observed to be raised upwards and the paragnaths made lateral movements. The labrum, paragnaths and mandibular palps all appeared to be involved in pushing food into the mouth.

Similar food manipulation was observed in the case of animal material. Soft-bodied food such as *Nephtys hombergii* was ingested more rapidly than equivalent-sized pieces of algae.

Of the food items offered, bivalve meat and the live *N. hombergii* were preferred to algae. When starved individuals were offered the choice of food noted above, the live nephtyid polychaete was captured and ingested preferentially. However, brittle stars and peacock worms were totally ignored by almost all of the experimental animals.

It is to be noted that the elongated chelae of the first pereiopods are principally forcipate and not adapted for crushing shells, etc., with the possible exception of the wide, curved chela type often seen in larger animals (section 2.3.6). Observations of feeding did not, however, shed any light on the functional significance of heterochely. The chelae were observed to be good for manipulating food items including algal thalli. *M. rugosa* was capable of

detecting buried food items. The chelipeds were used to probe the sand, and transferred food to the mouth. Pereiopods 2-4 could also help in the search for buried food. When picking up the food item, the chelar tips were observed to be held in an almost perpendicular (vertical) position against the substratum. Sediment taken with the food items was mostly ingested. The third maxillipeds were also apposed and used to shovel material upwards towards the mouth. Intact *Abra alba* were picked up by the chelae and transferred to the third maxillipeds . Then the second and the third maxillipeds grasped the shell and rotated and brushed it several times before transferring it to the opened mandibles. The mandibles were unsuccessful in breaking the shell and after further manipulation by the mouth parts, the mollusc was rejected.

The first pereiopods were used alternately to move food to the mouth when deposit feeding, but were often used in synchrony when lifting large food items to the mouth. Here the food was seized by the third maxillipeds which moved together or alternately. The second maxillipeds, however, were normally observed to work alternately. The second and third maxillipeds were observed to work co-operatively during feeding. The extension and flexion of the right third maxilliped was synchronous with similar movements of the second maxilliped of the left side. The second maxillipeds were important in manipulating and orientating food as it was presented to the mandibles, with the stout dactylar setae gripping macroscopic food items.

During feeding, the inner mouth parts (i.e. first maxillipeds, first and second maxillae), were observed to move in a horizontal plane and to all function in food manipulation and in pressing the food into the mouth opening. The first maxilliped pair were synchronized in their movements and operated in synchrony with the first maxillae. The synchronized movements of the second maxillae alternated with those of the first maxillae and the first maxillipeds. Thus, when the first maxillipeds and first maxillae were seen to move inwards

to meet at the curved mandibles, the outer endites of the second maxillae were seen moving away from the mandibles. These inner mouthparts appeared to function to hold the food against the mandibles and also to abrade softer food items.

Deposit feeding was often observed. The chelae of the first pereiopods were seen to convey sediment to the mouth; the chelar tips are pointed and slightly curved inwards which facilitated the collection of food from the substratum. The third maxillipeds, however, played the major role in sediment gathering, either directly from the substratum or by preening sediment from any of the pereiopods. Sometimes, even material cleaned by the setose and chelate fifth pereiopods from within the branchial chambers, the setae of the branchiostegite edge and elsewhere, was seen to be transferred to the third maxillipeds. The third maxillipeds were cleaned of entrapped sediment by the second maxillipeds and material was passed inwards towards the mouth. The third maxillipeds have a major role in preening and it was interesting to observe that preened material could be ingested.

M. rugosa is therefore a generalized feeder, capable of herbivory, carnivory and deposit feeding. It will both scavenge and predate and appears to have a preference for animal food over plant food. In addition, when overcrowded in an aquarium tank, the smallest individuals were seen to be attacked and eaten by the larger ones. Cannibalism has also been observed in the field (S. J. Anderson, pers. comm.).

2.3.6. Morphology of the mouth parts and pereiopods

The mouth parts of *M. rugosa* are illustrated in Figs 2.11 to 2.19.

Mouth.

The mouth opening lies posterior to the mandibles. A dorsally placed lobe,

the labrum, covers the mouth opening. On each side of the mouth opening is a lobate paragnath, located behind each mandible.

Mandibles. (Fig. 2.11)

The mandibles of *M. rugosa* are simple and appeared to be adapted for cutting rather than crushing. The molar process is smooth and convex. The incisor process is straight (i.e. not toothed), sharp and curved slightly inwards. The three segmented mandibular palp (Fig. 2.11 B) is provided with short, stout setae some of which are plumodenticulate while others appear to be triserrate. The setae are more dense on the distal segment which moves up and down to clean the face of the mandibles.

First maxillae. (Fig. 2.12)

The first maxillae are thinly chitinized. The contiguous coxa and precoxa form a semi-rectangular shape. The medial border of the basal and coxal endites of the first maxilla is fringed with dense stout plumodenticulate and cuspidate setae (Fig. 2.12 B). Pappose setae occur on the endites, particularly the coxal endite and both endites are provided with serrulate and possibly triserrulate setae. Proximally, the first maxilla bears a lobe (exite) which has long plumose setae arising along its ventral border.

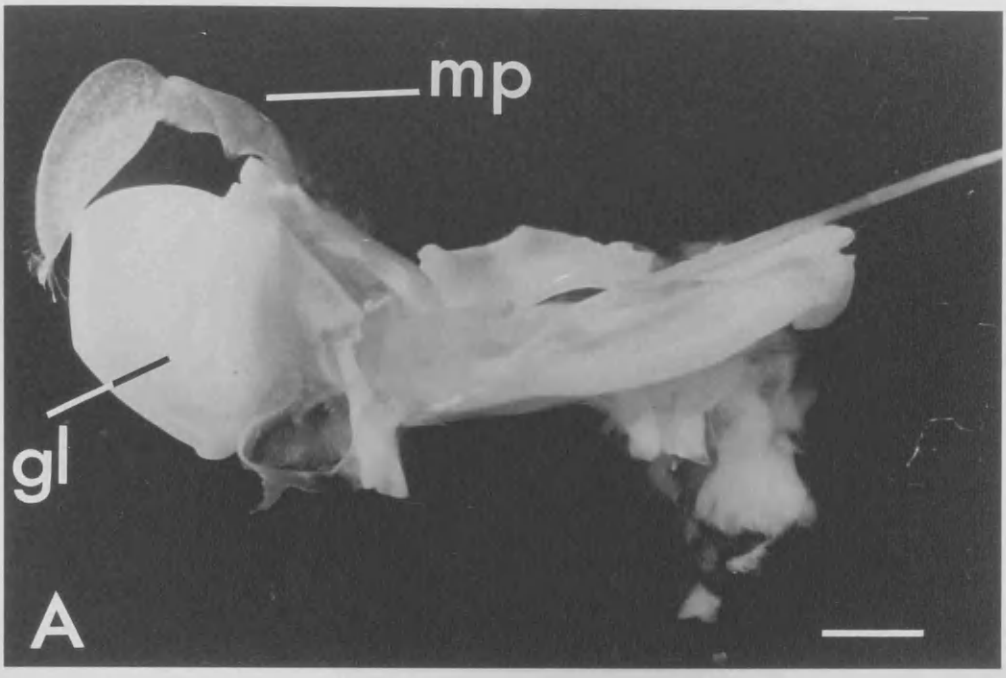
The basis of the first maxilla is roughly triangular in shape. An endopod is attached to the basis. Variously sized plumose setae were found on the endopod of the appendage.

Second maxillae. (Fig. 2.13)

Each appendage is thin and consists of a small endopod, bilobed basal and coxal endites and a large exopod. The exopod of this appendage is the sub-rectangular scaphognathite (Fig. 2.13 A, also Chapter 3) which generates branchial water flow. The edge of the scaphognathite is bordered with dense

Fig. 2.11

(A) Photograph of the right mandible (inner surface) and (B) scanning electron micrograph of the right mandibular palp of *M. rugosa*. Scale bars = 1000μm (A); 100μm (B) (the scale bar is the space between white bars - this should be remembered in all subsequent micrographs). gl = gnathal lobe; mp = mandibular palp.


mp
gl
A

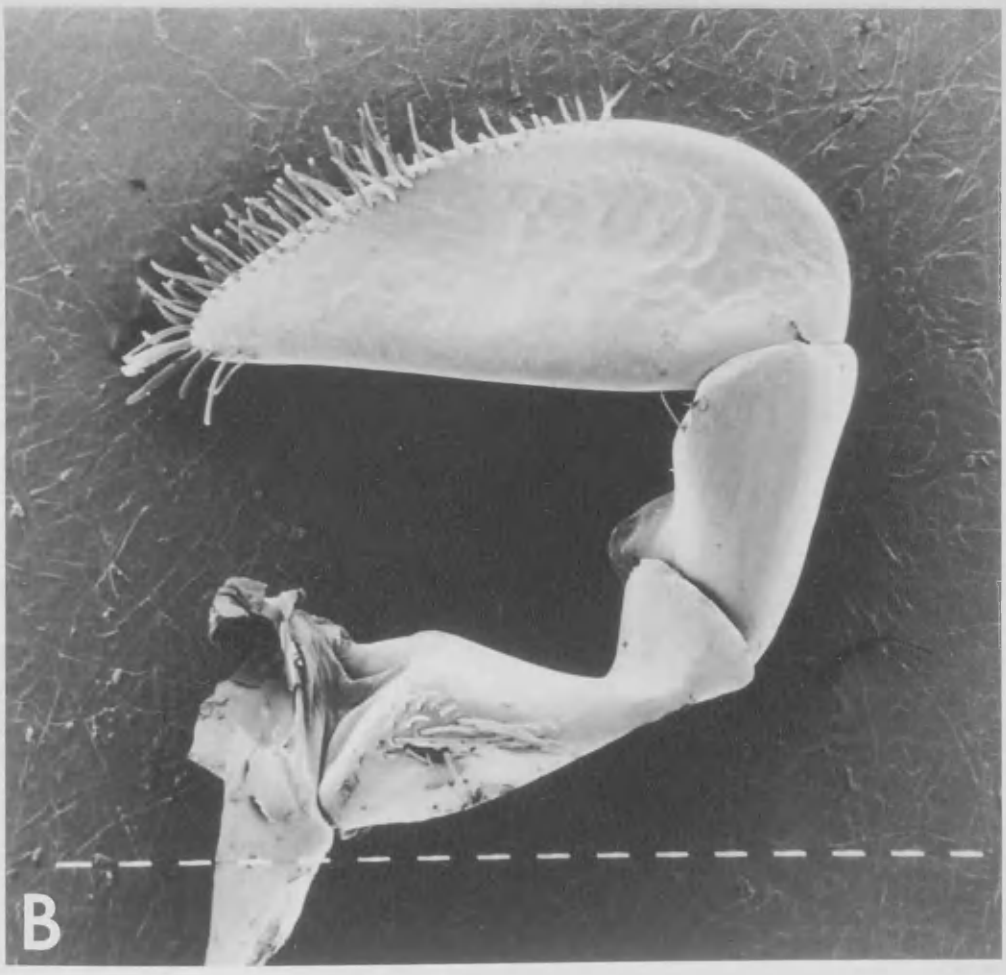
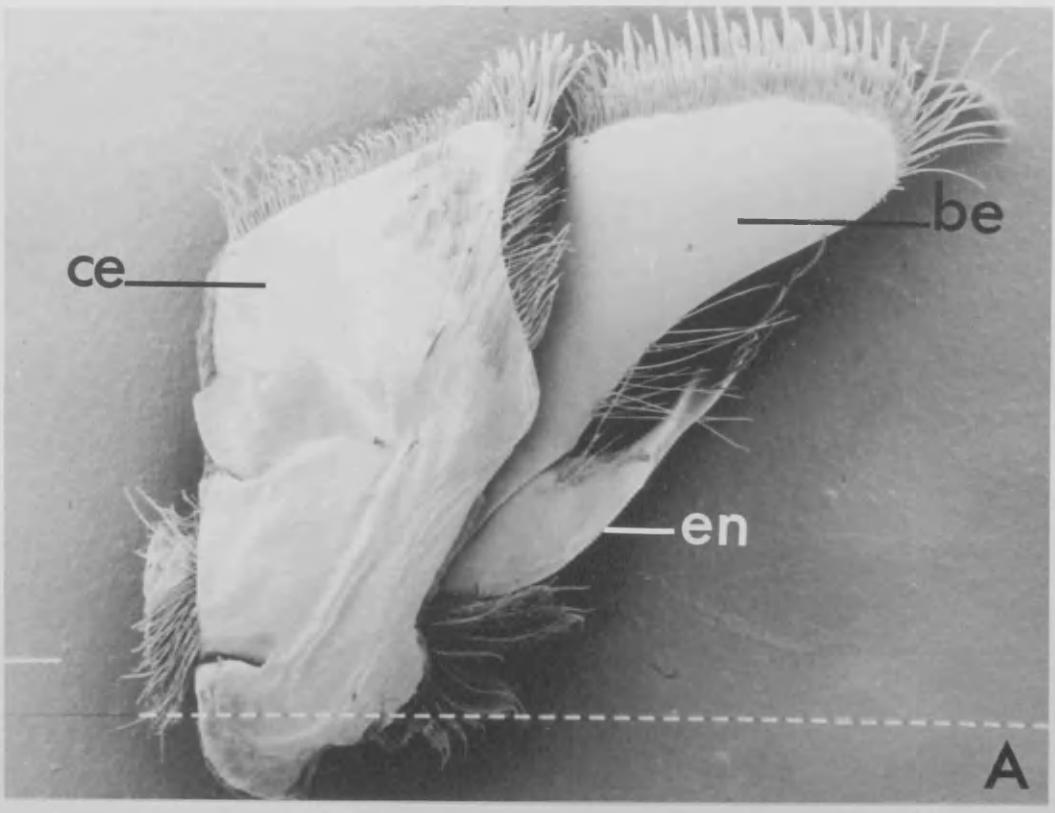

B

Fig. 2.12

Scanning electron micrographs of first maxilla of *M. rugosa*. (A), whole right maxilla (scale bar = 1000μm); (B) setae at edge of basal endite (be) of left appendage (scale bar = 100μm). ce = coxal endite; en = endopod.


ce
be
en
A

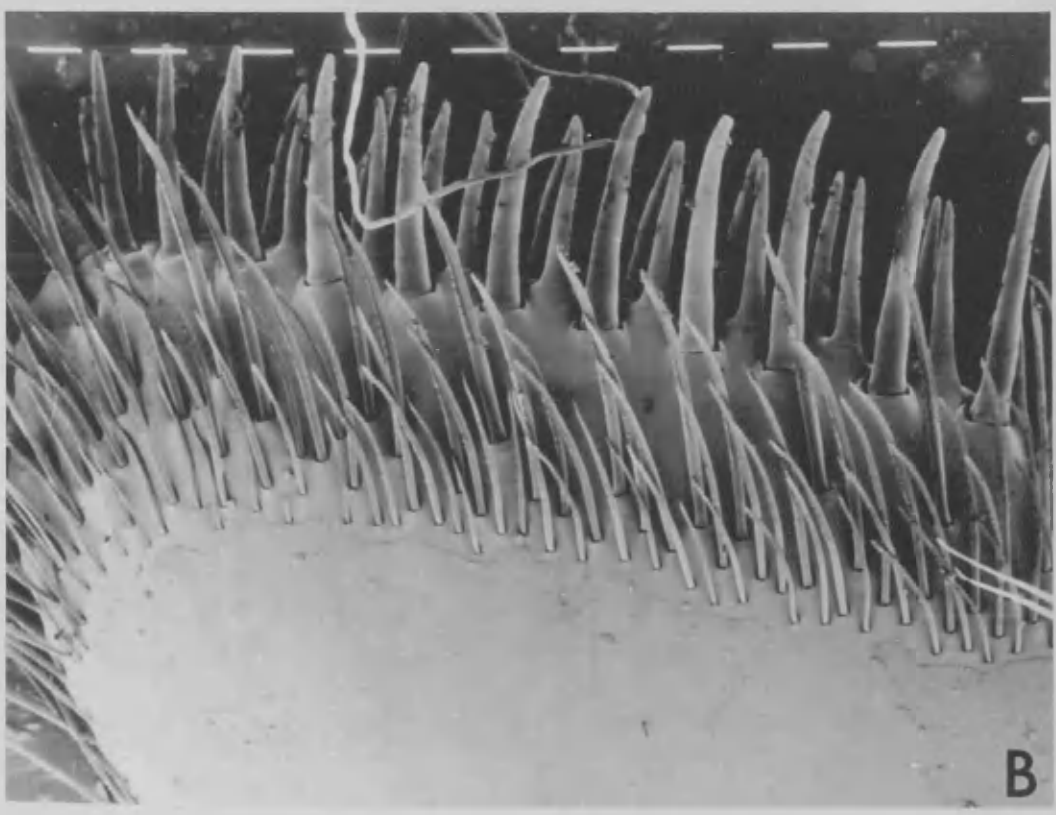
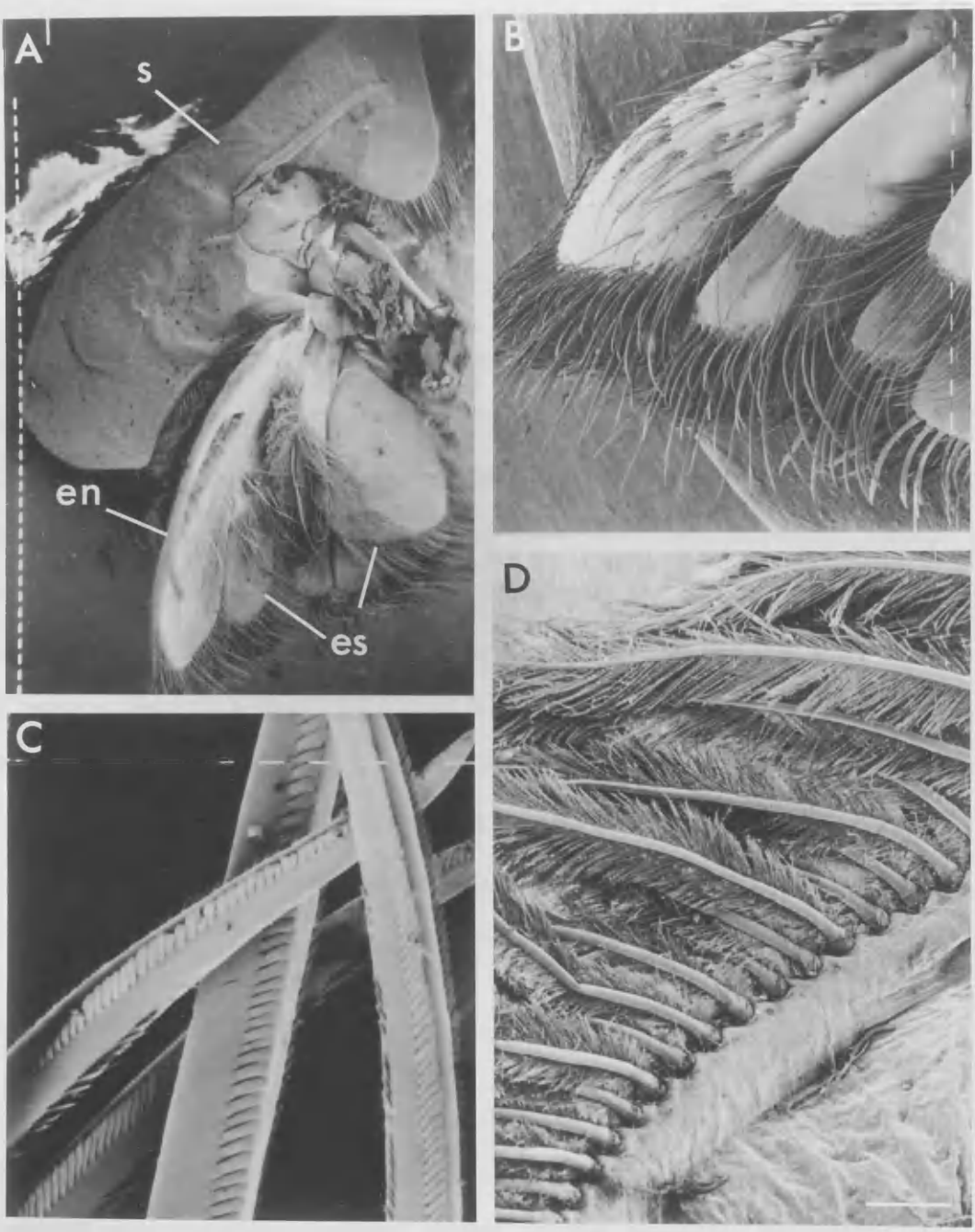

B

Fig. 2.13

Scanning electron micrographs of second maxilla of *M. rugosa*. (A), whole right appendage (inner surface); (B), outer surface of endites (es) showing setal fields; (C), details of triserrate endite setae (D), details of the plumose setae at the scaphognathite (s) margin. en = endopod. For (A), scale bar = 1000μm; scale bars for (B) & (D) = 100μm; scale bar for (C) = 10μm.


A
s
en
es
B
C
D

plumose setae (2.13 D).

Along the coxal and basal margins there are layers of setae which include long plumose and pappose setae together with stout cuspidate setae, and plumodenticulate and triserrate setae (Fig. 2.13 B & C). In addition, acute simple setae occur in groups of different sizes on the surface of the coxa and basis.

First maxillipeds. (Fig. 2.14)

The first maxillipeds are slightly thicker than the maxillae and each consists of an endopod, exopod and epipod. There are pronounced basal and coxal endites. The exopod articulates with a terminal flagellum. Dense plumose setae occur along the distal part of exopod and along the flagellum (Fig. 2.14 C). The blade-like epipod bears pappose setae along its margins and there are plumed setae along the endopod. The medial margins of the basal and coxal endites are densely setose (Fig. 2.14 B). Here, comb-like layers of setae comprise plumodenticulate and triserrate or triserrulate setae on the coxa, with plumodenticulate setae and pappose setae on the basis. In addition, short and long simple setae are present on the surface of the appendage, together with serrulate setae.

Second maxillipeds (Fig. 2.15)

The exopod of this appendage is longer than the endopod. There is no epipod in this species. The endopod is similar to that of a typical ambulatory leg in consisting of a coxa, basis, ischium, merus, carpus, propodus and dactyl (Fig. 2.15A). The exopod terminates in a jointed flagellum (Fig. 2.15 C). Setal fields occur along the entire surface of the appendage and are particularly dense on the distal part of the endopod.

The dactyl of the endopod bears long cuspidate setae and long-shafted setae

Fig. 2.14

Scanning electron micrographs of first maxilliped of *M. rugosa*. (A), whole left appendage (inner surface); (B), setae of basal endite (be) (see text) (C), plumose setae of exopod (ex). ce = coxal endite; en = endopod; ep = epipod. For (A), (B) & (C), scale bars = 100μm, 10μm & 10μm, respectively.

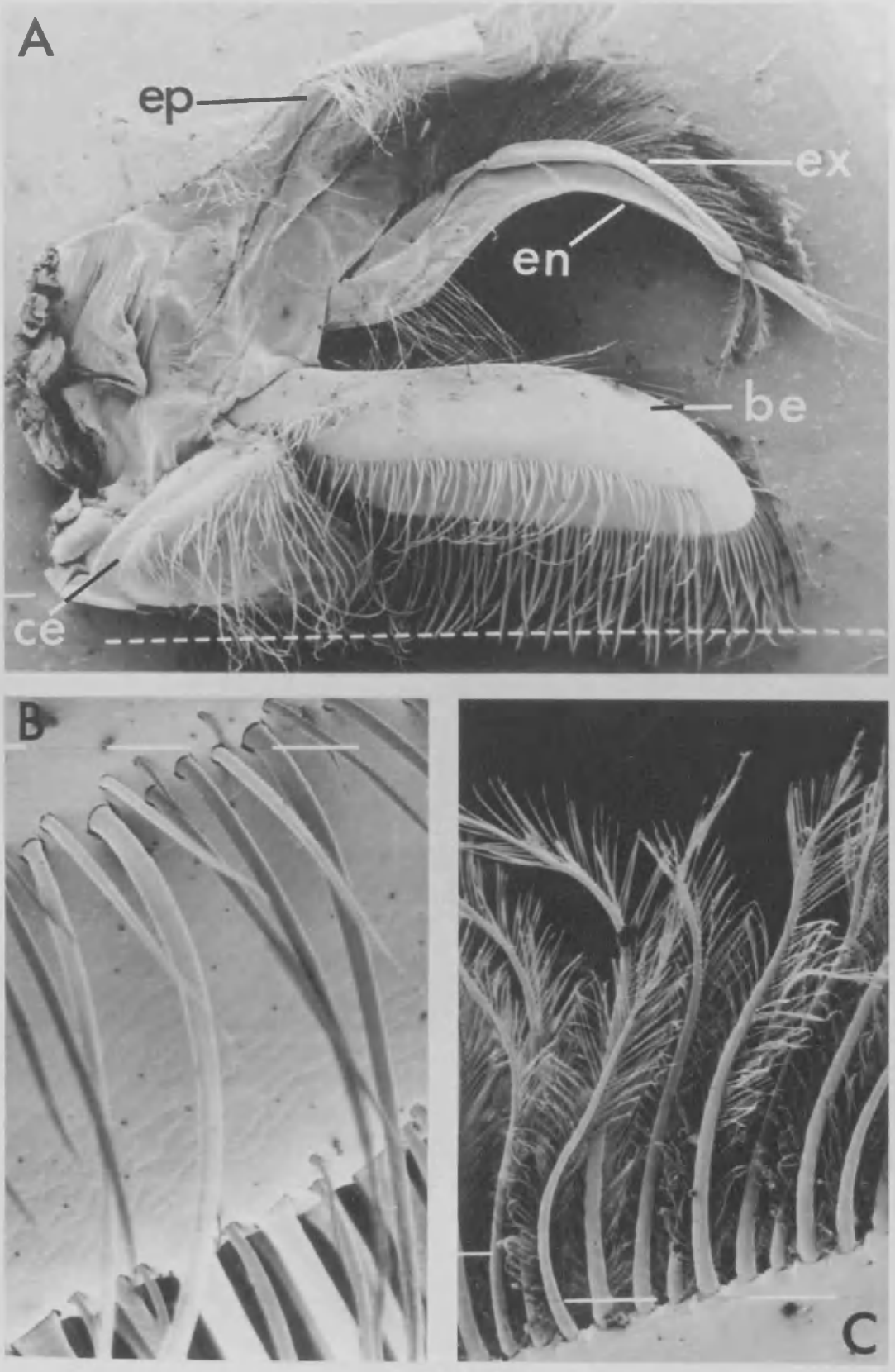
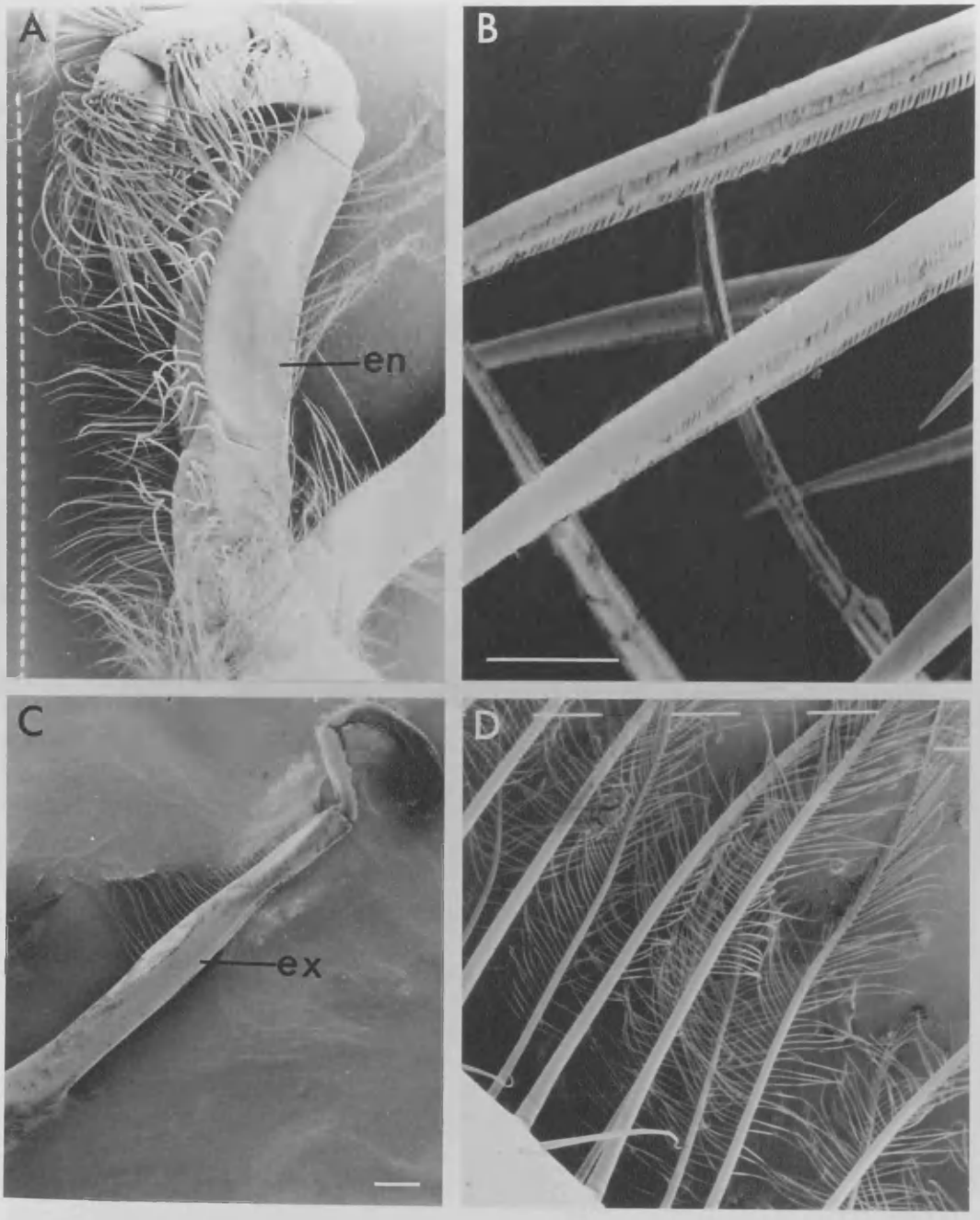

A
ep
ex
en
be
ce
B
C

Fig. 2.15

Scanning electron micrographs of second maxilliped of *M. rugosa*. (A), endopod (en), outer surface of left appendage; (B) triserrate setae of dactlyl; (C), exopod (ex), orientation as for (A); (D) plumose setae from exopod shaft. For (A), (B), (C), and (D), scale bars = 100μm, 100μm, 1000μm & 10μm, respectively.


A
en
B
C
ex
D

with triserrate tips (Fig. 2.15 B). Similar triserrate setae occur on the other endopod segments and plumodenticulate setae occur on the proximal segments. Serrulate or triserrulate setae appear to occur on all segments of the second maxilliped. Pappose setae are found on the basis and ischium. There is a stout spine on the ischium edge. The indentation which is formed as a consequence of the presence of the spine is filled with a dense tuft of pappose setae. Some pappose and simple setae are found amongst the other setal types elsewhere on the endopod. Long plumose setae occur on the flagellum of the exopod and, less densely, plumose and pappose setae, occur along the exopod shaft (Fig. 2.15 D).

Third maxillipeds. (Fig. 2.16)

In this appendage, the exopod is shorter than the endopod. An epipod is present which arises from the coxa. The ischium is longer than the merus in contrast to the second maxilliped. A large spine occurs on the medial distal edge of the ischium. Two large spines are present on the merus, one of which occurs on the lower part of the segment, on the medial edge, while the other is found at the top of the lateral edge, near the joint with the carpus. The latter spine is diagnostic for *M. rugosa* (Rice & de Saint Laurent, 1986) (Fig. 2.17 A)

The exopod again terminates in a flexible flagellum. Plumose setae occur densely on the flagellum and groups of pappose setae are found on the exopod shaft. Similar groups of pappose setae, and some plumose and plumodenticulate setae, occur on the lateral edge of the proximal segments of the endopod where simple setae are also found. Fan-shaped clusters of usually short, pappose setae are present on the outer surface of the exopod and endopod. In contrast to the second maxilliped, the dactyl and propodus of the third maxilliped endopod are covered with a dense field of serrate setae. Several robust setae occur at the tip of the dactyl and these may be long

Fig. 2.16

Scanning electron micrographs of the third maxilliped of *M. rugosa*. (A), whole right appendage, outer surface; (B), pappose setae of epipod (ep); (C), serrate setae of dactyl and propodus; (D) triserrate setae of ischium. en = endopod; ex = exopod. For (A), (B), (C), and (D), scale bars = 1000µm, 10µm, 10um & 10µm, respectively.

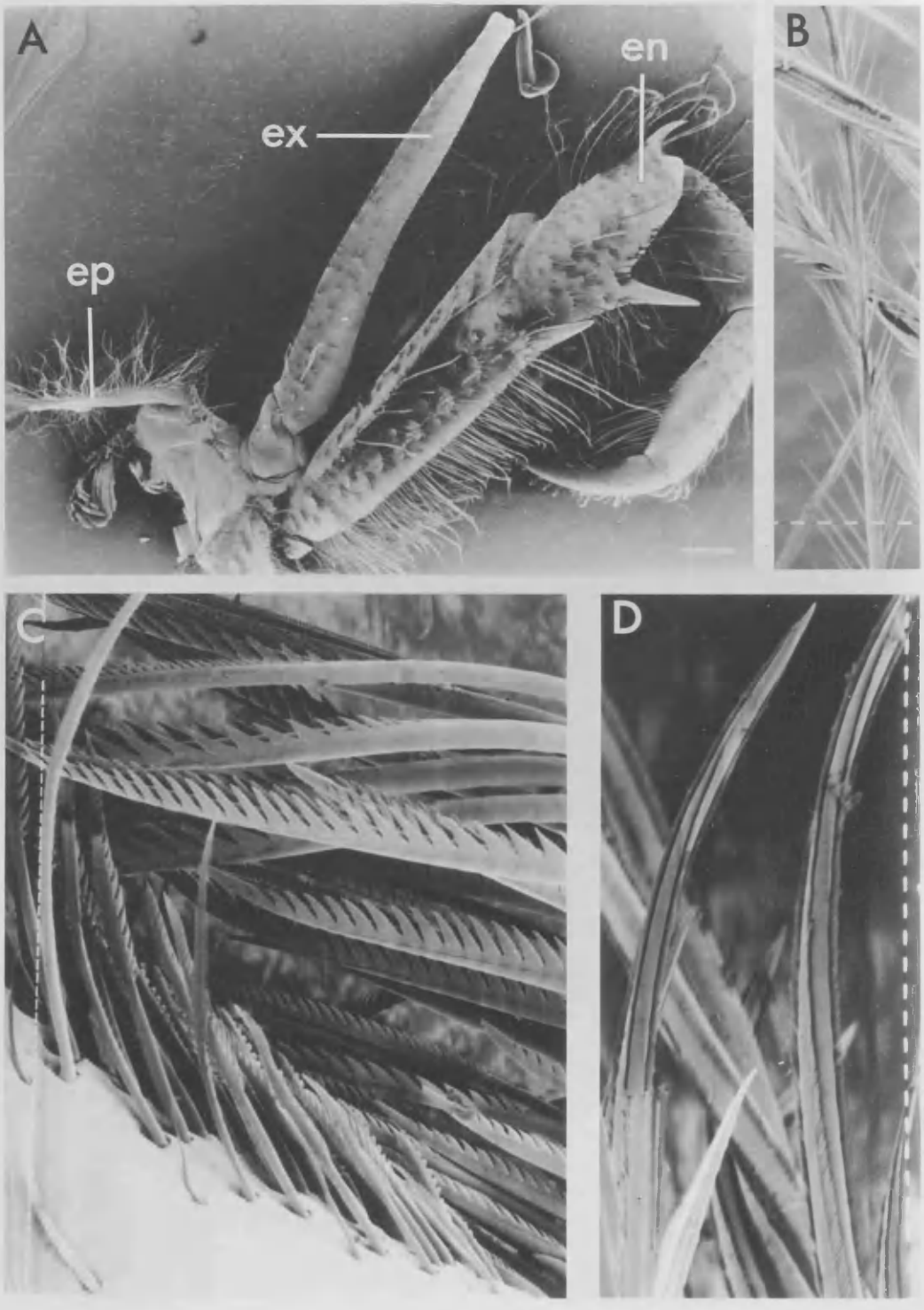
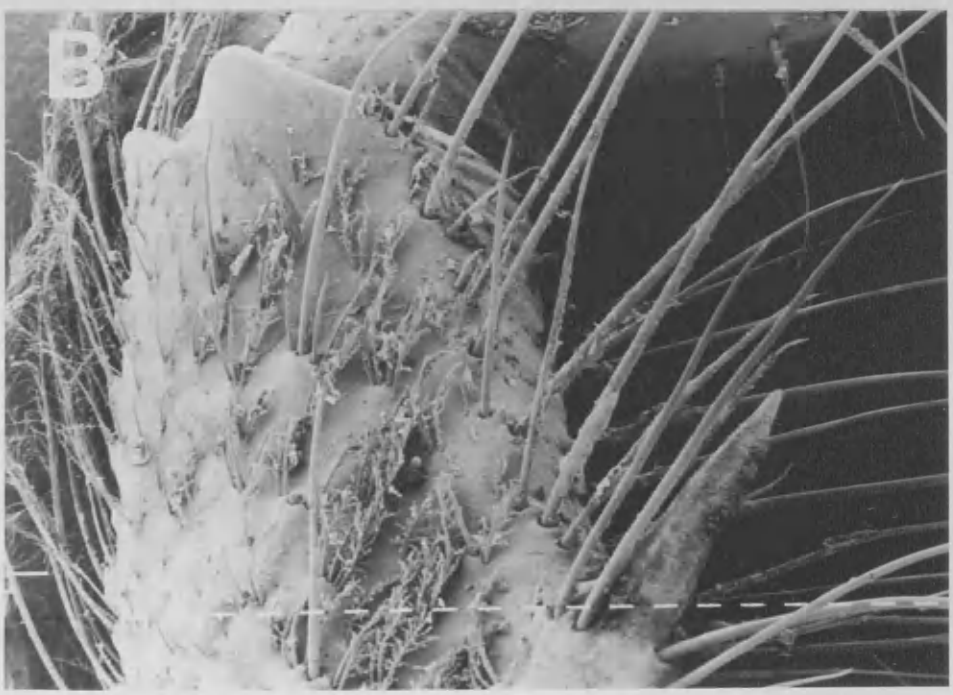

A
en
ex
ep
B
C
D

Fig. 2.17

Scanning electron micrographs of outer surface of merus of right endopod of the third maxilliped of *M. rugosa* (A) and *M. sarsi* (B). Scale bars = 100μm.


A


B

cuspidate setae. Triserrate setae also occur on the dactyl and propodus and these are the dominant setal type on the medial surfaces of the carpus, merus and ischium of the endopod. Triserrulate, serrulate, plumodenticulate and long pappose setae also occur on the medial surfaces of the merus, carpus and ischium and elsewhere. Plumodenticulate and pappose setae are also found on the basis and coxa.

First pereiopods (major chelipeds). (Figs. 2.18, 2.19)

Each major cheliped of *M. rugosa* is cylindrical and elongated. Numerous stout setae are present on all of the segments. A rudimentary epipod is present which arise from the coxa. The propodal extension and the dactyl form the chela which has 2-3 teeth and smaller denticulations along its inner edge. These denticulations of both edges of the chela meet imperfectly. Each chela is also covered with numerous simple setae and small spines. The tip of the dactyl terminates in a stout spine and the tip of the propodal 'finger' terminates in two spines (Fig. 2.19 C). Ideally, the dactylar spine fits between the propodal spines on chelar closure. However, they often fail to meet because of damage and regeneration. The spines of the tip of each claw are sharply pointed and inwardly curved. Because of these features, the claws of *M. rugosa* are poorly adapted for crushing or cutting but are forcipate in nature. There are, however, two types of chela (see Section 2.3.2 and Fig. 18 A-C). One form has long parallel 'fingers' while the other is broader with the propodal 'finger' being curved proximally, though the tip is still forcipate.

Pereiopods. 2-5

The second, third and fourth pairs of pereiopods are ambulatory and are well developed for clinging and gripping by possessing sharp pointed dactyls. They are covered with setae and spines. The fifth pereiopod is normally reflexed beneath the branchiostegite where it has a cleaning function. It is chelate and

Fig. 2.18

Photographs of chelipeds of *M. rugosa* (A-C), and *M. sarsi* (D-F) from different individuals. Chela types: (A) = left (L) 'arched', right (R) 'straight'; (B) = L 'straight' R 'arched'; (C) = both 'arched'; (D) = both 'straight'; (E) = as (B); F = as (C).

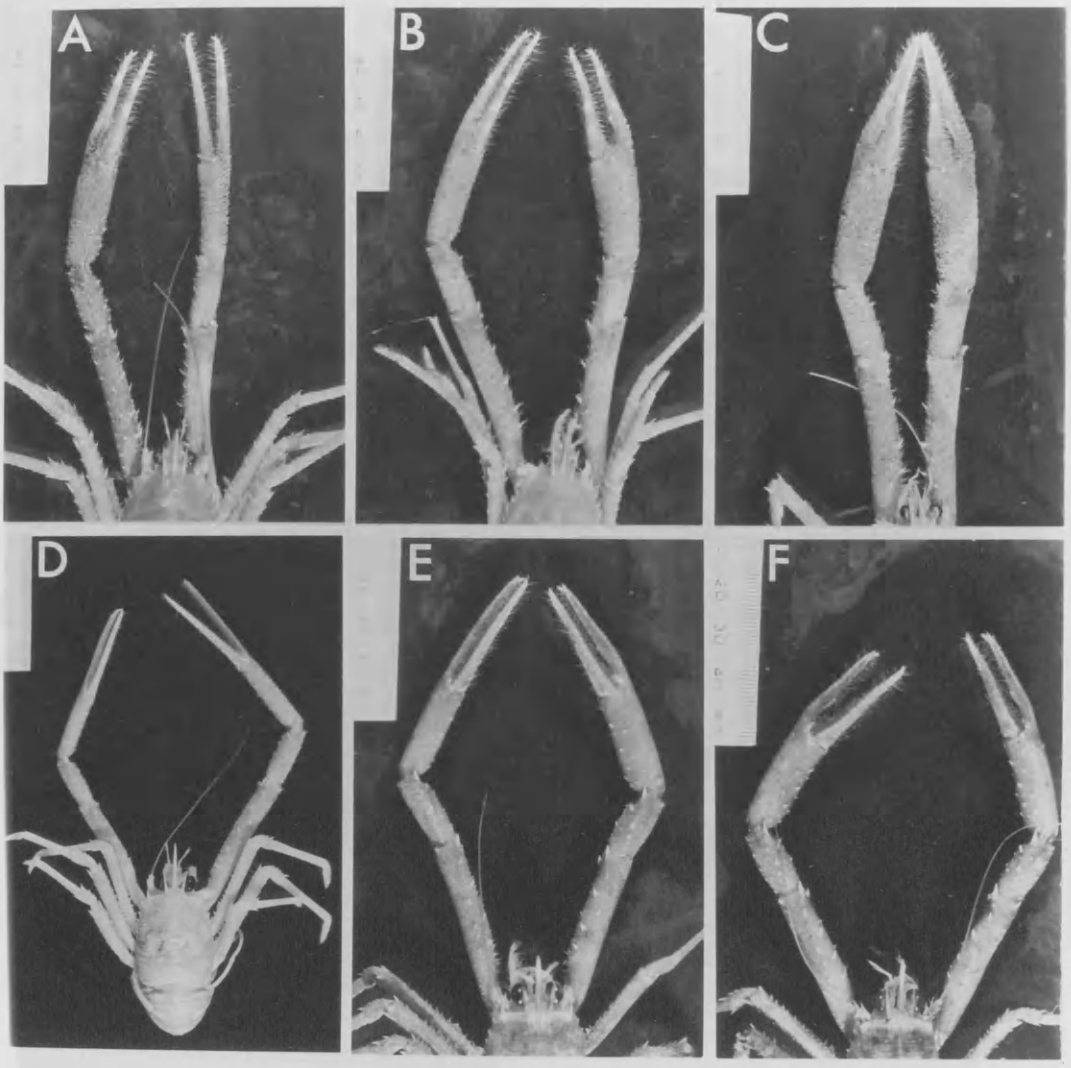
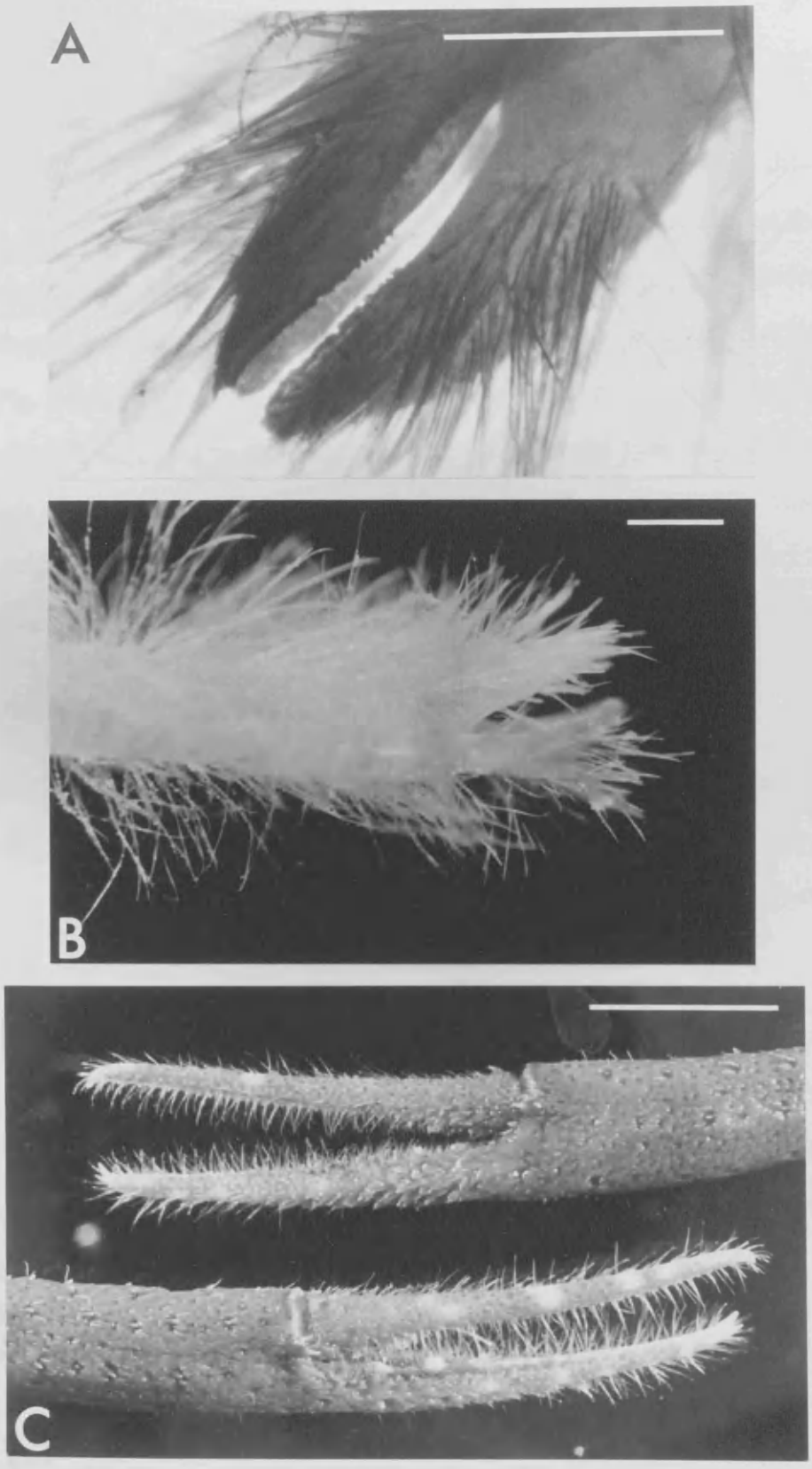

A
B
C
D
E
F

Fig. 2.19

Photographs of chelate fifth pereiopod (A) & (B), and first pereiopods (chelae) of *M. rugosa* (C). For (A) to (C), scale bars = 1mm, 1mm & 10mm, respectively.


A
B
C

densely setose (Fig. 2.19 A & B).

Antennules. (Fig. 2.20)

Although not feeding appendages, the sensory antennules were seen to be constantly in motion during feeding and were frequently cleaned by the third maxillipeds. Plumodenticulate, plumose and simple setae are present on the antennular base, with many of the pappose setae being arranged in clusters. There are three large dorsal spines and an additional fourth large spine arises ventrally (Fig. 2.20 A). A tuft of long pappose setae with thick shafts occur at the antennular tip, adjacent to the flagella. Two flagella are present. Between them is a tuft of long simple setae with annular shafts (Fig. 2.20 B). The basal region of each setal shaft is nodular. These latter setae are regarded as olfactory since they are innervated by the olfactory nerve (Pike, 1947).

2.3.7. Morphology of the stomach (Figs. 21-24)

The stomach of *M. rugosa* was examined by cutting along a longitudinal axis either through the cardio-pyloric valve (Fig. 2.21 A), or through the dorsal tooth so that, in this latter case, the intact cardio-pyloric valve could be seen (Fig. 2.21 B).

The stomach consists of two compartments: an anterior cardiac stomach into which the oesophagus opens and where grinding of the food occurs, and a posterior, pyloric stomach. Here filtering of the fine food particles occurs. The pyloric stomach leads into the hind-gut. The digestive gland opens into the pyloric stomach.

The cardiac stomach contains teeth carried by ossicles which form the grinding apparatus - the 'gastric mill' (Fig. 2.22). The stomach ossicles articulate with each other and are moved by a complex musculature. There is a median dorsal tooth, and on each side a lateral tooth and a lateral accessory

Fig. 2.20

Scanning electron micrographs of the antennules of *M. rugosa*. (A), whole appendage; (B), setae of flagella. ab = antennular base; ap = antennular peduncle; fl = flagella. For (A), and (B) scale bars = 1000μm, and 100μm respectively.

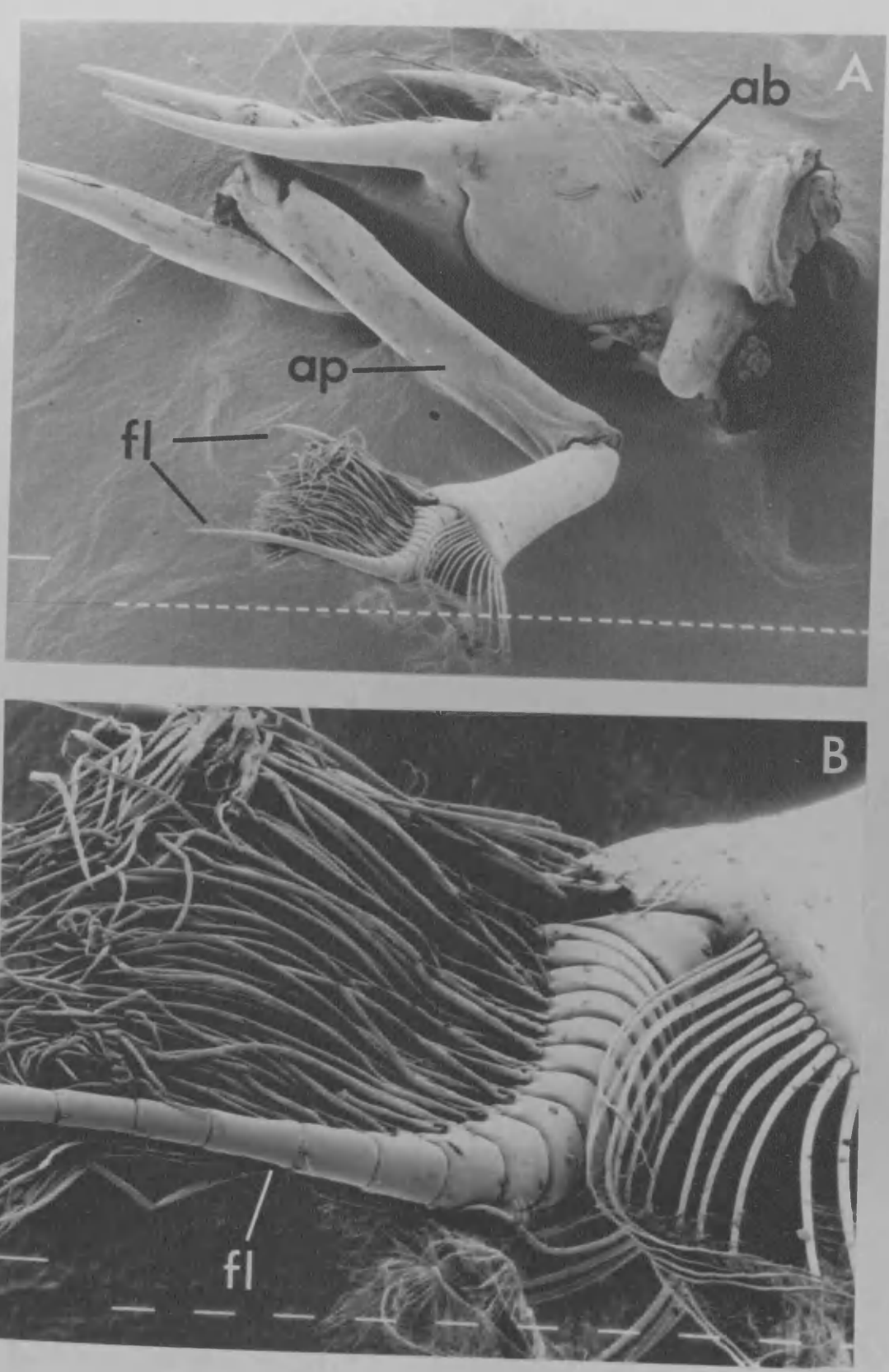
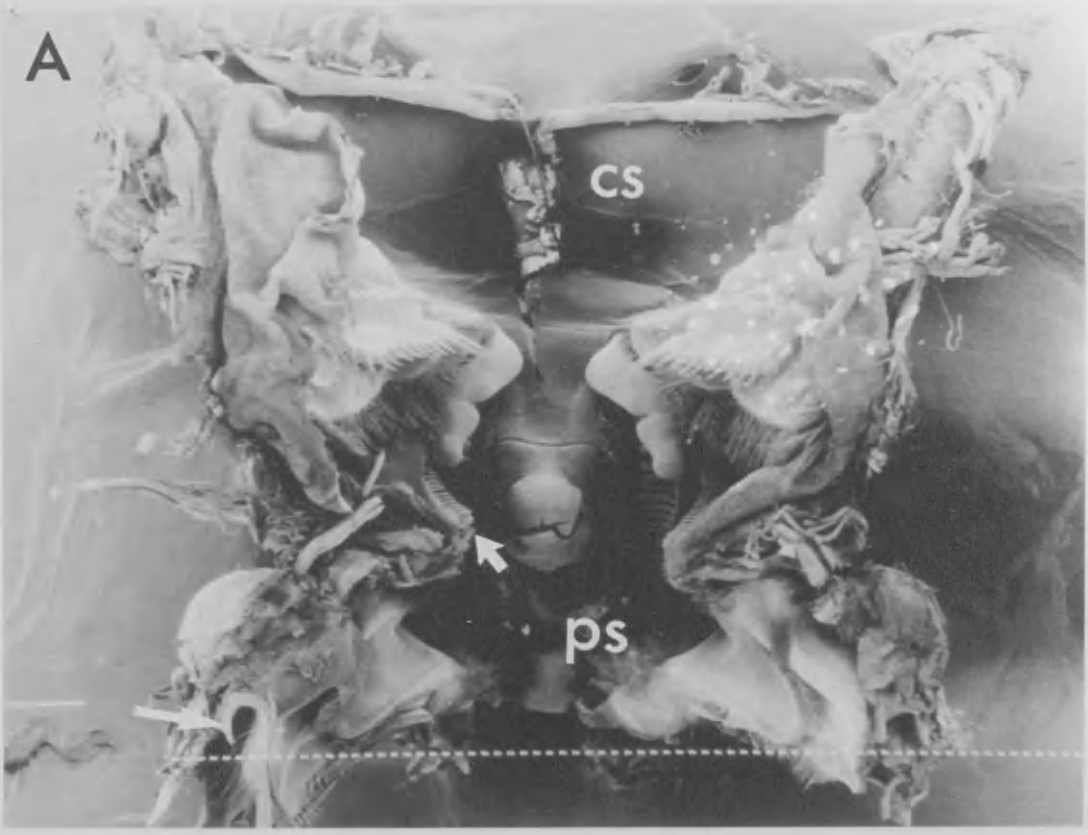

A
ab
ap
fl
B
fl

Fig. 2.21

Scanning electron micrograph of the whole stomach of *M. rugosa* (A)-stomach wall bisected ventrally to show internal structures: cs = cardiac stomach; ps = pyloric stomach. (B), cardiopyloric-valve revealed by a dorsal bisections of the stomach wall: ts = transverse striae; ch = channel; ct = cardio-pyloric trough. The long arrow marks the position of the detail shown in Fig. 2.24. The short arrow indicates part of the bisected cardio-pyloric valve. For (A) and (B), scale bars = 100μm.


