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Comparative Physiological Ecology of Some Mud-Burrowing Shrimps (Crustacea: Decapoda: Thalassinidea)

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A Thesis submitted for the degree of Doctor of Philosophy
to the Faculty of Science at the University of Glasgow
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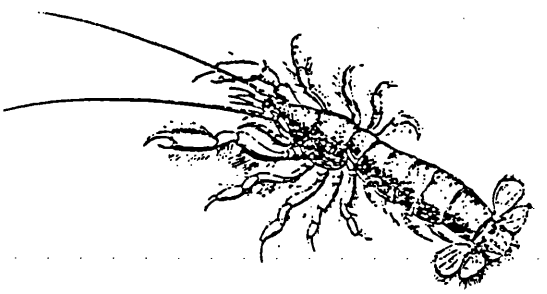
".... There is an extraordinary creature with a very long tail, short body, and small clawed legs, which has one of its fore claws as big as its body, and highly disproportionate. It has small eyes, small feelers, and is of a bright red colour when living. It lives buried up to its neck in sand or mud on the Devon coast, and can hide itself completely in its burrows. The skin is half membraneous. It is called Callianassa. Another of these burrowing crustaceans, called the Gebia, frequents the mud and sand of Plymouth harbour. It has not the great claw of the last, and it is said to burrow for a hundred feet or more! These diggers and burrowers kill and eat, protected by their sandy or muddy homes, but they are not able to move or crawl well, when they are turned out....."[1]



[1]A description of *Callianassa* and *Upogebia* .as given by Professor P.M. Duncan (1886) in *The Sea-Shore* (Natural History Rambles, p210; E & J.B. Young & Co., London).

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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.

C. M. Astall B.Sc (Hons).

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Specimens of *Upogebia pusilla* were kindly provided by Professor J.P. Truchot, Arcachon, France, and without the help of Ashley Rowden (Plymouth Marine Laboratories) I would have obtained very few shrimps.

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Recordings from burrows of the filter feeding upogebiid mud-shrimps *U. stellata* and *U. deltaura*, constructed in laboratory aquaria, showed a gradient of oxygen tension with depth. The burrow water at the deeper parts of the burrow was often severely hypoxic. The water pH also decreased through the burrow, reflecting an increase in the PCO_2 with depth. Long term changes in the burrow water PO_2 are dependent upon a number of factors of which burrow irrigation is the most important. Irrigation not only provides a feeding current but also serves to replenish the oxygen-depleted burrow water. The degree of exchange of burrow water is dependent both on the amount of time spent irrigating and the rate of irrigation. Large temporal fluxes in oxygen tension occur within the burrow. These fluxes in PO_2 are dependent upon the irrigatory behaviour, respiration of the occupant and of the meiofauna and microflora associated with the burrow wall and upon the diffusion of oxygen through the burrow system.

Differences in the gill structure were observed between a number of mud-shrimps. The branchial complement, gross gill morphology, gill ultrastructure and gill area to weight relationships were compared between the deeper-burrowing deposit feeders, *Callianassa subterranea* and *Jaxea nocturna,* which are often exposed to severe hypoxia or anoxia, and the filter feeding upogebiids *U. stellata, U. deltaura* and *U. pusilla.* A trend towards simplification of the gill formula in the higher thalassinidean families was found. Deposit feeders had larger gill areas than the filter feeders, with the larger gill area a result of flattening of the trichobranch gill filaments resulting in a phylloid trichobranch form. The phylloid trichobranch gills of the deposit feeders had a thinner chitinous cuticle and reduced diffusion distance. A larger gill area and reduced diffusion distance may be construed as functional adaptations to the more severe conditions that the deposit feeders *C. subterranea* and *J. nocturna* are likely to regularly experience.

As thalassinideans are so successful in a habitat where hypoxia and hypercapnia are regularly experienced, the respiratory and blood physiology was examined in more detail. In general, the weight-specific rate of oxygen consumption was significantly higher in the filter feeding upogebiids, *U. stellata* and *U. deltaura,* than in the deposit feeders *C. subterreanea* and *J. nocturna.* All four species of mud shrimp showed a high degree of respiratory independence and were able to maintain their rates of oxygen consumption approximately constant over a wide range of oxygen tension. For similar sized individuals, the oxygen tension at which respiratory independence was lost (Pc) was between 10-20 Torr for *J. nocturna* and *C. subterranea,* whereas the Pc range for the upogebiids was 30-40 Torr. The response of the heart and scaphognathite to hypoxia was examined in more detail for one of the shrimps, *U. deltaura.* Under normoxia, cardiac and scaphognathite activity was closely correlated. During progressive hypoxia there was no significant change in the heart rate. There was, however, a significant

increase in the scaphognathite rate below 80 Torr and down to 30 Torr but any further decrease in PO_2 resulted in a rapid decrease in scaphognathite activity.

The haemolymph of all four mud-shrimp species had high concentrations of magnesium ions and low calcium to magnesium ion ratios. Values of total haemolymph oxygen carrying capacity (C_{HCYO_2}) were, *U. deltaura*, 0.7±0.04 mmol.l^{-1}; *U. stellata*, 0.51±0.08; *J. nocturna* 0.55±0.02 and *C. subterranea*, 1.12±0.05. Oxygen dissociation curves, constructed at 10°C, showed that the haemocyanin of each of the mud-shrimps exhibited a high oxygen affinity. The P_{50}, calculated from *in vivo* pH values, for *Upogebia deltaura* was 5.8 Torr (pH=7.83); *U. stellata*, P_{50}=6.7 Torr (pH=7.83); *J. nocturna*, P_{50}=2.8 Torr (pH=7.83) and *C. subterranea*, P_{50}=0.24 Torr (pH=8.2). The haemocyanin of each species also showed a high significant Bohr coefficient (ϕ), *U. deltaura*, ϕ=-1.15; *U. stellata*, ϕ=-1.67; *C. subterranea* ϕ=-1.32; *J. nocturna* ϕ=-2.26. The cooperativity (n_{50}) remained independent of pH for *U. deltaura, U. stellata* and *J. nocturna;* however, a decrease in n_{50} associated with an increase in pH was observed for the haemocyanin of *C. subterranea* at pH values >8.1. In addition, the haemocyanin sub-unit composition and association states were also examined.

The sulphide oxidation activity of the tissues of three burrowing decapods and three decapods that either bury or are non-burrowing were examined. Those tissues showing Michealis-Menton kinetics for sulphide oxidation possessed a sulphide oxidase enzyme. Sulphide-oxidising activity in the hepatopancreas tissue was significantly higher than in the other tissues tested (gill, muscle or blood), which did not exceed that of non-specific oxidation activity. The oxidation activity of the hepatopancreas was highest in *Cancer pagurus* (3.77μmol min^{-1} g^{-1}.) and *Nephrops norvegicus* (3.44μmol min^{-1} g^{-1}). The active swimming crab, *Liocarcinus depurator*, had the lowest hepatopancreas oxidation activity (0.62μmol min^{-1} g^{-1}) of all the species.

Sulphide tolerance was determined, using a stepwise increase method, both for a decapod unlikely to be exposed to sulphide *in vivo*, *Liocarcinus depurator*, and the mud-shrimp *Calocaris macandreae*. More than 50% of *L. depurator* survived for <11h exposure to a total concentration of 2.3mmol.l^{-1} sulphide, after which the crabs rapidly succumbed with any further increase in sulphide concentration. *Calocaris macandreae* survived for >16h, with no mortality, at a final concentration of 2.9mmol.l^{-1}.

The concentration of sulphide and its oxidation products (thiols) following 12h exposure to sulphide (0.73-0.85mmol min^{-1}) in hypoxic and normoxic conditions was measured for the mud-shrimp *Calocaris macandreae*. The concentration of sulphide in all the tissues of individuals exposed to normoxic + H_2S or hypoxic + H_2S was significantly higher ($P<0.05$) than in those specimens exposed only to normoxic or hypoxic conditions. There were no significant differences ($P>0.05$) in the mean haemolymph sulphide concentrations of individuals exposed to either hypoxia+ H_2S or normoxia+ H_2S.

Abstract

Changes in the concentration of sulphide, thiosulphate, sulphite and GSH in the tissues of *Calocaris macandreae* exposed to a constant sulphide concentration of 0.93 ±0.06mmol.l^{-1} ;n=4) under conditions of hypoxia (Po_2<20 Torr) for a 12h period were determined. In addition, changes in thiol concentration in the blood, hepatopancreas and muscle tissue of *C. macandreae* were measured during recovery, under both normoxic and hypoxic conditions, following 12h exposure to constant sulphide concentration (0.93 and 0.73mmol.l^{-1} sulphide respectively)

Chapter 1

Introduction

Thalassinidean taxonomy and systematics

Borradaile (1903) divided the Thalassinidea into 4 families, the Axiidae, Laomediidae, Callianassidae (subfamilies, Callianassinae and Upogebiinae) and Thalassinidae, and later placed the Superfamily Thalassinidae within the Tribe Anomura (Sub-Order: Reptantia, Order: Decapoda) (Borradaile, 1907). The status of the group was later changed by Selbie (1914) to the Tribe Thalassinidea. Based on larval characteristics, Gurney (1942) suggested there was a natural division between the two family groups Axiidae and Callianassidae (which he called the Homarine group) and the Laomediidae and Upogebiidae (the Anomuran group).

The classification within the sub-order Reptantia, of the phylum Crustacea, is quite confusing and has previously been described as a 'morass' (see review by Schram (1986) and references therein). Even though this has led to a number of discussions as to the position of the Thalassinidea within the Decapoda, it is clear the Thalassinidea have affinities with both the anomurans and astacids (Schram, 1986). The following scheme of classification is based on Abele (1982), Kensley (1989), Schram (1986) and Manning & Felder (1991) and has been used throughout this study;

Phylum: Crustacea
Order: Decapoda
Infraorder: Thalassinidea Lattreille 1831
Superfamily: Thalassinoidea Lattreille 1831
Family: Axiidae Huxley 1879
Callianideidae Kossman 1880
Callianassidae Dana 1852
Ctenochelidae Manning & Felder 1991
Thalassinidae Dana 1852
Laomediidae Borradaile 1903
Upogebiidae Borradaile 1903
Axianassidae Schmidt 1924
Calocarididae Kensley 1989

British Thalassinideans

Members of the Thalassinidae found around the U.K. are placed in the following families;

Family: Axiidae Huxley 1879
  Genus: *Axius* Leach 1815
    Axius stirhynchus Leach 1815
Family: Calocarididae Ortmann 1891
  Genus: *Calocaris* Bell 1846
    Calocaris macandreae Bell 1846
Family: Callianassidae Dana 1852
  Genus: *Callianassa* Leach 1814
    Callianassa subterranea (Montagu 1808) (*C. pestae* [part], *C. helgolandica*)
    Callianassa tyrrhena (Petagna 1792) (*C. laticauda, C. stebbingi, C. candida*)
Family: Laomediidae Borradaile 1903
  Genus: *Jaxea* Nardo 1847
    Jaxea nocturna Nardo 1847
Family: Upogebiidae Borradaile 1903
  Genus: *Upogebia* Leach 1814
    Upogebia stellata (Montagu 1808)
    Upogebia deltaura (Leach 1815)
    Upogebia pusilla (Petagna 1792) (*U. litoralis*)

Owing to the deep burrowing habits of the Thalassinidea, an understanding of the distribution of many species is limited. The Upogebiidae is a relatively large, but uniform, family containing more than 101 species of which 87 burrow into sediments, 4 bore into corals and 10 live in siliceous sponges (Dworschak & Ott unpubl. obs). Until recently the Upogebiidae was one of the three sub-families that consituted the Family Callianassidae (Upogebiinae, Callianassinae and Callianidienae) (Borradaile, 1903; Balss, 1957). The numerous characteristics separating the Callianassinae and Upogebiinae indicated that these sub-families had distinct phyletic origins. As a result, the sub-family Upogebiinae was raised to distinct family status (de Saint Laurent, 1973).

Upogebia stellata (Fig.1.2) was first described as *Cancer Astacus stellatus* by Montagu (1808), Leach (1813) later renamed it *Upogebia stellata* and Leach (1815) described a second species under another genus, *Gebia deltaura* (Fig.1.1). *Upogebia stellata* and *U. deltaura* usually occur together and, as a consequence, have often been confused as the same species. For some time it was believed that any differences between these species were sexual and not specific

Fig. 1.1. *Upogebia deltaura*

Fig. 1.2. *Upogebia stellata*

(Bell, 1853). Borradaile (1903), however, recognised that these two species were indeed distinct and placed them into the separate subgenera *Upogebia* and *Gebiopsis*. De Morgan (1910) found males and females of both forms and later Webb (1919) described the larval development of both species, which are now clearly recognised as separate and distinct species. Although this division was retained by Selbie (1914), the genus *Upogebia* is now considered monogeneric and the subgenus *Gebiopsis* has been discarded (de Saint Laurent, 1973).

Upogebia stellata is considerably smaller than *U. deltaura*, the prodopus of the chela is much shorter than the dactylus and *U. stellata* has a small spine on the antero-lateral corner of the carapace, ventral and posterior to the eye-stalk (this feature is absent in *U. deltaura*). The living animal is covered with numerous orange-red stellate markings (hence the specific name *stellata*), compared with the uniform creamy-orange colour of *U. deltaura*.

Although compounded by the difficulty of distinguishing between the two species in early records, the distribution of both *U. stellata* and *U. deltaura* has been recorded as extending from the Swedish coast of Kattegat and Skagerak (Gustafson, 1934; Tunberg, 1986); west coast of Norway (Samuelson, 1974); northern Kattegat (Poulson, 1940); east coast of Britain (Stephenson, 1910), Irish sea (Selbie, 1914); southern North Sea (Adema *et al.*, 1982); west coast of Scotland (Selbie, 1914; Allen, 1967) and the English Channel (DeMorgan, 1910; Webb, 1919; Plymouth Marine Fauna, 1957). *Upogebia deltaura* has been found as far south as the Atlantic coast of western Europe and the Mediterranean Sea (Stephenson, 1910; Gustafson, 1934).

Upogebia stellata is also closely related to *U. pusilla* (=*U. litoralis*) a species which has a more southerly distribution. *Upogebia pusilla* is very common around the Mediterranean (de Saint Laurent & Le Loeuff, 1979; Dworschak, 1983) but may extend as far north as Norway (Bouvier, 1940). Upogebiids are principally found in the littoral zone with individuals recorded to depths of 40m (Adema *et al.*, 1982). Although the distribution pattern of *U. stellata* and *U. deltaura* with depth has not been extensively studied, it has been suggested that where these two species co-occur *U. stellata* is often found at greater depths (Webb, 1919; Gustafson, 1934). A substratum difference is also suggested - *U. deltaura* occurs in coarser deposits (R.J.A. Atkinson pers. comm.).

The family Callianassidae consists, at present, of more than 154 species distributed between 8-10 genera, the majority being found within the large genus of *Callianassa* (de Saint Laurent & Le Loeuff, 1979; Manning & Felder, 1991; Dworschak & Ott unpubl. obs.). The Callianassidae, as defined by Borradaile (1903), has since been redefined by a number of workers. De Saint Laurent (1973) divided the family Callianassidae into the two sub-families Callianideinae and Callianassinae, both of which were later modified by de Saint Laurent & Le Loeuff (1979). A recent revision of American thalassinideans highlighted the heterogenous nature of the genus and this finding led Manning & Felder (1991) to define one new family (for

Fig. 1.3. *Callianassa subterranea*

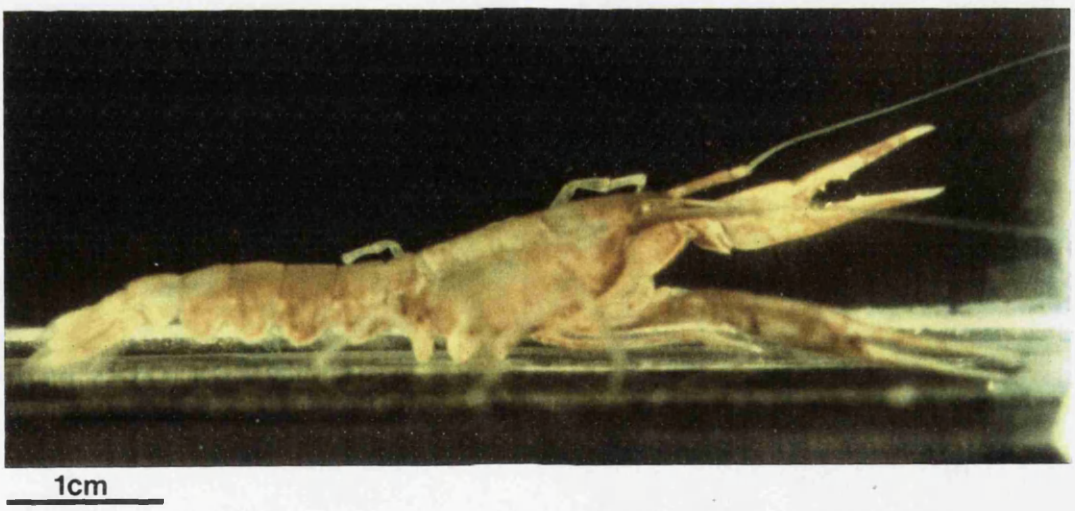


Fig. 1.4. *Jaxea nocturna*

taxa previously assigned to Callianassidae), the Ctenochelidae, and six new sub-families. De Saint Laurent (1979) also suggested that the families Callianassidae, Callianideinae and Axiidae all share a number of characteristics, indicating a link (or parentage) between the families, and as a result she placed these families within the superfamily Axoidea.

Two species of the genus *Callianassa* have been recorded from the North Sea and adjacent waters: *C. subterranea* (includes *C. helgolandica* Poulson (1940)) and *C. tyrrhena* (= *C. stebbingi* = *C. laticauda*) (Borradaile, 1903; Selbie, 1914; Lutze, 1938; Poulson, 1940). *Callianassa subterranea* has been recorded from the south coast of England, within the littoral zone and often among *Zostera* beds (Montagu, 1808; Allen & Todd, 1909; Plymouth Marine Fauna, 1957; Rowden & Astall unpubl. obs.). Although Selbie (1914) considered the distribution of *C. subterranea* to be restricted to the south coast of England, it has been taken in large numbers in several Scottish sea lochs and in the Clyde Sea area (Allen, 1967; Atkinson, 1986; Anderson, 1989; Nickell, 1992).

Callianassa subterranea (Fig.1.3) has also been recorded at depths of 30m around the Swedish coast (Gustafson, 1934), the south coast of Norway between 60-100m (Christiansen & Grere, 1982) and parts of the Kattegat, Skagerak and North Sea which border the Danish coast (30-60m depth) (Poulson, 1940). Further south, *C. tyrrhena* is more common, being found in the Mediterranean and as far as the west coast of Africa, from Mauritania to the south of Angola (de Saint Laurent & Le Loueff, 1979; Dworschak, 1987). *Callianassa tyrrhena* has also been recorded from Lundy and the Isle of Man (Atkinson & Schembri, 1981; Bruce *et al.*, 1963) and numerous specimens have been taken intertidally from the Channel Islands (Manning pers. comm.).

The Axiidae family are a group of thalassinideans which are characterised by the lack of the longitudinal groove in the cephalothorax, otherwise known as the *linea thalassinica*. Originally, two genera from this family were represented in British waters, *Axius* Leach 1815 and *Calocaris* Bell 1853. Boradaille (1903), and later De Mann (1925) established 5 subgenera for the genus *Axius* and 2 subgenera for *Calocaris*. Sakai & de Saint Laurent (1989), in their check list of Axiidae, highlighted the problems of discovering new species. Since the characteristics for the taxa imposed by Borradaile (1903) and de Mann (1925) were continually narrowing, it was becoming more difficult to relate these new species to their older descriptions. Indeed, in an earlier review, de Saint Laurent (1972) noted the lack of generic diagnosis in the Axiidae family and emphasised the need for a more complete description of the group (including the branchial and genital apparatus). A much required revision of the systematics of this group was later given by Kensley (1989) who reinstated the family Calocarididae, therefore *Axius* and *Calocaris* are now found in separate families.

Axius stirhynchus is rarely encountered as a result of its burrowing habit in often inaccesible near-shore sediments. The very few records are usually of single animals. This

species has been recorded in shallow sublittoral or littoral sediments from the south west coast of England (Bell, 1858; Plymouth Marine Fauna, 1957; Haywood & Ryland, 1990), in the Severn Estuary (Anderson, 1989), Norfolk coast (Ellis & Baker, 1972), Clyde Sea area (Allen, 1967), Channel Islands and Mediterranean (Sakai & de Saint Laurent, 1989).

Calocaris macandreae, a member of the Calocarididae Ortmann 1891, was first taken in Loch Fyne, Scotland by Mr McAndrew in 1845 and was later named after McAndrew by Bell (1853). It has since been recorded from depths of 30m-1100m (Buchanan, 1963) and densities of up to 20 per m^2 at 80m depth have recorded off the Northumberland coast (Buchanan, 1963). *Calocaris macandreae* is one of the more commonly thalassinideans and has a wide geographical range which extends from the eastern boreal Atlantic-Mediterranean including the south and west coasts of Norway, Sweeden, Kattegat and Skagerrak (Stephenson, 1910; Gustafson, 1934; Poulson, 1940), the coast of Britain, Holland and Belgium (Selbie, 1914; Buchanan, 1963; Allen, 1967; Haywood & Ryland, 1990) and as far as North America, the Arabian Gulf, Indian ocean and Pacific ocean (Sakai & de Saint Laurent, 1989).

The Laomediidae comprises 13 species distributed between 5 genera (Wear & Yaldwin, 1966; Pervesler & Dworschak, 1985; Dworschak & Ott unpubl. obs.). Only one species, *Jaxea nocturna* (Fig.1.4), has been recorded from the north and south coasts of Britain and the Irish Sea. (Selbie, 1914; Plymouth Marine Fauna, 1957; Allen, 1967). Recently, a large population has been discovered burrowing in soft mud in Loch Sween, Argyll (Atkinson, 1987) and recent underwater TV surveys have shown it to be abundant throughout the Clyde Sea area (R.J.A. Atkinson pers. comm.). Prior to these finding only single or very few specimens were recorded from around the U.K. possibly because of inappropiate sampling methods for this deep-burrowing species. *Jaxea nocturna* is also found in the Adriatic Sea (Hohenegger & Pervesler, 1984; Pervesler & Dworschak, 1985).

Thalassinidean biology and ecology

The majority of thalassinideans construct burrows within sediments, and as a result of this secretive habitat knowledge of their population structure, distribution, reproductive ecology and general biology is limited to only a few species. Information is particularly scarce for those species inhabiting sublittoral as compared to littoral areas.

The life span of thalassinideans, estimated using size frequency distributions, varies from at least 2-3 years for *Callianassa australiensis* (Hailstone & Stephenson, 1961) and *C. kraussi* (Forbes, 1977) to 4 years for *C. filholi* (Devine, 1966) and 10 years for *Neotrypaea* (as *Callianassa*) *californiensis* (MacGinitie, 1934). Dworschak (1988) reported a life span of over 5 years for *Upogebia pusilla*, the longest estimated for any member of the Upogebiidae. A life span of at least 3 years was suggested by Tucker (1930) for *U. pusilla* and by Gustafson (1934)

for *U. deltaura*. *Calocaris macandreae* appears to live for 5-10 years (Buchanan, 1963; Calderon-Perez, 1981), with regional variations.

The breeding season is mainly divided into two distinct breeding peaks, which are clearly separated into a major and minor episode, with a spring and summer division. Such a cyclical pattern has been shown for *Callianassa australiensis* (Hailstone & Stephenson, 1961), *C. kraussi* (Forbes, 1979), *Upogebia pusilla* (Dworschak, 1988) and *Calocaris macandreae* (Buchanan, 1963). Although the breeding periods were widely separated for most *U. africana*, Hanekom & Erasmus (1989) reported a single, prolonged breeding season in large individuals. A single breeding season has also been recorded for *Lepidopthalmus* (as *Callianassa*) *louisianensis* (Felder & Lovett, 1989) and *Upogebia deltaura* (Tunberg, 1986).

As thalassinideans are rarely observed outside their burrows, many authors have suggested that mating occurs in intersecting burrows (e.g. Pohl, 1946; Rodrigues, 1976; Felder & Lovett, 1989). However, the location, timing and frequency of mating is unknown for virtually all thalassinidean species. There is information for *Calocaris macandreae* which, unusually for decapods, is a protrandous hermaphrodite (Buchanan, 1963). Both the testes and ovaries develop at the same time for the first three years of life. During the fourth year the testes degenerate but the *vasa deferentia* are left filled with spermatophores. The ovaries continue to develop and mature, with the first batch of eggs being laid during year 5 in Northumbrian specimens (Buchanan, 1963).

Information on moulting has shown that *C. macandreae* moult annually from four years of age although for younger individuals several moults take place through the year (Buchanan, 1963). Tunberg (1986) found *U. deltaura* moult once during the year and there is a distinct difference in moulting period between the male and females. The females moult about one month earlier than the males. This may be expected if hardened males were to copulate with soft females (Tunberg, 1986; Hailstone & Stephenson, 1961).

The majority of thalassinideans lead a solitary life, with only one individual occupying a single burrow. Thalassinideans seldom leave the protection of the burrow, outside of which they are vunerable. Apart from a brief pelagic larval phase (which may be abbrieviated in some species e.g. *C. macandreae*) most thalassinideans rely on burrows for the provision of a site for protection from predators, localizing feeding activities, moulting, mating, egg incubation or juvenile recruitment (see review by Atkinson & Taylor, 1988). Although little is known of the biology of thalassinideans, probably as a result of their fossorial habitat, the burrow architecture has intrigued biologists for many years and, as a result, the burrow morphology of more than 44 species, distributed between 10 genera, is now known (Dworschak, 1983; de Vaugelas, 1990; Griffis & Suchanek, 1991). Thalassinidean burrow architecture has been shown to be species-specific, varying in complexity from a simple 'U' or 'Y' form to more complicated mosaics of galleries and networks of interconnected tunnels (e.g. Dworschak, 1983; Suchanek, 1985;

Bromley, 1990; de Vaugelas, 1990; Griffis & Suchanek, 1991). Currently, a number of models have been developed which relate burrow structure to the trophic feeding mode of the shrimp (e.g. Suchanek, 1985; de Vaugelas, 1990; Griffis & Suchanek, 1991; Nickell, 1992).

The bioturbatory activity of thalassinideans has long been recognised, with most studies concentrating on members of the Callianassidae. Burrowing shrimps play a dominant role in sediment turnover (bioturbation) and, as a result, have been shown to strongly influence the structure and composition of macrobiotic and microbiotic communities (e.g. Branch & Pringle, 1987; Suchanek, 1983; Posey, 1986; Griffis & Chavez, 1988; Posey *et al.*, 1991). The burrow also provides a site for the recycling of organic material (Suchanek *et al.*, 1986; Vaugelas & Buscail, 1990), nutrients (Aller *et al.*, 1983; Waslenchuck *et al.*, 1983; Koike & Mukai, 1983; Witbaard & Duniveld, 1989; Nickell, 1992) and trace elements (Pemberton *et al.*, 1976; Aller *et al.*, 1983; Abu-Hilal *et al.*, 1988).

One consequence of a burrowing habit, however, is the reduced oxygen tensions of the water often found within the burrow (see Atkinson & Taylor, 1988 for review; Anderson *et al.*, 1991). As a result of their burrowing habit, thalassinideans have adopted a range of behavioural and physiological adaptations to cope with life within their hypoxic burrow. Such adaptations have included reduced metabolism at lowered oxygen tensions and the ability to regulate respiration rate; tolerance of severe hypoxia and anoxia; a high affinity respiratory blood pigment and burrow irrigation behaviour related to oxygen tension (e.g. Farley & Case, 1968; Thompson & Pritchard, 1969; Miller & Van Holde, 1974; Torres *et al.*, 1977; Miller *et al.*, 1976; Felder, 1979; Hill, 1981; Mukai & Koike, 1984; Hanekom & Baird, 1987; Witbaard & Duniveld, 1989; Anderson *et al.*, 1991; Nickell, 1992).

Aims of thesis

1. To determine the burrow structure of the sublittoral species *Upogebia stellata* and *U. deltaura* and to examine the irrigation behaviour and physico-chemical fluxes within the burrow.

2. To examine the comparative respiratory physiology of the filter feeding upogebiids, *U. stellata* and *U. deltaura*, and the deposit feeders *Jaxea nocturna* and *Callianassa subterranea*. The comparative 'physiology' was divided into a number of discrete sections, namely;

    (a) comparative and functional branchial morphology
    (b) oxygen consumption and regulation
    (c) blood-gas transport and comparative haemocyanin structure and function.

These aspects of respiratory physiology were then discussed in relation to the burrowing habitat.

3. To investigate sulphide tolerance and detoxification ability of the mud-shrimp *Calocaris macandreae*.

Chapter 2

Upogebiid burrow morphology, environment and irrigation.

Introduction

Burrows are a conspicuous feature of marine sediments and thalassinidean shrimps are amongst the most common burrowing organisms of the marine environment; littoral or sublittoral (Griffis & Suchaneck, 1991). The primary function of the burrow is to provide protection, not only from predators but also from abiotic environmental fluctuations. The burrow also provides a site for localizing feeding activities, moulting, mating or juvenile recruitment (Atkinson & Taylor, 1988; Bromley, 1990).

The burrow morphology will reflect the life habit and ecology of the burrower, or its architect, with the dimensions being dependent on the size of the occupant (Dworschak, 1983). Burrowing will have important ramifications on the characteristics of the sediment (Suchaneck, 1983), nutrient recycling (Aller *et al.*, 1983; Koike & Mukai, 1983; Waslenchuck *et al.*, 1983) and local community composition (Suchaneck, 1983; Posey, 1986; Posey *et al.*, 1991).

Thalassinidean burrow morphology has been described for callianassids (Shinn, 1968; Ott *et al.*, 1976; Braithwaite & Talbot, 1972; Dworschak, 1983; de Vaugelas, 1984; Dworschak & Pervesler, 1986; Witbaard & Duniveld, 1989; Atkinson & Nash, 1990; Nickell, 1992), upogebiids (Frey & Howard, 1975; Ott *et al.*, 1976; Dworschak, 1983; de Vaugelas, 1990; Nickell, 1992), axiids (Pemberton *et al.*, 1976; Pervesler & Dworschak, 1985) and calocaridids (Nash *et al.*, 1984: Anderson, 1989). Recently, a model has been developed linking thalassinidean burrow structure, ecology and trophic feeding mode (Suchaneck, 1985; Griffis & Suchaneck, 1991). By analysing the burrow structure of 44 thalassinidean species, Griffis & Suchaneck (1991) derived a simple model classifying thalassinidean burrows into 6 major groups, based on a number of burrow morphological characteristics. Burrowing behaviour has been described for upogebiids (MacGinitie, 1930; Pearse, 1945; Ott *et al.*, 1976; Nickell, 1992), calocaridids (Nash *et al.*, 1984; Anderson, 1989) and callianassids (MacGinitie, 1934; Pohl, 1946; Devine, 1966; Ott *et al.*, 1976; Dworschak, 1987; Nickell, 1992).

Burrowing may affect the physical, chemical and biological characteristics of sediments, primarily through fluid bioturbation which allows the introduction of oxygen deep into the anoxic sediment (Aller & Yingst, 1985). Rates of fluid bioturbation, as burrow irrigation, through burrow structures are limited to theoretical or indirect evaluations for few species of thalassinideans (Dworschak, 1981; Koike & Mukai, 1983; Aller *et al.*, 1983) and an alpheid shrimp (Gast & Harrison, 1981). Direct measurements of water flow through the burrow and a complete irrigatory profile has only been determined for two thalassinidean shrimp species

(Nickell, 1992) and burrowing fish (Pullin *et al.*, 1980; Atkinson *et al.*, 1987).

Although the burrow may confer some advantages, aquatic species are faced with the potential problem of reduced oxygen tension (hypoxia) and elevated carbon dioxide (hypercapnia) (Atkinson & Taylor, 1988). Burrow irrigation will certainly influence the degree of oxygenation within the burrow and the complexity of burrow architecture will affect the spatial distribution of oxygen throughout the burrow.

This study was primarily concerned with detailing the burrow morphology of the upogebids *U. stellata* and *U. deltaura.* The degree of water flow and the irrigation profile for both species was measured directly using an electromagnetic flow probe and, coupled with measurements of spatial and temporal distribution of oxygen through the burrow, the burrow environment was discussed and compared with particular regard to the mode of feeding.

Materials and methods

Collection and maintenance of shrimps

Upogebia stellata were obtained by anchor dredging (depths to 25m) around the Isle of Cumbrae (Main Channel and White Bay), Scotland, UK (55°9'N, 5°11'W). *Upogebia deltaura* were collected with an anchor dredge (7-10m) from Plymouth Sound (50°20'N, 47°13'W), Plymouth, UK and from the North Sea (54°4'N, 4°50'W). Animals taken from Plymouth were placed individually into containers. The containers were then placed into plastic bags filled with 1l seawater. The plastic bags were sealed and the animals were transported to Scotland in a cool box.

Individuals, including the Plymouth animals transported to Scotland, were then transferred to Perspex tanks (35cm x 25cm x 12cm) filled to 25cm depth with sediment from the Cumbrae sample site. These tanks were maintained under red light conditions within a temperature controlled room (14±2°C). Recirculated, fully oxygenated seawater (salinity 32-35‰; temperature 10-13°C) was passed into each tank, through a pipe positioned above the sediment surface.

Although an animal often constructed a burrow within the first few days of being placed in a tank, the burrow was left undisturbed for 2-3 months prior to experimentation. Burrows constructed within the tanks often made contact with the clear Perspex walls and this allowed behavioural observations to be made and provided a site for water sampling.

Burrow morphology

Field studies

Polyester resin casts of the burrows were made at depths of 15-20m at Farland Point, Cumbrae using a method similar to that of Atkinson and Chapman (1984). Nylon marker lines were placed along the sea bed by divers using SCUBA equipment. Sections of plastic tubing 6.5cm in diameter and 5cm long were carefully placed over a number of burrow openings (usually 3 or 4 per casting session) and pushed into the sediment. The following day, low viscosity polyester resin (Trylon SP701PA) was mixed with an organic peroxide catalyst (1% by volume). If necessary, thinners (10% by volume) were added to the mixture. The mixed resin was then taken to the sea bed and carefully poured into the marked burrow openings. The resin was left to harden for more than 24h before the casts were removed from the sediment.

The Farland Point site was considered the best of the known areas around Cumbrae, however, limitations of the time allowed underwater and the depths at which the burrow openings were found restricted diving. Additionally, the small size of the burrow openings (3-10mm; Fig.2.1) made identification and pouring of resin very difficult.

Laboratory studies

Once recording had been completed, a number of burrows that had been constructed in the laboratory-maintained mud-tanks were cast with polyester resin. In addition, where the burrow made contact with the clear Perspex wall of the mud-tank, its structure could also be inferred from observations.

Burrow water sampling

To determine the oxygen tension, pH and ammonia concentration of the burrow water, samples of burrow water were taken from the burrow lumen using a method similar to that described by de Vaugelas & Jaubert (1989). Perspex plates (20mm x 20mm x 8mm) equipped with a plastic tube (3mm internal diameter) were fixed to the wall of the mud-tank. A 2mm hole was then drilled through the tube, plate and wall of the mud-tank and a 3-way luer valve attached to the end of the plastic tube to prevent water escaping from the burrow. Compared to the pre-drilled method of sampling (Koike & Mukai, 1983), this system is very flexible and holes can be drilled at any point on the mud-tank wall (de Vaugelas & Jaubert, 1989).

Spatial and temporal chemical fluxes within the burrow

Burrow water was taken from the sampling ports using either a lml or 5ml plastic syringe. The pH of a 0.5-1.0ml sample was measured at the environmental temperature using a pH microelectrode (Russel) connected to a pH meter (PHM83, Radiometer, Denmark).

The concentration of ammonia in the burrow water was measured using the method of Liddicoat *et al.* (1975). This method is based primarily on the Berthelot reaction where ammonia, in the presence of a hypochlorite donor, forms chloramine which reacts with phenol in the presence of a catalyst to form quinonechloramine. Indophenol is formed following subsequent reaction with another molecule of phenol. Since indophenol dissociates at a high pH to give a blue colour the absorbance can easily be measured.

The use of a complexing agent, sodium citrate, prevents the complextion of the magnesium and calcium in sea water at the high pH needed for the reaction (Solorzano, 1969). Other modifications to the method have involved changing the catalyst, source of hypochlorite ions and the technique of colour development (Solorzano, 1969; Gravitz & Gleye, 1975; Liddicoat *et al.*, 1975).

The oxygen tension (PO_2) of the burrow water was measured by passing a water sample into a thermostatted (13°C) microcell (MC100, Strathkelvin Instruments) and the PO_2 determined using a polarographic oxygen electrode (1302, Strathkelvin Instruments) connected to an oxygen meter (781, Strathkelvin Instruments).

For long-term measurements of oxygen tension, plastic tubing (internal diameter = 0.5mm) was connected from the luer valve (at the sample port) to the microcell. Burrow water was slowly siphoned through the microcell at a constant flow rate (< $lml.min^{-1}$ or at a rate that was not detectable by the flow probe) and the PO_2 continuously monitored using the oxygen electrode. Prior to each experiment the oxygen electrode was calibrated against a solution having a PO_2 of zero (sodium sulphite in 0.01M sodium tetraborate) and air saturated sea water. During long term PO_2 measurements the sampling port was positioned in that part of the burrow in which the occupant was previously observed to spend the majority of its time.

Irrigation activity

In addition to measuring the burrow water PO_2, movement of water through the same burrow was measured continuously using an electromagnetic blood flow transducer (Spectramed, SP2202 flowmeter). The flow probe, 1.5mm or 3.0mm diameter, chosen to match the diameter of the burrow opening as closely as possible, was mounted onto a plastic filter funnel (4cm base diameter).

The filter funnel was inverted, placed over one of the burrow openings and pushed gently into

the sediment to form a seal. Any movement of water through the burrow passed through the flow probe and the rate of water flow was detected by the flowmeter and recorded using a chart recorder (SE120, Belmont Instruments). Prior to use the flow probe was calibrated for flowing sea water by direct measurement. Seawater samples were passed through the flow probe and were collected over a known time period, the rate of flow was then calculated from the volume of seawater collected over the time period (Nickell, 1992).

Statistical analysis

Data were plotted and appeared normally distributed. Statistical analyses were carried out using analysis of variance, two-way analysis of variance, and Students *t*-test where appropiate (Sokal & Rohlf, 1981). Where the mean of a number of observations is given the deviation from the mean is expressed as standard deviation (SD). However, where the mean of a number of mean values are determined, the deviation is expressed as standard error (SE).

Results

Burrow morphology

A total of four *Upogebia* spp. burrows were cast at depths of 18-20m at Farland Point, Cumbrae. During the process of resin casting the shrimp occupying the burrow was trapped in three of the four burrows cast, indicating that each burrow was occupied by one individual and provided positive identification that the casts were indeed the burrows of *Upogebia* species. It was not possible to remove the shrimp from the resin cast, therefore, identification of the occupant to species level could not be made. As *Upogebia stellata* and *U. deltaura* had both been caught previously from the study site it was not possible to determine which species constructed the cast burrows. Laboratory observations indicated that the burrow morphology of each species was similar. Therefore, the following description of burrow morphology applies to both *Upogebia stellata* and *U. deltaura*.

The field casts of the burrows of *Upogebia* showed two openings which connected to form a basic U-shaped burrow (Fig.2.2). In one cast a perpendicular shaft originated from the U (Fig.2.2). Casts of the burrows of *Upogebia stellata* and *Upogebia deltaura* constructed in mud-tanks showed a similar U-shaped pattern with a main shaft originating from the lower part of the U and branching downwards, usually in a spiral manner (Fig.2.3).

The shape of the U varied between burrows depending upon the distance between the burrow openings. In burrow casts where the distance between the openings was small the U was pear-

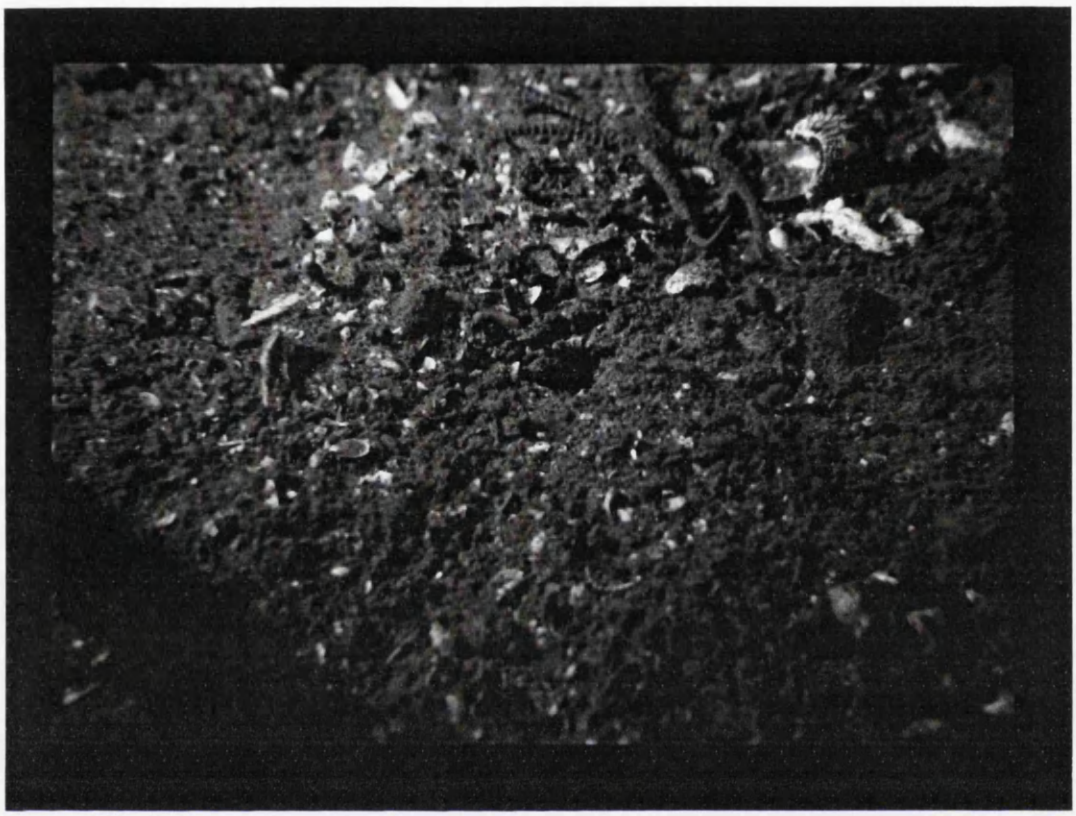


Fig. 2.1. Burrow opening of a *Upogebia* spp. at 16m depth off Farland Point, Cumbrae. The diameter of the burrow opening is 7mm.

shaped (Fig.2.2), whereas the U was flattened in those burrows in which the distance between the openings was greater compared to the depth (Fig.2.2). From observations of shrimp which had established burrows in mud-tanks, additional side branches were sometimes constructed from the main U prior to the shrimp moulting. The shrimp would retreat into this secondary tunnel during moulting and once moulting was complete the tunnel was backfilled so burying the exuvia (Fig.2.4).