A
cs
ps

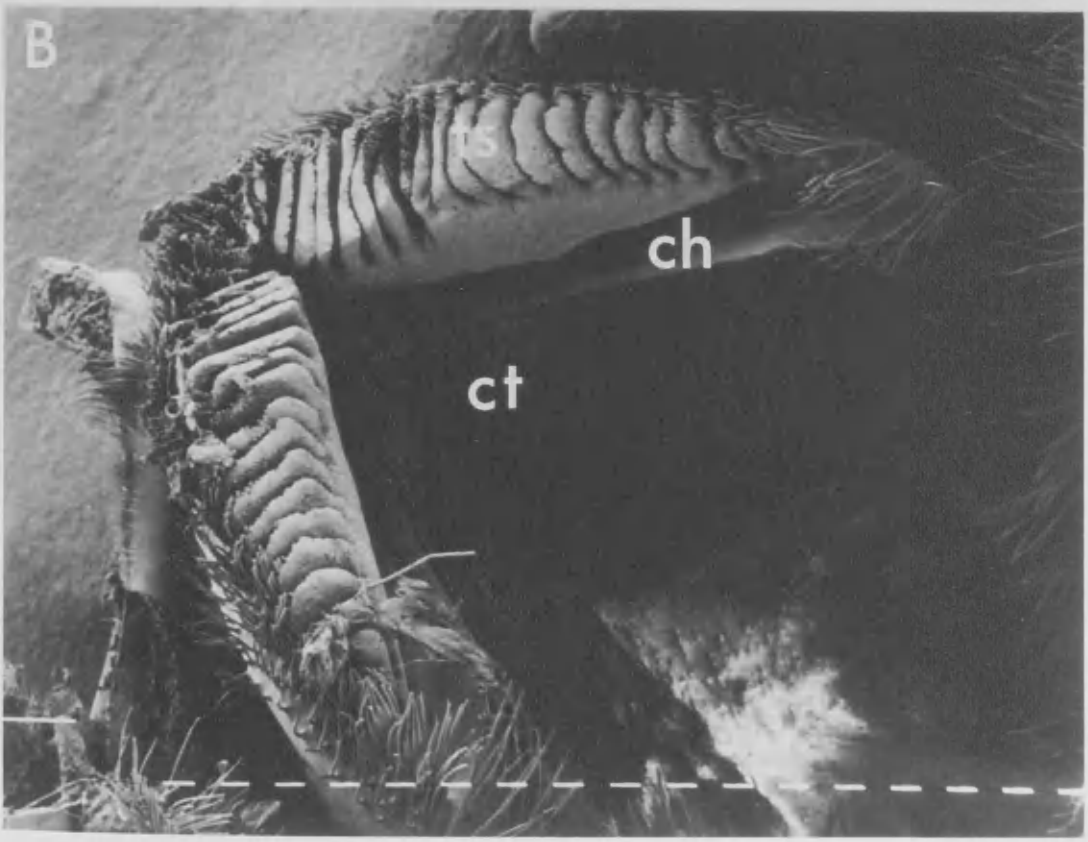
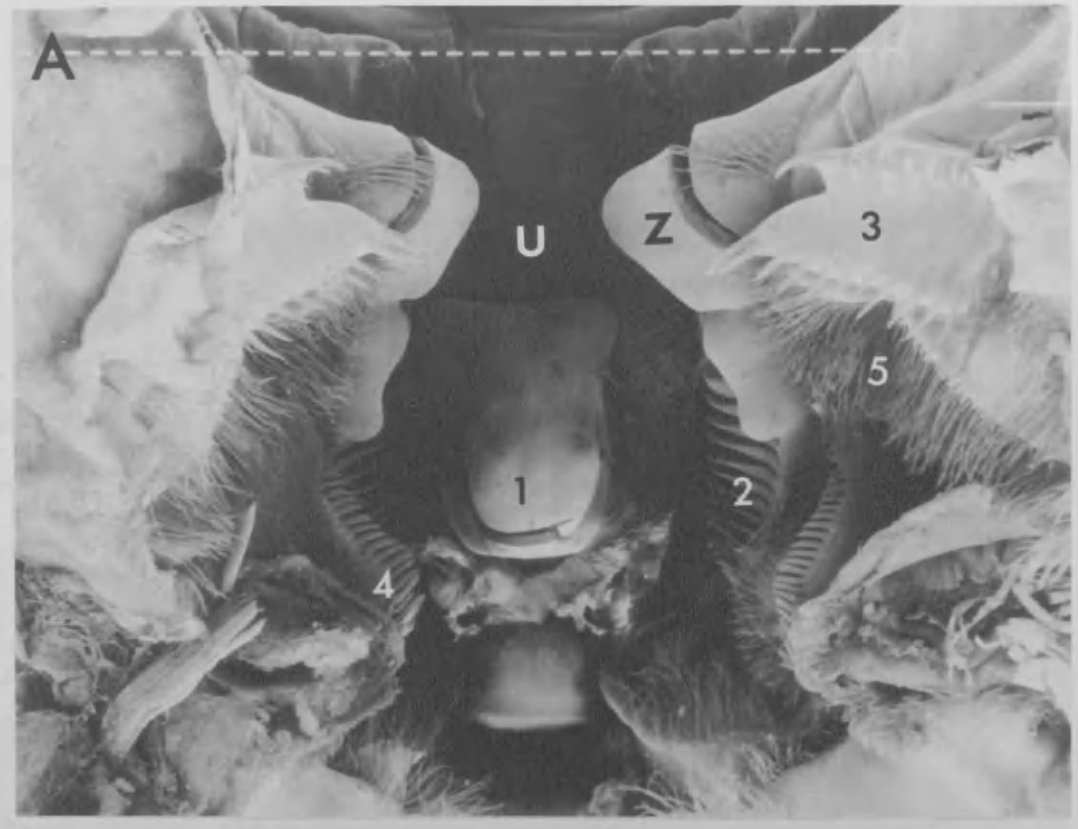

B
ch
ct

Fig. 2.22

Scanning electron micrograph of cardiac stomach of *M. rugosa* (A), the ventral stomach wall has been bisected and opened to reveal the internal structures; (B), left side wall of stomach opened to give lateral view of internal structures. U = urocardiac ossicle; Z = zygocardiac ossicle; 1 = dorsal tooth; 2 = lateral tooth; 3 = lateral accessory tooth; 4 = cut part of cardiopyloric valve; 5 = setal fields. For (A) and (B) scale bars = 100μm. The arrow indicates the position of detail shown in Fig. 2.23 C.


A
U
Z
3
5
1
2
4

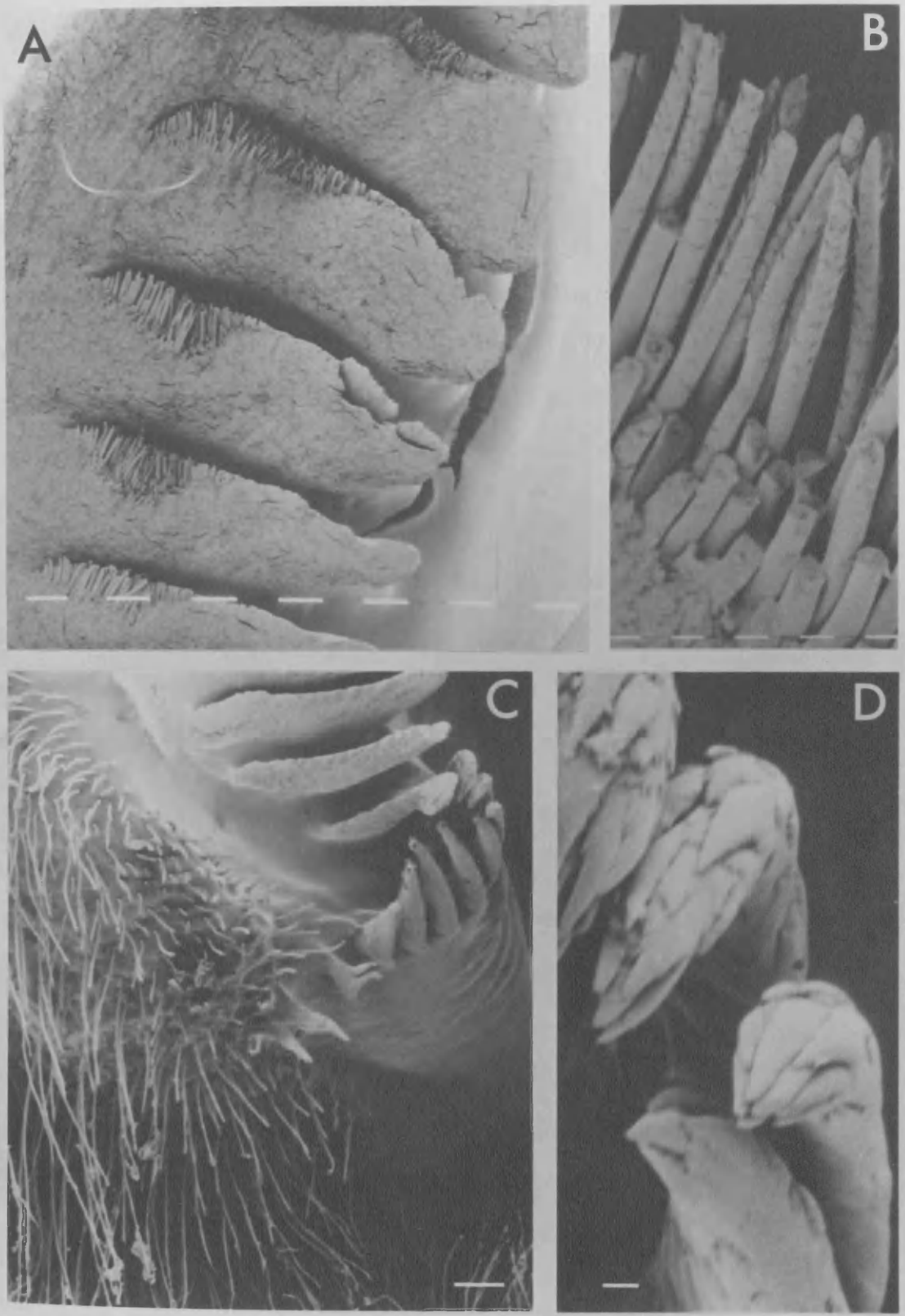

B
3
Z
U
5
4
1
J

tooth (see Fig. 2.22). The lateral accessory teeth probably serve to provide additional help in grinding and shredding food, and separate the food entering the stomach through the oesophagus from that which has passed through the gastric mill. Each accessory tooth has a semi-rectangular shape and is bordered with 12 stout spines (possibly derived from cuspidate setae) on its inner side and around 9 smaller, less distinct spines on the opposite edge, many of which bear thin apical setae. Each accessory tooth is also fringed posteriorly with numerous long setae. These setae, which occur between the lateral accessory teeth and the lateral teeth, are plumed (possibly plumodenticulate), with numerous setules which make an acute angle with the setal shaft and are longer and thinner towards the tip of the seta. The setules are apparent only at high magnification.

The lateral teeth are the major grinding teeth and occur one on each side of the dorsal tooth. Manipulation suggests that their movement is mainly lateral and towards the middle of the cardiac stomach (see Fig. 2.22), so that their grinding surfaces move against the dorsal tooth which itself moves against them. They are carried by the zygocardiac ossicles. Each lateral tooth is elongated and consists of a bilobed, projected, massive denticle with a depression formed between the lobes. It is also transversely divided into 13 denticulated ridges (Fig. 2.23 A). At higher magnifications, the anterior ridges are revealed to have setal combs projecting from their inner margins. These coarse setae are shown in Fig. 2.23 B and their shafts bear numerous triangular setules. In addition, the ridges of each lateral tooth decrease in size posteriorly and their outer edges become progressively more upturned, so that the digitate 13th ridge abuts with (and structurally grades into) a series of digitate projections orientated at right angles to the main series of ridges (Fig. 2.23 C). These smaller projections are sharply curved, denticulate (Fig. 2.23 D), and, together with the 13th ridge which is of similar structure, form a cup. From their appearance, the denticulations, and possibly the projections themselves,

Fig. 2.23

Scanning electron micrograph of the stomach of *M. rugosa*, (A), lateral tooth ridges; (B), setae from central region of a ridge; (C), posterior edge of the tooth showing ridges and setal pad; (D), detail from top right hand corner of (C). For (A), (B), (C), and (D), scale bars = 100μm, 10μm, 100μm, and 10μm respectively.


A
B
C
D

may be setal derivatives.

A setose pad occurs below and posterior to the lateral teeth and is provided with long plumed setae and rod-shaped setae. However, the rod-shaped setae may perhaps be the shafts of broken plumed setae (Fig. 2.23 C).

The other major grinding tooth is the median dorsal tooth and this is formed from the posterior end of the urocardiac ossicle (Fig. 2.22 B). The dorsal tooth has a smooth blunt surface with a depression at the junction with the remainder of the ossicle. The head of the tooth is rounded to fit the depression of the lateral teeth.

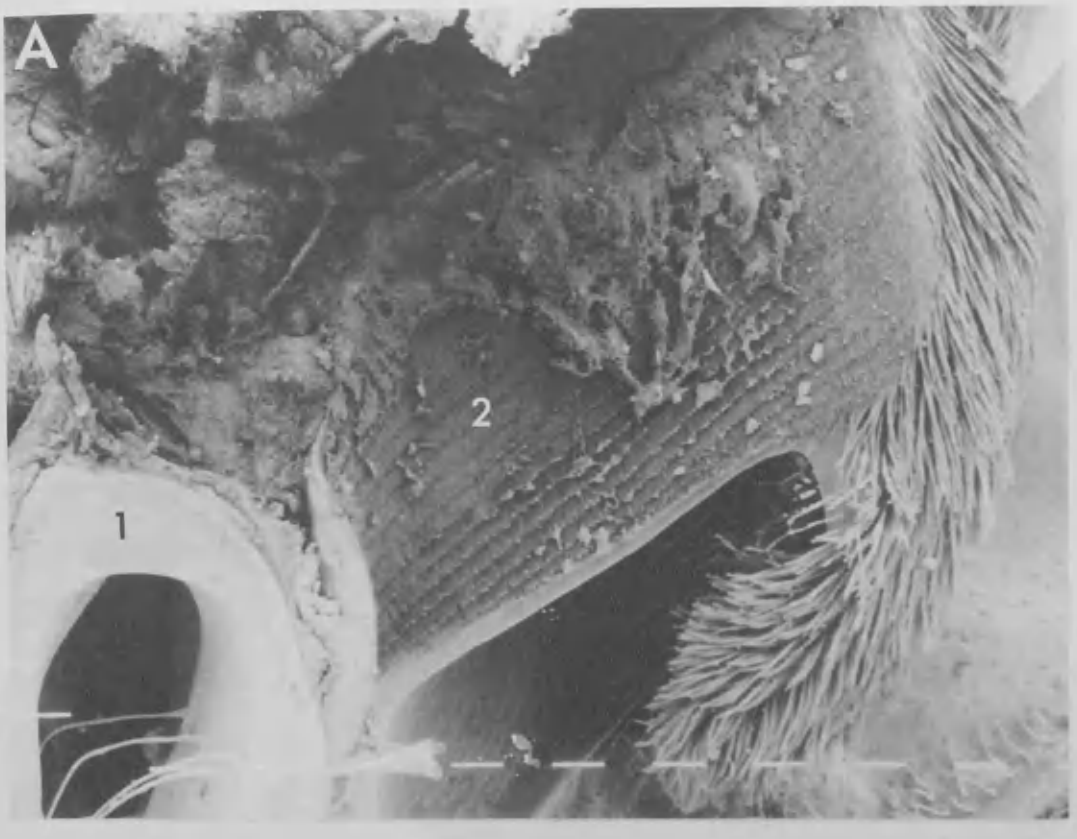
The wall of cardiac stomach is membranous and chitinized. On either side, posterior to the lateral accessory teeth, it is provided with many layers of dense setae particularly on the wall which opposes the dorsal tooth. Membranous folds with long setae also occur on both sides of the dorsal tooth (Fig. 2.22).

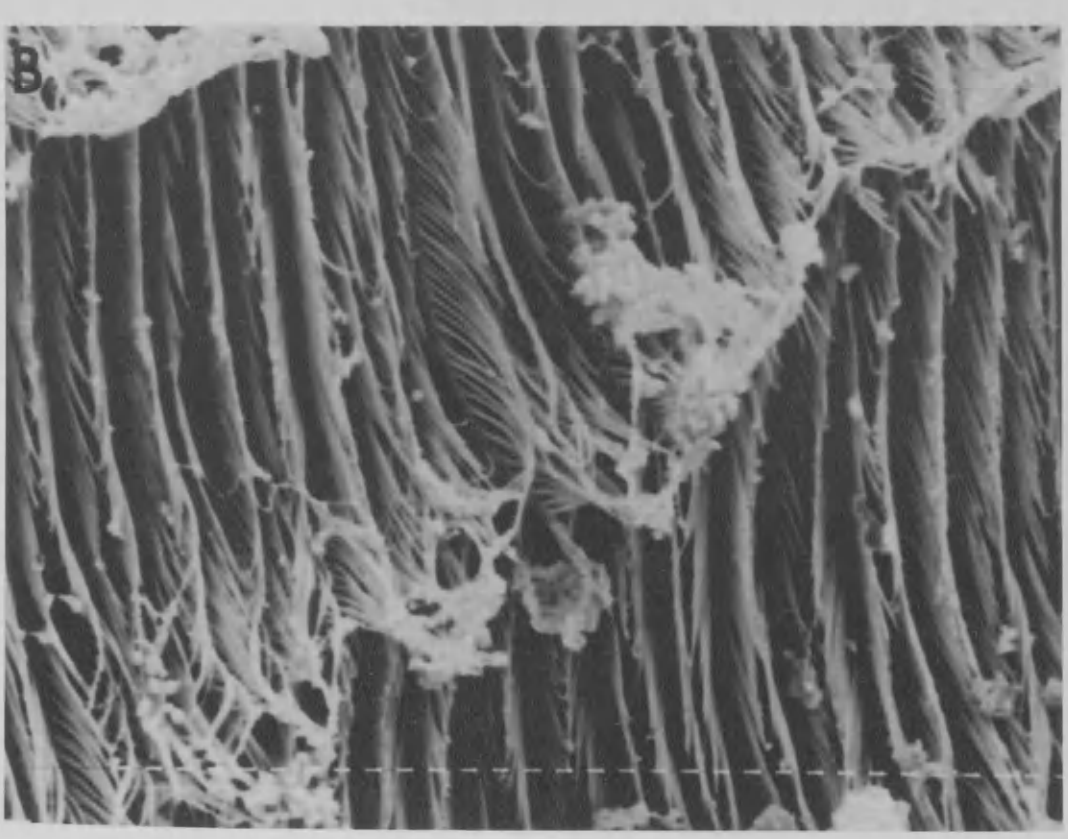
The cardiac stomach communicates with the pyloric stomach through the orange or brown coloured cardio-pyloric valve (Fig. 21 B). The cardio-pyloric valve is V-shaped in general appearance with the two arms of the valve slightly curved towards the centre. The two oblong oval-shaped arms meet to form the head of the V. The surface of the arms is chitinous and thickened with ridges similar to the lateral teeth but less dentate. There are 22 ridges on each arm (44 in total) with some having straight and some irregular crests. The crests were most prominent distally. Numerous, apparently simple, setae occur on the valve and these become more dense towards the head of the valve.

The pyloric stomach lies posterior to the cardio-pyloric valve. The wall of this chamber is less supported with ossicles than the cardiac chamber. It is composed of folds, grooves and membranes. The folding of the wall of the pyloric stomach is relatively simple (Figs. 2.21 & 24 A). There is not a

Fig. 2.24

Scanning electron micrograph of the wall of the pyloric stomach of *M. rugosa*. (A), pleuro-pyloric fold (1) and ampullar filter bed (2) (for position see long arrow on Fig. 2.21 (A). (B), plumose setae of filter (2). For (A) and (B) scale bars = 100μm, and 1μm respectively.


A
1
2


B

complexly folded pleuro-pyloric valve as in the genus *Galathea* (Pike, 1947; Ngoc-Ho, 1984). Dense setal fields occur in the pyloric stomach (Fig. 2.24 A). The finer setae are plumose, but the longer ones are more difficult to categorize. Some bear two irregular rows of denticular setules and probably conform to a serrate category: some appear to be pappose, others appear to be multidenticulate or plumodenticulate. The grooves lead to the filtering apparatus which projects ventrally as two lobes (ampullae). As in other decapods, (see Warner, 1977) this apparatus opens into the hind-gut. The details of the filter wall are shown in (Fig. 2.24 A & B). It bears several dense layers of fine plumose setae forming a sieve-like structure. The setal rows are parallel and are approximately 22 or more in number. The setae of each row appear to overlap those of the following row, though according to Schaefer (1970), this is not so and the effect is caused by setae overlying a series of ridges.

2.3.8. *Munida sarsi*

This species was collected only from deep water (95-115m) in the Main Channel between the Cumbraes and Bute where the substrata consisted of muddy sand, sandy mud and adjacent bedrock. The size-frequency distribution of the animals collected is given in Fig. 2.25. The peak carapace length is at approximately 25mm for males and at approximately 20mm for females. Very few females were caught, so little can be said about reproduction other than the observation that 'soft' (i.e. newly-moulted) animals were observed in April and May, and that ovigerous females were observed in February, March and April. Two 'soft' males were seen in January and others in March.

Length/ weight relationships and data on relative growth are given in Table 2.6. Since so few females were collected, a meaningful comparison between the sexes is difficult. In nearly all cases, the trends in the data were similar to those seen for *M. rugosa*.

Fig. 2.25

Size frequency histograms for the male and female *M. sarsi* used in the investigation of relative growth.

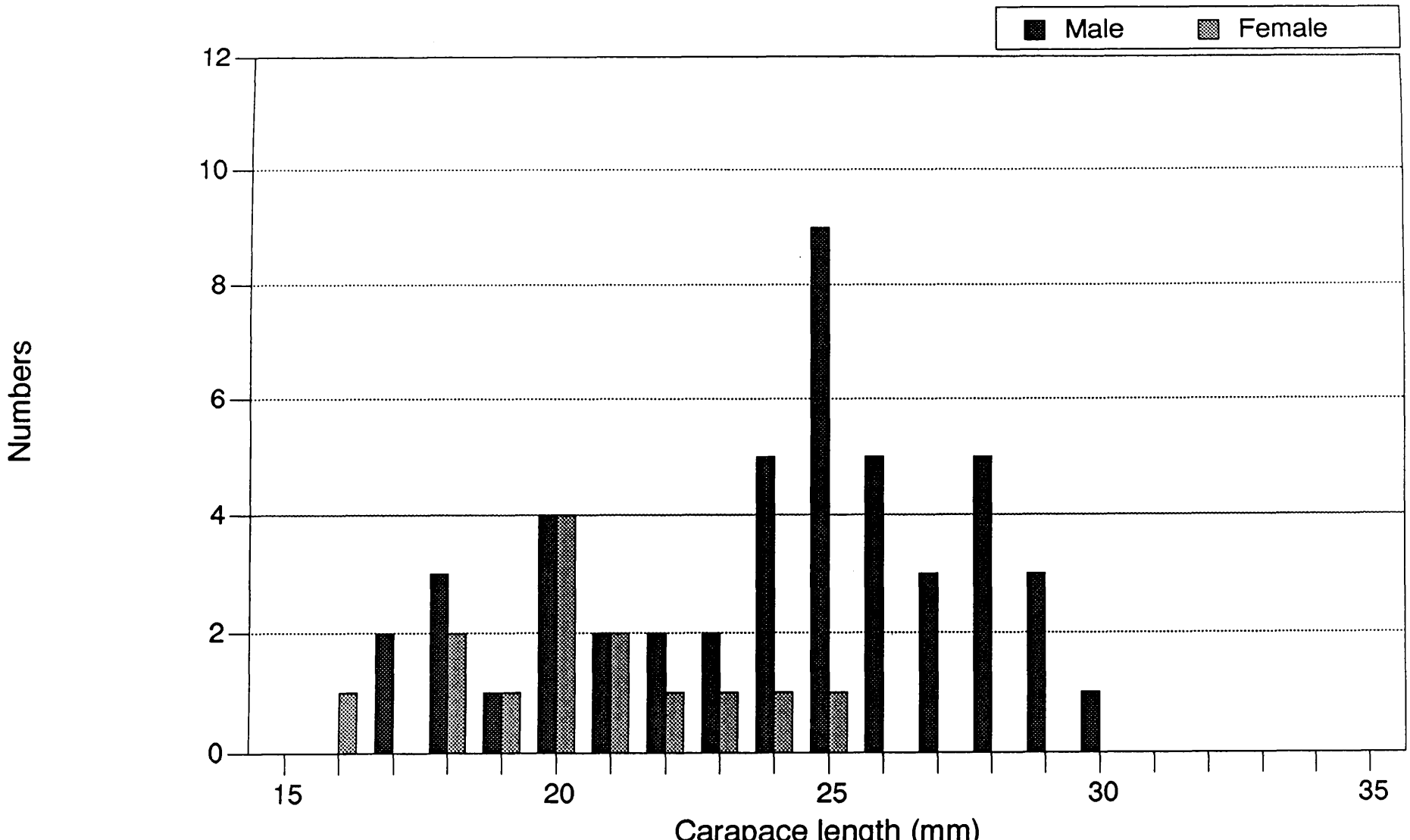

Male
Female
12
10
8
6
4
2
0
Numbers
15
20
25
30
35
Carapace length (mm)

Table 2.6. Regression coefficients for the relationships between different body measurements and the carapace length of male (M) and female (F) *Munida sarsi*. The regression lines calculated for the data for male and female animals have been compared using covariance analysis. The F values for the slopes (F_s) and elevations (F_e) of these lines are given and any significant difference between the slopes (P) is also indicated. A modified t-test was used to compare the slopes of the regression lines (b) with a value of 1. A value of 'b' which did not differ significantly from 1 indicates isometric growth; a value greater than 1 indicates positive allometry and a value of less than 1 indicates negative allometry. For further details see text.

cl = carapace length (mm); cw = carapace width; tlr = total length from tip of rostrum; tlo = total length from orbit; abdw = abdominal width; Lchl & Rchl = left and right chela length; Lpro & Rpro = left and right propodus length; Ldac & Rdac = left and right dactylus length; Lchw and Rchw = left and right chela width; wid. = wider chelae; nar. = narrower; lon. = longer; sho. = shorter; a = elevation and b slope of the regression equations; r = correlation coefficient; n = number of animals; t= t value; allo. = allometry. (Note: the t value for the weight/ carapace length was calculated but compared with a slope of 3).

Table 2.6.

	sex	a	b	r	n	F_s	F_e	P_s	t	allo.
wt/cl	M	-3.11	3.16	0.98	30	-	-	-	1.23	iso
cw/cl	F	-0.09	1.05	0.94	14	0.395	0.51	>0.05	0.40	iso
	M	-0.03	1.00	0.99	47				0.04	iso
tlr/cl	F	0.63	0.91	0.97	14	1.087	0.68	>0.05	-1.45	iso
	M	0.75	0.81	0.95	47				-4.59	-ve
tlo/cl	F	0.49	0.94	0.97	14	0.267	2.88	>0.05	-0.89	iso
	M	0.55	0.89	0.96	47				-2.89	-ve
abdw/cl	F	-0.10	1.07	0.98	14	6.15	86.97	<0.05	1.18	iso
	M	0.07	0.91	0.98	47				-3.60	-ve
Lchl/cl	F	0.30	1.16	0.84	06	1.45	35.4	>0.05	0.43	iso
	M	-0.07	1.52	0.95	38				6.39	+ve
Rchl/cl	F	0.30	1.16	0.86	07	0.64	56.4	>0.05	0.53	iso
	M	0.13	1.37	0.94	33				4.09	+ve
Lpro/cl	F	-0.14	1.25	0.89	05	0.93	31.3	>0.05	0.63	iso
	M	-0.46	1.57	0.95	36				6.24	+ve
Rpro/cl	F	-0.03	1.15	0.84	07	0.97	47.85	>0.05	0.47	iso
	M	-0.31	1.46	0.93	33				4.29	+ve
Ldac/cl	F	-0.62	1.42	0.94	06	0.25	41.27	>0.05	1.59	iso
	M	-0.71	1.56	0.95	38				6.83	+ve
Rdac/cl	F	-0.22	1.11	0.81	07	1.26	35.84	>0.05	0.31	iso
	M	-0.57	1.45	0.93	30				4.25	+ve
Lchw/cl	F	-1.27	1.50	0.85	07	0.20	6.84	>0.05	1.05	iso
	M	-1.57	1.80	0.82	37				3.80	+ve
Rchw/cl	F	-1.59	1.74	0.85	07	0.03	9.56	>0.05	1.53	iso
	M	-1.61	1.84	0.80	33				3.41	+ve

Pooled data of left and right for males and females *M. sarsi*.

chl./cl	F	0.19	1.25	0.88	12	1.06	78.68	>0.05	1.19	iso
	M	0.02	1.45	0.95	71				7.50	+ve
chw./cl	F	-1.38	1.58	0.83	12	0.26	15.86	>0.05	1.70	iso
	M	-1.60	1.82	0.81	70				5.20	+ve
wid./cl	M	-1.63	1.87	0.97	29	-	-	-	4.02	+ve
nar./cl	M	-1.27	1.57	0.80	29	-	-	-	2.52	+ve
lon./cl	M	0.05	1.44	0.97	29	-	-	-	-5.97	+ve
sho./cl	M	0.09	1.39	0.94	29	-	-	-	4.07	+ve

Regarding chela morphology, few females were caught (n = 14) and most of these (n = 11) were missing one or both chelipeds. Therefore the following comments relate to males only.

	Left cheliped	Right cheliped	Neither
longer	48.3%	44.8%	6.9%
wider	44.8%	48.3%	6.9%

An analysis of pooled data (n = 31) showed that in 48.4% of cases there was a longer, narrower cheliped paired with a shorter, wider one. In 41.9% of cases there was a longer, wider cheliped paired with a shorter, narrower one. 9.7% of animals had one or both of these dimensions equal. Cheliped length was not a reliable indicator of the 'arched' chela v. 'straight' chela type of heterochely.

The numbers of animals with unequal or equal chela widths are indicated by the ratio of left:right chela widths. Left handedness is indicated by a ratio greater than 1, right handedness by a ratio less than 1. Chelae with equal widths have a ratio of 1.

Ratio	>1	<1	1
Numbers	13	14	2

Thus, there is an approximately equal possibility of the left or the right chela being wider. How well this reflects the presence of the 'arched' form of chela is examined below using 37 animals (out of 61 collected) which possessed both chelipeds:

	L. arched R. straight	R. arched L. straight	Both arched	Both straight
MALE n = 34	16%	8%	16%	52%
FEMALE n = 3	0%	3%	0%	5%

The 'arched' and 'straight' types of chela are shown in F. 2.18 D-E. The 'arched' form of chela was present in males and females. Since so few females were examined it is impossible to make much of this. A further 14 animals had lost one cheliped. Of these, the remaining chela was the 'arched' type in only 3 animals (all males).

The smallest male which had a chela of the 'arched' type was 16.6mm in carapace length (its other cheliped was missing). The curvature of the propodal 'finger' of this specimen was only slight. The arching of the propodal 'finger' of the chela was more pronounced in larger specimens. The only female with an arched chela was of 25mm carapace length.

Mouthpart morphology is similar to that seen in *M.rugosa*, except that the maxillipeds appear to be slightly more setose in *M. sarsi.* In the material examined, the robust, curved triserrate setae on the inner median edge of the dactyl of the second maxilliped of *M. sarsi* are coarser than those in the equivalent position in *M. rugosa.*

The absence of a distinct distal tooth on the merus of the third maxilliped of *M. sarsi* is an important taxonomic character when distinguishing *M. sarsi* from other *Munida* species (Rice & de Saint Laurent, 1986) (Fig. 2.17 B).

The examination of a few stomachs of *M. sarsi* showed that they contained

highly digested organic matter which included fragments of both animal and plant material, together with sediment. No obvious differences could be seen in the stomach morphology of the two *Munida* species. Deposit feeding was observed in laboratory animals and some survived for 5 months by deposit feeding (macrobiotic food was withheld). Other experimental animals readily consumed mussel tissue which was used as a standard food for laboratory animals.

2.4. DISCUSSION

2.4.1. Ecology

Munida rugosa was found on a wide range of substrata at depths from 8-115m during this work. Although it was reported from burrows, independent burrowing behaviour could not be substantiated. Preferred substrata appeared to be muddy sands and sandy muds in the vicinity of rocks. This was also true of *M. sarsi*, except that this species was confined to deep water (95-115m). Also, it appeared to be confined to an area where there was good water exchange, i.e. at the south end of the channel between the Cumbraes and Bute. Most *M. sarsi* were taken from the area between the Little Cumbrae and the south end of Bute. Here there was good tidal exchange and rock outcrops constricted the channel such that it was only just possible to manoeuvre the trawl through this passage. Here it was caught together with *M. rugosa*. At equivalent depths to the SE of Bute, where water exchange is less (Admiralty Chart 2221; Edwards *et al.*, 1986) and where there may be some oxygen depletion in summer (K. Jones, pers. comm.), no *M. sarsi* were found, though *M. rugosa* occurred. This correlation is only apparent and other factors may underlie this difference in distribution.

2.4.2. Relative growth; heterochely

Crustacea usually change in shape as they grow, which is referred to as relative, allometric, or occasionally heterogonic growth (see review by Hartnoll, 1982). The theoretical problems of relative growth have been reviewed by Huxley (1972) and the quantitative analysis of allometric growth has been reviewed by Hartnoll (1982). The simple allometry formula $Y = aX^b$ is generally accepted where a is the Y-intercept and b is the allometric growth constant or relative growth rate. In the logarithmic form log y will produce a linear plot against log X. If growth was isometric, then a straight line relationship would be seen in data plotted on arithmetic axes, but where there is a change in proportion with growth (allometry), then a curve will result if the data are plotted on arithmetic axes and a logarithmic plot is necessary. In general terms allometry of size has been defined as changes in proportion of various organs (Teissier, 1960; Gould, 1966; Huxley, 1972). For a standard part of the body to be used as a reference dimension, carapace length is the most accurate and useful measurement (Farmer, 1974; Hartnoll, 1982).

Studies of relative growth have been used to identify secondary sexual characters in numerous Crustacea (Ingrand, 1937; Needham, 1950; Teissier, 1960; Gould, 1966; Pope & Thomas, 1967; Huxley, 1972; Hartnoll, 1974; Mori, 1986). Ingrand (1937) found a change in the relative rate of growth of the meri of the pereiopods of male and female *M. sarsi* (see Section 2.1.) at 11.5mm carapace length and this was assumed to mark the size at which sexual maturity occurred. Above this size the pereiopod meri showed marked positive allometric growth whereas the relative growth rate of the meri of the females decreased slightly. Attrill (1988), plotting cheliped length against carapace length, could demonstrate no equivalent positive allometry for males (though the significance of the positive deviation from 1 of slope of his regression line was not given). His data suggested a decreased relative growth rate of the

chelipeds of the larger females, though the significance of this was not tested. In the case of the *M. rugosa* examined in the present study, there was no indication of meral allometry in most of the pereiopods and a sexual difference was not apparent. The numbers of *M. sarsi* collected were too few to make a comparison of meral growth with the data presented by Ingrand (1937).

The lack of allometry in pereiopods 1-4 was in some ways surprising since positive allometry is evident when other cheliped dimensions of males are considered (Table 2.1.). Few small animals were collected and so it was not possible to use differences in relative growth to directly identify the size at which sexual maturity occurs in *M. rugosa*. It could, however, be estimated indirectly by extrapolation of relative growth regression relationships for male and females. This suggested a size of sexual maturity of approximately 17mm carapace length. This is rather larger than estimates by Brinkmann (1936) and Attrill (1988) for *M. sarsi* and *M. tenuimana*, based on the smallest size of egg bearing females (see Section 2.4.3.). Data collected for *M. sarsi* in the present study were too few to be properly analysed in this way, but suggested a value of close to 10mm carapace length.

Rios (1979) investigated length weight relationships in *Munida rugosa* from Dunstaffnage Bay, in western Scotland. Since the slopes (b) of the log weight against total length regressions were close to 3, he concluded that growth was isometric. This is possibly misleading, however, since the 'b' values given (2.59 - 2.81) are within the range that may indicate allometric growth (see Hartnoll, 1982). Also, since males and females were not dealt with separately by Rios, any allometry resulting from sexual dimorphism will be masked. In the present work, however, there were no significant differences between the sexes when weight\ length relationships were examined and 'b' values (Table 2.1) were close to those found by Rios (1979). According to Hartnoll (1982), the relationship between weight and length in crustaceans is such that weight is

rarely proportional to the cube of the length as it would be if growth was isometric. It must be remembered that although an isometric relationship based on length and weight may occur, subtle differences in relative growth may still be present as is clear in the present data.

It was observed for both sexes of *M. rugosa*, that there were usually slight differences between the lengths of the major chelipeds in a given individual. Such differences, however, were small and did not correlate with the differences in chela morphology noticed. Where large differences in length occurred these probably reflected limb regeneration. Terslin (1938) also noticed differences in cheliped length in individual *M. rugosa*. The sample size was small, however, and no firm conclusions on this would be drawn. Terslin (1938), in addition, noted that males had relatively longer chelipeds than females.

The most obvious bilateral differences in cheliped measurements were of chelar widths. The 'arched' type of chela was consistently wider than the 'straight' type. Prizibram (1905) used the term 'heterochely' to describe bilateral differences in cheliped morphology and noted that there were several different forms of this (see also Hartnoll, 1982). Ingrand (1937) observed a form of heterochely in several galatheids, including *M. sarsi*, and the phenomenon appears to be widespread in decapods, though more restricted in galatheids (Prizibram, 1905; Schäfer, 1954).

In her work on *M. sarsi* (see Section 2.1.), Ingrand (1937) showed that the 'fingers' of one chela tended to be long and parallel, while those of the other were comparatively truncate and were curved so that their apposed edges met only towards the tip. There was wide variation in this phenomenon and it occurred in both sexes, though it was more pronounced in large animals and in males. Ingrand did not quantify her observations of heterochely. This same

form of heterochely was observed in the present study of *M. rugosa* and *M. sarsi*, thus confirming Ingrand's observations of the latter species.

Attrill (1988) also observed this form of heterochely in *M. sarsi*. Again, it was not fully quantified (there were many cheliped losses during capture and preservation), though Attrill noted that it was found in specimens of greater than 17mm carapace length and a tendency towards it could be seen in some animals of 15-16mm carapace length. The curvature or arching of the chela was most pronounced in the largest individuals which caused Attrill to postulate that it took more than one moult to develop this chela form. Not all large specimens showed heterochely. It was more common in males and only the largest females were heterochelous. In Attrill's samples of intact animals, significantly more animals were left-handed (i.e. the left-hand chela was of the 'arched' type). Several animals had both chelae of the 'arched' type, but Attrill did not note the proportion of his samples in which the larger animals had both claws of the parallel-'fingered' type. He postulated that the 'arched' chela type might relate to predation of a new bivalve food resource, but could not substantiate this from a dietary comparison with specimens without the 'arched' form of chela.

Although the data obtained on heterochely in the present Firth of Clyde study are not extensive, the results for *M. sarsi* agree closely with those of Attrill (1988). In the case of *M. rugosa*, the 'arched' form of chela was seen in only a few of the largest males. There may be significant differences in the expression of heterochely in the two species and further sampling would be worthwhile in order to investigate this. Dr R.J.A. Atkinson provided the following information for a preserved sample of 72 *M. rugosa* that were found in the Specimen Supply Department of the University Marine Biological Station, Millport. The material had been creel-collected from approximately 40m depth, SW of Little Cumbrae in the Firth of Clyde, probably in 1985. The

sample contained 65 males and 7 females. One female (26.1mm carapace length) had both chelae just begining to show the 'arched' chela form. The remaining females all had chelae of the 'straight' type. Amongst the males, however, 28 had both chelae of the straight form, 17 had both chelae of the 'arched' form, and the remaining 20 had one chela of each type (10 were right handed : 10 were left handed). The smallest male with an 'arched' chela was of 23.3mm carapace length. The dominance of this chela form was evident in all size classes above this, but the two largest males both had straight chelae only.

Therefore, comparing the two sets of data for *M. rugosa*, there is wide variation in the expression of heterochely and further work is necessary to properly quantify this and to assess its functional significance. It was noticed that the chelae of regenerating chelipeds were always of the 'straight' type.

2.4.3. Aspects of reproduction

Egg number and the rate of egg production may be characteristic of a species, but intraspecific variation may also occur depending on animal size, environmental factors, temperature, and food availability (Sastry, 1983). Little can be said about this in the present study because sample size was so small and ovarian oocyte counts were not attempted.

Based on the limited data available (10 individuals), the correlation between egg diameter and carapace length was not significant. Thus mean egg diameter was judged to be representative (0.89mm $\pm$ 0.08, n = >600). A similar egg diameter was reported by Lebour (1930) (0.64 - 0.80mm) and Zariquey Alvarez (1968) (0.66 - 0.80mm). By way of comparison, Attrill (1988), for *M. sarsi* from the Porcupine Sea-bight, indicated mean egg diameters in two samples (early and late development) of eggs of 0.73mm and 0.80mm and, in the case of *M. tenuimana*, 0.86mm and 1.11mm for eggs in early and late stages of development.

The number of eggs carried by *M. rugosa* was variable (mean = 4778 ± 2355, animal number = 11) and (5507 ± 2385, animal number = 17 when frozen eggs were included). The correlation between total number of eggs and animal size is significant ($P<0.05$); large females carried more eggs than the small ones. Part of the variability in egg numbers in similar sized individuals can be accounted for in terms of egg loss during laying and capture. This is well documented for other species (e.g. Bailey *et al.* 1986). The smallest female used in the analysis was 20.6mm carapace length, but this does not mean that this is the minimum size of ovigerous females. Attrill (1988) found the smallest egg bearing females of *M. sarsi* and *M. tenuimana* to be 9.6mm and 11.4mm carapace length, respectively. This was taken to be the size of female sexual maturity. Brinkmann (1936) gave corresponding values of 11.5mm and 12.5mm, respectively.

For *M. sarsi* and *M. tenuimana*, Attrill (1988) found that egg number was very variable. In the case of *M. sarsi*, there appeared to be a significant correlation between animal size and egg number, though no statistics were given. No such correlation was apparent in *M. tenuimana*. Excluding one anomalous sample, Attrill (1980) gave egg numbers for *M. sarsi* of 1277 ± 566: the number was 2780 ± 1166 when the anomalous sample (animals significantly larger) was included. Attrill (1988) noted that *M. tenuimana* carried fewer eggs, though a mean value was not given.

Only a rough estimate can be given of the time required for the embryo to develop in *M. rugosa*. Ovigerous females were first observed in November and egg hatching occurred from March to May. This gave from five to seven months between laying and hatching. In addition, ovaries were in an intermediate stage during May, June and July while developed ovaries were observed from August. This is an indication of roughly six months for

maturation of the ovaries. These brief conclusions on the reproductive cycle of *M. rugosa* in the Firth of Clyde are similar to those deduced by Comely and Ansell (1989) for the same species in the Lynn of Lorne near Oban. Allen (1967) noted that ovigerous females were reported from the Clyde Sea Area in July, November and February. Presumably, the July animals were late in shedding their larvae. The presence of *M. sarsi* in this area was not realised at the time and it is possible that some of the records attributed to *M. rugosa* (as *M. bamffica*) by Allen (1967) may relate to *M. sarsi*. Breeding was said to take place from winter to early summer, with larvae taken from February to June. Attrill (1988) noted that in the Porcupine Sea-bight, *M. sarsi* with mature ovaries were seen in August and September. The pattern was much more variable in the deeper water *M. tenuimana*.

Attrill (1988) indicated that *M. rugosa* in the Irish Sea lay eggs in September/ October and that these hatched in March/ April. The reproductive cycles of Norwegian and Porcupine Sea-bight populations of *M. sarsi* and *M. tenuimana* appear to be similar to those of *M. rugosa* (Brinkmann, 1936; Attrill, 1988), except that, in the case of *M. tenuimana* from deep water, seasonal patterns become less clearly defined.

During the present study, few newly moulted animals were seen. Newly moulted males were first seen during February. Newly moulted females were observed in March, June and August. Presumably, in common with many other decapods, the females moult following larval hatching and the peak of moulting may be later in larger animals than in smaller ones (Bailey *et al.*, 1986).

Wenner (1982) concluded that two modes of breeding existed in the Galatheidae. The first, seen for example in *Munida* spp. involves the production of many small eggs (see also Benedict, 1903) from which pelagic larvae emerge. The second is characteristic of *Munidopsis* spp. and involves the production of few large eggs that develop into advanced larvae which remain

within the bathymetric realm of the parent (Lebour, 1930; Wenner 1982). In the Anomura, there are usually four or five zoeal stages (Calman, 1911; Lebour, 1930 a,b; Gore, 1979; Christiansen & Anger, 1990). The first stages of *M. rugosa* were observed during this study and, like others in the group, are characterized by long spines, particularly at the postero-lateral margins of the carapace (see Calman, 1911). The larvae of *M. rugosa* have been described by Lebour (1930a) and of *M. rugosa* and *M. sarsi* by Huus (1935).

2.4.4. Dietary analysis

In this study the stomach contents of 130 *Munida rugosa* were examined. The high proportion of highly digested material has perhaps resulted in an underestimate of the occurrence of soft-bodied prey in the diet and prevented anything but a general identification of dietary components. The dietary analysis, though crude, has revealed the feeding habit of the species. *M. rugosa* is an omnivore with a preference for animal food. It will scavenge and predate and, in addition, deposit feeding is indicated. Deposit feeding is, perhaps, only resorted to as a supplementary source of feeding. This was suggested by the laboratory observation that particulate material was ingested along with other food and that deposit feeding usually ceased when discrete food items were presented. It is to be noted that deposit feeding is here regarded as including the ingestion of detritus, etc. which was preened from general body setation. Interestingly, Nicol (1932) observing *Galathea dispersa*, saw that material cleaned from the fifth pereiopods and the antennules by the third maxillipeds could be passed to the mouth. This was seen in the present work on *M. rugosa*. Herbivory was also observed in the laboratory and algal fragments occurred in the stomachs of animals collected in the field. The few observations made on *M. sarsi* suggest similar feeding behaviour.

Nicol (1932) remarked that she had examined stomach contents of *M. rugosa*

(as *M. rondeletii*), and, in a general statement which also embraced *Galathea squamifera*, *G. strigosa* and *G. dispersa*, stated that "always the bulk of the stomach contents was found to consist of unidentifiable detritus, fine sand, small pieces of red and green algae, a few diatoms and unicellular algae, parts of crustacea, eggs, and small gastropods. In addition pieces of muscle and larger pieces of algae were found in small quantities....." For *M. rugosa* no further details were given. Samuelsen (1970) indicated that *G. intermedia* also fed on microscopic and macroscopic food items.

The food taken by galatheids is, therefore, basically of two sorts: large pieces of animal and vegetable material, or small particles of organic debris and micro-organisms from sea bed deposits (Nicol, 1932). From examination of the stomach contents, Nicol (1932) concluded that the deposit-feeding mode was the more usual in British species of the genus *Galathea*. During the present study, it appeared that *M. rugosa* opportunistically employed a variety of feeding modes. *Galathea squamifera* has been observed to filter food particles from self-induced (by maxilliped exopods) water currents (Pike, 1947; Ngoc-Ho, 1984). It is possible that this also occurs in *M. rugosa* and *M. sarsi*, but it was not observed.

2.4.5. Functional morphology of the mouth parts and chelipeds

The mouth parts of *M. rugosa* and *M. sarsi* are adapted for food manipulation rather than for mastication. In general the mouthparts were very similar to those described for other galatheids and for pagurids (Nicol 1932; Pike, 1947; Caine, 1975; 1978; Schembri, 1982a, b), with species differing mainly in the type and distribution of setae.

The mandibles are simple and work very slowly if the food is large and firm as in the case of algal thalli, though the cutting edges effectively slice through this material. Nicol (1932) also noted that the mandibles of *Galathea dispersa*

operated with a cutting action and not a tearing one, though she noted that the second maxillipeds could pull food against the grip of the inner mouthparts in order to tear 'tough' food items. The mandibular palp assists in pushing material into the oesophagus and in cleaning the mandible.

The first maxillae are very similar to those of *Galathea squamifera* (Pike, 1947) and *G. dispersa* (Nicol, 1932). The presence of cuspidate setae on the appendage help in maintaining the food against the mouth while the mandibles cut through it. Close interspecific similarities are also seen in the structure of the second maxillae. The scaphognathites, fringed with plumose setae, have a respiratory role (see Chapter 3), but the basal and coxal endites of the second maxillae function in food manipulation. Their robust setae assist in this. Cuspidate setae are effective in gripping food items, while finer setae will assist in the manipulation of particulate material.

In decapod crustaceans, the maxillipeds are usually biramous and the exopods frequently terminate in multiarticulate flagella (McLaughlin, 1982). The setose third maxillipeds have a major role in feeding. It has been shown that structural differences in the third maxillipeds of decapods may correspond with differences in the types of food collected (Orton, 1926; Caine, 1975). Deposit feeders and suspension feeders, for example, have very setose third maxillipeds (e.g. Nicol, 1932; Crane, 1941; Altevogt, 1976). In both of the *Munida* species under observation, these appendages were used in both feeding and preening, as is commonly the case in other species (e.g. Caine, 1975). Zimmermann (1913) observed that in *Galathea* spp., the third maxillipeds were said to function to gather food particles, preen the antennules and guard the prebranchial apertures. He divided the setae into long sweeping types and stouter combing types. Both Nicol (1932) and Pike (1947) noted that *Galathea* spp. took organic debris and microorganisms from the deposit by sweeping actions of the third maxillipeds, as well as seizing macrobiotic food with these

appendages. Particulate material swept-up by the third maxillipeds was brushed from these appendages by the second maxillipeds before transference to the inner mouthparts. The same sort of behaviour was observed in *M. rugosa* and *M. sarsi*.

The morphology of the mouth parts of *M. sarsi* was found to be similar to those of *M. rugosa*. However, some differences exist in the amount of setation present on the mouth appendages. In particular, the second and third maxillipeds are more densely setose in *M. sarsi*. Another difference relates to the dactyl of the second maxilliped where comparatively larger, inwardly directed (towards the mouth), triserrate setae occurred. However, it was not the purpose of the study to make a detailed morphological comparison between the two species. In general, both species are very similar except for the relative size of their eyes, those of *M. sarsi* being conspicuously larger, the presence of a lateral distal spine on the merus of the third maxilliped in *M. rugosa* (it is absent in *M. sarsi*) and the presence of a pair of spines on the fourth abdominal tergite of *M. sarsi* (absent in *M. rugosa*) (see Rice & de Saint Laurent, 1986).

In *Munida* spp. the major chelipeds are elongate and cylindrical. Their chelae are used to collect food material and to convey it to the third maxillipeds. Although the role of the chelae in the feeding is clear, the animal does not entirely depend on them for this purpose. Severely disturbed animals would often autotomize their chelipeds and a relatively large proportion of the catch included individuals with missing chelipeds. The role of the chelipeds in agonistic behaviour has not been assessed and neither has the functional role of the observed heterochely. Most, if not all, decapod chelipeds are regionally specialized multi-functional appendages. Large variations in the chelipeds between different groups of decapods reflect differences in diet and mode of feeding (Warner, 1977) and further work is necessary in order to assess the

functional significance of the chela morphologies seen in *Munida* spp. According to Brown (1979), elongated chelae like this, generate comparatively low pressures during cheliped closure. Whether or not the 'arched' chela is capable of generating greater pressures than the 'straight' form requires assessment.

Pike (1947) drew attention to the chelate nature of the galatheid 5th pereiopod. This is true in most Anomura (see Calman, 1909), though some (Zimmermann, 1913; Nicol, 1932; Eales, 1961) appear to have overlooked this feature. It is used to preen body setation and brush body surfaces, including the gills. The precise mode of operation of its chela was not observed, but this will presumably be used to seize particles as well as in combing setae as suggested by Pike (1947). The setose surface of the 5th pereiopod functions as a brush (Pike, 1947). Nicol (1932) noted that the fifth pereiopods of *G. dispersa* were cleaned by the third maxillipeds, as was seen in the observations of the *Munida* spp. reported here.

2.4.6. Functional morphology of the stomach

General descriptions of the functional morphology of the stomach of decapod crustaceans include those of Schaefer (1970), Warner (1977) and Dall & Moriarty (1983), with Pike (1947) and Ngoc-Ho (1984) providing detailed information for the genus *Galathea*. The stomachs contain very complicated masticatory, sorting and filtering structures whose morphology varies with the type of food eaten (Schaefer, 1970; Powell, 1974). Differences in stomach morphology are of taxonomic significance (Mocquard, 1880 cited from Patwardhan, 1935e; Pike, 1947). It has been noted that for each natural group, there is a given type of gastric mill, and if the gastric mill of a crustacean placed within that natural group does not conform to the type, then that species should be removed from that group (Mocquard, 1880). This was confirmed in the extensive studies of Patwardhan (1934, 1935 a, b, c, d, e).

Amongst decapods, the most complex gastric mills are found in Anomura and Brachyura (Patwardhan 1935e). A detailed comparative morphology of the gastric mills in decapods have been recently given by Felgenhauer & Abele (1983).

The stomach is divided into the anterior cardiac and posterior pyloric regions. The ossicles supporting the stomach walls are ten in total. They form two arches: an anterior arch formed by the **mesocardiac** ossicle in the middle and the lateral **pterocardiac** ossicles, and a posterior arch, formed by the pyloric ossicle, the paired **exopyloric** and the paired **zygocardiac** ossicles. The ossicles which connect the two arches are the anterior **urocardiac** and posterior **prepyloric** ossicles. The anomuran type of gastric mill resembles the macrurous type in the shape and disposition of the ossicles of the anterior arch (Patwardhan, 1935 b).

The shape of the stomach ossicles differs slightly within the Anomura (see Patwardhan, 1935 b). The **urocardiac** ossicle (dorsal tooth) is T-shaped in *M. rugosa*, has a depressed surface, and is smooth. Amongst Anomura, the dorsal tooth bears transverse ridges in its most complex form, while simpler dorsal teeth may possess small, lateral denticles or be unornamented (Patwardhan, 1934, 1935e). In *M. rugosa* (and *M. sarsi*), the dorsal tooth is simple i.e. without ridges. (The stomachs of *M. rugosa* and *M. sarsi* were very similar and no detailed comparison was attempted).

The gastric mill in Anomura differs in some respects from that of Brachyura (Patwardhan, 1935b). The mesocardiac ossicle is small in Brachyura and the pterocardiac ossicles are large and elongated. In the Anomura, the mesocardiac ossicle is relatively large and the pterocardiac ossicles are small. In addition, the cardio-pyloric valve is simple in Brachyura compared to the situation in Anomura (Patwardhan, 1935e).

Patwardhan (1935b) compared stomach structure between Brachyura and Anomura. He noted that in Brachyura, the lateral accessory teeth are usually present, but are more usually absent in Anomura. In the forms in which they are present e.g. *Munida* and *Galathea* spp., each tooth consists of a triangular or oval plate bordered with spines. In the reptant macrurous decapods, the lateral accessory teeth may be present or absent (Patwardhan, 1935b).