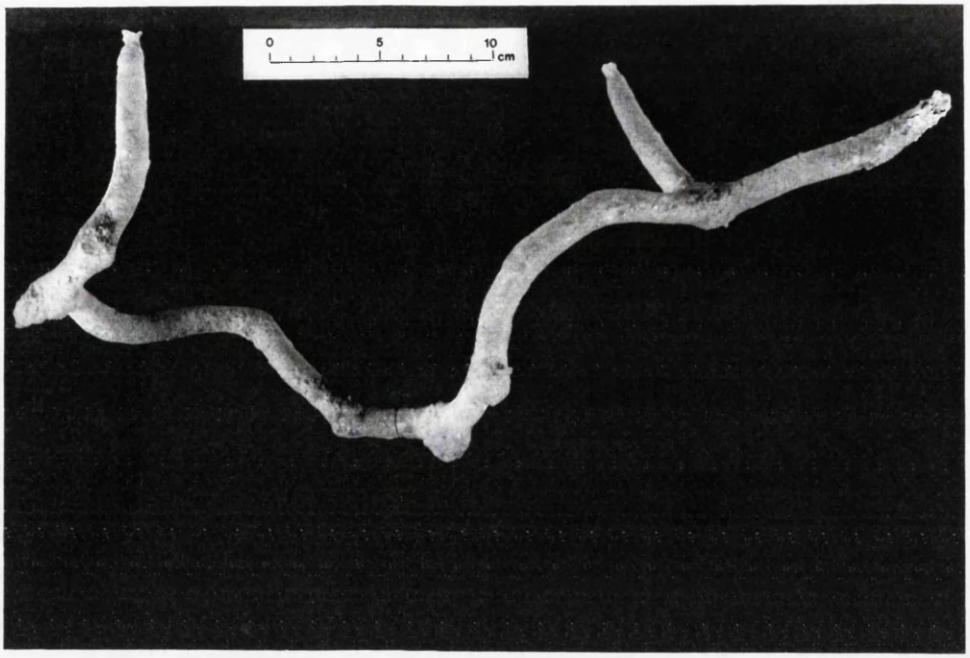
The burrow casts were circular in cross section and the diameter of the tunnels was usually between 13 to 17mm and from observations of burrows constructed in the mud-tanks the diameter of the lumen reflected the diameter of the extended telson and uropods (Fig.2.5). Measurements of individuals trapped within the resin casts and laboratory observations indicated that the diameter of the burrow was always found to be smaller than the carapace length of the individual shrimp (Fig.2.6).

Numerous enlargements (3-5 per burrow) of the lowermost part of the burrow lumen were observed in all the resin casts (Figs.2.2 and 2.3). These enlargements have been termed 'turning chambers' by Dworschak (1983) (Fig.2.7). A turning chamber was also formed in the region where the shaft ascended from the more horizontal U-shaped part of the burrow. As the shrimp has a carapace length larger than the burrow diameter the turning chamber allows the occupant to change direction within the burrow, this it does by performing a foreward somersault within the turning chamber (Fig.2.8). The turning chambers were pear-shaped in cross-section, with the narrow part pointing downwards, and measured 26-40mm at the widest point (Fig.2.7).

In all the complete field burrow casts, the uppermost 5-15mm of the burrow shaft was constricted to 6-8mm in diameter (Fig.2.9). Similar constrictions were seen in burrows cast from laboratory mud-tanks and during observations of burrows maintained in laboratory aquaria

The burrow wall was mostly smooth, but in the lower part of the turning chamber it was rougher, probably a result of sediment deposition (especially large particulate material). Observations of a number of shrimps within burrows constructed in laboratory mud-tanks have shown that the shrimps spend a large proportion of their time tending to their burrows. This often involved cleaning, compaction and maintenance of the burrow wall which in combination with the movement of the shrimp through the burrow, probably accounts for the smooth burrow wall. No direct observations of mucus secretions lining the burrow walls have been observed for either of the upogebiid species; however, a mucus lining is not improbable as the shrimps were often observed compacting sediment against the smooth Perspex wall of the mud-tank.

An oxidized layer of sediment surrounding the burrow lumen varied in thickness from approximately 2-13mm depending on the burrow size. The oxidized wall sediment of a *Upogebia deltaura* burrow, taken from Plymouth Sound, is shown clearly as a light brown layer adjacent to the burrow lumen (Fig.2.10), whereas the surrounding reduced sediment is black. Similarly, the wall sediment of upogebiid burrows constructed in laboratory mud-tanks appeared lighter in



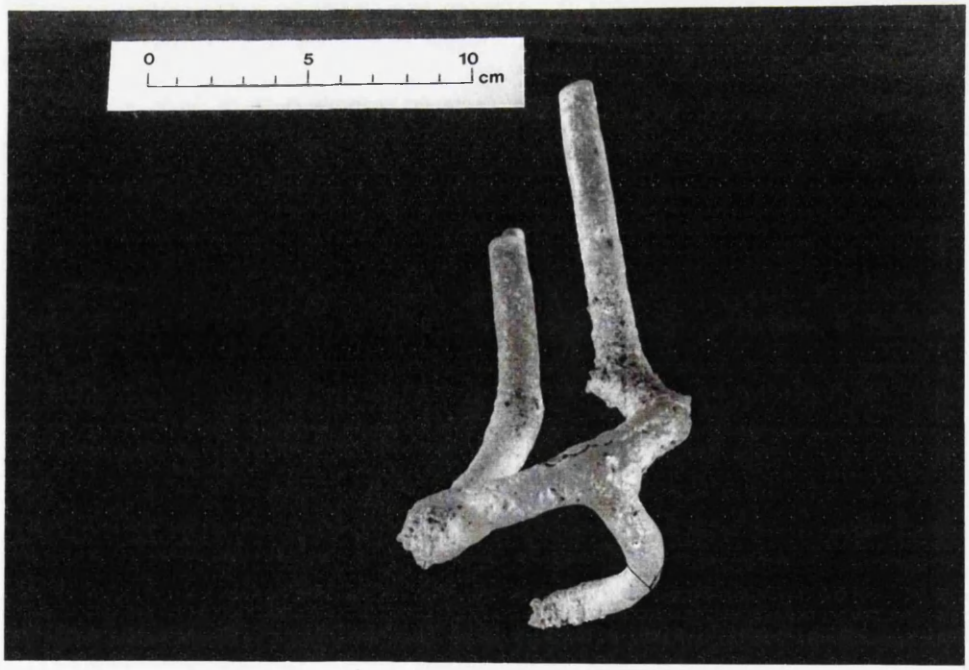


Fig. 2.2. Resin casts of *Upogebia* burrows taken at 18-20m at Farland Point, Cumbrae. Scale bar is in cm.

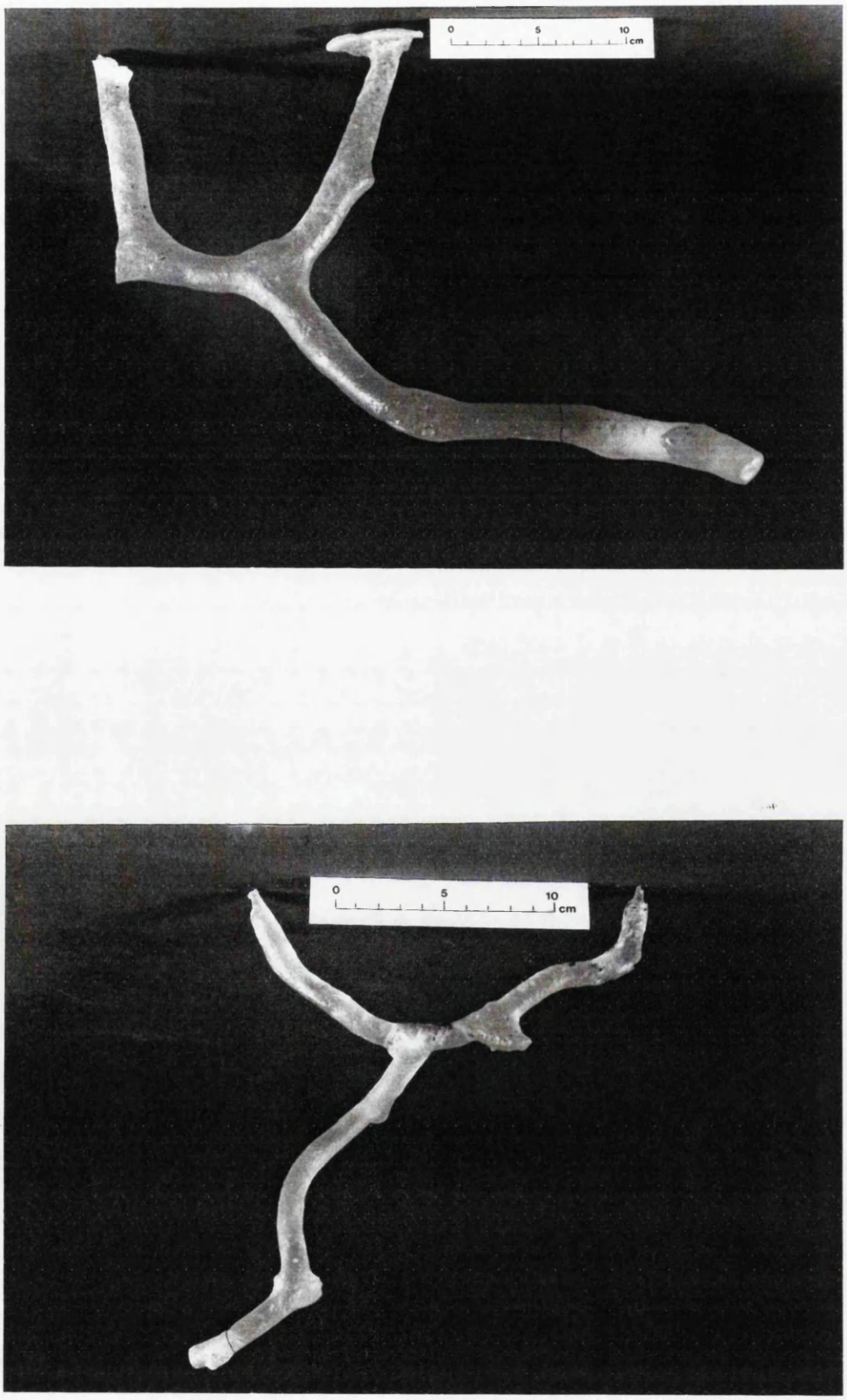


Fig. 2.3. Resin casts of *Upogebia* burrows constructed in mud-tanks. (a) *Upogebia deltaura,* (b) *Upogebia stellata.* Scale bar is in cm.

colour than the surrounding reduced sediments (Fig.2.4).

In burrows that had been occupied by the same individual for long periods of time (6-18 months) the gross structure changed very little once the burrow had been constructed. However, the shrimp constantly reworked the sediment within the burrow. Mud, collected from the burrow, was either carried to the sediment surface and expelled, or retained and used as building material to maintain the walls of the burrow. This suggests that the burrows of the upogebiids are relatively stable, permanent structures. Even the structure of burrows in which the inhabitant had died were retained for long periods. The sediment from the walls of such non-maintained burrows accumulated slowly in the lumen of the burrow but even after 6 months the structure of two burrows in which the occupants had died was still recognisable.

Burrow irrigation

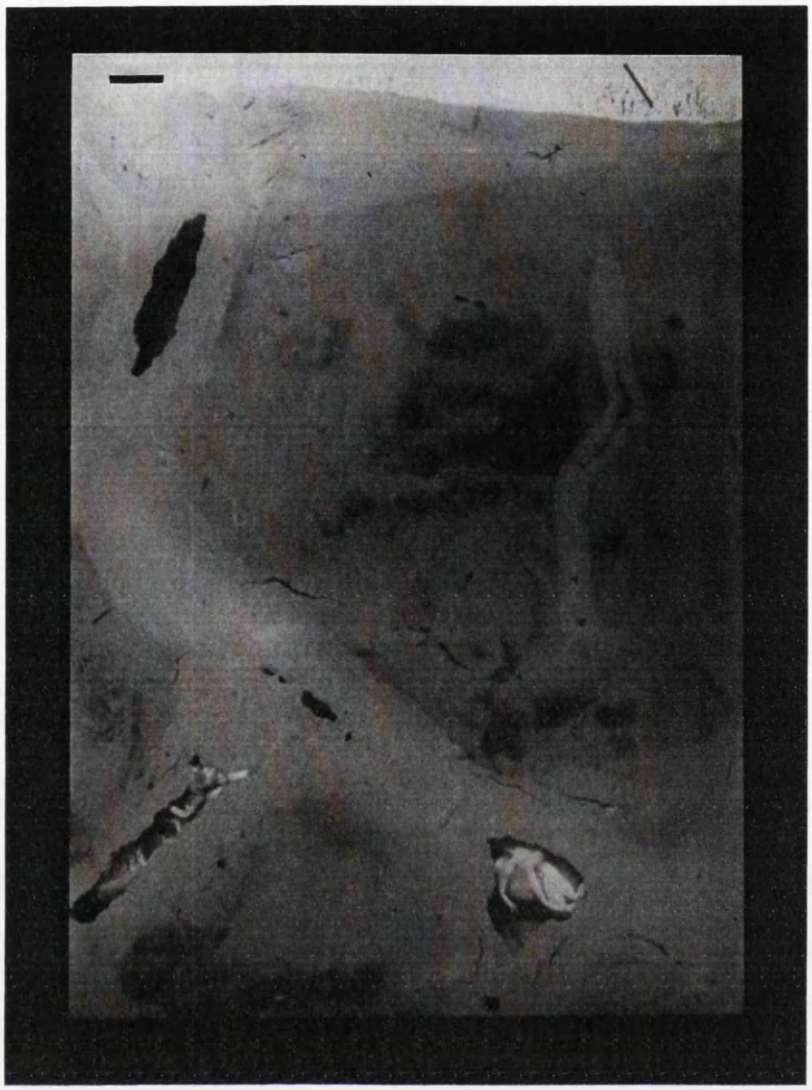
Irrigation of the burrow by the mud-shrimps *Upogebia stellata* and *U. deltaura* was achieved by the fanning effect of the abdominal appendages (pleopods) (Fig.2.11).

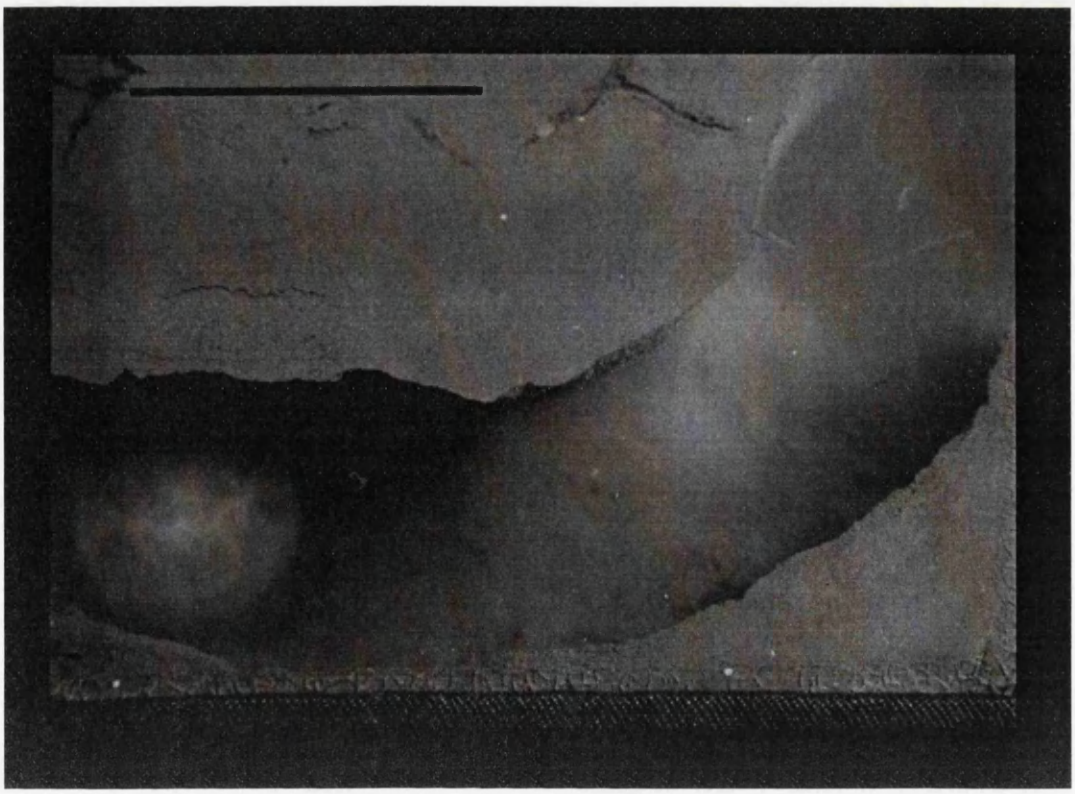
Table 2.1. *Upogebia deltaura* and *U. stellata*. Summary of the burrow irrigation profiles for two thalassinidean mud-shrimps. [a] and [b] are recordings made from the same burrow but on two separate occasions. The size of the shrimp was estimated using orbital length (anterior edge of the eye orbit to the posterior edge of the cephalothorax). Values given are means ±SD.

Burrow No.	Orbital length (mm)	Flow rate ($ml.min^{-1}$) during irrigation	% time irrigating	Irrigation time (min)	Time to next event (min)	Irrigation episodes	Recording duration (h)
U. deltaura							
1	10	4.6 ± 2.7	9.1	3.6 ± 2.8	35.6 ± 35.4	87	56.8
2	20	13.9 ± 11.7	4.1	7.7 ± 4.3	176.0 ± 92.4	13	39.7
3	10	34.1 ± 11.7	32.6	29.3 ± 40.5	60.5 ± 109.1	30	44.9
4[a]	25	25.1 ± 13.4	8.2	5.6 ± 7.3	61.6 ± 53.9	51	57.0
4[b]	25	13.6 ± 8.0	2.3	6.3 ± 6.6	265.0 ± 358.0	13	58.8
5[a]	11	18.0 ± 10.0	9.9	4.8 ± 4.9	36.2 ± 52.9	63	42.9
5[b]	11	19.9 ± 7.6	5.9	2.5 ± 2.0	57.9 ± 46.6	29	20.6
U. stellata							
1	8	4.6 ± 1.5	16.1	3.6 ± 7.2	18.5 ± 19.4	177	54.8
2	4	4.2 ± 3.4	7.5	2.1 ± 1.3	26.1 ± 40.0	94	44.1
3	8	7.4 ± 6.6	9.1	1.6 ± 0.6	16.5 ± 34.9	21	6.3
4	8	27.1 ± 22.8	38.6	5.8 ± 1.2	8.9 ± 4.6	39	9.5
5	10	18.0 ± 10.0	6.2	2.2 ± 3.1	33.3 ± 53.3	89	53.3
6	11	31.6 ± 17.9	6.7	1.4 ± 1.0	21.9 ± 15.2	24	9.4
7	10	15.4 ± 16.0	5.7	2.7 ± 1.9	46.7 ± 47.0	37	29.6

Fig. 2.4. Section of an established *Upogebia deltaura* burrow, constructed in the laboratory, which shows a secondary tunnel containing recent excuvia (arrowed). The shrimp can be seen moving past one of the turning chambers. Note the lighter oxidized burrow lining compared to the darker, anoxic, surrounding sediment. Scale bar = 5cm.

Fig. 2.5. Section of an established *U. deltaura* burrow. The diameter of the burrow lumen reflected the diameter of the extended telson and uropods. Note the smooth burrow wall. Scale bar = 5cm.





Pereiopods 3, 4 and 5 were held against the burrow wall and served to stabilize the shrimp during irrigation. The metachronal beat of the pleopods created a posteriorly directed water current through the burrow.

During periods of burrow irrigation both upogebiid species assumed the feeding position described previously for *U. pusilla* Dworschak (1987) and *U. pugettensis* (MacGinitie, 1930); pereiopods 1 and 2 were held upwards and outwards such that the numerous long setae on the propodi and meri overlaped and formed a basket. Suspended particles entering the burrow with the water current were trapped by the setate basket and the trapped material was then removed from the basket by the maxillipeds and transferred to the mouth where it was ingested.

Irrigation response of *U. stellata* to progressive hypoxia

The effect of progressive environmental hypoxia on the burrow irrigation activity of three individual *U. stellata* (mean fresh weight (±SD)=1.64±0.08g), measured using semi-closed respirometry, is shown in Fig.2.12. The beat frequency did not change significantly ($P<0.05$) over the range of PO_2 and was maintained at 30.4 ± 0.6 (SE) beats.min^{-1}. There was no significant difference ($P<0.05$) in the amount (%) of time spent irrigating, measured over three consecutive 15min periods, over the PO_2 range 150 to 60 Torr (40.3 ± 7.7%).

A further decrease in PO_2, however, resulted in a significant ($P>0.05$) increase in the % time spent irrigating until a maximum of 93.6 ± 6.5% was recorded at 35 Torr (Fig.2.12). Below a PO_2 of 35 Torr the amount of time spent irrigating decreased significantly ($P>0.05$) to 55 ± 25% at PO_2=6 Torr. Even at 0 Torr, pleopod irrigation did not cease for several hours.

Burrow irrigation profiles

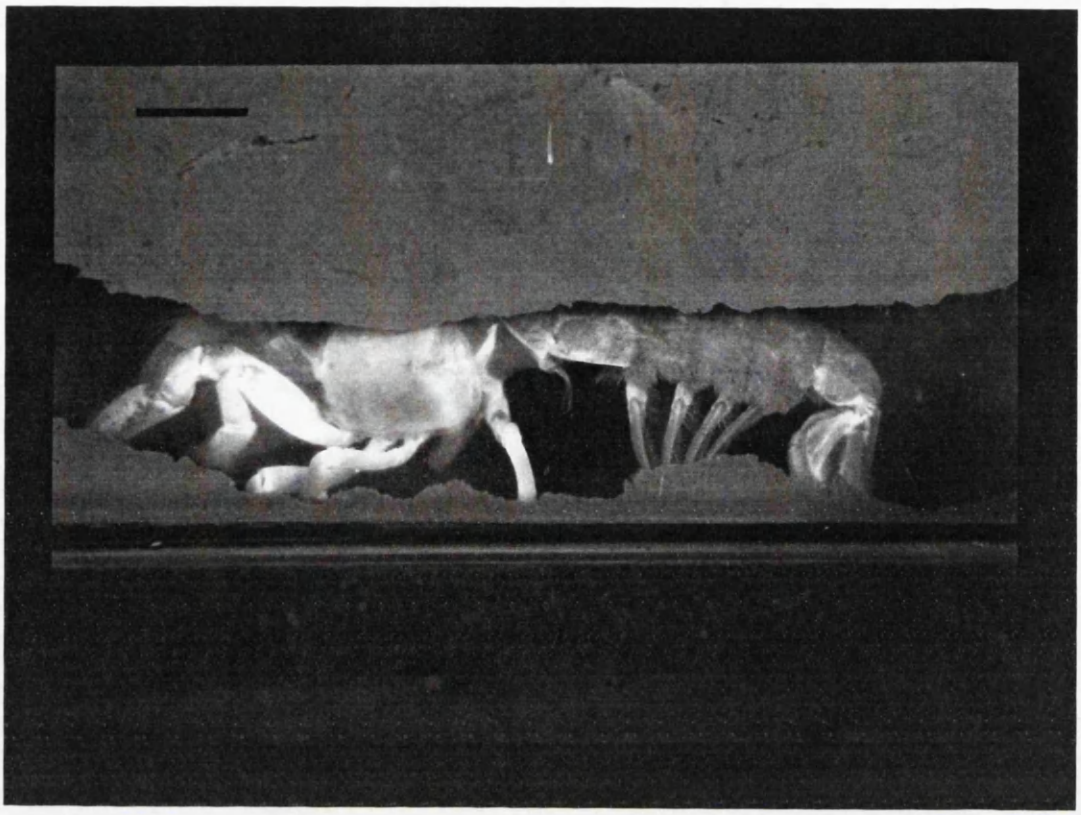
Seven continuous recordings of burrow irrigation activity were made for the mud-shrimps *Upogebia stellata* (n=7 individuals) and *U. deltaura* (n=5 individuals) for periods of time ranging from 6.3 to 57.1h, with 519h of recordings in total. In all individuals, episodes of burrow irrigation occurred at regular intervals, although the duration of irrigation, flow rate, amount of time spent irrigating the burrow and time between consecutive irrigation events varied between individuals in both species (Table 2.1).

A typical recording of water movement through a upogebiid burrow is shown in Figure 2.13 which illustrates burrow irrigation for *U. stellata*. Irrigation, water flow through the burrow, was achieved via pleopod activity. Periods of irrigation were interspersed with little activity or water movement through a burrow and the profile of each irrigatory event was similar in the majority of individuals.

The duration of each irrigation episode varied from 1.4min to 29.3min with a mean (±S.E.) of

Fig. 2.6. *Upogebia deltaura* within an established burrow. This individual is using the fifth perciopod to clean the gills situated in the left branchial chamber. Scale bar = 2cm.

Fig. 2.7. Field resin cast of a *Upogebia* burrow turning chamber. The turning chambers were pear-shaped in cross-section and were used by the shrimps to change direction within the burrow. Scale bar = 1cm.



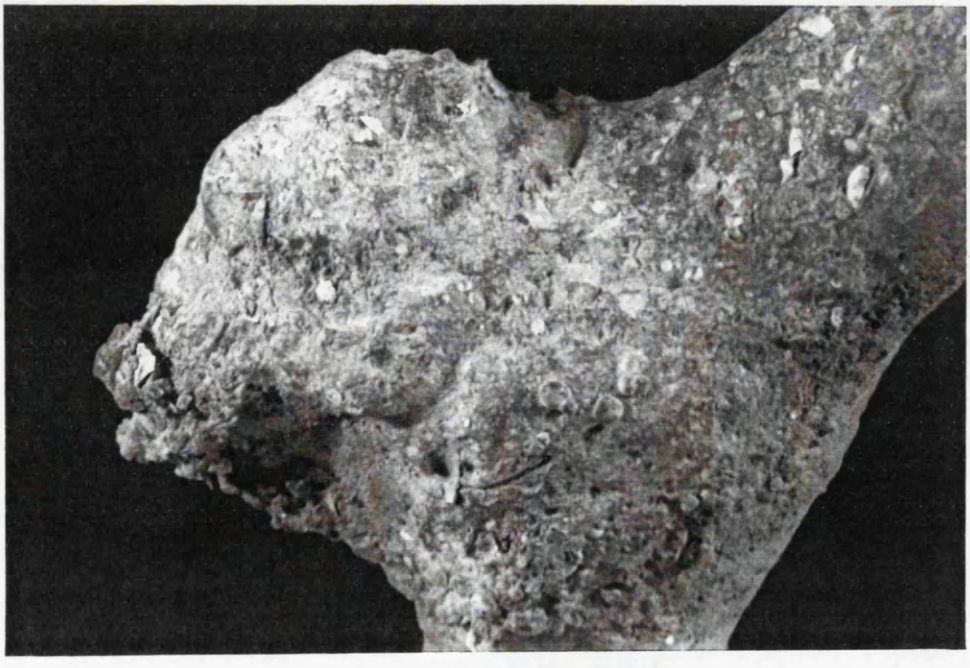
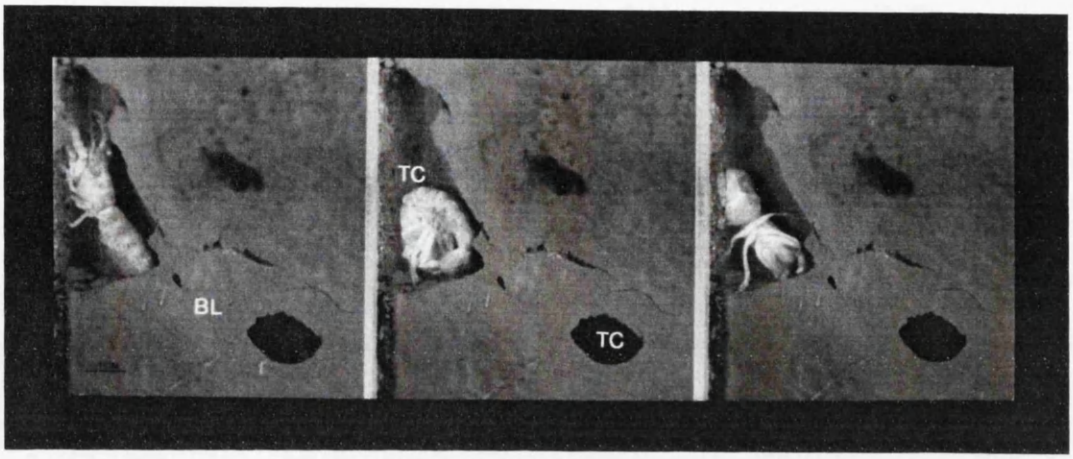


Fig. 2.8. Sequence showing *Upogebia stellata* changing direction within the burrow. By using enlargements of the burrow lumen, turning chambers, the shrimp was able to change direction by performing a forward somersault. Scale bar = 1cm.

Fig. 2.9. Close-up of the vertical shaft of a *U. stellata* burrow cast. In all complete field and lab burrow casts the uppermost section of the burrow was constricted. Scale bar = 1cm.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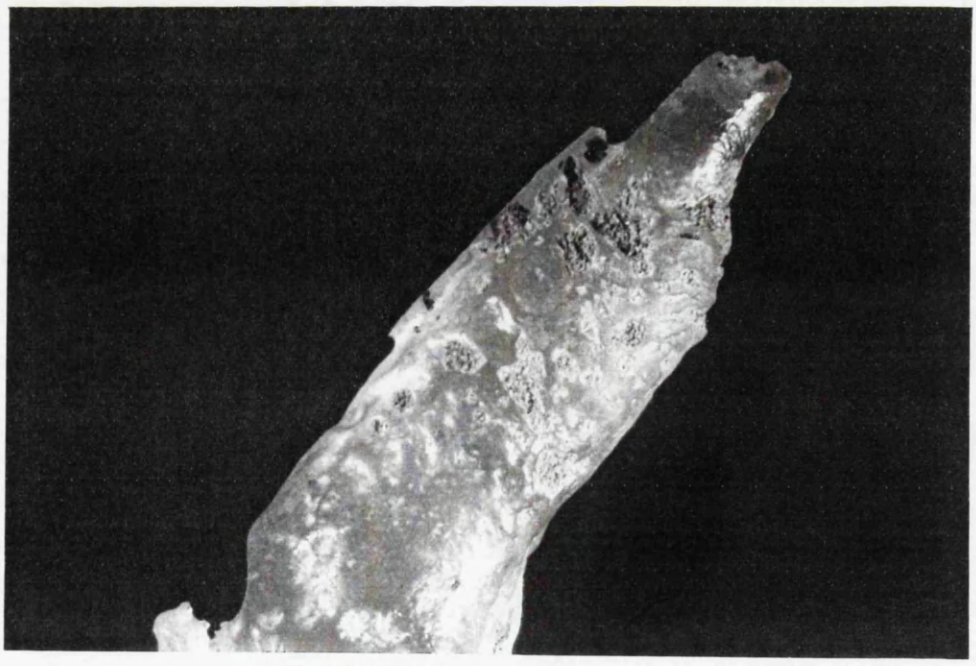

TC

8.5 (± 3.5min) for *U. deltaura* and 2.8 (± 0.5min) for *U. stellata*. The amount of time spent irrigating the burrow during the recordings ranged from 2.3 to 38.6% with mean values of 10.3 (± 3.8%) and 12.8 (± 4.5%) for *U. deltaura* and *U. stellata*, respectively. The average flow rate recorded during the irrigation episodes varied from 4.2ml.min^{-1} to 34.1ml.min^{-1} with *U. deltaura* and *U. stellata* having mean flow rates of 18.4 (± 3.5ml.min^{-1}) and 15.5 (±4.1ml.min^{-1}) respectively.

The long-term irrigation rates (ml.h^{-1}) were estimated for *U. deltaura* and *U. stellata* by multiplying the mean flow rate for the irrigation activity by the average time, in minutes, that the shrimp spent irrigating the burrow during a 1h period (determined from the % time spent irrigating). The minimum, maximum and mean long-term irrigation rates for both upogebiid species are shown in Table 2.2.

A constant flow rate was often maintained during the entire irrigatory event (Fig.2.13), however, within each irrigation episode the flow velocity may vary. Typically the flow rate may decrease (or increase) steadily during an irrigation period (Fig.2.14). Burrow irrigation was not restricted to one particular direction (Fig.2.14), since by utilizing the turning chambers or enlargements of the burrow lumen, the occupant was able to change direction within the burrow and, as a result, movement of water through the burrow could occur in either direction. Activity of the occupant and changes in direction often resulted in the movement of water through the burrow. Water movement was caused by pleopod activity during locomotion and the 'piston effect' - a result of the close fit of the shrimp to the burrow lumen.

Table 2.2. Long-term irrigation rates calculated for *Upogebia deltaura* and *U. stellata*.

	Long-term irrigation rate (ml.h^{-1})		
	min	max	mean ± SE
Upogebia deltaura	25.1	666.1	149.5 ± 5.7
Upogebia stellata	18.9	627.6	139.7 ± 5.7

Patterns of water flow through the burrow were, at times, quite complex (Fig.2.15) depending upon the behaviour of the mud-shrimp. Figure 2.15 shows the complex irrigation recordings from the burrow of *Upogebia deltaura*. After the initial period of burrow irrigation (Ir) the occupant showed an intense period of burrow maintenance behaviour. During burrow maintenance, loose sediment within the burrow was expelled or retained and used to repair the burrow wall. Expulsion of sediment from the burrow, which was achieved by rapid irrigation (E), and movements of the shrimp through the burrow (M) were also shown on the flow

Fig. 2.10. Section of sediment taken from Plymouth Sound using an anchor dredge. The burrow is shown with *Upogebia deltaura* still within the lumen. The stability of the burrow is illustrated as is the lighter oxidized burrow lining, which in places approaches 1cm in thickness. Scale bar = 5cm.

Fig. 2.11. *Upogebia deltaura* filter feeding. The shrimp has assumed the characteristic feeding position and by beating the pleopods water is drawn through the burrown in an anterior to posterior direction. The first and second pereiopods are setate and act as a net whilst the remaining pereiopods are used to stabilize the shrimp during burrow irrigation. Scale bar = 2cm.

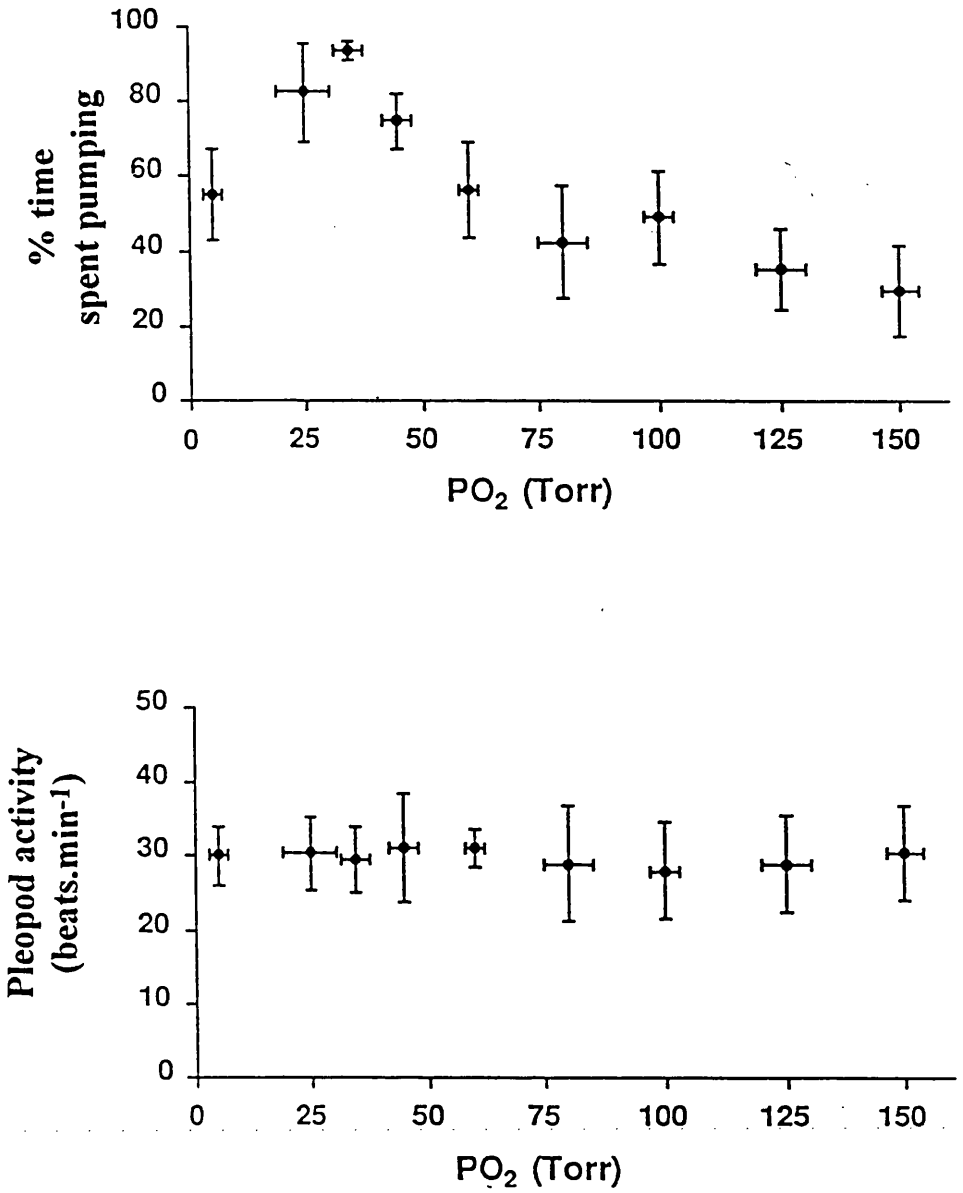
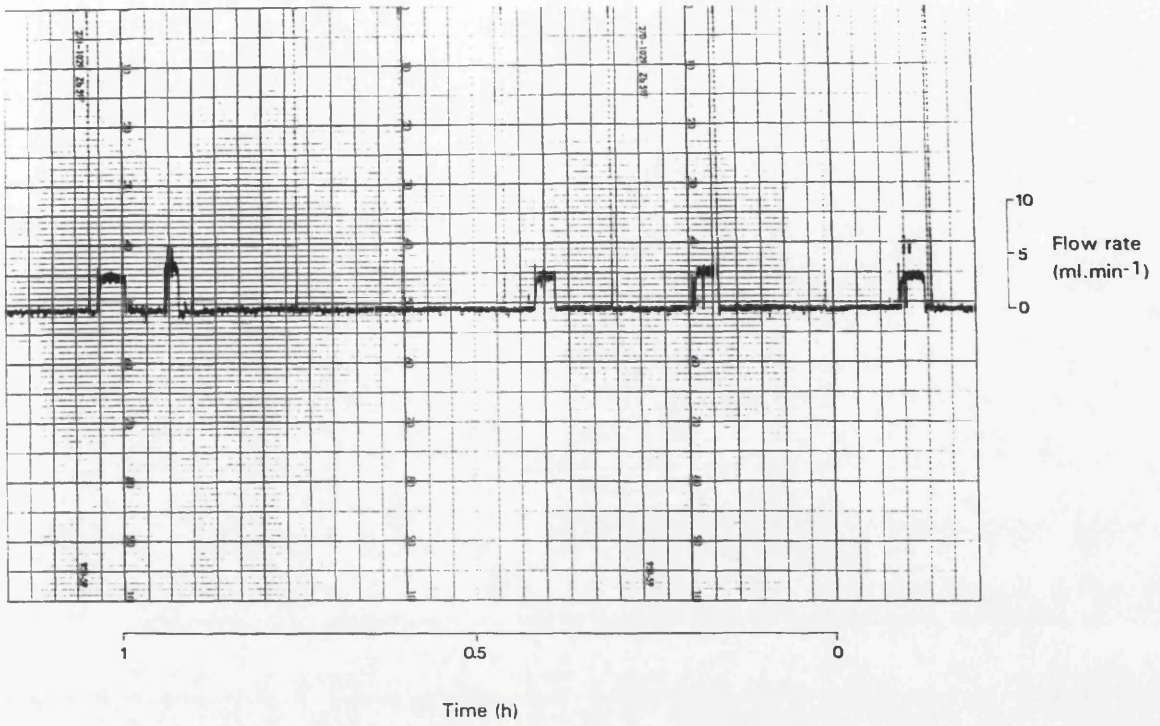
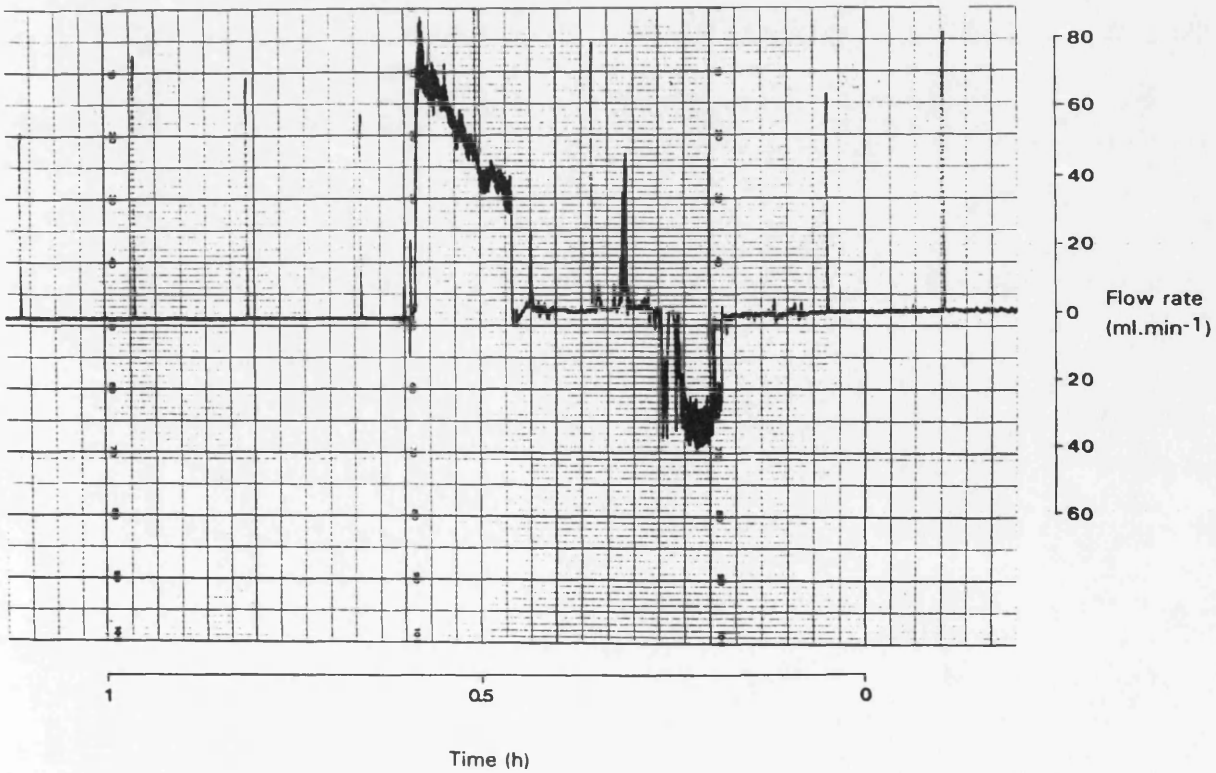


Fig. 2.12. Burrow irrigation response of *Upogebia stellata* to progressive hypoxia. Pleopod activity is expressed as; (a) the amount of time spent irrigating (%) and (b) pleopod beats per min. Irrigation activity was measured over three consecutive 15min periods at various oxygen tensions (Torr). The mean and standard error for each point is given (n=3).

Fig. 2.13. Part of a burrow irrigation profile for *Upogebia stellata*. Periods of irrigation activity are indicated (Ir). Note the direction of the x-axis.

Fig. 2.14. Section of a burrow irrigation profile for *Upogebia deltaura*. Water flow through the burrow may occur in either direction. During each irrigation episode, the water flow rate may vary or be maintained at a constant velocity. Note the direction of the x-axis.


10
5
0
Flow rate
(ml.min-1)
1
0.5
0
Time (h)


80
60
40
20
0
20
40
60
Flow rate
(ml.min-1)
1
0.5
0
Time (h)

recordings (Fig.2.15). Changes in PO_2 associated with the rate of oxygen consumption of the shrimp are shown in Fig 2.16 (R1 & R2). At this time, the shrimp was situated directly at the point where the burrow water was being sampled. The respiratory activity (oxygen consumption) of the shrimp resulted in localised oxygen depletion at this point, which was seen as a rapid decrease in the burrow water PO_2.

During the recordings for some individuals, the burrow openings beneath the flow probe were closed and a new opening established away from the probe. Similar flow patterns were seen during these periods of burrow maintenance and construction.

Spatial variation of PO_2 within the burrow

The spatial variation of oxygen tension within the burrows of *Upogebia deltaura* and *U. stellata* was examined by measuring the PO_2 and pH of the burrow water at various positions throughout a number of burrows constructed in mud-tanks (Fig.2.17 and 2.18). There was a trend for a gradient of PO_2 through the burrow, with higher PO_2 values being recorded near the burrow openings. The average PO_2 in the burrows of both the upogebiid species ranged from 15-95% saturation of the overlying sea water.

The pH profile of the burrow water was similar to the PO_2, with a lower pH recorded in the deeper parts of the burrow. Although there was variation between successive recordings of PO_2 and pH though the burrow, the spatial trend of the PO_2 gradient between the deeper levels and the surface was retained.

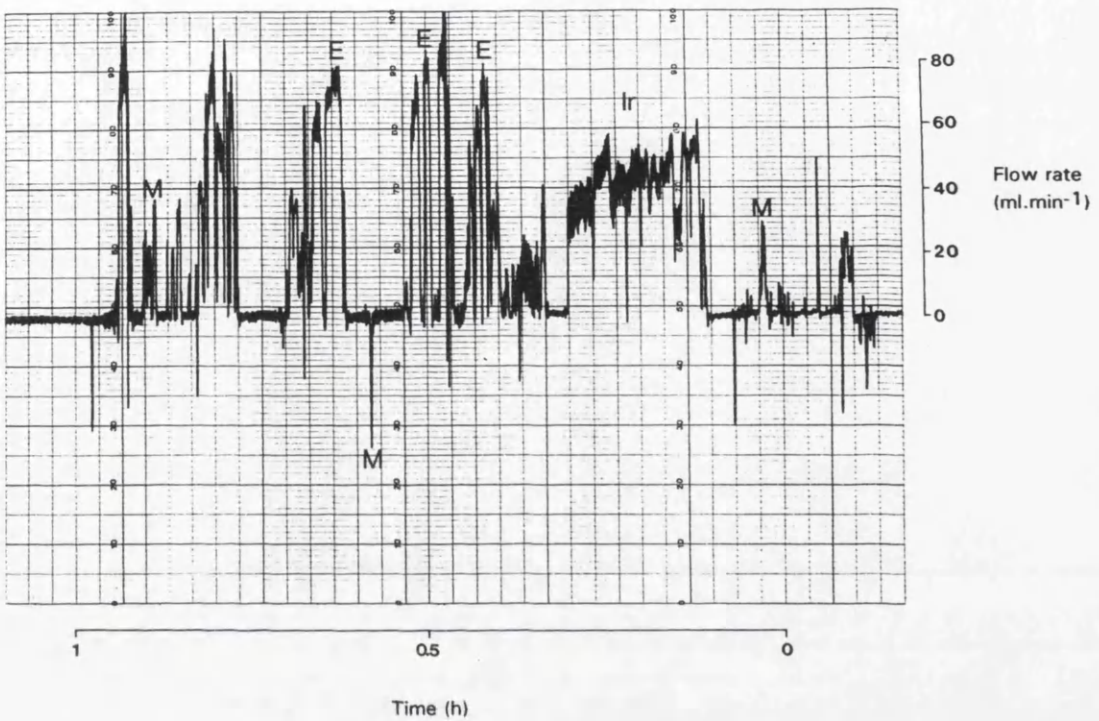
Table 2.3. Ammonia concentration of the burrow waters of four different *Upogebia* burrows.

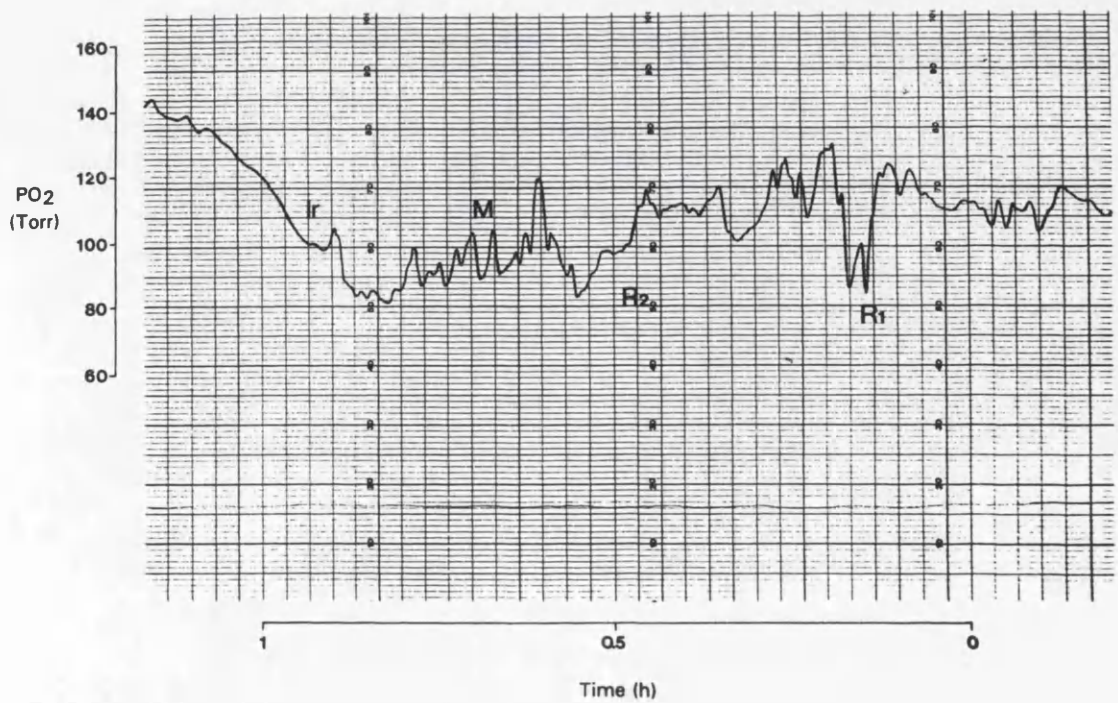
	Ammonia concentration ($\mu g\text{-at}NH_4\text{-}N.l^{-1}$)	
Burrow	*U. stellata*	*U. deltaura*
1	13.9	15.0
2	60.3	2.9
3	1.2	13.9
4	6.6	7.6
Overlying sea water	0.4	0.1

Ammonia concentrations of the sea water in the burrows of *U. stellata* and *U. deltaura* ranged from 1.2 to 60.3 $\mu g\text{-at-}NH_4\ l^{-1}$ (Table 2.3) and were higher than the values recorded for the overlying sea water.

Fig. 2.15. Irrigation patterns and water flow through the burrow can be quite complex as illustrated by this section of trace from *Upogebia deltaura*. Activity and movement of the shrimp results in velocity pulses through the burrow (M). After a period of irrigation (Ir), the shrimp underwent a period of intense burrow maintenance. Sediment was collected from within the burrow and was expelled in frequent irrigation episodes of high velocity and short duration (E). The expelled sediment was often seen to exit the burrow as a plume. Note the direction of the x-axis.

Fig. 2.16. Temporal variation of oxygen tension within a *U. deltaura* burrow. Changes in PO_2 associated with respiratory activity are shown (R1 & R2). The initiation of burrow irrigation (Ir) resulted in a steady increase in the PO_2 of the burrow water. Note the direction of the x-axis.


E
E
E
Ir
M
M
M
80
60
40
20
0
Flow rate
(ml.min-1)
1
0.5
0
Time (h)


160
140
120
100
80
60
PO2
(Torr)
Ir
M
R2
R1
1
0.5
0
Time (h)

Temporal variation of PO_2 within the burrow

Long-term PO_2 recordings of burrow water were made in conjunction with flow rate measurements for a number of *Upogebia stellata* and *U. deltaura* burrows. The PO_2 of the burrow water was found to vary, although between 25 to 146 Torr for most of the burrows examined. During periods when the shrimp was not irrigating the burrow, the overall trend was for the PO_2 of the burrow water to decline steadily (Figs.2.16, 2.19 and 2.20). Rapid changes, both increases and decreases, in burrow PO_2 levels were often observed during periods of irrigatory inactivity. For example, the sudden reductions in PO_2 as highlighted in Figure 2.16 (R), were the result of the respiratory activity(oxygen consumption) of the burrow occupant. At the point R1 (Fig.2.16) the shrimp was situated adjacent to the sample port and, due to localized depletion, a high rate of PO_2 reduction was recorded, whereas at the point R2 the shrimp was situated further away from the sample port and the rate of PO_2 reduction was lower. Movement of the shrimp through the burrow (Fig.2.16;M) and active irrigation activity (Fig.2.16; I) resulted in rapid increases in burrow water PO_2 levels, as fresh oxygenated sea water passed into the burrow.

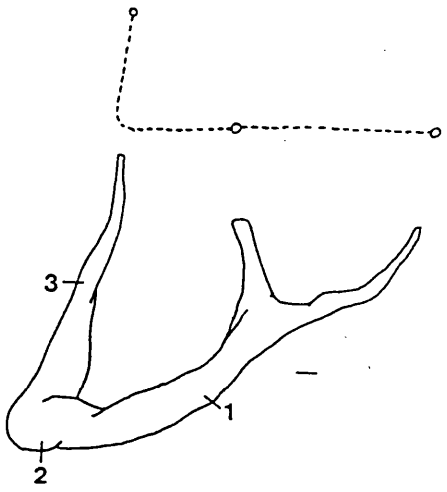
The main changes in burrow water PO_2 occurred during burrow irrigation. A typical recording showing irrigation activity and burrow water oxygen tension is shown in Figure 2.19. The PO_2 of the burrow water steadily decreased from 50 to 25 Torr over the first 1.5h of recording. During the 8min of irrigation activity the PO_2 of the burrow water increased to a maximium of 126 Torr. This was followed by a rapid decrease in the PO_2 of the burrow water in the 15min following cessation of irrigatory activity. The rapid decline probably occurred as a result of the re-equilibration of the oxygen gradient through the burrow. After this time, the rate of decline of PO_2 stabilized to a lower level and reflected the rates of oxygen consumption of the micro-biota and meiofauna of the burrow as well as that of the mud-shrimp.

Another example of the effect of irrigatory activity on burrow water PO_2 levels is shown in Figure 2.20. This is a recording of the same individual but clearly shows two irrigatory events, resulting in a two-step increase in the burrow water PO_2 . Both periods of irrigation activity were of a short duration and, as complete exchange of the burrow water did not occur, the PO_2 of the burrow water remained low.

The degree of exchange of burrow water and hence the large temporal changes in PO_2 is, therefore, dependent upon the amount of time the occupant spends irrigating the burrow and the flow rate during the period of irrigation activity.

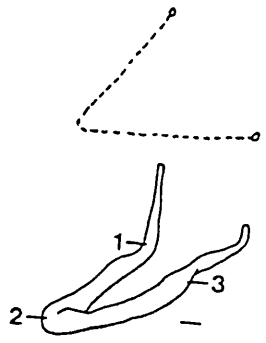
Discussion

Resin casts of the burrows of *Upogebia* spp taken *in situ* and casts of the burrows of *U. stellata*



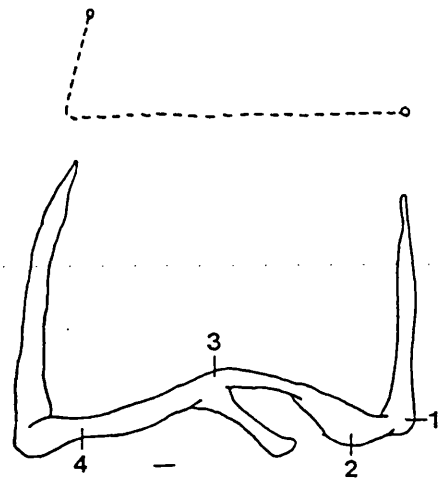
A

Sample port	pH		Po_2 (Torr)	
	a	b	a	b
1	8.10	7.94	104	91
2	8.12	7.90	103	65
3	8.11	7.96	113	115



B

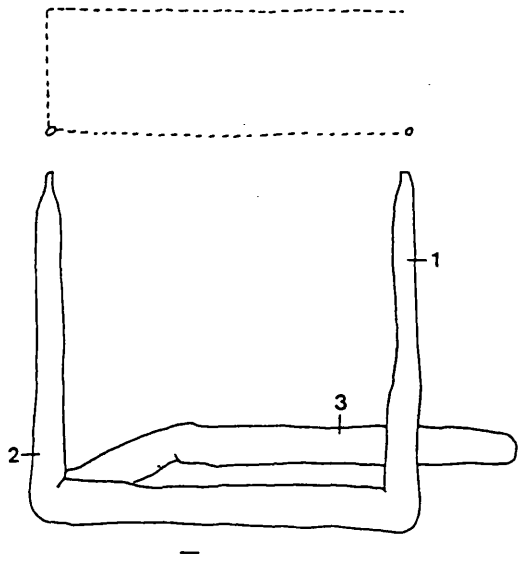
Sample port	pH	Po_2 (Torr)
1	7.67	154
2	7.95	128
3	7.99	137



C

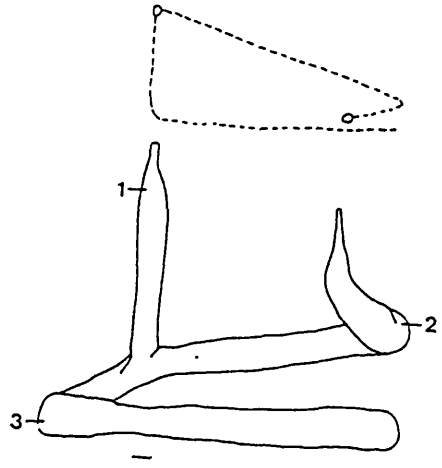
Sample port	pH		Po_2 (Torr)	
	a	b	a	b
1	7.63	7.72	39	34
2	7.68	7.86	31	42
3	7.70	7.81	72	39
4	7.69	7.84	71	46

Fig. 2.17. Diagram of a selection of *Upogebia stellata* burrows constructed in laboratory mud-tanks. The general shape of each burrow was inferred through casting and/or observations. Both side and plan views of the burrow are shown and the position of the sample ports are indicated (e.g. 1, 2, 3). The spatial variation in PO_2 and pH, measured on two different occasions at each of the sample positions is also shown. Scale bar = 1cm.



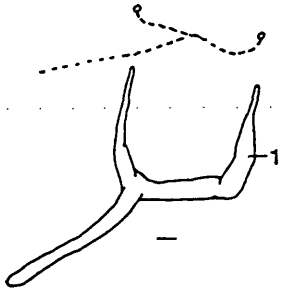
A

Sample port	pH		Po_2 (Torr)	
	a	b	a	b
1	-	-	-	-
2	7.61	7.86	25	117
3	7.63	7.94	48	109



B

Sample port	pH		Po_2 (Torr)	
	a	b	a	b
1	8.04	7.96	154	106
2	8.01	7.86	128	79
3	8.04	7.96	137	90



C

Sample port	pH		Po_2 (Torr)	
	a	b	a	b
1	7.52	7.75	64	67

Fig. 2.18. Diagram of a selection of *Upogebia deltaura* burrows constructed in laboratory mud-tanks. The general shape of each burrow was inferred through casting and/or observations. Both side and plan views of the burrow are shown and the position of the sample ports are indicated (e.g. 1, 2, 3). The spatial variation in PO_2 and pH, measured on two different occasions at each of the sample positions is also shown. Scale bar = 1cm.

and *U. deltaura* constructed within laboratory mud-tanks had a similar basic structure consisting of a U and a descending blind-ending shaft. The burrow morphology of *U. deltaura* was very similar to the description given by Dworschak (1983) based on burrows constructed in an aquarium. The burrows of *U. stellata* were similar to the resin casts taken for the same species by Nickell (1992) from the west coast of Scotland. Interestingly, one of the burrows cast by Nickell (1992) bifurcated a number of times giving rise to a structure made up of multiple U-s with a shaft originating from the deepest U, but having a total of seven openings. This burrow structure is, however, consistent with the decription given for *Upogebia* spp, in particular *U. pusilla*, by Dworschak (1983).

The simple burrow pattern of *U. stellata* and *U. deltaura* described in the present study are fairly typical of the upogebiids as a group (Dworschak, 1983). The burrows of *U. pusilla* consist of a single or double U plus a descending shaft (Ott *et al.*, 1976 (as *U. litoralis*); Dworschak, 1983). Similar descriptions of burrow morphology have also been given for *Upogebia pugettensis*, a species from the west coast of North America (MacGinitie, 1930; Swinbanks & Murray, 1981; Swinbanks & Luternauer, 1987) and *U. major* from Japan (Ohshima, 1967).

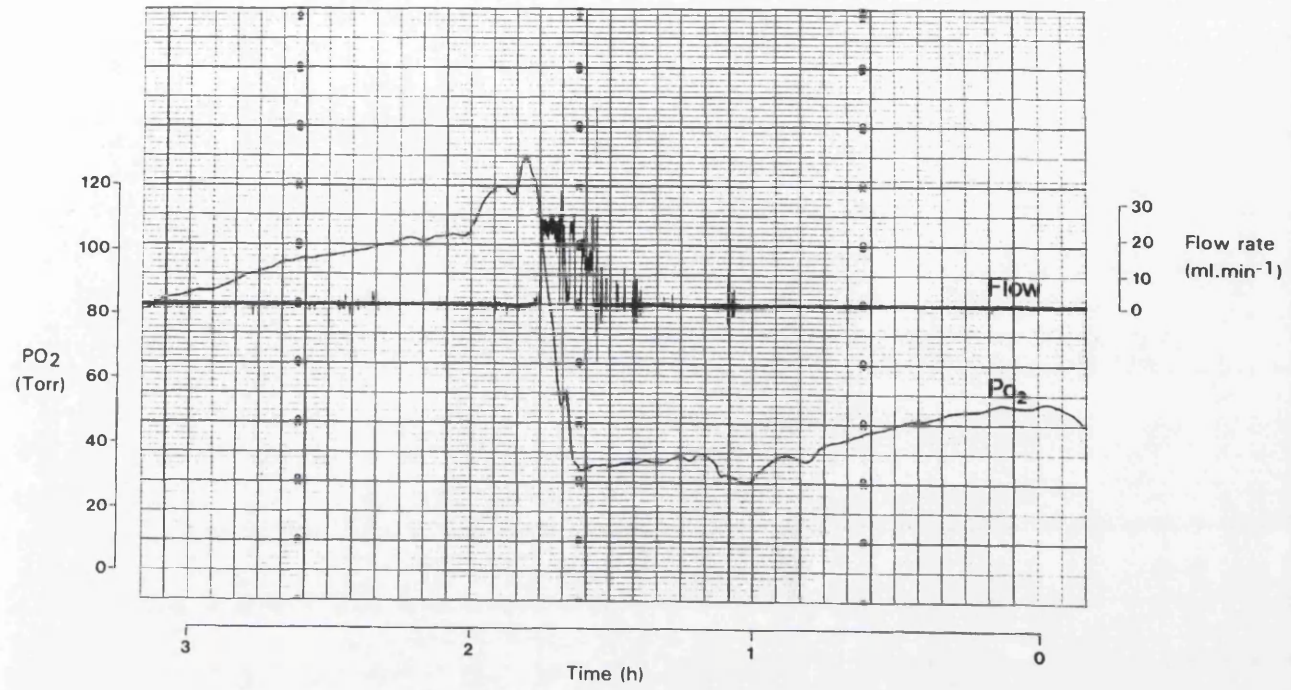
The burrow casts of *Upogebia affinis* from the east coast of North America had a number of distinctive smaller satellite burrows connected to the main burrow via constricted passages (Frey & Howard, 1975). These smaller, juvenile burrows still exhibited the typical U plus descending shaft structure, but were more ramified and originated at various places along the shaft and tunnels of the adult shrimp burrows. The basic upogebiid burrow morphology is even retained in *Upogebia operculata* and other tropical *Upogebia* spp which are able to construct burrows by actively boring into corals and siliceous demosponges on tropical reefs (Kleeman, 1984; Scott *et al.*, 1988).

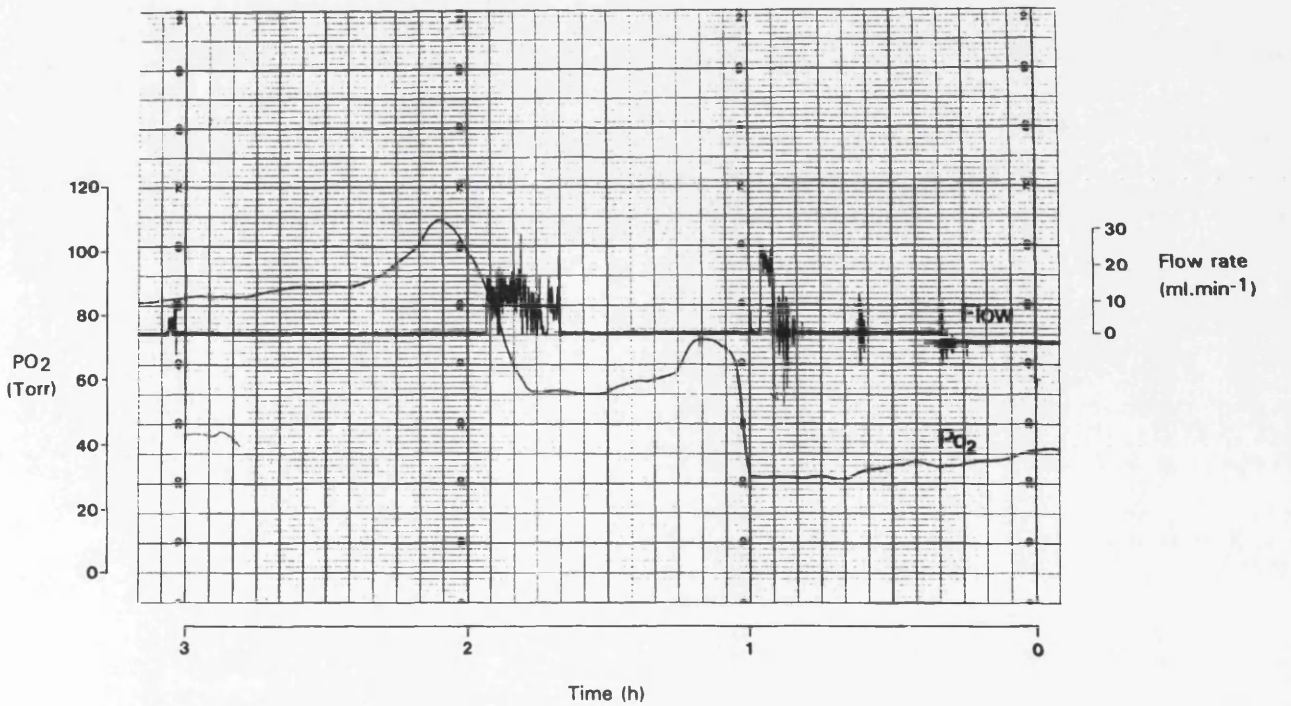
The burrows of *U. stellata* and *U. deltaura* were typically enlarged at a number of places within the burrow, the enlargements being more frequent at points were the burrow bifurcated. These enlargements are turning chambers and appear to be a common feature of upogebiid burrows (MacGinitie, 1930; Ohshima, 1967; Ott *et al.*, 1976; Frey & Howard, 1975; Dworschak, 1983; de Vaugleas, 1990; Nickell, 1992). Another feature common to upogebiid burrows is the constriction of the upper 5-10cm of the main shaft (Smith, 1967; Frey & Howard, 1975; Dworschak, 1983). The function of the constriction is unclear, but it may act as an anti-predator device (preventing the shrimp from being sucked from the burrow) or it may affect the velocity of water flow and mixing patterns within the burrow (Vogel & Bretz, 1971).

Upogebiid burrows are primarily compression structures, with less than half of the sediment corresponding to the volume of the burrow being transported to the surface and ejected during burrow construction (Dworschak, 1983). Once constructed, the burrow remains essentially unchanged (MacGinitie, 1930; Dworschak, 1983; Swinbanks & Murray, 1987; Nickell, 1992),

Fig. 2.19. The irrigation profile (flow rates) and oxygen tension of the burrow water were recorded simultaneously and are shown for *Upogebia deltaura.* Following an irrigation episode, the PO_2 of the burrow water rapidly increased from hypoxic to near normoxic levels. Note the direction of the x-axis.

Fig. 2.20. The irrigation profile for *U. deltaura* and burrow water PO_2 . Following the first irrigation event, the PO_2 of the burrow water remained severely hypoxic. This illustrated that the extent of burrow water exchange and hence temporal changes in PO_2 are dependent on the length of the irrigation episode and the flow rate during the irrigation period. Following the second period of irrigation, however, the PO_2 of the burrow water increased to nearly 125 Torr. Note the direction of the x-axis.


120
100
80
60
40
20
0
PO2
(Torr)
30
20
10
0
Flow rate
(ml.min-1)
Flow
Pa2
3
2
1
0
Time (h)


120
100
80
60
40
20
0
PO2
(Torr)
30
20
10
0
Flow rate
(ml.min-1)
Flow
Pa2
3
2
1
0
Time (h)

although it is constantly maintained. The constant locomotor, maintainance and irrigation activities result in a characteristic smooth, well-oxidized burrow wall, as seen in both *U. stellata* and *U. deltaura* burrows. MacGinitie (1930) recorded that the movement of *U. pugettensis* within its burrow led to the development of smooth burrow walls. Similarly, the constant attention and movement through the burrow was thought to create the characteristic smoothness of the burrow walls of *U. stellata* and *U. deltaura*. The burrow wall had a thicker oxygenated layer of sediment compared to the surrounding dark, anoxic sediment. The oxidation of the burrow wall is the result of sediment oxygenation, made possible by the exchange of oxygenated water through the burrow. This is a feature common to burrows of many thalassinideans (e.g Ott *et al.*, 1976; Dworschak, 1983).

The burrow walls of *U. major*, *U. africana* and *U. capensis* are reported to be mud-lined (Ohshima, 1967; Hill, 1971; Hill & Allanson, 1971), however, the burrow walls were not lined with a distinct membrane but may be enriched with organic matter (Thompson, 1972). Plant debris has been found in the burrow walls of *U. affinis* (Frey & Howard, 1975) and Ott *et al.* (1976) found that *U. pusilla* (as *U. litoralis*) worked decomposed seagrass into the burrow wall, which was used to support a growth of bacteria on which it could possibly feed. Although no distinct mucus burrow lining was observed for either *U. deltaura* or *U. stellata*, aquarium observations have shown that sediment was applied to the Perspex wall of the mud-tank where the burrows impinged against it. MacGinitie (1930) observed *U. pugettensis* similarly attaching sediment to the wall of a glass tube and suggested that the shrimp must have secreted some form of cementing material. Thompson (1972) later found that *U. pugettensis* did indeed use a mucopolysaccharide secretion from the hind-gut gland in plastering the burrow wall. Distinctive burrow linings consisting of mud or organic material have been observed for a number of *Callianassa* species (Pohl, 1946; Frey *et al.*, 1978; see review by Dworschak, 1983).

Although burrow linings may have a role in mediating the diffusion of specific ions within a deposit (Aller, 1983), Griffis & Suchaneck (1991) suggest that the burrow lining is an important aspect in the feeding biology of some species. Filter/suspension feeding is considered to be the primary method for obtaining food in the Upogebiidae (Griffis & Suchaneck, 1991). However, upogebiids may also obtain food by deposit feeding (e.g. *U. pusilla*; Dworschak, 1983), resuspending sediment in the burrow as observed in the present study by *U. stellata* and *U. deltaura* and there is speculation as to whether some species actively culture bacteria in the burrow (e.g. *U. pusilla* (as *U. litoralis*), Ott *et al.*, 1976; *U. affinis*, Frey & Howard, 1975).

In the present study, *in situ* observations indicated that the burrow openings of *Upogebia* spp were flush with the sediment surface, i.e. no mounds were formed around the openings. Similar observations have been recorded for other upogebid species (Ott *et al.*, 1976; Dworschak, 1983; Swinbanks & Leuternauer, 1987; Griffis & Suchaneck, 1991). Upogebiid burrows are considered to be primarily dwelling structures whose shape provides an efficient

path for the unidirectional flow of water for filter/suspension feeding purposes (de Vaugelas, 1990). Several authors have used the presence or absence of burrow mounds as a feature for constructing a model relating burrow architecture and trophic mode in thalassinidean shrimp (Suchaneck, 1985; de Vaugleas, 1990; Griffis & Suchaneck, 1991).

Burrow irrigation

Burrow irrigation rates, expressed as long term rates ($ml.h^{-1}$) were calculated by multiplying the short term irrigation rates in $ml.min^{-1}$ by the time in minutes that the shrimp was actively irrigation during a one hour period (Dworschak, 1983). The calculated long term irrigation rate for *Upogebia deltaura* ranged from 25.1 to 666.1 $ml.h^{-1}$ (mean ± SE, 149.5 ± 5.7 $ml.h^{-1}$) and 18.9 to 627.6 $ml.h^{-1}$ (mean ± SE, 139.7 ± 5.5 $ml.h^{-1}$) for *U. stellata*. Dworschak (1983) found that in *U. pusilla* the irrigation rate was dependent on the size of the individual, however, the relationship was exponential and only significant for individuals larger than 45mm total length. As a result of the small size range used, no allometric relationships were found for either species of upogebid in this study.

In a separate study, Nickell (1992), using the same experimental methods examined the burrow irrigation profile of two deposit feeding shrimps *Callianassa subterranea* and *Jaxea nocturna*. The long term irrigation rates, calculated using an integration technique, for *C. subterranea* were 19.8-90.9 $ml.h^{-1}$ (mean ± SD, 50.3 ± 33.6 $ml.h^{-1}$) and 60.5-134.6 $ml.h^{-1}$ (mean ± SD, 96.3 ±37.1 $ml.h^{-1}$) for *J. nocturna*. These values for both of the deposit feeders were lower than the long term irrigation rates calculated for the filter/suspension feeding upogebiids.

Dworschak (1981) found the irrigation rate of *U. pusilla*, determined using an overflow apparatus and from respiration measurements, ranged from 5-900$ml.h^{-1}$. The irrigation rates of the deposit feeders *Callianassa japonica* and the filter feeder *Upogebia major*, estimated from the oxygen balance of the burrow were 29-63$ml.h^{-1}$ and 14-33$ml.h^{-1}$, respectively (Koike & Mukai, 1983). In another, alternative approach, Aller *et al.* (1983) calculated the *in situ* irrigation rate for the intertidal *Upogebia affinis*, using solute flux rates, as 174$ml.h^{-1}$.

Comparison of the different irrigation rates in relation to feeding mode are, however, difficult since different experimental methods were used. Even so, from the above data it appears that the filter/suspension feeding upogebiids have a higher irrigation rate, in accord with their feeding style, than those thalassinideans which are primarily deposit feeders.

The short term irrigation rates of thalassinideans are within the range quoted for other marine, aquatic burrowing species. Irrigation rates of 10 and 40 $ml.min^{-1}$ have been recorded for the burrowing fish *Cepola rubescens* and *Lumpenus lampretaeformis*, respectively (Pullin *et al.*, 1980; Atkinson *et al.*, 1987). Gast & Harrison (1981) recorded a irrigation rate of 5.8$ml.min^{-1}$ for the alpheid shrimp *Alpheus mackayi*. The long term irrigation rate of thalassinideans,

however, were much lower than those of some invertebrates; *Alpheus mackayi* has a mean long term irrigation rate of 350ml.h^{-1} (Gast & Harrison, 1981).

Nickell (1992) found that although long term flow rates were similar, the irrigation profiles of *Callianassa subterranea* and *Jaxea nocturna* were quite different, and suggested that this may be due to morphological differences between the two species. The discoidal pleopods of *C. subterranea* match the diameter of the burrow and are capable of generating quite a measurable current at a low rate of pleopod beat. The slender pleopods of *J. nocturna* can, however, only irrigate the wide burrow lumen by rapid and vigorous beating and the high rate of flow generated by *J. nocturna* can only be maintained for a short period of time.

Both *U. stellata* and *U. deltaura* spent a similar amount of time irrigating the burrow, although the duration of each irrigation event was longer and the average rate of water flow for each irrigation episode matched that recorded for *J. nocturna*. Interestingly, the pleopods of upogebiids are, as in callianassids, discoidal and match the burrow lumen.

Dworschak (1981) found that *U. pusilla* spent 18-42% (mean 28%) of the time irrigating the burrow and the duration of each event ranged from 0.5-6min (mean 3.7) with a mean interval between episodes of 9.9 min. *Callianassa subterranea* spent between 8.1% and 12.3% of its time irrigating the burrow, whereas the values for *J. nocturna* were less than half of this (Nickell, 1992). Observing individual *Calocaris macandreae* in aquaria, Anderson *et al.* (1991) found comparatively little time (<2% for each 6h recording period) was spent irrigating the burrow. Torres *et al.* (1977) showed *Neotrypaea* (as *Callianassa*) *californiensis* spent between 27.3 and 77.2% of its time irrigating at a pleopod beat rate of 14-40 beats per minute.

Although both *U. stellata* and *U. deltaura* exhibited some degree of regular periodic pleopodal irrigation activity, it is not clear whether the stimulus to irrigate was respiratory or nutritional. As upogebiids are primarily filter/suspension feeders, periods of irrigation activity possibly fulfill both the individual's feeding and respiratory requirements simultaneously. In this study, the effect of PO_2 on the pleopod beat frequency and % irrigation activity was examined for *U. stellata*. The pleopod beat frequency was maintained between 21-38 beats.min^{-1};however, the amount of time spent irrigating increased significantly below 50 Torr and then decreased with any further decrease in PO_2.

The response of the pleopods to low oxygen tensions was similar to the response of the scaphognathites (see Chapter 4: Oxygen consumption). Similarly, Farley & Case (1968) observed an increase in pleopodal irrigation at low oxygen tensions in *Neotrypaea* (as *Callianassa*) *affinis*, with pleopod beat frequencies as high as 120 beats.min^{-1} being recorded in hypoxic water. The oxygen tension below which irrigation activity rapidly increased was less than 28 Torr. This value is lower than that recorded for *U. stellata* and probably reflects the more hypoxic burrow environment and tolerance of *N. affinis* to hypoxia (see Chapter 4: Oxygen consumption).

A similar response was recorded for *Lepidopthalmus* (as *Callianassa*) *jamaicense*, where pleopodal beat rate rapidly increased below a PO_2 of 30 Torr (Felder, 1979). Felder (1979) suggested that an increase in the rate of pleopod beating could represent an 'escape' response, although this is unlikely since it more probably represents an effort to replace severely hypoxic burrow water with more oxygenated overlying waters.

Observations on *U. stellata* and *U. deltaura* held under anoxia for >12h have shown that, following high pleopodal activity and increased irrigation rate, pleopod activity remains fairly constant at very low frequencies (10-15 beats.min^{-1}) for up to 3-4h. When exposed to longer periods of anoxia, two of the four individuals were observed to eventually leave their burrows, probably in an attempt to move away from the localised anoxic conditions. Farley & Case (1968) have shown that *Neotrypaea* (as *Callianassa*) *affinis* could detect and respond differentially to external oxygen concentrations and these authors hypothesized the presence of an oxygen receptor. Anderson (1991) suggested a neural PO_2 detector may be located in the vascular system of decapods, even so, direct evidence of such a receptor in thalassinideans is still lacking (Felder, 1979).

Whether to replenish hypoxic water within the burrow or for feeding purposes, many species of burrowing decapods do actively irrigate their burrows. However, water may also be drawn through the burrow without the need for active irrigation (Atkinson & Taylor, 1988). Burrows situated in an environment where water flows over the sediment surface may be subject to a considerable degree of passive ventilation (Vogel & Bretz, 1972; Allanson *et al.*, 1992). Indeed, in a study of the hydrodynamic properties of *Upogebia affinis* burrows, Allanson *et al.* (1992) found that water currents induced through the burrows from tidal flow over the sediment surface can considerably supplement active irrigation and result in quite a significant degree of energy saving by the occupant.

During this study, the irrigation profiles of *U. stellata* and *U. deltaura* were determined in a situation where water flow over the sediment surface of the aquaria was minimal. Although Allanson *et al.* (1992) found that the irrigation time of the intertidal estuarine shrimp *U. africana* was significantly greater in a static environment, i.e. no flow through the burrow, it is very difficult to compare this information with *U. stellata* and *U. deltaura*. Although both of these upogebiid species occur subtidally, the degree of water flow over the sediment surface *in situ* has not been quantified.

The degree of passive irrigation through a burrow system is dependent upon a reduced pressure gradient, created by water flow over the sediment surface, set up by differences in the height or diameter of the burrow openings (Vogel & Bretz, 1972; Allanson *et al.*, 1992). Field observations showed little evidence of distinct burrow mounds for either *Upogebia* species, unlike many estuarine upogebiid species (de Vaugelas, 1990). Interestingly, the burrow morphology of the majority of deposit feeders are characterised by large burrow mounds which

in some species can attain heights of over 3m above the sediment surface (de Vaugelas, 1990; Griffis & Suchaneck, 1991). This suggests that passive irrigation may provide the major source of water movement through the large, deep and complicated burrow structures of the deposit feeders, however, as yet there is no direct evidence that supports such a theory.

Narrowing of the initial 5-10cm of the burrow, a characteristic feature of the majority of upogebiid burrows, may result in 'viscous sucking', where fluid is drawn from a small tube into a more rapid stream crossing its orifice during periods of tidal movement (Vogel & Bretz, 1972). Clearly, since the work of Allanson *et al.* (1992), the importance of passive ventilation as a mechanism of supplementing both feeding and respiratory requirements should not be under-estimated.

Spatial and temporal distribution of oxygen (PO_2) within the burrow

Within the burrows of *Upogebia* stellata and *U. deltaura* there is a gradient of PO_2 with depth, with higher PO_2 values being recorded near the burrow openings. An oxygen content as low as 15% air-saturation was recorded and although there was variation between recordings from different burrows, the spatial trend of PO_2 gradient with depth was retained. Clearly, hypoxic conditions are a regular occurrence in the burrows of both these shrimp species.

A similar gradient of oxygen tension with depth has been recorded from burrows of *Calocaris macandreae* with the deeper levels being severely hypoxic (<20 Torr) (Anderson *et al.*, 1991). In the more complicated burrow system of *Callianassa subterranea*, however, the PO_2 of the burrow water is unevenly distributed throughout the burrow, with oxygen concentrations of 71% of the overlying seawater sometimes being recorded in the deeper parts of the burrow, resulting in a mosiac of micro-environments (Witbaard & Duiniveld, 1989; Nickell, 1992). Oxygen concentrations of 0.6% to 88% of air-saturation have been recorded from the burrow waters of *C. subterranea* (Witbaard & Duiniveld, 1989; Nickell, 1992; Astall unpubl. obs.) and burrow water oxygen levels of between 6.4% and 89.7% air-saturation have been found for the deposit feeder *Jaxea nocturna* (Nickell, 1992).

At low tide, when the burrow openings were uncovered, Thompson & Pritchard (1969) reported oxygen levels of between 0-14.9% saturation (mean 9.8%) from the burrows of *U. pugettensis*. The burrow water oxygen content of *U. major* ranged from 30-41% air-saturation (Koike & Mukai, 1983).

Felder (1979) measured oxygen content of 0-3.5% saturation from *Callianassa jamaicense* burrows at the edge of an estuarine pond and 7-76.8% saturation from burrows inside the pond. During low tide, Torres *et al.* (1977) determined burrow water oxygen content of 14% saturation for the intertidal shrimp *Neotrypaea* (as *Callianassa*) *californiensis* which rose to an average of 30% saturation during high tide conditions. An oxygen content of 12-45% air-saturation has

been recorded from the burrow waters of Lepidopthalmus (as *Callianassa*) *japonica* (Koike & Mukai, 1983).

The fairly simple burrow morphology of the upogebiids is adapted for the efficient unidirectional flow of water through it (de Vaugelas, 1990). The higher oxygen tensions of the upogebiid burrow waters are primarily a result of the periodic irrigatory activity of the shrimp, which not only provides a feeding current but also serves to replenish the oxygen deplete burrow waters. In contrast, the burrows of the deposit feeders can be quite complex structures (de Vaugelas, 1990), some burrows having numerous shafts and a matrix of interconnecting galleries.

As a result of their mining activities and continual digging through the largely anoxic sediment as part of their feeding strategy, the burrow waters of the deposit feeders often become hypoxic. Due to the complex nature of the burrow, only those tunnels directly connecting the burrow openings may be regularly irrigated and, as a result of poor circulation, extreme hypoxia or even anoxia may exist for long periods in many sections of the burrow system. In addition, the burrow waters of intertidal species are likely to be severely hypoxic during tidal emersion, as oxygen exchange with the overlying seawater will be limited.