The midgut of Anomura, unlike Brachyura, is large (Jackson, 1913; cited from Patwardhan, 1935e). The hepatopancreas is essentially similar in all decapods. According to Yonge (1924; 1931), digestive juices from the digestive gland enter the pyloric stomach and move forwards to the cardiac stomach where they mix with the food. The mixture is then ground to a pulp by the gastric mill. Large particles pass straight backwards into the hind-gut. Fine particles and liquids which can enter the ventro-lateral grooves move back into the filters via the cardio-pyloric valve. In the filters, the fine particles are separated and the finer are then also passed into the hind-gut while the liquids enter the digestive gland. Work on gut function in live preparations by Powell (1974) on thalassinids and Dall & Moriarty (1983) on a variety of decapods has shown that this functional interpretation of the foregut derived solely from morphology was incorrect in some respects. It was previously assumed that digestive fluids flowed ventrally from the posterior stomach to the cardiac stomach, but it now appears that the secretions take a dorsal route and are then mixed dorsally and ventrally with food circulating in the cardiac stomach before passing back to the pyloric stomach. Here liquids extracted from the solids directed towards the hindgut join secretions passing forwards to the cardiac stomach. Ventrally, in the filter press, fine particulate material is strained from solutions which then enter the digestive gland. According to this recent work, circulation of ingesta and fluids, food extraction and digestion are all more rapid than was previously supposed. Dall & Moriarty (1983) indicate that more

work is necessary in order to test this new concept of stomach function.

The mechanical action of the foregut is effected by a complex musculature (Pike, 1947; Schaefer, 1970; Ngoc-Ho, 1984). How the galatheid stomach works is indicated in the studies of Pike (1947) and Ngoc-Ho (1984) on *Galathea squamifera*. Ingested food passes down the oesophagus and into the cardiac stomach via the oesophageal valves. The lateral accessory teeth prevent the food from entering the ventral part of the cardiac stomach and direct it dorsally towards the gastric mill. Large particles pass each side of the dorsal tooth and pass into the pyloric stomach. Smaller particles which have been ground in the gastric mill accumulate in the cardio-pyloric trough where they are mixed with digestive juices and particles pass from here to the pyloric stomach by way of the cardio-pyloric valve to be filtered. Ngoc-Ho (1984) suggests that coarser particles pass through the cardio-pyloric valve ahead of finer ones and describes two different types of movement of the gastric mill and cardio-pyloric valve when achieving this.

In *Munida rugosa*, a masticatory role for the cardio-pyloric valve is possible since its ridged surfaces will make contact with the dorsal tooth during the postero-ventral movements of the tooth. However, Ngoc-Ho (1984) suggests that the function of this action may be to squeeze the food, releasing digestive fluids.

By analogy with *G. squamifera* (Pike, 1947; Ngoc-Ho, 1984) material entering the pyloric stomach includes that coarse material which has passed dorsally through the gastric mill, beside the dorsal tooth, and that which has been ground in the gastric mill and passed ventrally, through the cardio-pyloric valve. These materials are then packaged to form faecal pellets following moulding by pleuro-pyloric valve which is simple in structure in *Munida*. Apparently, fluids squeezed from the faecal matter pass back to the cardiac stomach. Fine particulate material is removed by the filter beds of fine setae of the ampullae

and the liquids and dissolved food pass to the digestive gland. Coarser setae brush particulate material from the filter beds towards the hindgut (Schaefer, 1970). Material coming through the cardio-pyloric valve which is too coarse for the filter beds, passes upwards and posteriorly into the hindgut (Ngoc-Ho, 1984). According to Schaefer (1970), the foregut functions to reduce food to a liquid state.

The faecal pellets of the anomurans were examined by Moore (1931) who found them to be rod-shaped which is common amongst Crustacea. In *Munida* spp., unlike other members of the infraorder, the pellets are simple rods, circular in section, and with no trace of either a cap of fine material or of canals, and the pellets are composed of extremely fine homogeneous material (Moore, 1932). This must reflect the treatment of the food in the stomach as well as its packaging in the hindgut. According to Pike (1947), the presence or absence of canals in the faeces reflects the morphology of the pleuro-pyloric valve, with folds in the valve producing canals in the faeces. The pleuro-pyloric region of the pyloric stomach of *Munida* is simple, with just a single fold, whereas that of *Galathea* has five folds (Mocquard, 1883; Pike, 1947; Ngoc-Ho, 1984), giving rise to faeces with canals.

M. rugosa possesses a robust gastric mill with a large, simple dorsal tooth and lateral teeth with well-developed ridges. This and the presence of lateral accessory teeth for directing food to the gastric mill suggests that the diet includes coarse items. Conversely, the mandible is simple and lacks well-developed molar processes. Patwardhan (1934, 1935a-e) hypothesised that such a relationship is to be expected, a simple mandible requiring a complex gastric mill, but this is not supported by work on penaeids (Dall & Moriarty, 1983). The simple nature of the pleuro-pyloric valve of *M. rugosa* is suggestive of a diet that does not over-rely on the ingestion of fine particles and probably indicates a diet primarily based on macroscopic food items. Dall & Moriarty

(1983) point out, however, that although there are correlations between foregut structure and diet in some species, in others no such correlations can be shown. They suggest that the configuration of the gastric mill has an evolutionary basis, with diet and animal size acting as modifying factors.

CHAPTER 3. BRANCHIAL MORPHOLOGY, VENTILATION AND CARDIAC ACTIVITY

3.1. INTRODUCTION

There have been numerous studies of branchial structure and function in decapod crustaceans, though such studies are restricted to a comparatively small number of species (Burggren & McMahon, 1988; McLaughlin, 1983). Gills vary in their structure and development according to the type of organism and its environment (Hughes, 1982). In decapods, there are three different positions from which gills may arise; podobranchs arise from epipods, arthrobranchs from the junction of the limb with the body, and pleurobranchs from the body wall. Morphologically, there are three types of gills: phyllobranchiate; trichobranchiate; and dendrobranchiate gills. Most of the anomuran aimals have phyllobranchiate gills and some have trichobranchiate gills (Mill, 1972). The branchial morphology of Crustacea has been reviewed by McLaughlin (1983). In caridean shrimps and brachyuran crabs (except dromiids) the gills are exclusively phyllobranchs; in lobsters and crayfishes they are trichobranchs and in the thalassinids, pagurids and galatheids the gills are variable. The branchial morphology of several thalassinids has been recently studied by Anderson (1989). Within this group both phyllobranchiate and trichobranchiate gills occur. Dendrobranchiate gills are found in the penaeid shrimps (Foster & Howse, 1978).

The number of gills is variable among decapods, for example, excluding epipods/mastigobranchs, some penaeid shrimps have 24 pairs of gills; nephropid lobsters have 20 pairs of gills; freshwater crayfish have 13-18 pairs of gills; pagurids have 10-13 pairs of gills, but there are only three pairs of gills in pinnotherid crabs whereas other brachyuran crabs have a greater number, though this may vary between families (6-20 pairs) (Calman, 1909; McLaughlin,

1983).

Several workers have studied gill area in decapod Crustacea. One of the most extensive studies was that by Gray (1957) who compared the gill areas of sixteen species of crabs. He correlated the gill areas with the size of the crabs, their activity level, and their habitats. Gray (1957) concluded that the active aquatic species, such as the Portunidae, have greater gill areas than sluggish bottom-dwelling species such as the spider crab, *Libinia emarginata* (Majidae).

In aquatic and most semi-terrestrial decapods, the respiratory current (either water or air) is generated by regular movements of the scaphognathite which is the exopod of the second maxilla situated in the prebranchial chamber (Makarov, 1938). In the semi-terrestrial species, *Parathelphusa transversa*, however, the scaphognathites are not used to create a flow of air through the branchial chambers (Greenaway & Taylor, 1976). Instead, movements of the membranous thoracic wall and roof of the branchial chamber are involved during which there is a slow lateral oscillation of the thoracic viscera. As a result of these movements air is drawn into one branchial chamber whilst being simultaneously expelled from the other. Some other Brachyura and Anomura use different, but analogous methods of branchiostegal pumping for ventilation of the epibranchial 'lungs' (Burggren & McMahon, 1988).

In the majority of aquatic decapods, the ventilatory currents flow through the branchial chambers in a posterior to anterior direction except during brief periods of reversal of the ventilatory current (see below). In crayfishes, for example, the ventilatory current is drawn through openings at the posteroventral angle of the branchiostegites and is expelled anteriorly (Lochhead, 1950 cited from McLaughlin, 1983). Similarly, in the thalassinids, the ventilatory current is mainly in a postero-ventral to antero-dorsal direction (Anderson, 1989). In the pagurids, the branchiostegite is thin and less heavily calcified and water is free to enter the branchial chamber from any point, but

flow is in the normal posterior to anterior direction (Makarov, 1983). In the brachyuran crabs, the branchiostegite is expanded laterally and fits closely near the bases of the gills. The ventilatory current enters mainly via the Milne-Edwards openings at the base of the chelipeds and via smaller openings at the base of each leg (Borradaile, 1922). Among burying carbs, however, there may be some modifictaion of the direction of the branchial current. In species such as *Corystes cassivelaunus* (Garstang, 1897; Bridges, 1979) and *Atelecyclus rotundatus* (Garstang, 1897; Taylor 1984) the direction of water flow through the branchial chambers may be reversed when the animals are buried and represents an adaptation to ensure that ventilation of the gills can be maintained even when the crab is buried in the sediment. In addition, the crab, *Ebalia tuberosa*, and other members of the Leucosiidae, show more complex morphological adaptations. In these crabs which bury in coarse sediments, the inhalant respiratory currents flow down channels formed by the apposition of the buccal appendages and enter the branchial chambers at the base of the third maxillipeds (Schembri, 1982c). As a result, water enters and leaves the branchial chambers anteriorly.

In many decapods, the direction of the respiratory current is occasionally reversed for short periods (Boradaile, 1921; Wilkens & McMahon, 1972; Wilkens, 1976; Bridges, 1976; Warner, 1977). Reversals of forward flow are associated with an increase in the pressure within the branchial chamber. The function of these reversals has been the subject of much discussion but it appears that one of their main functions is to clean the gills (Borradaile, 1922), but other workers have suggested a respiratory function (Arudpragasam & Naylor, 1964; Hughes *et al.*, 1969). The frequency of reversals appears to be lowest following disturbance or activity and highest in resting animals (McDonald *et al.*, 1977). Taylor (1976) reported an increased incidence of reversals in response to hypoxia and decreased salinity in the crab *Carcinus*

maenas.

Only a limited amount of information is available concerning the morphological and respiratory adaptations of squat lobsters. The morphology of the respiratory system and the respiration of *Galathea strigosa* have been studied by Bridges (1980). The monograph of Pike (1947) on *Galathea squamifera* dealt only with morphological aspects of the respiratory system.

The characteristics of the gills of *Munida quadrispina* from fjordic habitats having differing oxygen availabilities have been studied by Burd (1983, 1985, 1987) and Burd & Brinkhurst (1984). These studies showed that the slope (b) of the relationship between dry gill weight and body weight for animals from sites which regularly experienced hypoxic conditions was higher than the slope obtained for similar data for animals occurring in normoxic areas (Burd, 1987). In addition, only large *Munida quadrispina* were found in regions of low oxygen availability, and they had greater relative gill weights than their normoxic counterparts (Burd, 1987). Similarly, in another galatheid, *Pleuroncodes planipes*, it has been found that, although the adults are benthic and are regularly exposed to very low oxygen levels, the juveniles are pelagic and may live at even lower oxygen levels (Boyd, 1967).

During the present study it was possible to obtain *Munida rugosa* from two sites in the Firth of Clyde which differed in depth (c40 & 95-115m) (Chapter 2). Comparative studies of the gill morphology and gill area were carried out on animals from these two sites to investigate whether there were any differences between the two populations. In addition, comparative studies were also carried out on a related species, *Munida sarsi* which occurs only in deep water in the Firth of Clyde (95-115m). The aim of this work was therefore to carry out both interspecific and intraspecific comparisons of the gill morphology of these species.

3.2. MATERIALS AND METHODS

Munida rugosa collected at depths of c40m & 95-115m around the Cumbraes in the Clyde Sea area (Lat 55° 43.7'N Long 4 57.5' W) were used. Some animals were also obtained at shallower depths of 8-20m in Loch Fyne. Specimens of *M. sarsi* were obtained from a depth of 95-115m from a site north-west of Little Cumbrae in the Firth of Clyde. The animals were maintained at 10°C in the sea water aquarium in the Zoology Department and were fed on either fresh or frozen mussels (*Mytilus edulis*) at least once a week.

The morphology of the branchial chambers and the gills was studied using light, scanning and transmission electron microscopy. The gills were carefully dissected from the branchial chambers of freshly-killed animals of differing sizes (fresh weight range = 2-56g) and histological sections prepared using the following procedures.

3.2.1. Preparation of gills for light microscopy

Gills were fixed in formal saline (100ml of 40% formalin and 8.5g of NaCl in 900ml of distilled water) for 24h. They were transferred to Histokine (Shandon 2L Processor MK11) with a series of 70%, 90%, absolute alcohol, Histoclear 1, Histoclear 2, Wax 1, and Wax 2 for 2h in each, respectively. Blocks of paraffin wax containing the gills were made and mounted on a rotary microtome (Leitz no.1512) for sectioning. Longitudinal and transverse sections (6µm) were cut and mounted on glass slides using egg albumen (one drop of pure egg albumen in 25ml of distilled water). The slides were then allowed to dry prior to staining. The staining protocol was as follows: the slides were held in a rack and placed in Histoclear 1 and absolute alcohol (5 minutes in each). The slides were then transferred to an alcohol series of 90%, 70%, 50%, and 30% (3 minutes in each). The slides were rinsed and dipped in Haemalum for 5 minutes and washed in Scott's tap water for one minute before they were transferred to an

alkaline solution of sodium bicarbonate and magnesium sulphate. The slides were washed again, and dehydrated in an alcohol series 30%, 50%, 70%, and 90% (3 minutes in each). Finally, the slides were transferred to eosin (1 min), absolute alcohol (2 min), and Histoclear 2 (5 min) then mounted in Histomount.

3.2.2. Preparation of gills for scanning electron microscopy

Specimens were fixed in a solution of glutaraldehyde and sodium cacodylate in sea water for 2h, then rinsed in buffer and fixed in 1% osmium for 1h. The gills were washed in water before being dehydrated using an acetone series. Finally, the specimens were critical point dried for 1h and placed on stubs and coated with a thin layer of gold.

3.2.3. Preparation of gills for transmission electron microscopy

Samples were fixed as in the SEM preparation, rinsed with water and covered with 0.5% uranyl acetate. The samples were kept in the dark for 30 minutes, then rinsed in water and dehydrated using an alcohol series. The samples were embedded in freshly made Araldite and placed in an oven at 60^{o}C. Sections of between 60-100 nm thick were cut using a microtome (LKB MK1 Ultratome). The sections were examined under a transmission electron microscope (Zeiss AE1 801) operating at 80 KV.

The sections prepared for light microscopy were used to examine the arrangement and the dimensions of the gill lamellae. The latter were obtained using an eyepiece graticule calibrated with a stage micrometer. The thickness of the gill cuticle and the minimum distance from the water to the blood spaces were measured from the TEM photographs, with reference to the print magnification.

3.2.4. Gill area measurements

The gill areas of fresh *Munida rugosa* and *Munida sarsi* were determined using a dissecting light microscope fitted with a camera lucida. Animals were weighed, and measurements of carapace length (posterior rim of orbit to posterior mid-line of dorsal carapace), and carapace width (maximum) were taken before the animals were killed by exposure to low temperature (in a freezer at -20^{o}C). Each gill from one of the branchial chambers was dissected and mounted in sea water. The number of gill lamellae on each gill was recorded.

Since the diameters of the lamellae varied along the gill axis, it was decided to measure the mean values of at least three representative lamellae selected from near the top, middle and base of the gills. Three transverse sections were made for each gill and an outline drawing made of the lamellar cross section at each of these positions. The drawings were calibrated using a stage micrometer and the areas calculated by tracing the outline of each lamella using a digitising pad (Cherry digitiser) connected to a BBC computer. The area of each lamella was multiplied by 2 to obtain the area for both sides of the lamella. This value was then multiplied by the total number of lamellae present on that gill. The total gill area of the animal was then calculated by adding the areas for each of the 14 gills present and then multiplying by 2 to obtain the total gill area for both sides of the animal. The total number of gill lamellae, the total gill area and the weight specific gill area (i.e. total gill area per gram fresh body weight) were then plotted against the fresh weight of the animal.

3.2.5. Branchial ventilation and scaphognathite activity

The patterns of water flow through the branchial chamber for both species of *Munida* were studied by releasing diluted ink at different sites around the margins of the branchial chambers and at the bases of the legs. The circulation of

the ink was observed and the passage of the inhalant and exhalant water was determined visually. The movements of the scaphognathites of *M. rugosa* were studied by placing an animal on its dorsal side in a dish of sea water and observing them with the aid of a low power microscope.

Recordings of scaphognathite and heart activities were made using an impedance technique (Trueman, 1967). A small hole was made in the carapace using a dentist's drill with final penetration of the carapace being effected by using a hypodermic needle. The electrodes used consisted of shellac-coated copper wire (diam. = 0.28mm) with the shellac removed from the tips. The electrodes were inserted through the carapace close to the pericardial membrane with care being taken not to cause any damage to the heart. The electrodes were held in position by cyanoacrylate adhesive.

Scaphognathite activity was recorded using a similar technique but, in this case, the electrodes were inserted through a small hole drilled into the branchiostegite near the scaphognathite and held in position using the cyanoacrylate adhesive. The heart and scaphognathite electrodes were connected to impedance units (Strathkelvin Instruments) contained within a pen recorder (Washington Oscillograph 400 MD/2, Searle Bioscience).

Recordings of the pressure changes inside the branchial chambers were carried out using a pressure transducer (Washington PT400). Narrow bore cannula tubing (diam. = 1.3mm) was inserted into a hole made in the hyperbranchial region at the anterior of the branchial chamber. The cannula tubing was fixed in position with the adhesive, with care being taken to ensure that there were no leaks around the cannula. In most cases, the pressure inside one of the branchial chambers was monitored while concurrent recordings of scaphognathite activity were made using the impedance technique. Recordings were carried out on male and female animals of differing sizes. Only animals in

the intermoult stage were used. However, the emphasis was on observing the branchial pressure changes and not on quantifying the pressures involved.

3.2.6. Recordings of ventilatory activity under normoxic conditions

Animals were placed in tanks provided with well-aerated sea water having a salinity of 32‰ and temperature of 10°C. Each tank was covered to avoid any visual disturbance. The animals were allowed to recover from the electrode implantation for 12-24h. The recordings were continued for several hours and in some cases were continued for several days. On those occasions the water was changed daily.

Initially, recordings were made from both the right and the left scaphognathites and their activity compared to establish the degree of synchrony between them. In many other recordings, however, the activity of only one of the scaphognathites was monitored.

3.2.7. Effects of disturbance on ventilatory and cardiac activity

Recordings of both heart and scaphognathite activity were made for several hours until the rates had decreased to lower, and approximately constant, rates which were taken to be representative of resting animals. In all subsequent experiments, the resting rates of heart and scaphognathite activity were established before the effects of experimental disturbances (both visual and tactile) were examined.

3.3. RESULTS

3.3.1. Branchial morphology

As in other decapods, the branchial chambers are located on either side of the thorax and consist of two parts, an anterior prebranchial chamber which is occupied by the gill bailer (scaphognathite), and the post-branchial chamber in which the gills are situated (Fig. 3.1). The branchial chambers are bounded by the extension of the carapace on either side (branchiostegites). In *Munida rugosa* the branchiostegite (Fig. 3.2 A & B) has a triangular shape and has a dense layer of plumose setae along the ventral edge. The setae are particularly dense along the posterior margin of the branchiostegite. The setae along the anterior edge of the branchiostegite near the mouth are less dense than those in other regions. The inner surface of the branchial chamber is smooth with some horizontal ridges in the region of the body wall, whereas the out-lying inner surface of the branchiostegite is uniformly smooth. In squat lobsters, the branchiostegites are not fused to the thorax. The fifth walking leg could be inserted inside the chamber for cleaning purposes during which the branchiostegite was often seen to flex.

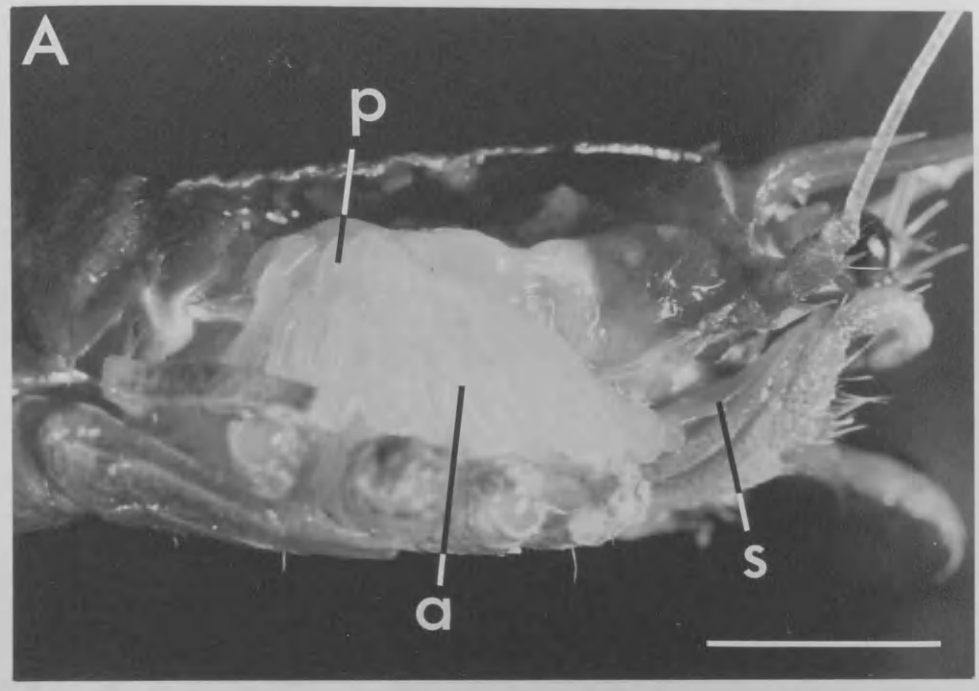
The gills of *Munida rugosa* are of the phyllobranchiate type i.e. they are lamellate (Fig. 3.3 A & B). The lamellae occur on both sides of a central axis in which the afferent and the efferent blood vessels are located. The larger lamellae occupy the central region of the gills with the diameter of the lamellae decreasing gradually towards both ends of the gill. The reduction in the size of the lamellae was most pronounced towards the distal region of the gill.

Transverse sections of gill lamellae taken from different regions along the gill axis are shown in Fig.3.4 . The overall shape of the lamellae is heart shaped with the indented edge directed towards the branchiostegite. The morphological function of this is perhaps associated with channelling the water

Fig. 3.1

(A) Photograph of the right branchial chamber of *Munida rugosa* after removal of the branchiostegite. The positions of the pleurobranch (p) and arthrobranch (a) gills and the right scaphognathite (s) are shown. Scale bar = 10 mm.

(B) Inner surface of the right second maxilla of *Munida rugosa*. s = scaphognathite, en = endopod and es = endites. Scale bar = 1 mm.


A
p
a
s

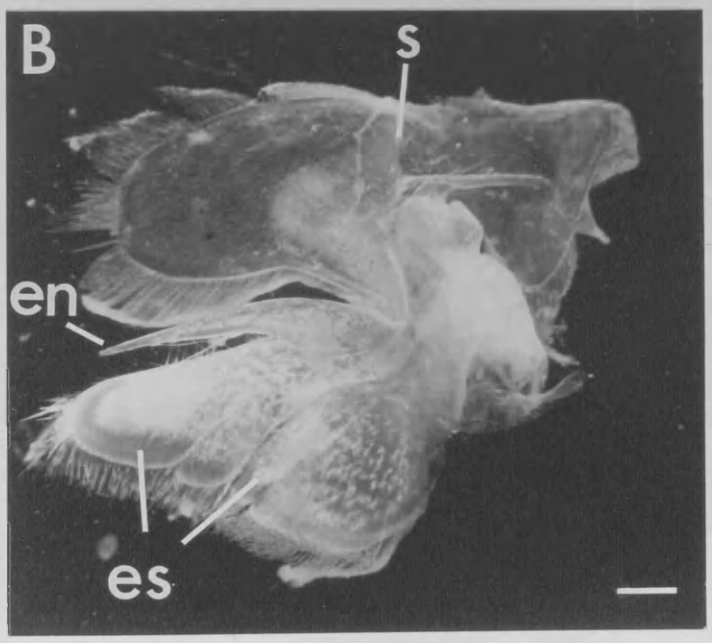
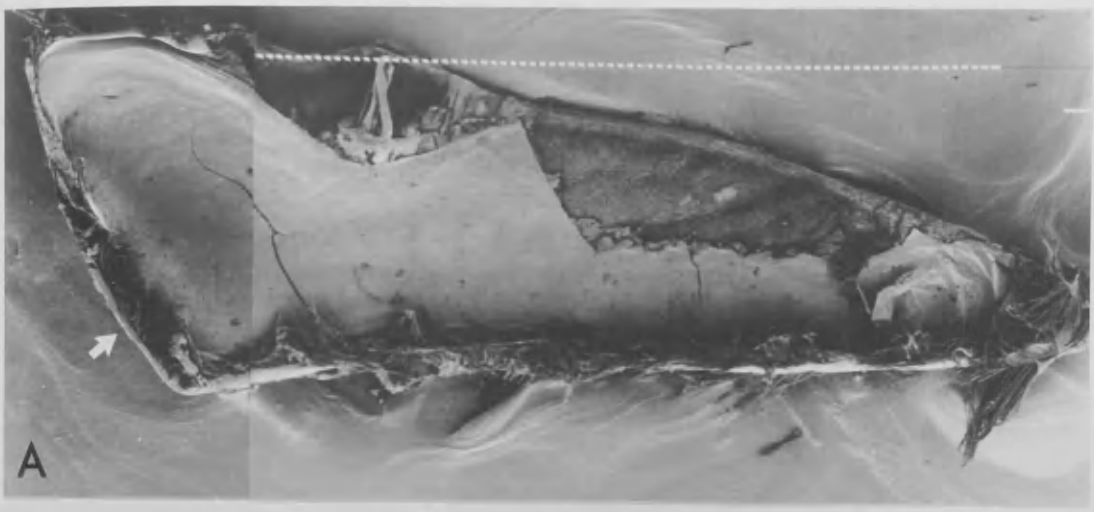

B
s
en
es

Fig. 3.2

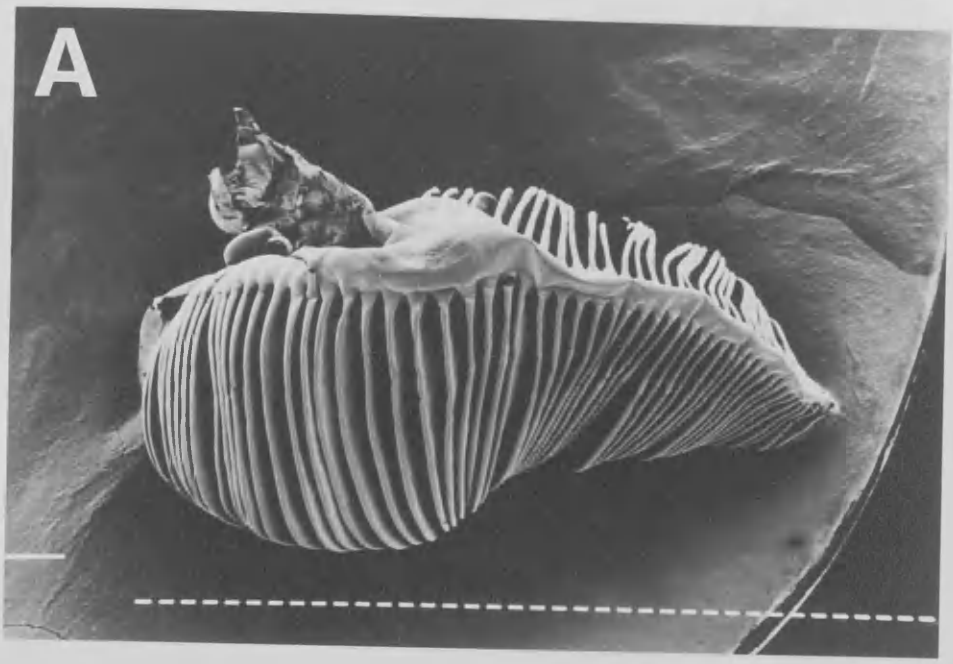
(A) Scanning electron micrograph of the inner surface of branchiostegite of the branchial chamber of *M. rugosa*. Black scale bar = 100 µm. (B) Scanning electron micrograph of the setae on the edge of the branchiostegite. Scale bar = 100 µm.


A


B

Fig. 3.3

Scanning electron micrographs of the first gill (A1) of *Munida rugosa*. A = outer view, black scale bar = 100 µm. B = inner view, black scale bar = 100 µm.


A

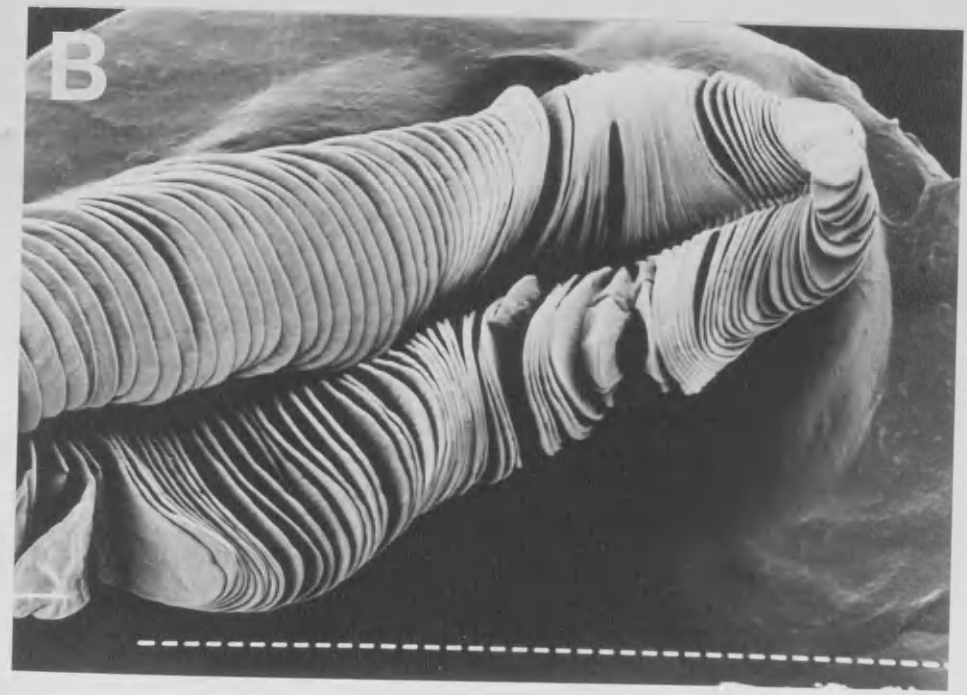
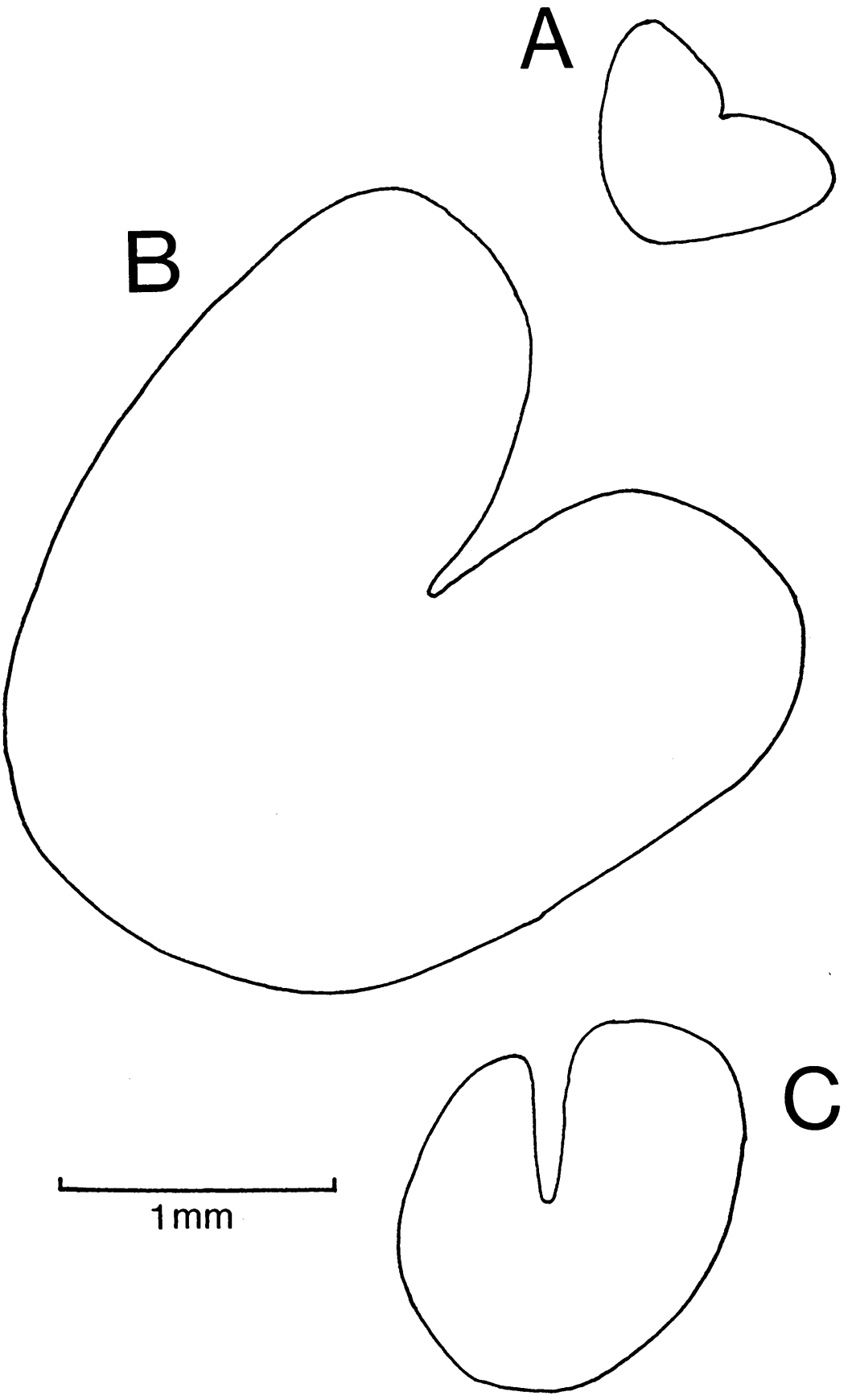

B

Fig. 3.4

Outline drawings made using a camera lucida of gill lamellae from one of the pleurobranch gills (P1) of *Munida rugosa* . The lamellae were taken from the tip (A), middle (B), and base (C) of the gill. Scale bar = 1 mm.


A
B
C
1mm

stream (Borradaile, 1922).

The gills are arranged in two rows in each branchial chamber (see below). A total of 28 gills are present, 14 in each branchial chamber. Ten are arthrobranchs since they are attached to the joints of the limbs near the body wall and 4 are pleurobranchs as they attached directly to the body wall. In one specimen, however, a total of 15 gills was found on each side. In this individual, there was an abnormal growth of the arthrobranchs; gills 1 and 2 were united at their bases in an unusual way and an additional gill was joined to them. This resulted in a total of 11 arthrobranchs in each chamber and the total number was 30 gills.

The normal gill formula of *Munida rugosa* is as follows:

Thoracic appendages	:	1	2	3	4	5	6	7	8
Pereiopods	:	0	0	0	1	2	3	4	5
Arthrobranchs	:	0	0	2	2	2	2	2	0
Pleurobranchs	:	0	0	0	0	1	1	1	1
Epipods	:	1	0	1	1	0	0	0	0

The gill formula of *Munida sarsi* was found to be the same as *M. rugosa* except that the epipod on the base of the cheliped was absent.

The gills were numbered in sequence; the most anterior gill being gill A1 to form a series as follows: A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 (i.e. arthrobranchs) in the first row, which is located ventrally in the branchial chambers, and P1 P2 P3 P4 (i.e. pleurobranchs) which form a second row lying in a more dorsal position to the arthrobranchs (see Fig. 3.1A).

The arthrobranchs are grouped into five pairs and each pair is joined at its base by a membrane. This membrane was cut carefully in order to separate the pair of gills since it was found that pulling them caused damage to at least one

of the gills. The largest arthrobranchs are those which occur mid-way along the thorax, namely gills A4 and A5. The smallest gill of all is the arthrobranch A1, situated at the base of the 3rd maxilliped. The pleurobranchs, however, are arranged singly. The largest gills are the pleurobranchs, in particular P4 which is situated dorsally and more posteriorly than the rest. The pleurobranchs P1, P2, & P3 are partly covered by the arthrobranchs A7, A8, A9, & A10.

3.3.2. Thickness of the lamellar cuticle

From transverse and longitudinal sections of the arthrobranchs A1 and A2, the following measurements were taken from the tip, middle and bottom of the gills: the distance between the adjacent lamellae = 71.8 ± 24μm, n = 86 (this variability may be attributable to the displacement of the lamellae during preparation) (Fig. 3.5); the maximum distance between the two inner walls = 44.3 ± 9μm, n = 41, the minimum distance between the two walls = 12.8 ± 4.6μm, n = 63; the distance between the epicuticle and the blood space = 6.8 ± 3μm, n = 62. The transmision electron micrographs revealed the fine structure of the lamellar wall. It consists of an outer layer of epicuticle, below which is the endocuticle which in turn consists of an outer and inner layers. A basal membrane is also present beneath the inner layer of the endocuticle. The electron micrograph (Fig. 3.6) also shows the presence of cells known as pillar cells or 'trabeculae' (Hughes, 1982) which divide the lumen of the lamellae into a series of blood spaces (haemocoel).

From the transmission electron microscope (TEM) photographs (Fig. 3.6), the following measurements were obtained: the thickness of the cuticle = 1.7 ± 0.6μm, and the shortest pathway through the tissue (pillar cell flange) = 5.37μm ± 1.4, the distance from the water (i.e. outer surface of gill) to the centre of the haemocoel = 20.6 ± 3μm. The values obtained from both the histological sections and the TEM photographs did not differ significantly.

Fig. 3.5

Transverse sections of gill lamellae from the first arthrobranch gill (A1) of *Munida rugosa*. The photographs were taken using a light microscope. Scale bars for (A) and (b) = 1000μm and 10μm respectively.

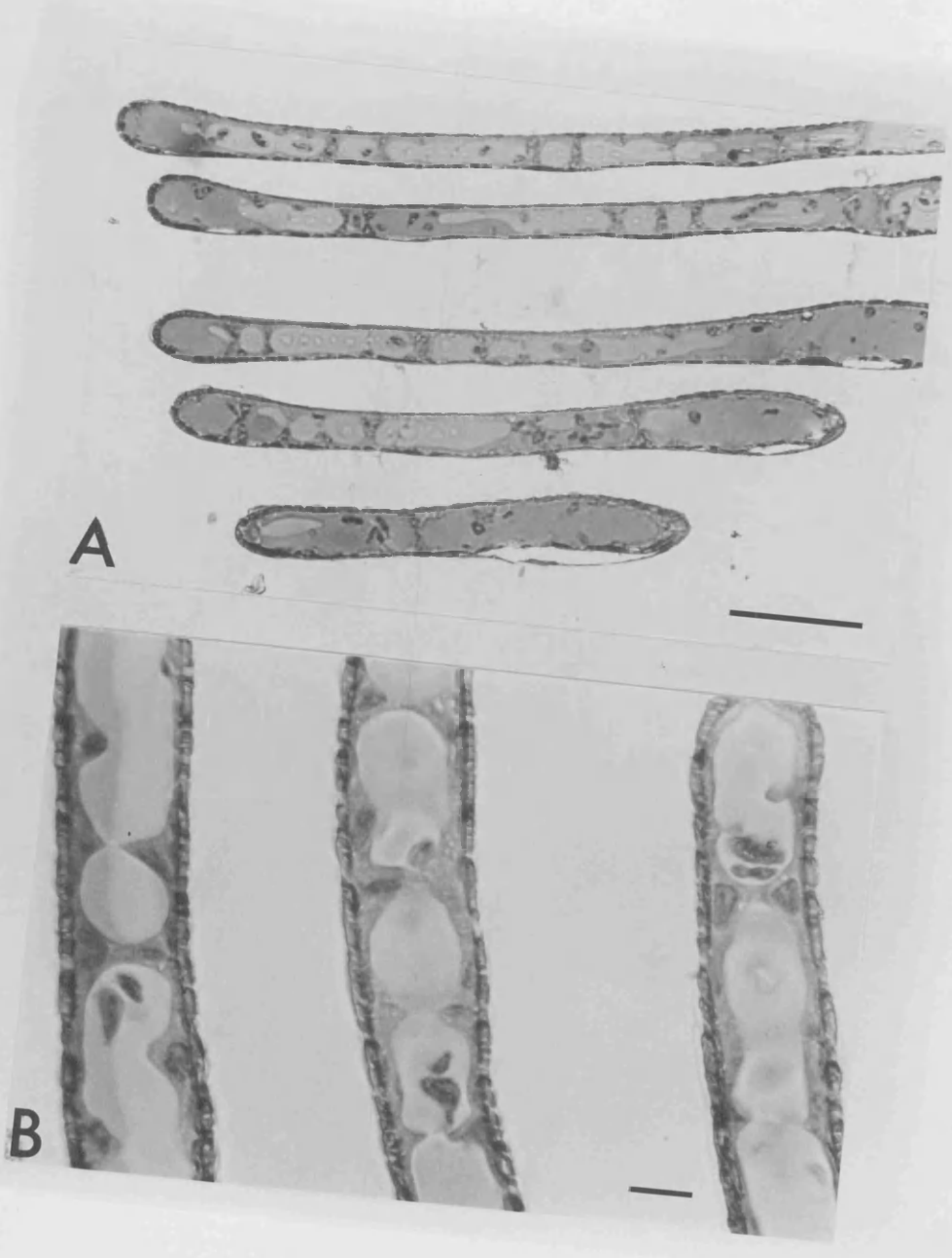
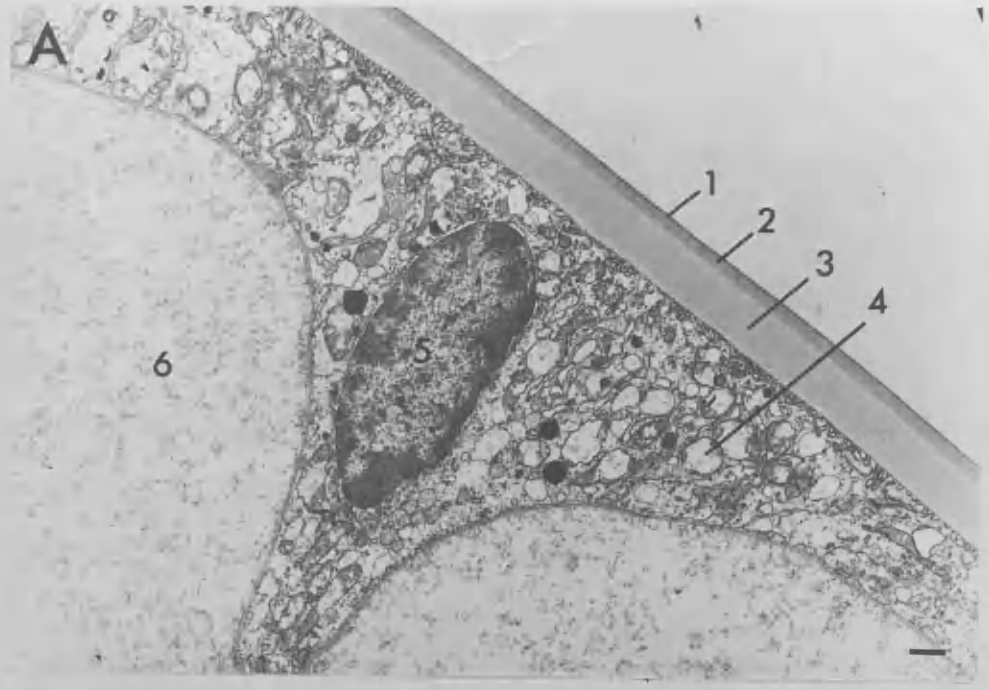

A
B

Fig. 3.6

(A) Transmission electron micrograph of a transverse section of an individual lamella from the first arthrobranch gill (A1) of *Munida rugosa*. 1 = epicuticle; 2 = outer endocuticle; 3 = inner endocuticle; 4 = pillar cell; 5 = nucleus of pillar cell; 6 = haemocoel space. Scale bar = 1 µm.

(B) Part of the same section seen at higher magnification. 1 = epicuticle; 2 = outer endocuticle; 3 = inner endocuticle. Scale bar = 100 nm.


A
1
2
3
4
5
6


B
1
2
3

3.3.3. Gill area measurements

The relationships between gill area and body weight for each of the individual gills of *Munida rugosa* from both the shallow and deep water sites and for *M. sarsi* are shown in Fig. 3.7. There is a greater proportional increase in the gill area with increasing body weight for the pleurobranch P4, which is situated at the most posterior end of the branchial chamber, than for the other gills. The increase in gill area is associated with an increase in the number of the lamellae (Fig. 3.8). In addition, the correlation between the areas of individual gills and their position showed that the areas and the number of lamellae varied with the position of the gill within the branchial chamber (Fig. 3.9).

The average values of gill area measurements in mm^2 of 32 individuals of *Munida rugosa* were determined. Data for males and females (body weight = 2-56g) were pooled. The relationship between the total gill area and body weight (Fig. 3.10A) for *Munida rugosa* from shallow water is expressed by the regression equation: $\log y = 0.771 \log x + 2.851$. When these data were expressed as weight specific gill area ($mm^2 . g^{-1}$ body weight) the expected negative relationship was obtained (regression equation:

$$\log y = -0.228 \log x + 2.850)$$ (Fig. 3.10B).

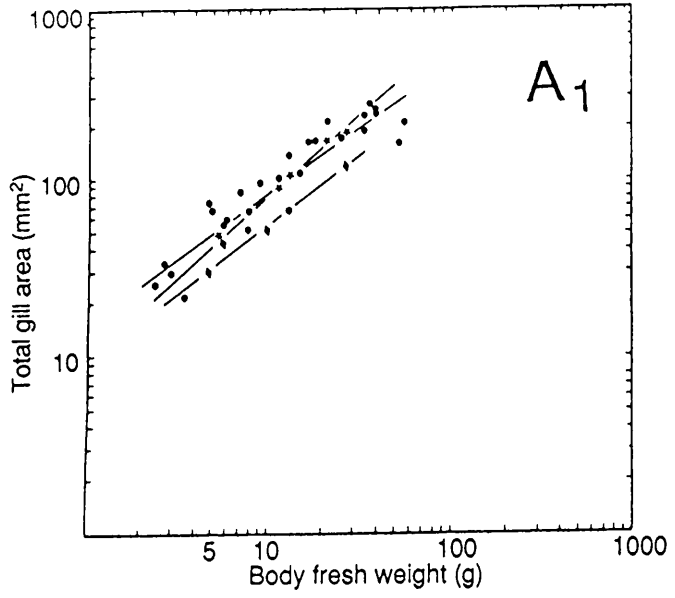
The relationship between the total number of the lamellae and fresh body weight is plotted in (Fig. 3.11). The regression equation fitted to these data was:

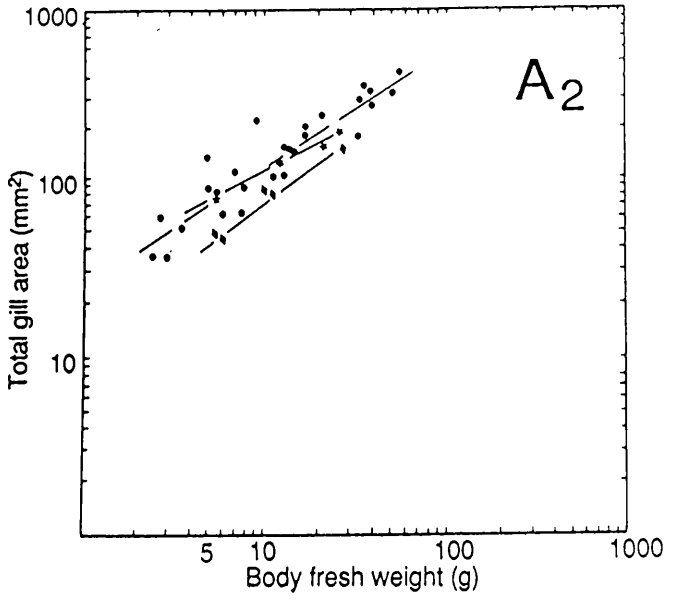
$$\log y = 0.099 \log x + 3.184 \quad r = 0.517 \ (P<0.01).$$

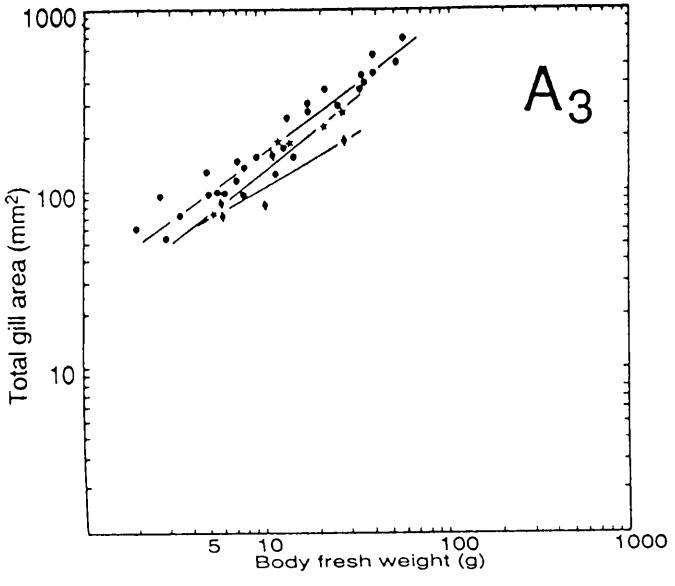
Similar correlations were obtained for deep water *M. rugosa* and *M. sarsi* (see Table 3.1). Covariance analysis of the data for *M.rugosa* from both sites and for *M.sarsi* were carried out. There was no significant difference ($P > 0.05$) between either the slopes or the elevations of the regression lines between the

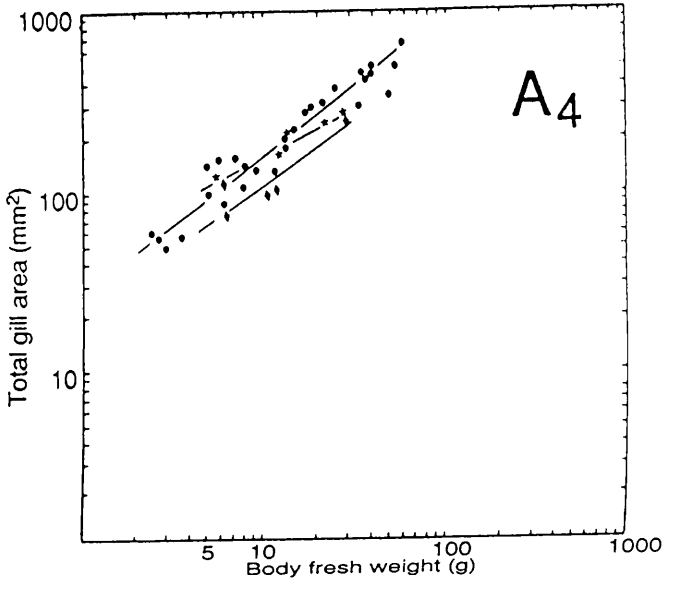
Fig. 3.7

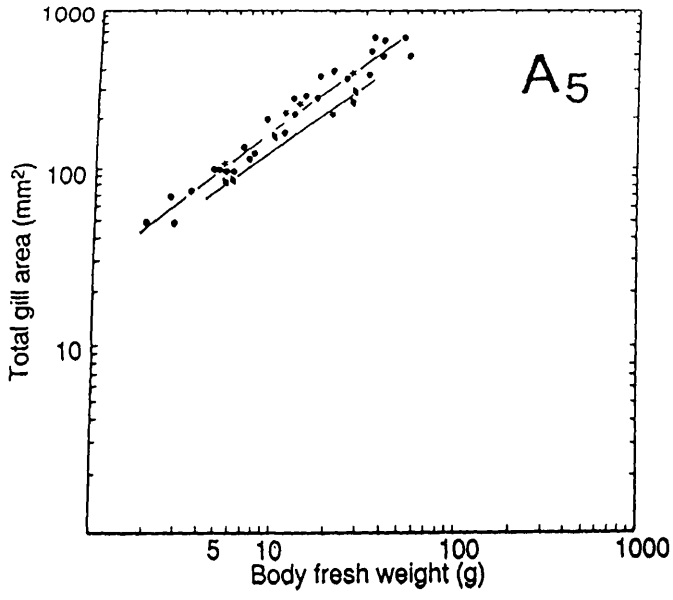
The relationships between total area and body fresh weight for each of the individual gills of *Munida rugosa* from the shallow (●) and deep water (★) sites, and for *M. sarsi* (◆). A1-A10 = arthrobranchs 1-10 and P1-P4 = pleurobranchs 1-4. The regression equations of the lines fitted to these data are given in Table 3.3.