Low burrow water PO_2 values have also been recorded for other crustaceans (Innes, 1985; see review by Atkinson & Taylor, 1988) and for fish (Pullin *et al.*, 1980; Atkinson *et al.*, 1987). Although clear spatial gradients of oxygen tension exist in the burrows of *U. stellata* and *U. deltaura*, long term recordings (upto 45h) have indicated a degree of temporal variation and regulation. These temporal fluxes in oxygen tension are dependent upon the respiratory and irrigatory behaviour of the occupant, the oxygen demands of the burrow wall biota, the decomposition of organic matter and the diffusion of oxygen through the burrow system.

Koike & Mukai (1983) recorded narrow temporal variation in burrow water PO_2 for both *Lepidopthalmus* (as *Callianassa*) *japonica* and *Upogebia major*. Within the burrow, the oxygen content was maintained at a constant level by irrigation for both species of shrimp, although the burrow water of *L. japonica* was maintained at half the oxygen tension as was the case for *U. major*. Similarly, Nickell (1992) recorded temporal fluctuations in the PO_2 of the burrow water of *Callianassa subterranea*. Only parts of the burrow were maintained at higher PO_2 levels with most of the burrow remaining hypoxic. Temporal fluctuations in PO_2 for *C. subterranea* were caused by irrigation activity, movements of the shrimp through the burrow and burrow expansion or maintenance (Nickell, 1992).

Although the PCO_2 could not be determined within the burrow waters, the pH was measured at a number of positions within the burrows of both *U. stellata* and *U. deltaura*. There was some indication the the pH decreased with depth through the burrows of both these species, thus indicating that hypercapnia (burrow water CO_2 enrichment) does indeed occur.

The burrows of *U. stellata* and *U. deltaura* were both enriched in terms of ammonia, with

burrow water values being ten fold those of overlying waters. Accumulation of ammonia in burrow waters has also been reported for *Lepidopthalmus japonica*, *C. subterranea*, *Upogebia major* and *U. affinis* (Aller *et al.*, 1983; Koike & Mukai, 1983; Witbaard & Duniveld, 1989; Nickell, 1992). Ammonia enrichment in the burrow is the result of benthic animal and micro-organism metabolism, with the major input of nitrogenous material being derived from the shrimp. The recycling of nitrogenous nutrients from the burrow waters and sediment depends on the exchange of burrow water with overlying waters (Koike & Mukai, 1983).

To conclude, *U. stellata* and *U. deltaura* are two filter feeding thalassinidean mud-shrimps which occupy U-shaped burrows within sublittoral sediments. Within the burrow there is a spatial gradient of oxygen tension with depth and burrow water PO_2 values ranged from 15-95% saturation. Temporal changes in burrow water PO_2 are dependent upon burrow irrigation, movement of the animal within the burrow, respiration of the occupant and the meiofauna and microflora of the burrow wall and diffusion of oxygen from the overlying seawater.

There were few differences between the irrigatory profiles of *U. deltaura* and *U. stellata*. For both species, the amount of time spent actively irrigating the burrow varied between 2-38% (mean 11%). Long-term irrigation rates of *U. deltaura* ranged from 25.1 to 666.1 $ml.h^{-1}$ and 18.9 to 627.6 $ml.h^{-1}$ for *U. stellata*. When exposed to progressive hypoxia, *U. stellata* showed pronounced pleopodal irrigation below 50 Torr. Active irrigation not only provided a feeding current for both upogebiid species, but also served to replenish the oxygen-depleted burrow water. The degree of water exchange was, however, dependent on the amount of time spent irrigating and the rate of irrigation.

Chapter 3

Comparative gross branchial morphology, giill area and gill ultrastructural studies of some thalassinidean mud-shrimps.

Introduction

In their review of crustacean ventilation and perfusion, MIcMahon and Wilkins (1983) stated that oxygen uptake may be broken down into a number of smaller processes namely; ventilation of the gill, oxygen uptake over the exchange surface, removal of oxygen from the gill by perfusion and transport of oxygen to the tissues. Although this clearly illustrates the importance of the gill as the primary site of gas exchange, little information is available concerning the functional morphology of the gill system in relation to the respiratory physiology of decapod crustaceans.

Structural observations of the gill as a respiratory organ are limited to only a few decapod species (Fisher, 1972; Burggren *et al.*, 1974; Goodmam & Cavey, 1992). Compared to the amount of information available on the physiology amd mechanisms of gas exchange, gill structure have not received "comparable attention...to provide the pertinant data to facilitate extensive extrapolation of the functional observations" (Maina, 1990). The majority of structural studies are primarily based on gills as organs of ionoregulation and excretion (for example, Morse *et al.*, 1970; Talbot *et al.*, 1972; Foster & Howse, 1978; Finol & Croghan, 1983; Barra *et al.*, 1983; Martello *et al.*, 1986; Compere *et al.*, 1989; Maina, 1990; Dickson *et al.*, 1991).

Even since the early, extensive work of Gray (1957), who correlated gill area with habitat and level of activity in sixteen species of crab, there have only been a limited number of studies on comparative gill morphology within the decapods (for example, Hawkins & Jones, 1982; Scammell & Hughes, 1982; Hughes, 1983; Burd, 1988;; Swain *et al.*, 1988; Johnson & Rees, 1988).

Thalassinidean mud-shrimp typically construct burrows within hypoxic marine sediments and, as a result of their burrowing habit, they have adopted a range of physiological adaptations to cope with life within burrows (see reviews by Atkinson & Taylor, 1988; Taylor & Atkinson, 1990).

Some of the behavioural and physiological features of the respiratory adaptations to a burrowing habit have been described for a number of intertidal mud-shrimps. Such adaptations have included a low rate of oxygen consumption, a high degree of respiratory independence, the possession of a respiratory pigment with a high oxygen affinity and burrow irrigation behaviour related to oxygen tension (Farley & Case, 1961; Thompson & Pritchard, 1969; Roxby *et al.*, 1974; Miller & Van Holde, 1974; Miller *et al.*, 1976; Torres *et al.*, 1977; Felder, 1979; Hill,

1981; Koike & Mukai, 1983; Mukai & Koike, 1984).

As the functional morphology of the gills of thalassimideans has received little attention and to provide a greater understanding of the respiratory adaptations in this group, the comparative relationships between gill structure and function were investigated for a number of burrowing thalassinidean shrimps. The gill formulae, gross gill morphology, gill area and gill ultrastucture are described and the findings discussed with particular regard to the different burrowing life-styles of the shrimps.

Materials and Methods

Collection and maintenance of mud-shrimps

The thalassinidean mud-shrimps *Upogebia stellata, Jaxea nocturna* and *Calocaris macandreae* were obtained both by anchor dredging (depths to 50m) and with a bait pump ('slurp gun') by SCUBA diving (20-25m), from the waters around the Isle of Cumbrae; Main Channel and White Bay (55°9'N, 5°11'W) and from Loch Sween (10-25m), west coast of Scotland, UK (56°2'N, 5°36'W). *Callianassa subterranea* were taken by anchor dredge from Loch Sween and by box core from the North Sea (54°34'N, 4°50'W). *Upogebia deltaura* were collected with an anchor dredge (7-10m) from Plymouth Sound (50°20'N, 4°13'W), Plymouth, UK. *Upogebia pusilla* were caught intertidally at Arcachon, France (45°6'N, 1°25'W).

Animals captured alive were taken to Glasgow University Zoology Department where they were held in darkness within aquaria supplied with re-circulating sea water (33‰, 10°C) until required. Animals that died were immediately placed into 5% buffered formalin.

Gross branchial morphology

The fresh weights of the mud-shrimps were determined after removal of all surface water. Following dissection of the branchial chamber, the position, number, type and structure of gills was noted. The nomenclature of McLaughlin (1980, 1983) has been followed throughout this study.

Gill area measurement

Individual gills were removed, placed on a microscope slide in 5% buffered formalin, and the length, basal diameter and number of filaments per row was recorded. Measurements were made using an eyepiece graticule calibrated with a stage micrometer. Two estimates of

gill area were used in this study, direct measurement (calculations based on solid geometry; the gills are considered to resemble cones and cylinders) and pictorial measurements (calculations based on plane geometry; the gills are considered as two dimensional shapes).

Direct estimation

For direct estimation of gill area each individual gill filament had to have a shape from which the surface area could be easily calculated. This was possible with *Upogebia deltaura, U. stellata,* and *U. pusilla.* Following observations using scanning electron microscopy (SEM) and light microscopy, the gill filaments of the upogebiids were regarded as cylinders, whose surface area was determined from measurements of the average length (*L*) and diameter (*D*) of the cylinder. The total surface area of one gill:

$$\text{Surface area} = L \times D/2 \times 2 \times \pi \times N \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

(N = total number of filaments).

Preliminary observations of several individuals confirmed that, in each species, gill areas on each side were identical. In all subsequent studies, only the gills from the left side of specimens were measured and the total gill area calculated by multiplying the area for one side by two.

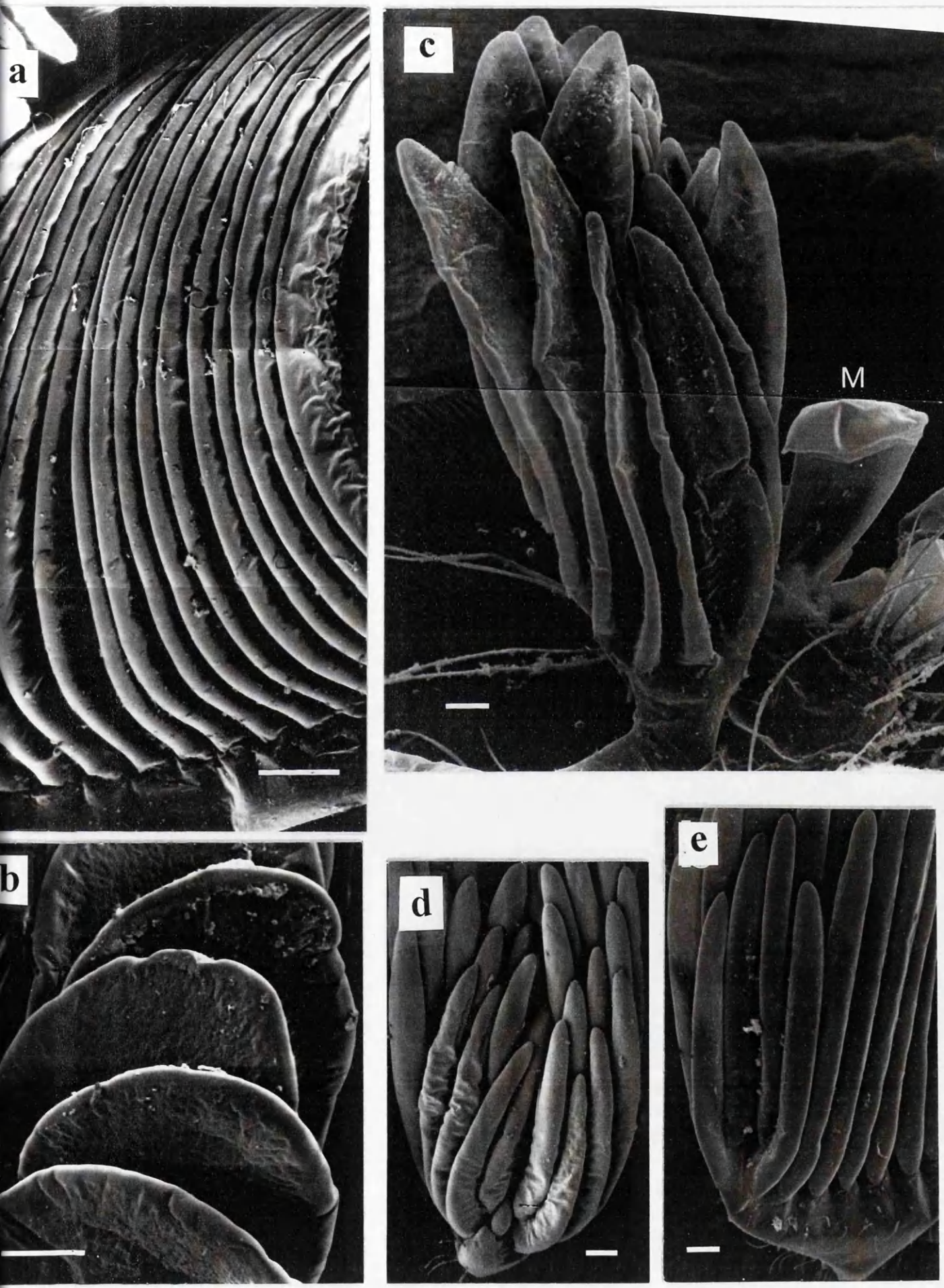
Pictorial method

Gill filaments were removed and placed on a slide and covered with a cover slip. The projected outlines of each gill were drawn with the aid of a dissecting microscope, fitted with a camera lucida. The surface area was then measured using a digitising pad and image analysis software, written for a BBC microcomputer. The area of an individual gill filament was calculated as twice its planar projection (Moore & Taylor, 1984).

All gill areas were expressed as either total gill area (mm^2) or as weight specific gill area ($mm^2.g^{-1}$ fresh body weight). To compare the accuracy of the two methods, the area of the gills from one side of a number of individuals of *U. stellata* were estimated using both the direct and pictorial methods.

One problem associated with the use of preservatives is shrinkage of the gill filaments and subsequent under-estimation of gill surface area (Hughes, 1984). To determine the effects of these preservatives, the gill areas of both preserved (48h) and fresh material of some species were measured using the direct method. No significant difference (t-test, $P < 0.05$), less than 4.5% shrinkage, was found between the gill areas of fresh and preserved

Fig. 3.1. *Callianassa subterranea* has two rows of plate-like lanceolate gill filaments (a) which arise from a central gill axis. The gill filaments are extremely flattened (b) but retain an ultrastucture analogous to the trichobranch gill filaments of *Upogebia* spp.(d & e). The gill type is intermediate in gross structure between trichobranch and branchyuran phyllobranch and is termed phylloid trichobranch. Similarly, *Jaxea nocturna* also has phylloid trichobranch gill filaments (c). Note the epipod and associated mastigobranch (M) of the podobranch of *J. nocturna*. *Upogebia stellata* (d) and *U. deltaura* (e) have trichobranchiate gills. The cylindrical gill filaments arise from a central gill axis. Scale bar = 100 μm.


a
c
M
b
d
e

material.

As each gill possessed numerous filaments it was too time consuming to calculate the area of each individual filament. Therefore, a sub-sampling technique was employed. Every third filament (including the first and last) was taken from the posterior row of each gill for *J. nocturna* and *C. subterranea*, and along the ventral row on the posterior side of each gill for *Upogebia* spp. The validity of this method of sub-sampling was tested using one species, *U. stellata*. The mean gill area, calculated by sub-sampling, was compared with the mean gill area determined by measuring all the filaments of the same gill. This was repeated for all the gills from one side of three individuals. No significant differences in gill area were found using either method (t-test, $P > 0.05$). Therefore, it was concluded that this particular method of sub-sampling provided a more rapid method of gill area estimation without a significant loss of accuracy.

Electron microscopy

The tissues used for both scanning and transmission electron microscopy were fixed in a 1:10 solution of gluteraldehyde in 0.1mol.l^{-1} sodium cacodylate/sea water buffer at pH 7.6 for 3h. Following initial fixation the tissue was post-fixed in 0.1% osmium tetroxide for 2h at room temperature. The tissue was then thoroughly rinsed with distilled water and fixed in 0.5% uranyl actetae for 1-2h in the dark.

The tissues for scanning electron microscopy (SEM) were dehydrated in a graded acetone series, critical point dried in liquid carbon dioxide, mounted on aluminium stubs, sputter coated with gold-palladium and viewed on a Philips 500 SEM at an accelerating voltage of either 3 or 6kV.

The tissues for transmission electron microscopy (TEM) were dehydrated to 70% alcohol then transferred to a 1% solution of 70% alcohol and p-phenylenediamine for 1-2h. The tissue was rinsed and then further dehydrated to absolute dry alcohol, passed through epoxy-1,2 propane and then infiltrated and embedded in epoxy resin (araldite) overnight. 'Semi-thin' sections (1μm) were cut using a LKB3 microtome and stained using 1% borax/toluidine blue solution. Gold sections were cut using a diamond knife on a Reichart UltracutA ultramicrotome. The gold sections were harvested on 75μm formvar coated copper grids, stained with uranyl acetate and lead citrate then viewed on a Zeiss 902 TEM at an accelerating voltage of 80kV

Branchial ventilation

Mud shrimps were placed singly into small tanks of sea water (33°/oo,10°C) and the patterns

of water flow through the branchial chambers were examined. Dye (blue pen ink) was released from a pasteur pipette at several locations close to the margin of the branchiostegite and the pereiopods and the resulting flow patterns were observed with the aid of a Wild dissecting microscope.

Statistical analysis

Data were plotted and appeared normally distributed. Statistical analyses were carried out using analysis of variance, two-way analysis of variance, and Students *t*-test where appropiate (Sokal & Rohlf, 1981). Where the mean of a number of observations is given the deviation from the mean is expressed as standard deviation (SD). However, where the mean of a number of mean values are determined, the deviation is expressed as standard error (SE).

Results

Branchial formulae

The gills were attached either directly to maxillipeds 1-3 and pereiopods 1-5 (podobranchs), to the articulations between these appendages and the body walls (arthrobranchs), or to the body wall of each pleuron (pleurobranchs). The gills occupied most of the volume of the branchial chamber (formed between the body wall and the branchiostegite). The gill formulae are given below for a number of thalassinideans.

Upogebia stellata

Two trichobranchiate arthrobranchs were present on maxilliped 3 (M3) and pereiopod 1-4 (P1-4). Pereiopod 5 carried a simple trichobranchiate pleurobranch which, in smaller specimens, appeared rudimentary.

Table 3.1. *Upogebia stellata.* Branchial formula

	Maxilliped			Pereiopod				
Gill	1	2	3	1	2	3	4	5
Pleurobranch	-	-	-	-	-	-	-	1
Arthrobranch	-	-	2	2	2	2	2	-
Podobranch	-	-	-	-	-	-	-	-
Epipod	-	-	-	-	-	-	-	-
Mastigobranch	-	-	-	-	-	-	-	-

The trichobranchs consisted of two rows of paired filaments (approximately circular in cross section), with one member of the pair arising from a more dorsal position, attached to the central main gill axis (Fig.3.1). The anterior and posterior arthrobranchs were joined close to the point of attachment to the arthrodial membrane. The gill formula is shown in Table 3.1 (number of individuals (n) = 33).

Only three individuals (6%) showed variation from the normal branchial complement, these were; a) No Plp. Rudimentary M3 as well as M3a and M3p, b) No M3p, extra P3p, c) No P1p (nomenclature: P1p indicates pereiopod 1, posterior arthrobranch of the pair. P1a indicates anterior arthrobranch, M3 indicates maxilliped 3)

Upogebia pusilla

Two paired trichobranchiate arthrobranchs were found on M3 and P1-P4 (n=13). No pleurobranch was found on P5. The external gill morphology was similar to that of *U. stellata* (Fig.3. 1).

Upogebia deltaura

The branchial arrangement (n=12) and gill type (Fig.3.1) were similar to that of *U. stellata*, except that no pleurobranch was found on pereiopod 5. One individual lacked an anterior arthrobranch on P3, but this individual had an extra posterior arthrobranch on P4.

Jaxea nocturna

Anterior and posterior arthrobranchs were present on M2-M3 and P1-P4. The branchial formula (showing the maximum number of gills found) for *Jaxea nocturna* (n=10) is given in Table 3.2. The posterior arthrobranch, which was much reduced in length, arose from a more dorsal position on the pleuron than the anterior gill.

Table 3.2. *Jaxea nocturna.* Branchial formula

	Maxilliped			Pereiopod				
Gill	1	2	3	1	2	3	4	5
Pleurobranch	-	-	-	-	-	-	-	-
Arthrobranch	1	2	2	2	2	2	2	-
Podobranch	-	1	1	1	1	1	-	-
Epipod	-	1	1	1	1	1	1	-
Mastigobranch	-	1	1	1	1	1	1	-
Setobranch	-	-	1	-	-	-	-	-

The podobranch fitted into the space immediately below the posterior arthrobranch. The epipod, onto which the podobranch was attached, projected posteriorly dorsal to the gills. The arthrobranch and podobranch both possessed 2 rows of flattened, lanceolate gill filaments arising from a central gill axis (Fig.3.1).

Although the gill filaments are flattened to such an extreme the ultrastructure of the filaments is still analogous to trichobranch gill filaments. Since this particular type of gill filament is intermediate, in gross structure, between a trichobranch and phyllobranch it will now be referred to as a phylloid trichobranch (hereafter referred to as the phylloid gill).

A small flattened respiratory process, the mastigobranch, arose from the basal region of the epipod posterior to the podobranch (Fig.3.1). Epipods were found on M2, M3 and P1-P4, mastigobranchs arose from the epipod only on P1-P4. No podobranch was found on P4 or P5. The number of gills present on Ml and M2 was very variable. A setobranch was found anterior to the podobranch on M3. The setobranch consisted of approximately 15-30 denticulate setae arising from a ridge on the base of the coxa.

Callianassa subterranea

Callianassa subterranea had a simplified gill formula, with paired arthrobranchs present only on M3 and P1-4. There were neither pleurobranchs nor podobranchs (including epipods and mastigobranchs) on any appendage. There were no differences in the gill formulae between *C. subterranea* from Loch Sween and from the North Sea.

Table 3.3. *Callianassa subterranea.* Branchial formula

	Maxilliped			Pereiopod				
Gill	1	2	3	1	2	3	4	5
Pleurobranch	-	-	-	-	-	-	-	-
Arthrobranch	-	2	2	2	2	2	2	-
Podobranch	-	-	-	-	-	-	-	-
Epipod	-	-	-	-	-	-	-	-
Mastigobranch	-	-	-	-	-	-	-	-

The arthrobranchs were similar in structure to the gills of *Jaxea nocturna,* with two rows of filaments arising from a central axis (Fig.3.1). The gill filaments of *C. subterranea* were larger and flattened to a greater extent than those of *J. nocturna* and were also considered to be phylloid. The branchial formula (showing the maximum number of gills found) for *C. subterranea* (n=22) is given in Table 3.3.

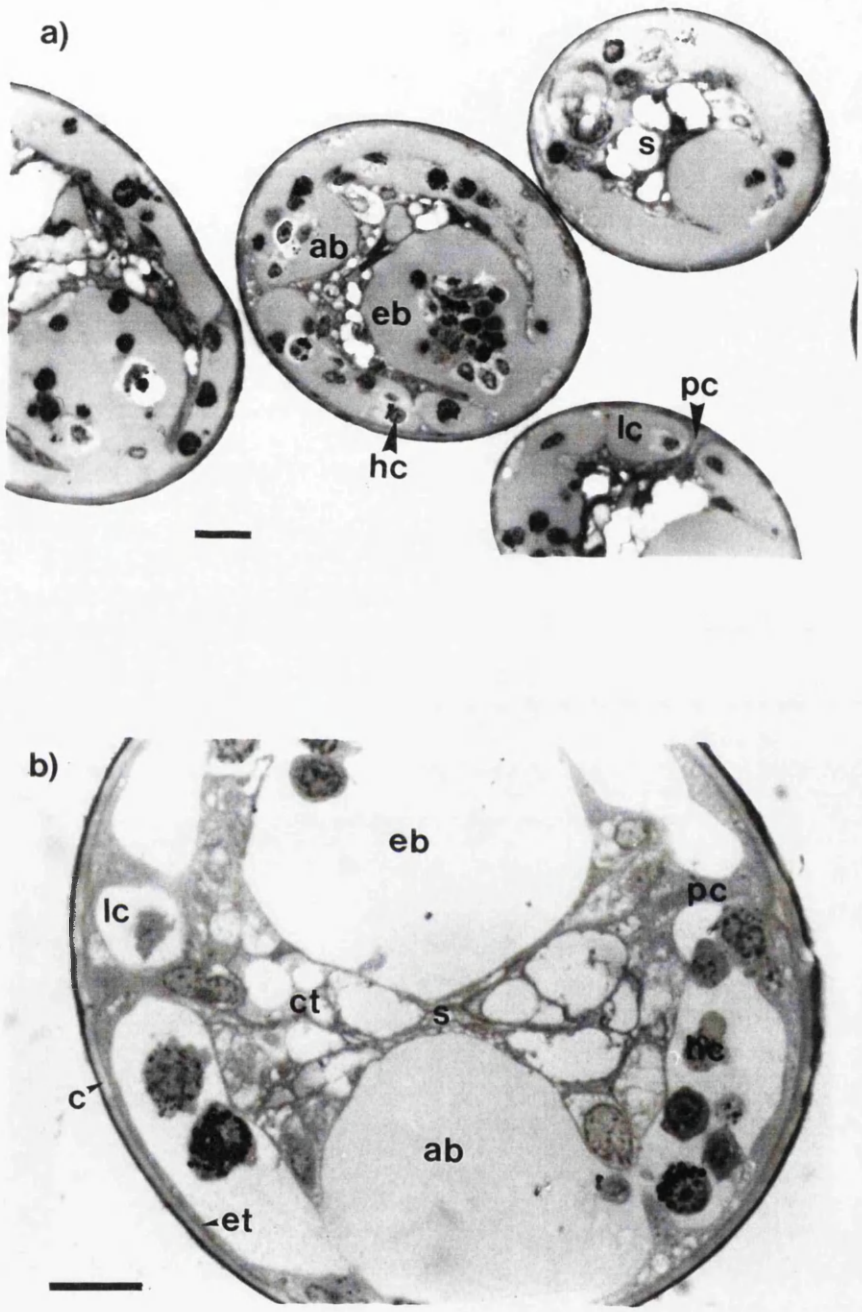


Fig. 3.2. (a) Light micrograph of a semi-thin transverse section of the trichobranch gill filaments of *Upogebia* spp. Afferent blood vessel (ab), efferent blood vessel (eb), pillar cells (pc), lacunae (lc), haemocyte (hc). Scale bar = 10μm.

(b) Close up of a single gill filament showing the afferent (ab) and efferent (eb) blood channels separated by the septum (s). The connective tissue (ct) is supported by the pillar cells (pc) which are extensions of the epithelial layer (et). The spaces between succesive pillar cells are termed lacunae (lc). Note the chitinous cuticle (c) covering the surface of the gill filament. Scale bar = 10μm.

Gross gill morphology and blood flow within the gill filament

Upogebia stellata and *U. deltaura*

The trichobranchiate arthrobranchs of the mud-shrimps *Upogebia stellata* and *U. deltaura* consisted of two rows of paired gill filaments, with one member of the pair arising from a more dorsal position, attached to the central gill axis (Fig.3.1).

The central gill axis is divided into an afferent and efferent vessel, and each gill filament is divided longitudinally, by a septum of connective tissue, into an afferent and efferent blood channel (Fig.3.2a). As well as the main blood channels, numerous peripheral lacunae or secondary blood spaces were observed (Fig.3.2b). The septum separating the gill filament does not continue for the length of the filament but terminates at a position short of the distal end, forming a large lacuna. Numerous secondary blood channels were also observed at the distal end of the filament. Although flow of blood within the gill filaments could be observed, albeit with great difficulty, the resulting pattern of blood flow is hypothesized from observations on the ultrastructure of the gill.

The deoxygenated blood leaves the main gill axis, via the afferent vessel, and is carried into the afferent channel of the most dorsal of the paired gill filaments. The blood passes toward the distal end of the filament, returns via the efferent blood channel and instead of passing directly into the main gill axis, it is carried towards the distal end of the second, lower gill filament of the pair. The oxygenated blood then returns to the main gill axis via the efferent channel of the lower gill filament. In addition to the blood passing through the gill filaments via the afferent and efferent blood channels, blood also traverses the gill filament through the network of lacunae.

Callianassa subterranea and Jaxea nocturna

The phylloid gill filaments of the mud-shrimps *Jaxea nocturna* and *Callianassa subterranea* are unlike phyllobranch gills where a central gill raphe bears paired plate-like lamellae, but are similar to trichobranch gill filaments in their ultrastucture and in the way they arise from the gill axis.

The margins of the gill filaments were expanded to form efferent and afferent marginal channels (Fig.3.3a; 3.4a). A central septum, supported by pillar cells, ran the width of the gilll filament. The septum did not extend the complete width of the lamellae, but was delimited at the marginal channels; nor did it extend the length of the filament, but terminated in a position short of the distal tip (Figs.3.3 & 3.4). Thus the marginal channel

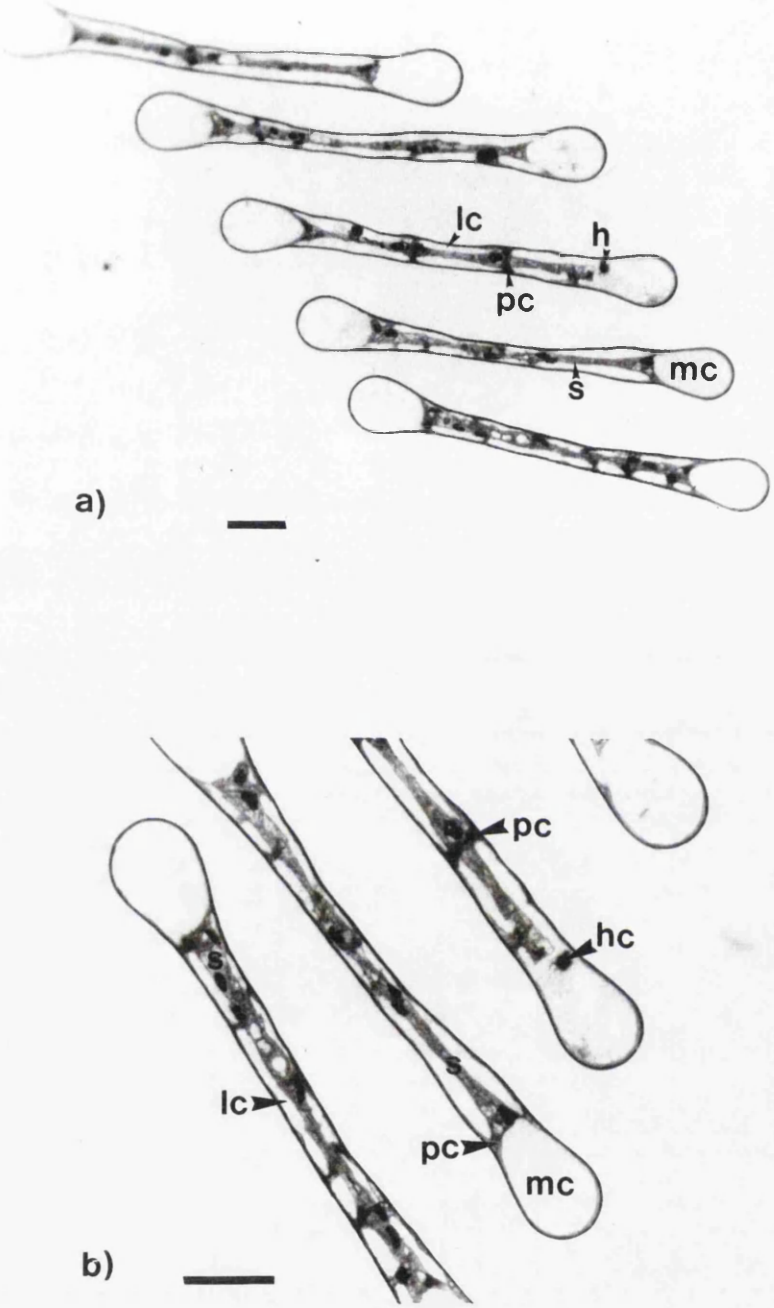


Fig. 3.3. (a) Light micrograph of a semi-thin transverse section through a series of phylloid trichobranch gill filaments of *Jaxea nocturna*. Note the central septum (s) which extends to the marginal channels (mc). Pillar cells (pc) support the septum and create a series of lacunae (lc) or 2° blood spaces. Haemocytes (hc) are found throughout the gill filament. Scale bar = 10 μm.

(b) Close up of a number of gill filaments. Note the septum (s) was not always delimited at the marginal channels (mc). Pillar cells (pc), lacunae (lc), haemocytes (hc). Scale bar = 10μm.

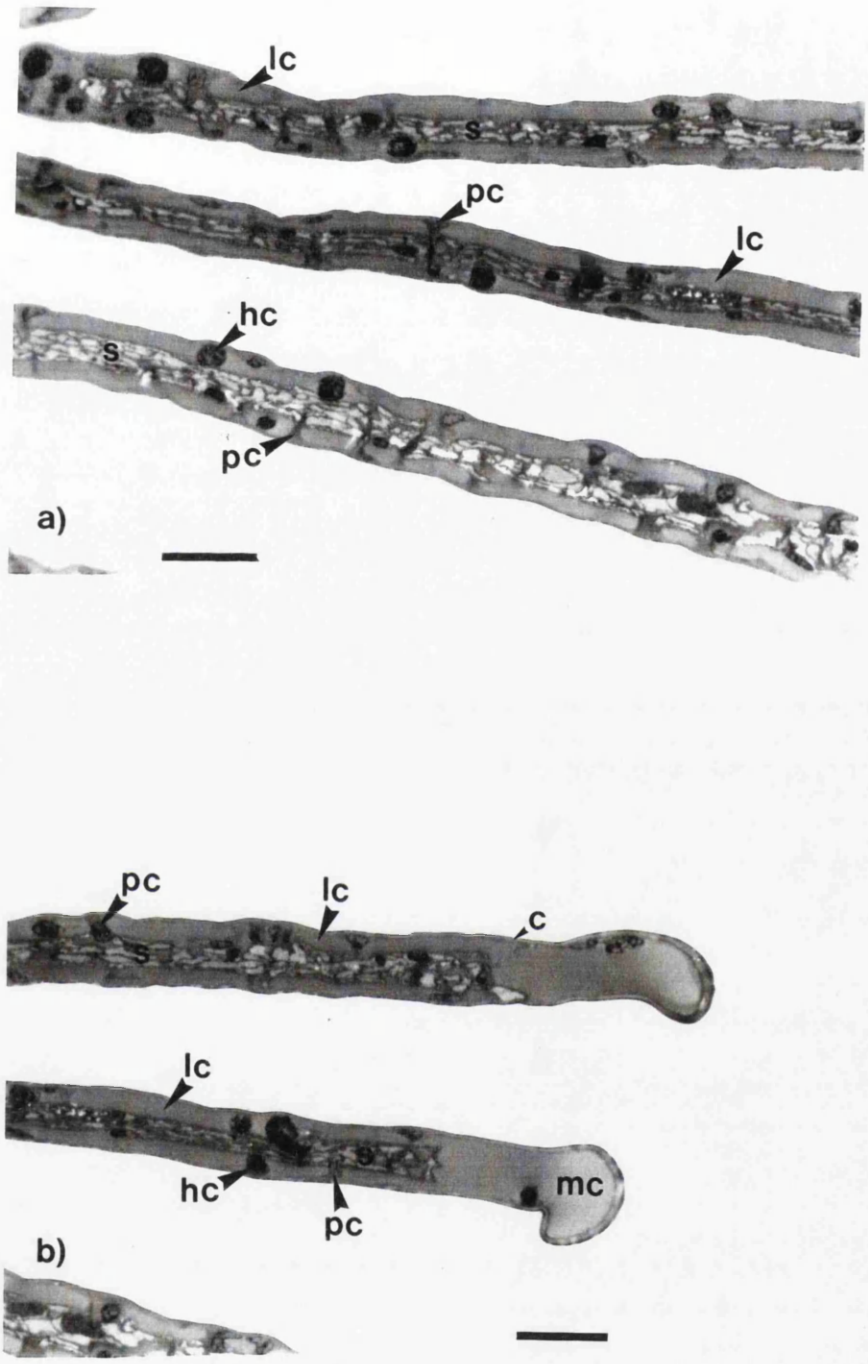


Fig. 3.4. (a) Light micrograph of a semi-thin transverse section through a series of phylloid trichobranch gill filaments of *Callianassa subterranea*. Pillar cells (pc) supporting the septum (s) are clearly seen. Haemocytes (hc), lacunae (lc). Scale bar = 10μm. (b) Close-up of the gill filaments. Note the distended marginal channel (mc), the cuticle (c) of which is noticibly thicker than the rest of the filament. Pillar cell (pc), lacunae (lc), haemocyte (hc). Scale bar = 10μm.

extended around the complete periphery of the gill filament.

From ultrastructural observations it is hypothesized that blood flows from the afferent blood vessel of the gill axis into the afferent marginal channel of the gill filament. Blood then passes through the gill filament and back into the efferent vessel of the gill axis via the marginal channel. At numerous positions blood will pass from the afferent marginal channel into the network of secondary spaces created between the pillar cells and the septum and across to the efferent marginal channel.

Gill filament ultrastructure

Upogebia stellata, U. deltaura and *Calocaris macandreae*

The basic ultrastructure of the trichobranch gill of each species of mud-shrimp was very similar. In the *Upogebia* spp. the non-calcified cuticle was of a uniform thickness around the circumference of each gill filaments (approximately 1.4μm) and composed of three distinct layers; a thin outer epicuticle ($<0.1\mu$m), an exocuticle (approximately 0.3μm) and a thicker endocuticle (approximately 1.1μm) (Fig.3.5). The exocuticle is composed of a number of closely spaced lamellae, whereas the lamellae of the endocuticle are more broadly spaced and easily distinguishable (Fig.3.5c). Pore canals, extensions of the underlying epithelial cells, were observed in some of the sections (Fig.3.5a) but were not observed to penetrate the epicuticle. The mean cuticle thickness of the gill filament for *C. macandreae* was approximately 0.9μm.

For each species the subcuticular epithelium varied in thickness from 0.8 to 3.6μm, but was thicker in those regions adjacent to pillar cells. The apical surface of the epithelium was often folded; the folds form villus-like projections that extend up to 1.0μm into the epithelial cells (Fig.3.5b and 3.5c). Some regions of the filaments are very dense with microvillar processes, in particular the apical epithelial surface of pillar cells. Infoldings were also observed at the basal region of the epithelium. The membranes of adjacent epithelial cells are connected by junctional complexes (Fig.3.5c and 3.6).

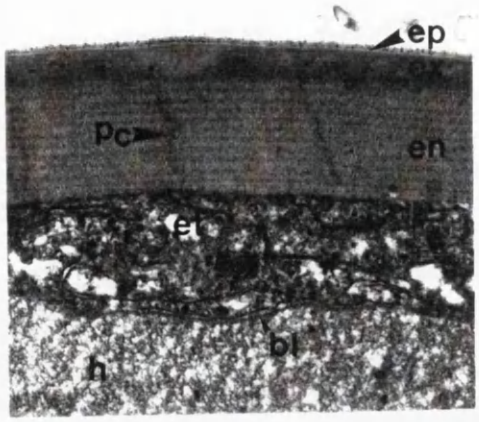
In some regions of the filament, extensions of the epithelial cells project across to the central septum and associated connective tissue to form pillar structures (Fig.3.2 and 3.5). The basal portions of the pillar cells were embedded into the loose connective tissue forming the septal region. The spaces that were formed between successive pillar cells, the subcuticular epithelium and connective tissue are termed lacunae. These lacunae provide a series of secondary blood spaces, or channels, that ramify throughout the gill filament and serve to connect the main afferent and efferent blood channels.

The haemolymph within the blood spaces has a flocculant appearance. Numerous

Fig. 3.5. (a) Transmission electron micrograph (TEM) showing the cuticle of a gill filament from *Calocaris macandreae*. Typically the cuticle is composed of three distinct layers; the epicuticle (ep), exocuticle (ex) and endocuticle (en). Pore canals (pc) extend from the epithelial cells (et) and penetrate the endocuticle. Note the basal lamina (bl) of the epithelial cell layer and the haemolymph space (h). Mag x 19,050.

(b) TEM of a pillar cell of *Upogebia* spp. Note the numerous microtubules (mt) which are orientated in a perpendicular fashion to the cuticle and apical infoldings (ap) of the cell surface. The pillar cell projected across to the loose connective tissue (ct) and the spaces formed between successive pillar cells, lacunae (lc), create a complex matrix of blood channels. Mag x 9,850.

(c) *Upogebia* spp. TEM showing a pillar structure separating two lacunae (lc). Note the convoluted nucleus (n), apical folding (ap) and the cuticle which is composed of three distict layers; epicuticle (ep), exocuticle (ex) and endocuticle (en). Mag x 12,425.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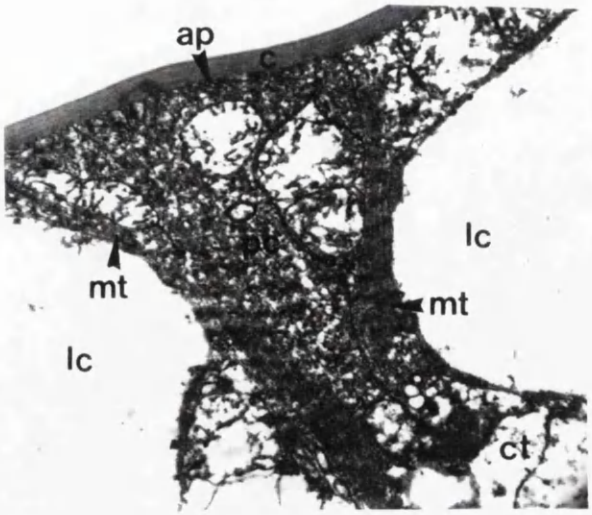
ap
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lc
mt
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bundles of microtubules, orientated in a perpendicular fashion to the cuticle, are distributed throughout the pillar cell (Fig.3.5) and, in addition to being rich in mitochondria, granular and agranular endoplasmic reticulum and free ribosomes, each pillar cell is characterized by a highly convoluted basal nucleus (Fig.3.6). Few mitochondria and associated structures were found in the epithelial layer other than within the pillar cells.

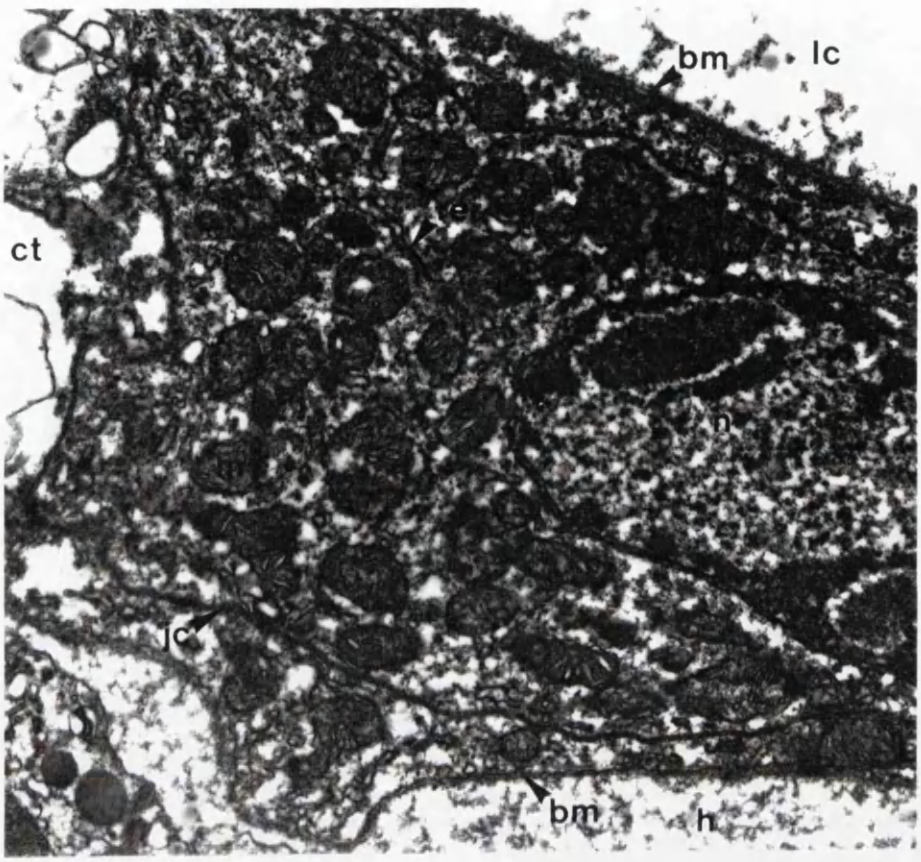
The septum traversed the blood space of the gill filament, dividing it into distinct afferent and efferent blood channels, and was connected to the periphery of the filament by the pillar cells. The septum consisted of loose, generally anucleate connective tissue which varied in width throughout the length of the filament. At the distal region of the filament the septum became shorter and was often absent. The afferent basal membrane of the septum was usually thinner than the efferent side but showed extensive basal folding (Fig.3.2).

Table 3.4. Summary of the statistics derived from regression analyses of mean filament surface area (mm^2) and the number of filaments per gill against fresh body weight (g) for each of the gills from the thalassinidean shrimp *Jaxea nocturna*. Both gill area and the number of filaments were related to fresh weight (g) by the equation $\log_{10}Y=\log_{10}a+b\log_{10}X$, where Y is the mean filament area or number of filaments and X is fresh body weight (g); r is the correlation coefficient; * indicates not significant at $P>0.05$. Values of a are $\log_{10}$ numbers. Key: M1, maxilliped 1; P2, pereiopod 2; a, anterior arthrobranch; p, posterior arthrobranch.

	Jaxea nocturna					
Gill type	Mean filament area (mm^2)			Number of filaments		
	a	*b*	*r*	*a*	*b*	*r*
M1	-1.04	0.27	0.96	3.61	0.25	0.87
M2a	-0.60	0.19	0.87	1.79	0.05	0.77
M2p	-0.87	0.37	0.84	1.58	0.21	0.93
M2podo	-0.44	0.21	0.96	1.67	0.10	0.90
M3a	-0.31	0.33	0.93	1.85	0.05	0.65
M3p	-0.42	0.42	0.94	1.71	0.06	0.92
M3podo	-0.53	0.32	0.93	1.59	0.07	0.86
P1a	-0.27	0.30	0.98	1.85	0.04	0.60*
P1p	-0.30	0.35	0.97	1.67	0.07	0.82
P1podo	-0.66	0.23	0.84	1.43	0.08	0.88
P2a	-0.21	0.29	0.97	1.89	0.06	0.90
P2p	-0.24	0.39	0.97	1.66	0.07	0.95
P2podo	-0.65	0.38	0.93	1.45	0.11	0.93
P3a	-0.22	0.29	0.99	1.88	0.07	0.91
P3p	-0.30	0.39	0.97	1.64	0.08	0.83
P3podo	-0.71	0.42	0.97	1.42	0.13	0.94
P4a	-0.27	0.35	0.99	1.82	0.08	0.92
P4p	-0.43	0.31	0.96	1.54	0.07	0.72

Fig. 3.6. *Upogebia* spp. Pillar cells are typically characterized by numerous mitochondria (m) and rough endoplasmic reticulum (rer). Note the large nucleus (n), junctiona; complexes (jc), basement membrane (bm). Connective tissue (ct), lacunae (lc), haemolymph (h). Mag x 20,525.

Fig. 3.7. TEM of a section through a gill filament of *Callianassa subterranea.* The thin cuticle (c) of the gill filament is underlined by epithelial cells (et). Extensions of the epithelium, pillar cells (pc) projected across to the central septum (s). The pillar cells were characterized by numerous mitochondria (m) and bundles of microtubules (mt). Spaces between successive pillar cells, lacunae (lc) form a complex sinus system. Mag x 8,900.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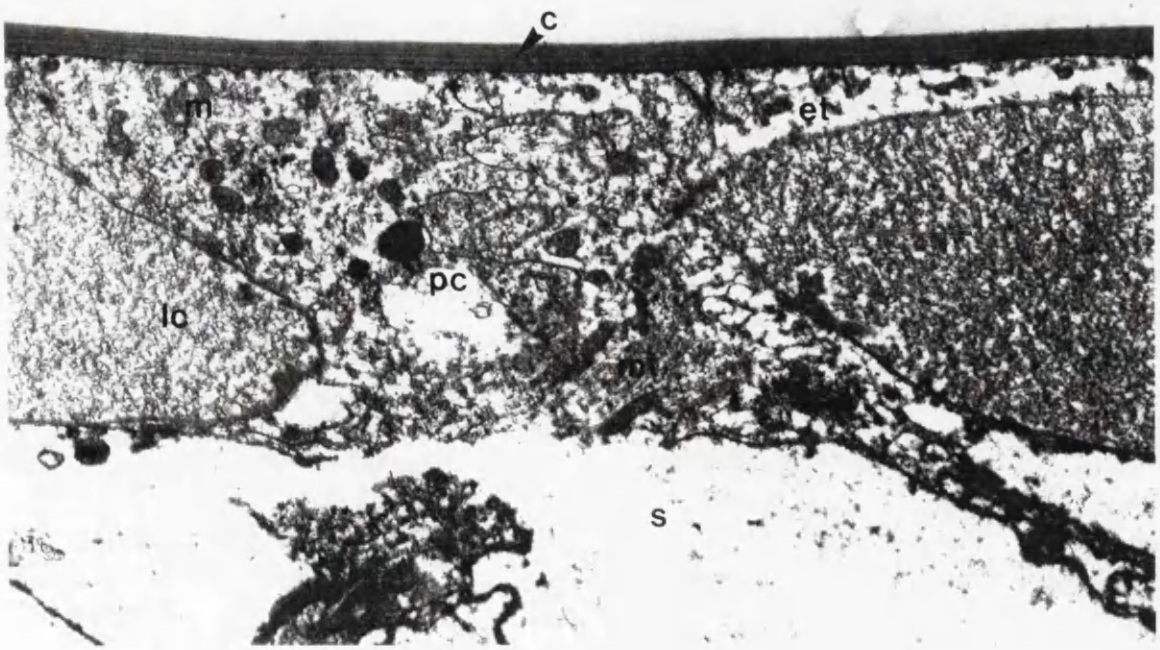
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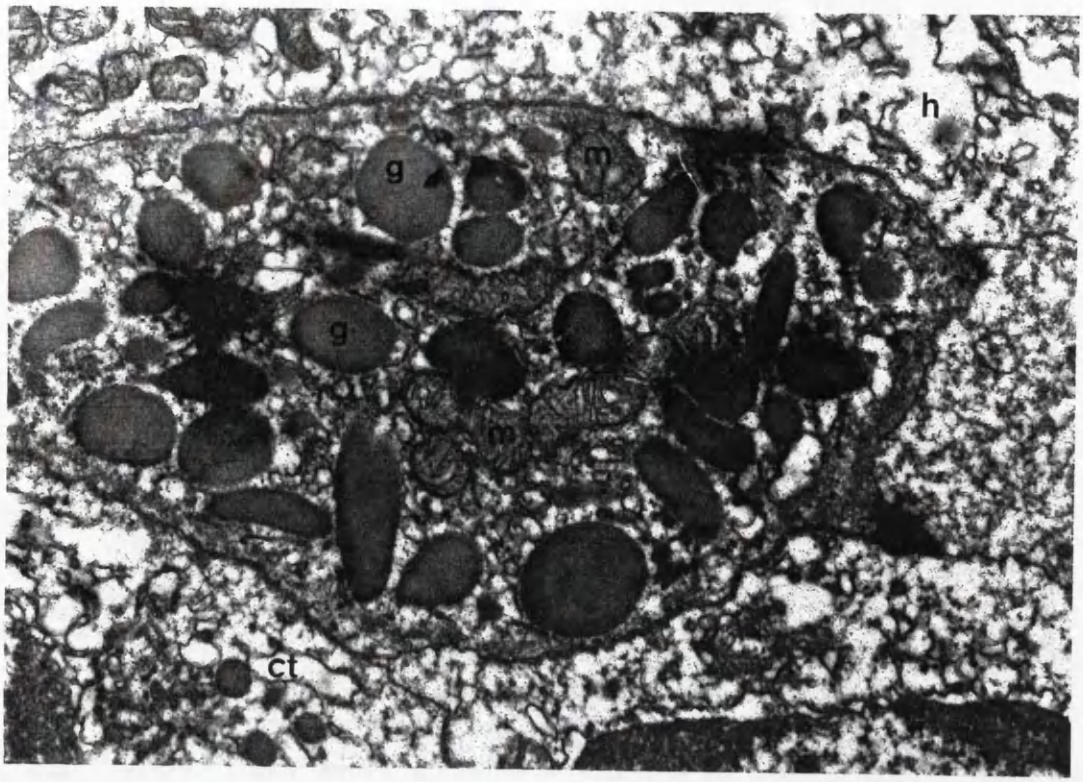
Fig. 3.8. A glycocyte adjoining loose connective tissue (ct) in the haemolymph space of the gill filament of *Upogebia* spp. The majority of the cell is packed with large electron dense glycogen granules (g) and a few mitochondria (m). Mag x 20,525.

Fig. 3.9. *Jaxea nocturna*. Glycocyte in the haemolymph space (h) of a gill filament. Note the large nucleus (n), vacuoles (v) and the small electron-dense vesicles (vs). Mag x 9,610.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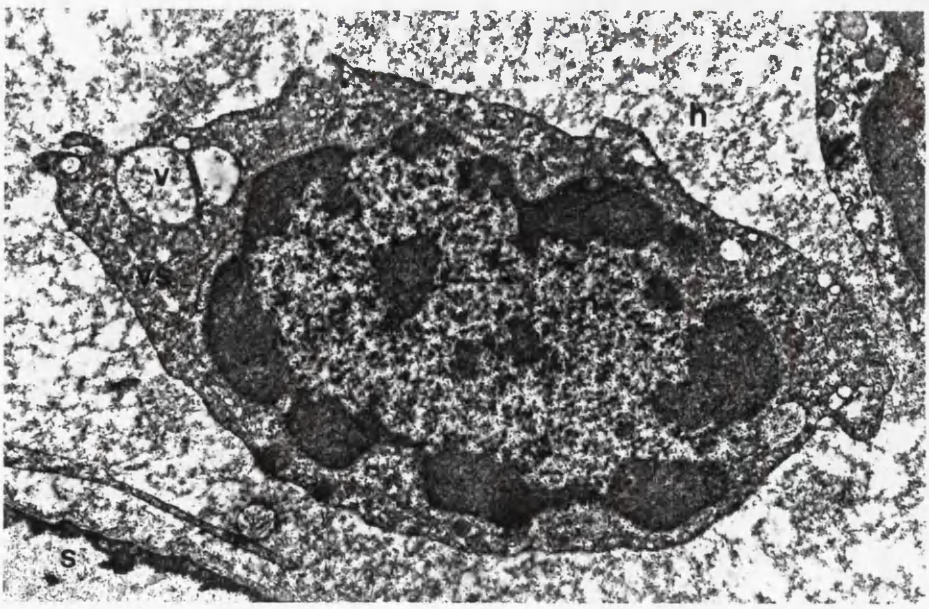
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Haemocytes were regularly observed in the blood spaces of the gill filaments (Fig.3.2). Many were found attached to the subcuticular epithelium, septum or pillar cells. The haemocytes are large, mainly spherical cells with a diameter of 8-15μm. More than one type of haemocyte was observed (Johnson, 1980). Glycocytes had a large, central nucleus that occupied most of the cell, the remainder was packed with electron dense glycogen granules and a few mitochondria (Fig.3.8 and 3.9). Another type, podocytes, had a large nucleus, a number of satellite vacuoles and a series of highly folded cytoplasmic processes called pedicels. The presence of the vacuole and the pedicels are the distinguishing characteristics of the podocyte (Fig.3.10) (Strangeways-Dixon & Smith, 1970).

Callianassa subterranea and *Jaxea nocturna*

The gill filaments consisted of an outer non-chitinous cuticle which was composed of three layers; an epicuticle, exocuticle and endocuticle (see above). In *Callianassa subterranea* the cuticle of the marginal channel was thicker (2.0μm) than the rest of the filament (0.23μm), presumably to provide support. In *Jaxea nocturna* the cuticle thickness over the marginal channel was approximately 0.5μm compared with a cuticle thickness of 0.3μm throughout the rest of the filament (Fig.3.3 and 3.4).

Although the structure of the cuticle was as described for the *Upogebia* spp. and *Calocaris macandreae,* it was significantly thinner in the phylloid gill type than in the standard trichobranch gill filament (Fig.3.11). The cuticle of the gill filaments was underlined by epithelial cells varing in thickness from 0.2μm to 2.1μm, but epithelial layer was thicker in those regions adjoining the pillar cells. In a number of regions extensions of the subcuticular epithelium, pillar cells, projected across to the central septum. These pillar cells had an ultrastucture similar to that described for the upogebiids and were characterised by bundles of microtubules within the cytoplasm (Fig.3.7).

Few apical or basal microvilli were observed in the epithelial cells, with the majority of the cells containing large mitochondria (Fig.3.7). The septum consisted of generally anucleate connective tissue and varied in width along the length of the filament. The septum extended across the complete width of the basal part of the gill filament, and decreased in width nearer to the distal end. The spaces formed between the pillar cells and the septum create a complex matrix or catacomb arrangement of blood channels. The haemocytes found in the gill filaments and main axis were similar to those described for the *Upogebia* spp and *Calocaris macandreae* (Fig.3.8, 3.9 and 3.10).

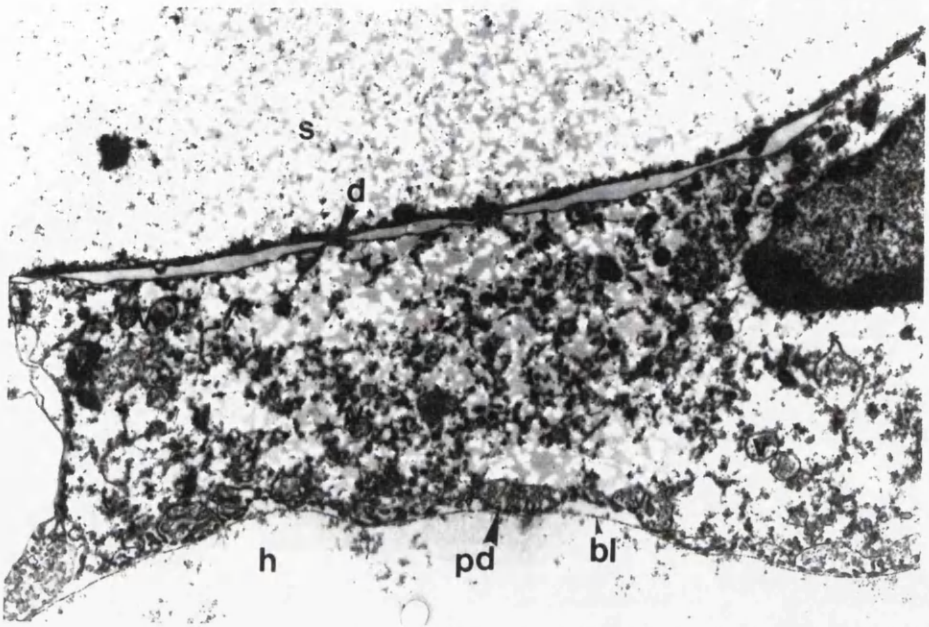


Fig. 3.10. A podocyte in the haemolymph space (h) adjoining the gill septa of *Upogebia* spp. At intervals the lateral cell margins are linked by desmosomes (d). The cytoplasm contains numerous small coated vesicles and a number of larger vacuoles (v). Conspicuous podocyte foot processes (pd) are seen beneath the basal lamina (bl). Mag x 7,120.

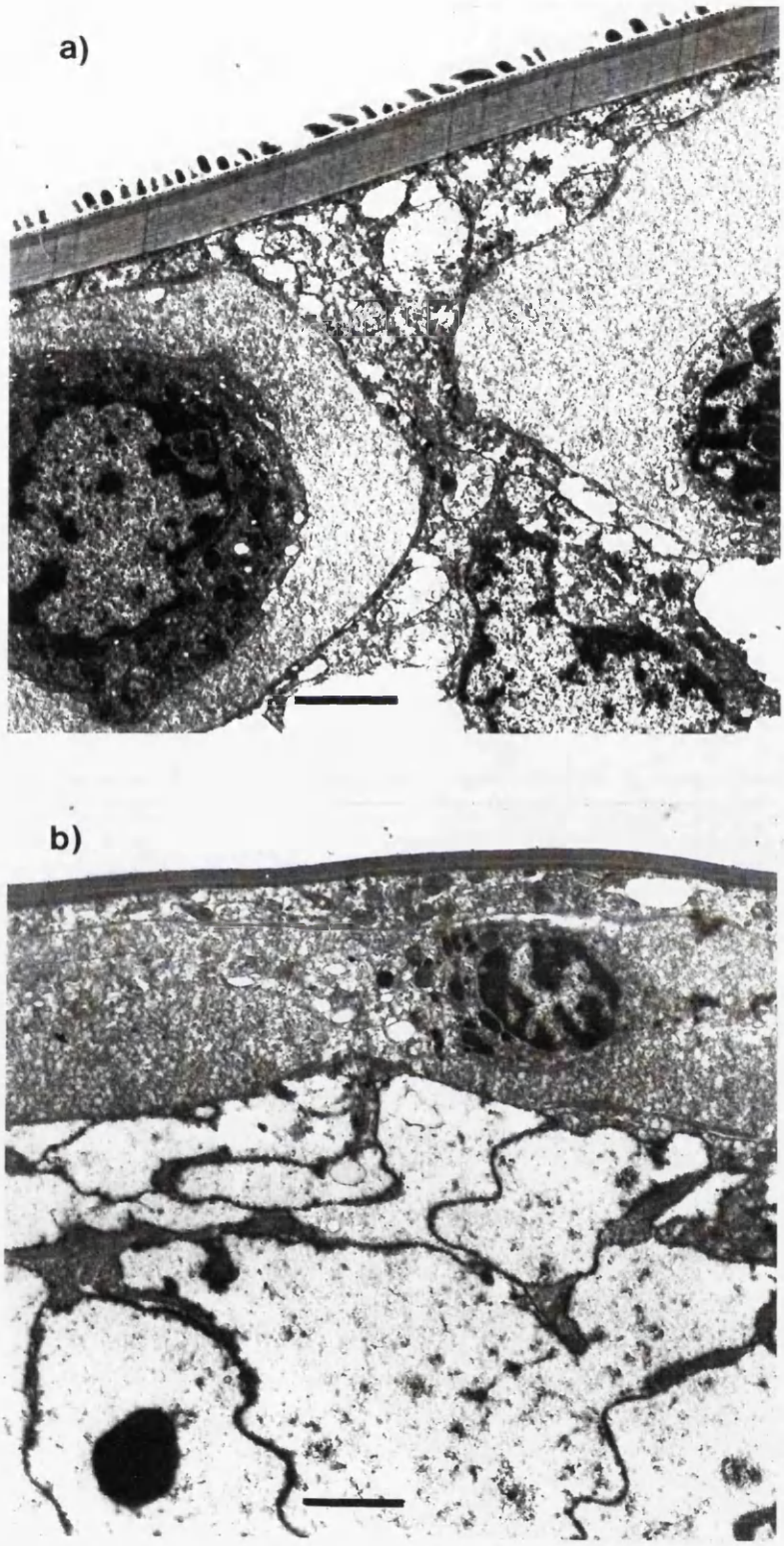


Fig. 3.11. TEM of a section through a trichobranch gill filament of *Upogebia* spp. (a) and phylloid trichobranch of *Callianassa subterranea* (b). The effective diffusion barrier is minimised by reducing the thickness of the chitinous cuticle covering the gills. Note the difference in thickness between the two gill types; the cuticle of *C. subterranea* is much thicker than that of *Upogebia* spp., although the epithelial layer is of similar thickness. Scale bar = 2μm.

Branchial distribution

For each species of mud-shrimp examined, the percentage contribution to the total gill area of individual gills within the branchial chamber is illustrated in Figure 3.12. In *Jaxea nocturna,* a shrimp that has podobranchs (here, for simplicity, the term podobranch refers to the epipod, mastigobranch and podobranch gill as a single branchial entity), the gills found in the central region of the branchial chamber have the largest percentage contribution to the total gill area; i.e. the gills on P2 and P3 (Fig.3.12). The percentage contribution of the podobranch, in *Jaxea nocturna,* was greater in the anterior gills. The gill areas of the anterior and posterior arthrobranchs on appendages M3 to P4 were significantly different (t-test, $P<0.05$), with the anterior arthrobranch having a greater area. The distribution of gill areas within the branchial chamber of an individual *J. nocturna* can he explained primarily as a result of differences in the mean filament area (Table 3.4). There were, however, differences in the number of filaments per gill with gill type, particularly in the podobranchs (Table 3.4); the podobranchs that are situated more posteriorly have fewer gill filaments. The contribution of each particular gill type to the total gill area for *Jaxea nocturna* is shown in Table 3.5.

Table 3.5. *Jaxea nocturna.* The mean percentage contribution of each gill type to the total gill area. (number of observations = 10).

	Gill area	
Gill	% area	±1SD
Arthrobranch	83.2	3.4
Podobranch	11.6	3.3
Epipod	4.7	2.0
Mastigobranch	0.5	0.2

In both *Callianassa subterranea* and *Upogebia* spp, the percentage contribution of each gill to the total gill area increases through M3 to P4, with the majority of the gill area represented by the gills on P2-P4 (Fig.3. 12).

Although there were no significant differences between the contributions of the anterior and posterior gills of each arthrobranch pair on M3 and P2-P4 (t-test, $P<0.05$), there was, however, some difference between Pla and Plp. The posterior gill had the greater gill area contribution of the pair (t-test, $P< 0.05$). The contribution to the total gill area of P5, in *U. stellata* was very small. This increase in gill area contribution from the gills on M3 through

to the gills on P4, within both *C. subterranea* and the upogebiids, is due mainly to an increase in both the filament area (which, in the upogebids, is seen as an increase in the length of the gill filament) and number of filaments per gill (Table 3.6).

Table 3.6. Summary of the statistics derived from regression analyses of mean filament surface area (mm^2) and the number of filaments per gill against fresh body weight (g) for each of the gills from the thalassinidean shrimps *Upogebia deltaura, U. pusilla, U. stellata* and *Callianassa subterranea*. Both gill area and the number of filaments were related to fresh weight (g) by the equation $\log_{10}Y=\log_{10}a+b\log_{10}X$, where Y is the mean filament area or number of filaments and X is fresh body weight (g); r is the correlation coefficient; * indicates not significant at $P>0.05$. Values of a are $\log_{10}$ numbers. Key: M1, maxilliped 1; P2, pereiopod 2; a, anterior arthrobranch; p, posterior arthrobranch.

	Upogebia stellata						*Upogebia pusilla*					
Gill type	Mean filament area (mm^2)			Number of filaments			Mean filament area (mm^2)			Number of filaments		
	a	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*
M3a	-1.11	1.66	0.58*	1.52	-0.31	0.35*	-0.86	0.16	0.2*	1.50	-0.04	0.09*
M3p	-0.75	0.41	0.24*	1.28	0.66	0.35*	-0.87	0.51	0.61	1.64	0.03	0.17*
P1a	-0.86	1.07	0.48	1.69	0.11	0.14*	-0.67	0.61	0.78	1.84	-0.07	0.27*
P1p	-0.67	0.57	0.36*	1.77	0.18	0.24*	-0.59	0.50	0.80	1.98	0.16	0.57*
P2a	-0.60	0.90	0.53	1.86	0.02	0.14*	-0.50	0.63	0.84	2.03	0.10	0.62
P2p	-0.51	0.64	0.39*	1.93	-0.97	0.15*	-0.47	0.63	0.88	2.06	0.05	0.26*
P3a	-0.46	0.57	0.35*	1.86	0.23	0.53	-0.40	0.49	0.63	2.08	0.08	0.33*
P3p	-0.53	0.86	0.57	1.90	0.09	0.23*	-0.45	0.67	0.69	2.08	0.08	0.40*
P4a	-0.48	0.71	0.34*	2.01	-0.38	0.18*	-0.34	0.37	0.57	2.10	0.04	0.20*
P4p	-0.59	1.23	0.58*	1.96	-0.16	0.16*	-0.39	0.59	0.75	2.08	0.07	0.29*
P5	-0.78	0.43	0.10	0.96	0.95	0.20*						

	Upogebia deltaura						*Callianassa subterranea*					
Gill type	Mean filament area (mm^2)			Number of filaments			Mean filament area (mm^2)			Number of filaments		
	a	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*
M3a	-0.87	0.28	0.24*	1.25	0.00	0.00*	-1.02	0.49	0.65	1.27	0.30	0.57
M3p	-0.96	0.47	0.42*	1.35	0.02	0.03*	-0.76	0.71	0.65	1.50	0.30	0.67
P1a	-0.88	0.78	0.79	1.75	-0.02	0.07*	-0.76	0.72	0.90	1.81	0.13	0.59
P1p	-0.64	0.54	0.73	1.87	0.08	0.49*	-0.38	0.78	0.95	1.92	0.13	0.69
P2a	-0.69	0.85	0.84	1.91	0.15	0.43*	-0.22	0.76	0.94	2.04	0.13	0.89
P2p	-0.64	0.80	0.90	1.97	0.10	0.75	-0.18	0.76	0.92	2.05	0.14	0.89
P3a	-0.63	0.82	0.93	2.01	0.10	0.67	-0.14	0.82	0.93	2.05	0.13	0.85
P3p	-0.59	0.81	0.97	2.03	0.05	0.35*	-0.12	0.79	0.95	2.06	0.12	0.88
P4a	-0.46	0.61	0.91	2.02	0.09	0.52*	-0.10	0.83	0.94	2.06	0.15	0.85
P4p	-0.46	0.72	0.88	2.00	0.09	0.45*	-0.06	0.81	0.93	2.05	0.14	0.85

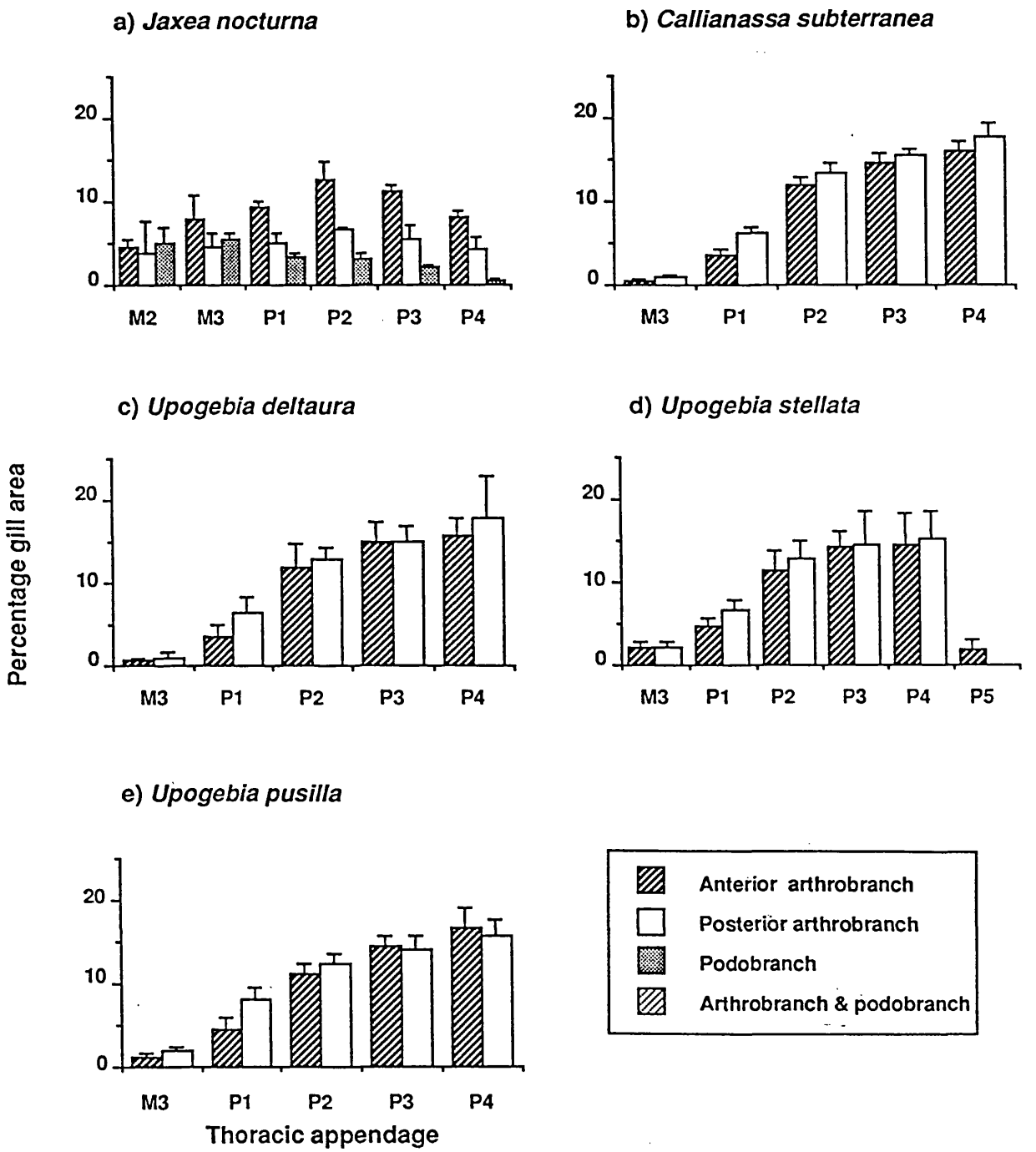


Fig 3.12. The percentage contribution to the total gill area of individual gills within the branchial chambers of a number of thalassinidean mud-shrimp. Mean values are shown ±SD, the number of observations for each species is shown in parenthesis.

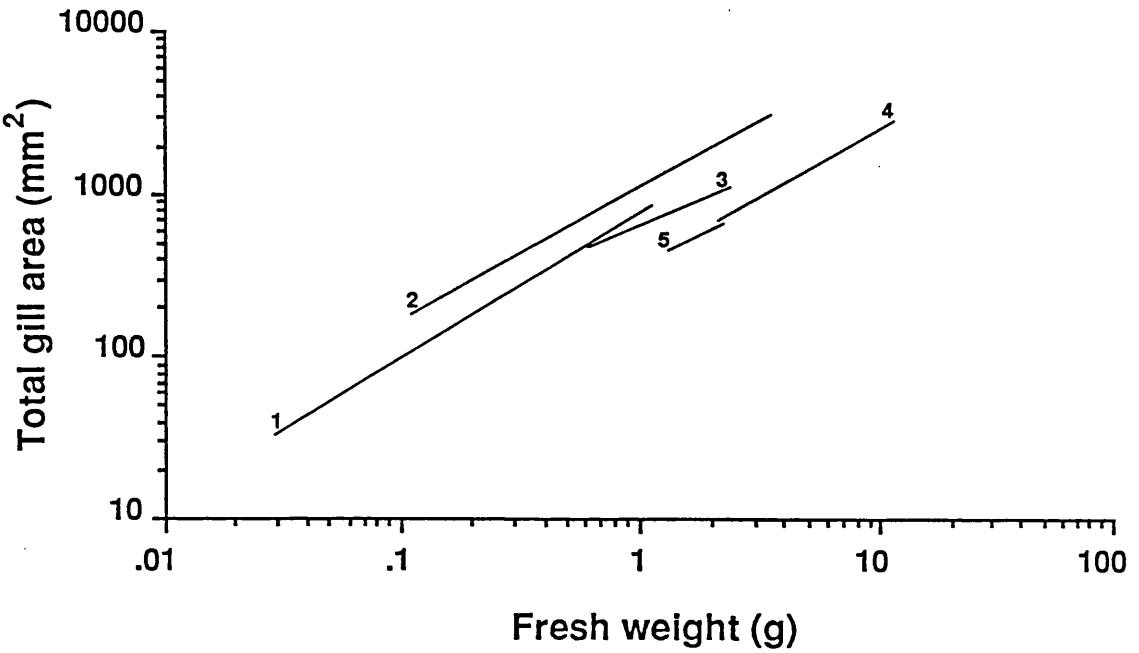


Fig. 3.13. Relationship between gill area (mm^2) and fresh body weight for five thalassinidean mud-shrimp. 1. *Jaxea nocturna*; 2. *Callianassa subterranea*;3. *Upogebia pusilla*; 4. *Upogebia deltaura*; 5. *Upogebia stellata*. Fitted regression lines are described in Table 3.7 . Individual points have been omitted for clarity.

Gill area

The $\log_{10}$ transformed relationships between total gill area and fresh body weight, for five species of thalassinidean shrimp, *Jaxea nocturna, Callianassa subterranea, Upogebia stellata, U. deltaura,* and *U. pusilla* are presented in Figure 3.13. A significant relationship existed between log gill area and log fresh body weight for all the species examined. The regression equations of the lines fitted to all the data for all five species are presented in Table 3.7.

As a result of the mean size differences between *Callianassa subterranea* obtained from Loch Sween (1.56g $\pm$0.94, n=10) and those obtained from the North Sea (0.49g $\pm$0.27, n=8), both groups were, initially, treated separately. However, covariance analysis showed no significant difference between the slopes (F_s=4.00; d.f.,1, 13; $P>0.05$) or elevations (F_e=0.9; d.f., 1, 14; $P>0.05$) of the regression lines fitted to the data (combined regression coefficient = 0.78), therefore, the data obtained from the two groups were combined for subsequent analysis.

Table 3.7. Summary of the relationship between fresh body weight and total gill area for some thalassinidean shrimps. Fitted regressions are of the form $\log_{10}Y=\log_{10}a+b\log_{10}X$, where Y is the total gill area and X is fresh body weight (g). Values of a are $\log_{10}$ numbers.

	Regression constants		Correlation coefficient	Level of significance	Number of observations
Species	a	b	r	P	n
Jaxea nocturna	2.89	0.89	0.99	0.001	9
Callianassa subterranea	3.05	0.82	0.97	0.001	17
Upogebia stellata	2.57	0.71	0.56	0.014	19
U. deltaura	2.58	0.83	0.96	0.001	10
U. pusilla	2.81	0.61	0.82	0.001	12

Covariance analysis demonstrated that there were no significant differences in the slopes of the regression lines for all the species investigated (combined regression coefficient = 0.80); however, there were differences in the elevations (F_e = 100.02; d.f., 6, 74; $P<0.01$). The total gill areas of *Upogebia stellata* and *U. deltaura* were very similar. The elevations of their regression lines were significantly different from that of *U. pusilla* (F_e = 7.22; d.f.,1, 24; $P<0.01$), indicating that individuals of *U. pusilla* have a greater total gill area than the other upogebiids of an equivalent weight (Fig.3.13). *Callianassa subterranea* and *Jaxea nocturna,* species which both have the phylloid gill forms, had greater total gill areas than the thalassinideans with the standard trichobranch gill type (e.g. *C. subterranea* / *U. pusilla*

F_e = 70.84; d.f., 1,27; $P<0.01$). The gill area of *C. subterranea* was significantly larger than that of *J. nocturna* (F_e = 32.15; d.f., 1,23; $P<0.01$), indeed *C. subterranea* had the largest gill area of all the mud-shrimps investigated.

Between species, the differences in total gill area were attributable mainly to differences in mean filament area and not to changes in filament number (Tables 3.4 & 3.6). For example, *Upogebia stellata* and *U. deltaura* both had similar, but smaller, mean filament areas than *U. pusilla*, although the filament number remained relatively constant (Table 3.6). The greater filament area of *C. subterrranea* and *J. nocturna* was attributable to the flattened shape of their individual gill filaments.

Branchial ventilation

The movement of seawater through the branchial chamber was observed for *Upogebia* spp., *Callianassa subterranea* and *Jaxea nocturna.* A similar pattern of water movement through the branchial chamber was observed for all the species examined. Beating of the scaphognathites within the prebranchial chambers induced water to flow through the branchial cavity in a posterior-anterior direction and out via the scaphognathite channel.

Inhalant water entered the branchial chamber primarily via the ventro-lateral margins of the branchiostegite and, to a lesser extent, through the spaces between the limb bases. Water then passed over the gills and into the prebranchial space, where it was expelled anteriorly. There appeared to be a gradient of water tlow into the branchial chamber, the flow rate was greatest at the anterior edge of the branchiostegite (nearest to the scaphognathite) and was least at the most posterior part of the branchiostegite.

Discussion

Gill complement and gross morphology

The number and morphology of the gills has regularly been used as a major characteristic for the classification of the Decapoda (e.g. Huxley, 1878; Bate, 1888; Borradaile, 1907; Selbie, 1914; Burkenroad, 1981), but such features may vary from species to species even in a family. Within the thalassinideans, the number or even presence of gills on the maxillipeds appears to be quite variable. Selbie (1914) and Caroli (1946) both recorded 17 gills in *Jaxea nocturna*, but disagreed as to their distribution. Although the branchial formulae of 18 gills as given in this study agrees with that of Wear & Yaldwyn (1966) for *J. nocturna* and *J. novaezealandiae*, the number of gills present on maxillipeds 1 and 2 was found to be quite variable.

In the calocaridid *Calocaris macandreae* only 8% of individuals examined had an arthrobranch on maxilliped 2 (Anderson, 1989), and in *Upogebia stellata* and *U. deltaura* there was some variation in both the number and type of gills particularly the gills on the maxillipeds. Indeed, Stebbing (1900) noted that a single gill on maxilliped 2 was recorded for the genus *Upogebia* (as *Gebia*) by H. Milne-Edwards. This was not seen in the present study. Such variability in the number of gills has also been shown for other decapods; for example, Hartnoll (1964) found that in majid crabs one or more of the gills on maxilliped 2 were either rudimentary or absent in 8 of the 17 genera examined and suggested that a reduction in gill number had occurred several times in independent stocks.

Thalassinideans that have been recorded as having pleurobranchs are found only in the sub-genus *Axius* (*Axius*) (Borradaile, 1903; De Mann, 1925; Gurney, 1942) [it was this characteristic that led De Mann to divide the genera *Axius* into sub-genera *Axius* (*Axius*) (pleurobranchs present) and *Axius* (*Neaxius*) (pleurobranchs absent) and in two South African *Upogebia* species (Stebbing, 1900; de Saint Laurent & Le Loeuff, 1979). The pleurobranch found on pereiopod 5 of *U. stellata* was not found in earlier observations (Borradaile, 1903; de Saint Laurent, 1973). It was suggested by Borradaile (1903) that a new sub-genus should be erected specifically for the South African upogebiid, *U. capensis* and any other upogebiids that have a pleurobranch on the fifth perionite. The importance placed on such taxonomical features is illustrated in another decapod group, the Astacidae (crayfish). Clarke (1936) used the presence or absence of pleurobranchiae as a major argument for the erection of the genus *Parastacoides*.

The possession of a pleurobranch on a upogebiid is interesting because members of the Upogebiidae and Callianassidae are considered advanced groups (de Saint Laurent, 1972) whereas a pleurobranch is regarded as a primitive characteristic (Gurney, 1942; de Saint Laurent & Le Loeuff, 1979). Upogebiids and callianassids, in addition to usually lacking pleurobranchs (Biffar, 1971; de Saint Laurent, 1973; Rodrigues, 1978) have podobranchs (and associated mastigobranchs) that are either rudimentary, if present, or absent. This has led to an overall reduction in the maximum number of gills in each branchial chamber (*Jaxea nocturna* has 18 gills; *Calocaris macandreae* has 16; *Upogebia* spp have 11 or 10; *Callianassa subterranea* has 12 gills) indicating a transition to a more simplified gill arrangement in the higher families.

During the evolution of the Decapoda there has been a trend towards the reduction in the number of gills (Calman, 1909; Gurney, 1942; Burkenroad, 1981; Swain *et al.*, 1988).Within different decapod groups various explanations have been given to try to account for the trend of a reduction in gill number. Amongst brachyurans and some anomurans, loss of gills has been associated with an increasing adoption of terrestial habit (e.g. Gray, 1957; Hawkins & Jones, 1982). In another case, reduction in gill number in pinnotherids may have resulted from adaptation of the species to a commensal way of life (Hong, 1988).

Previously, significant taxonomical emphasis has been placed on various branchial characteristics (Borradaile, 1903; Burkenroad, 1981). It has been suggested, however, that the value of some of the characters used in taxonomy should be reconsidered (Felgenhauer & Abele, 1983). The variability in gill number between species has been discussed, but Felgenhauer and Abele (1983) specifically draw particular attention to gill morphology. Three basic gill forms are recognised based on their external morphology (McLaughlin, 1983); the trichobranchiate gill which has a series of filamentous lateral branches arising from the main branchial axis (as found in most lobsters and crayfish); the phyllobranchiate gill with paired lamellar filaments arising from the central gill axis (as in caridean shrimp and most brachyuran crabs); and the dendrobranchiate gill which is similar to the trichobranch gill but has subdivided secondary rami arising from each lateral branch (found only in sergistoids and penaeoids). Although these three basic gill types are recognised, many species possess gills which appear to be transitional between the basic trichobranchiate and phyllobranchiate form (as in thalassinideans, pagurids and galatheids) and this has led to difficulty and inconsistancy in determining the gill type, though de Saint Laurent (1992) has recently shown how this diversity of structure may have arisen within the Decapoda.

Thalassinideans exhibit a large degree of morphological variation in gill form both between families and even within a genus which has led to confusion as to the classification of gill form. Variation in gill form is also quite common amongst a number of decapods as, for example, in the dromiid genus *Dicranodromia* (Bouvier, 1940). In the present study the three upogebiids and the calocaridid had distinct trichobranchiate gills, whereas the callianassid and laomediid had phylloid gills (i.e. having flattened gill filaments which, in ultrastructure, are analogous to the trichobranchiate gill). The thalassinidean phylloid gill structure is analogous to the palaemonid phyllobranch gill type which represents a situation intermediate between the trichobranchiate and brachyuran phyllobranch gill types (Taylor & Taylor, 1992)

Huxley (1878) stated that the gills of *Upogebia* (as *Gebia*) and *Callianassa* were phyllobranchiate, but Stebbing (1900) disagreed and called them trichobranchiate. In his classification of the Thalassinidea, Borradaile (1903) noted that the gills of the axiids (including the calocaridid) were trichobranchiate and those of the laomediids were trichobranchiate but "with somewhat broadened filaments". The family Callianassidae was characterised as having gills that were trichobranchiate or had filaments that broadened in various degrees, but both the sub-families Callianassinae and Upogebinae were included in the Callianassidae (Borradaile, 1903). Felgenhauer and Abele (1983) believed the gills of *Upogebia* (*U. pugettensis*) and axiids were phyllobranchiate.