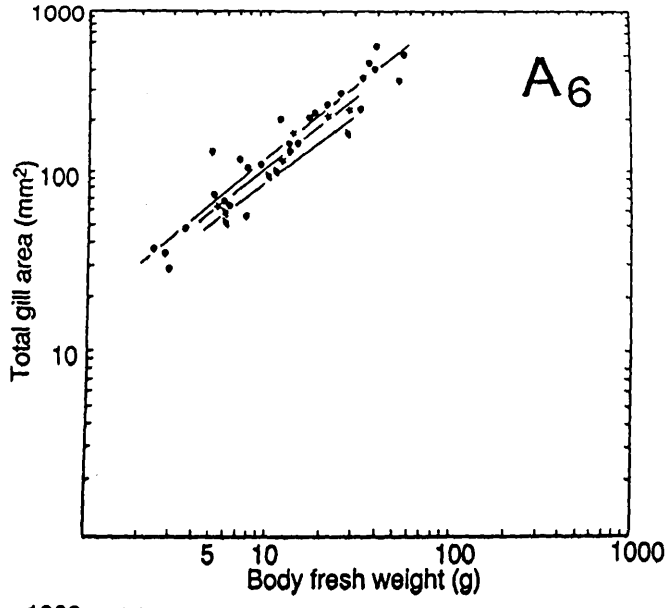

A1
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

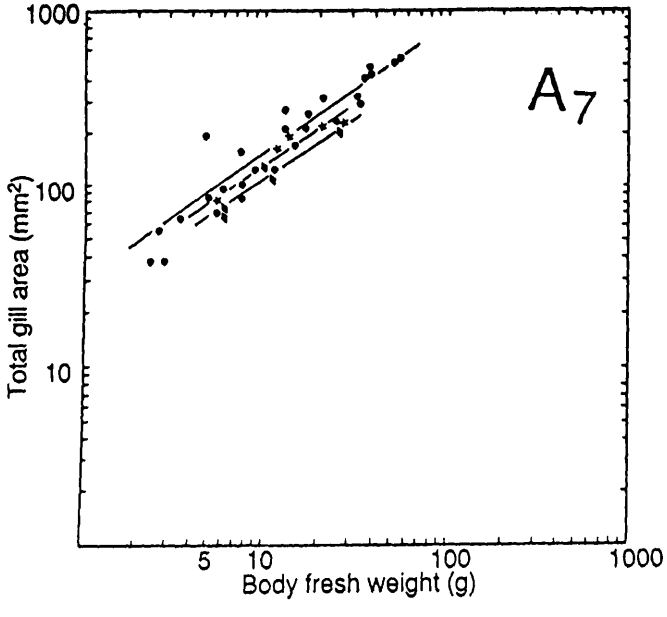

A2
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

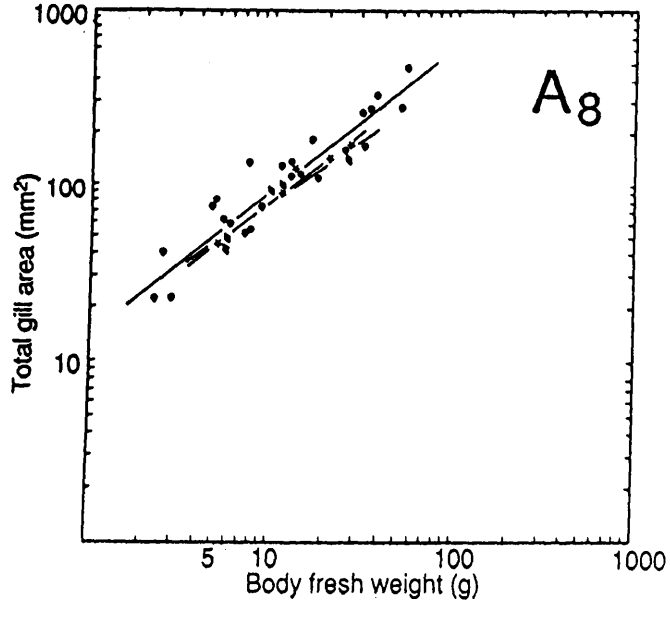

A3
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

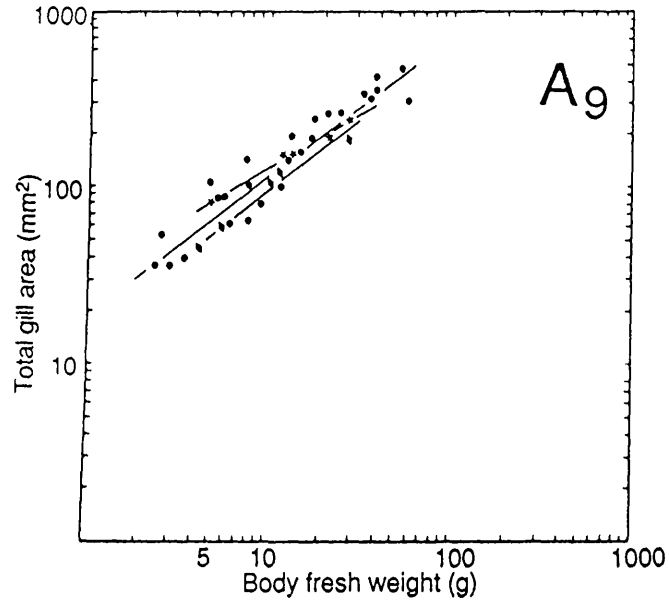

A4
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

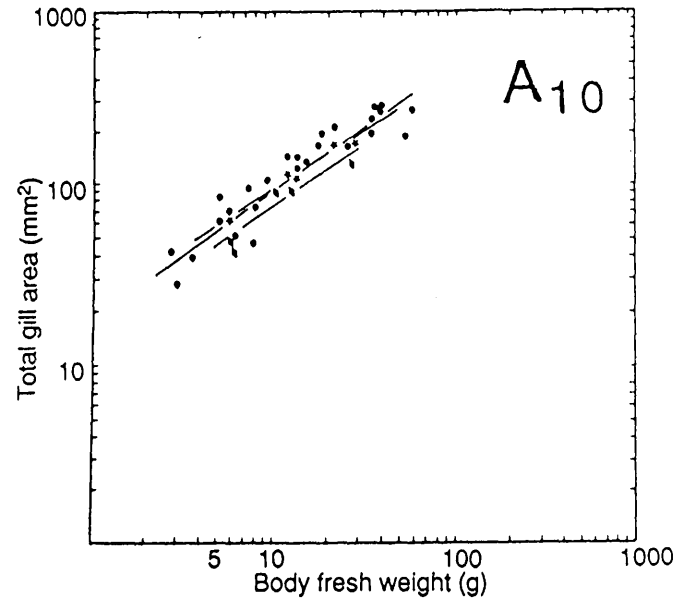

A5
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)

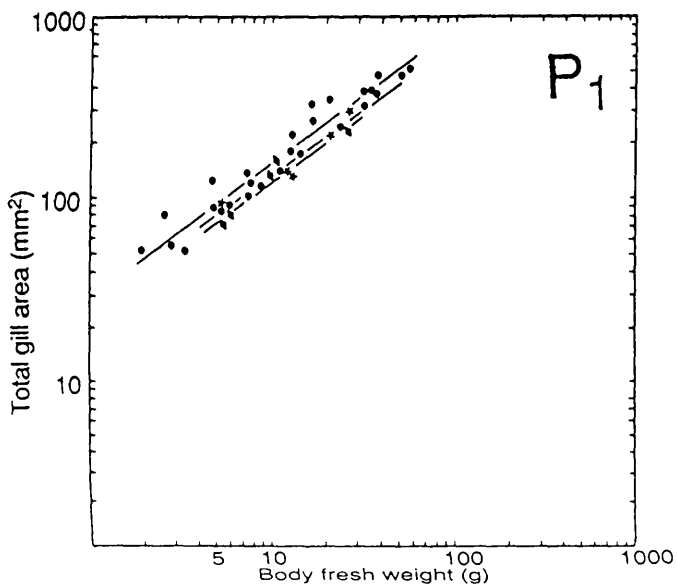

A6
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)

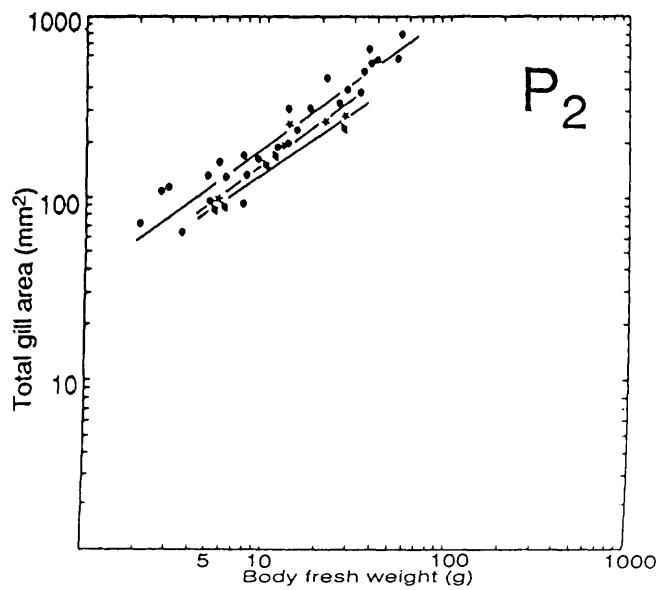

A7
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)

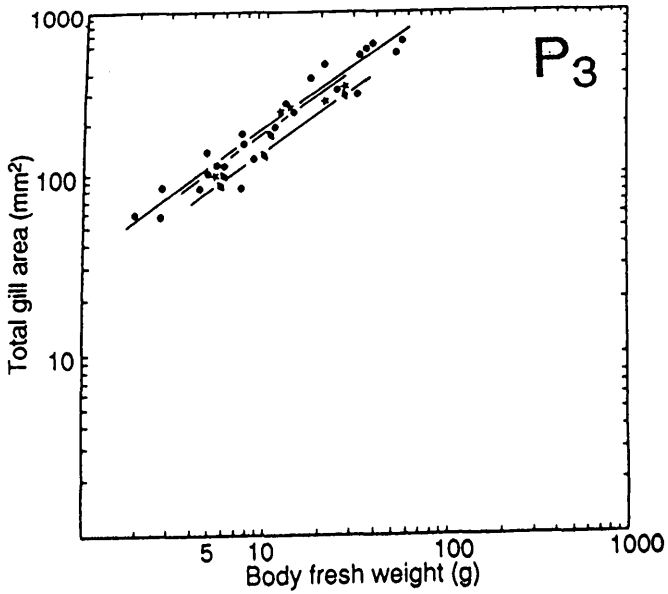

A8
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)


A9
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)


A10
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)


P1
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)


P2
Total gill area (mm²)
1000
100
10
5
10
100
1000
Body fresh weight (g)


P3
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

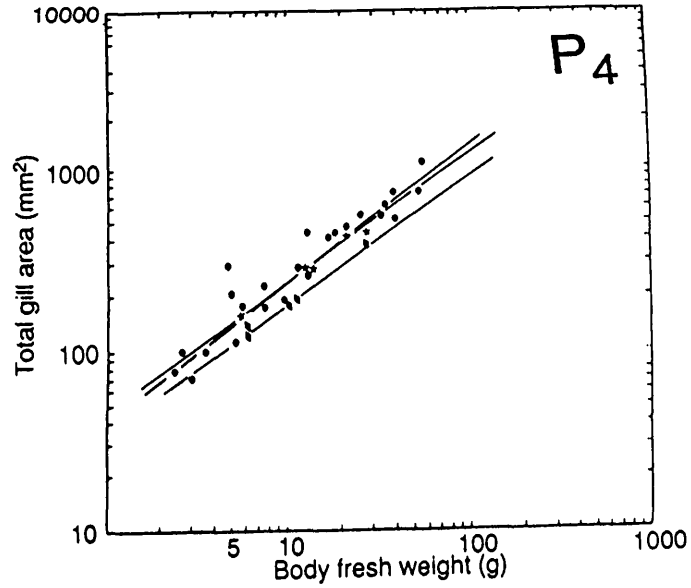

P4
10000
1000
100
10
Total gill area (mm²)
5
10
100
1000
Body fresh weight (g)

Table 3.3

Regression coefficients for the relationship between the area of individual gills and fresh body weight. a = intercept and b = slope of the regression lines fitted to these data; r = correlation coefficient, n = number of animals.

Munida rugosa (shallow)

Gill No.	a	b	r	n
A 1	15.49	0.74	0.93	27
A 2	24.60	0.69	0.92	
A 3	29.04	0.75	0.96	
A 4	28.12	0.78	0.97	
A 5	26.49	0.82	0.98	
A 6	16.79	0.87	0.95	
A 7	30.20	0.72	0.80	
A 8	13.55	0.83	0.96	
A 9	19.10	0.80	0.95	
A 10	18.92	0.73	0.94	
P 1	27.99	0.74	0.97	
P 2	37.76	0.73	0.95	
P 3	31.05	0.78	0.96	
P 4	41.98	0.77	0.97	

Munida rugosa (deep)

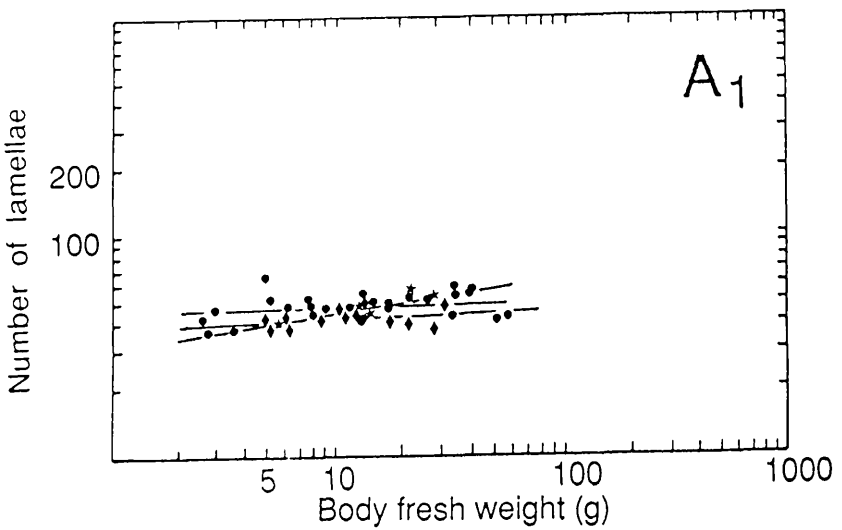
Gill No.	a	b	r	n
A 1	09.46	0.93	1.00	5
A 2	33.50	0.53	0.90	
A 3	20.42	0.81	0.97	
A 4	50.58	0.52	0.97	
A 5	26.98	0.77	1.00	
A 6	16.90	0.82	0.97	
A 7	25.94	0.71	0.96	
A 8	12.27	0.82	0.97	
A 9	30.34	0.62	1.00	
A 10	20.70	0.67	0.97	
P 1	26.18	0.71	0.97	
P 2	31.55	0.71	0.93	
P 3	34.67	0.71	0.95	
P 4	48.31	0.60	0.99	

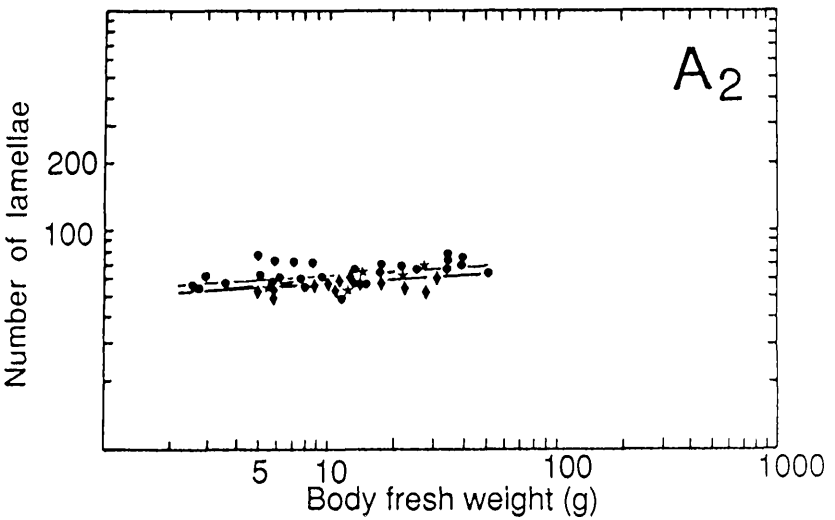
Munida sarsi

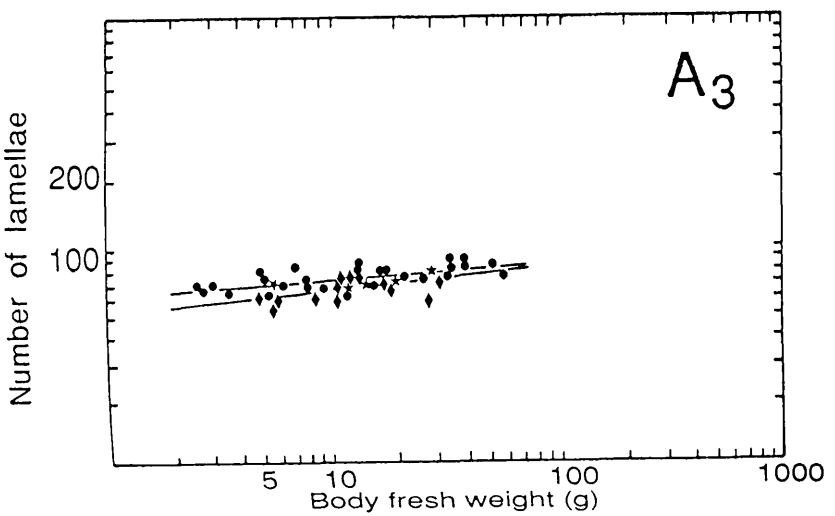
Gill No.	a	b	r	n
A 1	09.57	0.75	0.94	5
A 2	12.56	0.77	0.98	
A 3	27.29	0.60	0.84	
A 4	22.86	0.70	0.86	
A 5	22.49	0.78	0.96	
A 6	16.22	0.74	0.99	
A 7	23.82	0.67	0.96	
A 8	13.93	0.75	0.93	
A 9	15.28	0.79	0.97	
A 10	17.10	0.67	0.88	
P 1	22.23	0.75	0.95	
P 2	30.55	0.66	0.96	
P 3	24.38	0.78	0.97	
P 4	35.73	0.71	0.99	

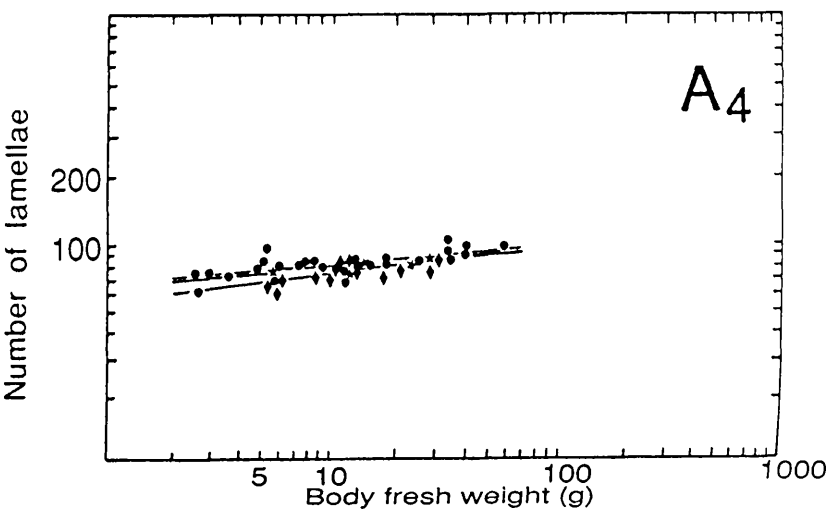
Fig. 3.8

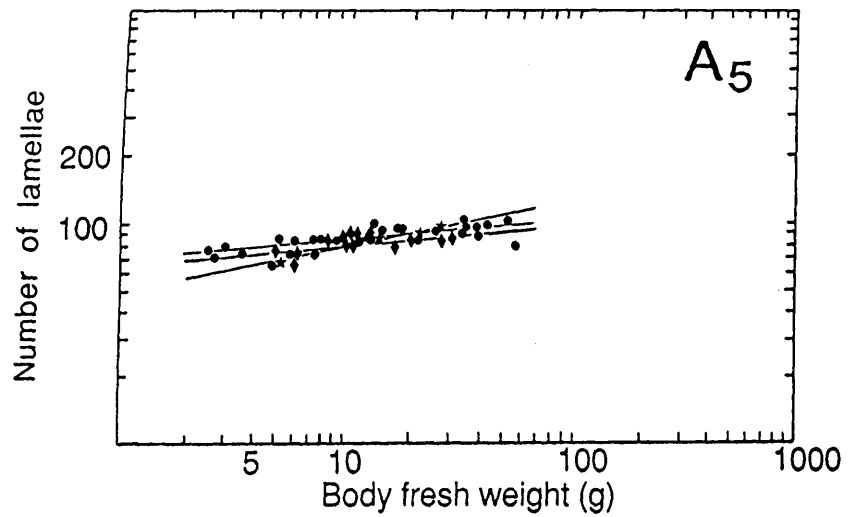
The relationships between the number of gill lamellae and body fresh weight for each of the individual gills of *Munida rugosa* from the shallow (●) and deep water (✖) sites, and for *M. sarsi* (◆). A1-A10 = arthrobranchs 1-10 and P1-P4 = pleurobranchs 1-4. The regression equations of the lines fitted to these data are given in Table 3.4.

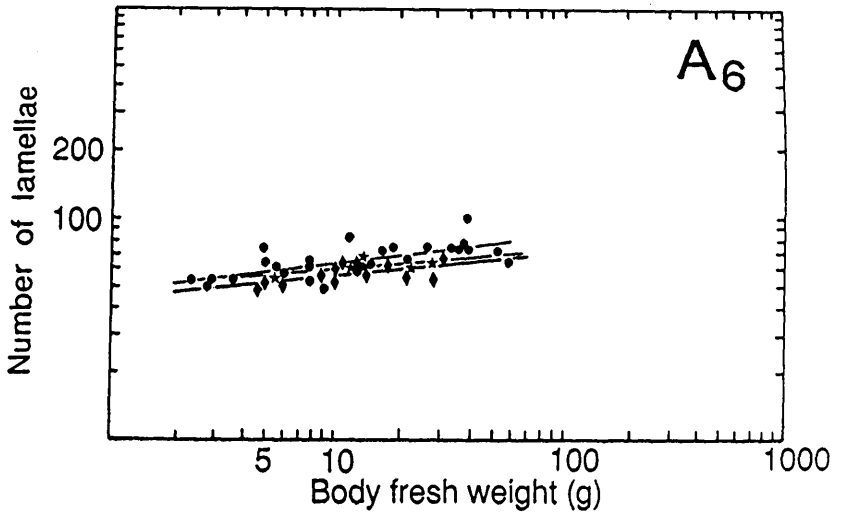

A1
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

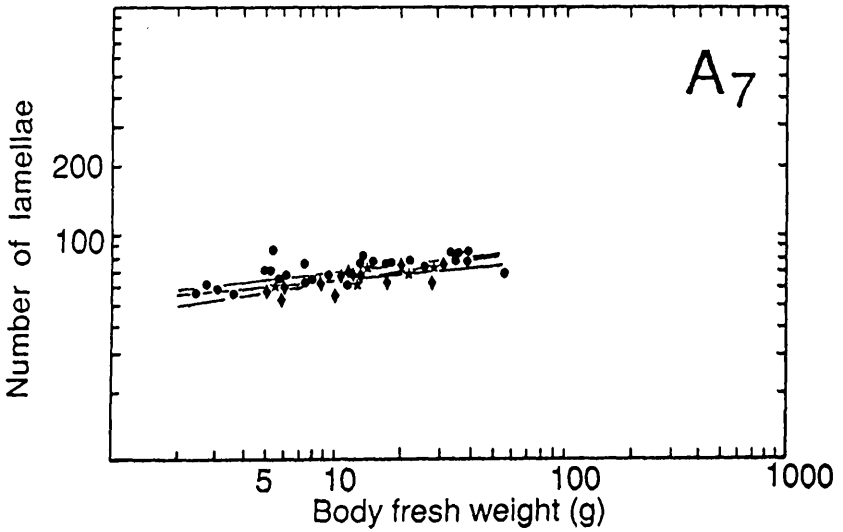

A2
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

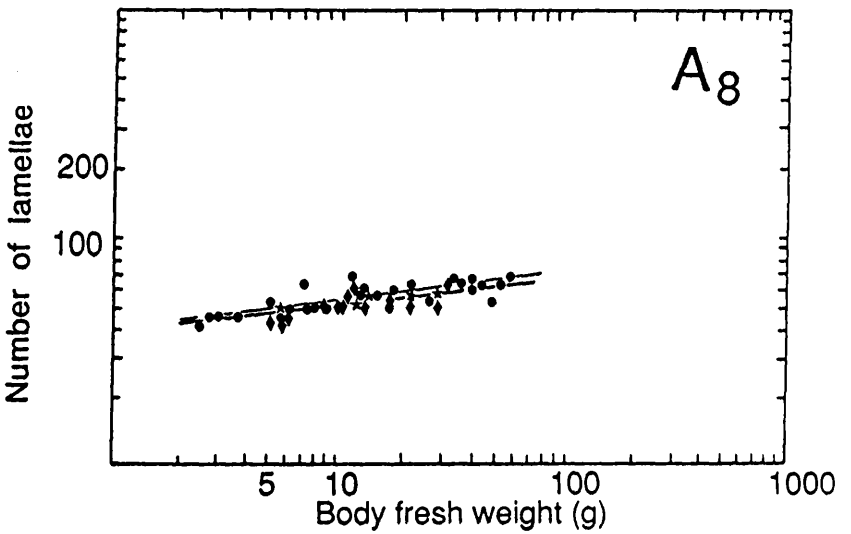

A3
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

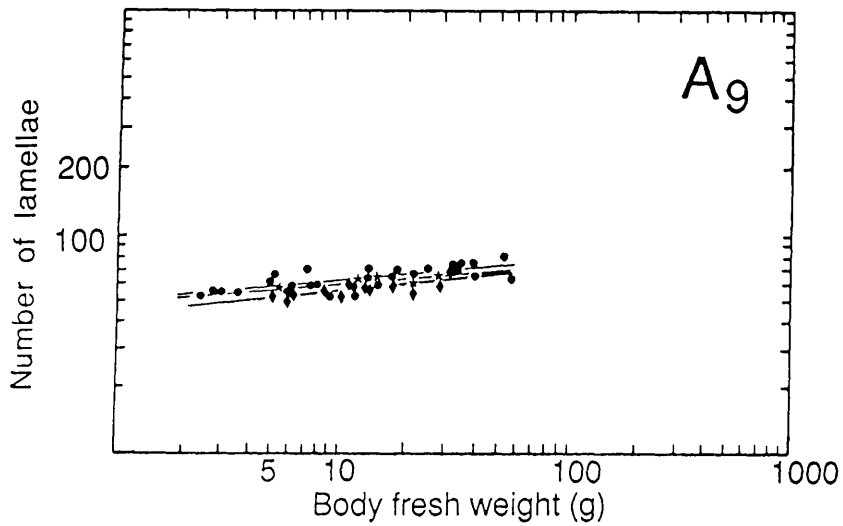

A4
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

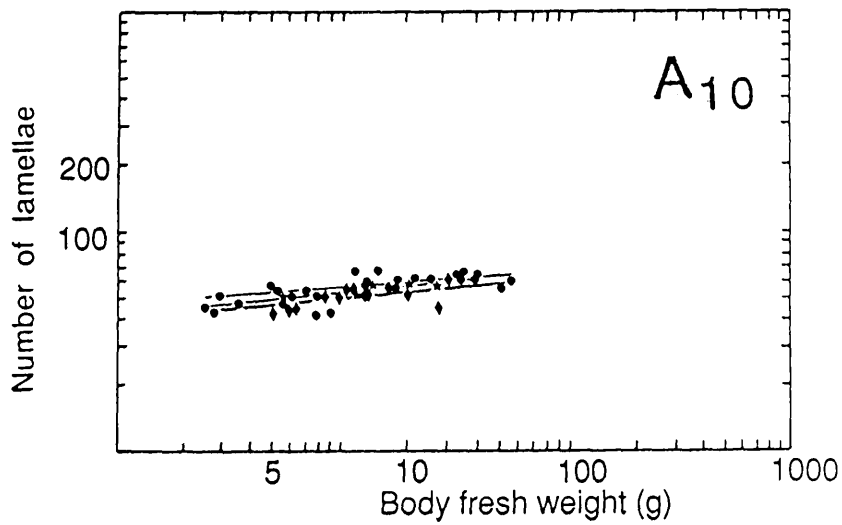

A5
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

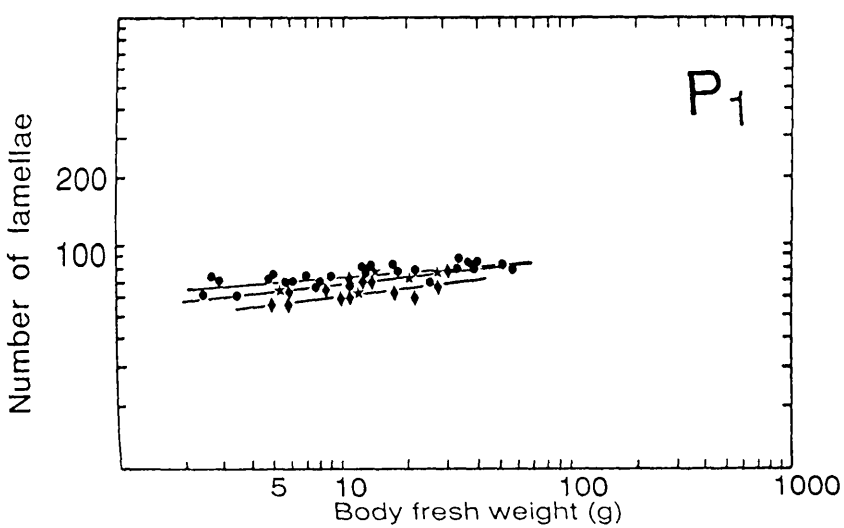

A6
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

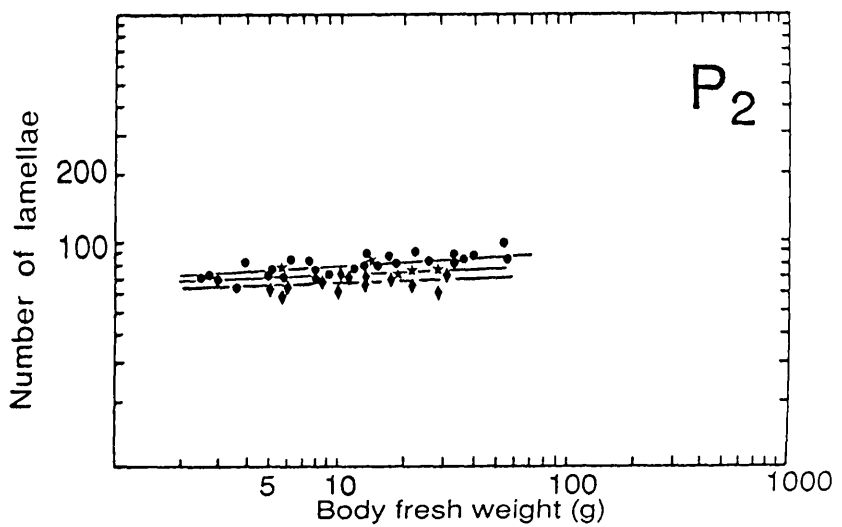

A7
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

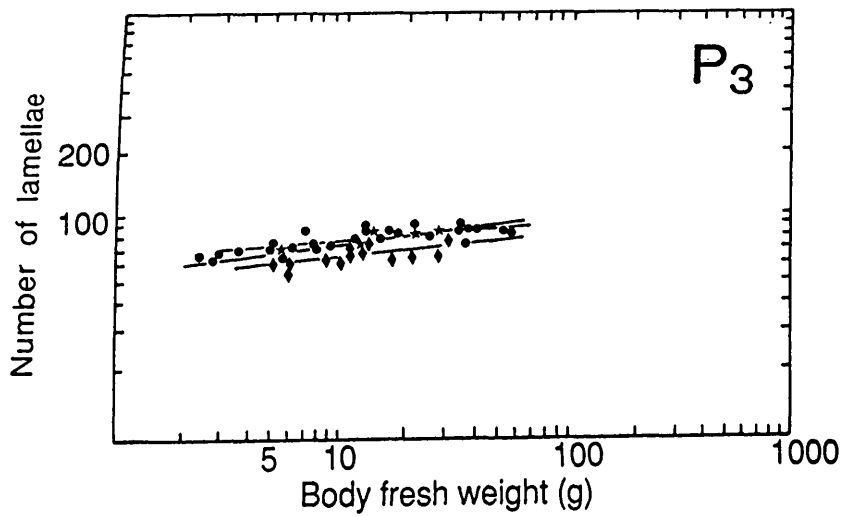

A8
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)


A9
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)


A10
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)


P1
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)


P2
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)


P3
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

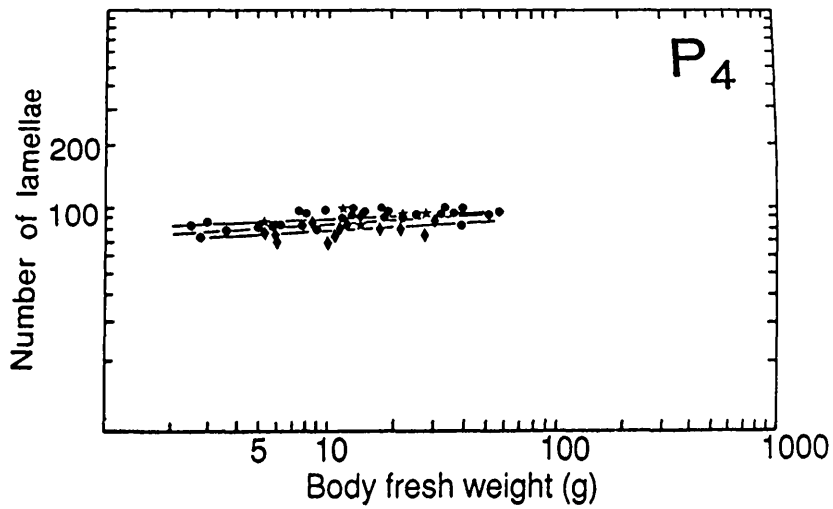

P4
Number of lamellae
200
100
5
10
100
1000
Body fresh weight (g)

Table 3.4.

Regression coefficients for the relationships between the number of lamellae on individual gills against fresh body weight. a = intercept and b = slope of the regression equations fitted to these data; r = regression coefficient; n = number of animals.

M. rugosa (shallow)

Gill No.	a	b	r	n
A 1	44.16	0.04	0.23	27
A 2	55.34	0.07	0.53	
A 3	64.57	0.08	0.65	
A 4	66.07	0.10	0.78	
A 5	66.68	0.10	0.72	
A 6	48.08	0.13	0.69	
A 7	54.58	0.10	0.77	
A 8	40.36	0.13	0.80	
A 9	50.12	0.10	0.75	
A 10	43.15	0.11	0.70	
P 1	62.81	0.08	0.71	
P 2	67.92	0.08	0.75	
P 3	61.52	0.10	0.83	
P 4	76.91	0.07	0.70	

M. rugosa (deep)

Gill No.	a	b	r	n
A 1	30.97	0.17	0.88	5
A 2	47.64	0.11	0.75	
A 3	62.66	0.07	0.61	
A 4	67.14	0.08	0.87	
A 5	48.41	0.21	0.97	
A 6	47.53	0.10	0.82	
A 7	54.20	0.10	0.59	
A 8	39.90	0.12	0.83	
A 9	49.66	0.10	0.91	
A 10	48.19	0.06	0.68	
P 1	55.08	0.11	0.71	
P 2	71.12	0.03	0.29	
P 3	55.08	0.14	0.99	
P 4	72.61	0.07	0.95	

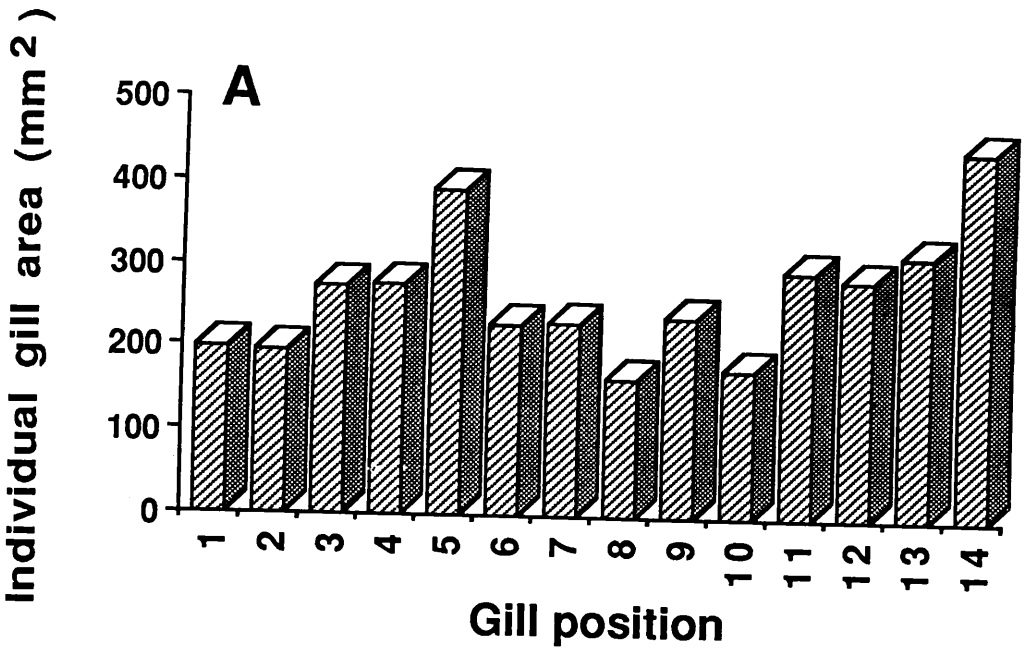
M. sarsi

Gill No.	a	b	r	n
A 1	38.73	0.04	0.26	13
A 2	50.12	0.06	0.41	
A 3	53.70	0.10	0.55	
A 4	56.23	0.13	0.64	
A 5	64.57	0.09	0.60	
A 6	45.71	0.11	0.80	
A 7	43.65	0.16	0.74	
A 8	36.31	0.15	0.70	
A 9	42.66	0.12	0.76	
A 10	39.81	0.11	0.56	
P 1	50.12	0.11	0.62	
P 2	64.57	0.03	0.20	
P 3	52.48	0.10	0.61	
P 4	69.18	0.07	0.42	

Fig. 3.9

Histograms showing the variation between the gill area (A) and the total number of lamellae (B) of each individual gill in one of the branchial chambers of *Munida rugosa*. The gills are numbered from anterior to posterior. For further details see text.

(Note: the numbers 1-10 = Arthrobranchs A1-A10; 11-14 = Pleurobranchs P1-P4


A
Individual gill area (mm 2)
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Gill position

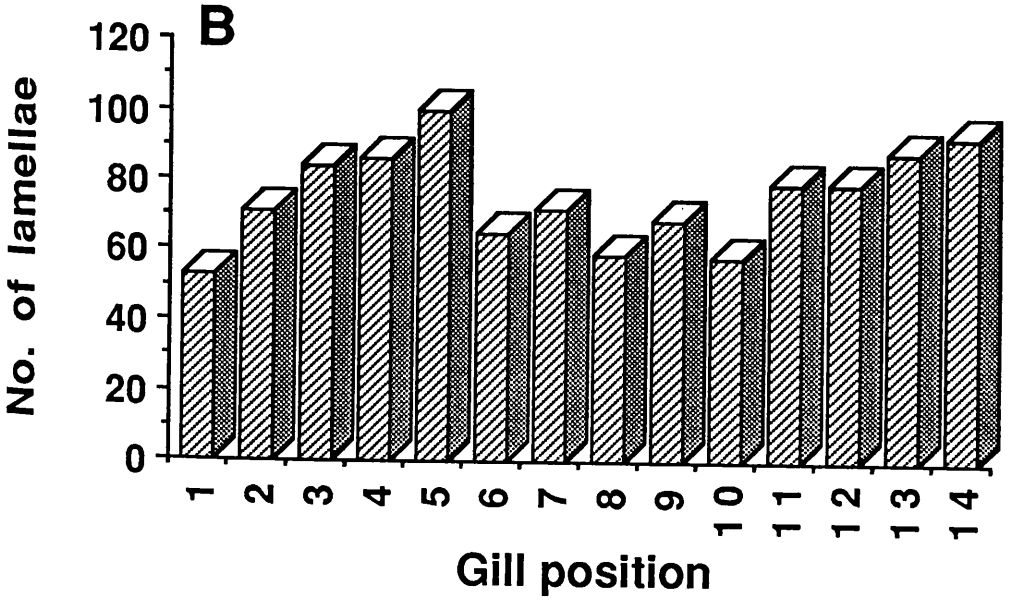
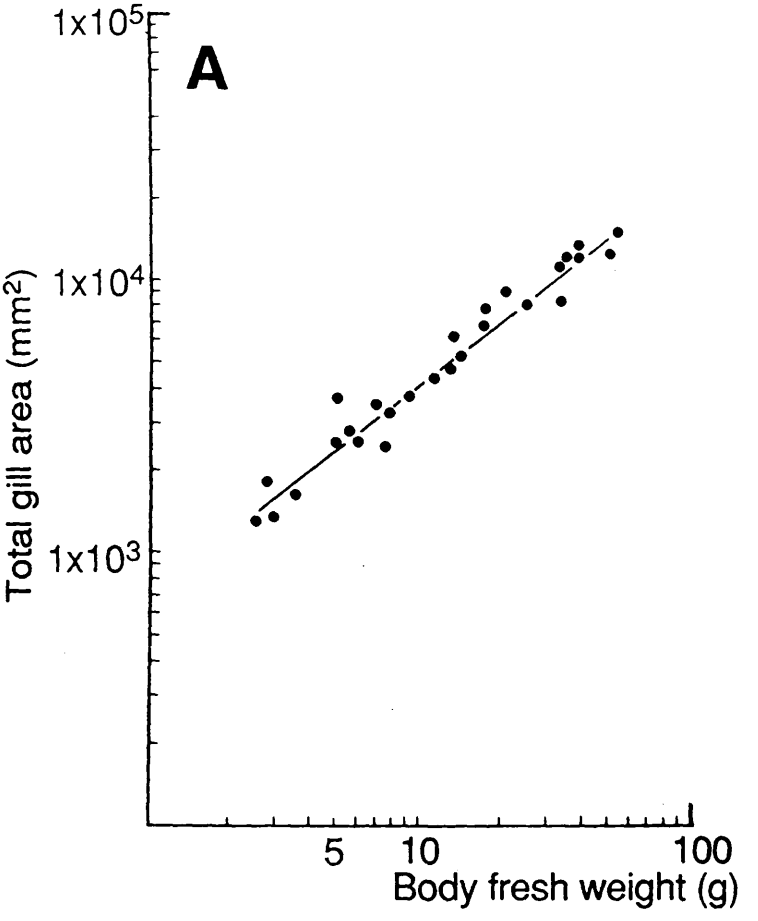

B
No. of lamellae
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Gill position

Fig. 3.10

The relationships between total gill area and body fresh weight (A) and weight specific gill area and body fresh weight (B) of *M. rugosa*.


A
1x10^5
1x10^4
1x10^3
Total gill area (mm^2)
5
10
100
Body fresh weight (g)

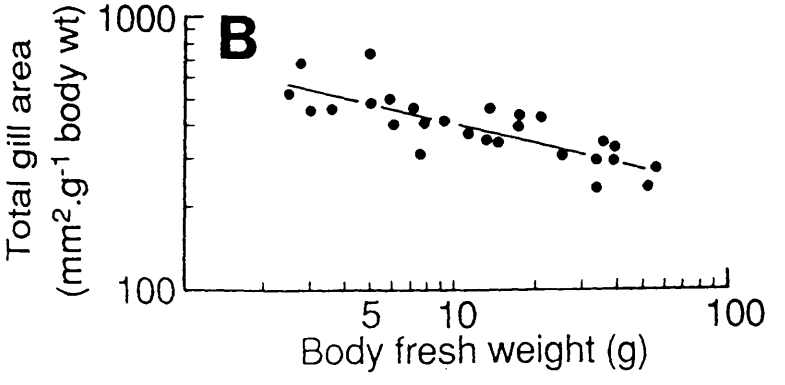

B
1000
100
Total gill area
(mm^2.g^-1 body wt)
5
10
100
Body fresh weight (g)

Fig. 3.11

The relationship between the total number of the lamellae and body fresh weight of *M. rugosa*.

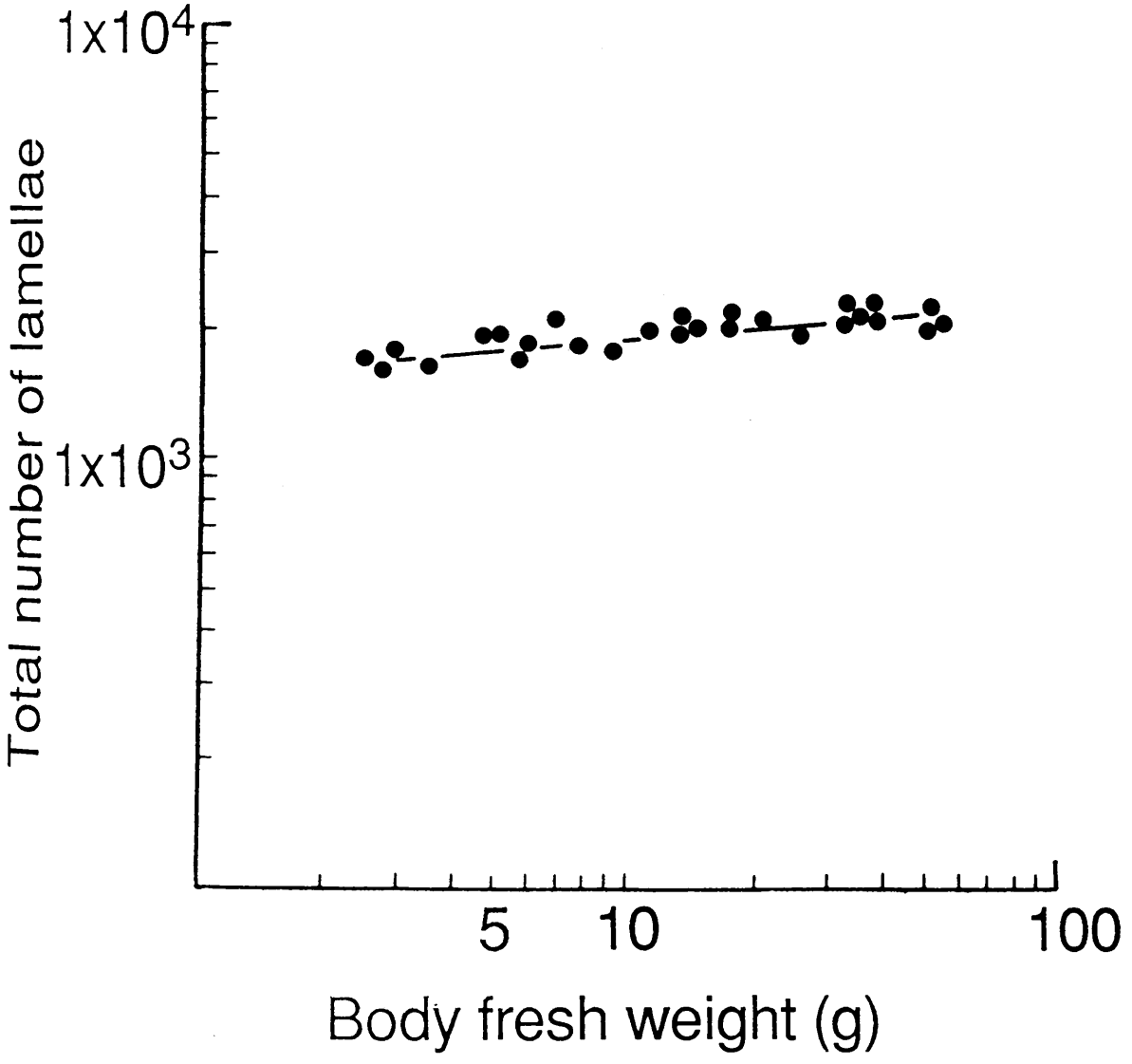

1x10^4
1x10^3
Total number of lamellae
5
10
100
Body fresh weight (g)

Table 3.1.

Regression coefficients for the relationship between total gill area ($mm^2.g^{-1}$) and fresh body weight of *M. rugosa* from the two sites and for *M. sarsi*. a = intercept and b = slope of the regression equations fitted to these data; r = correlation coefficient; n = number of animals.

	a	b	r	n	P
Munida rugosa (shallow).	707.9	-0.228	-0.798	27	<0.05
Munida rugosa (deep).	758.6	-0.296	-0.966	5	<0.05
Munida sarsi.	539.5	-0.255	-0.879	5	<0.05

Table 3.2.

Regression coefficients for the relationship between the total number of gill lamellae and fresh body weight. a = intercept and b = slope of the regression lines fitted to these data; r = correlation coefficient; n = the number of animals; * = significant at 1% level.

	a	b	r	n	p
Munida rugosa (shallow).	1528	0.099	0.517	27	<0.05
Munida rugosa (deep).	1493	0.101	0.928	5	<0.05
Munida sarsi.	1589	0.054	0.476	13	<0.05*

three sets of data.

Analyses of covariance of the relationship between individual gill areas and body weight for *M. rugosa* from both depths and for *M. sarsi* indicated that, although there was no significant difference between the slopes of the lines fitted to these data, there was, however, a significant difference ($P<0.01$) between the elevations of the lines for the fourteen gills.

Covariance analyses of the relationships between the lamellar number of the individual gills and body weight of *M. rugosa* from the shallow site indicated that there was no significant difference between the slopes and the elevations of the regression lines fitted to these data (see Table 3.2). However, the regression line for arthrobranch A1 had a slightly different slope than those for the rest of the gills. Similarly, the analysis for *M. rugosa* from deeper water and for *M. sarsi* also showed no significant difference between the regression lines in their slopes and elevations.

3.3.4. Ventilation

As in nearly all decapods, ventilation of the branchial chambers is brought about by the complex movements of the scaphognathites (Fig. 3.1B), which are the exopods of the second maxillae (McMahon & Wilkens, 1983). The beating action of the scaphognathites draws the inhalant current into the branchial chamber where it flows over the gills before been forced out of the chambers via the exhalant openings on either side of the mouth.

The scaphognathite movements could be seen more clearly after the second and third maxillipeds had been removed. The scaphognathite action was similar to that observed in other decapods (Wilkens, 1976) i.e. the scaphognathite beats in a complex sinusoidal motion. Although the general observable movement consists of up and down strokes, the exact action of the

scaphognathite was seen more clearly when part of the branchiostegite was also removed to expose the posterior end of the blade.

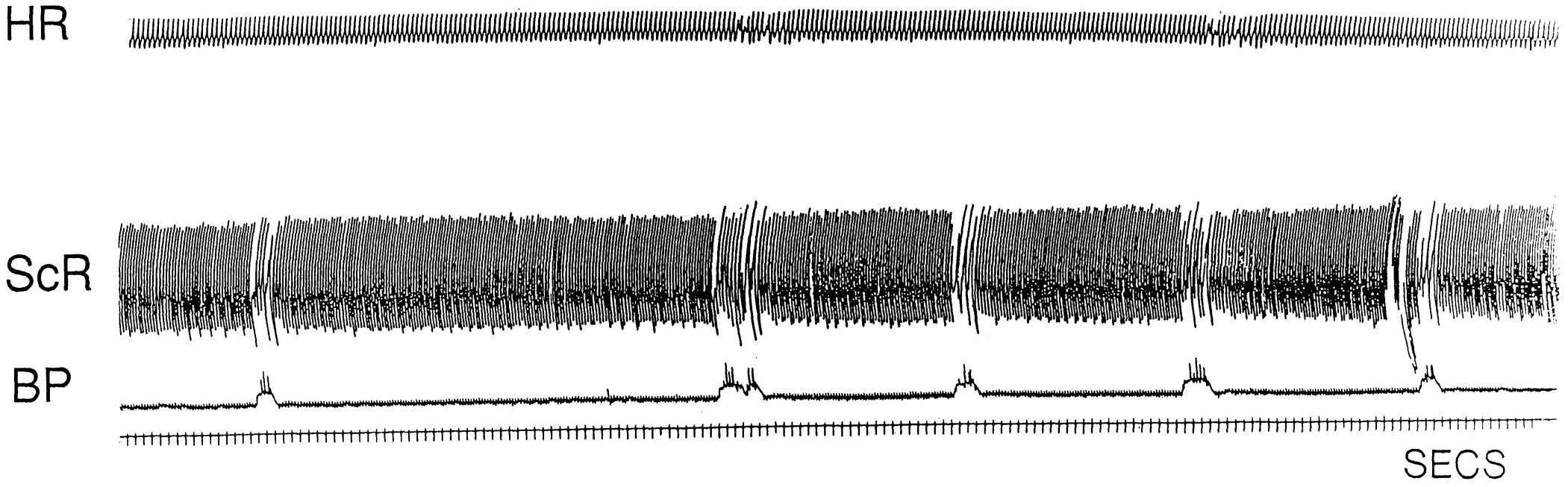
In both species of *Munida*, water enters each branchial chamber posteriorly as the inhalant current and leaves anteriorly after passing between the gill lamellae. Observations using diluted ink indicated that the main site of water entry into the branchial chamber was at the posterior region, whereas the lateral and anterior regions were much less important. A stream of exhalant water was observed from the openings near the mouth. Reversals of the current, however, were not clearly observable using this method. The ventilatory currents were mainly forward and the pressure inside the branchial chamber was negative. Very weak positive pressures were recorded, however, and these were coincident with a transitory change in the direction of the beat of the scaphognathites (Fig. 3.12). It is possible that the failure to record significant changes in pressure within the branchial chambers during periods of reversal may have been due to the fact that the branchiostegites do not fit closely around the bases of the walking legs as in many Brachyura. The pressure recordings obtained indicated, however, that the frequency of reversals in *M. rugosa* was approximately 1-2.min^{-1} and they were of limited duration (2.5-30 sec). In disturbed animals, however, the frequency of reversals normally increased in response to the presence of particulate matter.

The ventilatory pattern of *Munida sarsi* was similar to that of *M. rugosa*. Periods of respiratory pausing were also recorded in *M. sarsi* and were of similar frequency and duration. Periods of reversed beating of the scaphognathites appeared to occur even less frequently in *M. sarsi* than in *M. rugosa*. Even when particulate matter was added to the water or when the animal was disturbed, this often failed to induce reversals.

The highest rates of beating of the scaphognathites recorded were over 280 beats. min^{-1}. Such high rates were recorded mainly in animals which had been

Fig. 3.12

Recordings of the heart beat (HR) and right scaphognathite beat (ScR) from an individual male *M. rugosa* (25 g fresh weight) made using the impedance technique. Concurrent recordings of the pressure (Bp) within the right branchial chamber are also shown. The recordings were carried out at 10^{O}C.


HR
ScR
BP
SECS

stressed by electrode implantation and handling. This high ventilation rate, together with heart rate, decreased over approximately the next 12h (Fig. 3.13). In completely quiescent animals which had remained undisturbed for many hours, scaphognathite rates of < 10 beats. min^{-1} were recorded. In inactive animals, the scaphognathite rate was always higher than the heart rate. There was also some evidence of a diurnal rhythm of both the heart and scaphognathite rates, for during the night the rates recorded were frequently higher than during the day. Simultaneous recordings of both scaphognathites showed that, in the majority of cases, there was no significant difference between their rates of beating (t-test) (Fig. 3.14). Beating of the scaphognathites was normally closely coupled during periods of activity or during pausing. Occasionally, however, it was possible to record differences between the activity of the two scaphognathites. In addition, the activity of the scaphognathites and the heart was also closely coupled, with changes in heart rate invariably accompanying changes in scaphognathite rate (Fig. 3.13). This was most clearly demonstrated during 'pausing' behaviour. Respiratory pausing was characterized by periods of apnoea, during which scaphognathite activity ceased, and was accompanied by a pronounced bradycardia and sometimes by complete cardiac arrest. The duration of these respiratory pauses was very variable and sometimes lasted for only a few seconds although occasionally much longer pauses, lasting up to 8 minutes, were recorded. Ventilatory pausing was normally synchronous between the two scaphognathies, but again it was sometimes observed that the duration of the pause was not always exactly the same in both branchial chambers i.e. one scaphognathite would recommence pumping activity before the other.

Fig. 3.13

Recordings of the rates of the heart beat (HR) and right scaphognathite (ScR) from an individual *Munida rugosa* made using the impedance technique. The recordings were made soon after electrode implantation (A) and after approximately 12 h (B) and 18 h (C). All recordings were made at 10^{o}C.

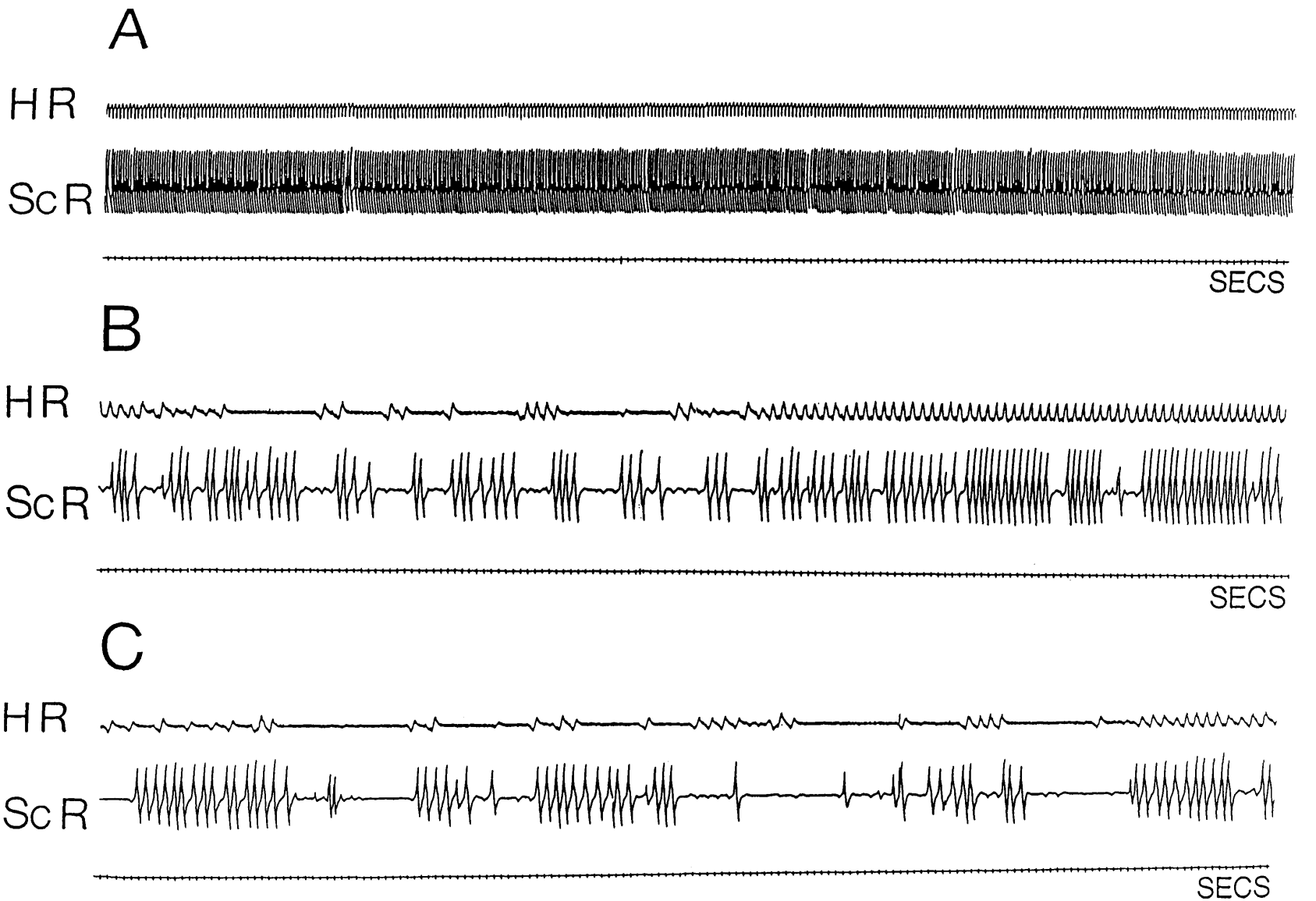
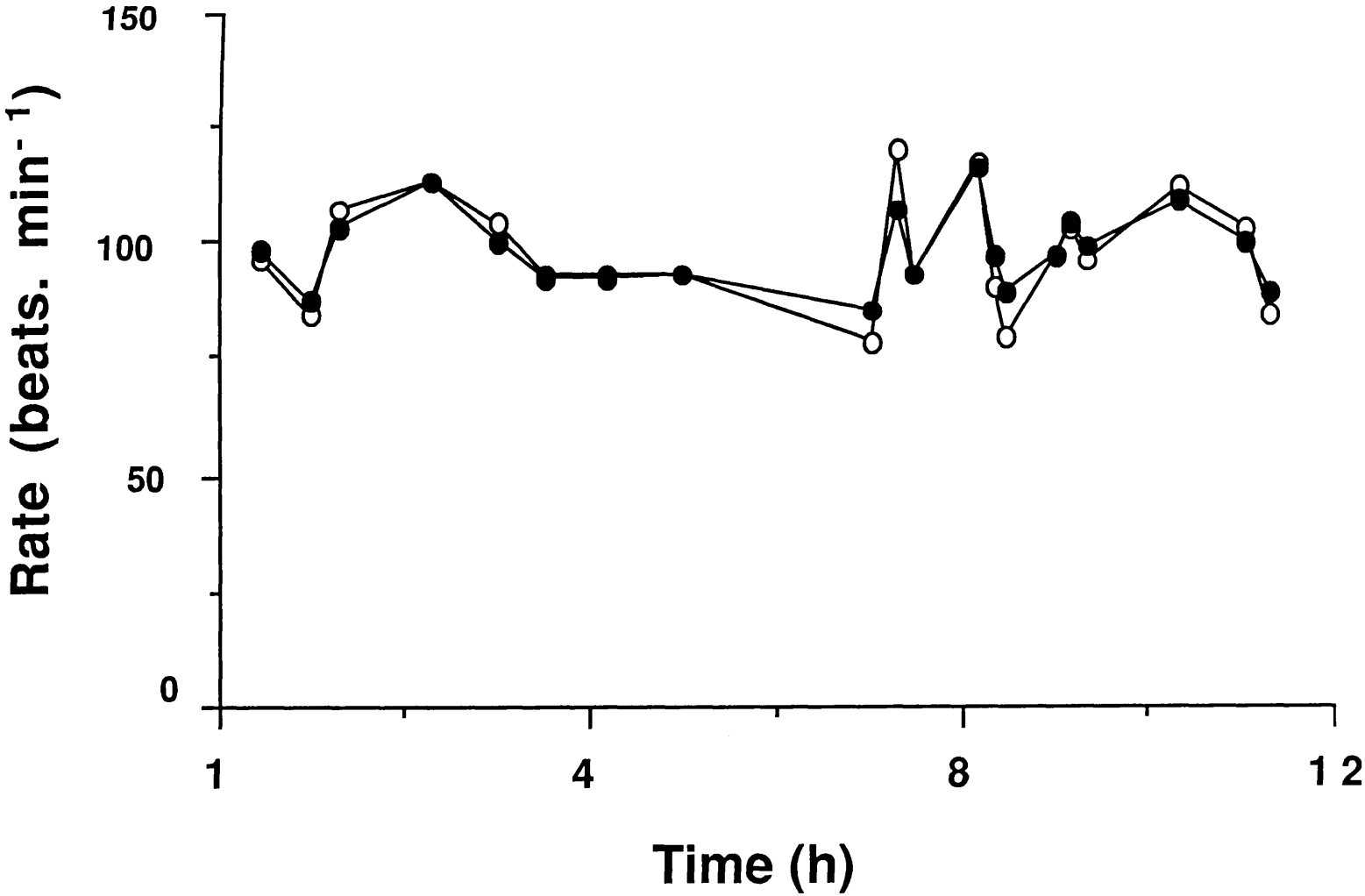

A
HR
ScR
SECS
B
HR
ScR
SECS
C
HR
ScR
SECS

Fig. 3.14

Simultaneous recordings of the rate of beating of the left (○), and right (●) scaphognathites of *Munida rugosa*. The recordings were made at 10^{o}C.

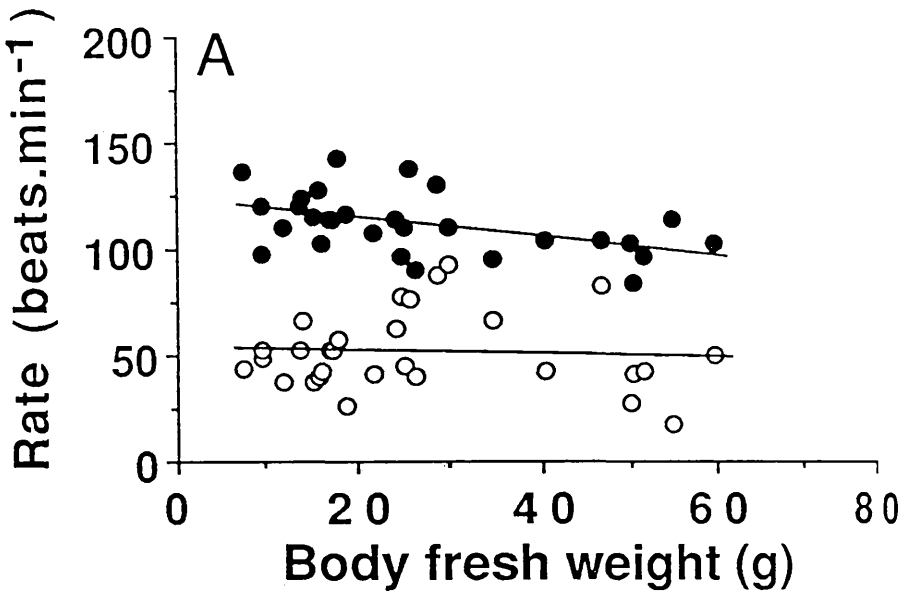

150
100
50
0
Rate (beats. min-1)
1
4
8
12
Time (h)

3.3.5. The effect of disturbance on cardiac and ventilatory activity

The respiratory pauses described above were only recorded in quiescent animals and appeared to occur spontaneously. However, sudden disturbance of the animals also resulted in brief periods of respiratory pausing. For example, any sudden visual or vibrational disturbance invariably resulted in a sharp decrease in heart and scaphognathite rates and sometimes resulted in complete cardiac and ventilatory arrest. These disturbance-induced pauses were of short duration, however, and usually lasted for only a few seconds. It was also noted that during these pauses the heart would resume beating before the scaphognathites.

3.3.6. Body weight relationships for heart rate and scaphognathite rate

The relationships between heart rate and body weight in both quiescent and active *M. rugosa* are plotted in Fig. 3.15A. It was interesting to note that although there was a significant correlation between heart rate and body weight ($r = 0.473$; $n = 29$; $P < 0.05$) for active animals, this relationship was not significant when data for quiescent animals were analysed ($r = 0.071$; $n = 29$; $P > 0.05$). Similarly, no significant correlation was found between scaphognathite rate and body weight in either resting or active animals (Fig. 3.15B).


A
Rate (beats.min-1)
200
150
100
50
0
0
20
40
60
80
Body fresh weight (g)

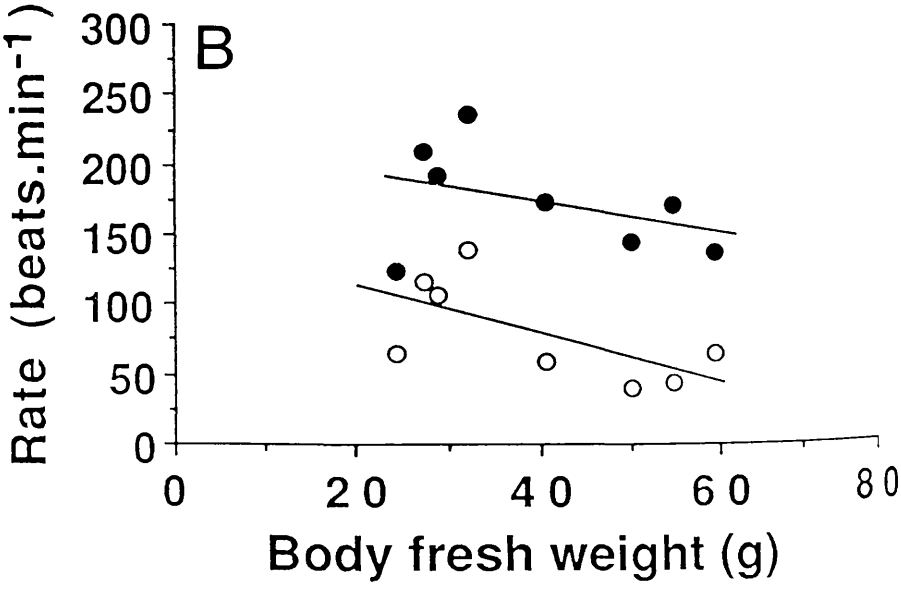

B
Rate (beats.min-1)
300
250
200
150
100
50
0
0
20
40
60
80
Body fresh weight (g)

Fig. 3.15

The relationships between heart rate and fresh body weight (A) and scaphognathite rate and fresh body weight (B) of active (●) and inactive (○) *Munida rugosa*. The recordings were made at 10° C.

3.4. DISCUSSION

3.4.1. Branchial chamber morphology

In the two *Munida* species studied, the main opening to each branchial chamber is along the posterior edge of the branchiostegite. In contrast, in the closely related *Galathea squamifera* most water is admitted at the bases of the chelae (Pike, 1947). In both genera the respiratory current is mainly in a forward direction, though weak reversals may occur in *G. squamifera* (Zimmerman, 1913) and in *Munida* spp.

The branchial chamber morphology of the Galatheidae conforms to the general decapod pattern. In *M. rugosa*, the lamellae at both ends of the gill have smaller areas than those in the middle region where the respiratory current is apparently strongest (Scammell & Hughes, 1982). This is also a reflection of the importance of the middle part of each gill in terms of gas exchange. In addition, the lamellae are arranged with their heart-shaped edge directed towards the branchiostegite and to face the ventilatory current. The morphological function of this arrangement is perhaps associated with channelling the water stream (Borradaile, 1922). In *Carcinus maenas* the position of the largest gills tends to reflect the region where maximum water flow has been observed (Scammell & Hughes, 1982). In *M. rugosa* and *M.sarsi*, the posterior pleurobranchs are the largest gills. The arthrobranchs situated midway along the thorax (gills 4 & 5) are also relatively large gills which, if the views of Scammell & Hughes (1982) are correct, might suggest that this region of the branchiostegite also admits a significant amount of respiratory water. The smallest gills (A1 & A2) were situated in the exhalant path.

The number of the gills in most, if not all, the Galatheidae and Porcellanidae is fourteen on each side and, in general, podobranchiae are absent (Henderson, 1888). Generally speaking, the number of the gills can be correlated with

lifestyle. In the Paguridae (Anomura), there is a correlation between gill number and increasing terrestrialness; there are fewer gills in the most terrestrial species (Edney, 1960; Wolvekamp & Waterman, 1960) as is also the case in Brachyura (Gray, 1957; Hughes, 1982; Scammell & Hughes, 1982).

Within the Galatheidae there is also variation in the number of branchial epipods. *G. squamifera* has five pairs of epipods (on the first and third maxillipeds, and pereiopods 1-3) (Calman, 1909). *Munida rugosa* has three pairs (on the first and third maxillipeds and the chelipeds). *Munida bellior* and *M. eleganti* have three pairs of epipods (Baba, 1977). *M. sarsi* has two pairs (on the first and third maxillipeds) (present study). *G. strigosa* has one pair (on the third maxillipeds). *Munidopsis lentigo*, *M. nitida*, *M. pilosa*, *M. scabra* and *M. verrili* all lack epipods on the pereiopods, whereas *M. eringana*, *M. ciliata* and *M. latriostris* have them on the chelipeds (Williams & Van Dover, 1983). Epipods are completely absent in the Porcellanidae (Pike, 1947).

In the brachyuran crabs the epipods are well developed. Compared with the Anomura, the epipods are larger and, by their sweeping action, appear to be used to clean the gills (Borradaile, 1922). For example, in the shore crab, *Carcinus maenas* there are three pairs of epipods one pair on each of the first, second & third maxillipeds. The functional significance of the epipods in the Galatheidae is unclear because of their rudimentary structure. It is to be noted, however, that the epipods of the third maxillipeds are relatively larger, more setose and reach to the first and second arthrobranchs. They perhaps help in cleaning these small, anterior gills. In the case of *Munida sarsi*, these epipods are larger and more setose than in *Munida rugosa*. In *Munida* species, the problem of cleaning the gills is mainly solved by the activity of the fifth pereiopods which, being small, thin, setose and chelated, are well adapted for this purpose (Fig. 2.19 A,B Chapter 2). They were occasionally seen removing detritus from inside the branchial chamber, especially when the animal was

placed in a tank with sediment, or when any particulate matter such as milk or ink was added to the water. In addition, the plumose setae along the branchiostegite edges also play a role in preventing particulate matter from entering the branchial chambers. These setae are more dense postero-laterally where most water is admitted to the branchial chambers (Fig. 3.2A).

Active, aquatic anomurans and brachyurans have larger gill areas than inactive or semi-terrestrial species (Gray, 1957; Hughes 1982; Scammell & Hughes, 1982; Greenaway, 1984). The respiratory surface area and the thickness of the lamellar cuticle are informative indicators of respiratory function. The efficiency of gas transfer could be facilitated by having a large gill area, and a thin barrier between the water and the blood. The thickness of the cuticle governs diffusion distances across the gills in crustaceans. The cuticle thickness in *Munida rugosa* can be regarded as being thin (see below). This species is characterised by having a comparatively small gill area.

A number of studies have been made of the phyllobranchiate gills of some decapod crustaceans (squat lobsters and crabs) e.g. *Galathea squamifera* (Pike, 1947), *Carcinus maenas* (Taylor & Butler, 1978; Hughes, 1982), *Holthuisana transversa* (Taylor & Greenaway, 1984), *Eriocheir sinesis* (Barra *et al.*, 1983) and of the trichobranchiate gills of marine and fresh water crayfishes e.g. *Astacus pallipes* (Fisher, 1972), *Procambarus clarkii* (Burggren *et al.*, 1974; Scammell & Hughes, 1982), *Panulirus argus* (Filshie & Smith, 1980).

Histological preparation is likely to modify cuticular thickness so that the measurements carried out can provide only an approximation of the true diffusion distance. TEM studies indicated that the cuticle was composed of an epicuticle and a stratified endocuticle. In *Munida rugosa*, the diffusion barrier (cuticular thickness of the gill) was approxmately 2 μm which is similar to the values obtained for the marine crayfish *Panulirus argus* (2 μm) (Filshie &

Smith, 1980), and for the fresh water crayfishes (*Procambarus clarkii* (1.5-2.5 μm) (Burggren *et al.*, 1974) and *Astacus pallipes* (1.5-2.5 μm) (Fisher, 1972). The value is higher than 0.78μm reported for the fresh water African crab *Potamon niloticus* (Maina, 1990), and only slightly higher than that reported for the brown shrimp, *Penaeus aztecus* (1.0-1.5μm). This value may also be compared with that of shore crab *Carcinus maenas* (5-6 μm) (Taylor & Butler, 1978; Hughes, 1982).

Generally, invertebrate gills are similar to fish gills in that the surface is divided into two epithelia joined together at intervals by pillar cells or 'trabeculae' (Pike, 1947; Hughes, 1982). The detailed morphology of the surface of the gill of *M. rugosa* is similar to that of *Galathea squamifera* (Pike, 1947) and some other decapods. Mean diffusion distances in *M. rugosa* are comparable with those of fish and mammals (0.36-2.5 μm) (Gordon, 1972). The fish with the largest gill surface areas tend to have the thinnest cuticle which gives a high diffusing capacity (Hughes, 1982), but data of comparable detail do not appear to be available for decapod Crustacea. Nevertheless, the thickness of the cuticle will be important in affecting the diffusion capacity of their respiratory system (Hughes, 1982).

The mean values for the gill areas of *Munida rugosa* and *M. sarsi* were found to be smaller than in the majority of aquatic decpods for which data are available (Gray, 1957; Hughes, 1982, 1983; Scammell & Hughes, 1982) (see Table 3.5).

The values of the regression coefficients (slopes) of the relationships between total gill area (mm^2) and body weight for *M. rugosa* from both depths and for *M. sarsi* (b = 0.77, 0.71, and 0.75 respectively) were within the range of values obtained for both British and American species of decapods (Hughes, 1982 & 1983). The slopes of the regression equation relating lamellar number to body size (b = 0.1 and 0.05 for *M. rugosa* and *M. sarsi* respectively), were

Table 3.5.

Interspecific comparison of weight specific gill area ($mm^2. g^{-1}$) of some decapod Crustacea. The slopes (b) of the regression equations describing the relationship between gill area ($mm^2.g^{-1}$) and fresh body weight are also given where available. The values for all species, with the exception of *Munida rugosa* and *M. sarsi*, were taken from Hughes (1982, 1983).

	Mean gill area ($mm^2.g^{-1}$)	Slope (*b*)
Callinecties sapidus.	1260	0.94
Menippe mercenaria.	771	0.82
Carcinus maenas.	765	0.96
Libinia dubia.	736	0.67
Libinia emarginata.	550	0.78
Uca minax.	496	0.54
Pagurus bernhardus.	466	1.00
Cancer pagurus.	423	0.93
Munida rugosa (shallow).	415	0.77
Maia squinado.	395	0.99
Austropotamobius pallipes.	380	0.54
Munida rugosa (deep).	348	0.70
Munida sarsi	301	0.75
Ocypode quadrata.	295	0.79
Homarus gammarus.	158	0.57
Nephrops norvegicus.	130	0.61

also within the range for other decapods (Hughes, 1982). It is to be noted that the values obtained for the *Munida* species were closer to those of the macrurous species which were characterised by having lower values for the slope 'b' than the brachyurous species (Hughes, 1982).

It has been claimed that increase in gill area in decapod Crustacea is related more to an increase in lamellar area than to an increase in the number of gill lamellae (Hughes, 1982, 1983; Greenway, 1984). In the present study, the correlation between the gill area and fresh body weight was found to be similar to that in other decapods. However, the correlation between the total number of lamellae and body weight indicated a significant ($P<0.01$) increase in lamellar number with increasing body weight. This was also found to be true for *Munida sarsi*. This means that the increase in the total gill area with increasing body size is partially dependent on the increase in the number of lamellae.

There was a considerable similarity between the two *Munida* species examined in this study in terms of the relationships between either the total gill area; the total lamellae number with increasing body weight or the data for the individual gills. In addition, the relative position of each gill inside the branchial chamber was also similar.

The respiratory surface area can be a limiting factor for oxygen uptake when oxygen demand is high (Steen, 1971). The thin epithelia and the small gill areas of the species studied may be associated with inactivity and a correspondingly low demand for oxygen (see Chapter 4). Aquarium observations indicate that *M. rugosa* (and also *M. sarsi*) are normally rather inactive. Both species will remain almost motionless for long periods and when locomoter activity occurs, movements are slow unless an animal is disturbed.

The gill areas of 8 decapod species (4 brachyurans, 3 macrurous species and

an anomuran) studied by Scammell & Hughes (1982) ranged between 300-900 mm^2 per gram of body weight . The gill area of their anomuran, *Pagurus bernhardus*, which inhabits molluscan shells, was similar to the gill area of the two *Munida* species. The gill areas of these *Munida* species were also similar to those of the relatively inactive brachyuran *Maia squinado*. There may, however, be dangers in making comparisons with non-anomurans since the weight specific gill areas of *Munida* are similar to those of the crayfish *Astacus fluviatilis*, which Scammell & Hughes (1982) regarded as an active species, and greater than those of the fairly active astacidean *Nephrops norvegicus* (Scammell & Hughes, 1982) (Table 3.5). Therefore, the value of an interspecific comparison is questionable.

3.4.2. Ventilation and cardiac activity

In *M. rugosa* the movement of the scaphognathite was such that the anterior and the posterior edges alternate their up and down strokes while the action of the middle part was more complex and difficult to assess by visual observations. The movement patterns of different regions of individual scaphognathites have been analysed in *Carcinus maenas* (Hughes, Knights & Scammell, 1969; Young, 1975; Mercier & Wilkens, 1984) and in *Crangon crangon* (Dyer & Uglow, 1978). Impedance electrodes were used to monitor the movement of different regions of the scaphognathite. Dyer & Uglow (1978) have concluded that, in *C. crangon*, a beat comprises equally effective depression/levation phases but the timing sequence of the movements differs slightly from that of *Carcinus maenas*. From the analysis of the forward pumping of *Carcinus maenas*, the axial portion of the scaphognathite moves up and down sinusoidally whereas the anterior and the posterior tips of the blade move in trapezoidal fashion (Young, 1975). The analysis of the scaphognathite movements during forward and reversed beating in the lobster *Homarus americanus* have been studied by Wilkens & McMahon (1972) using photographic analysis. They concluded that

during forward pumping the scaphognathite first closes the posterior end of the pre-branchial chamber followed by the remainder of the blade to drive the trapped water out. Reversed pumping is effected by a reverse angle of attack of the scaphognathites during the upstroke and down strokes of the beat (Wilkens & McMahon, 1972; Wilkens, 1976).

In *M. rugosa* and *M. sarsi*, as in other Anomura, the carapace is loosely fitted to the thorax and is occasionally seen to move. Therefore, the change in the hydrostatic pressure recorded inside the branchial chamber was very small. Bridges (1976), using the same method of a cannula planted close to scaphognathite, successfully recorded branchial pressure changes in *Carcinus meanas*, *Corystes cassivelaunus*, *Atelecyclus rotundatus* and *Homarus gammarus*, but for *Galathea strigosa*, Bridges indicated that the general changes in the hydrostatic pressure within the chamber were beyond the resolution of this method. This is probably the main reason for the difficulties experienced in obtaining good pressure recordings during the present study.

The rhythmic motor output of ventilation in hermit crabs and lobsters appears to be controlled by a pair of neurons, one in each half of the subesophageal ganglion (Mendelson, 1971). The heart beat in decapods is generated by the endogenously active cardiac ganglion located within the dorsal wall of the heart and it is under the control of the cardio- regulatory nerves that originate from the suboesophageal (Astacidea) or thoracic (Brachyura) ganglion (reviewed by Wilkens, 1976).

Quiescent *Munida rugosa* have much in common with other decapods in respect of the correlations between cardiac and ventilatory activity (Wilkens & Young, 1975; Wilkens, 1976; McMahon & Wilkens, 1977; Cumberlidge & Uglow, 1977; McDonald, McMahon & Wood, 1977; Coyer, 1979; Rios, 1979; Bradford & Taylor, 1982; Taylor, 1984). The cessation of heart beat and

scaphognathite beat following applied stimuli was also found in other species (Larimer, 1964; Ashby & Larimer, 1965; Mislin, 1966; Blatchford, 1971). The scaphognathite beat rate was found to be faster than the heart rate under resting conditions but, when animals were disturbed, the heart rate increased to that of the scaphognathite and a close coupling between the two systems was demonstrated. Ventilatory activity was regular and there were alternating periods of apnoea and active pumping. The present observations on cardiac and ventilatory activity in *M. rugosa* are very similar to those made by Rios (1979) for this species.

In *M. rugosa*, the short periods during which the rate of scaphognathite beat was increased were not connected with reversals of the respiratory current. During reversals, the scaphognathites were found to beat more slowly than during forward pumping. This contrasts with the transitory increase in rates recorded during reversals in some other decapods (Young, 1975; Cumberlidge & Uglow, 1977; Dyer & Uglow, 1978).

The predominant forward pumping found in the majority of decapods allows counter-current gas exchange between haemolymph and branchial water (McMahon & Wilkens, 1983). However, because the carapace is rather 'loosely-fitted' in lobsters and crayfishes, it has been suggested that the counter-current cannot be as efficient as in brachyurans (McMahon & Wilkens, 1983), but this remains to be clearly established.

Reversals of the respiratory current have been recorded in a wide variety of decapod Crustacea e.g. *Corystes cassivilaunus* (Garstang, 1896), *Galathea squamifera* (Pike, 1947), *Homarus americanus* (McMahon & Wilkens, 1972; Wilkens & Young, 1975), *Cancer pagurus*, *Liocarcinus puber*, *Corystes cassivelaunus*, and *Homarus gammarus* (Arudparagasam & Naylor, 1966), *Cancer magister* (McDonald *et al.*, 1977), *Carcinus maenas*, (Arudpragasam & Naylor, 1964; Hughes *et al.*, 1969; Newell *et al.*, 1972; Young, 1975; Berlind,

1977; Cumberlidge & Uglow, 1977; Taylor *et al.*, 1973; Taylor & Butler, 1978), *Cancer productus* (McMahon & Wilkens, 1977), *Crangon crangon* (Dyer & Uglow, 1978), *Astacus pallipes* (Massabuau *et al.*, 1980), *Atelecyclus rotundatus*, (Taylor, 1984). It is interesting to note, however, that in a few species, e.g. the crayfish *Orconectes virilis* (McMahon *et.al.*, 1974) and the prawn, *Palaemon adspersus* (Hagerman & Uglow, 1979), reversals have not been recorded.

The role of reversals of respiratory current in decapods from different habitats has been reviewed by Wilkens (1976). Reversals and ventilatory pauses could be the result of modulation of a pre-programmed motor output from the CNS (McMahon & Wilkens, 1977; McDonald *et al.*, 1977). Reversals may also be a general response to all unusual stimuli to the respiratory system (Wilkens & McMahon, 1972). As in other decapod crustaceans, reversals of the ventilatory currents in *M. rugosa* are perhaps helpful in cleaning particulate matter from the gills since an increase in reversal frequency was observed when particulate matter was added to the water. Reversal of the respiratory current may also have a respiratory role. Reversals were observed to be more frequent in recently disturbed *M. rugosa* which were placed in fresh, well-aerated sea water. As the animals gradually recovered from this disturbance, the frequency of the reversals slowly diminished with time. There is no clear explanation for the occurrence of reversals under these conditions. In these animals, the ventilatory rate is likely to be high following disturbance and a correlation between ventilatory rate and reversal frequency has been seen in other decapods (Arudpragasam & Naylor, 1964, 1966; Hughes, Knights & Scammell, 1969; McDonald, *et al.*, 1977; Taylor, 1977; Mercier & Wilkens, 1984).

In *Crangon crangon*, it has been observed that there was a connection between the rhythmic increase in the rate of beating of the scaphognathites and the occurrence of reversals (Dyer & Uglow, 1978). That is, during reversals scaphognathite rate was much higher. In *Munida rugosa*, however, this increase

in the scaphognathite rate during reversals was not observed.

In disturbed animals, the elevated rates of ventilation associated with tachycardia serve to increase the oxygen supply to the respiring tissues. The relationship between the heart and respiratory system is reflected in their common control by the CNS (reviewed by Wilkens, 1976). Spontaneous cardiac activity is co-ordinated with ventilatory activity in that periods of apnoea coincide with periods of bradycardia or cardiac arrest. Similar co-ordination has been observed in many other decapods e.g. *Homarus americanus* (Wilkens & Young, 1975), *Cancer magister* (McDonald, *et al.*, 1977), *Carcinus maenas* (Cumberlidge & Uglow, 1977), and *Nephrops norvegicus* (Young, 1978). Periods of apnoea and cardiac arrest lasting for several minutes have been observed only in quiescent decapods; the duration of these pauses appears to be greatest among some burying species (Ansell, 1973; McMahon & Wilkens, 1977; Bradford & Taylor 1982; Taylor, 1984). In *Munida rugosa*, as in many decapods e.g. *Cancer magister* (McMahon & Wilkens, 1972; Florey & Kriebel, 1974; McDonald *et al.*, 1977) and *Cancer productus* (Florey & Kriebel, 1973; McMahon & Wilkens, 1977), sudden cessation of heart and scaphognathite activity was also observed in disturbed animals, but the duration and frequency of these pauses was generally much briefer than when they occur in quiescent animals. A distinction should be made, however, between the brief cardiac and ventilatory pauses which result from disturbance, for these may last for only a few seconds and are often followed by a transient increase in the heart and scaphognathite rates, and those which occur in quiesent animals which are of much longer duration. Pauses may have a defensive role since stopping the respiratory current, may represent a response to reduce the risk of detection by a potential predator (Wilkens, 1976).

Spontaneous pausing in quiescent *M. rugosa* is often of much longer duration (1-8 min.). Rios (1979) also noted the occurrence of periods of cardiac and

ventilatory pausing during his study of *M. rugosa*. Although he gives no values for the average duration of these pauses, the traces which he presents indicate that they were of short durtion. In the majority of species studied, the average pause duration is between 5 and 20 minutes (Taylor, 1984). It is now generally accepted that this type of pausing occurs to enable the animal to make a saving in metabolic energy during periods of inactivity by reducing the energy cost of pumping water and blood (McDonald *et al.*, 1977; McMahon & Wilkens, 1977; Burnett & Bridges, 1981; Bradford & Taylor, 1982; Taylor, 1984).

McMahon & Wilkens, (1972) have recorded in *Homarus americanus* that, following a pause, ventilatory activity was increased over the pre-pause levels. In the burrowing crab, *Atelecyclus rotundatus* there was a brief period of increased scaphognathite activity before and after the pause (Taylor, 1984). In *M. rugosa*, the ventilatory rate was higher before the onset of a pause but there was no corresponding increase when ventilation recommenced. Following the disturbance-induced pauses, however, an increase in rate was recorded.

The neural control of the scaphognathite function has been reviewed by McMahon & Wilkens (1983). Several crustaceans show a similar pattern of bilateral coordination between scaphognathites in which phase constancy alternates with periods of drift during which the scaphognathites demonstrate the independence of their respective pattern generators (Wilkens & Young, 1975; Young & Coyer, 1979). Different types of stimuli (optical, mechanical, chemical, and osmotic) are known to alter scaphognathite rhythms (Arudpragasam & Naylor, 1966; Larimer, 1964; Berlind, 1977; Wilkens and McMahon, 1972; Taylor & Butler, 1973; Florey & Kriebel, 1974; Taylor, 1977). The most commonly seen response is a pronounced reduction in scaphognathite rate and sometimes a complete cessation of scaphognathite activity accompanied by cardiac arrest (McDonald *et al.*, 1977; Young & Coyer, 1979).

CHAPTER 4. RESPIRATION

4.1. INTRODUCTION

There have been numerous studies of the respiratory physiology of a wide range of decapod crustaceans from a variety of habitats (reviewed by Herreid, 1980; McMahon & Wilkens, 1983). Compared with the vast literature on the respiratory physiology of the Brachyura, however, the information available for the Anomura is restricted to only a few species e.g. *Pagurus hirsutiusculus*, (Young, 1963), *Diogenes bicristimanus* (Sarojini & Nagabhushanam, 1968), *Pleuroncodes planipes* (Quetin & Childress, 1976). *Pagurus bernhardus* (Shumway, 1978; Davenport *et al*, 1980; Bridges & Brand, 1980), *Clibanarius vittatus* (Wernick & Penteado, 1983), *Birgus latro* (Greenaway *et al.*, 1988; Morris *et al.*, 1988; Morris & Greenaway, 1989). Despite the wide distribution of the Galatheidae, studies of their respiratory physiology have been carried out only on *Galathea strigosa*, (Bridges & Brand, 1980), *Munida quadrispina* (Burd, 1985; Burd & Brinkhurst, 1985) and *M. rugosa* (as *M. bamffica*) (Rios, 1979).

In the previous chapter the morphology of the gills and gill area measurements in *Munida rugosa* from shallow and deep water sites and for *M. sarsi* were investigated. This chapter presents the results of a study of the respiratory physiology of *M. rugosa* and *M. sarsi*. In particular, their ability to withstand exposure to low oxygen availability and their respiratory responses to hypoxia have been investigated. In addition, the effects of temperature and feeding on oxygen consumption rates of *M. rugosa* were examined.

4.2. MATERIALS AND METHODS

M. rugosa and *M. sarsi* were obtained from the U.M.B.S. at Millport. They were caught by trawling and by creel in the Clyde Sea Area at a depth of ca 40m, but some were caught from deeper water (95-115m). The animals were transported to the Zoology Department at Glasgow University where they were maintained in a recirculating sea water aquarium at a salinity of 32‰, a temperature of 10^{o}C and exposed to a 12:12h light:dark regime. The animals were fed regularly on fresh mussels (*Mytilus edulis*) but were starved for at least two days before being used in the experiments.

Oxygen consumption was measured using closed system respirometers. No antibiotics were added to the respirometer as has been done by some other workers (e.g. Burd, 1985; Quetin & Childress, 1976) since during this study the background respiration was found to be negligible. However, to ensure that bacterial respiration remained insignificant, the system was flushed with an antiseptic solution (sodium hypochlorite) then rinsed thoroughly with distilled water between experiments.

The volume of the respirometers used varied between 80ml and 1600ml according to the size of the animal. The respirometer chambers were made of 'perspex' and were provided with a removable lid which was fixed to the chamber using screws. A rubber 'O' ring was used to ensure an effective seal between the lid and the chamber. An oxygen electrode (E5046, Radiometer, Denmark) was fitted through a rubber bung inserted into the lid of the chamber. Care was taken to ensure that there were no leaks around the electrode and that no air bubbles were trapped inside. This was achieved by sealing the respirometer while it was submerged in a tank of sea water. The oxygen electrode was connected to an oxygen meter (Stathkelvin Instruments, model 781) which was in turn connected to a pen recorder (Servogor, SE120).

The respirometer was also provided with a magnetic stirrer bar to maintain the circulation inside the chamber and to prevent stagnation of water around the oxygen electrode. Inlet and outlet taps were fitted to the respirometer and a submersible pump was used to circulate aerated sea water through the chamber from a reservoir tank. The respirometer and the reservoir tank were both placed in a thermostatted water bath. All recordings were made at $10^{o}C$ although some investigations of the effect of temperature on oxygen consumption were also made.

The oxygen electrode was calibrated using a solution of 0.01M sodium tetraborate and sodium sulphite to provide a solution having a Po_2 of zero, and against air-saturated water. This was obtained by bubbling air through distilled water at the experimental temperature. The Po_2 of the water was calculated according to the following equation:

$$Po_2 = (BP - WVP) \times 0.2095 \text{ Torr}$$

where BP is the barometric pressure and WVP is the water vapour pressure at the experimental temperature.

4.2.1. Oxygen consumption rates under normoxic conditions

Rates of oxygen consumption of *M. rugosa* and *M. sarsi* under normoxic conditions were measured in animals of differing size (fresh weight range = 4 - 52g). Recordings were made on individuals of both sexes but only animals in the intermoult stage were used. The animals were placed in the respirometer and were left undisturbed for 12 - 24 hours before the start of the experiment to allow time for acclimatization to the experimental conditions. During this period, aerated sea water was circulated continuously through the respirometer. Care was taken to avoid visual or vibrational disturbance at any time during the experiments. The former was avoided by partly covering the respirometer with a polystyrene sheet.

At the start of the experiment, the circulation of water through the respirometer was stopped so that it functioned as a closed system with the Po_2 of the water being reduced by the animal's oxygen consumption. During these recordings, the Po_2 of the water was not allowed to decline below approximately 140 Torr before the chamber was flushed through with aerated sea water to ensure that the animals were not subjected to hypoxia. Flushing of the respirometer was carried out automatically using a pump and solenoid valves (RS Components Ltd.) controlled by an automatic time switch (Sangamo). The time switch was programmed to switch on and off at 30 minute intervals. Under the control of the time switch, the circulating pump was switched on and the solenoid valves opened to allow water to circulate through the respirometer for a period of 30 min. At the end of this period the pump was switched off and the solenoid valves closed to enable the respirometer to function as a closed system for a further 30 min. This procedure enabled the rates of oxygen consumption to be monitored continuously for up to 24 h. The weight specific metabolic rate was calculated from the trace of the reduction in the Po_2 in the respirometer. As time taken (min.) for a reduction of 10 Torr.

$\dot{M}o_2$ = 10 (Torr) X α X resp.vol./1000 X 1/22.4 X 60/time X 1/wt

where $\dot{M}o_2$ = oxygen consumption rate ($\mu mol.g^{-1}.h^{-1}$), α = solubility coefficient ($ml.l.^{-1}Torr^{-1}$) for O_2 in sea water at $10^{o}C$, resp.vol. = respiratory volume (ml) and wt = fresh weight (g).

The rates of oxygen consumption recorded during these experiments were those of quiescent animals. Some further recordings were also carried out, however, to determine the $\dot{M}o_2$ of active animals. It was found that the disturbance caused by electrode implantation and handling resulted in the highest rates of oxygen consumption and of heart rate and scaphognathite rate being recorded immediately after the animals had been placed in the respirometer. Such rates were taken to be active rates of oxygen consumption.

In addition, however, other recordings were made during which the animals were exposed to a variety of stimuli e.g. tactile or changes in light intensity in an attempt to determine the maximum rates of oxygen consumption of individual animals. The relationship between $\dot{M}o_2$ and body weight was investigated in both *M. rugosa* and *M. sarsi*.

4.2.2. Effect of temperature on rates of oxygen consumption

Animals were kept undisturbed at 10°C in well-aerated sea water under constant light in the laboratory and allowed to acclimatize to the experimental conditions for at least 12h before recordings were made. Recordings of the rates of oxygen consumption of quiescent animals were made at 10°C and then further recordings were carried out at a range of different temperatures (5-20 °C). On each occasion, the animal was exposed to a given temperature for only 1 h before recordings commenced to ensure that there was no possibility of acclimation to the new temperature.

Measurements of heart and scaphognathite rates under the same conditions were also recorded either simultaneously or in a series of separate experiments. In these experiments, up to four animals of differing size (fresh weight = 7-69 g) were prepared as described in Chapter 3 and were placed in a large 'perspex' tank contained within a water bath. The animals were allowed to recover from the disturbance of electrode implantation for 24 h at 10 °C and then recordings of heart rate were made at a range of different temperatures as described above for measurements of oxygen consumption. The temperature coefficient Q_{10} was calculated according to the Van't Hoff equation:

$$Q_{10} = (k_2/k_1)^{(10/t2-t1)}.$$

4.2.3. The effect of feeding on rates of oxygen consumption

Some experiments were also carried out to examine the effect of feeding on rates of oxygen consumption in *M. rugosa* in an attempt to carry out a preliminary investigation of the Specific Dynamic Action (SDA) in this species. Animals were starved for 2 days before the experiments and then placed in the 'perspex' respirometer and left for a further 12 h to recover from the disturbance caused by handling. During this time the respirometer was flushed continuously with aerated sea water. Recordings of the rates of oxygen consumption were then carried out using the procedure described above in which the respirometer acted as a closed system. Changes in the Po_2 of the water were recorded over a 15 min period after which the respirometer was flushed with aerated sea water for a further 15 min under the control of a time switch. Recordings of $\dot{M}o_2$ were made at 15 min intervals for a period of 24 h before and after the animal was fed.

The animal was fed by inserting a pre-weighed amount of mussel tissue (*Mytilus edulis*) into the respirometer through a large hole drilled in the lid. The animal was then left undisturbed for approximately 15 min before any unconsumed food was carefully removed from the respirometer. This was then re-weighed to obtain the actual weight of food consumed. The opening in the lid of the chamber was then resealed with a rubber bung and further measurements of $\dot{M}o_2$ were made over the following 24 h. At the end of the experiment the respirometer was dismantled and thoroughly cleaned with a dilute solution of sodium hypochlorite then washed several times in water to ensure that there was no accumulation of bacteria that would affect background rates of oxygen consumption in subsequent experiments.

A total of 18 individuals of both sexes (fresh weight range = 22-32 g) were used in these experiments. The data from 6 individuals of similar size (mean weight = 28.4 $\pm$ 2.0 g) were pooled to enable a detailed analysis of the changes

in the rate of oxygen consumption following feeding to be carried out. In some experiments changes in heart rate and scaphognathite rate as well as oxygen consumption were also recorded.

4.2.4. Effects of hypoxia on oxygen consumption rate

The effect of hypoxia on rates of oxygen consumption in *M. rugosa* and *M. sarsi* was investigated using a closed system respirometer. The experimental procedures used were similar to those described above except that the respirometer was not flushed at regular intervals so that the Po_2 of the water in the respirometer gradually decreased until very low oxygen tensions (Po_2 < 10 Torr) were reached.

Concurrent recordings of heart rate and scaphognathite rate were also made during these experiments. Recordings of the rates of oxygen consumption for *M. rugosa* under conditions of declining oxygen tension were made for animals of differing size (4-52 g). All recordings were carried out at 10^{o}C.

Further experiments were carried out to monitor the rate of oxygen consumption as well as heart rate and scaphognathite rate during the period of recovery from exposure to severe hypoxia in the respirometer. After the Po_2 of the water in the respirometer had declined to very low levels, the respirometer was flushed with aerated sea water. The automatic flushing system was then used to enable the rates of oxygen consumption to be recorded at 15 min intervals as described above. Recordings of oxygen consumption, heart rate and scaphognathite rate were made throughout the recovery period.

In addition, some recordings of heart rate and scaphognathite rate were made on animals exposed to severe hypoxia or anoxia in tanks in the aquarium. The animals were exposed to these conditions for up to 6 h after which time the Po_2 of the water was increased to normoxic levels by aeration and additional

recordings were made for a further 15-20h. *M. rugosa* and *M. sarsi* of similar sizes were compared for their maximum tolerance to withstand anoxia.

4.2.5. Survival experiments under anoxic condition

As part of a study of the ability of these species to withstand exposure to low oxygen tensions, experiments were carried out to determine to what extent they could survive exposure to oxygen tensions below the Pc value and to total anoxia. The experiments were carried out on 110 *M. rugosa* and 33 *M. sarsi* of differing size (5-45g). The experiments were carried out in a sea water aquarium maintained at a constant temperature of 10^{o}C. Groups of 5-10 animals were placed in a large tank (vol. = 63 l) fitted with a 'perspex' lid. An oxygen electrode (E5046, Radiometer, Copenhagen) was fitted through the lid to enable the Po_2 of the water to be continuously monitored. The Po_2 of the water was maintained at the required level by bubbling a gas mixture (nitrogen, oxygen and carbon dioxide) produced by a precision gas mixing system through the water via an 'air stone'. A small amount of CO_2 was added to the gas mixture to maintain the pH of the water constant throughout the experiment. Control animals were maintained in aerated sea water at the same temperature. Care was taken to disturb both groups of animals as little as possible during the experiments.

During the initial experiments, the animals were examined every 15 min to determine mortality rates. Animals were classified as dead if they failed to respond to tactile stimuli. It proved quite difficult to clearly establish if the animals were actually dead, however, since the animals usually became motionless and appeared torpid. The experimental procedures were therefore modified to overcome this problem. Groups of animals were exposed to the experimental Po_2 for varying periods of time (30 min to 8 h). At the end of these periods the animals were carefully transferred to other tanks containing aerated sea water and were left for several hours during which they were

observed at 15 min intervals to determine the number that recovered from exposure to hypoxia. Although this did not allow precise records to be established of the time that individual animals died, this procedure did ensure that animals were not falsely classified as dead.

4.3. RESULTS

A number of preliminary experiments were carried out during which the rates of oxygen consumption of *M. rugosa* were monitored continuously under natural light:dark cycles for several days to establish the time taken for the animals to recover from handling disturbance and to become acclimatized to the experimental conditions. Immediately after the animals had been placed in the respirometer, $\dot{M}o_2$ was initially high but declined significantly during the next few hours until, after approximately 12 hours, a much lower $\dot{M}o_2$ was recorded. The $\dot{M}o_2$ remained approximately constant at this low level throughout the rest of the recording period. There was some evidence of a circadian rhythm of oxygen consumption in this species for although the rate of oxygen consumption remained at this low level for many hours, a significant increase in the rate of oxygen consumption was frequently recorded during the night. This diurnal variation in the rate of $\dot{M}o_2$ was only observed, however, when recordings were carried out under a 12:12 h light:dark regime. When recordings were carried out in constant light, the circadian rhythm was not sustained for long. Similar diurnal variations in the heart rate and scaphognathite rate were also recorded (see Chapter 3).

To overcome the problem of diurnal fluctuations in metabolic rate, all other experimental recordings were made under conditions of constant illumination using animals which had been maintained in the laboratory for few days under constant light.

Although maximum $\dot{M}o_2$ values were recorded in animals immediately after

handling or following the stress of electrode implantation, an increase in the rate of oxygen consumption of quiescent animals was observed following any form of disturbance (Fig. 4.1). It was also observed, however, that in many animals there was an initial sudden decrease in $\dot{M}o_2$ following such disturbance after which $\dot{M}o_2$ increased significantly. The increased rate of oxygen consumption following disturbance generally lasted for only a short period. A sudden disturbance also caused a short-lived reduction in heart and scaphognathite rates which followed by an increase in rates (see Chapter 3).

4.3.1. The relationship between $\dot{M}o_2$ and body weight

The relationship between whole animal oxygen consumption and body weight can be described by the equation:

$$Y = aX^b \quad \text{.....(1)}$$

where Y = oxygen consumption of the whole animal and X is the body weight (see reviews of Zeuthen, 1953; Von Bertalanffy, 1957; Hemmingsen, 1950, 1960). This equation can be written in its linear form as:-

$$\log Y = \log a + b \log X \quad \text{.....(2)}$$

A straight line relationship is then obtained when the data are plotted on logarithmic axes, where 'b' is the slope and 'a' the intercept on the Y axis.

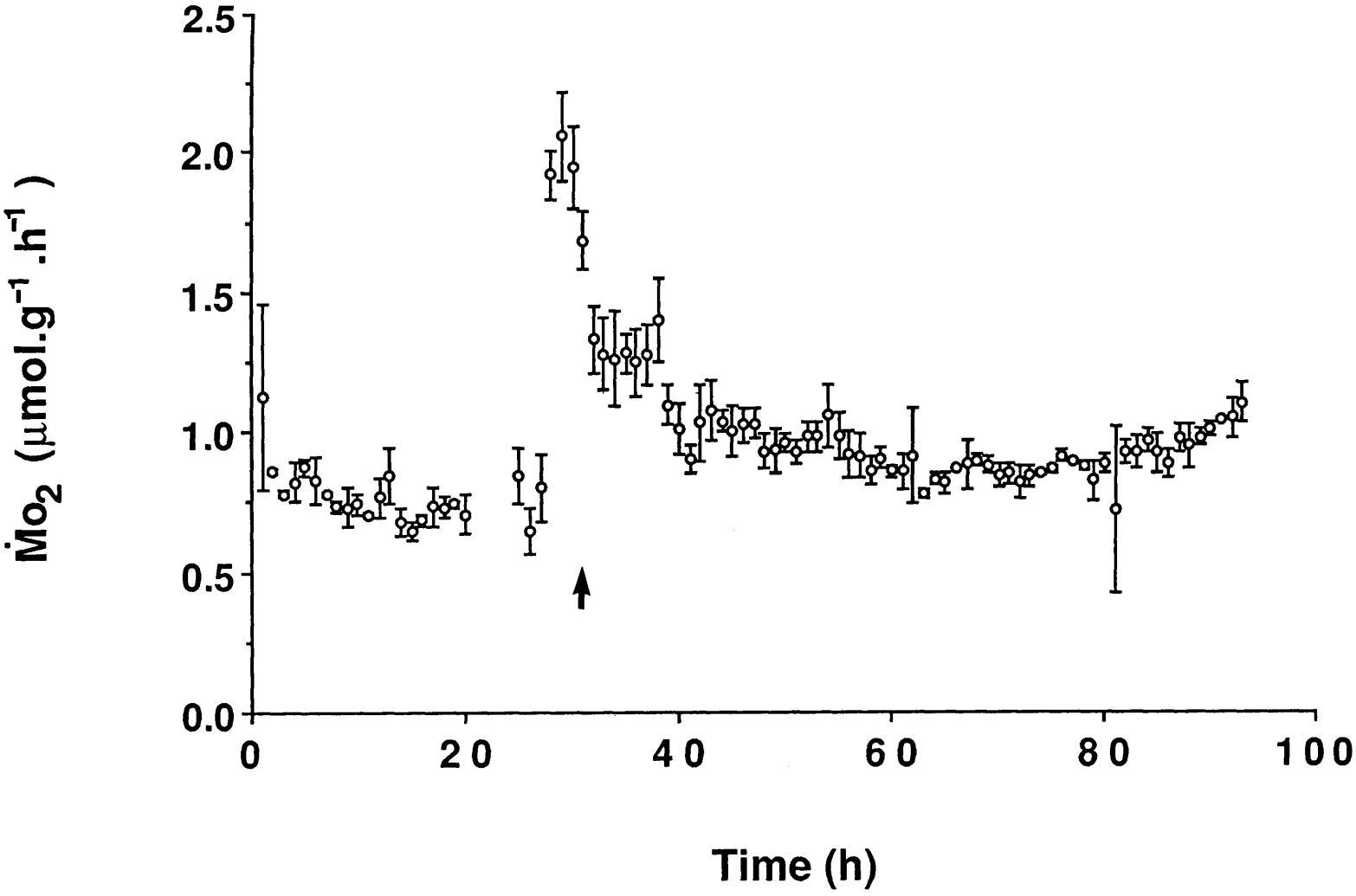
Much of the published data relating oxygen consumption to body weight are expressed as oxygen consumpion per unit weight ($\dot{M}o_2$). The relationship between weight specific oxygen consumption ($\dot{M}o_2$) and body weight can be expressed as:

$$\frac{Y}{X} = aX^{b-1} \quad \text{.....(3)}$$

Davies (1966) has drawn attention to the confusion which can occur over the term 'b'. Since the value of 'b' in equation 1 normally has a value of less than 1,

Fig. 4.1

The effect of disturbance on the rate of oxygen consumption ($\dot{M}o_2$) (μmol O_2. g^{-1}. h^{-1}). Following disturbance (arrow), there was significant increase in $\dot{M}o_2$ after which $\dot{M}o_2$ returned to normal 'resting' levels during the next few hours. Values are means ± S.D. of four determinations of $\dot{M}o_2$ made during each hour of the experiment.


2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
100
$\dot{M}o_2$ (μmol.g^{-1}.h^{-1})
Time (h)

the value 'b-1' in equation 3 will therefore have a negative value. Confusion can arise since 'b-1' is often written as '-b' or just 'b'. In order to avoid this problem Davies (1966) suggested that the equation relating $\dot{M}o_2$ to body weight should be rewritten as:

$$Y' = aX^{b'} \quad \text{.....(4)}$$

where Y' = weight specific oxygen consumption (Y/X) (or $\dot{M}o_2$)

X = body weight

a = a constant

b' = the weight exponent (b-1)

To avoid the confusion between '-b' and 'b', the notation of Davies (1966) will be used in this chapter. The relationship between $\dot{M}o_2$ and fresh body weight in *M. rugosa* is shown in (Fig. 4.2). As in most animals (Hemmingsen, 1950 ; Zeuthen, 1953; Schmidt Nielson, 1979), a negative correlation was found between the weight specific rate of oxygen consumption and fresh body weight.

In *M. rugosa*, the following values were obtained:

b = 0.856; b-1 = -0.144; a = 1.174; r = -0.338; n= 44. The correlation coefficient (r) was significant ($P<0.05$). There was no significant difference in this relationship between quiescent males and females (covariance analysis) (see below):

Including ovigerous females:

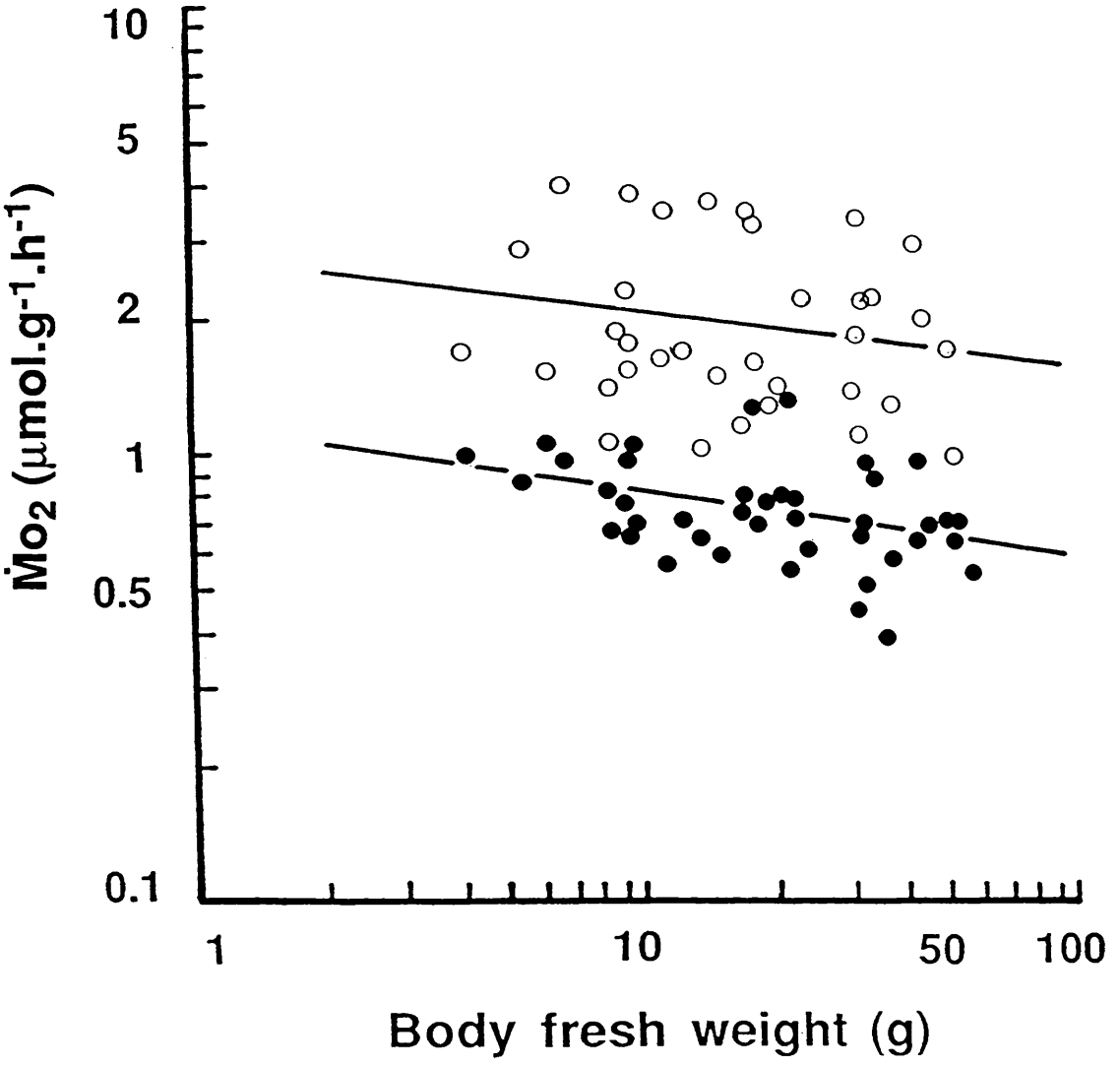
	b'	a	r	n
Resting:	-0.144	1.17	-0.338	44
Active:	-0.129	2.78	-0.203	37

Excluding ovigerous females:

Resting:	-0.082	1.054	-0.281	35
Active:	-0.067	2.213	-0.107	28

Fig. 4.2

The relationships between quiescent (●) and active (○) rates of oxygen consumption ($\dot{M}o_2$) (μmol O_2. g^{-1}. h^{-1}) and fresh body weight (g) for *M. rugosa*. The regression equations of the lines fitted to these data are given in the text.