From the scarce descriptions of thalassinidean gill type in the literature, the gills of the upogebiids have been described so far as; trichobranchiate with a) two tubular filaments arising from each side of the central gill axis (*U. stellata, U. deltaura, U. pusilla, U. balmorum, U.*

baweana), or b) a single tubular filament arising from each side of the axis (*U. laemanu, U. gracilis, U. pugrax, U. hexaceras*) and as "lamellar" (a possible interpretation would be phylloid-trichobranchiate) with a flattened single filament arising from each side of the central axis (*U. octoceras, U. pugettensis*) (Drach, 1930; Felgenhauer & Abele, 1983; Ngoc-Ho, 1990). De Saint Laurent and Le Loueff (1979) in their description of *Upogebia* from the West African coast, describe the gills of *U. furcata, U. nitida, U. demani, U. crosieri, U. anstata, U. poensis, U. contigua and U. talsimani* as 'des lamelles branchiales divisées...'. Although the previous interpretation of lamellar has been that of phylloid-trichobranch, de Saint Laurent and Le Loueff (1979) have also given the same branchial description for the thalassinideans *U. pusilla and U. deltaura*. Clearly both these species have tubular gills, thus the description of the gills given by these authors should be interpreted as tubular and not lamellar.

It is clear that until the terminology of the gills has been re-evaluated (both in terms of external morphological features and the ultrastructure of the gills) and until more complete descriptions of gill formulae and structure are given, such confusion is going to remain and the validity of utilizing the gill form as a taxonomical character may be questionable. The classification of gill morphology is also very important if the evolution of the trichobranchiate gill type gave rise to both the dendrobranchiate and phyllobranchiate gills or if the dendrobranchiate gill was the starting point (Burkenroad, 1981; Felgenhauer & Abele, 1983).

The gill form may also be of some use when considering the phylogenic relations between various decapod groups, especially within a group such as the Thalassinidea where there is a lot of disagreement as to the evolutionary relationships (de Saint Laurent pers. comm.).

Surface area of gills

Respiratory gas exchange will be limited by the functional morphology of the branchial system. Apart from the control of perfusion and ventilation, the major charactersitic of an efficient organ of gas exchange is the combination of a maximum surface area available for gaseous diffusion and a minimum diffusion distance across the gill from the external medium to the circulating haemolymph. The variability in the type, number and morphology of the gills between decapod species will significantly influence one particular aspect of branchial system, the area available for gaseous diffusion, which in turn will effect respiratory gas exchange.

Differences in gill areas between the thalassinidean species in this study was primarily a result of differences in gill filament area and not the number of filaments per gill; *Jaxea nocturna* had fewer filaments per gill but a greater number of gills, whereas the *Upogebia* spp. and *Callianassa subterranea* had a similar number of gills. The larger weight-specific gill areas of the mud-shrimp *C. subterranea* and *J. nocturna*, compared to that of the *Upogebia* spp., was primarily a result of the lamellar shape of the phylloid-trichobranch gill fillaments. This

contrasts with the gill areas recorded for the crabs *Carcinus maenas*, *Liocarcinus depurator* and *Hyas araneus* where the differences in gill area between the species were primarily related to changes in the total number of gill filaments (Johnson & Rees, 1988).

The increase in gill area with body weight for all the species of thalassinidean shrimp was mainly a result of an increase in the average area of the gill filaments. This is also the situation for a number of other decapods, both aquatic and semi-terrrestial (Hughes, 1982; Scammell & Hughes, 1982; Greenaway, 1984; Johnson & Rees, 1988; Zainal, 1990). For some species, however, the increase in gill area with body size has been attributed more to an increase in the total number of filaments and number of filaments per gill rather than changes in filament area (Hughes, 1957; Swain *et al.*, 1988). Swain *et al.* (1988) suggested that the way changes in gill area with body size are determined (e.g. an increase in the number of filaments, or a larger filament area) may reflect fundamental differences between phyllobranch and trichobranch gills. Caution should be exercised when making such statements, however, as an increase in filament area with body size has been shown for both gill types.

Table 3.8. Weight-specific gill area for five thalassinidean mud-shrimps. Values given are for a 'standard' 1.5g specimen, calculated from regression equations relating body weight with weight-specific gill area given previously (Table 3.7).

Species	Area ($mm^2.g^{-1}$)
Callianassa subterranea	1043
Jaxea nocturna	742
Upogebia pusilla	551
U. deltaura	354
U. stellata	330

Although the gills of the mud-shrimp *Callianassa subterranea* and *Jaxea nocturna* are not truely phyllobranch it is important to remember that gill area comparisons between different groups of decapods should, primarily, be retained within those groups that possess similar basic gill types. The majority of allometric differences have been correlated with the level of activity or habitat, but such disparity could simply be the result of comparing decapods with different basic branchial morphologies, such as phyllobranchiate and trichobranchiate gills (e.g. Gray, 1957; Scammell & Hughes, 1982). Once this has been recognised allometric comparisons between branchial groups may be made with more confidence.

The weight-specific gill surface areas, calculated for an 'average' sized individual (1.5g), for five species of thalassinidean mud-shrimp are shown in Table 3.8. The differences in weight-specific gill areas between species appear to reflect the different burrowing strategies employed

by the shrimp and hence the respiratory conditions within the burrow.

The burrow conditions of the deposit feeders *C. subterranea* and *J. nocturna* are typically severely hypoxic and even anoxic, whereas because of irrigation activity during feeding, the burrows of the filter feeding upogebiids are more oxygenated (Atkinson & Taylor, 1988; Witbaard & Duiniveld, 1989; Nickell, 1992). The larger gill area of the deposit feeders may be interpreted as an adaptation to the extreme hypoxic conditions that regularly occur within the burrows. Although a deposit feeder, the small gill area of the mud-shrimp, *Calocaris macandreae*, probably reflects the more oxygenated burrow conditions, created by the shallower, large lumen burrow system (Atkinson & Taylor, 1988; Anderson, 1989). Similarly, Swain *et. al.* (1988) found that the burrowing crayfish *Parastacoides tasmanicus* had a significantly larger gill area than the non-burrowing stream-dwelling *Astacopsis franklinii*. The difference in gill area was interpreted as being associated with the prolonged periods of hypoxia that *P. tasmanicus* regularly experienced.

Table 3.9. Weight-specific gill area for a number of decapod crustacea having trichobranch or phyllobranch basic gill types. The values shown are calculated from regression equations, from previously published data, using a 'standard' sized (10g) specimen. Although the weight chosen represents the upper size range for some of the species and the lower range for others it still provides a useful means of comparing gill area between species.

Species	Area ($mm^2.g^{-1}$)	References
Trichobranch gill		
Callianassa subterranea	741	This study
Jaxea nocturna	602	This study
Pagurus bernhardus	459	Hughes (1983)
Nephrops norvegicus	420	Hughes (1983)
Upogebia pusilla	264	This study
U. deltaura	257	This study
U. stellata	190	This study
Calocaris macandreae	107	Anderson (1989)
Homarus gammarus	101	Hughes (1983)
Phyllobranch gill		
Callinectes sapidus	1476	Hughes (1983)
Carcinus maenas	810	Hughes (1983)
Cancer pagurus	564	Hughes (1983)
Munida rugosa	418	Zainal (1990)
Munida sarsi	299	Zainal (1990)

Functional adaptations, such as large gill area, to low environmental oxygen conditions have also been recorded for the squat lobster *Munida quadrispina* from a low-oxygen fjordic habitat

(Burd, 1987) and in the hypoxia-tolerant bathypelagic mysid *Gnathophausia ingens*, which lives in the oxygen depleted waters off the Californian coast (Belman & Childress, 1976). Interestingly, changes in gill area with habitat are not just restricted to crustaceans. As well as substantially increasing the functional gill area during exposure to acute or short term hypoxia by lamellar erection, fish from different habitats have also been shown to have different gill areas, for example oxyphilic compared to sluggish species (for review see Laurent & Perry, 1991 and references therein).

Aside from correlating gill area with differences in habitat oxygen levels, gill area in crustaceans has also been correlated with activity. When decapod Crustacea of a similar body mass are compared, active species that possess a similar gill type (e.g. phyllobranchiate or trichobranchiate gills) tend to have larger gill areas (Gray, 1957; Scammell & Hughes, 1982; Hughes, 1983; Johnson & Rees, 1988; Henry *et al.*, 1990a; Zainal, 1990).

Mud-shrimps are considered to be relatively inactive species, as indicated by their low rates of oxygen consumption (Anderson, 1989; Witbaard & Duiniveld, 1989) and low behavioural activity (Anderson, 1989; Nickell pers. comm.; Stamhuis, pers. comm.), but the gill area of *J. nocturna* and *C. subterranaea* is much greater than that of the active hermit crab, *Pagurus bernhardus*, and even comparable to the surface area of the phyllobranch gills of crabs (Table 3.9). Therefore, this suggests that, within the thalassinideans, a large gill area is primarily an adaptation to the hypoxic conditions found within the burrow.

Additionally, changes in gill allometry; such as fewer number of gills, smaller gill areas and an increase in inter-lamallar spacing, have also been related to the transition from an aquatic to semi-terrestial habitat (Gray, 1957; Hawkins & Jones, 1982; Hughes, 1983; Spicer & Taylor, 1986). Such changes, however, are in response to a different set of physiological constraints imposed by aerial, rather than aquatic, respiration.

The position of the largest gills, or maximum gill area, in those thalassinideans that possess a simple gill arrangement (the *Upogebia* spp. and *Callianassa subterranea*) were associated with periopods 2-4 (P2-P4). In *Jaxea nocturna* and *Calocaris macandreae*, species that possess podobranchs, the region of maximal gill area was displaced anteriorly to P1-P3 (Anderson, 1989).

Scammell and Hughes (1982) found that for a number of decapods, the largest gills occupied a position in the branchial chamber which reflected the region of greatest ventilatory flow. It is not clear, however, whether in the thalassinideans, this displacement of maximum gill area is due to variations in contact between the branchiostegite and limb bases, which in turn will affect the flow of water through the branchial chamber (this was the reason given by Scammell and Hughes (1982) for the observed differences of gill area distribution between brachyurans, anomurans and macrurans), or is simply as a result of the more complicated (primitive) branchial arrangement.

Fine gill ultrastructure

The fine structural organisation of the trichobranchiate gills of the mud-shrimp, *Upogebia stellata, U. deltaura* and *Calocaris macandreae* and the phylloid gills of *Callianassa subterranea* and *Jaxea nocturna* were similar to the descriptions given for a number of other decapods (Morse *et al.*, 1970; Fisher, 1972; Burggran *et al.*, 1974; Foster & Howse, 1978; Compere *et al.*, 1989; Goodman & Cavey, 1992; Maina, 1990; Dickson *et al.*, 1991). The phylloid gills of *C. subterranea* and *J. nocturna* are analogous to compressed trichobranch gill filaments. The phylloid gill filament is very similar in ultrastructure to the brachyuran phyllobranch lamellae, the major difference is that in the phylloid gill type the intralamellar septa is present in every gill filament and orientated 90° to that in the trichobranchiate gill (Taylor & Taylor, 1992), whereas in the phyllobranch gill it is present in only some of the lamellae, and when it is present it is often perforated (Barra *et al.*, 1983; Goodman & Cavey, 1992).

Table 3.10. Cuticle and epithelium thickness for trichobranch and phyllobranch gills of some decapod Crustacea

Species	Cuticle (μm)	Epithelium (μm)	Total (μm)	Reference
Trichobranch				
Procambarus clarkii	1.5-2.5	1.5-6.0	3.0-8.5	Burggren *et al.* (1974)
P. clarkii	0.5-2.0	0.7-3.0	1.2-5.0	Dickson *et al.* (1991)
Astacus pallipes	0.8-2.5	0.7-3.1	1.5-5.6	Fisher (1972)
Upogebia spp.	1.2-1.4	0.8-3.6	2.0-5.0	This study
Calocaris macandreae	0.90	0.7-4.0	1.6-4.9	This study
Callianassa subterranea	0.23	0.2-2.1	0.4-2.5	This study
Jaxea nocturna	0.30	0.2-2.5	0.5-2.8	This study
Phyllobranch				
Carcinus maenas	5.0	1.0	6.0	Taylor & Butler (1978)
C. maenas	2.1	0.5-5.5	2.6-7.6	Goodman & Cavey (1992)
Munida rugosa	1.1-2.3	2.8-3.5	3.9-6.8	Zainal (1990)
Eriocheir sinensis	1.0	2.0-4.0	3.0-5.0	Barra *et al.* (1983)
Potamon niloticus	0.75	6.0	6.7	Maina (1990)

The epithelial cells of the thalassinidean gills contained numerous mitochondria and showed a high degree of apical infolding. The majority of the mitochondria were found, however, within

distinct epithelial cells termed pillar cells (Drach, 1930). Although morphologically distinct ionoregulatory and respiratory regions (mitochondrially rich) have been observed in a number of decapods with trichobranch gills (e.g. *Procambarus clarkii,* Dickson *et al.*, 1991; *Pacifastacus leniusculus,* Morse *et al.*, 1970) and phyllobranch gills (e.g. *Callinectes sapidus,* Copeland & Fitzjarrell, 1968; *Carcinus maenas*, Compere *et al.*, 1989; *Eriocheir sinensis*, Barra *et al.*, 1983) in this study, no such distinct sites could be discerned from the micrographs of the gills of the thalassinideans. Mitochondria were, however, often found within most of the gill filaments examined. Although the salinity range experienced by these shrimps is likely to be quite constant, thus negating the need for large ares of iono-regulatory sites within the gill filament, the mitochondria may be involved with other functions such as acid-base balance. Similarly, no distinct ionoregulatory areas were found in the gills of the freshwater crab, *Potamon niloticus* (Maina, 1990). As there is no discrete predominance of ionoregulatory or respiratory function among different gill filaments, it is assumed that the gills serve a dual role and that there must be some sort of structural compromise in the design of the gill.

Structural changes recorded in the gill epithelium, in particular extensive folding of the apical and basolateral membrane, for a number of decapod species exposed to hypo- or hyperosmotic salinities (Foster & Howse, 1978; Martelo & Zanders, 1986; Compere *et al.*, 1989) illustrate the plastic nature of the gill (in relation to structure, function or both). Although no specific sites of regulatory tissue have been found in the gills of thalassinideans, it would be interesting to examine the gills of a number of intertidal or estuarine mud-shrimps for specific ionoregulatory sites and compare them to the gills of species that live in more stable, sublittoral habitats. Specialized epithelial cells, pillar cells, were found in both the trichobranch and phylloid gill filaments. The pillar cells (also termed 'pillaster' (Copeland & Fitzjarrell, 1968), 'pilaster' (Compere *et al.*, 1989) and 'trabecular' (Nakao, 1974) cells), characterized by the numerous mitochondria and bundles of microtubules, extend across the blood space to the central septum. The pillar cells are an intergral part of the gill ultrastructure and serve a number of important structural and physiological functions.

The extensive microtubular system of the pillar cell anchors the epithelial layer to the cuticle forming an extension of the cytoskeleton (Compere *et al.*, 1989; Dickson *et al.*, 1991). In addition to maintaining the uniform width of the haemocoel (Cioffi, 1984), it has been suggested that the pillar cells provide support and help to stabalise the gill lamellae against the hydrostatic pressure and shear forces generated during haemolymph flow. (Finol & Croghan, 1983; Dickson *et al.*, 1991).

Spaces between successive pillar cells form an extensive network of secondary lacunae which in turn creates a complex sinus around the periphery of the gill filament. By altering the functional length of the gill filament via lateral shunting of haemolymph, the sinus will not only affect the exchange capacity of the gill filament (Burggren *et al.*, 1974) but will also facilitate

gaseous diffusion. The numerous lacunae will effectively increase the surface area of the gill filament and also reduce the water-blood diffusion barrier. The diffusing capacity of the gill filaments is, in addition to ventilation and perfusion, dependent upon the diffusing surface area, structural organistaion of the gill filament and on the haemolymph-water diffusion distance. The tissue diffusion barrier, cuticle and epithelium thickness, for both the thalassinidean gill types is shown in Table 3.10 and is comparable to values recorded for other decapods which possess either trichobranch or phyllobranch gills.

The chitinous cuticle covering the surface of the gill filaments may comprise up to 80% of the diffusion path (Table 3.10). As the diffusion of oxygen through chitin is 10-fold slower than through other tissues (Krogh, 1919), chitin will comprise a large barrier to oxygen uptake. Taylor & Butler (1978) calculated that the thickness of chitin covering the gill lamellae of the crab, *Carcinus maenas*, effectively increased the diffusion barrier by five to ten times the barrier presented by the secondary lamellae on a fish gill. The effective diffusion barrier may be minimized by reducing the thickness of the chitinous cuticle covering the gills. This may be achieved by simply reducing the width of the cuticle or by extensive infiltration of the cuticle by pore canals, which can penetrate up to 75% of the cuticular thickness (Burggren *et al.*, 1974).

The cuticle thickness of the phylloid gill filaments of the deposit feeding thalassinideans was significantly reduced compared to the trichobranch gill filaments of *Calocaris macandreae* and *Upogebia* spp. This difference in cuticle thickness is consistant between the two gill forms and appears to reflect the poorly oxygenated burrow habit of *Jaxea nocturna* and *Callianassa subterranea*. Similarly, in *Gnathophausia ingens*, a mysid from the hypoxic oxygen minimum layer off the Californian coast, the cuticle is much thinner than most other crustaceans and it is this reduced effective diffusion distance that, coupled with the large gill area, provides the mysid with a gill that has a high diffusion capacity (Belman & Childress, 1976).

Gill perfusion

Perfusion patterns through trichobranch gill filaments have been described by Drach (1930), Morse *et al.* (1970), Fisher (1972), Burggren *et al.* (1974) and Massabuau (1983). They describe co-current flow between the haemolymph and water in the afferent channel of the gill filament and then countercurrent flow as the haemolymph passes back down the filament via the efferent channel. The majority of haemolymph oxygenation probably occurs as haemolymph is forced from the afferent channel through the complex sinus created by the lateral lacunae to the efferent channel (Fisher, 1972). Some oxygenation may also take place along the length of the both haemolymph channels (Burggren *et al.*, 1974; Dickson *et al.*, 1991). The potential for effective gas exchange will be limited, however, by the possibility of cross diffusion of gases via the septum or by the admixture of afferent and efferent blood passed across the lacunae

(Burggren *et al.*, 1974). The possibility of cross diffusion of gases is reduced in the crayfish, *Procambarus clarkii* (Dickson *et al.*, 1991), as the predominant respiratory gill filaments have thickened septa separating the afferent and efferent blood channels, compared with the thinner septa separating the haemolymph channels in the transporting filaments.

As *Upogebia* spp. have a single row of paired filaments emanating from each side of the gill axis, haemolymph flow through the gill is more complicated and probably less efficient since blood must pass through both filaments in succession before passing into the axial efferent vessel.

Although the phylloid gills of *Callianassa subterranea* and *Jaxea nocturna* are simply flattened trichobranchs it is thought that the haemolymph flow patterns are similar to those recorded in the palaemonid phyllobranchiate gill (Taylor & Taylor, 1992) and the phyllobranch gill (Hughes *et al.*, 1969; Barra *et al.*, 1983; Maina, 1990). The main differences between the brachyuran phyllobranchiate gill and the thalassinidean phylloid gill form is that in the phylloid gill both the axial blood vessels are located in the ventral aspect of the gill shaft, whereas there is a dorsal afferent and ventral efferent axial vessel in the brachyuran phyllobranch gill. The phylloid gill also has very flattened, non-distinct afferent and efferent filament channels compared to the trichobranch gill. Therefore, as in the phyllobranch gill, the majority of blood will flow through the gill filament via the marginal channel.

It is speculated that haemolymph travelling around the marginal channel will traverse from the inner to the outer part of the filament in a combined cross-current and counter-current manner. The majority of haemolymph oxygenation will probably occur as blood percolates through the central aspect of the gill lamellae via the sinus created by the pillar cells and the septum. In addition to increasing the lamellar transit time, and thus facilitating oxygenation (Maina, 1990), the marginal channel in the phylloid gill type, as in the phyllobranch gill, probably acts as a 'spacer' keeping apart the closely packed lamellae and making a clear space for water to circulate between adjacent gill filaments (Compere *et al.*. 1988; Goodman & Cavey, 1992; Maina, 1990).

To summarize, there was a trend towards simplification in gill formula in the 'higher' thalassinidean families. The deeper burrowing deposit feeders that are regularly exposed to prolonged periods of hypoxia, *Callianassa subterranea* and *Jaxea nocturna*, had significantly larger gill areas than thalassinideans which occupied more oxygenated burrows. The increase in gill area was a result of flattening of the trichobranch gill filaments giving rise to a phylloid gill form. The efficiency of gas transfer, and hence diffusing capacity, was enhanced in the phylloid gill by the larger gill area and, because of the reduced cuticle thickness, shorter effective haemolymph-blood diffusion distance. The increased diffusion capacity of the phylloid gill is interpreted as a functional adaptation to the more severe conditions within the burrow waters that deposit feeders *Callianassa subterranea* and *Jaxea nocturna* are likely to regularly experience

Chapter 4

Comparative Respiratory Physiology: Oxygen Uptake

Introduction

The burrow morphology and burrow environment of the filter feeding upogebiid shrimps, *U. deltaura* and *U. stellata* were described in detail in Chapter 2. Within these simple burrows a gradient of oxygen tension with depth was often recorded, with the extent and duration of hypoxia experienced being dependent upon the irrigation profile. Compared with the filter feeders *U. deltaura* and *U. stellata*, deposit feeders such as *Callianassa subterranea* and *Jaxea nocturna* generally construct deeper, more complex burrows which regularly become severely hypoxic or even anoxic.

In general, burrowing aquatic decapods are highly tolerant of hypoxia and their ability to maintain respiratory independence under these conditions is well developed (see reviews by Atkinson & Taylor, 1988; Taylor & Atkinson, 1991). However, comparative information on aspects of physiological adaptation to life within burrows is scarce. In the previous chapter, the comparative relationship between gill structure and function was described with particular regard to the burrowing life-style. It was found that those species that are regularly exposed to prolonged periods of hypoxia (*C. subterranea* and *J. nocturna*) had significantly larger gill surface areas compared to those shrimps that occupied more oxygenated burrows (*Upogebia stellata* and *U. deltaura*). The larger gill area and shorter effective haemolymph-blood diffusion distance (a result of the reduced cuticle thickness) resulted in an increased diffusion capacity (the capacity for gas transfer through a system will be dependent on the area available for gaseous diffusion, the diffusivity of the gas and thediffusion medium).

This chapter investigates the comparative ability of four species of burrowing mud-shrimp, *Callianassa subterranea, Jaxea nocturna, Upogebia stellata* and *U. deltaura* to maintain oxygen uptake during progressive hypoxia. In addition, some of the compensatory mechanisms employed to maintain oxygen uptake during hypoxia, namely the ventilatory and perfusion responses, were examined in more detail for one of the shrimps, *U. deltaura*.

Materials and methods

Collection and maintenance of mud shrimps

The thalassinidean mud-shrimps used for this study were collected by anchor dredge. *Upogebia*

stellata were caught from White Bay, Isle of Cumbrae, Scotland (55°9'N, 5°11'W) at depths of 25-30m. Individuals of *U. deltaura* were obtained from Plymouth Sound (50°20'N, 4°13'W), Plymouth, U.K., the North Sea (54°35'N, 4°50'W) and the Irish Sea (54°7'N, 3°27'W). *Callianassa subterranea* and *Jaxea nocturna* were taken from Loch Sween (10-25m), west coast of Scotland, U.K (56°2'N, 5°36'W). All shrimps were then taken to the University of Glasgow Zoology Department aquarium, placed within small (volume=1litre) holding pots containing sediment from the White Bay sample site and maintained with recirculating sea water (salinity=33-35‰, 10-14°C) until required. During this holding time the animals fed on the sediment within the holding pots. This method was very succesful as some individuals were kept for upto one year under these conditions. During prolonged holding the shrimp were observed to moult at least once.

Measurement of oxygen consumption during normoxia and hypoxia

Measurements of respiration rates during normoxia and hypoxia were made using 'closed' and 'semi-closed' respirometry. The acrylic respirometers were supplied with sea water from a reservoir containing fully aerated, ultraviolet sterilized, artificial sea water (Instant Ocean, Aquarium Systems, Ohio) at a salinity of 33 ±2‰) The respirometer and reservoir were placed within a water bath which was maintained at 10.0 ±0.2°C for the duration of the experiment (10-16h). The chamber was fitted with an isolated magnetic stirrer bar, and the water gently stirred to prevent stratification and local oxygen depletion around the electrode.

The partial pressure of oxygen (PO_2) of the sea water was measured using a polarographic oxygen electrode (E-5046, Radiometer or 1302, Strathkelvin Instruments) inserted directly into the respirometer. Prior to each experiment, the electrode was calibrated against a zero oxygen solution (sodium sulphite in 0.01 mol l^{-1} borax) and air-saturated sea water. The oxygen electrode was connected to an oxygen meter (781, Strathkelvin Instruments) and the PO_2 of the sea water recorded with a chart recorder (SE120, Belmont Instruments). For all respiration experiments only individuals in the intermoult stage were used. The weight range for each species and the number of shrimps used to determine oxygen consumption rates are given in Table 4.4.

Since thalassinideans appear stressed outside of a burrow environment (MacGinitie, 1930; 1934; Witbaard & Duinveld, 1989; Astall *pers obs*), they were placed within perforated glass tubes, 50-100 mm long, 10-18mm diameter (2mm size classes) and the ends covered with plankton gauze before each experiment. Tube diameter has previously been shown to affect the pleopod activity and performance in the burrowing shrimp, *Neotrypaea* (as *Callianassa*) *affinis* (Farley & Case, 1968), therefore, the diameter of the tube into which each individual was placed was selected according to the relationship between the burrow diameter and carapace length established previously for *Upogebia pusilla* (Dworschack, 1983). The tubes were then placed

into the respirometer with sea water flowing through.

Rates of oxygen consumption were measured using both 'closed' and 'semi-closed' respirometry in order to assess the viability of both techniques. For measurements using 'closed' respirometry, an individual was placed within the respirometer and left to acclimatize for at least 12h, with aerated sea water pumped from the reservoir through the chamber. After this time, the flow of water to the respirometer was stopped and, with the use of one way valves, the chamber was isolated. The shrimp was then allowed to deplete the available oxygen in the respirometer down to near anoxic levels (<10 Torr).

For measurements using 'semi-closed' respirometry, sea water of different oxygen tensions was passed through the respirometer. The oxygen tension of the sea water in the reservoir was altered by bubbling gas mixtures, supplied by a gas mixing apparatus, having different concentrations of O_2, N_2 and CO_2 through the water in the reservoir. CO_2 was included in the gas mixture (0.01%) to maintain the pH of the sea water at 8.00-8.10. After acclimatization, sea water having a reduced PO_2 was then passed through the respirometer for 30-60min. The chamber was isolated and the decrease in PO_2 of the sea water over a period of lh was monitored. After this time the chamber was carefully flushed with sea water having a lower PO_2 and the procedure repeated. Using this method the shrimps were exposed to increasing levels of hypoxia in a progressive manner.

For both methods, the respirometer was maintained in darkened conditions and the shrimp kept as free as possible from external disturbances. During a number of preliminary trials, the pH and ammonia levels (Davies, 1988) were monitored in both the 'semi-closed' and 'closed' systems. During the experiments the maximum variation in pH was 0.6 pH units and changes in the ammonia concentration were negligible. The bacterial oxygen consumption was determined by running a blank control and this value was then subtracted from that measured for each individual shrimp. The apparatus was sterilized with a hypochlorite solution between experiments to reduce bacterial contamination. The fresh body weight was determined after removing excess water and rates of oxygen consumption (as $\mu molO_2\ g^{-1}\ h^{-1}$) calculated according to the equation; ,

$$MO_2 = ß(P_iO_2 - P_tO_2)\ V/1000\ 60/T\ 1/W \quad \text{.....(1)}$$

Where; ß is the oxygen solubility coefficient ($\mu mol.l^{-1}.Torr^{-1}$) at the appropriate temperature and salinity (Weiss, 1970); P_iO_2 (Torr) is the initial partial pressure of oxygen in the respirometer; P_tO_2 (Torr) is the final partial pressure of oxygen in the respirometer at time *t* (The values for P_iO_2 and P_tO_2 were only calculated when the rate of oxygen depletion was linear); *V* is the volume of water (ml) within the respirometer; *T* is the duration (min over which P_iO_2 and P_fO_2 were measured; *W* is the wet weight of the shrimp (g), The symbol for the weight-specific rate

of oxygen consumption is given (using SI units) as MO_2 , however, it will be referred to throughout the thesis as MO_2 hereafter.

Ventilatory and heart rate measurements

Heart and scaphognathite activity were measured in more detail for one species of mud-shrimp, *Upogebia deltaura*, using an impedance technique modified from Hoggarth and Trueman (1967) (Dyer & Uglow, 1977). Shellac-coated copper wire (S.W.G. 44), with the shellac removed from the last millimetre, formed the electrode and the bared ends were bent to form a hook. Cardiac activity was measured by hooking one electrode over the dorsal posterior part of the cephalothorax thus positioning the electrode close to the heart.

The single scaphognathite electrode was hooked over the anterior margin of the branchiostegite. Both electrodes were anchored to the cephalothorax of the shrimp with cyanoacrylate adhesive. The reference electrode was constructed from a piece of aluminium plate (60mm x 30mm) and placed within the respirometer. The electrodes were connected to a George Washington impedance pneumograph and pen recorder. Once the electrodes were firmly attached, the shrimp was placed within an artificial burrow inside the respirometer and left to recover with aerated sea water flowing through the respirometer for a minimum of 12h. Using 'closed' respirometry, the heart and scaphognathite rates were measured during normoxia, progressive hypoxia and anoxia with minimal disturbance.

Results

Oxygen consumption and body weight

The relationship between the weight specific rate of oxygen consumption (MO_2) and fresh body weight was determined using closed respirometry for each species. It was assumed that the rate of oxygen consumption measured under steady state conditions was the 'routine' rate, when the animal was in spontaneous activity, rather than standard or active rate (Wycliffe & Job, 1977).

High initial MO_2 levels, recorded for 15-30min following initiation of the experiment, were thought to be the result of both a small decrease in the temperature (1-2°C) and alterations in water flow patterns within the respirometry chamber following isolation of the respirometer. Such apparent increases in MO_2 were considered artificial and not representative of true MO_2 levels and were therefore not taken into account when calculating the rate of oxygen consumption during normoxic conditions.

The relationships between the weight specific rate of oxygen consumption (MO_2) and fresh

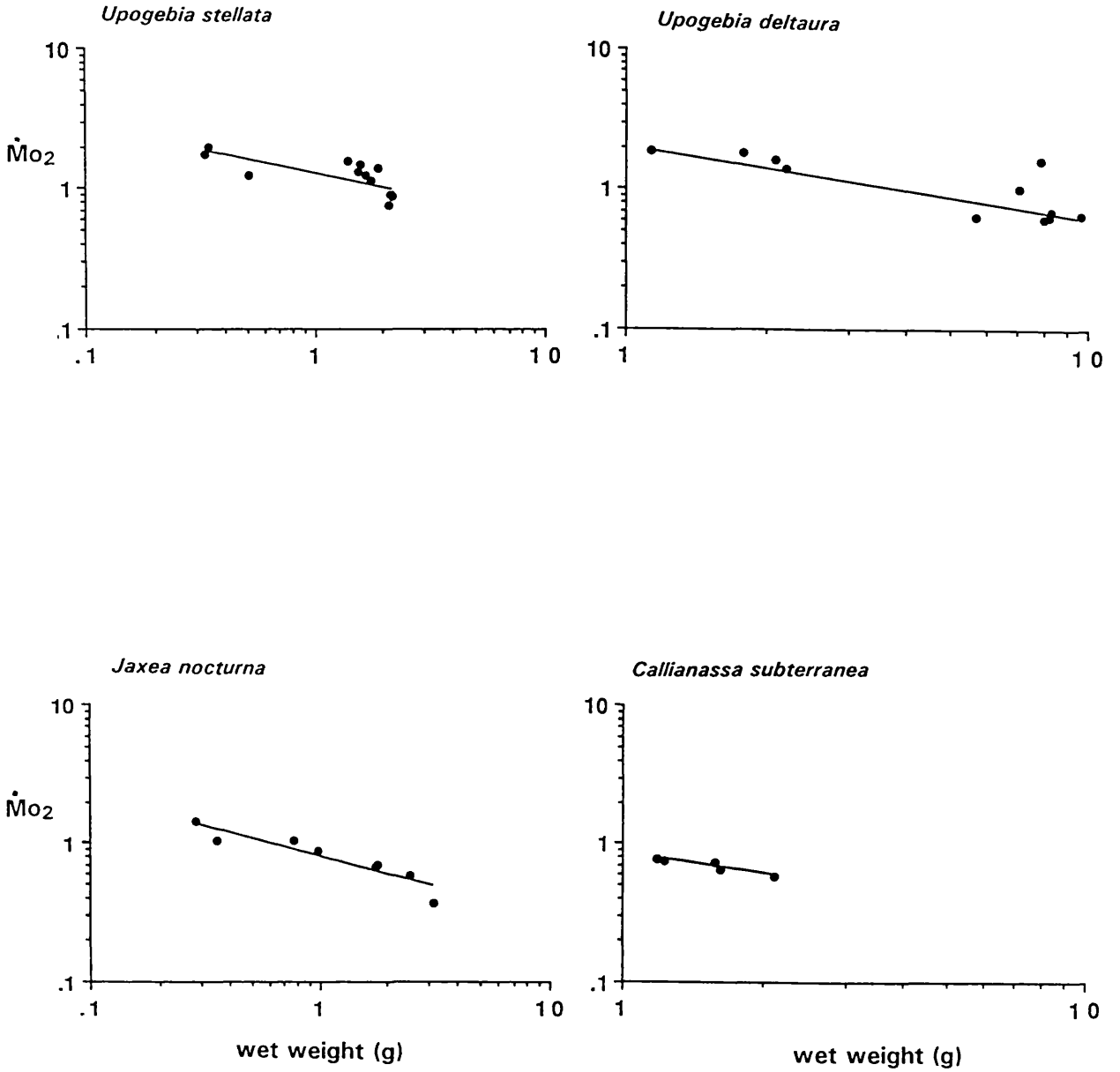
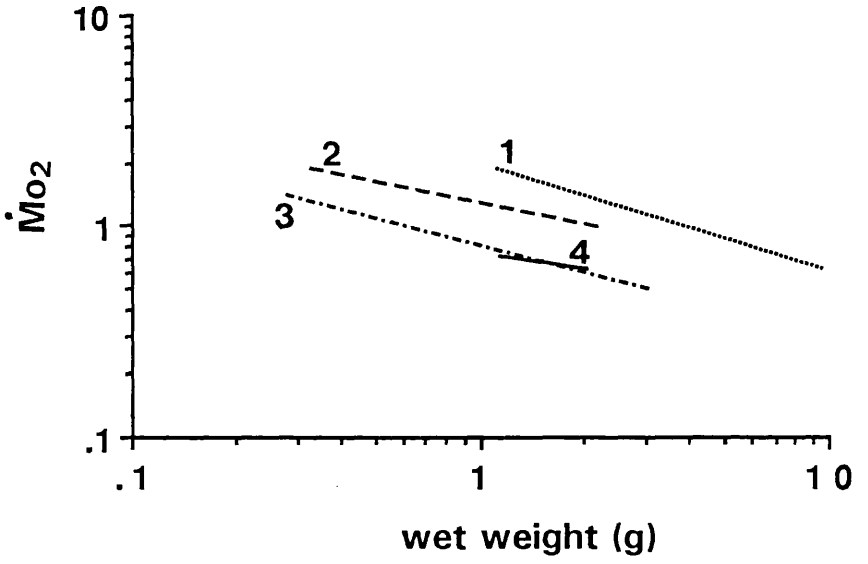


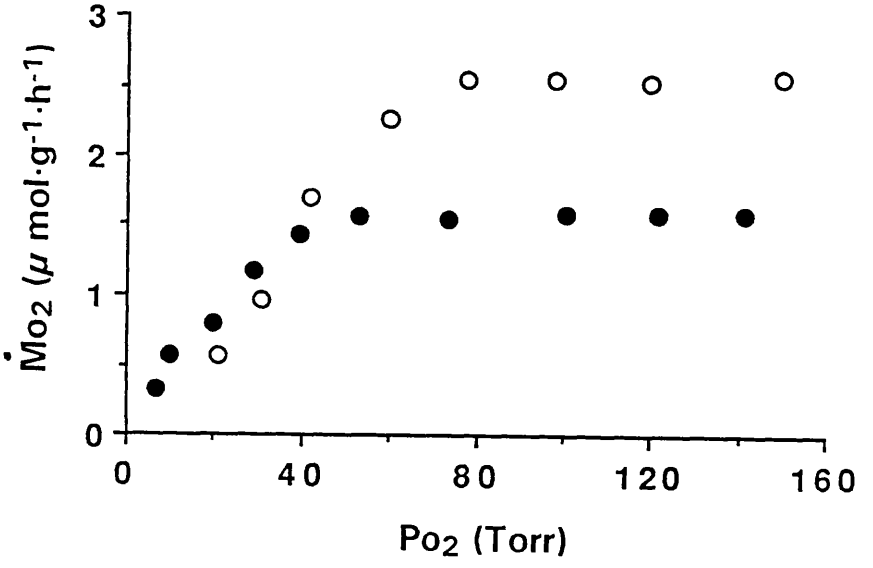
Fig. 4.1. The relationship between the rate of oxygen consumption (MO_2) and wet weight at 10°C for four species of thalassinidean shrimp. Oxygen consumption was measured using closed respirometry techniques. The equations of the regression lines describing the data are given in Table 4.1.

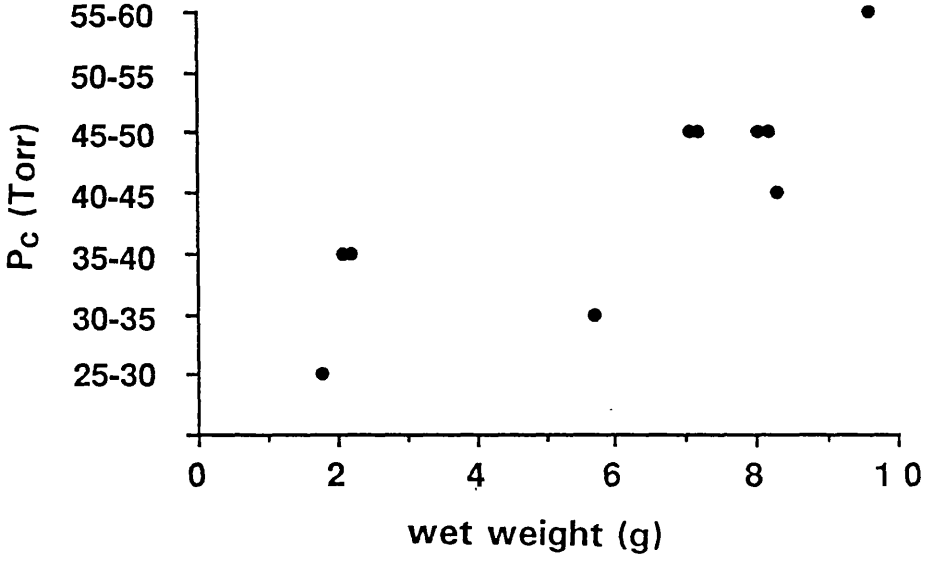
Fig. 4.2. Summary of the relationship between the rate of oxygen consumption (MO_2) and wet body weight at 10°C for *Upogebia deltaura* (1), *U. stellata* (2), *Jaxea nocturna* (3) and *Callianassa subterranea* (4).

Fig. 4.3. The effect of declining oxygen tension on the rate of oxygen consumption (MO_2) of the same individual *Upogebia stellata* (wet body weight 1.61g), determined using closed respirometry (•) and semi-closed respirometry (o) at 10°C.

Fig. 4.4. The relationship between the critical oxygen tension (Pc range) and wet body weight for *Upogebia deltaura* at 10°C.


10
1
.1
$\dot{M}o_2$
2
1
3
4
.1
1
10
wet weight (g)


3
2
1
0
$\dot{M}o_2$ (μ mol·g^{-1}·h^{-1})
0
40
80
120
160
Po_2 (Torr)


55-60
50-55
45-50
40-45
35-40
30-35
25-30
P_c (Torr)
0
2
4
6
8
10
wet weight (g)

body weight for each of the four species of thalassinidean shrimp are shown in Fig.4.1. The data for some, if not all, species are limited due to the availability of individual shrimps, even so for each species an inverse relationship between MO_2 and body weight was observed. This relationship can be described by the equation:

$$MO_2 = aW^{b'} \quad \text{.....(2)}$$

where; MO_2 is the weight specific rate of oxygen consumption; W is the fresh body weight, b' is the slope of the relationship and a is a constant.

A summary of the regression equations describing the relationship betwen MO_2 and body weight for the four species of mud-shrimp is given in Table 4.1.

Table 4.1. Relationships between the weight-specific rates of oxygen consumption (MO_2) and body weight range (g) for four species of thalassinidean mud-shrimps, described by the expression $MO_2 = aW^{b'}$ Number of observations (n), correlation coefficient (r), significance of correlation coefficient (P) and b =slope of non weight-specific relationship

Species	Wet weight (g)	MO_2 ($\mu molO_2\ g^{-1}\ h^{-1}$)	b	n	r	P
Jaxea nocturna	0.28-3.13	$0.806W^{-0.439}$	0.561	8	-0.915	0.001
Callianassa subterranea	1.15-2.06	$0.738W^{-0.249}$	0.751	5	-0.917	0.018
Upogebia deltaura	1.13-9.61	$2.117W^{-0.479}$	0.521	9	-0.793	0.003
Upogebia stellata	0.33-2.19	$1.132W^{-0.282}$	0.718	12	-0.698	0.011

Covariance analysis showed no significant differences ($P<0.05$) between the slopes of the regression lines for any of the four species. There were no significant differences (ANCOVA, $P<0.05$) between the elevations of the regression lines for *U. deltaura* / *U. stellata* and *C. subterranea* / *J. nocturna*, although the elevations of the regression lines for *U. deltaura* and *U. stellata* were significantly higher (ANCOVA, $P> 0.05$) than those of *C. subterranea* and *J. nocturna* (Fig.4.2).