10
5
2
1
0.5
0.1
$\dot{M}o_2$ (μmol.g^{-1}.h^{-1})
1
10
50
100
Body fresh weight (g)

The relationships between $\dot{M}o_2$ and body weight of both active and quiescent *M. rugosa* under normoxic conditions are shown in (Fig. 4.2) together with the regression lines fitted to these data. Covariance analysis indicated that, although there was no significant difference ($P>0.05$) between the slopes of the regression lines, there were highly significant differences between their elevations ($P<0.01$). The active rates of oxygen consumption were obtained mainly from recordings made immediately after the animals had been placed in the respirometer since it was found that this form of disturbance often resulted in the highest rates of $\dot{M}o_2$. There was a considerable variation between individuals in their active rates. The active rate in terms of percentage increase over the resting level varied between 40% to 282%. Generally, the active rates of $\dot{M}o_2$ in *M. rugosa* were approximately twice the quiescent rates.

There was no significant difference (t-test) in the rate of the oxygen consumption of similar sized animals (see the following comparisons):

	Wt (g)	$\dot{M}o_2$ ($\mu mol.g^{-1}.h^{-1}$)	n
M. rugosa (shallow):	26.2 ± 6.0	0.73 ± 0.19	22
M. rugosa (deep):	25.3 ± 1.9	0.85 ± 0.07	4
M. sarsi:	19.8 ± 2.6	0.71 ± 0.13	6

Due to the limited size range of *M. sarsi* that was available, it was not possible to determine the relationship between the $\dot{M}o_2$ and fresh body weight in this species.

4.3.2. Effect of temperature on $\dot{M}o_2$, heart rate, and scaphognathite rate

As in most other ectothermic animals, oxygen consumption, heart rate and scaphognathite rate increased with increasing temperature. There was a pronounced increase in each of the rates as the temperature was increased progressively up to 20°C (Fig. 4.3). At higher temperatures (20-25°C), most animals showed a progressive decrease in the $\dot{M}o_2$ and in the heart and scaphognathite rates. It was clear that the animals were stressed by such temperatures which are outside the range normally experienced in their natural environment and prolonged exposure to such temperatures usually resulted in death.

The patterns of the heart and scaphognathite beat of *M. rugosa* during exposure to increasing temperature are shown in (Fig. 4.4). At the higher temperatures to which the animals were exposed there was a gradual reduction in these rates, often associated with frequent periods of cardiac arrest and ventilatory pauses. At that stage, if the temperature was slowly reduced to 'normal' (10°C), the animals usually recovered and the $\dot{M}o_2$ and the heart and scaphognathite rates returned to normal levels within a few hours (~6-10h). Recovery from exposure to temperatures as high as 20°C was dependent upon the duration of the exposure to that temperature. It was found that at 20°C *M. rugosa* could survive exposure to this temperature for short periods (< 90 min.) but with increasing duration of exposure to such temperatures there was an increase in mortality rate.

Values for the temperature coefficient (Q_{10}) for $\dot{M}o_2$, heart rate and scaphognathite rate in *M. rugosa* determined at different temperature intervals over the range of temperatures studied (5-20°C) are presented in Table 4.1. These data are the mean values for Q_{10} calculated for animals placed in different size classes since it was found that the Q_{10} values varied between

Fig 4.3

Changes in oxygen consumption rate ($\dot{M}o_2$) (C), scaphognathite rate (A) and heart rate (B) with increasing temperature. Values are means ± S.D. weight range = 7-60g.

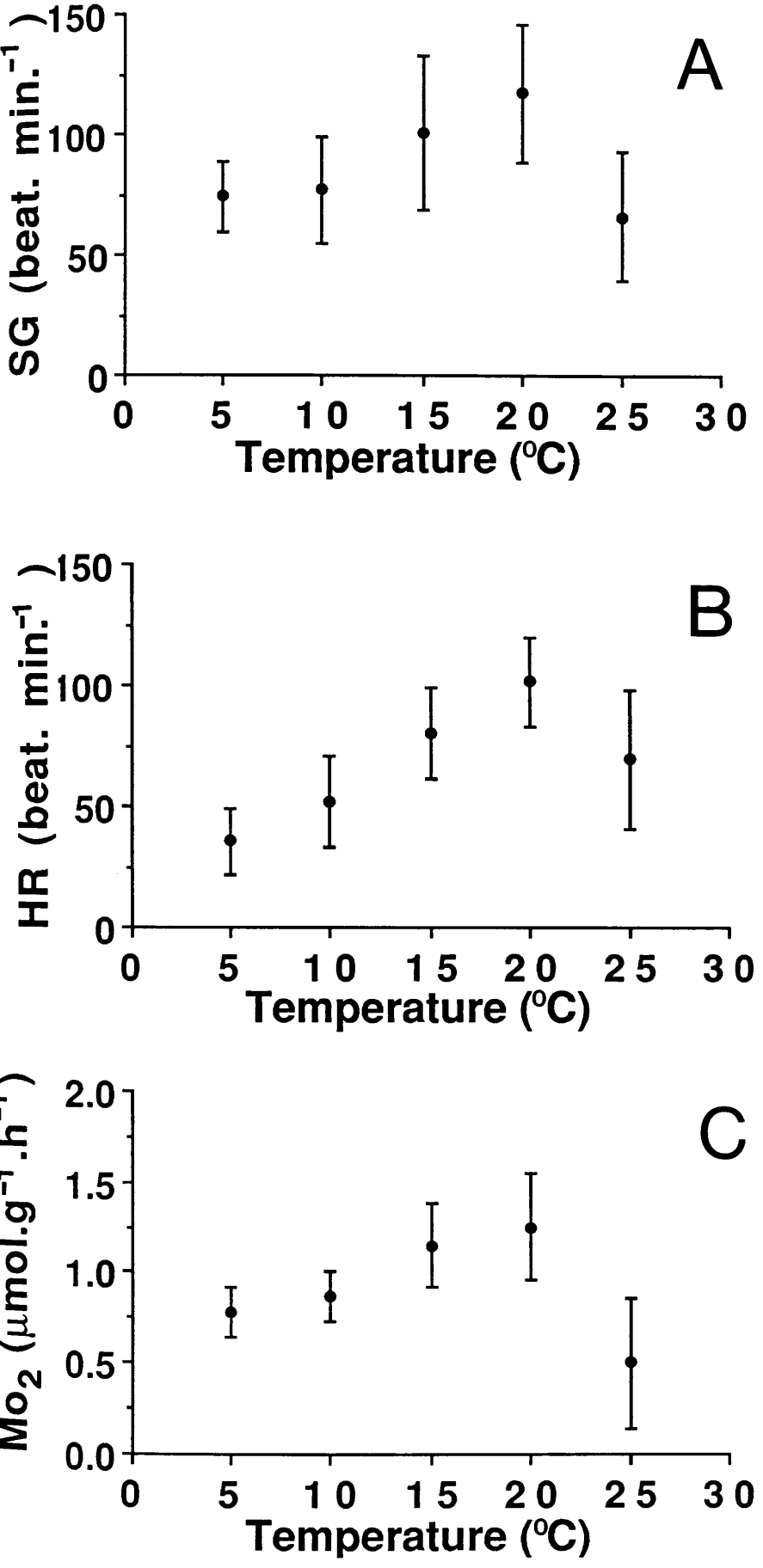

A
SG (beat. min.-1)
150
100
50
0
0 5 10 15 20 25 30
Temperature (°C)
B
HR (beat. min.-1)
150
100
50
0
0 5 10 15 20 25 30
Temperature (°C)
C
Mo2 (μmol.g-1 .h-1)
2.0
1.5
1.0
0.5
0.0
0 5 10 15 20 25 30
Temperature (°C)

Fig. 4.4

Recordings of the heart (HR) and right scaphognathite beat (ScR) of an individual male *Munida rugosa* (fresh wt = 40.4g) during exposure to an increase in temperature. A = 10^{o}C, B = 15 ^{o}C, C = 20^{o}C, D = 25^{o}C.

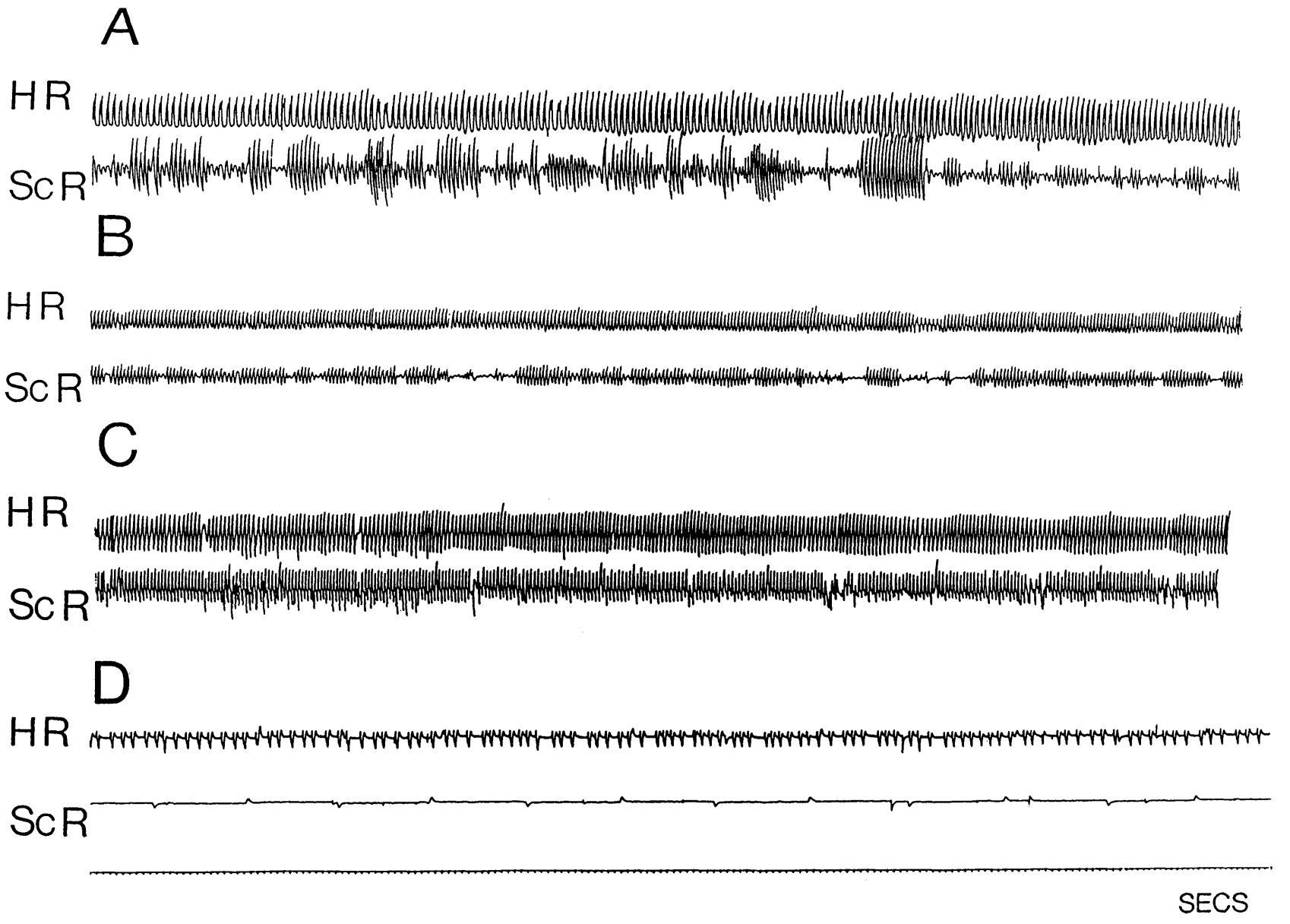

A
HR
ScR
B
HR
ScR
C
HR
ScR
D
HR
ScR
SECS

Table 4.1. Values for the temperature coefficient (Q_{10}) for oxygen consumption, heart and scaphognathite rates of *M. rugosa* Q_{10} values have been calculated for different temperature ranges and for animals of differing fresh weight. The number of animals (n) in each size class is also given.

$\dot{M}o_2$ Q_{10}:

Temperature (°C)	Fresh weight (g)						
	(<10)	(10-20)	(20-30)	(30-40)	(40-50)	(50-60)	mean(7-60)
05-10	-	-	1.53	1.08	1.40	-	1.34±0.19
10-15	-	-	1.95	1.86	1.61	-	1.81±0.14
15-20	-	-	1.12	1.04	1.18	-	1.11±0.06
20-25	-	-	-	-	0.69	-	0.69
n=	-	-	5	4	3	-	12

Heart rate Q_{10}:

05-10	-	2.37	1.48	2.44	2.92	2.49	2.34 ±0.4
10-15	2.8	2.25	1.86	1.32	2.65	3.69	2.43 ±0.7
15-20	1.7	2.10	1.58	1.97	1.58	2.49	1.91 ±0.3
20-25	-	1.0	0.30	0.50	0.55	0.81	0.64 ±0.3
n =	3	10	9	5	5	5	37

Scaphognathite rate Q_{10}:

05-10	-	-	1.19	1.32	1.54	-	1.35 ±0.14
10-15	-	-	1.56	1.49	1.98	4.14	2.29 ±1.0
15-20	-	-	1.62	1.40	1.44	1.43	1.47 ±0.08
n =	-	-	6	5	1	3	15

animals of differing size (7-60g). The changes in Q_{10} for $\dot{M}o_2$, heart and scaphognathite rates with increasing temperature are plotted in (Fig. 4.5).

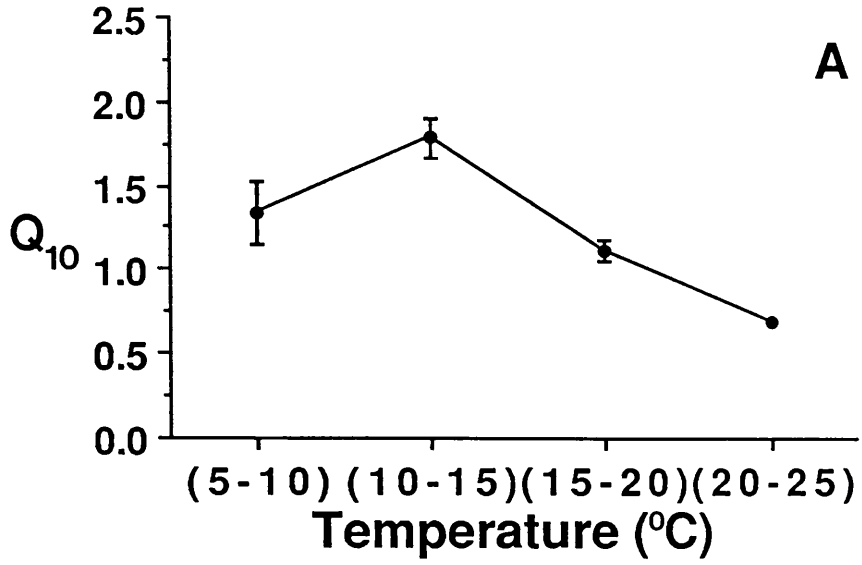
The Q_{10} values for the heart rate were less variable over the temperature range studied, than those for the $\dot{M}o_2$ and the scaphognathite beat.

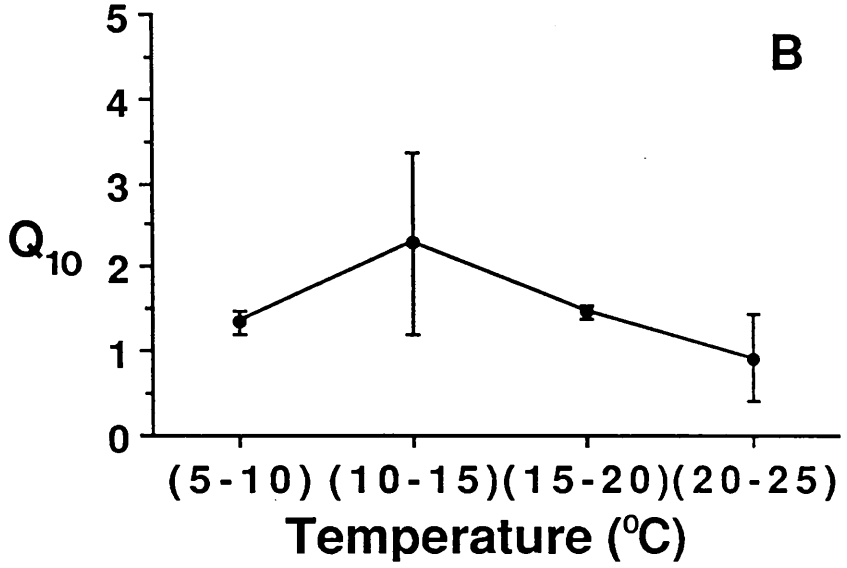
4.3.3. Effects of feeding on metabolic rate

Experiments to investigate the SDA effect in *M. rugosa* showed that, when starved animals were fed, there was a significant increase in both $\dot{M}o_2$ and in scaphognathite rate. In contrast, however, there was no significant increase in heart rate. The changes in $\dot{M}o_2$ of an individual *M. rugosa* (male, fresh weight = 27.5g) during one such experiment are plotted in (Fig. 4.6). The animal consumed 0.38g of the food (fresh mussel) provided and soon after feeding there was a 55% increase in $\dot{M}o_2$ above that recorded prior to feeding. The $\dot{M}o_2$ returned to approximately the pre-feeding level within 4-5h. Mean values of the percentage increase in $\dot{M}o_2$ of 6 individuals (fresh weight = 28.4 $\pm$ 2g) following feeding are presented in (Fig. 4.7). The data were calculated as the mean values of $\dot{M}o_2$ during the twelve hours prior to feeding and mean values of the post-fed (mean for the elevation until the decline to the normal resting level was reached. The amount of food eaten by these animals was between 0.29 and 0.63 g. The time taken for $\dot{M}o_2$ to return to normal levels following a meal was very variable and ranged from 2-8 hours. The percentage increase in $\dot{M}o_2$ following a meal varied between 21% to 57.8%. No clear correlation was obtained between the amount of food consumed and either the duration or the magnitude of the SDA.

Fig. 4.5

Changes in Q_{10} for oxygen consumption ($\dot{M}o_2$) (C), scaphognathite rate (A) and heart rate (B) with increasing temperature. Values are means $\pm$ S.D. Weight range = 7-60 g.


A
2.5
2.0
1.5
1.0
0.5
0.0
Q_{10}
(5-10) (10-15)(15-20)(20-25)
Temperature (°C)


B
5
4
3
2
1
0
Q_{10}
(5-10) (10-15)(15-20)(20-25)
Temperature (°C)

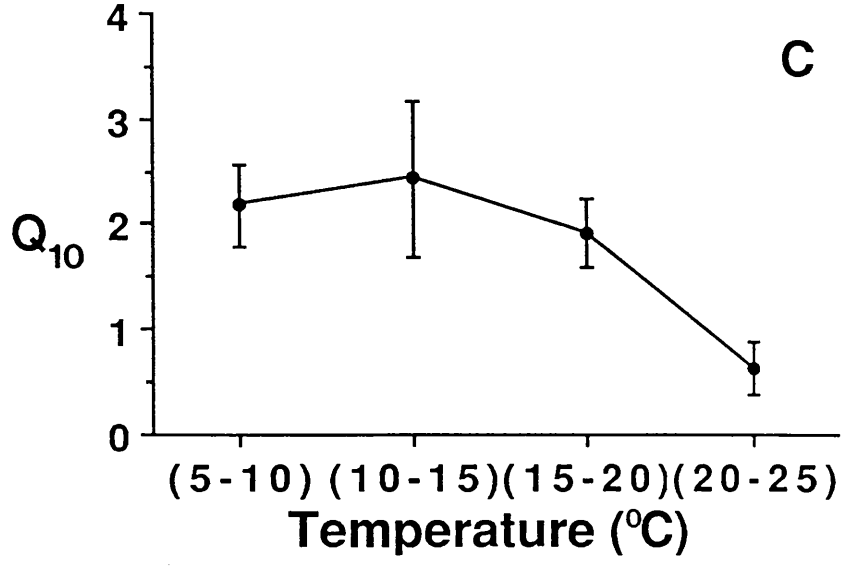

C
4
3
2
1
0
Q_{10}
(5-10) (10-15)(15-20)(20-25)
Temperature (°C)

Fig. 4.6

The effect of feeding on the rate of oxygen consumption ($\dot{M}o_2$) of an individual male *Munida rugosa* (fresh wt = 27.5g) maintained at 10° C. The animal was fed at the point indicated (F) with a small quantity of fresh mussel flesh. For further details see text.

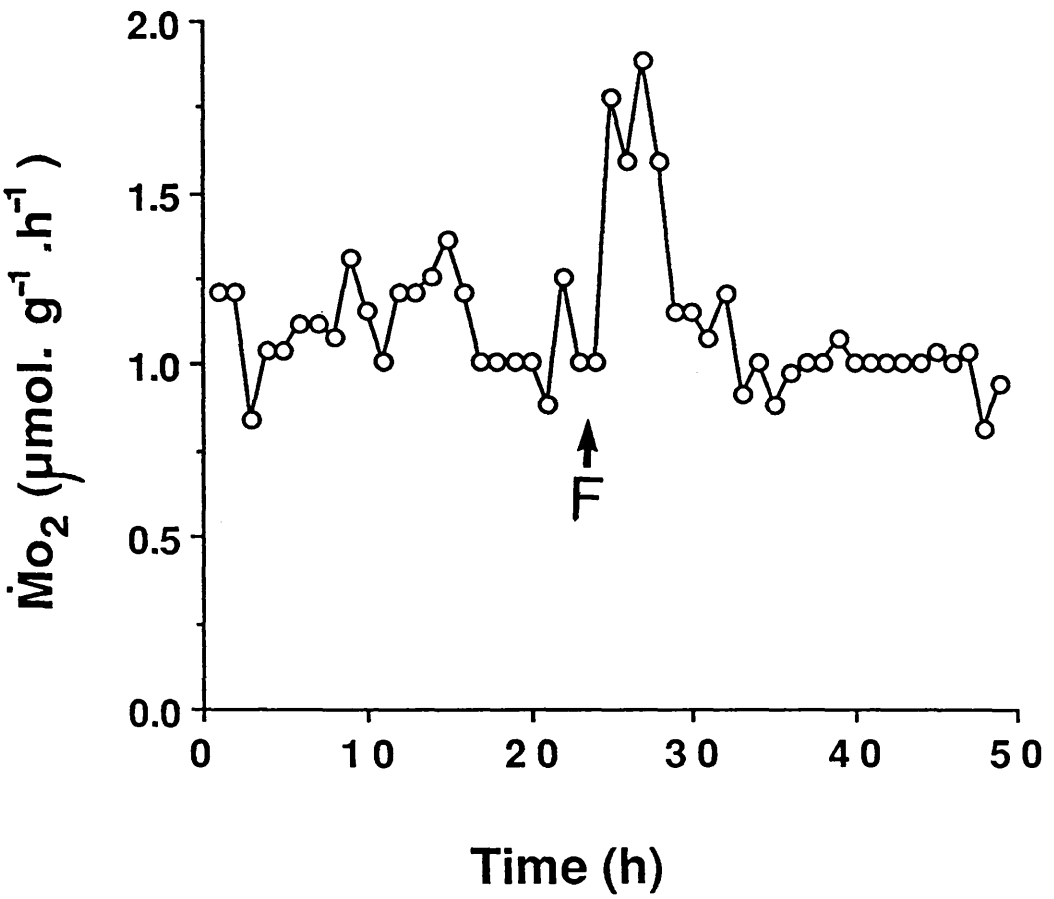
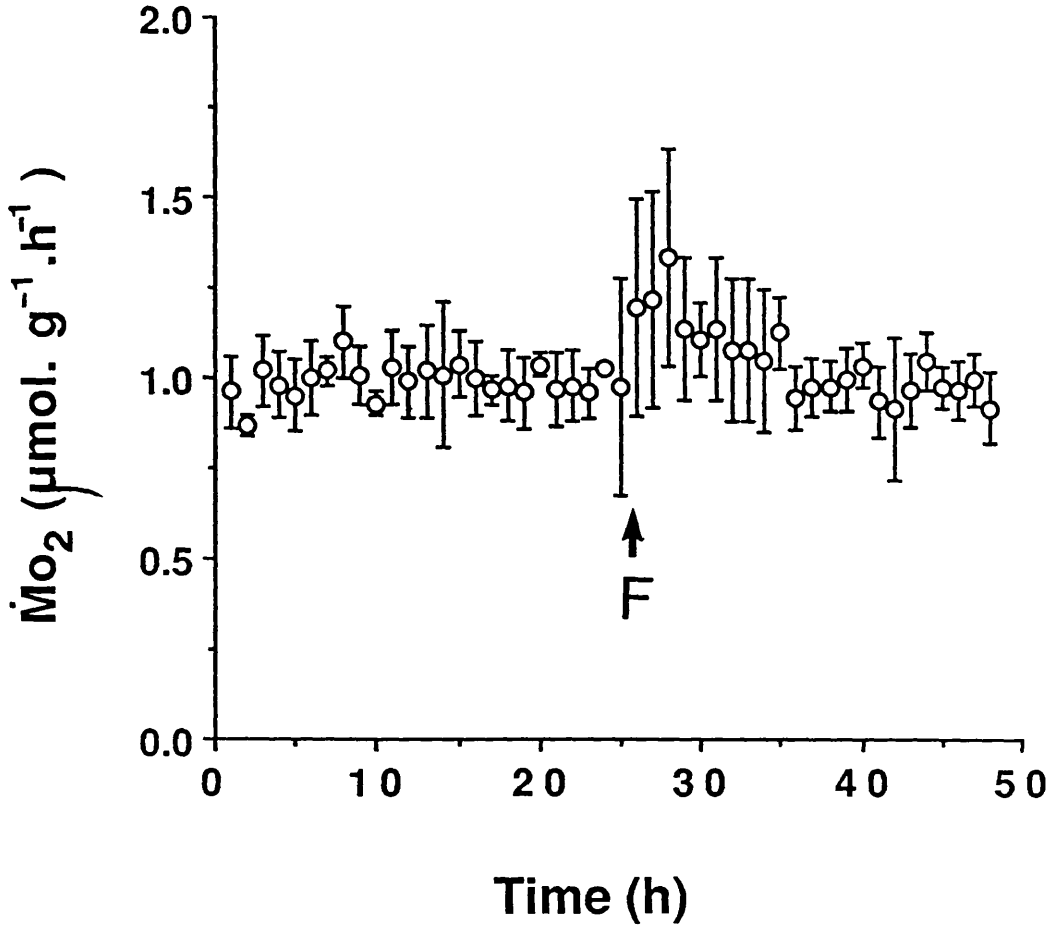

2.0
1.5
1.0
0.5
0.0
$\dot{M}o_2$ (µmol. g^{-1} .h^{-1})
F
0
10
20
30
40
50
Time (h)

Fig. 4.7

The effect of feeding on the rate of oxygen consumption ($\dot{M}o_2$) of six *Munida rugosa* (mean fresh wt = 28.4 ± 2g). The animals were fed at the point indicated (F) with a small quantity of fresh mussel flesh. Values are means ± S.D. For further details see text.


2.0
1.5
1.0
0.5
0.0
$\dot{M}o_2$ (µmol. $g^{-1}.h^{-1}$)
F
0
10
20
30
40
50
Time (h)

4.3.4. Effect of hypoxia on the rates of oxygen consumption and on heart and scaphognathite rate

Both *Munida rugosa* and *M. sarsi* showed a considerable ability to maintain their rates of oxygen consumption independent of the ambient oxygen tension. The rates of oxygen consumption together with the heart rates and scaphognathite rates under conditions of declining oxygen tension of *M. rugosa* from the shallow site are shown in Fig. 4.8 . Similar data for an individual *M. sarsi* are also presented (Fig. 4.9). Both species were able to maintain their rates of oxygen consumption approximately constant over a range of oxygen tensions. The Po_2 at which respiratory independence was lost (the critical point, or Pc) varied only slightly between individuals of the same species and even between the two species of *Munida*. The Pc values (Torr) were 49.7 ± 7 (n = 22); 38.7 ± 11 (n = 4); 55.8 ± 17 (n = 6) for similar sized (19-26g) *M. rugosa* from shallow and deep waters and *M. sarsi* respectively. The differences between these values were found to be not significant ($P > 0.05$)

The relationship between the Pc and body size for *M. rugosa* from the shallow collection site is shown in Fig. 4.10. This relationship had a significant correlation coefficient:

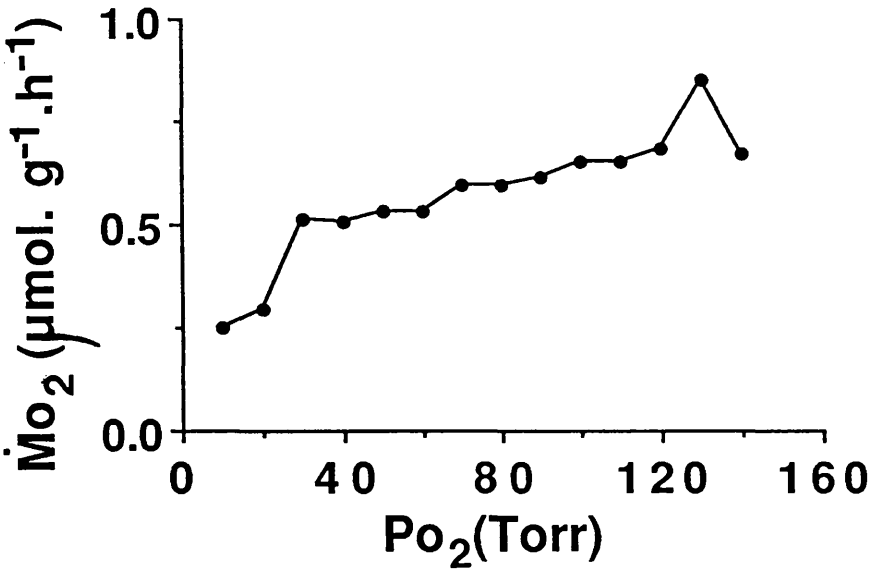
$\log Y = 0.17 \log X + 1.46$ ($r = 0.475$; $n = 33$; $P < 0.01$), and indicates that the smaller sized individuals are characterized by having lower Pc values. During this study, it was not possible, however, to obtain animals of a very wide size range. Additional information for a much wider size range of animals is needed to confirm this relationship.

In both *M. rugosa* and *M. sarsi* there was a progressive increase in scaphognathite rate during exposure to declining oxygen tension (Figs. 4.8 & 4.9). In the majority of animals, the scaphognathite rate increased by a factor of approximately 2 during the period of exposure to hypoxia. At oxygen tensions

Fig. 4.8

The effect of declining oxygen tension (Po_2) on the rate of oxygen consumption ($\dot{M}o_2$) of an individual *M. rugosa* (fresh wt = 14g) from the shallow water site (A). Simultaneous recordings of the heart (●) and scaphognathite (○) rate of the same individual are also shown (B). The recordings were carried out at 10°C.

A


1.0
0.5
0.0
$\dot{M}o_2$ (µmol. g^{-1}.h^{-1})
0
40
80
120
160
Po_2(Torr)

B

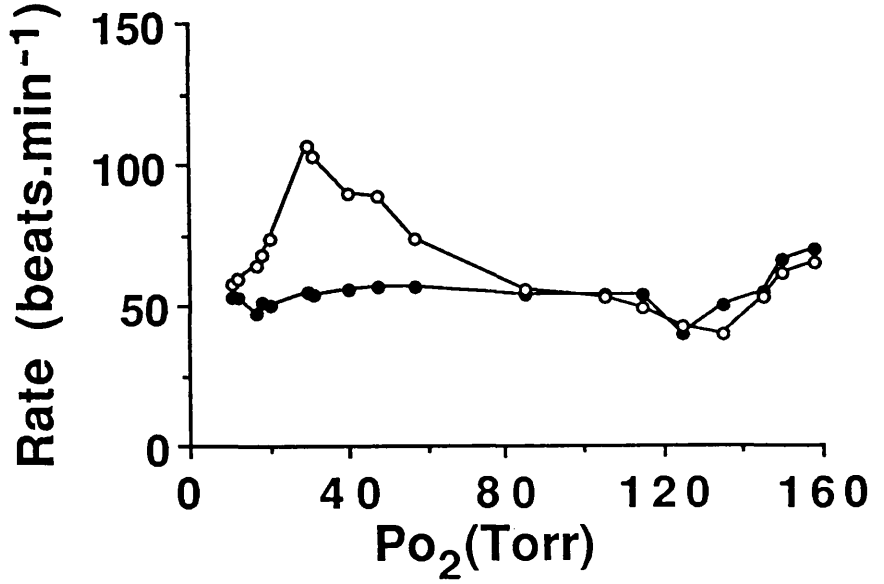
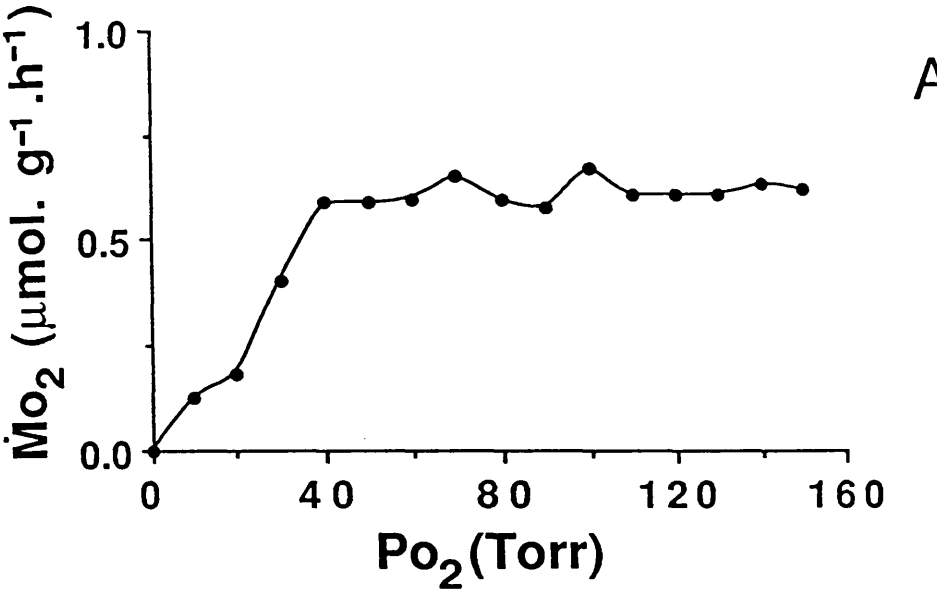

150
100
50
0
Rate (beats.min^{-1})
0
40
80
120
160
Po_2(Torr)

Fig. 4.9

The effect of declining oxygen tension (Po_2) on the rate of oxygen consumption ($\dot{M}o_2$) of an individual *M. sarsi* (fresh wt = 16.8g) (A). Simultaneous recordings of the heart (○) and scaphognathite (X) rate of the same individual are also shown (B). The recordings were carried out at 10 °C.


A
$\dot{M}o_2$ (μmol. $g^{-1}.h^{-1}$)
1.0
0.5
0.0
0
40
80
120
160
Po_2(Torr)

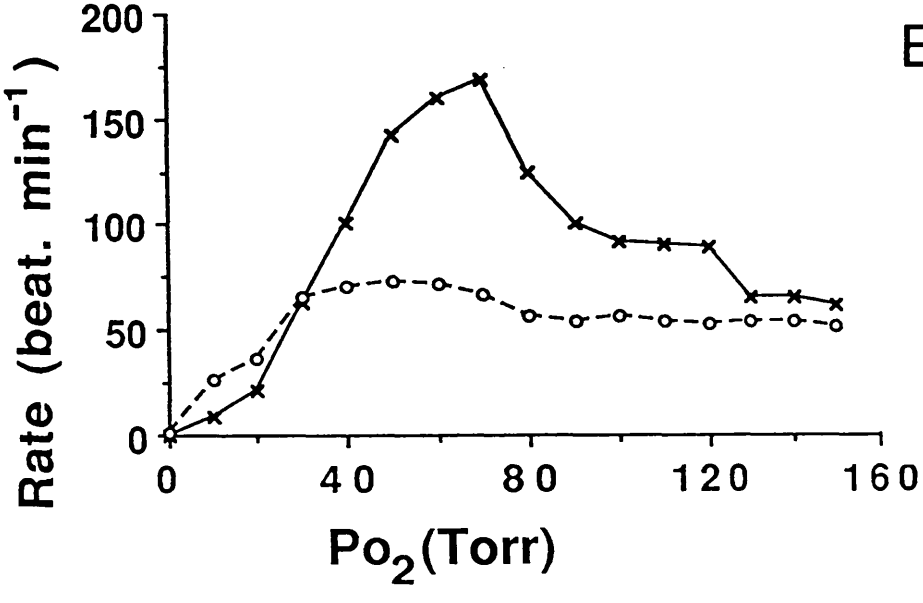
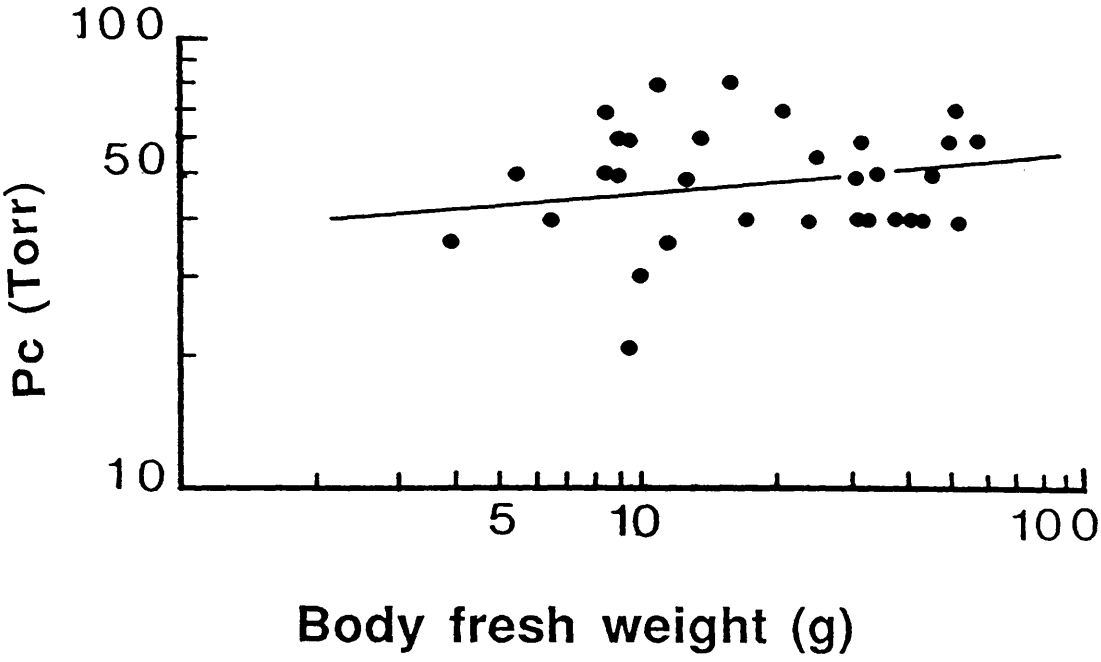

B
Rate (beat. min^{-1})
200
150
100
50
0
0
40
80
120
160
Po_2(Torr)

Fig. 4.10

The relationship between the 'critical' Po_2 (Pc) and body fresh weight for *M. rugosa* from the shallow water site. All recordings were carried out at 10°C. The regression equation of the line fitted to these data is given in the text.


100
50
10
Pc (Torr)
5
10
100
Body fresh weight (g)

close to the Pc, however, the ventilation rate began to gradually decline until at extremely low Po_2 ventilatory activity ceased.

In contrast to the changes recorded in scaphognathite rate during hypoxia, the heart rate of the majority of animals remained approximately constant over a wide range of Po_2 (Figs. 4.8 & 4.9). Below the Pc, however, heart rate declined progressively until, under anoxic conditions, the heart rate was often reduced to only 1-2 beats. min^{-1} (Fig. 4.11).

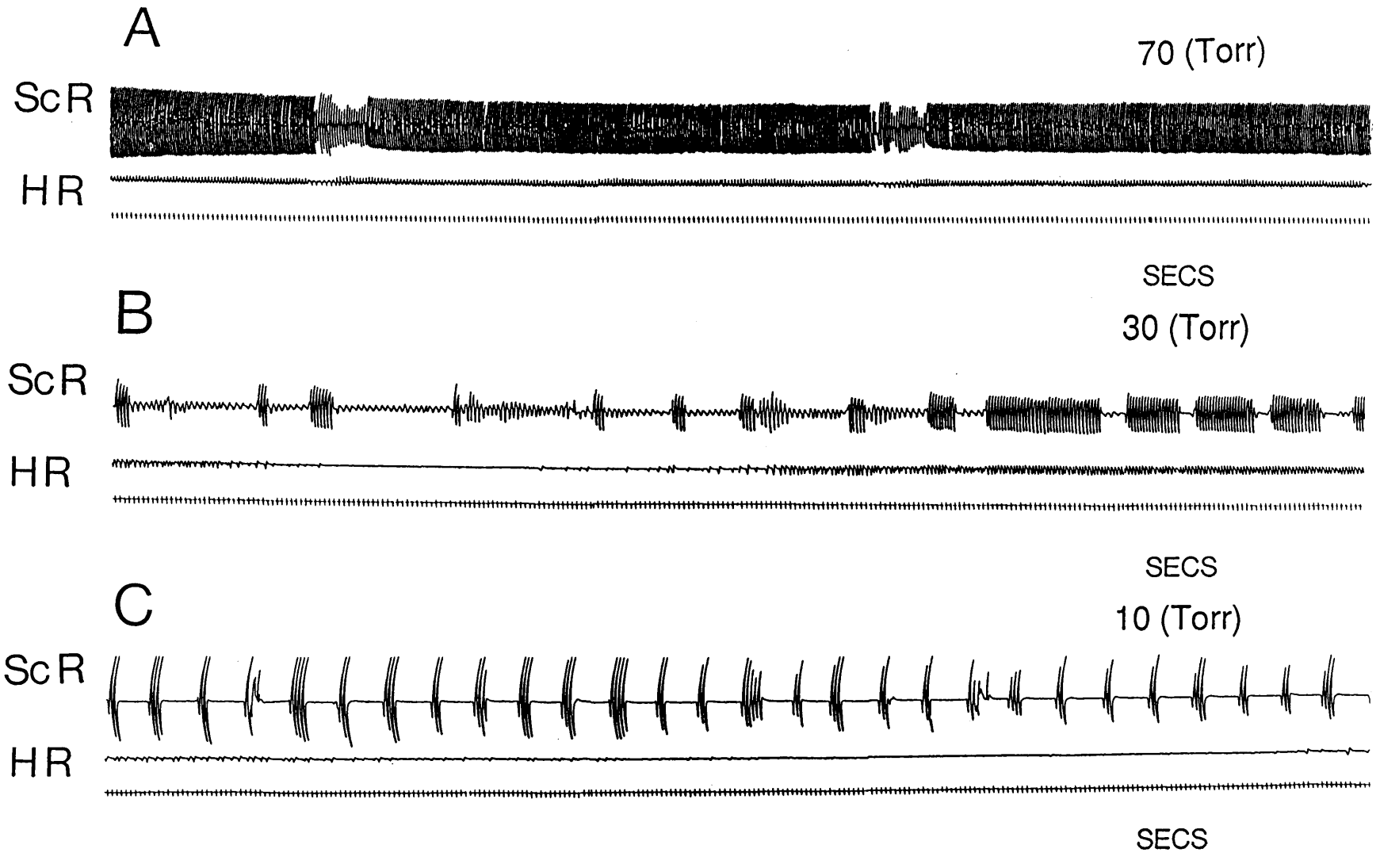
The ability of *M. rugosa* and *M. sarsi* to regulate their rate of oxygen consumption was observed only when the Po_2 of the water was reduced at a slow rate (e.g. by the animal's own respiratory activity when confined in the respirometer). When the Po_2 was reduced rapidly by bubbling nitrogen through the water, the rate of oxygen consumption, the heart rate and scaphognathite rate showed initial elevation followed by a progressive decline. Under these conditions, there was no evidence of any regulation of oxygen consumption rates.

4.3.5. Anoxia tolerance

During the experiments to record the effect of declining Po_2 on the rates of oxygen consumption, it was found that *M. rugosa* was able to survive exposure to oxygen tensions below the Pc (e.g. 15-20 Torr) for up to 11h. When exposed to completely anoxic conditions, however, survival times were considerably reduced. Although no attempts were made to establish a value for the LT_{50} in these species, the available data showed that approximately 50-60% of *M. rugosa* survived for 8 h under anoxia. *M. sarsi* appeared to be less tolerant of anoxia, however, since few animals survived for longer than 4 h.

Fig. 4.11

Recordings of the heart (HR) and right scaphgnathite (ScR) beat in an individual *M. rugosa* (fresh wt = 25.0g) under conditions of declining oxygen tension. Recordings are shown for heart and scaphognathite activity at oxygen tensions of 70 (A), 30 (B) and 10 (C) Torr. The recordings were carried out at 10 °C.


A
70 (Torr)
ScR
HR
SECS
B
30 (Torr)
ScR
HR
SECS
C
10 (Torr)
ScR
HR
SECS

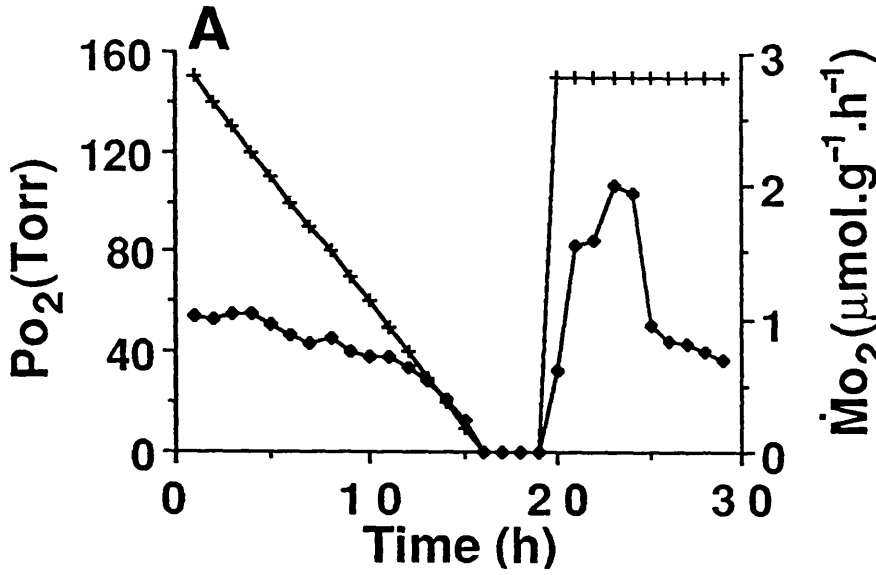
4.3.6. Oxygen consumption, heart and scaphognathite rate during recovery

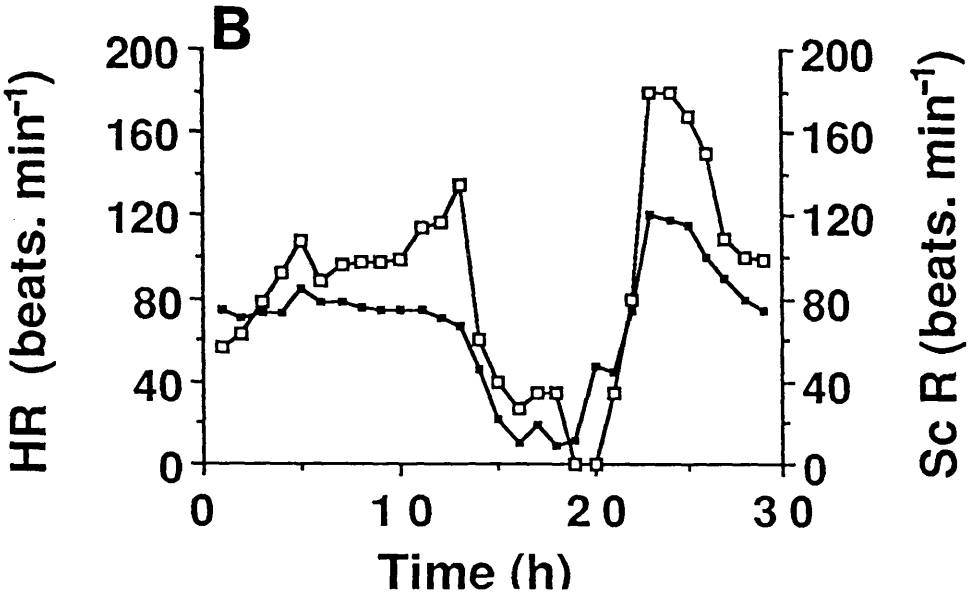
Changes in oxygen consumption rate, heart rate and scaphognathite rate during anoxia and during subsequent periods of recovery from anoxia of an individual of *M. rugosa* are shown in Fig. 4.12. During the first few hours of the recovery period, there was a pronounced increase in the rate of oxygen consumption and in the rates of beating of the heart and scaphognathites. During the remainder of the recovery period there was a gradual reduction in $\dot{M}o_2$ and in the heart rate and scaphognathite rate until they returned to 'normal' resting levels after approximately 12-24 hours. Following exposure to anoxia for periods of up to 4 h, the percentage increase in $\dot{M}o_2$ (above the resting level) during the first hours of recovery ranged from 65% to 267% for *M. rugosa* and from 52% to 63% for *M. sarsi*. This 'overshoot' in the rate of oxygen consumption following periods of exposure to extreme hypoxia or anoxia is thought to represent the repayment of an 'oxygen debt' (Bridges & Brand, 1980). There was some evidence, based on the magnitude of the 'overshoot' in $\dot{M}o_2$ and on the duration of the recovery period, that the size of the 'oxygen debt' was correlated with the duration of exposure to anoxia.

Some animals which had been exposed to anoxia for periods greater than 4h, did not show an 'overshoot' of the $\dot{M}o_2$. These animals often failed to show any signs of recovery; $\dot{M}o_2$ and the heart and scaphognatite rates remained at low levels for a few hours until the animals died.

Fig. 4.12

Recordings of the rate of oxygen consumption ($\dot{M}o_2$) (◆) of an individual *Munida rugosa* (fresh wt = 25.8g) during exposure to conditions of declining oxygen tension and during the period of recovery under normoxic conditions at 10°C (A). Simultaneous recordings of the heart (■), and scaphognathite (□) rates of the same individual are shown in (B). The animal was kept at a Po_2 of 0 Torr for 4 h before the water was reoxygenated. The changes in the Po_2 of the water throughout the experiment are also shown (X).


A
160
120
80
40
0
Po_2(Torr)
0
10
20
30
Time (h)
3
2
1
0
$\dot{M}o_2$(μmol.g^{-1}.h^{-1})


B
200
160
120
80
40
0
HR (beats. min^{-1})
0
10
20
30
Time (h)
200
160
120
80
40
0
Sc R (beats. min^{-1})

4.4. DISCUSSION

There is an extensive literature on rates of oxygen consumption of decapod Crustacea (reviewed by McMahon & Wilkens, 1983). It is difficult, however, to make meaningful comparisons between species since different workers have often used different experimental procedures. It is now well established that the conditions under which the animals were maintained prior to the experiments e.g. the feeding regime (which may affect nutritional levels), their reproductive state, their stage in the moult cycle as well as the recording procedures themselves have important effects on the rates of oxygen consumption recorded (McMahon & Wilkens, 1983). For many studies, such information is not available and prevents valid interspecific comparisons being made.

Despite these difficulties, attempts were made to compare the rates of oxygen consumption of *M. rugosa* and *M. sarsi* with those of other decapods. It was found that, in general, the galatheid crabs appear to be characterized by having lower metabolic rates than many macrurous and brachyuran decapods (Table 4.2). An alternative method for making such interspecific comparisons is to compare their metabolic scope for activity or, more accurately, their aerobic metabolic scope (McMahon & Wilkens, 1983). Metabolic scope for activity is defined as the difference between the minimum and maximum metabolic rates under a given set of conditions (Fry, 1947; Gordon, 1968). Oxygen uptake is not only affected by activity but also by disturbances, physiological status and by changes in many environmental parameters (McMahon & Wilkens, 1983).

4.4.1. Relationship between $\dot{M}o_2$ and body size

As has been shown in a wide variety of animals (see reviews by Prosser, 1973; Schmidt-Nielsen, 1979, 1984; Eckert & Randall, 1983; Robinson *et al.*, 1983), small individuals have a greater weight specific rate of oxygen consumption

Table 4.2. Rate of oxygen consumption ($\dot{M}o_2$) of a selected range of decapod crustaceans. The temperature at which the recordings were carried out were also shown.

Species	T°C	$\dot{M}o_2$ ($\mu mol.\ g^{-1}.\ h^{-1}$)	Reference
Cancer magister	08-10	1.5-3.66	McMahon *et al.*, 1979
Cancer productus	10-12	1.62	deFur, 1978
Carcinus maenas	15	1.2-1.32	Taylor, 1978
Homarus americanus	12	1.32	McMahon *et al.*, 1975
Homarus gammarus	15	1.02	Butler *et al.*, 1978
Corystes cassivelaunus	10	1.07	Bridges *et al.*, 1980
Nephrops norvegicus	10	1.65	"
Pagurus bernhardus	10	3.13	"
Galathea strigosa	10	2.28	"
Upogebia africana	10	3.13	Hill, 1981
Pleuroncodes planipes	10	0.63	Quetin & Childress,
Munida quadrispina	10	0.89-3.13	Burd, 1985
Munida rugosa	10	0.73-1.9	This study
Munida sarsi	10	0.71	This study
M. rugosa	15	1.34	Rios, 1979

than larger individuals. The slopes (b-1) of the regression lines fitted to the data obtained for *M. rugosa* and *M. sarsi* are compared to values obtained for some other decapods in Table 4.3.

The significance of the value of the exponent 'b' or 'b-1' in these equations has been the subject of much speculation (Zeuthen, 1947, 1953; Hemmingsen, 1950, 1960; Von Bertalanffy, 1957). As early as 1883, Rubner observed that the metabolism of homeothermic animals, when expressed per unit body weight, decreases with increasing size, although it is approximately constant when calculated per unit surface area (Rubner's surface law). Zeuthen (1947, 1953) was of the opinion that the metabolism of cells was related to their surface area, so that the total metabolism of metazoans would be related to the aggregate of their cell surfaces; in which case metabolism would be expected to increase with the 0.67 power of the weight. This concept was later modified by Hemmingsen (1950, 1960) who showed that metabolism varies more closely with the 0.75 power of the body weight. He therefore suggested that metabolism may not be proportional to the cell surface as such, but to other factors such as vascularization and the development of more complex respiratory surfaces.

Both Zeuthen (1947, 1953) and Hemmingsen (1950, 1960) have shown that the common regression line relating log $\dot{M}o_2$ to log body weight for a wide range of organisms from protozoans to larger poikilothermic metazoans has a slope of 0.75. More recently, McMahon (1973) has produced a mathematical model to try to explain why the weight exponent should have a value of 0.75.

Despite these theoretical considerations, many studies have obtained values for the weight exponent 'b' which differ slightly or, occasionally, considerably from this value. Although there may be valid biological reasons for these differences there is considerable evidence that, in many studies, data from only a limited size range of animals have been obtained with the result that the

Table 4.3. Values of the weight exponents b and b obtained for the calculated relationships between $\dot{M}o_2$ and dry weight for some decapod crustaceans. For further details see text.

Species	b	b	Reference
Ebalia tuberosa	-0.37	0.63	Schembri, 1979
Carcinus maenas	-0.56	0.44	Wallace, 1971
Clibanarius vittatus	-0.25	0.78	Wernick, 1980
Gecarcinus lateralis	-0.53	0.47	Taylor & Davies,1980
Corystes cassivelaunus	-0.12	0.88	Bridges & Brand,1980
Nephrops norvegicus	-0.14	0.86	"
Galathea strigosa	-0.26	0.74	"
Pagurus bernhardus	-0.25	0.75	"
Pagurus bernhardus	-0.33	0.67	Shumway 1978
Pagurus herbstii	-0.20	0.80	Leffler, 1973
Pagurus hirsutiusculus	-0.39	0.61	Young, 1963
Munida rugosa	-0.14	0.86	This study
Munida sarsi	-0.45	0.55	"

Table 4.4. Q_{10} values for oxygen consumption of some decapod crustaceans. Values in brackets represent the temperature range over which the Q_{10} values were calculated.

Species	Q_{10}	Reference
Carcinus maenas	2.36 (10-18)	Taylor *et al.*, 1977
Panulirus interuptus	3.25 (13-16)	Winget, 1969
Galathea strigosa	3.41 (07-11)	Bridges, 1980
Galathea strigosa	3.71 (11-15)	Bridges, 1980
Palaemon elegans	1.40 (05-10)	Morris & Taylor,1984
Palaemon elegans	1.83 (10-15)	Morris & Taylor,1984
Munida rugosa	1.85 (10-15)($\dot{M}o_2$)	This study
Munida rugosa	2.43 (10-15)(HR)	"

regression line fitted to these data may not be an accurate description of the relationship between oxygen consumption and body size in that species. This may provide an explanation for the low value of 'b' (or high b-1 value) obtained for *M. sarsi* since the data obtained for this species were for a restricted size range of individuals. In contrast, the weight exponent obtained for *M. rugosa* (calculated from data from a much greater size range of animals) is much closer to the expected value. The value for the weight exponent obtained during the present study is identical to that obtained by Rios (1979) (at 15°C) for this species. Rios (1979) quotes an average rate of oxygen consumption of 0.0434 $mg.g^{-1}.h^{-1}$ (1.34 $\mu mol.g^{-1}.h^{-1}$) for animals of 5.9-24.7 g fresh weight at a temperature of 15°C. During the present study, the average $\dot{M}o_2$ for animals of 4-52 g fresh weight was 0.73 $\mu mol.g^{-1}.h^{-1}$ but it is impossible to make any meaningful comparisons since the values of $\dot{M}o_2$ reported here were determined at 10°C. There is little value, however, in quoting average rates of oxygen consumption of animals of such a wide size range since $\dot{M}o_2$ varies inversely with body weight. If such comparisons are to be carried out, a better procedure is to determine the $\dot{M}o_2$ of a 'standard' animal of a certain weight from the relationship between $\dot{M}o_2$ and fresh body weight. Since this relationship in *M. rugosa* was determined only at 10°C during this study it is impossible to carry out valid comparisons with the data presented by Rios (1979).

The difference between the resting and active rates of $\dot{M}o_2$, heart and scaphognathite rates (see Chapter 3) for *M. rugosa* was highly significant. Similarly, much higher rates of oxygen consumption were recorded in animals soon after handling. This increase in $\dot{M}o_2$ following disturbance has also been recorded in *M. rugosa* (Rios, 1979) and in other species (e.g. Sutcliffe *et al.*, 1975). Other studies have investigated the relationship between $\dot{M}o_2$ and activity measured in terms of walking speed (e.g. Houlihan & Mathers, 1984).

Such studies have shown that $\dot{M}o_2$ is often correlated with the walking speed. During the present study, however, no attempts were made to correlate $\dot{M}o_2$ with locomotor activity. Instead, active rates were taken to be those recorded in animals subject to disturbance since the highest rates of oxygen consumption were usually recorded under such conditions. In *M. rugosa* active rates of oxygen consumption were found to be more than double the resting rate. According to the present data the average metabolic scope for activity (maximum - minimum) (Fry, 1947) for medium sized *M. rugosa* (wt = 25.6 ± 6g) was 1.14 μmol. g^{-1}. h^{-1}. The $\dot{M}o_2$ (active)/(quiescent) ratio was equivalent to 2.3 which is lower than that for other decapods (4-5) (McMahon & Wilkens, 1983).

4.4.2. Effect of Temperature on $\dot{M}o_2$

Several studies have included the effect of temperature on the rate of oxygen uptake, in particular, for those species which are likely to be exposed to temperature fluctuations in their natural environments (Davies, 1966; Newell, 1972; Marsden *et al.*, 1973; Florey & Kriebel, 1974; Burton, *et al.*, 1980; Dye *et. al.*, 1980; Bridges & Brand, 1980; Taylor, 1981; Morris & Taylor, 1984). It is unlikely that *M. rugosa* or *M. sarsi* are normally exposed to extreme conditions; they do not normally experience temperatures above about 10°C nor are they frequently exposed to sudden changes in temperature (Chapter 2). The values of Q_{10} obtained for these species were found to be within the range calculated for many other decapods (see Table 4.4). Calculation of the Q_{10} is useful because it aids the quantitative description of the relationship between metabolic rate and temperature and enables predictive calculations at temperatures other than those at which the process was measured (Cossins & Bowler, 1987).

Measurement of high and low lethal temperatures after acclimation to

to different temperatures characterizes species as to their zone of tolerance (Prosser, 1973). *M. rugosa* can survive temperatures of $<5^{o}C$ for several hours but the upper lethal temperature was found to be approximately $>20^{o}C$. If the animals were kept at this temperature for periods of up to 2 h, the respiratory rates declined progressively, heart beat became irregular and the scaphognathite activity became very erratic. Prolonged exposure to this temperature resulted in the death of the animal.

4.4.3. Effect of feeding on $\dot{M}o_2$

A number of studies have shown that the metabolic rate of decapod crustaceans may be correlated with feeding level (e.g. Aldrich, 1975). Starvation generally results in a gradual reduction of metabolic rate (Aldrich, 1974; Marsden *et al.*, 1973; Wallace, 1973). In *Cancer pagurus*, starvation resulted in a progressive decrease (up to 50%) in the total daily oxygen consumption from the rate normally recorded in fed animals. Subsequent feeding resulted in a period of increased metabolic rate, the duration of which was correlated with the amount of food given (Ansell, 1973). Starvation of *Carcinus maenas* led to similar results (Marsden *et al*, 1973; Wallace, 1973).

When *C. maenas* were fed after a short period of starvation, a significant increase in metabolic rate was recorded which remained elevated for a period of up to 5 days (Wallace, 1973). In *M. rugosa*, however, the metabolic rate (measured as oxygen consumption) remained elevated for a much shorter time (2-8h). This increase in metabolic rate following feeding has been known for many years and has now been recorded in a wide variety of animals (see review by Jobling, 1983). Rubner (1902) called this effect the 'specific dynamic action' (SDA) but the reason for the observed increase in metabolic rate after feeding has been the subject of considerable debate and a variety of explanations have been put forward. For example, some workers consider this effect to be due to the increased metabolic demands associated with digestion and absorption of a

meal (see review by Jobling, 1983). Others believe that the SDA effect may represent a post-absorptive phenomenon. It has been suggested that the increase in metabolic rate may be associated with a short-term increase in the rate of protein synthesis (Rubner, 1902 cited from Lusk, 1931; Ashworth, 1969; Jobling, 1983; Tytler & Calow, 1985). Jobling (1983) has also suggested that a close relationship may exist between thyroid hormone secretion, protein synthesis and metabolic rate which may provide a clue to their involvement in the SDA effect. More recent work in this field (e.g. Houlihan *et al.*, 1988; McMillan & Houlihan, 1988) supports the view that the increase in oxygen consumption following a meal may be due principally to the stimulation of protein synthesis.

As in many other animals, an obvious specific dynamic action was recorded in *Munida rugosa* immediately following feeding. The size of the SDA effect was measured as the percentage increase in $\dot{M}o_2$ over the pre-fed rate recorded in resting animals. The size of SDA effect varied considerably among individuals (20 to 58%). This variability in the size of the SDA effect has also been recorded in other animals. For example, in some fish, the magnitude of the SDA effect varied between 2-45% (Du Preez, 1987).

It has been suggested that, although environmental factors such as temperature may affect the size and duration of the SDA effect, the most important factor is probably the amount of food consumed (Jobling, 1981). For example, in the fish *Pleuronectes platessa*, the size and duration of the SDA effect were found to be correlated with the meal size and also with increasing protein content of the meal (Jobling & Davies, 1980). During the present investigation, however, no significant correlation was obtained between the amount of food consumed and the magnitude or duration of the SDA effect.

It was not intended to carry out a detailed study of the SDA effect in *Munida rugosa* but to briefly investigate the effect that another variable i.e. feeding

level can have on the rate of oxygen consumption of this species. The results presented here therefore provide only some preliminary information on the occurence of the SDA effect in this species. Further work is needed to investigate this more fully.

4.4.4. Effect of declining oxygen tension

The respiratory responses of decapod Crustacea to hypoxia and the interactions between ventilation, perfusion and oxygen uptake have been reviewed by McMahon and Wilkens (1983). The results of some early studies on a variety of aquatic organisms suggested that species could be classified as either 'regulators' or 'conformers' according to whether they could maintain their respiratory rate independent of ambient Po_2. There is now evidence that some of the differences between species recorded by earlier workers may have been due to the experimental procedures used which often did not ensure that the animals were fully quiescent before recordings were carried out. Later experiments appeared to suggest that most animals have some ability to regulate their respiratory rate during exposure to hypoxia and that this ability could be considerably affected by factors such as disturbance or activity, (e.g. Belman & Childress, 1976; McMahon *et al.*, 1979; Herreid *et al.*,1979; Bridges & Brand, 1980; Rutledge, 1981), temperature (e.g. Taylor, *et al.*, 1973; McMahon *et al.*, 1978; Bridges & Brand, 1980), salinity (e.g. Taylor, 1977) and the nutritional status of the animal (e.g. Ansell, 1973; Hamada & Ida, 1973; Marsden *et al.*, 1973; Wallace, 1973; Aldrich, 1975; Jobling & Davies, 1980; Jobling, 1981, 1983; Santos, 1985; Du Preez, 1987). It is now generally accepted that the terms 'regulator' and 'conformer' represent the two extremes of what is probably a variable capacity for maintaining respiratory independence (Taylor & Brand, 1975).

Decapod crustaceans vary considerably in their ability to maintain respiratory independence during hypoxia, even when care has been taken to control for the

above factors. Of those species investigated, the majority of decapod crustaceans are able to maintain their rate of oxygen consumption independent of declining oxygen tension (Arudpragasam & Naylor, 1964; Taylor, 1976; McMahon & Wilkens, 1977; Batterton & Cameron, 1978; Butler *et al.*, 1978; Burnett, 1979; Bradford & Taylor, 1982; Morris & Taylor, 1985). The mechanisms by which the regulation of oxygen uptake under conditions of declining oxygen tension is achieved are reviewed by McMahon & Wilkens (1983).

The most important respiratory response to enable decapods to maintain their rate of oxygen consumption during exposure to hypoxia is to increase the frequency of scaphognathite beating (i.e. hyperventilation) and therefore the ventilatory flow rate across the gills (Taylor & Butler, 1973; Taylor, 1976). This hyperventilation helps to maintain the supply of oxygen to the respiratory surfaces despite the reduced oxygen content of the medium (Young, 1973; Taylor, 1976; Taylor *et al.*, 1973, 1977; Batterton & Cameron, 1978; Butler *et al.*, 1978; Burnett, 1979; McMahon *et al.*, 1978a). However, an increase in ventilatory activity will itself increase the oxygen demand of the animal until eventually the oxygen supplied is insufficient even to meet the energy requirement of the ventilatory pump. It is at this point, which corresponds to the critical oxygen tension (Pc), that respiratory independence can no longer be maintained (McMahon & Wilkens, 1975; Taylor, 1976; Bradford & Taylor, 1982).

The cardiovascular responses to hypoxia have been studied in a number of decapods. Most of the data currently available relate to recordings of changes in heart rate during exposure to hypoxia. In the majority of species studied, heart rate remains approximately independent of Po_2 until at very low oxygen tensions heart rate declines (Blatchford, 1971; Ahsanullah & Newell, 1971;

Taylor *et al.*, 1973; Taylor, 1976; Spaargaren, 1976; Batterton & Cameron, 1978; McMahon *et al.*, 1979; Burnett *et al.*, 1980; Wood & Randall, 1981; Bradford & Taylor, 1982; McMahon & Wilkens, 1983; Massabuau *et al.*, 1984). Some workers, however, have reported an increase in the heart rate during hypoxia (e.g. McMahon *et al.*, 1974).

Accurate data on changes in cardiac output, however, are almost totally lacking. In a few studies, cardiac output has been calculated using the Fick principle. In *Carcinus maenas*, it was found that cardiac output may decline during hypoxia (Taylor, 1976) but in other studies it would appear that there may be an increase in stroke volume of the heart so that cardiac output may actually increase under these conditions (McMahon & Wilkens, 1975; Burnett, 1979).

In many previous studies on a variety of animals, regulatory ability has been expressed in terms of the critical point or Pc, a low value for the Pc indicating a greater ability to maintain respiratory independence under conditions of declining oxygen tension. During the present study, the Pc was determined by visual inspection of the graph relating $\dot{M}o_2$ to changes in ambient Po_2 and was taken to be the point at which $\dot{M}o_2$ began to decline. This technique is obviously subjective and there have been attempts to produce mathematical models to obtain, more objectively, a value for the Pc (Tang, 1933; Bayne, 1971; Mangum & Van Winkle, 1973). Recently, Yeager & Ultsch (1988) have developed a computer programme for the determination of the Pc. This programme divides the data into two sets and calculates the best-fit regression lines which can be fitted to the two data sets. The Pc is then derived as the mid-point of the transition range between these two lines. This technique was applied to the data obtained for *M. rugosa* and *M. sarsi* to compare the results obtained using these two procedures. It was found that the values for the Pc obtained using this mathematical procedure were very similar to those obtained

by simple inspection of the graphs. As might be expected, the agreement was best when the data showed good evidence of regulation of the respiratory rate and when there was a clear change in this rate at low oxygen tensions (Fig. 4.13 A) . When this technique was applied to other sets of data, for example those in which the rates of oxygen consumption were initially higher than expected (at high Po_2), probably because the animal had not yet become completely quiescent, the mathematical procedure often gave values for the Pc which were slightly higher than those estimated visually (Fig. 4.13 B).

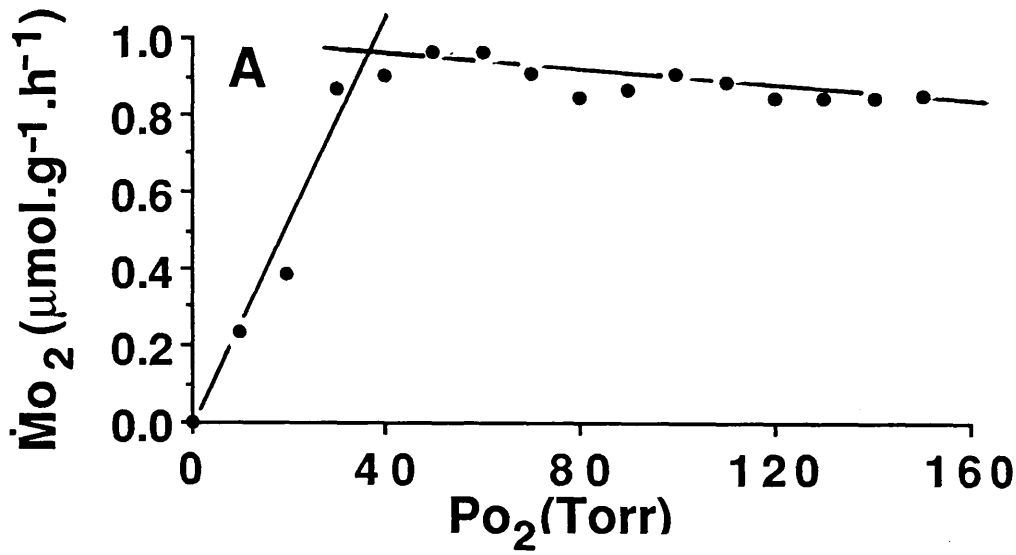
4.4.5. Oxygen debt

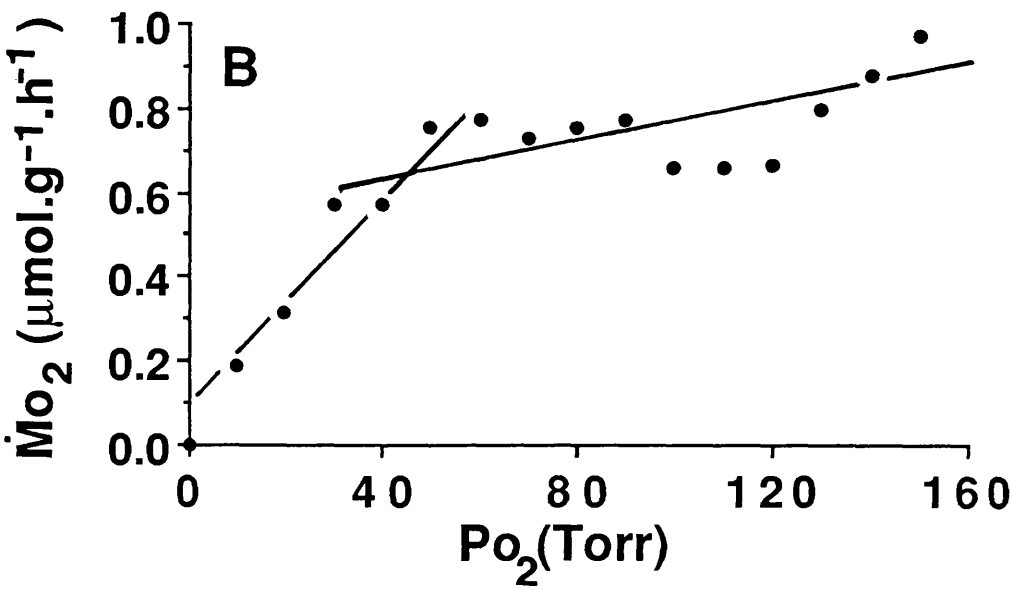
Animals exposed to periods of hypoxia or anoxia incur an 'oxygen debt' due to the use of anaerobic metabolism to meet their energy requirements. The repayment of this 'oxygen debt' was first investigated by Hiestand (1931) and the processes that occur in invertebrates during this recovery period have recently been reviewed by Ellington (1983). The repayment of the 'oxygen debt' involves a significant increase in the rate of oxygen consumption during the recovery period. In decapods, the increase in $\dot{M}o_2$, and in the heart and scaphognathite rates compared with the rates recorded in quiescent animals during the pre-hypoxic period are indications of an increased demand for oxygen during this time (Butler *et al.*, 1978; Bridges & Brand, 1980; Hill, 1989). Similarly, the results of the present study on *Munida rugosa* clearly indicated a significant increase in the metabolic rate of these animals during the recovery period; a pronounced increase in the rate of oxygen consumption and in the heart rate and scaphognathite rate were observed.

This increased oxygen demand appears to be due to a number of factors. Firstly, there is a need to replenish the blood oxygen stores and also stores of arginine phosphate but, most importantly, to metabolize the accumulated end-products of anaerobic metabolism. In decapods, the main end-product is L-lactate (Von Brand, 1946; Bridges & Brand, 1980; Gäde, 1984; Van Aardt &

Fig. 4.13

Comparison between the Pc values estimated visually (A) and by a mathematical method (B). For details see text.


A
1.0
0.8
0.6
0.4
0.2
0.0
0
40
80
120
160
$\dot{M}o_2$ (μmol.g^{-1}.h^{-1})
Po_2(Torr)


B
1.0
0.8
0.6
0.4
0.2
0.0
0
40
80
120
160
$\dot{M}o_2$ (μmol.g^{-1}.h^{-1})
Po_2(Torr)

Wolmarans, 1987; Van Aardt, 1988). The metabolic fate of L-lactate during the recovery period has been the subject of some discussion but there is now growing evidence to show that very little lactate is excreted during recovery (Bridges & Brand, 1980; Gäde *et al.*, 1986). Among decapod crustaceans, excretion of lactate has only rarely been reported. For example, de Zwaan & Skjoldl (1979) found that between 27 and 52% of the total amount of lactate produced by the isopod *Natatolana* (= *Cirolana*) *borealis* during periods of anoxia was excreted into the surrounding water.

Recent studies have now shown that, although some lactate may be completely oxidised to carbon dioxide during periods of recovery (Hill, 1989), most of the lactate produced is converted back into glycogen via gluconeogenic pathways (Gäde *et al.*, 1986; Van Aardt, 1988; Hill, 1989). So far, however, the site(s) at which gluconeogenesis takes place has not yet been identified. There is some indication that the hepatopancreas may be the most important site for gluconeogenesis (Munday & Poat, 1971; Giles *et al.*, 1975) but other possible sites such as the gills and the abdominal muscles have also been suggested (Thabrew *et al.*, 1971; Eichner & Kaplan, 1977).

There is growing evidence to suggest that there may be differences between decapods in their ability to metabolize lactate during the recovery period. For example, Bridges & Brand (1980) observed that the decrease in the blood lactate concentration was much slower in *Galathea strigosa* and *Homarus gammarus* than in the burrowing crab *Corystes cassivelaunus*. In addition, studies of the intertidal prawns, *Palaemon elegans* and *P. serratus* have shown that the former is able to re-metabolize lactate more rapidly during periods of recovery from hypoxia (Taylor & Spicer, 1987). This indicates that there may be differences between species not only in their ability to tolerate periods of exposure to hypoxia or anoxia but also in the time taken to recover from periods of anaerobiosis.

CHAPTER 5. RESPIRATORY PROPERTIES OF THE BLOOD

5.1. INTRODUCTION

In the previous chapter, the results of a number of studies to investigate the effects of several factors on the rates of oxygen consumption of *Munida rugosa* and *M. sarsi* were presented. This work represented part of a study of the respiratory physiology of these species. Such a study would not be complete, however, without a detailed investigation of the oxygen and carbon dioxide transporting properties of the blood.

Our knowledge of the role of the blood of decapod crustaceans in oxygen transport has increased enormously over the past 20 years. Of particular importance has been the improvement in our understanding of the molecular structure of haemocyanins and also of the importance of both inorganic ions and organic molecules in affecting haemocyanin oxygen affinity (Truchot, 1975, 1980; Booth *et al.*, 1982; Mangum, 1983; Bridges *et al.*, 1984; Bridges & Morris, 1986; Lallier & Truchot, 1989; Morris & McMahon, 1989). These studies have clearly demonstrated that a number of factors may directly or indirectly affect the ability of the haemocyanin to transport oxygen. Unfortunately, the exact mechanisms by which these effects are brought about remain unclear and await further investigation.

A study of the role of organic molecules in the modulation of the oxygen affinity of the haemocyanin of *Munida* was considered to be beyond the scope of the present investigation. Instead, work has concentrated on describing the oxygen and carbon dioxide transporting properties of the blood of *Munida rugosa*. Due to problems of animal availability, it has not been possible to carry out such a detailed study on the blood of *M. sarsi*. Nevertheless, attempts have been made to make interspecific comparisons where data are available for both

species.

5.2. MATERIALS AND METHODS

Munida rugosa and *M. sarsi* were collected from sites in the Firth of Clyde and maintained in the sea water aquarium in the Department of Zoology as described previously (Chapter 4). The animals were maintained at a temperature of 10^{o}C and all experiments were carried out at this temperature.

5.2.1. Ionic composition of the blood

The ionic composition of the blood of *Munida rugosa* was determined using blood samples from freshly-collected animals. The blood samples were taken from the arthrodal membrane at the base of the chela or the second walking leg using a 1ml syringe and hypodermic needle. Blood samples from several animals were pooled, mixed well and centrifuged (1000g) for 10 minutes to remove haemocytes and any clotted protein. The pooled sample was then divided into 1ml aliquots and frozen at -20^{o}C.

The concentrations of sodium, potassium and magnesium in the blood were determined using an Atomic Absorption Spectrophotometer (Philips PU9820). Blood samples were diluted with deionized water following the dilution scheme shown in (Fig. 5.1) to ensure that the concentrations of each ion were in the optimum detection range for this instrument. Calcium concentrations were also determined using Atomic Absorption Spectrophotometry after dilution of the blood and the addition of lanthanum chloride (1:5 v/v). Calibration curves were constructed using a series of appropriate standards. Chloride concentrations were determined by electrochemical titration using a chloride meter (Jenway PCLM3).

Fig. 5.1

The dilution scheme used to dilute the blood for the determination of the concentrations of Na, K, Ca, Mg by Atomic Absorption Spectrophotometry and Cl by electrochemical titration in the blood of *Munida rugosa* and *M. sarsi.*

DILUTION SCHEME - BLOOD

							dilution factor
blood	0.2ml	+	dist water 2ml	=	soln. 1	Cl	x11
soln. 1	0.5ml	+	dist. water 4.45ml	+	LaCl 0.55ml	Ca	x121
soln. 1	1ml	+	dist. water 10ml	=	soln. 3	K	x121
soln 3	0.2ml	+	dist. water 10ml			Na	x6171
soln 3	1ml	+	dist. water 2ml			Mg	x363

5.2.2. Lactate concentration of the blood

The concentration of L-lactate in the pooled blood samples was determined since it is now known that L-lactate can act as a modulator of haemocyanin oxygen affinity (Truchot, 1980; Booth *et al.*, 1982; Johnson *et al.*, 1984; Bridges & Morris, 1986). The method used was based on that of Gutman & Wahlefeld (1974) with the modifications suggested by Engel & Jones (1978). A 100µl sample was taken from the pooled blood and added to 100µl of 0.3M perchloric acid (PCA). The resulting solution was mixed thoroughly and centrifuged (10 min at 10000g) to remove the coagulated protein. The supernatant was transferred to a second Eppendorf tube and neutralized by the addition of a small volume of potassium bicarbonate. The pH was checked using Whatman indicator paper (pH range = 1-14). The final reaction mixture was as follows:

Hydrazine Glycine buffer (pH 9)	1000µl
NAD (40 mM)	50µl
Lactate Dehydrogenase	6µl
Blood sample or standard	100µl

A blank was run in which the blood sample was replaced by an equal volume of distilled water. A calibration curve was constructed using a series of L-lactate standards. After thorough mixing, the tubes were placed in a water bath (37°C) and the reaction allowed to proceed for 1-2 h. The absorbance of each solution was then read at 340nm using a spectrophotometer (Philips PU8620).

5.2.3. *In vivo* Po_2 and pH of the blood

The Po_2 of the pre- and post-branchial blood of *Munida* was determined in quiescent animals maintained under normoxic conditions. Pre-branchial blood (P_vO_2) samples were obtained from the arthrodal membrane at the base of the chela or the second walking leg. Post-branchial blood (P_aO_2) samples were

obtained by inserting the needle of a 1 ml hypodermic syringe into the pericardium through the membrane between the cephalothorax and the abdomen. Care was taken to ensure that the samples were collected as rapidly as possible and with minimal disturbance to ensure that the Po_2 values obtained represented as closely as possible the *in vivo* oxygen tensions of the pre- and post-branchial blood. In addition, the syringes used were flushed with nitrogen gas prior to sampling to replace any oxygen in the dead space of the syringe. If the sample was not obtained quickly, or if the animal showed any signs of disturbance, the sample was discarded. The Po_2 of each blood sample was determined immediately by injecting the sample into a thermostatted cuvette (at $10^{o}C$) containing an oxygen electrode (E5046, Radiometer, Copenhagen) which was in turn connected to an oxygen meter (Strathkelvin Instruments).

The pH of the same blood samples were measured using the microcapillary pH electrode of a Radiometer BMS2 connected to a pH meter (Corning 255 Ion Analyzer).

5.2.4. Oxygen carrying capacity of the blood

The oxygen carrying capacity of the blood is the amount of oxygen that can be carried per unit volume of the blood when the respiratory pigment is saturated. Generally, the oxygen carrying capacity of blood is determined by equilibrating the blood with air then measuring the total oxygen content. The total oxygen carrying capacity of whole blood was determined for individuals of *M. rugosa* (both males and females of differing sizes). The oxygen carrying capacity was also determined for the pooled blood sample. The total oxygen carrying capacity includes oxygen which is bound to the haemocyanin plus that physically dissolved in the blood. The total oxygen content of the blood (cO_2) was determined using the method of Tucker (1967) as modified by Bridges *et*

al. (1979). The equipment used for this procedure was a small thermostatted perspex chamber (volume = 300 µl) containing an oxygen electrode (E5046, Radiometer, Copenhagen) and a small magnetic stirrer bar to ensure complete mixing of the solution. The electrode was connected to an oxygen meter (Radiometer PHM73 Blood gas analyser) which was in turn connected to a pen recorder (Tekman). The chamber was filled with a 1% solution of potassium cyanide and the Po_2 of the solution recorded. The blood sample (10 µl) was then injected into the chamber using a Hamilton microsyringe. The KCN reacted with the haemocyanin to displace all the oxygen bound to the respiratory pigment with the result that the Po_2 of the solution increased. The total oxygen content of the blood was then calculated from the difference between the initial Po_2 of the solution and Po_2 after the introduction of the blood sample using the following equation (Bridges *et al.*, 1979):

$$cO_2 = Po_2 \times \alpha \times cv \times 100 / 760 \times sv$$

where ΔPo_2 = Po_2 (final) - Po_2 (initial) x (cv-sv/cv)

and α = solubility coefficient for O_2 in the cyanide solution (at 30^oC α = 0.0261 ml.100 ml^{-1}); cv = chamber volume; sv = sample volume.

The haemocyanin-bound O_2 content of the blood sample was then calculated from the equation:

Oxygen bound to haemocyanin = total O_2 content - physically dissolved O_2.

The results obtained using this procedure were also compared with those obtained using an indirect method of calculating the oxygen carrying capacity based on the copper concentration of the blood. A 10µl blood sample was diluted with 1ml of distilled water in a cuvette and the absorbance (at 335 nm) of the solution recorded using a spectrophotometer (Philips PU8620). The carrying capacity of the haemocyanin was then calculated using the extinction coefficient for the blood of *Carcinus maenas* ($E^{1\%}_{1cm}$ = 2.33) (Nickerson & Van Holde, 1971) and by assuming that the molecular weight of the oxygen binding

site in *M. rugosa* is 75000 Dalton as in many other decapods (Magnum 1983a).

An alternative procedure was also used to calculate the carrying capacity of the haemocyanin. In this method the protein concentration of the blood was measured by recording the absorbance (at 280nm) of the diluted blood samples. The oxygen carrying capacity was then calculated using the extinction coefficient (at 280 nm) determined for the blood of *Carcinus maenas* (Nickerson & Van Holde, 1971). Both methods are subject to certain errors, however, since the actual extinction coefficients for the blood of *M. rugosa* were not determined during this study and these may vary slightly from those of *C. maenas*. In addition, the former method assumes that all the copper in the blood is present in the haemocyanin molecule and the latter method assumes that all the protein present in the blood is haemocyanin. Although copper is present primarily in the haemocyanin molecule it is now known that some copper may also be present bound to other proteins e.g. metallothioneins (Horn & Kerr, 1963; Williams, 1984; White & Rainbow, 1985; Rainbow, 1988). Similarly, although most of the protein content of the blood is known to be haemocyanin, other proteins are also present. The haemolymph proteins of the crab *Carcinus maenas* have been studied by Uglow (1969). The three major proteins found were haemocyanins (fast and slow) and an intermediate type (apohaemocyanin). The apohaemocyanin protein has no respiratory role but it is transferrable to haemocyanin (see Horn & Kerr, 1963). In addition, the haemolymph of decapod crustaceans also contain glycoproteins, lipoproteins and fibrinogen-like clotting proteins (see Manwell & Baker, 1963; Horn & Kerr, 1969; Uglow 1969; Rochu & Fine, 1979). It has been estimated that in *Callinectes sapidus*, 68-69% of the haemolymph protein is haemocyanin, the remaining protein being apohaemocyanin (Horn & Kerr, 1963).

The oxygen carrying capacity of the blood of 31 individuals was determined from blood samples which were taken as soon as the animals arrived in the

department, since it is known that starvation or poor feeding conditions can affect the total protein content of the blood and therefore its carrying capacity (e.g. Uglow, 1969). Measurements of the oxygen carrying capacity were also carried out on another group of 24 animals which had been kept in the aquarium for a few days before the blood samples were taken to determine whether this had an effect on the oxygen carrying capacity of the blood.

5.2.5. Oxygen affinity of the haemocyanin

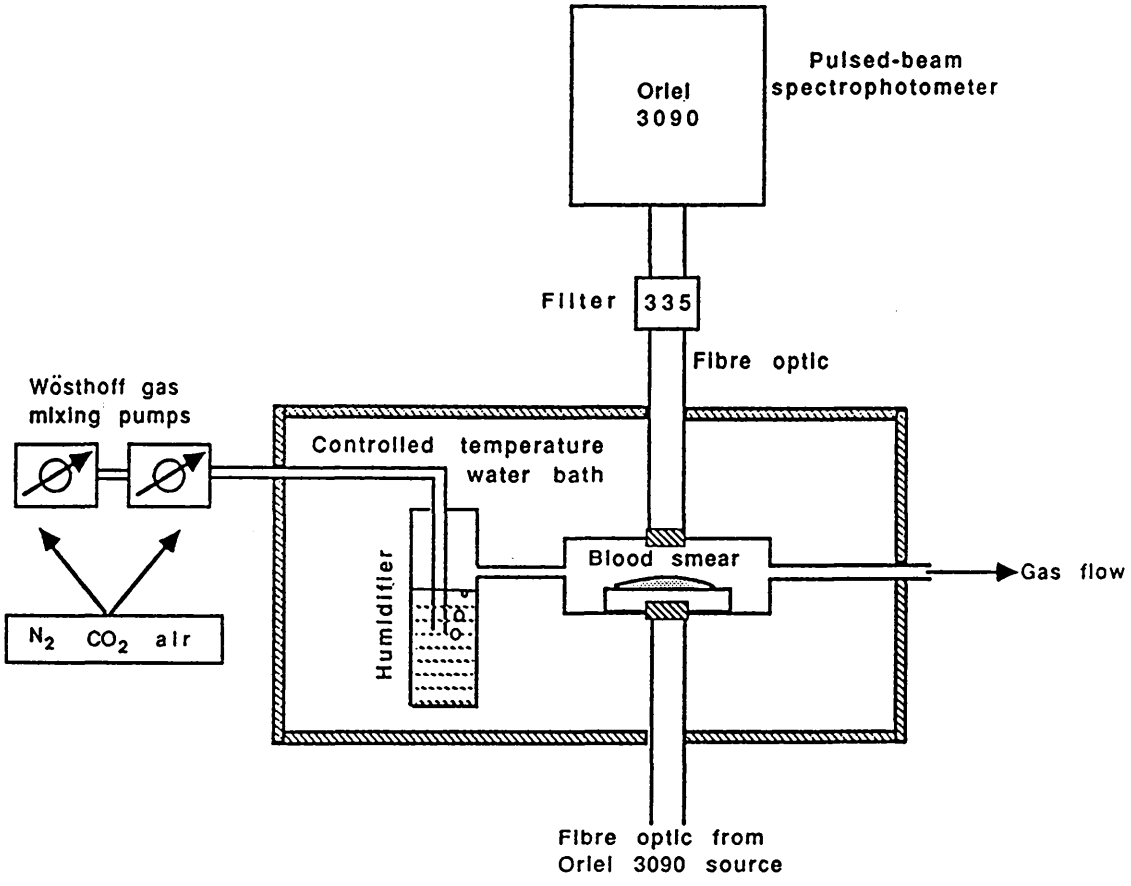
In vitro oxygen dissociation curves for the blood of *M. rugosa* and *M. sarsi* were constructed spectrophotometrically using a diffusion chamber (Fig. 5.2) (Sick & Gersonde, 1969). The changes in the absorbance (at 335 nm) associated with changes in the saturation of the haemocyanin was detected by means of two fibre optics cables connected to a spectrophotometer (Oriel Scientific 3090). A small sample (3-5 µl) taken from the pooled blood samples was placed on a glass slide which was then placed inside the diffusion chamber situated inside a thermostatted housing. The blood was equilibrated against gas mixtures, supplied by Wösthöff gas mixing pumps (M301), having the required partial pressures of oxygen and carbon dioxide. The Po_2 of the gas mixture was increased in a step-wise manner while the Pco_2 was kept constant for each curve determined. The gas mixture was saturated with water vapour before entering the diffusion chamber to prevent the blood sample from drying out.

The blood was allowed to equilibrate to the experimental temperature for 20 minutes prior to the start of each determination. It was also important that sufficient equilibration time was allowed to ensure that the blood was in complete equilibrium with the gas mixture at each of the Po_2 increments.

Dissociation curves were constructed at different pH's to examine the magnitude of the Bohr factor in these species. The pH of the blood was altered by varying the proportion of carbon dioxide in the gas mixture. The pH of the

Fig. 5.2

Diagram of the diffusion chamber used to construct oxygen dissociation curves for the blood of *Munida*.


Orlel
3090
Pulsed-beam
spectrophotometer
Filter
335
Fibre optic
Wösthoff gas
mixing pumps
Controlled temperature
water bath
Blood smear
Gas flow
Humidifier
N_2 CO_2 air
Fibre optic from
Orlel 3090 source

blood was measured by tonometering another blood sample (100 µl) in a Radiometer BMS2 against the same gas mixtures supplied to the diffusion chamber. The pH of the blood was determined at the P_{50} using the microcapillary pH electrode of the BMS2 connected to a pH meter (Corning model 255 ion analyser).

The P_{50} (i.e. the Po_2 at which the pigment is 50% saturated) was estimated from saturation values between 25% and 75% according to the Hill equation. The data were also plotted in the form of Hill plot in which the O_2-affinity (P_{50}) of the haemocyanin may be determined as the intercept on the X axis of the regression line fitted to these data. According to the Hill plot, the slope of this regression line represents the numerical value for the cooperativity (n_{50}).

The effect of temperature on the oxygen affinity of the haemocyanin was also investigated by constructing oxygen dissociation curves over a range of temperatures between 5 and 20°C. The effect of temperature on the oxygen affinity of the haemocyanin can be quantified by calculating the change in enthalpy (ΔH) accompanying oxygenation of the haemocyanin at a constant pH (7.8) using the following equation:

$$\Delta H = -2.303 \times \frac{R \times \Delta \log P50}{1/T_1 - T_2} \quad (kJ.mole^{-1})$$

5.2.6. Carbon dioxide equilibrium curves

The blood transports carbon dioxide in three forms: CO_2 bound directly to the haemocyanin, as bicarbonate ions and as physically dissolved CO_2. The total carbon dioxide content (cCO_2) of the blood of *Munida rugosa* was measured using the method of Cameron (1971). This technique is similar to the Tucker method used to measure the total oxygen content of the blood but, in this case, the chamber is filled with dilute acid to ensure that all the carbon dioxide is

converted into the physically dissolved form.

The chamber was filled with dilute hydrochloric acid (0.01N) and allowed to equilbrate to the experimental temperature (30°C). This temperature was used to increase the speed of the reaction. At the end of the equilibration period the Pco_2 of the solution was recorded using a carbon dioxide electrode (E5037, Radiometer, Copenhagen) connected to a Radiometer PHM73 blood gas analyser.

During this time, blood was tonometered in a Radiometer BMS2 against gas mixtures, supplied by Wösthöff gas mixing pumps, having a known carbon dioxide content. After the blood had been tonometered against the gas mixture for a period of 20 minutes, a small sample (10 µl) was removed and injected into the Tucker chamber using a Hamilton microsyringe. The increase in the Pco_2 of the solution in the chamber, resulting from the conversion of the different forms of carbon dioxide into gaseous CO_2, was recorded by the CO_2 electrode.

The system was calibrated by injecting a 10µl sample of a freshly-prepared solution of sodium bicarbonate (10 $mmol.l^{-1}$) into the chamber and recording the resulting change in Pco_2. The total CO_2 contents of replicate blood samples which had been tonometered against gas mixtures containing differing concentrations of CO_2 were determined using this procedure. In addition, the pH of the blood was measured at each determination.

The total CO_2 content of the blood (i.e. CO_2 bound to the haemocyanin plus dissolved CO_2) was calculated as follows:

ΔPco_2 = initial Pco_2 (acid only) - final Pco_2 (with blood)

cCO_2 ($mmol\ l^{-1}$) = ΔPco_2 (blood) / ΔPco_2 (standard)

Since the ability of the haemocyanin to combine with CO_2 is also known to be

affected by its oxygenation state (Haldane effect). Carbon dioxide equilibrium curves were constructed for both oxygenated (Po_2 = 150 Torr) and deoxygenated (< 1 Torr) blood. The magnitude of the Haldane effect, which may be defined as the difference in the total CO_2 content between oxygenated and deoxygenated blood at a specific Pco_2, can be estimated from the following ratio $\Delta cCO_2 / \Delta cO_2$ (Truchot, 1976a). The capacitance coefficient (b) which is defined as the ratio of the increment of CO_2 concentration to the increment of Pco_2 ($b = \Delta cCO_2 / \Delta Pco_2$) was also calculated (Piiper *et al.*,1971; Truchot, 1976b).

The effect of temperature on the CO_2 equilibrium curves was also examined since the CO_2 solubility coefficient is known to be temperature dependent (Truchot, 1983). *In vitro* carbon dioxide equilibrium curves for the blood of *M. rugosa* were therefore obtained for oxygenated and deoxygenated blood at 5, 10, 15, & 20^{o}C. In addition, CO_2 equilibrium curves (at 5^{o}C and 10^{o}C) for the blood of *M. sarsi* and for *M. rugosa* from the shallow and deep sites were also compared.

The concentration of bicarbonate ions (HCO_3^-) was calculated from the following relationship:

$$cCO_2 = [HCO_3^-] + [CO_3^{2-}] + \alpha CO_2 . Pco_2$$

where αCO_2 is the solubility coefficient for CO_2 obtained from the nomogram of Truchot (1976a) at the appropriate temperature and salinity. The amount of carbonate in blood is always very small in aquatic animals at low temperatures and can be neglected (Truchot, 1983). The calculated HCO_3^- concentrations were plotted against the corresponding pH values for the blood since the slope of the regression line fitted to these data is equivalent to the buffering capacity of the blood.

5.3. RESULTS

5.3.1. Ionic composition of the blood

The concentrations of the major ions in the blood of *M. rugosa* are presented in Tables 5.1 & 5.2. There was no significant difference (t-test) between the mean values for the individual blood sample and the pooled blood. In addition, there was no significant variation between individual animals ($P > 0.05$).

The concentration of L-lactate in the pooled blood sample was low (0.25 mM). Higher values (2.5 mM) were obtained, however, in individuals which were slightly stressed.

5.3.2. Po_2, pH and oxygen carrying capcity of the blood

Mean values for the Po_2 of the pre- and post-branchial blood of *M. rugosa* were 38 ± 9 (n = 23) and 86.2 ± 11 (n = 22) Torr respectively. The mean values for the pH of the pre- and post-branchial blood were 7.73 ± 0.2 (n = 20) and 7.89 ± 0.15 (n = 10) respectively. A pH of 7.8 was taken to represent the physiological pH in this species (see below).

No significant differences were obtained in the values for the oxygen carrying capacity of the blood determined using the three different techniques. The mean value for the oxygen carrying capacity of the blood of freshly caught *M. rugosa* was 1.7 ± 0.34 ml O_2. 100 ml $blood^{-1}$ (n = 31) whereas animals which had been kept in the aquarium for several days had a slightly lower oxygen carrying capacity 1.5 ± 0.37 (n = 24). The difference between these two values, however, was found to be not significant (t-test; $P > 0.05$). After subtraction of the amount of oxygen which is present in physical solution, the oxygen carrying capacity of the haemocyanin of *M. rugosa* was calculated to be 1.6 ml.100 ml $blood^{-1}$. In addition, the oxygen carrying capacities of the blood of *M. rugosa*

Table 5.1. The concentrations of major ions in blood samples from individual *M. rugosa*. Values are means ± S.D (n = 6).

		$mg.ml^{-1}$	mM
Na^+	=	8.14 ± 0.24	354.3 ± 10.90
K^+	=	0.40 ± 0.01	010.3 ± 0.29
Mg^{2+}	=	0.98 ± 0.02	040.3 ± 0.97
Ca^{2+}	=	0.43 ± 0.01	010.7 ± 0.33
Cl^-	=	4.73 ± 0.36	416.0 ± 10.00

Table 5.2. Ionic concentrations of the pooled blood:

		$mg.ml^{-1}$	mM
Na^+	=	10.50	456.30
K^+	=	0.44	11.14
Mg^{++}	=	1.02	41.81
Ca^{++}	=	0.52	12.98
Cl^-	=	15.02	425.10

from the deep water and for *M. sarsi* were 1.7 and 1.9 ml.100 ml^{-1} respectively.

5.3.3. O_2 dissociation curves

Oxygen dissociation curves for the blood of *M. rugosa* from the shallow water site are shown in (Fig. 5.3). The P_{50} value for each of these curves was obtained by linear transformation of the data using the Hill plot (Fig. 5.4). The values for P_{50} together with values for the cooperativity (n_{50}) and the Bohr factor (θ) for the blood of *M. rugosa* from both the shallow and deep water sites and for *M. sarsi* at the four experimental temperatures are given in Table 5.3. At 10°C and a pH of 7.85, the P_{50} was 20.3 Torr for *M. rugosa* from the shallow water and 38.5 Torr for deep water animals . This indicates that the haemocyanin of *M. rugosa* has a low oxygen affinity. The haemocyanin of *M. sarsi* was found to have an even lower oxygen affinity (P_{50} = 49.6 Torr, pH = 7.9; 10°C).

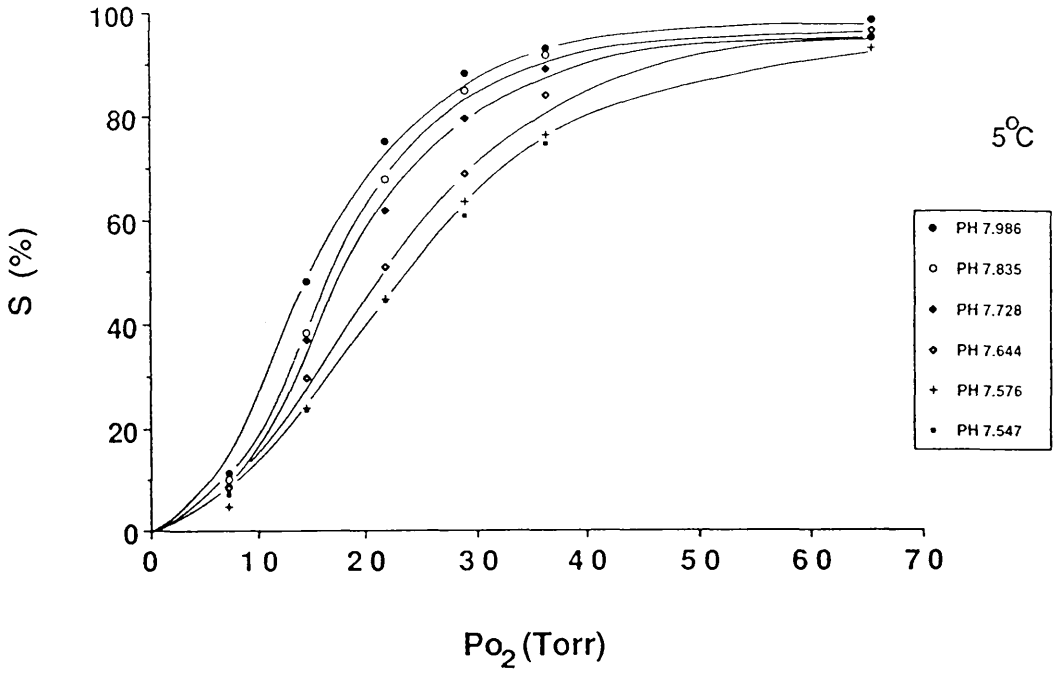
Both species demonstrated a normal Bohr effect since there was an inverse relationship between log P_{50} and pH (Fig. 5.5A). The slope of the regression line fitted to these data indicates the size of the Bohr shift. Values for the Bohr factor (θ) and for n_{50} for the haemocyanin of *M. sarsi* (θ = -0.43; n_{50} = 3.63 ± 0.09) and for *M. rugosa* from deep water (θ = -0.39; n_{50} = 3.68 ± 0.04) did not differ significantly from those values obtained for *M. rugosa* from shallow water sites (n_{50} = 3.33 ± 0.14).

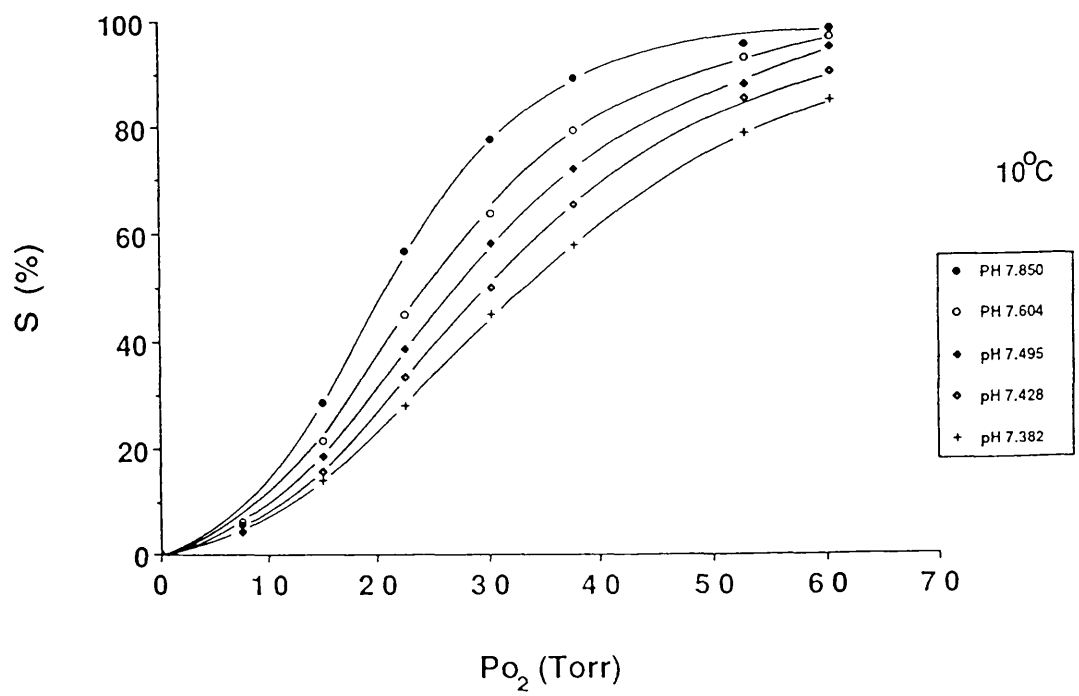
The cooperativity of the haemocyanin remained approximately independent of pH although at very low pH there was a noticeable reduction in the value of n_{50} in both *M. sarsi* and *M. rugosa* from both deep and shallow water sites (Fig. 5.5B).

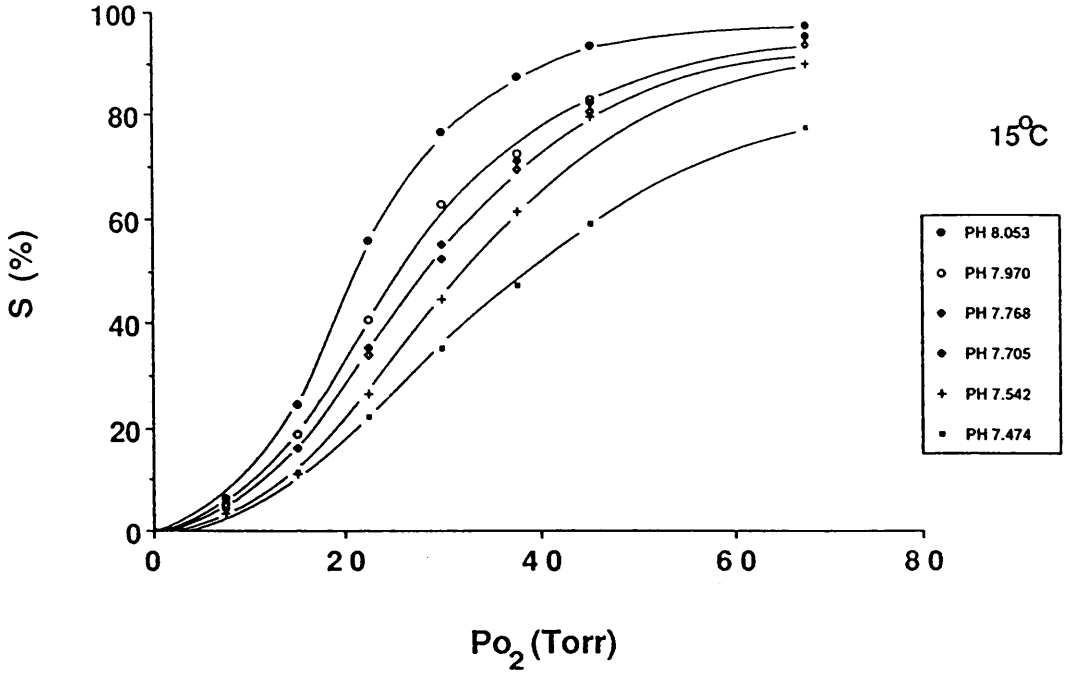
As in many other decapods, temperature had a pronounced affect on the oxygen affinity of the haemocyanin; an increase in temperature resulted in an

Fig. 5.3

Oxygen dissociation curves for the whole blood of *M. rugosa* at 5, 10, 15 and 20°C. The pH of the blood was altered by adjusting the proportion of CO_2 in the gas mixtures with which the blood was equilibrated. For further details see text.


100
80
60
40
20
0
S (%)
0 10 20 30 40 50 60 70
Po2 (Torr)
5°C
PH 7.986
PH 7.835
PH 7.728
PH 7.644
PH 7.576
PH 7.547


100
80
60
40
20
0
S (%)
0 10 20 30 40 50 60 70
Po2 (Torr)
10°C
PH 7.850
PH 7.604
pH 7.495
pH 7.428
pH 7.382


100
80
60
40
20
0
S (%)
0
20
40
60
80
Po2 (Torr)
15°C
PH 8.053
PH 7.970
PH 7.768
PH 7.705
PH 7.542
PH 7.474

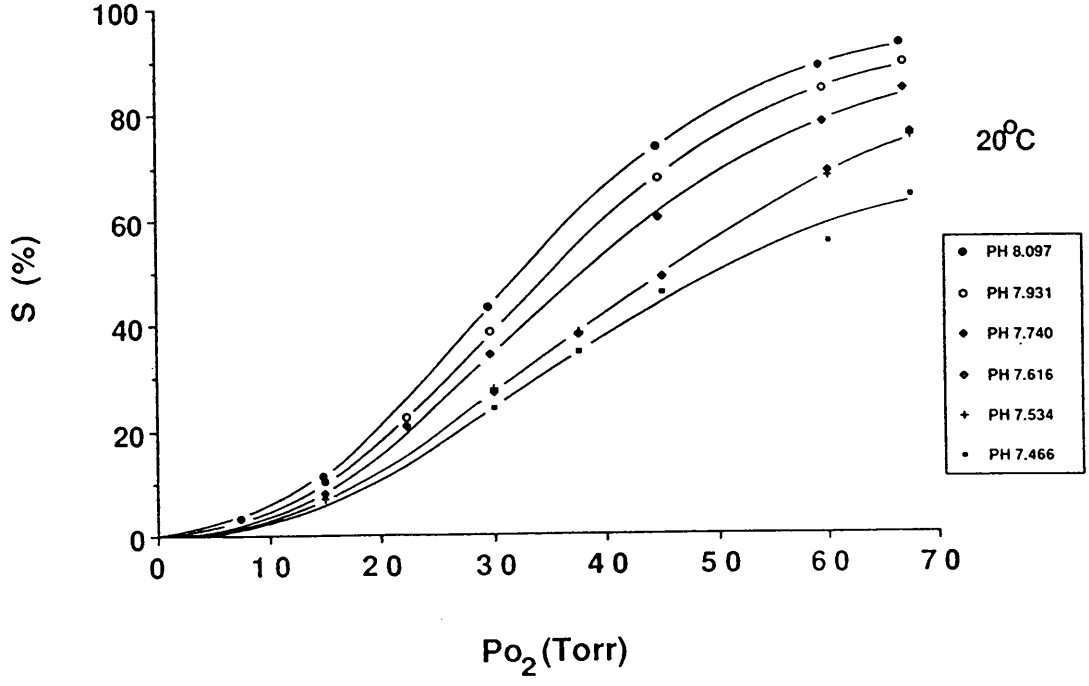

100
80
60
40
20
0
S (%)
0
10
20
30
40
50
60
70
Po2 (Torr)
20°C
PH 8.097
PH 7.931
PH 7.740
PH 7.616
PH 7.534
PH 7.466

Fig. 5.4

A Hill Plot of the oxygen dissociations curves constructed at 10°C for the blood of *Munida rugosa* (see Fig. 5.3). The P_{50} for each curve is the Po_2 at which the value of log S/1-S = 0. The slope of the regression line fitted to the data represents the cooperativity (n_{50}) of the haemocyanin.