To enable comparison between species, the MO_2 was calculated for a 'standard' 1.5g specimen (Table 4.2). This weight was chosen as it represented a value typical for each species that was used to determine the regression lines of the relationships between MO_2 and body weight. However, 'standard' size is not ideal for the smaller species.

Table 4.2. Weight specific rates of oxygen consumption (MO_2) calculated for a 'standard' 1.5g

individual using the relationships established previously (Table 4.1).

Species	MO_2 ($\mu molO_2\ g^{-1}\ h^{-1}$)
Jaxea nocturna	0.67
Callianassa subterranea	0.66
Upogebia deltaura	1.74
Upogebia stellata	1.01

The upogebiid shrimps had higher rates of oxygen consumption than either *J. nocturna* or *C. subterranea*, both of which had similar values, throughout the weight range (Fig.4.2) and for 1.5g individuals. The MO_2 of a 'standard' 1.5g *U. deltaura* was greater than *U. stellata* (Table 4.2). Similarly the MO_2 of *U. deltaura* was higher than that of *U. stellata* throughout the weight range studied (Fig.4.2).

Oxygen consumption during hypoxia

Initially, the routine rate of oxygen consumption during hypoxia for *Upogebia stellata* was measured using both 'closed' and 'semi-closed' respirometry. The MO_2 determined using 'semi-closed' respirometry, however, was significantly higher ($P<0.05$) than that recorded for the same individuals using the 'closed' respirometry system (Fig.4.3; Table 4.3).

Table 4.3. *Upogebia stellata*. Weight specific rate of oxygen consumption at normoxia (MO_2) and critical point (Pc) determined using 'semi-closed' (SC) and 'closed' (C) respirometry. The Pc was determined by eye.

		MO_2 ($\mu molO_2\ g^{-1}\ h^{-1}$)		Pc (Torr)	
Shrimp number	Wet weight (g)	SC	C	SC	C
1	1.58	1.40	1.32	45-50	35-40
2	2.19	1.16	0.88	55-60	30-35
3	1.61	2.50	1.49	55-60	35-40
4	1.74	3.25	1.60	60-65	35-40

In addition, the point at which the MO_2 ceased to be independent of the environmental PO_2 (Pc) was significantly higher in those individuals whose rate of oxygen uptake was measured using the 'semi-closed' system. Intermittent flushing of the 'semi-closed' system appeared to disturb the

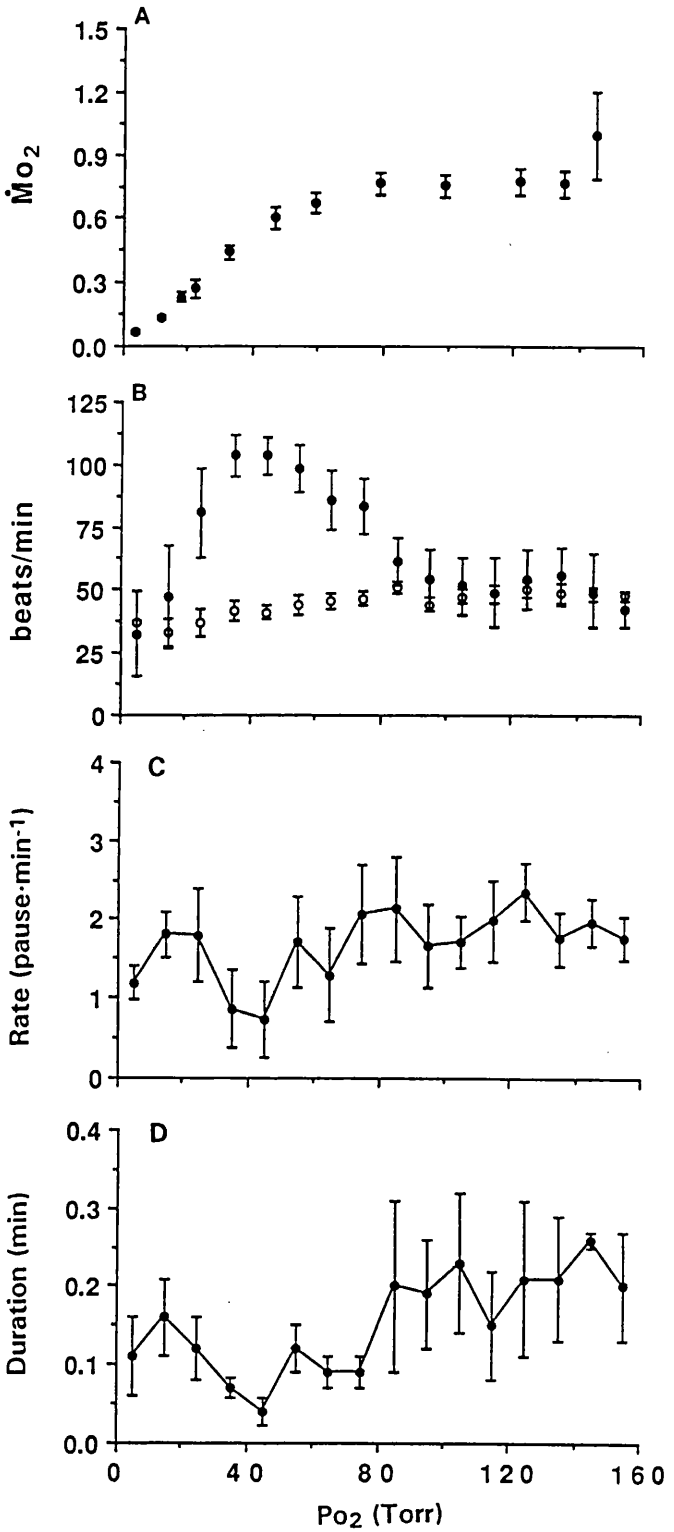


Fig. 4.5. Changes in (a) oxygen consumption (MO_2), (b) the heart (o) and scaphognathite (•) rate (c) the frequency of scaphognathite pausing and (d) the duration of each pause episode as a result of declining oxygen tensions for the mud shrimp *Upogebia deltaura* (mean wet weight 8.4g ±0.4g; n=5) at 10°C. All data points are given as means ±1SE.

shrimp and the subsequent elevated MO_2 and Pc values suggest that 'active' not 'routine' rates of oxygen consumption were being measured by the system. Subsequent determinations of MO_2 during progressive hypoxia for all four species of thalassinidean mud-shrimp were therefore determined using only 'closed' respirometry.

All four species of mud-shrimp were shown to be able to maintain a constant rate of oxygen consumption independent of oxygen tension, until a critical oxygen tension (Pc) was reached. Below the Pc the rate of oxygen consumption decreased with a further reduction in oxygen tension (Fig.4.3).

All of the mud-shrimps used for this study had a high degree of respiratory independence, being able to maintain their rates of oxygen consumption over a wide range of environmental PO_2. For similar sized individuals, the Pc range for *Jaxea nocturna* and *Callianassa subterranea* was extremely low (10-20 Torr), whereas the Pc range for the upogebiids was between 30-40 Torr (Table 4.4).

Table 4.4. Regulatory ability (Pc) of four species of mud-shrimp.

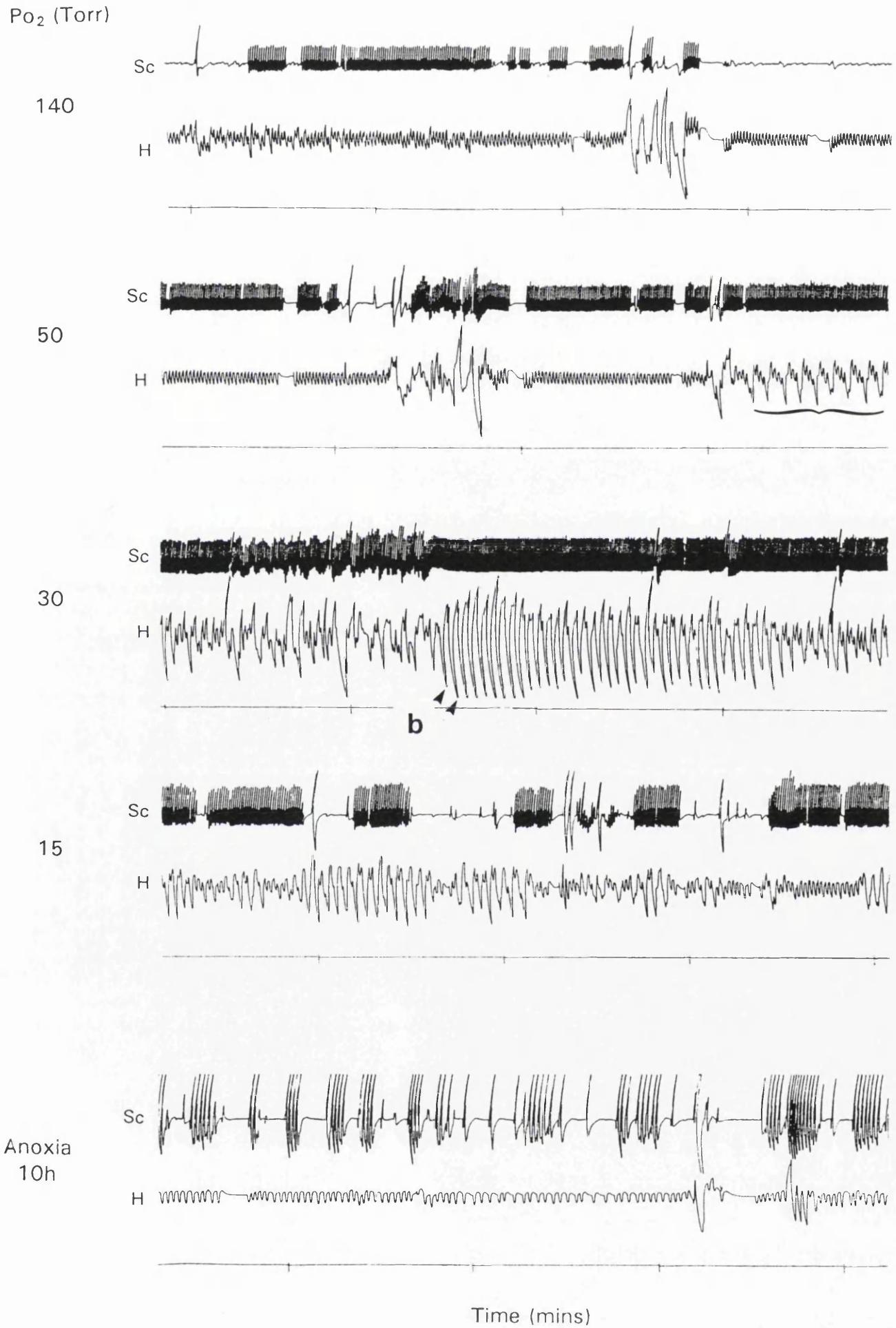
	Pc (Torr)		
Species	Range	Number of observations	Weight range (g)
Jaxea nocturna	10-20	8	0.28-3.13
Callianassa subterranea	10-20	5	1.15-2.06
Upogebia stellata	30-40	10	1.60-2.18
Upogebia deltaura	35-50	10	1.70-9.61

The size range of *Upogebia deltaura* was sufficiently large for the relationship between body size and Pc to be examined. Larger individuals had a higher Pc range and hence lower regulatory capacity than smaller specimens (Fig.4.4). However, because of the number of observations and spread of the data this relationship is very tenuous. For the other species of mud-shrimp no significant relationship between body size and regulatory ability could be determined, probably due to the limited size range of animals used.

Ventilatory and cardiovascular responses during hypoxia

The ventilatory and cardiovascular responses to hypoxia were examined in more detail for one species of mud-shrimp, *Upogebia deltaura*. The mean rate of oxygen consumption (MO_2) and

Fig. 4.6. Recordings of right scaphognathite (Sc) and heart (H) activity for one individual *Upogebia deltaura* (wet weight 9.6g) during declining oxygen conditions. At low oxygen tensions (PO_2<50 Torr) movements of the abdomen, which correlate with pleopod beating, are superimposed on the recordings of heart activity (a,b).


Po_2 (Torr)
140
Sc
H
50
Sc
H
30
Sc
H
b
15
Sc
H
Anoxia
10h
Sc
H
Time (mins)

the response of the heart and scaphognathite during progressive hypoxia was measured for five individuals (fresh body weight; 8.4 ±0.4g) and is shown in Fig.4.5. *Upogebia deltaura* maintained its rate of oxygen uptake constant during progressive hypoxia until a critical oxygen tension of approximately 50-60 Torr. Below this Pc there was a pronounced reduction in MO_2 as the PO_2 declined (Fig.4.5A).

During normoxic conditions ventilation is periodically interrupted by scaphognathite pausing or apnoea (Fig.4.6) which is usually coupled with bradycardia or cardiac arrest. Although coupling between the scaphognathite and heart rate was regularly observed, periods of cardiac arrest lasting longer than 7s were infrequent. The duration of respiratory pausing was variable and lasted from several seconds to 1-2min, no pauses longer than 2.5min were recorded.

Therefore, during long periods of apnoea (>60s) the heart may arrest a number of times (Fig.4.6). No reversals were observed using the impedance technique in any of the individuals examined. A better method of detecting reversals is to measure pressure changes within the branchial chamber (e.g. Hughes *et al.*, 1969) but, because of the small size of the mud shrimps, this was not attempted.

The scaphognathite rate was maintained over the PO_2 range 155 to 80 Torr at a mean frequency of 52.2 ±5.6 beats.min^{-1} (Fig.4.5; B). Below 80 Torr the scaphognathite rate increased steadily until a maximum rate of 103.7 ±16.8 beats.min^{-1} was recorded at 45 Torr. This hyperventilation response declined rapidly following any further reduction in PO_2. During anoxia, the scaphognathite rate was maintained between 20-30 beats.min^{-1} with little variation and this rate was sustained for up to 10h of anoxic exposure (Fig.4.6). No individual showed complete arrest of ventilatory activity during 10h anoxia.

The increase in scaphognathite rate below 80 Torr (Fig.4.5; B, and Fig.4.6) occurred as a result of a combination of factors. In addition to the scaphognathite beat frequency increasing, the number of pauses and pause duration both decreased (Fig.4.5; C, D and Fig.4.6) resulting in a more continuous pattern of scaphognathite beat, which was maintained down to a PO_2 of approximately 40 Torr. The scaphognathite beat frequency declined below 40 Torr and the rate and duration of pausing increased resulting in an overall decrease in scaphognathite activity.

There was no significant change ($P>0.05$) in the heart rate of *U. deltaura* over the range of oxygen tensions to which they were exposed (Fig.4.5 & 4.6). Cardiac activity continued during anoxia, albeit at the slightly lower rate of 10-20 beats.min^{-1} and this beat frequency was maintained for up to 10h anoxic exposure in all of the individuals studied (Fig.4.6). Stroke volume was not measured directly but changes in the amplitude of contractions were observed using impedance recordings (Depledge, 1977). As the PO_2 approached 20-30 Torr the amplitude increased by 20-100%, but below this PO_2 and under long term anoxia the amplitude decreased. However, there is no direct evidence for changes in cardiac output.

At low oxygen tensions the rate of pleopod irrigation in the upogebiids has been shown to

increase (Chapter 2). This irrigation activity was detected via the heart electrode, since large abdominal movements were clearly correlated with pleopod irrigation, and were recorded on the impedance traces (Fig.4.6). At 50 Torr small movements of the pleopods produced regular negative spikes in the heart recordings (Fig.4.6). At lower PO_2 levels, abdominal flexion by *U. deltaura* caused the extended pleopods to move together with the uropods and telson. The resultant pleopod movements, generated via abdominal flexion, are clearly illustrated on the impedance traces at 30 Torr (Fig.4.6). This type of irrigation activity was maintained, albeit at rates of less than 10 beats.min^{-1}. during progressive hypoxia and in anoxia for the majority of individuals (Fig.4.6).

Discussion

Assessment of respirometry systems

The measurement of oxygen consumption in crustaceans has been approached in a number of different ways. A plastic mask placed over the anterior of the animal has been used to measure oxygen consumption in addition to ventilation volume and oxygen utilization (Johansen *et al.*, 1970; Taylor, 1976; McMahon *et al.*, 1979; Bradford & Taylor, 1982; Greenaway *et al.*, 1983). This method suffers, however, because of the possible effects of restraining the animal, the stress caused by wearing the mask and the tendency of many Crustacea to periodically reverse the direction of ventilation (Titulaer, 1991). Also, it can only be used on comparatively large (decapods which have a very rigid carapace (e.g. *Carcinus maenas*; Taylor, 1976).

Oxygen consumption is more typically determined using closed, open or semi-closed respirometry systems. There is, at present, considerable disagreement in the literature over the most suitable method for measuring the oxygen consumption of aquatic organisms. Closed system respirometry (e.g. Torres *et al.*, 1977; Felder, 1979; Bridges & Brand, 1980b; Anderson *et al.*, 1991; Erdman *et al.* 1991, among others) is regarded to be the simplest in terms of construction and operation (Kaufmann *et al.*, 1989). The rate of oxygen consumption is measured by placing an individual into a sealed chamber and monitoring the decrease in PO_2 with time. To prevent PO_2 stratification and gradients within the chamber, the water has to be thoroughly mixed but with minimal disturbance of the occupant. This technique is very useful when determining oxygen consumption in individuals that have low rates of oxygen consumption.

The measurement of oxygen consumption using the closed system has, however, frequently been criticised (Tang, 1933; Kamler, 1969; von Oertzen, 1984; Steffensen, 1989). The main problem with the closed system is that metabolic products, such as carbon dioxide, will accumulate within the respirometer and may, in turn, affect the oxygen consumption of the

animal. Although von Oertzen (1984) found that accumulation of these metabolites resulted in a change in the degree of respiratory independence shown by the prawn, *Palaemon adspersus,* other studies (including this one) have shown little effect of metabolite accumulation on oxygen consumption (e.g. Bridges & Brand, 1980; Morris & Taylor, 1985; Anderson, 1989).

In the open-flow system an individual is placed within a respirometer chamber which has water constantly flowing through it. The rate of oxygen consumption is determined by measuring the difference in PO_2 of the water flowing into and leaving the chamber as well as the rate of water flow. Measuring oxygen consumption using an open-flow system (e.g. Taylor *et al.*, 1977; Taylor & Butler, 1978; Massabuau & Burton, 1984; Swain *et al.*, 1987; Wheatly, 1989; Henry *et al.*, 1990b; Anderson *et al.*, 1991; Rosas *et al.*, 1992) avoids the potential problem of metabolite accumulation as observed in the closed system. It has been shown that when using this system, however, base-line errors accumulate with time, complicated corrections for wash-out have to be made and it is often very difficult to measure low rates of oxygen consumption with any degree of accuracy (Kaufmann *et al.*, 1989; Steffensen, 1989).

Closed systems are also frequently employed to measure oxygen consumption during hypoxia. When the MO_2 of *Upogebia stellata* was measured during progressive hypoxia it was found that the rate of oxygen consumption was elevated when the semi-closed system was used, compared to the closed system. The higher MO_2 was due to disturbance of the shrimp caused by the periodic flushing of the semi-closed system with hypoxic water and probably reflected the 'active' and not 'routine' rates of oxygen consumption (Fry, 1971).

In the semi-closed system, an individual is placed within a respirometer chamber and the chamber is flushed with water at the required PO_2 and then sealed. The decline in PO_2 is measured for a short period of time prior to the chamber being flushed again with fresh sea water.

The semi-closed system has been considered a good compromise between the closed and open-flow systems (Steffensen, 1989; Kaufmann *et al.*, 1989). This is because the measuring period and the flushing period are kept separate, therefore the semi-closed system exhibits the simplicity of the closed system and the stable conditions of the open system. Although problems were encountered using this system during the present study, the semi-closed system has, however, been used successfully for determing the rates of oxygen consumption in fish (Jobling, 1982; Steffensen, 1989) and in decapod crustaceans (Zainal *et al.*, 1992).

The disturbance state, and hence activity level, affects both the degree of respiratory independence (disturbed animals showed poorer regulatory ability) and the rate of oxygen consumption (a higher MO_2 is recorded in disturbed animals) (Spoek, 1974; Taylor, 1976; Herreid, 1980; McMahon & Wilkens, 1983). Nichols (1975) suggested that respiration rates measured in the laboratory can be nearly twice those of rates measured in the field because animals measured in the laboratory were stressed. Even so, experimental stresses may be

reduced during respirometry measurements (indeed every effort should be made to minimize any stress likely to affect the respiratory performance of the subject) by, for example, providing shelter, a substratum (artificial or real) or burrow for the occupant (e.g. Schembri, 1979; Bridges & Brand, 1980; Witbaard & Duiniveld, 1989). In this study, thalassinidean shrimps were placed within artificial 'burrows' since they appeared stressed outside a burrow environment (McGinitie, 1930; 1934; Witbaard & Duiniveld, 1989). This was confirmed in preliminary experiments, since a higher MO_2 was recorded in those shrimps not placed within artificial 'burrows'. Similarly, Bridges & Brand (1980) showed that the presence of a substratum within the respirometer had a significant affect on VO_2 (O_2 consumption as $\mu l.g^{-1}.h^{-1}$) and on the regulatory ability of the masked crab *Corystes cassivelaunus*. A much lower VO_2 and higher regulatory ability was shown for a crab that was buried under sand, than when the same individual was exposed in a clear respirometer.

Acclimatization to the experimental conditions is important as it provides a method of simply reducing stress (McMahon & Wilkens, 1983). A number of studies have shown, however, that oxygen uptake of individuals was significantly reduced and regulatory ability increased following a period of acclimatization (3-48h) to the experimental conditions (e.g. McMahon *et al.* 1974; Spoek, 1974; Taylor, 1976; Taylor & Butler, 1978; Butler *et al.*, 1978; Innes, 1985). Therefore, although rates of oxygen uptake may vary with the type of experimental conditions and physiological state of the animal, albeit between limits, values obtained under stated conditions and comparable activity states may provide a basis for meaningful comparison between species (McMahon & Wilkens, 1983).

Oxygen uptake

Low rates of oxygen uptake, compared to a range of decapod species (McMahon & Wilkens, 1983) have also been recorded for the burrowing thalassinideans *Lepidopthalmus* (as *Callianassa*) *jamaicense* (Felder, 1979), *Neotrypaea californiensis* (Torres *et al.*, 1977), *Axius acanthus* (Mukai *et al.*, 1989) and *Calocaris macandreae* (Anderson *et al.*, 1991).

In this study, the upogebiid species had a higher rate of oxygen consumption, compared with the shrimps *Callianassa subterranea* and *Jaxea nocturna*. This probably reflects the more oxygenated burrow habitat of *Upogebia* species, their active filter feeding lifestyle and, therefore, their greater metabolic requirements. Similarly, Thompson and Pritchard (1969) reported significantly higher rates of oxygen uptake in the intertidal *Upogebia pugettensis* ($2.6\mu molO_2.g^{-1}.h^{-1}$ at 10°C for an average 5.7g wet weight individual) compared to the deposit feeding *Neotrypaea* (as *Callianassa*) *californiensis* ($1.3\mu molO_2\ g^{-1}.h^{-1}$ for a mean 5.3g wet weight). Hill (1981) recorded a rate of $2.2\mu molO_2\ g^{-1}.h^{-1}$ for an average 3g *Upogebia africana* at 10°C (assuming a Q_{10} of 2) and Koike and Mukai (1983) found *Upogebia major* had a rate of

oxygen uptake almost twice that of *Callianassa japonica* ($0.52 ml O_2 g^{-1}.h^{-1}$ and $0.28 ml O_2 .g^{-1}.h^{-1}$ respectively at 20°C).

The mass exponent (b) for expressing standard rate of oxygen consumption with respect to body mass (derived from the relationship between oxygen consumption and body mass, conventionally expressed by the equation $Y=aM^b$; where M is the weight of the organism), measured in the present study for four species of mud-shrimp varied, from 0.521 to 0.751 (Table 1). The value for *b*, however, agreed well with the interspecific values for the mud-shrimps *Upogebia africana* (*b*=0.84, 18°C; Hill, 1981), *Calocaris macandreae* (*b*=0.67, 10°C; Anderson *et al.*, 1991), *Callianassa subterranea* (*b*=0.59-0.67, 8-12°C; Witbaard & Duniveld, 1989), *Callianassa kraussi* (*b*=0.59-0.89, 15-25°C; Hanekom & Baird, 1987) and *Axius acanthus* (b=0.115, 28°C; Mukai *et al.*, 1989). Values of b<1 have been recorded for many crustaceans (e.g. Wolvekamp & Waterman, 1960; Bridges & Brand, 1980) and a collective interspecific mass exponent of 0.877 has been calculated by Wheatly (1989) for 15 species of aquatic decapods.

Wolvekamp & Waterman (1960) stated that '... the reason that a relatively simple exponential relationship between metabolism and size provides an adequate approximation for many animals remains controversial'. Since their early review, there is still no convincing explanation of the mass exponent for oxygen uptake (Wheatly, 1989). Originally, it was thought that the total metabolism of metazoans was proportional to the total cell surface area, resulting in a mass exponent (*b*) of 0.67 (Zeuthen, 1953). Hemmingson (1960) concluded, however, that metabolism varied more with the 0.75 power of the body weight and attributed this difference to additional factors such as the development of complex respiratory surfaces and increased vascularization.

Although the value for the mass exponent is now thought to have little to do with the surface law (Wheatly, 1989), deviations in the mass exponent have been attributed to activity states (Thomas, 1954; Newell, 1979; Mukai *et al.*, 1989), environmental conditions such as temperature (Witbaard & Duniveld, 1989; Taylor & Brand, 1976; Newell, 1979) and salinity (Hanekom & Baird, 1987), the small range of body size used (Bayne *et al.*, 1976; Jones, 1972) and supplementation of diffusion by ventilation (Calow, 1981).

Using chemical engineering theory to account for the metabolic scaling exponents found in aquatic invertebrates, Patterson (1992) suggested that, given the physico-chemical limitations of removing metabolites from moving water, the mass exponents would not be expected to cluster around a specific value (0.67 or 0.75). In this alternative approach he used dimensionless relations (Sherwood-Reynolds number functions) to demonstrate that organism size and water motion have a direct influence on the predicted value of the mass exponent by affecting mass transfer and ultimately metabolic rate. Interestingly, Patterson (1992) found that the range of mass exponents predicted by this approach encompassed the range given previously for a number

of aquatic invertebrates determined using respirometry.

The biological and ecological significance of this mass exponent is, at present, unclear. Bridges & Brand (1980) found that the value for *b* was significantly higher in burrowing species compared to non-burrowing species. Higher activity levels depress the value for *b*, indeed Mukai *et al.* (1989) attributed the unusually low *b* value for the mud shrimp *Axius acanthus* (*b*=0.11) to disturbance during measurements causing increased animal activity in the respirometer. However, a lowered value of *b* would occur if only small animals reacted to stress. If all sizes responded similarly, the value for *b* would be retained and only a difference in the mass coefficient would be recorded.

In this study, the effect of body size on the oxygen uptake of the different deposit and filter feeding mud-shrimps is limited due to the narrow weight range and small sample sizes used, which may affect the accuracy of *b* value determinations. This is a common problem inherent in studies of burrowing Crustacea; the shrimps are very difficult to catch, sample sites are scarce and often the method of capture resulted in some form of damage to the shrimps (though every care was taken to work only with apparently healthy animals in the present study).

Interestingly, Heusner (1991) considered variation in the mass coefficient, rather than the mass exponent, more central to studies in comparative physiology. Clearly from these data, the differences in the mass coefficients (the value of *a* from the equation relating oxygen consumption and body mass) between the filter feeding upogebiids and the deposit feeding shrimps (Table 4.1) appear more significant than differences in the mass exponent. An intraspecific comparison of the mass coefficient shows that both deposit feeding mud-shrimps, *Jaxea nocturna* and *Callianassa subterranea* had much lower values of *a* compared to the filter feeding upogebiids. The significance of the mass coefficient in this study illustrates a difference in their rates of oxygen consumption; the slopes of the relationship between body weight and oxygen consumption are similar (i.e. the rate of increase in oxygen consumption with increasing body size) but the mass coefficient (rate of oxygen consumption) is lower in the deposit feeders.

Personal and published observations (see chapter 2) suggest that those thalassinideans which generally construct deep, complex burrows constantly mine the anoxic sediments for food material. As a consequence, they are often exposed to long periods of hypoxia within their burrows, compared with their filter feeding counterparts. Even though *Upogebia* spp. live in a habitat with reduced oxygen levels, as a filter feeder its metabolic requirements are greater than those for *Callianassa subterranea* and *Jaxea nocturna*, i.e. deposit feeding shrimps. Hence a lower rate of oxygen consumption in the deposit feeders may be considered to be an adaptation to the hypoxic burrow environment.

Compared with other decapod Crustacea, thalassinideans appear to have rates of oxygen uptake lower than those of many non-burrowing species. These low rates have been interpreted both as an adaptation to the hypoxic conditions often prevalant in the burrow habitat (Thompson

& Pritchard, 1969; Felder, 1979; Hill, 1981) and to the reduced metabolic demands associated with low levels of activity (Anderson *et al.*, 1991).

Similarly, in a study of two burrowing and two non-burrowing decapods, Bridges & Brand (1980) found that oxygen consumption was lower in the burrowing crustaceans compared with the non-burrowing species. They attributed this difference to the sedentary life style of the burrowing forms.

Regulation of oxygen uptake in hypoxia

In their early review, Wolvekamp and Waterman (1960) noted that the regulatory ability of crustaceans was limited to only rather few species. More recent reviews have, however, concluded that the majority of malacostracan Crustacea show considerable ability to regulate oxygen uptake down to low environmental oxygen tensions although the Pc may vary considerably between species (McMahon & Wilkens, 1983; McMahon, 1988; Morris, 1991).

There have been a number of attempts to quantify the ability of an individual to regulate oxygen consumption over a range of environmental oxygen tensions (Bayne, 1971; Magnum & Van Winkle, 1973; Herreid, 1980). The simplest, and most widely used method, is to define the range of oxygen tensions below which a constant rate of oxygen uptake can no longer be maintained and that below this PO_2, the 'critical' oxygen tension (or Pc), the rate of oxygen uptake becomes dependent upon ambient PO_2 (Herreid, 1980). The Pc provides a good indication of the regulatory ability of an organism, although it is very difficult to assess accurately as a specific point. A number of investigators have used computer programs in an attempt to assess the Pc more accurately (Yeager & Ultsch, 1989), or have provided different mathematical procedures for quantifying regulatory ability (Bayne, 1971; Mangum & Van Winkle, 1973).

Although the critical PO_2 (Pc) is highly variable between species and is affected both by endogenous and environmental factors (Herreid, 1980; McMahon & Wilkens, 1983; McMahon, 1988), it now appears that there is a strong correlation between the ability of a species to maintain respiratory independence and the extent and duration of hypoxia normally experienced (Morris, 1991).

Under conditions of declining oxygen tension the thalassinideans examined in this study showed a high degree of respiratory independence; the Pc values recorded were 30-40 Torr in *Upogebia stellata,* 35-50 Torr in *U. deltaura.* 10-20 Torr in *Jaxea nocturna* and *Callianassa subterranea.* These Pc values were similar to those reported for other thalassinideans; 10-25 Torr in *Neotrypaea californiensis* (Thompson & Pritchard, 1969; Miller *et al.*, 1976; Torres *et al.*, 1977), 10-25 Torr in *Lepidopthalmus* (as *Callianassa*) *jamaicense* (Felder, 1979), 45-50 Torr in *Upogebia pugettensis* (Thompson & Pritchard, 1969) and 10 Torr in *Calocaris*

macandreae (Anderson *et al.*, 1991).

Similarly, those mud-shrimps that are regularly exposed to severe hypoxia, such as the deposit feeders *Callianassa subterranea*, *Jaxea nocturna* and *Calocaris macandreae* tend to have lower Pc values than those species that adopt a primarily filter feeding lifestyle. The deposit feeders are constantly mining the anoxic sediments for food and are, therefore, regularly exposed to severely hypoxic conditions. Conversely, a high water exchange rate through the simpler burrow structure of the filter feeders results in a more oxygenated burrow environment. In addition, reduced water exchange through the more complex burrow system is likely to enhance the hypoxic conditions found within the burrow (see chapter 2). Therefore, those species regularly exposed to severe hypoxic conditions within the burrow appear to have both lower rates of oxygen consumption and a greater degree of respiratory independence.

Regulatory ability is also well developed in other burrowing decapods; for example Pc values are approximately 20-40 Torr in *Goneplax rhomboides* (Taylor & Atkinson, 1991), 40 Torr in *Nephrops norvegicus* (Hagerman & Uglow, 1985) and 30 Torr in *Parastacoides tasmanicus* (Swain *et al.*, 1987). The relative ability to maintain respiratory independence during exposure to hypoxia is not just restricted to burrowing species, but is a frequent adaptive response shown by many Crustacea from a diverse range of habitats regularly exposed to reduced oxygen levels; for example, palaemonid prawns from rockpools (Morris & Taylor, 1985; Taylor & Spicer, 1989), midwater crustaceans from zones of oxygen depletion (Childress, 1975), the hydrothermal vent crab *Bythograea thermydon* (Mickel & Childress, 1982) and the deep-sea crab *Chaecon ferreri* (Henry *et al.*, 1990b).

Mechanisms of the regulatory response: ventilation and perfusion

The mechanisms by which respiratory independence was maintained during exposure to hypoxia, in particular the compensation responses of the scaphognathite and heart rate, were examined in more detail for one of the mud-shrimps, *Upogebia delatura*. *Upogebia deltaura* was chosen primarily because it is the largest of the mud-shrimps found in U.K. waters, and an easily accessible, high density sample site was discovered off Plymouth.

Under conditions of declining oxygen tensions, *U. deltaura* was able to maintain a constant heart rate down to the Pc, below which there was a distinct bradycardia. Thompson and Pritchard (1969) found that the heart rate of *Neotrypaea* (as *Callianassa*) *californiensis* was maintained during progressive hypoxia and that the hypoxia-induced bradycardia occurred at a PO_2 corresponding to the Pc for oxygen consumption. In other decapods that exhibit good respiratory independence, heart rates showed little response to hypoxia and a correlation between the onset of bradycardia and the Pc for MO_2 was observed for a number of species (McMahon & Wilkens, 1975; Taylor, 1976; Coyer, 1977; Butler *et al.*,1978; Morris & Taylor, 1985; Henry *et*

al., 1990b; Anderson *et al.*, 1991).

In contrast, those species which show poor regulatory ability characteristically exhibit a progressive bradycardia, e.g *Chaecon quinqedens* (Henry *et al.*, 1990), *Austropotamobius pallipes* (Wheatly & Taylor, 1981) and *Homarus gammarus* (as *H. vulgaris*) (McMahon *et al.*, 1978).

Cardiac output has been measured indirectly using the dimensions of the heart and the heart rate (deFur & Magnum, 1979), flow velocity and arterial dimensions (Belman, 1975) and the Fick principle (e.g. Taylor, 1976). Although cardiac output has been measured directly in some Crustacea (Belman & Childress, 1976; Burnett *et al.*, 1981), during the present study it was found to be virtually impossible to determine cardiac output directly or indirectly in such small shrimps. The use of impedance recordings, however, may provide an indirect means of determining stroke volume. Depledge (1977) showed that the amplitude of the heart beat recordings, obtained using the impedance technique (Trueman, 1967), of the crab *Carcinus maenas* were a reasonable indication of the stroke volume. Similarly, in this study, the amplitude of the impedance recordings for the heart of *Upogebia deltaura* were used as an indication of changes in stroke volume. During progressive hypoxia there appeared to be little change in stroke volume, as determined using impedance recordings, for *Upogebia deltaura.*

An increase in heart rate may result in enhanced gill perfusion, if the stroke volume of the heart is maintained or increased. During hypoxia, oxygen uptake at the gills may be facilitated by the maintenance of a high diffusion gradient for oxygen at the gill surface, possibly as a result of increased gill perfusion. An increase in heart rate (tachycardia) in response to progressive hypoxia is rarely observed and has only been recorded for the crabs *Libinia emarginata* (DeFur & Magnum, 1979) and *Ebalia tuberosa* (Schembri, 1979). However, for some species of decapods it has been shown that in the absence of an increase in heart rate, or during bradycardia, gill perfusion may be enhanced by an increase in stroke volume, e.g. *Cancer magister* (Johansen *et al.*, 1970; Jorgensen, *et al.*, 1982) and *Homarus americanus* (McMahon & Wilkens, 1975). Similarly, McMahon *et al.* (1979) found that cardiac output doubled in *Cancer magister* following exercise, even though the heart rate had increased by only 18%; the majority of the change resulting from an increase in stroke volume.

For other decapods, however, cardiac output changes little or may even decrease during progressive hypoxia, making increases in perfusion unlikely, e.g. *Carcinus maenas* (Taylor, 1976) and *Cancer pagurus* (Bradford & Taylor, 1982).

To compensate for the reduction in oxygen available at the respiratory surface during declining external PO_2, *Upogebia deltaura* also increased the rate at which water was passed over the gills. Increased scaphognathite activity, hyperventilation, is a compensation response utilised by many crustaceans (McMahon & Wilkens, 1975; Taylor, 1976; Butler *et al.*, 1978; McMahon *et al.*, 1978; Batterton & Cameron, 1978; Burnett & Bridges, 1981; Wheatly &

Taylor, 1981; Bradford & Taylor, 1982; Morris & Taylor, 1985; Taylor & Spicer, 1989; Henry *et al.*, 1990b; Anderson *et al.*, 1991). Similarly, burrowing fish also show an increased opercular ventilation rate when exposed to hypoxia (Taylor & Atkinson, 1991).

Compensatory hyperventilation will itself increase the oxygen demand of the animal and there will be a point at which it is energetically too costly to maintain a high scaphognathite rate. At this point, which corresponds closely to the Pc, the oxygen supplied is sufficient only to meet the energy demands of ventilation. Any further decrease in oxygen tension will, therefore, result in a decrease in scaphognathite rate and respiratory independence can no longer be maintained (McMahon & Wilkens, 1975; Taylor, 1976; Bradford & Taylor, 1982; Morris & Taylor, 1985; Anderson *et al.*, 1991).

During exposure to long-term hypoxic stress, the crayfish *Orconectes virilis* exhibited an initial hyperventilation response followed by a slow recovery over 7-10 days to near pre-hypoxic ventilation rates (McMahon *et al.*, 1974). During this period, it was suggested that adaptive mechanisms which serve to increase the effectiveness of oxygen uptake during hypoxic exposure (such as acclimatory changes to the circulatory system, changes in haemocyanin concentrations or increased efficiency of branchial pumping) may develop. As a result of the high energetic cost, increased ventilation may only be of use, therefore, as a short-term response to hypoxic conditions enabling *U. deltaura* to survive short periods of hypoxia or allowing time for other adaptive mechanisms to develop (McMahon & Wilkens, 1975).

Although ventilation volume was not measured directly for *Upogebia deltaura*, the scaphognathite rate and ventilation volume have been shown to be correlated for a number of decapod crustaceans (e.g. McMahon *et al*, 1974; Batterton & Cameron, 1978; Bradford & Taylor, 1981). The increase in the volume of water passing over the gills (ventilation volume) during hypoxic exposure in *Upogebia deltaura* was not simply as a result of increasing the scaphognathite rate, but was a combination of factors. In addition to the scaphognathite beat frequency increasing, there was a reduction in the pause duration and the number of pauses. Cardiac and ventilatory pausing have been described for many decapod species (see review by McMahon & Wilkens, 1983), although the duration of the pauses may vary widely between species; in brachyurans pauses occur infrequently but may last for long periods (McMahon & Wilkens, 1972; Batterton & Cameron, 1978; Bridges 1979; Bradford & Taylor, 1982; Taylor, 1984). The frequency of cardiac pausing in *U. deltaura* decreased during progressive hypoxia, but then the pause frequency increased at low oxygen tensions.

Ventilatory pausing may enable the animal to make a considerable energy saving during periods of inactivity. A reduction in both the frequecy and duration of ventilatory and cardiac pausing during progressive hypoxia has been recorded for a large number of decapods, e.g. *Homarus gammarus* (as *H. vulgaris*) (Butler *et al.*, 1978), *Palaemon elegans* (Morris & Taylor, 1985), *Ebalia tuberosa* (Schembri, 1979), *Atelecyclus rotundatus* (Taylor, 1984) and in the

thalassinidean *Calocaris macandreae* (Anderson, 1989).

Although pauses are less frequent, they may still occur in some species during hypoxia and it has been suggested that even though haemolymph oxygen stores will be reduced, ventilatory pausing during hypoxia could still lead to substantial energy savings (Burnett & Bridges, 1981).

Behavioural responses and long-term tolerance to hypoxia

Those species that are regularly exposed to more severe environmental conditions, e.g. deposit feeders, have a better developed regulatory ability. This is also true for the tolerance of mud-shrimps to severe hypoxia or anoxia. For example, the filter feeding upogebiids are able to survive total anoxia for many hours; e.g. *U. major,* 9h (Mukai & Koike, 1984); *U. africana,* 18h (Hill, 1981); *U. pugettensis,* 79h (Thompson & Pritchard, 1979). Even longer survival times have been recorded for the deeper burrowing, primarily deposit feeders; *Callianassa japonica,* 40h (Mukai & Koike, 1984); *Neotrypaea* (as *Callianassa*) *californiensis,* 136h (Thompson & Pritchard, 1979) and 60h (Zebe, 1982); *Lepidopthalmus* (as *Callianassa*) *jamaicense,* 80h (Felder, 1969); *Calocaris macandreae,* 43h (Anderson, 1989).

In addition to the respiratory responses and physiological adaptations to hypoxia previously described for the thalassinideans, a number of behavioural adaptations have been recorded. For example, during periods of low tide, when burrow irrigation is not possible, intertidal mud-shrimps may experience severe hypoxia or even anoxia. Hill (1981) found that during simulated low tide some *Upogebia* spp. from South Africa moved up to the air-water interface of their burrows so that the cephalothorax was above the water level. Water pumped through the gill chamber, whilst the animal was in this position, was passed back over the ventral surface of the shrimp where it was partly reoxygenated. Similarly, *Neotrypaea* (as *Callianassa*) *californiensis* was reported to move near the burrow openings during low tide (Farley & Case, 1969) and Felder (1979) has found *Lepidopthalmus* (as *Callianassa*) *jamaicense* in burrows up to 1m above the water table in mud. It is clear that there are differences in metabolic activity and in the degree of regulation even between thalassinidean species.

In conclusion, all four species of mud-shrimp examined in this study showed a high degree of respiratory independence (low Pc) and were able to maintain their rates of oxygen consumption over a wide range of oxygen tensions. The low MO_2 and Pc exhibited by the mud-shrimps *U. stellata, U. deltaura, C. subterranea* and *J. nocturna* are obvious adaptations to a burrow habitat in which burrow water oxygen tensions often fall to low levels.

The MO_2 was significantly higher in the filter feeding upogebiids, *U. stellata* and *U. deltaura* than the deposit feeders, *C. subterranea* and *J. nocturna.* The oxygen tensions at which respiratory independence was lost were between 10-20 Torr for *J. nocturna* and *C. subterranea*, whereas the Pc range for the upogebiids was 30-50 Torr. The greater degree of

regulation, in response to hypoxic stress, shown by *J. nocturna* and *C. subterranea* is thought to be an adaptation to the more restricted oxygen conditions found within the burrows of these deposit feeders, compared to the more oxygenated burrows of the upogebiids.