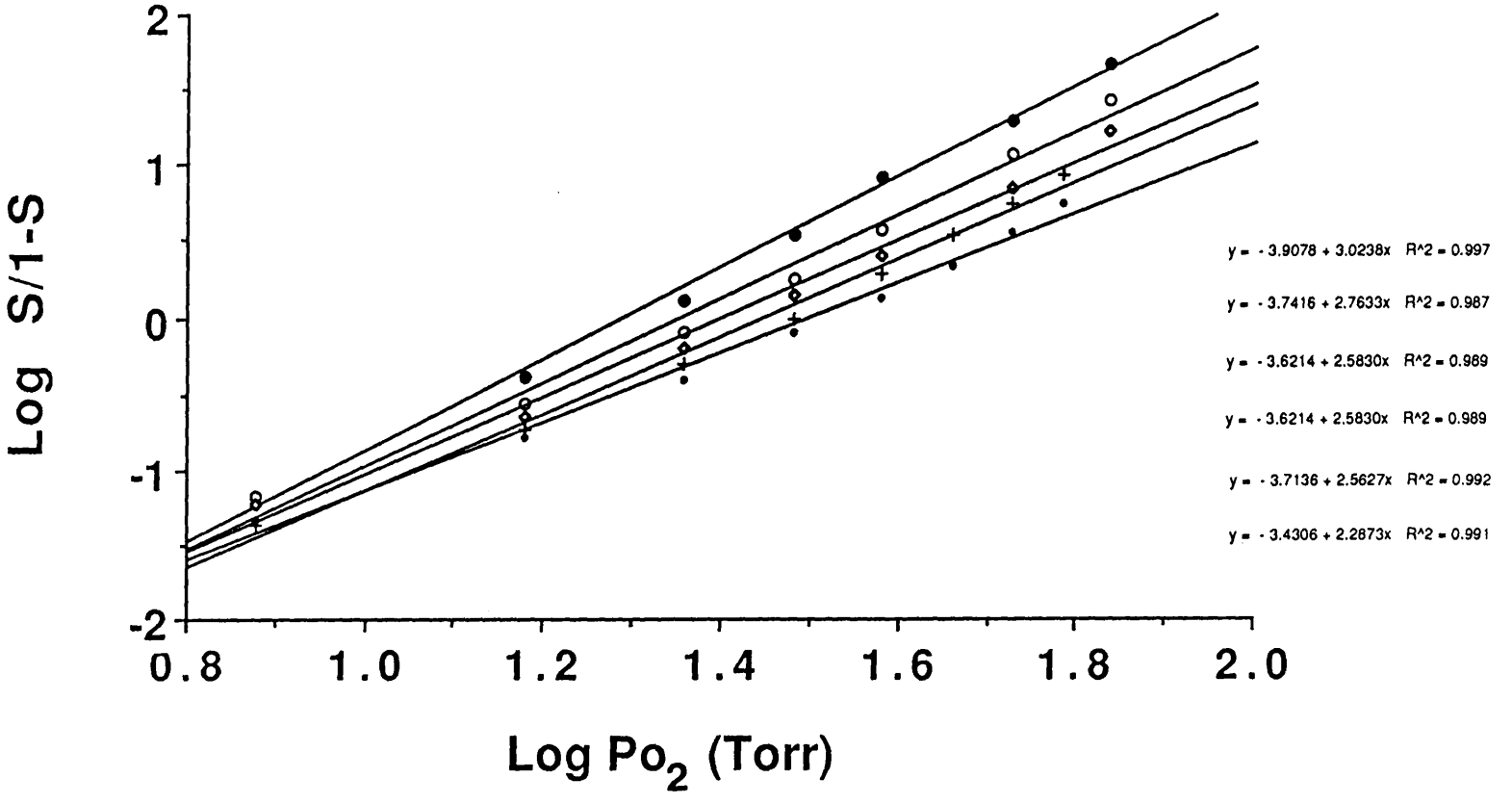

Log S/1-S
2
1
0
-1
-2
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Log Po2 (Torr)
y = -3.9078 + 3.0238x R^2 = 0.997
y = -3.7416 + 2.7633x R^2 = 0.987
y = -3.6214 + 2.5830x R^2 = 0.989
y = -3.6214 + 2.5830x R^2 = 0.989
y = -3.7136 + 2.5627x R^2 = 0.992
y = -3.4306 + 2.2873x R^2 = 0.991

Fig. 5.5

The relationship between oxygen affinity (P_{50}) and pH (A) and cooperativity n_{50} and pH (B) at 10°C for the whole blood of *M. rugosa* from both shallow (●) and deep water (○) sites and for *M.sarsi* (△). The Bohr values are equivalent to the slopes of the regression lines fitted to these data.

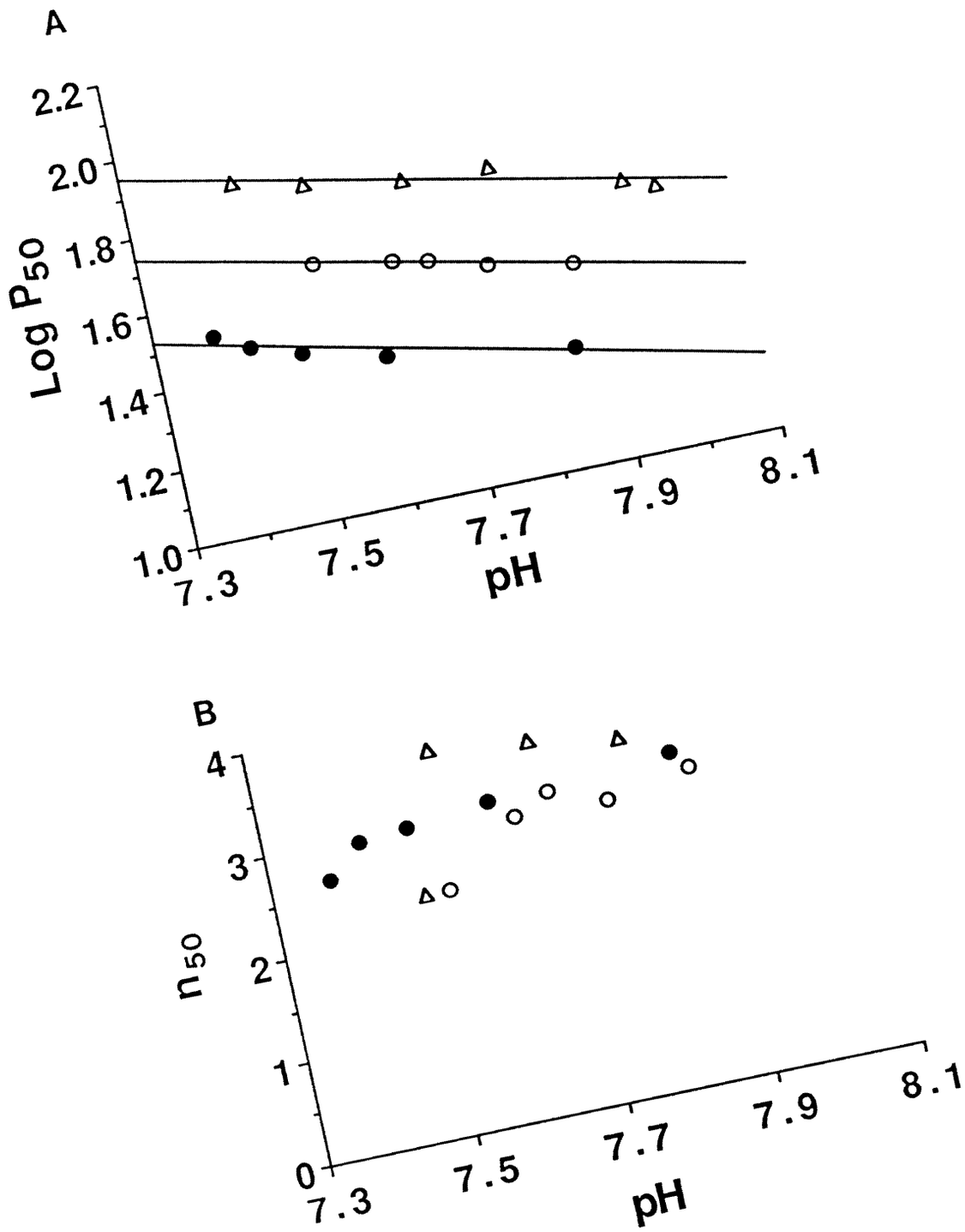

A
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Log P50
7.3
7.5
7.7
7.9
8.1
pH
B
4
3
2
1
0
n50
7.3
7.5
7.7
7.9
8.1
pH

Table 5.3. The effect of temperature on the Bohr value (θ), P_{50}, and n_{50} (at pH = 7.8) of the blood of *Munida rugosa* from shallow and deep water sites and for *M. sarsi*.

T°C	θ	P_{50}	log P_{50}	n_{50}
M. rugosa (shallow)				
5	-0.61	16.7	1.22	2.96
10	-0.42	20.3	1.31	3.19
15	-0.37	27.9	1.45	3.36
20	-0.28	34.1	1.53	3.10
M. rugosa (deep)				
5	-0.62	23.1	1.36	3.20
10	-0.39	38.5	1.59	3.00
M. sarsi.				
5	-0.57	38.33	1.59	3.42
10	-0.43	49.56	1.70	3.53

increase in P_{50} (Fig. 5.6), (Table 5.3). Analysis of covariance was used to compare the regression lines fitted to the data showing the relationship between log P_{50} and pH at different temperatures. These analyses indicated that there was no significant difference in the slopes of the lines ($P > 0.05$) but the elevations of the regression lines did differ significantly. The calculated values for ΔH were as follows:

$\Delta H_{(5\text{-}10)}$ = -17.6

$\Delta H_{(5\text{-}15)}$ = -42.9

$\Delta H_{(10\text{-}20)}$ = -43.1

$\Delta H_{(5\text{-}20)}$ = -89.3

Δ H calculated for a temperature change between (5-10^{o}C) for *M. rugosa* from deep water sites and for *M. sarsi* were -21.3 kJ.mole^{-1} and -20.7 kJ.mole^{-1} respectively. The ΔH for *M. rugosa* from deep water and *M. sarsi* did not differ significantly, although Δ H for *M. rugosa* from the shallow water had a slightly smaller value (see above).

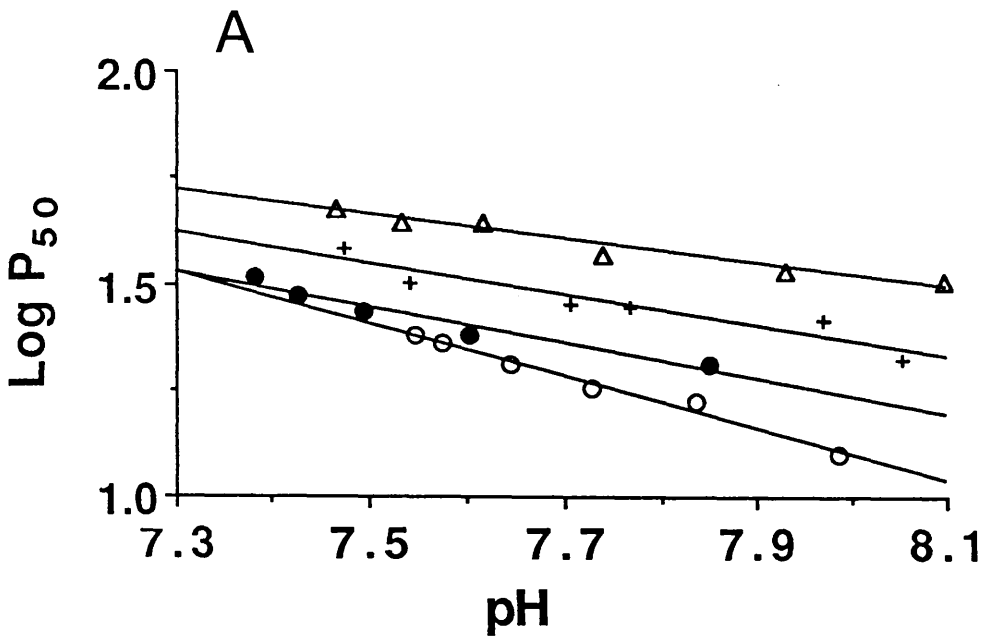
Temperature also appeared to affect the size of the Bohr factor since there was an inverse relationship between the Bohr factor and temperature (Table 5.3). In contrast, the cooperativity of the haemocyanin (n_{50}) remained approximately constant.

5.3.4. Co_2 equilibrium curves

In vitro carbon dioxide equilibrium curves for the blood of *M. rugosa* collected from the shallow water site are shown in Fig. 5.7. The blood showed a moderate capacitance for CO_2 transport ($\beta = \Delta cCO_2 / \Delta Pco_2$ = 3.45 and 3.37 mmol.l^{-1}.Torr^{-1} under deoxygenated and oxygenated conditions respectively). The blood of *M. rugosa* exhibited a Haldane effect i.e. at constant Pco_2, the CO_2 content of the deoxygenated blood was greater than that of the oxygenated blood. The Haldane coefficient ($=\Delta cCO_2$ / $cHcyO_2$) calculated at

Fig. 5.6

The effects of temperatures, 5°C (○), 10°C (●), 15°C (+), and 20°C (△) on the relationship between oxygen affinity (P_{50}) and pH (A) and between cooperativity (n_{50}) and pH (B) for the whole blood of *M. rugosa* from the shallow water site.


A
2.0
1.5
1.0
Log P_{50}
7.3
7.5
7.7
7.9
8.1
pH

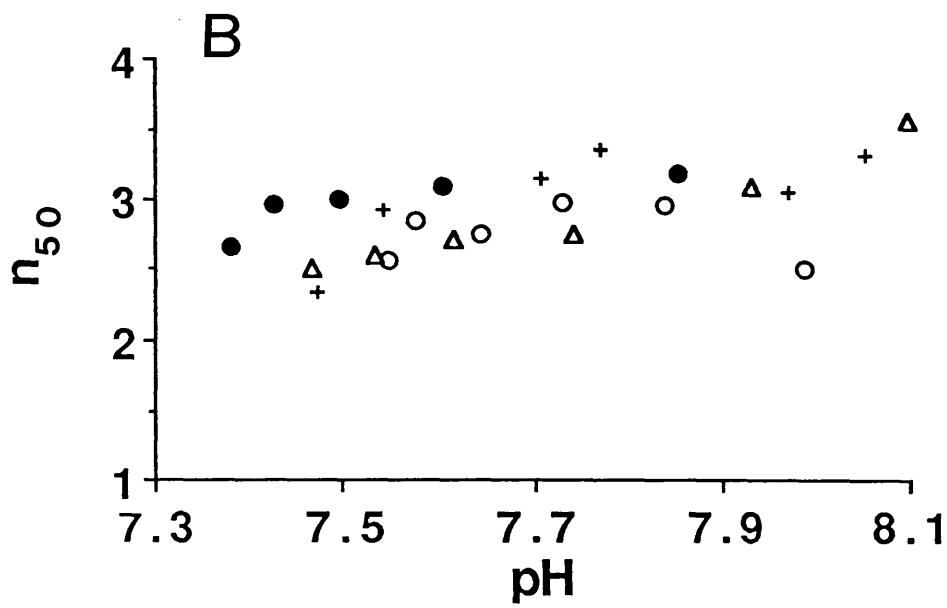
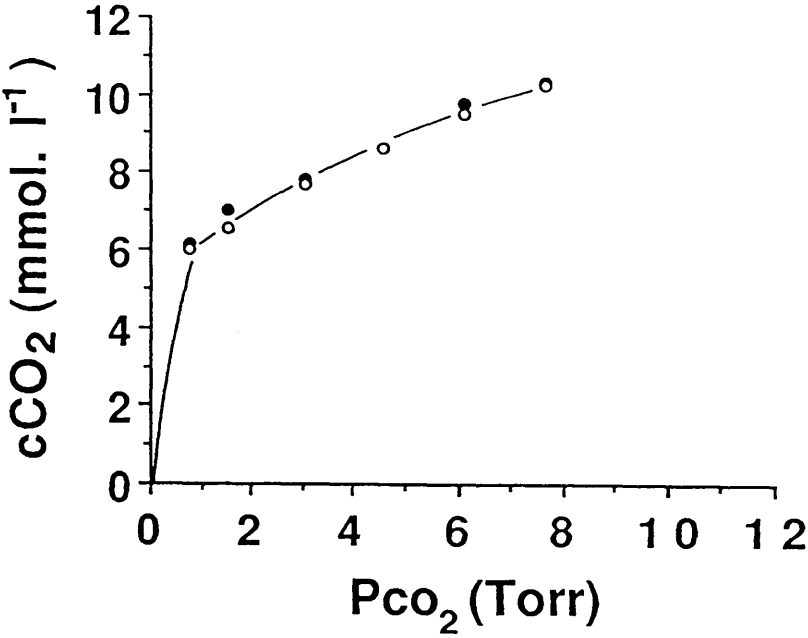
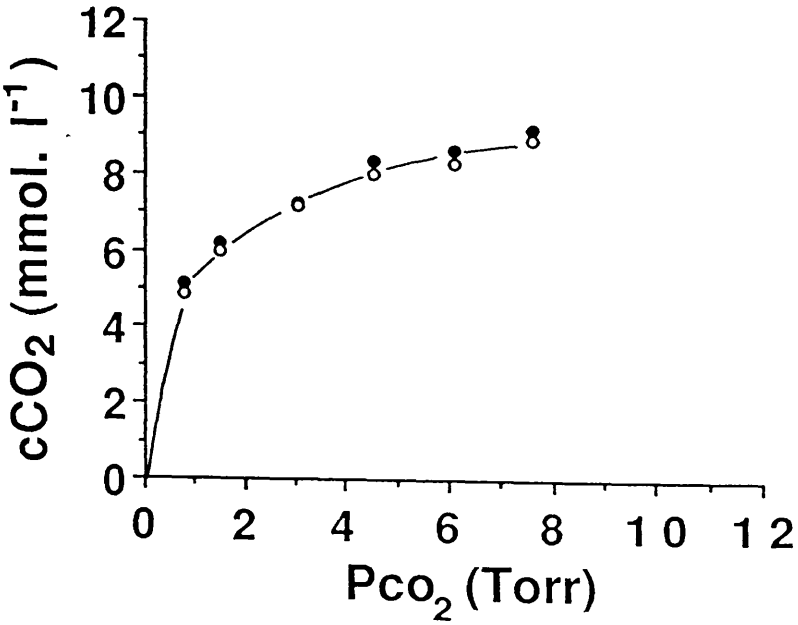
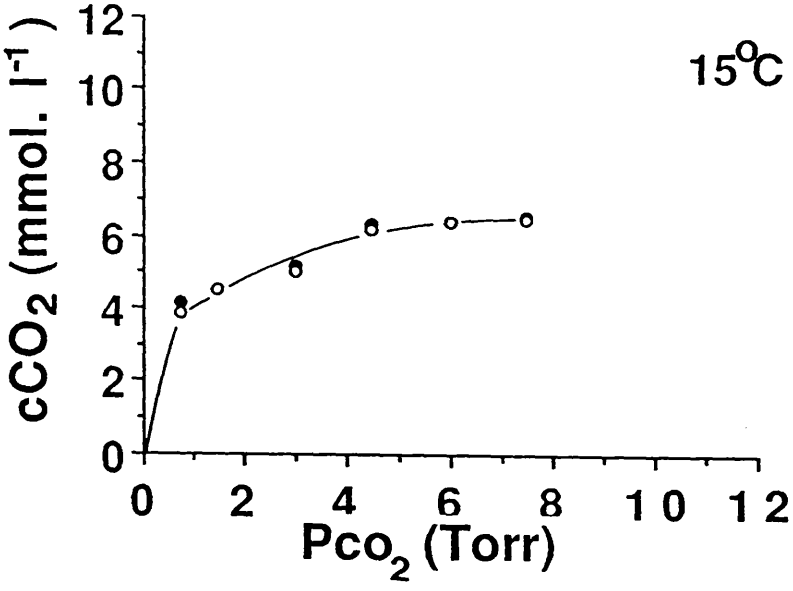

B
4
3
2
1
n_{50}
7.3
7.5
7.7
7.9
8.1
pH

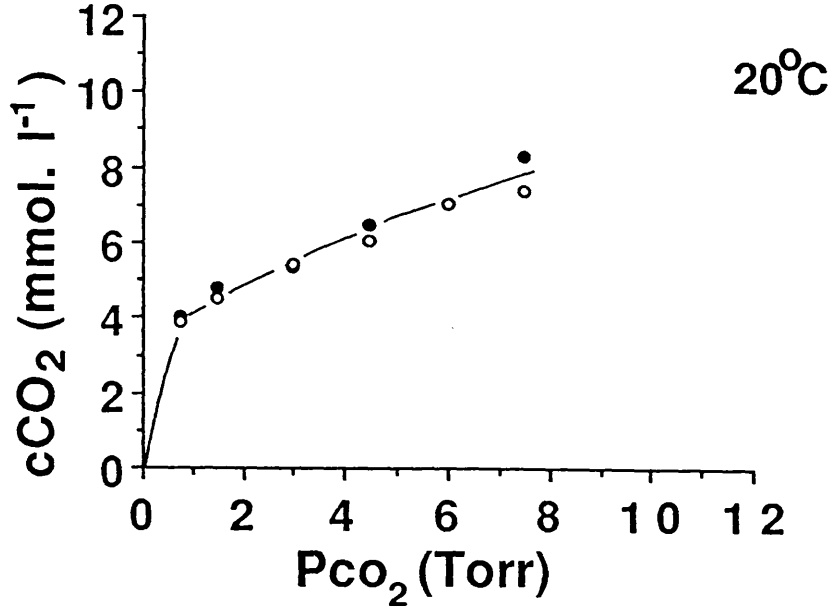
Fig. 5.7

Carbon dioxide equilibrium curves for oxygenated (○) and deoxygenated (●) blood of *M. rugosa* from the shallow water site. The curves were constructed at 5, 10, 15, and 20°C.


12
10
8
6
4
2
0
cCO2 (mmol. l-1)
0
2
4
6
8
10
12
Pco2 (Torr)
5°C


12
10
8
6
4
2
0
cCO2 (mmol. l-1)
0
2
4
6
8
10
12
Pco2 (Torr)
10°C


15°C
cCO2 (mmol. l-1)
12
10
8
6
4
2
0
0 2 4 6 8 10 12
Pco2 (Torr)


20°C
cCO2 (mmol. l-1)
12
10
8
6
4
2
0
0 2 4 6 8 10 12
Pco2 (Torr)

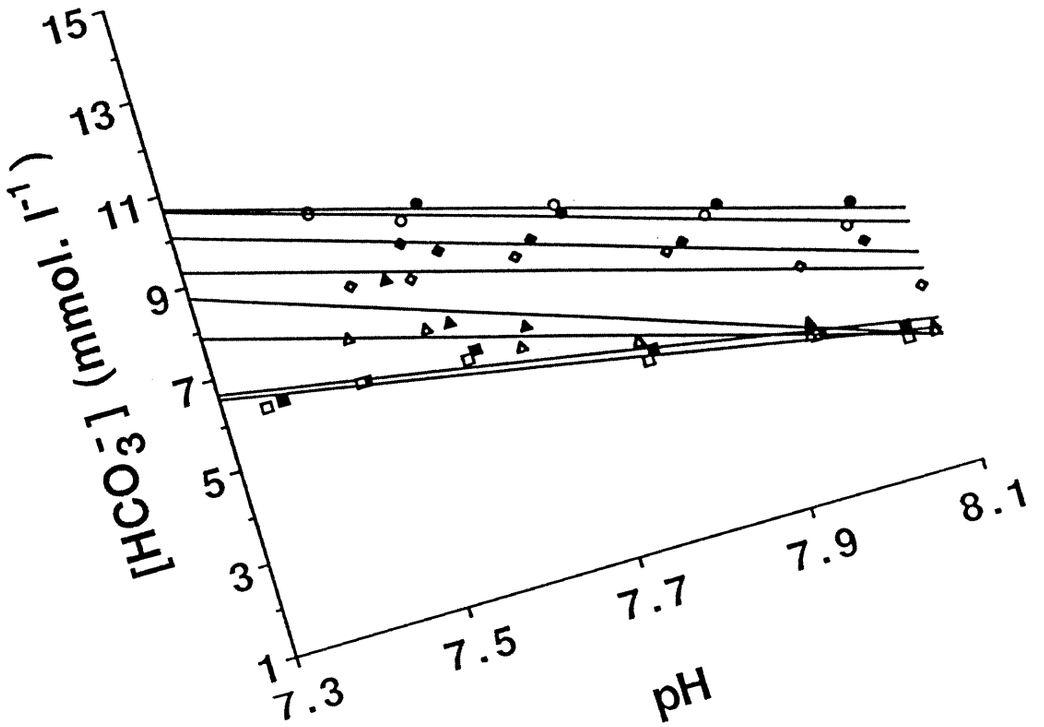
Pco_2 = 6 Torr was 0.56, 0.69, and 0.68 for *M. rugosa* from shallow and deep waters and for *M. sarsi* respectively.

The relationships between the calculated [HCO_3^-] and the pH of the blood of *M. rugosa* at the four experimental temperatures are shown in Fig. 5.8. Covariance analysis of these data showed that there was no significant difference between the slopes of the regression lines for the data at 5, 10 and 20°C ($P>0.05$) indicating that there was no significant effect of temperature on the buffering capacity of the blood. Values for the buffering capacity of the deoxygenated blood were -5.51, -5.35 and -6.29 mmol.l^{-1}. pH $unit^{-1}$ at temperatures of 5, 10 and 20°C respectively. The slope of the regression line fitted to the data obtained at 15°C, however, differed from the slopes of the other regression lines which suggests that the buffering capacity of the blood (-3.37 mmol.l^{-1}. pH $unit^{-1}$) was lower at 15°C than at the other temperatures.

The carbon dioxide equilibrium curves for the blood of *M. rugosa* from deep water and for *M. sarsi* were similar to those obtained for *M. rugosa* from the shallow water. Values for the buffering capacity of the deoxygenated blood of *M. rugosa* from deep water and for *M. sarsi* (at 10°C) were similar to those of *M. rugosa* from shallow water sites namely, -7.98 and -5.31 mmol.l^{-1}. pH $unit^{-1}$.

Fig. 5.8

The relationships between the calculated [HCO_3^-] and the pH of the blood of *M. rugosa* from the shallow water site at 5 (●), 10 (◆), 15 (■), and 20^oC (▲). The opened and closed symbols are for oxygenated and deoxygenated blood respectively.


15
13
11
9
7
5
3
1
[HCO3-] (mmol. l-1)
7.3
7.5
7.7
7.9
8.1
pH

5.4. DISCUSSION

5.4.1. Ionic composition of the blood

The ionic composition of the blood did not differ significantly between *M. rugosa* and *M. sarsi*. The blood of both species, however, had high concentrations of Mg^{2+} ions. This result is in agreement with the data obtained by Walter & Uglow (1981) for *M. rugosa*. In addition, in *M. sarsi*, the blood Mg^{2+} concentration was slightly higher (50.8 mM) and had a lower Ca^{2+} concentration (7.55 mM). It is now well established that, in decapods, the concentration of Mg^{2+} ions in the blood may vary considerably between species (Robertson, 1949, 1953, 1960; Walters & Uglow, 1981; Mantel & Farmer, 1983).

The concentration of Mg^{2+} ions in the blood of decapods has been correlated with the level of activity normally shown by different species; more active species were found to have low concentrations of Mg^{2+} ions (Robertson, 1949). In addition, there is evidence that the concentration of other ions e.g. Ca^{2+} may also influence activity (Robertson, 1949). Robertson has concluded that the ratio of Ca^{2+} / Mg^{2+} ions may be correlated with the degree of activity generally exhibited. For example, a low value for this ratio (0.19 - 0.31) was characteristic of species which are normally very inactive such as *Lithodes maia*, *Dromia personata* (= *D. vulgaris*), *Maia squinado* and *Hyas araneus*. In contrast, more active species had higher values for this ratio (0.39 - 2) (Robertson, 1953). The Ca^{2+} / Mg^{2+} ratio obtained for *M. rugosa* during the present study was 0.27 and is similar to that obtained previously for less active decapods. This provides further evidence to support the correlation between the value of the Ca^{2+} / Mg^{2+} ratio and the activity level since *M. rugosa* is normally a very inactive species (see Chapters 3 & 4).

During the present study of the oxygen transporting properties of the blood of

Munida, it was important to determine the ionic composition of the blood since it is now clearly established that the concentrations of certain ions may have pronounced effects on the oxygen affinity of the haemocyanin and on the size of the Bohr factor in decapod Crustacea (Larimer & Riggs, 1964; Truchot, 1973, 1975; Mangum & Towle, 1977; Brouwer *et al.*, 1978; Mason *et al.*, 1983; Morris *et al.*, 1986, 1988). Ca^{2+} and Mg^{2+} ions are of particular importance in this respect. Low concentrations of Ca^{2+} ions are known to affect the aggregation state of the haemocyanin molecule. Low concentrations of divalent ions increase the degree of dissociation of the subunits and, as a result, may have important effects on the oxygen affinity of the haemocyanin and on the size of the Bohr factor (reviewed by Mangum, 1983). Such effects may be of minor significance in species like *Munida* which normally do not experience significant changes in environmental salinity and therefore are unlikely to show changes in the ionic composition of the blood. In other species which inhabit estuaries or intertidal rock pools in which they may frequently be exposed to changes in salinity, however, changes in the ionic composition of the blood may have important effects on the ability of the blood to transport oxygen. Although such species may attempt to regulate the concentrations of these ions, exposure to reduced salinity may be accompanied by an increase in blood pH which appears to oppose any decrease in haemocyanin oxygen affinity caused by a reduction in blood ion concentrations (Truchot, 1973, 1975; Weiland & Mangum, 1975; Mangum & Towle, 1977; Cameron, 1978).

5.4.2. Oxygen carrying capacity of the blood

Information on the oxygen carrying capacities of the blood of decapods has been reviewed by Magnum (1983). The oxygen carrying capacity of the blood of the majority of decapods is low (1-3.5 ml O_2. 100 ml^{-1}). Higher values do occur in some decapods such as semi-terrestrial species and also in some of the thalassinids which may regularly experience hypoxic conditions in their burrows

(see reviews by Mangum, 1983; Atkinson & Taylor, 1988). It has been suggested that one of the main reasons for the low oxygen carrying capacity in decapods is that greater concentrations of haemocyanin in the haemolymph would result in high colloid osmotic pressures which may have important consequences for fluid balance between the intra- and extracellular compartments (Mangum & Johansen, 1975; Mangum & Lykkeboe, 1979).

The total oxygen-carrying capacities of the blood of *M. rugosa* and *M. sarsi* determined during the present study were 1.7-1.8 and 2.0 ml O_2. 100 ml^{-1} respectively. These values were within the range recorded for other decapods (Mangum, 1983) but comparative data for other galtheids are limited (Bridges & Brand, 1980)

No significant differences were recorded in the oxygen carrying capacity between male and female *M. rugosa*. Among other species of decapod crustaceans, however, some variation in the oxygen carrying capacity has been observed between males and females e.g. in the blue crab *Callinectes sapidus* (Horn & Kerr, 1963) and in *Palinurus elephas*, (Bellelli *et al.*, 1988). Other factors are also known to affect the oxygen carrying capacity of the blood of decapods. For example, oxygen carrying capacity may vary seasonally e.g. in *Carcinus maenas* (Uglow, 1969), and in *Callinectes sapidus* (Horn & Kerr, 1963) and can be affected by starvation (e.g. Stewart *et al.*, 1967; Uglow, 1969; Lynch & Webb, 1973), and by the stage of the moulting cycle e.g. in *Homarus gammarus*, (Laufer & McNamara, 1962; Spoek, 1974 Hagerman, 1983), and in *Carcinus maenas* (Busselen, 1970) and by salinity change (Péqueux *et al.*, 1979). In addition, prolonged exposure to hypoxia has also been shown to result in a significant increase in the oxygen carrying capacity e.g. in *Crangon crangon* (Hagerman, 1986) and in *Carcinus maenas* (Lallier & Truchot, 1989).

5.4.3. *In vivo* blood pH and Po_2

Early studies of decapod Crustacea indicated that the Po_2 of the post-branchial blood was low (Redmond, 1955; Wolvekamp & Waterman, 1960). Such low values were interpreted as indicating that there was a high diffusion resistance across the respiratory surfaces and suggested that the haemocyanin was not saturated at the gills. This caused doubts about the efficiency of oxygen transport by the haemocyanin of decapods (Wolvekamp & Waterman, 1960). Since these early studies, however, there is growing evidence from a wide range of species which suggests that, in quiescent individuals at least, the Po_2 of the post-branchial blood is quite high and is usually sufficient to ensure that the pigment is saturated as it passes through the gills (e.g. Johansen *et al.*, 1970; Taylor & Butler, 1973; Taylor *et al.*, 1973; Mangum & Weiland, 1975; McMahon & Wilkens, 1975; Taylor, 1976; 1984). The situation is further complicated, however, since it is now well established that many inactive decapods undergo periods of ventilatory and cardiac arrest (McMahon & Wilkens, 1975, 1977; Butler *et al.*, 1978; Bridges, 1979; Schembri, 1979; McMahon & Burrgren, 1979; Burnett & Bridges, 1981; Bradford & Taylor, 1982; Taylor, 1984; Morris & Taylor, 1984). The variation in gill perfusion that will result is likely to affect the Po_2 of the blood so that care must be taken that the values are representative of 'normal' animals. In the present study this was not a major problem since when periods of respiratory pausing occur in *Munida*, they are of only limited duration. Care was taken, however, to ensure that the blood samples were taken only from undisturbed animals.

Accurate measurements of the pH and Po_2 of the pre- and post-branchial blood of resting animals together with data on the *in vitro* oxygen dissociation curves are essential in establishing the role of the haemocyanin in oxygen transport. By interpolating the values for P_aO_2 in *M. rugosa* (86.2 ± 11 Torr) on the oxygen dissociation curves at the *in vivo* pH (7.8), it would appear that the

post-branchial blood is fully saturated on leaving the gills. In addition, the fairly high value for P_vO_2 (38 ± 9 Torr) indicates that, in quiescent animals under normoxic conditions, the haemocyanin gives up only a limited amount of oxygen as it flows through the tissues. A similar situation is thought to occur in other decapods (Taylor, 1976; McMahon *et al.*, 1979). Under normoxic conditions a significant quantity of the oxygen supplied to the tissues appears to come from the oxygen in physical solution in the blood and the oxygen bound to the haemocyanin acts as a venous oxygen reserve and is used to meet the increased demands of the tissues during periods of activity, or may be released during exposure to hypoxia.

Values for the *in vivo* Po_2 of the pre- and post-branchial blood of *M. sarsi* showed that, although there was no significant difference between the values of P_aO_2 in this species and *M. rugosa*, the values for P_vO_2 in *M. sarsi* were much lower (18.5 Torr) which gives a Po_2 difference of 62.5 Torr. This Po_2 difference between pre- and post-branchial blood is larger than the value obtained for *M. rugosa* and when the oxygen dissociation curves for *M. sarsi* are examined, it would indicate that a greater amount of oxygen is released from the haemocyanin of this species during its passage around the body. The true significance of this is difficult to assess, however, since the data for *M. sarsi* were obtained from only a few individuals and may not accurately reflect the true *in vivo* values in this species.

5.4.4. O_2 affinity of the blood

The haemocyanin of *Munida rugosa* from both depths and that of *Munida sarsi* is characterised by having a very low oxygen affinity (P_{50} = 20, 39 Torr and 50 Torr respectively (at pH 7.85) and 10°C. Covariance analysis of the relationships between P_{50} and pH indicated that there was no significant difference ($P > 0.05$) between the slopes of the regression lines fitted to these data but the elevations were significantly different. This demonstrates that,

although the oxygen affinity of the pigment differs significantly between the two species, there was no significant difference in the size of the Bohr effect. It is also interesting to note that the blood of *M. rugosa* collected from the deep water site appears to have a lower oxygen affinity than that of animals from shallow water. The P_{50} value for the haemocyanin of *M. sarsi* is one of the highest values recorded for decapod crustaceans from a variety of habitats (see review by Mangum, 1983).

There is considerable variation in the oxygen affinity of the haemocyanin among different crustaceans. In general, a low oxygen affinity pigment is characteristic of many air-breathing crustaceans e.g. in terrestrial isopods (Mangum, 1983) and also in semi-terrestrial crabs (Burnett, 1979). In aquatic decapods, however, the oxygen affinity of the haemocyanin is usually somewhat higher but even among these species values for P_{50} may vary quite widely e.g. 12 Torr in *Callinectes sapidus* (Bonaventura *et al.*, 1974); 18 Torr in *Cancer magister* (McMahon *et al.*, 1979); 21 Torr in *C. pagurus* (Truchot, 1971); 15 Torr in *Carcinus maenas* (Truchot, 1971); 10 Torr in *Homarus gammarus* (as *H. vulgaris*) (Butler *et al.*, 1978); 24 Torr in *Liocarcinus depurator* (Taylor *et al.*, 1985) 25 Torr in *Liocarcinus puber* (Truchot,1971); 23 Torr in *Pagurus bernhardus* (Bridges, 1986); 12.6 Torr in *Galathea strigosa* (Bridges, 1986); 16 Torr in *Palaemon adspersus* (Weber & Hagerman, 1981); 3.6 Torr in *P. elegans* (Morris *et al.*, 1985); 1.6 Torr in *Calocaris macandreae* (Anderson, 1989). The data for several species from a range of habitats are also summarized by Mangum (1983). The accumulating data suggest, however, that there may be a correlation among aquatic decapods between the oxygen affinity of their haemocyanin and the availability of oxygen in their normal environment. Species which are regularly exposed to hypoxia, such as mud-burrowing shrimps (Thalassinidae), have haemocyanins showing much greater oxygen affinities (see review by Taylor & Atkinson, 1990). Similarly, species which are rarely

exposed to hypoxia tend to have much lower oxygen affinities (see review of Mangum, 1983). The low oxygen affinities for the haemocyanin of *M. rugosa* and *M. sarsi* obtained during this study would appear to support this relationship since the available information suggests that neither species appears to be exposed for long periods of hypoxia in its natural environment.

It is very difficult, however, to make valid interspecific comparisons of oxygen affinity since it is now well established that the oxygen affinity of the haemocyanin can be affected by a number of organic modulators such as L-lactate and urate (Truchot, 1980; Booth *et al.*, 1982; Graham *et al.*, 1983; Bouchet & Truchot, 1985; Morris *et al.*, 1985; Bridges & Morris, 1986; Lallier & Truchot, 1989) and by the concentrations of inorganic ions such as calcium and magnesium (Truchot, 1975; Brouwer *et al.*, 1978). In many of the earlier studies, variations in the concentrations of these ions were not controlled. The concentration of L-lactate in the blood is of particular importance since this compound is produced as a result of anaerobic metabolism perhaps induced by stress during blood sampling and an increase in the concentration of L-lactate is known to result in a pronounced increase in haemocyanin oxygen affinity in decapods. In addition, it has recently been shown that some hormones (e.g. dopamine) may also affect the oxygen affinity of the haemocyanin (Morris, 1988).

Recently, an additional difficulty in making interspecific comparisons has been pointed out by Morris (1988). Morris (1988) found that storage of blood samples in a deep freeze could affect the cooperativity (n_{50}) of the haemocyanin although there was little evidence of a change in P_{50}. This has also been confirmed in another recent study (Lallier & Truchot, 1989). It would appear from these studies that long term storage especially at very low temperature (e.g. -80^{o}C) causes a reduction in the value of n_{50} due to the dissociation of the subunits of the haemocyanin molecule.

During the present study, oxygen dissociation curves were also constructed using blood that had been frozen (-20^{o}C). This was unavoidable since it was essential that all curves were constructed using the same pooled sample to overcome the effects of variations in the concentrations of blood ions or L-lactate between samples taken on different occasions. Nevertheless, comparisons of the value for n_{50} for freshly collected blood did not differ significantly from those for the pooled samples which had been stored in a deep freeze. The values of n_{50} obtained for the blood of *M. rugosa* and *M. sarsi* at 10^{o}C were 3.3-3.7 and 3.6 respectively are well within the normal range for other decapods (2.1-4.5) (Mangum, 1983).

The physiological significance of the cooperativity of the haemocyanin has been reviewed by Rochu & Fine (1980). Its primary function would appear to be to increase the amount of oxygen released from the haemocyanin to the tissues for a given P_aO_2-P_vO_2 difference, and effectively decreasing the amount of pigment required to transport a given volume of oxygen to the tissues (Rochu & Fine, 1980). A number of previous studies have shown that the n_{50} is independent of pH within the physiological range (Morris *et al.*, 1985). This was also observed during the present study but it was noted that the value of n_{50} of both species decreased at low pH.

The oxygen affinity of decapod haemocyanins is also affected by temperature (Mangum, 1983). In *M. rugosa* and *M. sarsi*, as in other decapods, oxygen affinity decreases with an increase in temperature. This may be of particular importance when animals are exposed to an increase in environmental temperature since a decrease in the oxygen affinity of the haemocyanin will facilitate the release of oxygen to meet the increased metabolic demands of the tissues.

The oxygenation of both haemoglobins and haemocyanins is exothermic with

values for the heat of oxygenation (ΔH) ranging from 0 to -158 $kJ.mol^{-1}$ (Bridges *et al.*, 1983). Values forΔH (between 5-10^{o}C) were -17.6, -21.3 and -20.7 $kJ.mol^{-1}$ in *M. rugosa* from shallow and deep water and for *M. sarsi* respectively. In both species, these values increased when calculated over a higher temperature range and indicate that the haemocyanin of these species is quite sensitive to changes in temperature. The temperature sensitivity of haemocyanin oxygen affinity varies between species but appears to be greatest in those species which do not regularly experience wide temperature fluctuations in their natural environment (Jokumsen & Weber, 1982; Taylor *et al.*, 1985; Bridges, 1986). The fact that the haemocyanin of *M. rugosa* and *M. sarsi* is fairly sensitive to temperature is perhaps not surprising since neither species is normally subjected to wide fluctuations in temperature; at the depth range at which they occur the annual variation in temperature is normally between 5-15^{o}C.

As in all other decapods studied so far, both *M. rugosa* and *M. sarsi* show a positive Bohr effect i.e. the oxygen affinity of the haemocyanin decreases with a reduction in pH. The value of the Bohr factor, like oxygen affinity, also varies considerably among decapods (Mangum, 1983). In decapods the Bohr factor ranges from very low values e.g. -0.19 in *Astacus leptodactylus* (Angersbach & Decker, 1978); -0.27 in *Cancer magister* (Johansen *et al.*, 1970); -0.2 in *Thalassina anomala* (Mangum, 1982) and -0.13 in *Holthuisana transversa* (Morris *et al.*, 1988) to much higher values of -1.5 in *Carcinus maenas*, (Taylor & Butler, 1973) and -1.5 in *Palaemon adspersus* (Weber & Hagerman, 1981). Values for many other decapod crustaceans are tabulated in the review by (Mangum, 1983). The values for the Bohr effect obtained during the present study did not vary greatly between *M. sarsi* and *M. rugosa* from both shallow water and deep water sites (θ = -0.42 to -0.62) and were within the normal range for decapods.

Both *M. rugosa* and *M. sarsi* appear to be inactive species. The blood of these species is characterised by having a moderately high oxygen carrying capacity. The oxygen affinity of the respiratory pigment is, however, quite low and exhibits a moderate Bohr effect. The properties of the haemocyanin of these species are typical of species which have an inactive mode of life but in which the haemocyanin may act as a 'store' of oxygen during periods of activity or stress (Taylor *et al.*, 1985).

5.4.5. CO_2 transport and acid-base balance

Acid-base balance and the influence of physiological and enviromental factors on acid-base status in decapod crustaceans has been reviewed by Truchot (1983). As in many other animals, the respiratory pigment of decapods plays a major role in buffering changes in blood pH (Truchot, 1983). Buffering by the haemocyanin is usually termed 'non-bicarbonate buffering' and the buffering capacity of the blood can be determined from the slope of the relationship between the bicarbonate concentration and the pH of the blood. In decapods, maintenance of acid-base balance during environmental stress such as hypoxia involves metabolic compensations such as the release of bicarbonate ions into the surrounding medium (Truchot, 1975; Johnson & Uglow, 1987). The decrease in blood pH associated with the release of protons when L-lactate is produced must be buffered by such metabolic compensations.

The formation of bicarbonate ions from carbonic acid and their subsequent conversion back to CO_2 at the respiratory surfaces is catalysed by the enzyme carbonic anhydrase. In vertebrates, carbonic anhydrase occurs in the erythrocytes. In decapods, however, this enzyme is not present in the haemolymph but in the epithelial membranes of the gills and in the cytoplasm (Henry & Cameron, 1982, 1983; Henry, 1988).

It has been recognized for many years that, in animals which possess

calcareous exoskeletons e.g. molluscs and arthropods, the carbonates in the exoskeleton could be important in buffering pH changes in the body fluids. During recent years, however, there has been some controversy over the role of exoskeletal carbonates in buffering these pH changes. A number of authors have suggested that this mechanism may be important in decapods (Truchot, 1979; 1983; Wood & Randall, 1981) but Cameron (1985) has calculated that, when the crab, *Callinectes sapidus* is exposed to hypercapnic conditions, only about 7.5 % of the total compensation for the resulting acid-base disturbances could be attributed to buffering by exoskeletal carbonates. There is evidence that buffering of acid-base disturbances by exoskeletal carbonates may be more important in semi-terrestrial decapods or in aquatic species exposed to air since they may lack ready access to a source of water to aid the loss of H^+ ions (DeFur *et al.*, 1980; Henry *et al.*, 1981).

The relationship between the amount of CO_2 carried by the blood and the Pco_2 is usually hyperbolic in shape. Truchot (1976b) was one of the first people to describe *in vitro* CO_2 equilibrium curves in decapods. Subsequent studies on decapods have shown that, as in *M. rugosa* and *M. sarsi*, the blood exhibits a moderately high capacitance for CO_2.

The blood of both species of *Munida* was found to exhibit a distinct Haldane effect i.e. the CO_2 equilibrium curves showed clear differences in the total CO_2 content of the haemolymph between oxygenated and deoxgenated blood and resulted in a difference in pH at constant Pco_2. This is due to the fact that oxygenation affects the buffering function of the respiratory pigment. At any given Pco_2 the deoxygenated haemocyanin buffers the blood at a higher pH than the oxygenated haemocyanin. As a result, this enhances the formation of bicarbonate ions and increases the total CO_2 content of the blood.

The Haldane effect shows that oxygen transport is linked to carbon dioxide transport and depends on the dissociation of Bohr protons during oxygenation

(Truchot, 1976). The blood of many decapods has been shown to exhibit a Haldane effect (e.g. Truchot, 1976b; Randall & Wood, 1981; Taylor *et al.*, 1985) but, interestingly, a Haldane effect does not appear to exist in the amphibious crab, *Holthuisana transversa* (Morris *et al.*, 1988).

The size of the Haldane effect can be expressed as the ratio of cCO_2 difference to the oxygen carrying capacity of the blood (ΔcCo_2 / $_{c}Hcy O_2$) which represents the number of moles of CO_2 released, at constant Pco_2, per mole of oxygen bound by the haemocyanin (Truchot, 1976). The magnitude of the Haldane coefficient varies between different species and appears to be correlated with the protein concentration of the blood (Truchot, 1976).

The Haldane coefficient is closely correlated with the magnitude of the Bohr effect (see Wyman, 1948; 1964). The Haldane coefficients obtained during the present investigation ranged from 0.56 to 0.69 in *M. rugosa* and 0.68 in *M. sarsi*. These values are similar to that for *Carcinus maenas* (Truchot, 1976) in which the Bohr value, as in these *Munida* species, is also moderate. In the prawn, *Palaemon elegans*, however, the blood exhibits both a high Bohr value (-1.7) and also a large Haldane coefficient (-1.25) (Morris *et al.*, 1985). A similar relationship was also found in three species of burrowing crab (Taylor *et al.*, 1985). These data confirm the linkage between the Bohr and Haldane effects (Wyman, 1964).

CHAPTER 6. GENERAL DISCUSSION

Taxonomic confusion within NE Atlantic *Munida* has been clarified following the revision by Rice & de Saint Laurent (1986). These authors support the contention that *M. rugosa* and *M. sarsi* are indeed separate species. The separation is largely based on standard morphological criteria and so it was useful to be able to compare aspects of the physiology of these two species in the present work.

Interestingly, although *M. sarsi* was confined to deeper water, there was overlap between the two species in that *M. rugosa* was collected from the same grounds that yielded *M. sarsi*. Such overlap has been noted by others (see Rice & de Saint Laurent, 1986), though Attrill (1988), who sampled a bathymetrically varying series of *Munida* spp. in the Porcupine Sea-bight, noted that the degree of overlap between the different species was small. It is therefore interesting to speculate on the possible reasons for such separation. Attrill (1988), reflecting on the work of Berrill (1970), speculated that *M. sarsi* was a competitive and aggressive species which prevented *M. tenuimana* from extending its range upwards. In other areas, however, these species show considerable overlap in range (Brinkmann, 1936). Brinkmann (1936), from studies in Norwegian fjords, suggested that small *M. sarsi* settled in shallow water and migrated into deep water following sexual maturity. Attrill (1988) could not substantiate this for the Porcupine Sea-bight and it is not apparent in the Firth of Clyde either. Attrill (1988) encountered one location where the *M. sarsi* were much larger than at other stations. He was unable to account for this difference. In fact, these large animals (up to 34mm carapace length) compare with a maximum size of 29mm carapace length for specimens found in the Firth of Clyde sample area. Brinkmann's largest specimen was 28mm carapace length. Size may simply reflect good feeding conditions. Brinkmann (1936) and

Attrill (1988) both found that size distribution of *M. sarsi* peaked at carapace lengths of between *c.* 12 - 18mm.

In the present work, both *M. rugosa* and *M. sarsi* were encountered in the same sample area and therefore, in general terms, on the same substrata. Specific differences in habitat utilization could not be assessed, but both species appeared to occur on a variety of bottom types. Locally, both appeared to favour coarse muddy deposits in proximity to rock; they favoured coarser sediments than those occupied by the Norway lobster (*Nephrops norvegicus*) (see Bailey *et al.*, 1986). This is well-known by creel fishermen (C.J. Chapman, pers. comm.). Attrill (1988) indicated that, in the Porcupine Sea-bight, the sediment is generally a coccolith-foraminifera marl, with clinker and glacial erratics. At 510m depth (near the peak abundance of *M. sarsi*), the median grain size is 4.4 phi (47μm), decreasing with increasing depth. Organic carbon accounts for 0.5% of sediment dry weight; Firth of Clyde values are higher (Pearson *et al.*, 1986). Thus, it would appear that *M. sarsi* extends to finer grained sediments than does *M. rugosa*.

Also, in general terms, both species consumed similar diets. These indicated a mixture of deposit feeding and macrophagy with both plant and animal material being consumed. The more setose maxillipeds of *M. sarsi* suggest that it may rely more on deposit feeding than does *M. rugosa*. The presence of algal fragments in the stomach of *M. sarsi* is a function of the relatively shallow water and the close proximity to land. The source of algal material would have been detached macrophytes which are known to accumulate on the sea bed in the Firth of Clyde sample area and such material would not be expected to feature in the diet of offshore animals. At deep offshore sites, deposit feeding may be relatively more important than at inshore sites where more macroscopic food is likely to be available.

The large eyes of the deeper water NE Atlantic *Munida* spp. (excluding the

deepest species - *M. micropthalama*) suggest that visual behaviour is important, but nothing is known about this.

In physiological terms there is little to separate *M. rugosa* and *M. sarsi*. Gill areas were similar in *M. rugosa* and *M sarsi*. By comparison with other decapod crustaceans, these gill areas were small. The low values obtained for the gill areas probably reflect the inactivity of the animals. This was also suggested by their low metabolic rates. Although capable of 'tail flick' swimming when disturbed or subjected to adverse environmental conditions, both species were observed to have long periods of almost total inactivity. Gill areas and activity levels have been correlated in various studies (e.g. Gray, 1957; Hughes, 1983). Within limits, both *Munida* species were able to regulate their metabolic rate (oxygen consumption and heart rate) independently of the ambient oxygen tension. The critical oxygen tensions (Pc's) were, however, unexceptional. Animals hyperventilated under hypoxic conditions, but below the Pc the rates of beating of the scaphognathites declined.

It was found that *M. rugosa* was more tolerant of hypoxia than *M. sarsi*. This may be significant, since environmental oxygen conditions are more likely to be variable in shallow water. The site from which the *M. sarsi* were obtained is probably well-oxygenated throughout the year. Deep offshore waters are also likely to be well-oxygenated and relatively stable. This, however, is not always true of fjords which may experience oxygen depletion in summer (Edwards, *et al.*, 1986. Burd (1985, 1987) was able to show that those *M. quadrispina* which experienced oxygen depletion had greater gill areas than those which lived elsewhere and were more tolerant of hypoxia. It would be interesting to investigate whether or not this is true of *M. sarsi* and *M. rugosa*. In the present work, the separation of *M. rugosa* into those collected from relatively shallow and deep water was an attempt to control for any depth effect that may influence the comparison with *M. sarsi*. No major differences were

demonstrated. An investigation in fjordic sites known to experience seasonal oxygen depletion would, however, be instructive.

Both *M. rugosa* and *M. sarsi* possess haemocyanins having low oxygen affinities. The oxygen affinities are amongst the lowest reported in decapods. The haemocyanin of *M. rugosa* from shallow water had a higher oxygen affinity than that of animals from deep water. The haemocyanin of *M. sarsi* had the lowest oxygen affinity. The blood of both species is characterized by having moderate oxygen carrying capacities and the values were within the range recorded for other decapods (Mangum, 1983). Normal, moderate Bohr and Haldane effects were observed and there were no significant differences between the two species in the magnitude of these effects. The occurrence of a Haldane effect shows that oxygen transport is linked to carbon dioxide transport and depends on the dissociation of Bohr protons during oxygenation (Truchot, 1976). The magnitude of the Haldane coefficient varies between different species and is correlated with the protein concentration of the blood (Truchot, 1976). The data obtained confirm the linkage between the Bohr and Haldane effects (Wyman, 1964).

It is now well established that the concentrations of certain ions, particularly Ca^{2+} and Mg^{2+} may have pronounced effects on the oxygen affinity of the haemocyanin and on the size of Bohr factor in decapod Crustacea (Mangum, 1983). The ionic concentration of the blood of the two *Munida* species is very similar and is characterized by having high Mg^{2+} concentrations which may be related to their low activity since, in other species, a correlation has been found between the concentrations of Mg^{2+} ions in the blood and the degree of activity (Robertson, 1949, 1953, 1960; Wolvekamp & Waterman, 1960; Walters & Uglow, 1981; Mantel & Farmer, 1983).

The *in vivo* blood parameters investigated were limited to the Po_2 and pH of

the pre- and post-branchial blood of *M. rugosa*. The values obtained were interpolated on the oxygen dissociation curves, and indicated that the post-branchial blood is fully saturated on leaving the gills. In addition, the pre-branchial blood saturation level was fairly high which indicates that, in quiescent animals under normoxic conditions, the haemocyanin gives up only a limited amount of oxygen as it flows through the tissues. Under normoxic conditions, a significant quantity of the oxygen supplied to the tissues appears to come from the oxygen in solution in the blood and the oxygen bound to the haemocyanin acts as a venous oxygen reserve to be used to meet the increased demands of the tissues during periods of activity, or to be released during exposure to hypoxia.

The degree of hypoxia experienced by *M. rugosa* and *M. sarsi* in the environment remains unknown. It has been postulated that the animals burrow (Brinkmann, 1936; Rice & de Saint Laurent, 1986; Attrill, 1988). Independent burrowing behaviour could not be substantiated in the present work, although *M. rugosa* at least may occupy vacated burrows of other species (see Chapter 2). *M. rugosa* is known often to occupy crevices in bedrock and particularly crevices at the sediment/ rock interface (see Chapter 2). Both hypoxia and hypercapnia may be experienced in burrows and crevices, but the two species are not particularly adapted to withstand prolonged or extreme conditions of hypoxia or hypercapnia. Observations suggest that those specimens that do occur in crevices and burrows, remain at or near the opening (R.J.A. Atkinson, pers. comm.). Should conditions become extreme, however, they can tolerate even several hours of anoxia. An interesting association has been observed for *M. tenuimana* which may use the oscula of glass sponges (*Pheronema* sp.) as 'burrows' (Attrill, 1988). They also occupy shallow burrows in the substratum, but whether or not they excavate these is unsubstantiated. Attrill (1988) considers that they and other *Munida* do form their own burrows. Further work on this topic would be useful.

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