The response of the heart and scaphognathite to hypoxia was examined in more detail for *Upogebia deltaura*. Under normoxic conditions, cardiac and scaphognathite activity was closely correlated. During progressive hypoxia respiratory independence was maintained down to low oxygen tensions, primarily through increased scaphognathite activity (hyperventilation) and maintained perfusion.

Chapter 5

Branchial parasitism in upogebiid mud-shrimps

Introduction

Bopyrids comprise a large group of parasitic epicaridean isopods (Bopyridae) belonging to the super-family Bopyrina, and are typically found within the brancial cavity, or occasionally clinging to the abdomen, of many decapod species. In his monograph, Bonnier (1900) divided the Bopyrina into four families. For the three families found infesting the decapods, the classification depended on the position of infestation; the Entoniscidae (found in the visceral cavity), the Bopyridae (the branchial cavity) and the Phyrxidae (attached to the abdomen).

Currently, the group of branchial parasites or Bopyridae are composed of more than 50 genera which have been divided into six sub-families; Pseudioninae, Orbioninae, Ceponinae, Bopyrinae, Atheleginae and Hemiartrinae, according to the external morphology of the male and female parasites (Shiino, 1965; Bourdon, 1968).

The bopyrids are highly modified parasites with the adults showing extreme specialization and loss of body form, a result of the evolutionary degeneration of organs which in the free-living isopod are much better developed (Shiino, 1965). The smaller males retain the free-swimming cryptoniscus form and are hyper-parasites of the large females. The male is typically found in the brood chamber or attached to the ventral surface of the female's pleon with the sole purpose of spermatic impregnation (Vallejo & Tristán, 1989).

Whilst examining the branchial morphology of the upogebiids, *Upogebia stellata* (Montagu), *U. deltaura* Leach and *U. pusilla* (Petagna) as part of a wider study of the physiological ecology of some thalassinidean mud-shrimps, some shrimps were observed to have a branchial gall (distended branchiostegite) containing a bopyrid parasite. In this chapter the bopyrids found infesting the branchial cavity of the shrimps are described in more detail.

Although bopyrid infestation of the branchial cavity is regularly recorded amongst decapods, the effect the parasite has on the gills and the respiratory physiology of the host has not been explored. As burrowing thalassinideans are regularly exposed to low oxygen tensions, a preliminary study was carried out on the effect of branchial parasitism on the respiratory response to progressive hypoxia in the mud-shrimp *Upogebia stellata*.

Materials and methods

Upogebia stellata were collected subtidally, with an anchor dredge, from White Bay, Isle of

Cumbrae,Scotland (55°9'N, 4°11'W) at depths of 25-30m. *U. deltaura* were taken from the Irish Sea (54°7'N, 3°27'W) with a box core and by anchor dredge from White Bay, Isle of Cumbrae. *Upogebia pusilla* were collected intertidally from the Arcachon Basin, France.

Prior to examination, the mud-shrimps were preserved in 10% buffered formalin, the parasites removed, rinsed with distilled water and transferred to 70% ethanol.

Material examined

Infested *Upogebia stellata* (Montagu) from the Isle of Cumbrae (3 male, 2 female), *Upogebia deltaura* Leach from the Irish Sea (1 female) and Isle of Cumbrae (1 male), *Upogebia pusilla* (Petagna) from Arcachon, France (1 male, 1 female).

Oxygen consumption

During this study the rates of oxygen consumption (MO_2) of three parasitised *Upogebia stellata* were measured during normoxia and progressive hypoxia. For one individual, the parasite was dislodged from the branchial cavity by gently folding back the branchiostegite. Once the parasite had been carefully removed, without damaging the gills, the branchiostegite was gently replaced and the shrimp was allowed to recover for 48h. After this time the MO_2 was re-determined. Measurements of oxygen consumption at 10°C were made using 'closed' respirometry as described in Chapter 4.

Statistical analysis

Data were plotted and appeared normally distributed. Statistical analyses were carried out using analysis of variance, two-way analysis of variance, and Students *t*-test where appropiate (Sokal & Rohlf, 1981). Where the mean of a number of observations is given the deviation from the mean is expressed as standard deviation (SD). However, where the mean of a number of mean values are determined, the deviation is expressed as standard error (SE).

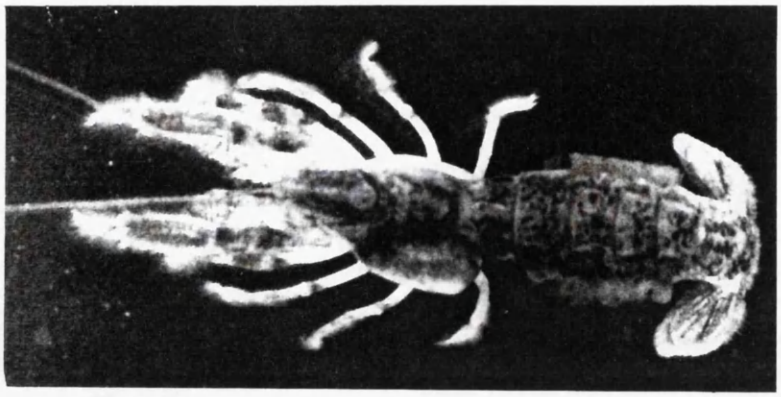
Results

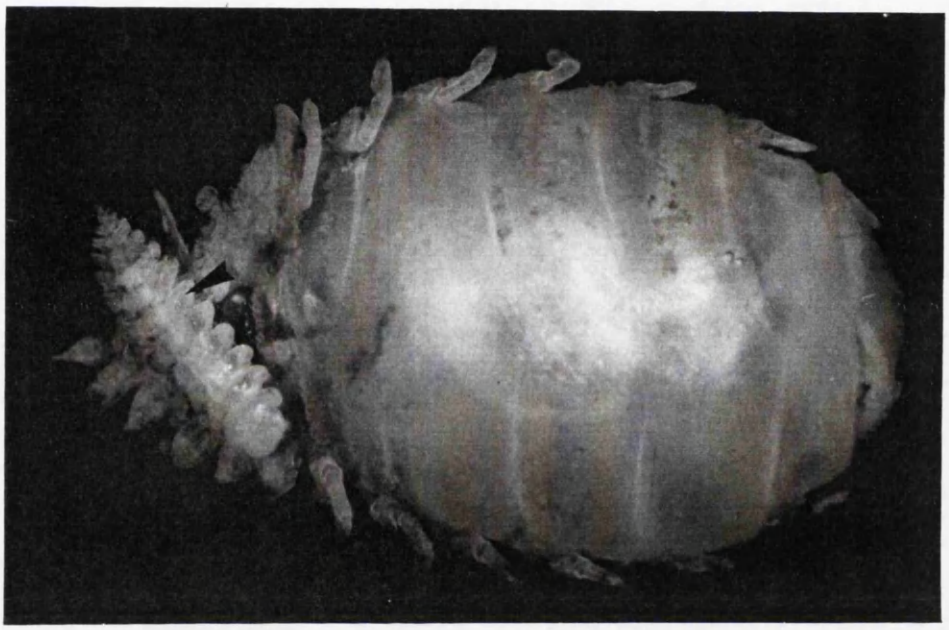
Bopyrid parasites were found within the branchial chamber, the space between the body wall and the branchiostegite, of the shrimp. Individuals parasitized had an enlarged branchial chamber, or branchial gall, a result of branchiostegite deformation (Fig.5.1). Apart from the branchial gall, the shrimp showed no other obvious effects of parasitism. On removing the parasite from the

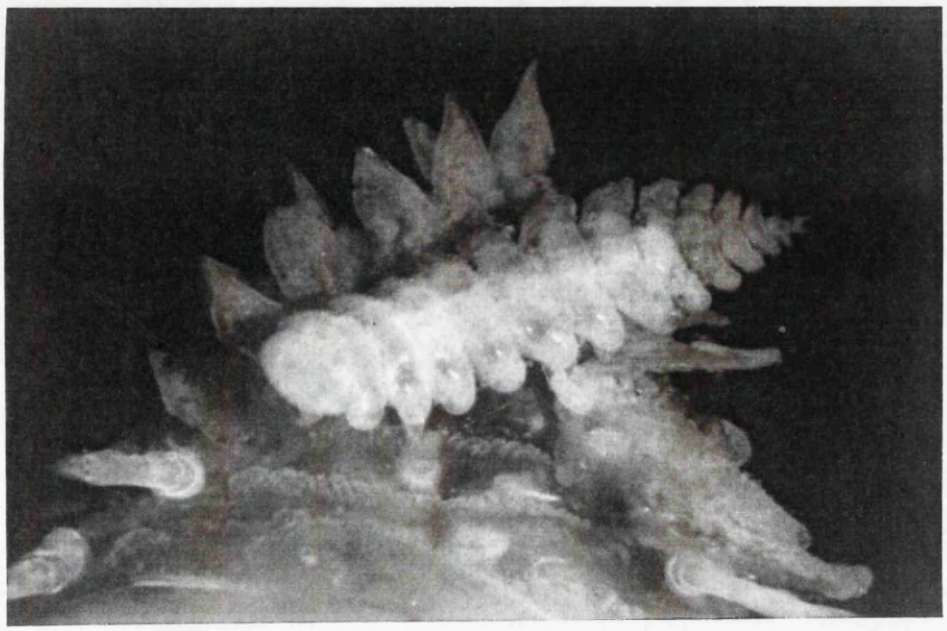
Fig 5.1. (a) *Upogebia stellata*. Mud-shrimp showing the 'face ache' condition. The left branchial chamber (arrowed) is grossley distorted, through deformation of the branchiostegite, in order to accomodate the female bopyrid isopod. This particular individual was parasitzed by *Progebiophilus euxinicus*. Scale bar = 2cm.

(b) P*rogebiophilus euxinicus*. Female. Ventral view, the male hyperparasite (arrowed) is attached to the ventral metasome. Scale bar = 2mm.

(c) *Progebiophilus euxinicus*. Male. Dorasl view of the male attached to the pleopods of the female. Note the tuberculate biramous pleopods of the female which project beyond the epimeral plates of the metasome. The male is not permanently attached, but is able to move freely over the pleopods of the female. Scale bar = 0.5mm.







branchial chamber, the gills appeared splayed and flattened but showed no signs of damage.

The two parasite species that were found infesting *Upogebia stellata*, *U. deltaura* and *U. pusilla* were identified as *Progebiophilus euxinicus* (Popov, 1929) and *Gyge branchialis* (Cornalia & Panceri, 1858). Although Bourdon (1968) has previously given a description of both parasite species in his excellent monograph, the following descriptions of *P. euxinicus* and *G. branchialis* are based on personal observations.

Bopyridae Rafinesque 1815
Pseudioninae (Codreanu 1967)
***Progebiophilus* (Codreanu & Codreanu 1963)**
***Progebiophilus euxinicus* (Popov 1929)**

The dimensions of the female and the male hyperparasite are given in Table 5.1. In each case, the male was found on the ventral pleon of the female.

Female. Body not asymmetrical, short metasome. Outline ovoid, pale pink or white in appearence (Fig.5.1 and 5.2; A, B;). Head, 1.5 times broader than long with two lateral processes extending beyond the margin of the first pereonite. Two small distinct eyespots below the anterior margin of the cephalon, below the line of the antennae. Antennae five-jointed, antennules three jointed. Each antenna distally setose with some setae on the other segments.

Pereon and pleon with distinct somites; seven pairs of pereiopods and five pairs of oostegites. Pereonites well defined with narrow coxal plate and lobulate margins. Dorsolateral bosses conspicuous on pereonites 1-5. Oostegites overlap on ventral side, completely enclosing the brood chamber. First oostegite bilobed with a posterior (superior) and anterior (inferior) lobe (Fig.5.2; C). Superior lobe with posteriodistal expansion supported with basal rib. Internal ridge of superior lobe has tuberculiform processes, with larger tubercules being branched (Fig.5.2; D). First and second oostegites with smooth lateral borders, oostegites 3-5 with setose dorso-posterior median ridge. Fifth oostegite posterior margin is more heavily setose.

Pereiopods are all similar with no scales or setae (Fig.5.2; E). Dactylus smooth and fused with propodus. The pleon made up of six pleonites with the last one reduced. Five pairs of biramous pleopods, which project beyond the epimeral plates of the metasome when seen trom the dorsal side. The first pair appear tuberculate, pleopods 2-5 are smooth and appear concave with raised margins (Fig.5.2; F). Uropods single, not paired and similar in character to the pleopods although somewhat elongated.

Male. All body segments were clearly separated (Fig.5.1 and 5.2). The metasome was three times longer than the metasome. The head was broad, oval with small, distinct posterio-lateral

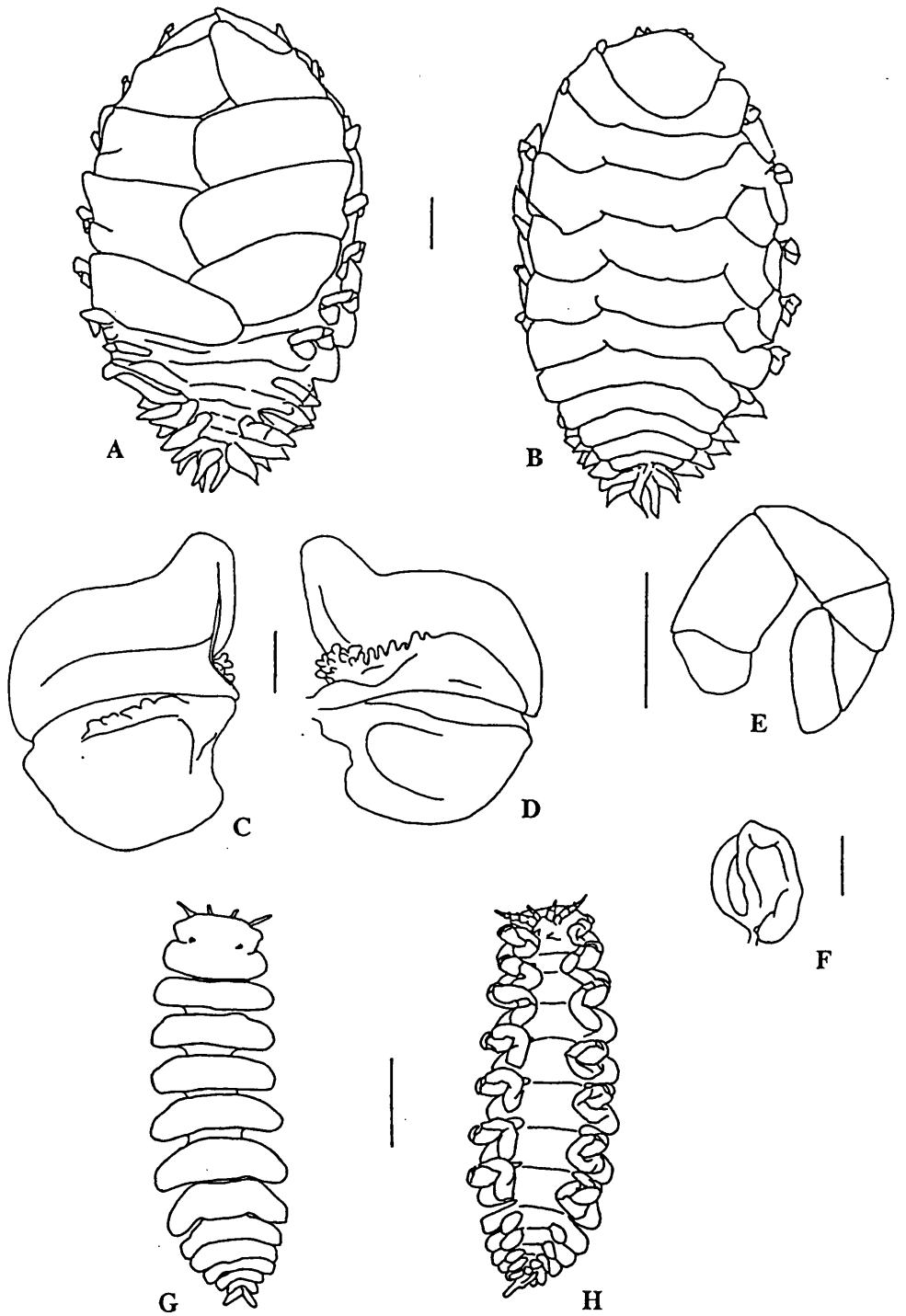


Fig. 5.2. *Progebiphilus euxinicus*. Female. A, ventral view; B, dorsal view; C, dorsal view oostegite 1; D, ventral view oostegite 1; E, left pereiopod; F, biramous pleopod from ploenite 1; G, male dorsal view; H, male ventral view. Scale bar 1mm; A, B, G, H; 0.5mm, D, E, F.

processes. Two small distinct eyes on the dorsal surface.

Antennules are short, three-jointed. Antennae twice as long and five-jointed with the last two joints extending beyond the cephalon. Seven distinctly separated pereonites, laterally rounded. Mid-ventral tuhercules absent.

Pleon had six pleonites and five pairs of small, rounded pleopods. Uropods smooth and elongate extending beyond the fifth pleon.

Bopyridae Rafinesque 1815

Pseudioninae (Codreanu 1967)

***Gyge* Cornalia & Panceri 1858**

***Gyge branchialis* (Cornalia and Panceri 1858)**

Dimensions of both the femal and male hyperparasite found on *Upogebia stellata* and *U. deltaura* are given in Table 5.1. The male was found either within the brood chamber formed by the overlapped oostegites (*U. stellata*) or on the ventral pleon of the female (*U. stellata, U.deltaura*).

Female. Body broadly oval, at least 3/4 as wide as long (Fig.5.3; A, B). Head is relatively large with distinct frontal lamina. Eyes absent. Antennae and antennules three-jointed. Pereon and pleon with distinct somites; seven pairs of pereiopods and five pairs of oostegites. Dorso-lateral bosses of pereiomeres are distinct and elongated. First oostegite sub-rectangular, internal ridge of superior lobe simple with few tuberculate proccesses (Fig.5.3; C). Inferior lobe has elongated distal lobe (Fig.5.3; C, D); other oostegites overlap enclosing brood pouch.

Pereiopods are relatively small. The pleon is short and broad; five pairs of small ovoid uniramous pleopods which do not project beyond the epimeral plates (Fig.5.3; E). Pleopods are smooth with raised margins (Fig.5.3; E). Pair of slender uropods, very much reduced and just visible.

Male. All body segments clearly separated. Cephalon rounded anteriorly and has two distinct, small eyespots (Fig.5.3; F, G). Antennules and antennae are three- and five-jointed respectively. Seven distinct pereonites, rectangular and laterally rounded.

Mid-ventral tubercules absent. Pleon with six segments, lacking pleopods. Uropods absent, although the posterior border of the last pleonite has two small projections (Fig.5.3; F, G).

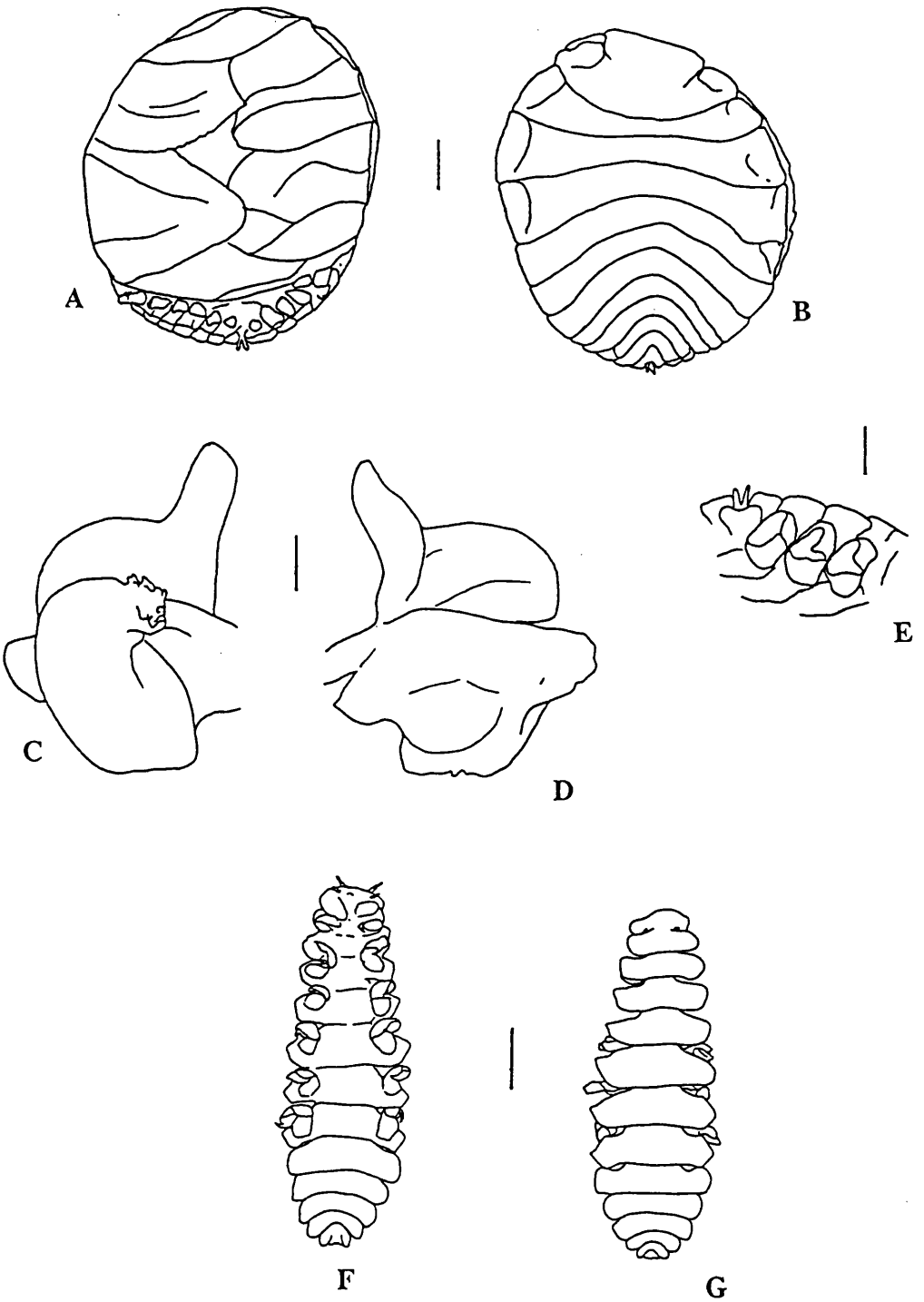


Fig. 5.3. *Gyge branchialis.* Female. A, ventral view; B, dorsal view; C, ventral side oostegite 1; D, dorsal side oostegite 1; E, view of uniramous pleopods 1-3 and uropods; F, male ventral view; G, male dorsal view. Scale bar 1mm, A, B; 0.5mm, C, D, E, F, G.

Table .5.1 Summary of parasite species taken from the branchial chamber of upogebiid mud-shrimps. The host species, date of capture, parasite species found [*Gyge branchialis* (G.B.) or *Progebiophilus euxinicus* (P.E.)] and which branchial chamber was parasitised is shown. In addition, the maximal length and width of both the male and female parasites are given as well as the angle of asymmetry (the degree of distortion of the head from the centre line) for the female. Each of the male hyperparasites was found with the female.

			Parasite dimensions (mm)				
			Female			male	
Host species	Branchial chamber	Parasite species	Length	Width	Angle of asymmetry	Length	Width
U. stellata (9.3.90)	left	G.B.	11.2	9.1	26° (right)	3.6	1.3
U. stellata (21.6.90)	right	G.B.	9.7	7.6	18° (left)	3.3	1.3
U. stellata (13.8.90)	left	P.E.	9.8	5.6	6° (right)	4.1	1.5
U. stellata (14.3.91)	left	P.E.	9.4	6.7	12° (right)	4.3	1.5
U. stellata (5.4.91)	left	P.E.	6.6	4.2	9° (right)	2.6	1.0
U. deltaura (20.5.91)	left	G.B.	13.6	11.6	15° (right)	6.5	2.0
U. deltaura (26.4.93)	right	G.B.	11.8	9.4	14° (left)	3.9	1.4
U. pusilla (29.7.91)	left	P.E.	9.6	6.6	33° (right)	3.1	1.3
U. pusilla (29.7.91)	left	P.E.	8.3	5.9	25° (right)	3.1	1.2

Oxygen consumption

The rate of oxygen consumption (MO_2) of three individuals of *Upogebia stellata*, parasitised with *Progebiophilus euxinicus*, are shown in Table 5.2.

Table 5.2. *Upogebia stellata*. The rate of oxygen consumption (MO_2) for three mud shrimp parasitised with the bopyrid isopod, *Progebiophilus euxinicus*. The MO_2 was calculated over a PO_2 range of 150-90 Torr and is shown as mean ±lSD. The number of measurements taken over the PO_2 range is shown in parenthesis. The critical point (Pc) is also given.

Specimen	MO_2 ($\mu molO_2.g^{-1}.h^{-1}$)	Pc (Torr)	Wet weight (g)
1	2.48 ±0.21 (7)	32-38	1.48
2	1.27 ±0.02 (9)	30-37	1.81
3	1.48 ±0.08 (8)	29-35	1.90

The critical oxygen tension (Pc), the point at which respiratory independence could no longer be maintained, was found to be between 30-40 Torr for the parasitised species. For one individual *U. stellata* the bopyrid parasite was carefully removed and, after allowing 48h for the upogebiid to recover, the MO_2 was re-measured. The mean MO_2 of this individual with the parasite removed was 1.57 +0.17μmol.0_2 g^{-1} h^{-1} (n=7 observations) and the shrimp had a Pc of 30-35 Torr.

The MO_2 of the shrimp after the parasite had been removed from the branchial cavity was significantly higher ($P<0.05$) than the MO_2 prior to removal. The Pc also increased from 30-35 Torr when the parasite was present to 50-55 Torr following removal of the parasite.

Discussion

The specimens of *Gyge branchialis* infesting *Upogebia stellata* and *U. deltaura* conformed well with the characters recorded previously for this species (Bourdon, 1968). The other bopyrid. *Progebiophilus euxinicus*, infesting the mud-shrimps *Upogebia stellata* and *U. pusilla* was first recorded as *Psedione euxinica* Popov, 1927. The genus *Progebiophilus* R. & M. Codreanu now contains nine described species all of which parasitise upogebiids (Bourdon, 1981; Vallejo & Tristán, 1989).

Gyge branchialis has previously been recorded on *U. deltaura* and *U. stellata* from the English Channel, the eastern North Atlantic and the Mediterranean coast of France (Normon, 1907; Plymouth Marine Fauna, 1933; Pike, 1953; Bourdon, 1963; 1967; 1968). Further south, *G. branchialis* has been recorded only on *U. pusilla* in the northern Mediterranean Sea, Adriatic and Black Sea (Popov, 1929; Bourdon, 1968).

With the present records from *U. stellata* from the Firth of Clyde, the geographical range of *Progebiophilus euxinicus* is extended further north. Previously there was only one record of *P. euxinicus* infesting *U. stellata* and that was from the west coast of Ireland (McGrath & Atkins, 1979). However, *P. euxinicus* has been recorded on *U. deltaura* from the English Channel and the Mediterranean coast of France (Bourdon, 1968) and on *U. pusilla* from the English Channel, northern Mediterranean, Adriatic and the Black Sea (Popov, 1929; Caroli, 1945; Catalano & Restivo, 1965; Bourdon, 1967; 1968).

Records of parasitic bopyrids infesting other thalassinidean species from the U.K. waters are scarce and limited to; *Ione thoracica* (Montagu) on *Callianassa subterranea* from western Ireland (McGrath & Atkins, 1979), the south coast of England and the Channel Islands (Pike, 1953; Plymouth Marine Fauna, 1957); *Pseudione borealis* Caspers, 1939 on *C. subterranea* from the North Sea and the Atlantic coasts of Britain, France and Portugal (Bourdon, 1981a; b); and *Pseudione callianassae* Kossman on *C. subterranea* from the south coast of England and

the Channel Islands (Pike, 1953; Plymouth Marine Fauna, 1957).

It was not possible to determine infestation rates as often only limited numbers of shrimp were collected and a programme of systematic collection was not carried out at any of the sample sites. Bourdon (1968) has, however, recorded infestation rates of 1.0% to 9.1% for *Progebiophilus euxinicus* parasitizing *Upogebia* from the Atlantic coast of France and 2.6% from Brittany. An infestation rate of 2.5% to 16.3% was recorded for *Gyge branchialis* parasitizing *Upogebia* from the north coast of Brittany (Bourdon, 1968; Bourdon pers. comm.), 15.5% to 31.4% from Naples (Tucker, 1930) and 2.5% to 3.5% from the northern Mediterranean Sea (Bourdon, 1963).

Where the two parasites *G. branchialis* and *P. euxinicus* do occur in the same geographical area *G. branchialis* is a far more common parasite of *Upogebia* spp. (Caroli, 1945). Generally, low infestation rates (<5%) of bopyrids parasitising decapod crustaceans are quite common. In a study of decapods from the Atlantic French coast, Bourdon (1967) found that 24 epicarideans from a total of 31 different species had an infestation rate of less than 5%.

The bopyrid lifecycle is interesting if not extrordinary. After hatching from the egg the first larval stage, or epicaridium, enters the planktonic stage where it then attaches to the primary host, usually a pelagic copepod, using its well developed pereiopods. Whilst attached to the primary host the epicaridium undergoes a number of ecdyses to the microniscus stage before swimming free as the cryptoniscus. The first cryptoniscus to settle on the final host becomes the female whilst any further larvae settling attach to the female and, retaining the cryptoniscus form, become dwarf male hyper-parasites (Naylor, 1972)

This description accounts for the life-cycle of the majority of bopyrids; however, *Progebiophilus euxinicus* has an unusual complication. Caroli (1946) and Catalano & Restivo (1965) both found that the cryptoniscus of *P. euxinicus* did not attach itself directly onto the host, *Upogebia pusilla*, but settled instead on *Gyge branchialis*, another bopyrid parasite already infesting *U. pusilla*, dislodging the resident male. The cryptoniscus transformed into a female and then dislodged the female *Gyge* from *U. pusilla*. The next *P. euxinicus* cryptoniscium to settle joined the female and became the definitive male of the pair.

The female bopyrid caused no obvious injury to the upogebiid host. The gills of each host were, however, splayed to accommodate the female parasite, but they were not damaged. The bopyrid does not appear to feed on the gills but presumably ingests particulate material which had been drawn into the branchial chamber with the ventilatory current, or mucus secretion from the gills. The only sign of obvious deformity was the distended branchiostegite. Although there are no reports of branchial parasites causing damage to the gills, Pike (1953), commenting on how the branchial bopyrid *Pleurocryta galatheae* retained its position in the branchial cavity whilst the host *Galathea squamifera* was moulting, suggested that prior to the moult the parasite pierced the cuticle at the base of the gills and ate its way through the integument. It then took up

its new position in preparation for the coming moult. The abdominal parasite *Hemiarthus abdominalis*, however, was shown to cause cessation of host reproductive activity and gonad development (Allen, 1966).

Although parasitism by *Gyge branchialis* or *Progebiophilus euxinicus* had no obvious effect on the gross morphology of the host other than the branchial gall, the position of the parasite within the gill cavity could affect the respiratory physiology of the shrimp. For example, water flow through the branchial chamber may be impaired, or the gills may be in competition with the parasite for any available oxygen. The presence of the parasite may also place additional stresses on the host which in turn may affect oxygen uptake and regulatory ability. To examine this aspect in more detail the weight-specific rate of oxygen consumption (MO_2) of three shrimp parasitized with *P. euxinicus*, was measured over a range of oxygen tensions. The MO_2 for two of the three parasitized shrimp was higher, although within the 95% confidence limits of an individual estimate determined previously from the regression of MO_2 against weight for unparasitized *U. stellata* (Chapter 4; Table 4.1). The MO_2 of the third shrimp was much greater and outwith the 95% confidence limits for unparasitized *U. stellata* as determined previously.

Since it was possible to make recordings for so few individuals, the results are inconclusive but may suggest that the host has slightly elevated respiration rates. Oxygen uptake by the gills of the host may be impaired, either by impeding water flow through the branchial chamber, or the gills may be in competition with the parasite for the available oxygen. The host could also incur additional stress through the presence of the parasite, thereby increasing oxygen uptake and placing a greater demand on the mechanisms of oxygen uptake of the host.

Both parasitized and unparasitized *U. stellata* exhibited a high degree of respiratory independence (Pc 30-40 Torr). For one shrimp the parasite was removed and the MO_2 remeasured. Following removal of the parasite the MO_2 of the shrimp was significantly higher and the Pc increased to 50-55 Torr. The elevated MO_2 and the concommitant poorer regulatory ability may have resulted from the additional stress caused by removing the parasite. The distended branchiostegite of the shrimp did not collapse following the removal of the parasite and probably created unusual water flow patterns through the enlarged branchial cavity. The gills were also splayed. Hosts previously infested and found lacking the parasite are quite uncommon, the only record being five *Munida rugosa* (as *M. bamffica*) from the Plymouth collection that had outlived their branchial parasites. In each of these specimens the vacated gill chambers had partially collapsed (Pike, 1953).

The findings from this preliminary study suggest that although possibly stressed, the host, *U. stellata*, was still able to regulate its oxygen consumption down to relatively low Po_2 values. Clearly there is a need for further information not only on the physiology of the bopyrid but also on the effects of branchial parasitism which may have repercussions on the respiratory

physiology of the host. This is especially so in species such as thalassinidean shrimp that construct burrows and are regularly exposed to prolonged periods of hypoxia (Atkinson & Taylor, 1988).

Chapter 6

Thalassinidean haemolymph oxygen transporting properties and comparative haemocyanin structure and function

Introduction

In their review, McMahon & Wilkens (1983) divided the function of oxygen uptake into a number of smaller processes some of which have been examined in previous chapters; the transport of oxygen from the environment to the area of respiratory exchange (see chapter 2); the uptake of oxygen from the environment across the respiratory exchange area (chapter 3) and the removal of oxygen from the exchange area (chapter 4). This chapter examines some aspects of oxygen transport, from the site of gas exchange to the tissues, by the haemolymph, and in particular the role of the respiratory pigment haemocyanin.

The possession of a respiratory pigment such as haemocyanin will strongly influence the physiological characteristics of respiratory gas transport (e.g. increased oxygen carrying capacity); particularly since one major function of haemocyanin is to maintain a large oxygen diffusion gradient across the respiratory surface (Redmond, 1968). Crustacean haemocyanins are multiples of 6-48 loosely linked polypeptide subunits. Each subunit has a molecular weight of between 65-80,000 daltons and a single active site containing two copper atoms. The subunits may polymerize to typically form aggregates of six (hexamers or 16S), 12 (dodecamers or 24S) or 24 (eikositetramers or 37S) subunits. The degree of polymerization may also influence oxygen binding and the physical properties of the haemolymph (Mangum, 1983). Since the early studies of Svedberg (1933), the haemocyanins of thalassinideans have been noted for their exceptionally high molecular weight polymers (24-mers).

The understanding of haemolymph oxygen transporting properties and the role of haemocyanin in gas exchange in decapods has developed at a rapid pace over the last twenty years, especially with the introduction of more advanced measuring techniques (e.g. Weiss & Weber, 1990). Even though the properties of the haemocyanin have been investigated in a number of crustaceans (see Mangum, 1983; Taylor, 1988; Atkinson & Taylor, 1988 and Morris 1990 for reviews), there is still little information (especially comparative studies) on burrowing species (Bridges, 1986).

It is now clear that the function of crustacean haemocyanin (in particular oxygen affinity) is affected by inorganic ions, temperature and organic compounds (in particular L-lactate and urate), resulting in a series of mechanisms for 'fine tuning' the oxygen affinity in response to changing physiological, behavioural and environmental conditions (Morris, 1991). The relationship between many of the characteristics of the haemocyanin as determined *in vitro* and

the actual *in vivo* function is still not clear (Anderson, 1989). This is particularly evident in small species such as thalassinideans where current *in vivo* measuring techniques of, for example, haemolymph pH, PCO_2, PO_2 are not practical.

The effects of pH, temperature and divalent cation concentration on the oxygen binding characteristics and structure of haemocyanin have been examined in the thalassinidean burrowing shrimps *Neotrypaea* (as *Callianassa*) *californiensis* (Roxby *et al.*, 1974; Miller & Van Holde, 1974; Blair & Van Holde, 1976; Van Holde *et al.*, 1977; Miller *et al.*, 1977; Arisaka & Van Holde, 1979; Miller & Van Holde, 1981a,b; Sanders & Childress, 1992), *N.* (as *Callianassa*) *gigas* (Miller *et al.*, 1977), *Upogebia pugettensis* (Miller *et al.*, 1977) and *Calocaris macandreae* (Svedberg, 1933; Anderson, 1989). Information on respiratory adaptations for other burrowing Crustacea have been provided by Taylor *et al.* (1985) and Bridges (1986).

During the present study, the haemolymph ionic composition, haemocyanin subunit configuration and association state were investigated for a number of thalassinidean species. The oxygen transporting properties, in particular the oxygen affinity and carrying capacity, of the haemolymph of the shrimps *Callianassa subterranea*, *Jaxea nocturna*, *Upogebia stellata* and *U. deltaura* and the effect of one modulator, hydrogen ion concentration, were also examined. Although this study was limited by the small volume of haemolymph obtainable from each shrimp species and the difficulty of determining *in vivo* haemolymph gas parameters (e.g. P_aO_2, P_VO_2, pH), the probable functional properties of the haemocyanin of these thalassinidean shrimps are discussed. Particular attention is paid to the role of the haemocyanins in oxygen uptake and transport in species such as these which inhabit a potentially severely hypoxic burrow habitat.

Materials and methods

Collection and maintanence of mud shrimps

Upogebia deltaura were collected with an anchor dredge at depths of 6-7m from Plymouth Sound, Devon, England (50°20'N, 4°13'W) and from the Irish Sea (54°7'N, 3°27'W). *Upogebia stellata* were obtained at depths of 20-30m with an anchor dredge from the north end of Isle of Cumbrae and *Calocaris macandreae*, with a beam trawl, from the Main Channel (between Cumbrae and Bute), Firth of Clyde, Scotland (55°9'N, 5°11'W). Specimens of *Jaxea nocturna* and *Callianassa subterranea* were ohtained from Loch Sween, Scotland (56°2'N, 5°36'W). All specimens were transported in plastic containers back to the Zoology Department at the University of Glasgow. Individual mud shrimps were placed into small containers (1-2 litres) with mud from the sample sites and allowed to construct a burrow. The containers were kept

within larger tanks in a recirculating sea water aquarium (S, 32‰; T, 10°C). Using this method very low mortality rates were recorded.

Collection of haemolymph

Haemolymph was extracted from quiescent shrimps using a 100µl syringe (Hamilton), the needle (22g) of which was inserted either into the arthrodial membrane at the base of the walking legs, or beneath the cephalothorax and into the pericardium. Haemolymph samples (10-50µl) were then transferred to 0.5ml Eppendorf tubes and kept on ice. As a result of the small volume of haemolymph obtained from each individual (10-50µl) and the irregularity of specimen capture as well as the paucity of individuals, it was necessary to pool the haemolymph samples to obtain sufficient blood. Pooled samples were mixed thoroughly, centrifuged at 13,000 xg for 10min to remove cells and particulate material and kept at 4°C or immediately frozen (-20°C). Although storage of the haemolymph at low temperatures (-80°C) has been shown to affect the cooperativity in some Crustacea (Morris, 1988), the determination of the oxygen transporting properties of haemolymph previously frozen was unavoidable.

Haemolymph ionic composition

Following dilution with deionised water, the concentration of Na^+, K^+, Mg^{2+} and Ca^{2+} in pooled, frozen haemolymph samples was determined using an Atomic Absorption Spectrophotometer (PU 9820, Philips). Lanthanum chloride was added (1:5 v/v) (as a stabilizer) when determining Ca^{2+} concentration. The total haemolymph Cl^- concentration was determined by electrochemical titration using a chloride meter (PCLM3, Jenway). From these data a physiological saline solution (Thalassinidean Ringer) was prepared having the following composition ($mmol.l^{-1}$): NaCl, 393; CaCl, 10: KCl, 15; $MgSO_4$, 20; $MgCl_2$, 25; $NaHCO_3$, 2; and adjusted to pH 7.8 with NaOH.

As lactate has been shown to be a modulator of oxygen affinity (Truchot, 1980; Bridges & Morris, 1986), the concentration of L-Lactate in pooled haemolymph samples was determined, using the method of Gutmann and Wahlefeld (1974) with the modifications of Engel and Jones (1978).

Construction of oxygen binding curves

In vitro oxygen binding curves were constructed on pooled whole haemolymph samples (3µl) using the spectrophotometric diffusion chamber technique (Sick & Gersonde, 1969). Changes in haemocyanin saturation were determined by measuring the absorbance (at 335nm) of the

haemolymph smear (5µl) using a fibre optic pulsed light source and detector connected to a spectrophotometer (3090, Oriel Scientific, Kingston-upon-Thames, England) (Zainal *et al.*, 1992). A haemolymph smear was equilibrated with varying humidified gas mixtures supplied to the diffusion chamber by precision gas mixing pumps (M301, Wösthoff, Bochum, F.R.G.). Oxygen dissociation curves were constructed for the blood of 4 species of mud-shrimp at 10°C by increasing the PO_2 of the gas mixture in a stepwise manner while maintaining a constant PCO_2 for each curve. To determine the Bohr effect, the dissociation curves were constructed at different pH values. The pH of the haemolymph was varied by altering the proportion of CO_2 in the gas mixture.

Separate samples of haemolymph (100µl) were simultaneously tonometered, in a BMS2 (Radiometer) at 10°C, against the same gas mixture as supplied to the diffusion chamber. The pH of the tonometered blood sample was determined at half saturation (P_{50}) using the microcapillary electrode of the BMS 2 connected to a pH meter (215 Ion Analyser, Corning). The P_{50} value and cooperativity (n_{50}) of the blood was estimated using regression analysis of the saturation values (between 25 and 75%) calculated previously from the Hill equation. Where possible, the pH of fresh haemolymph samples (100µl) from individual shrimp were also determined at 10°C using the above method.

Oxygen carrying capacity

The total oxygen content of the haemolymph (C_{O_2}) was measured using blood samples from individual mud shrimps. The oxygen content of duplicate 10µl haemolymph samples, tonometered in the BMS2 against air at 10°C, was determined using the method of Bridges *et al.* (1979). The oxygen carrying capacity of the haemocyanin (C_{HCYO_2}) was calculated by subtracting the physically dissolved fraction of oxygen in the haemolymph from the measured total oxygen content, using an oxygen solubility coefficient of 0.002mmol.l^{-1}.$Torr^{-1}$ (Altman & Dittmar, 1971)

Haemocyanin sub-unit composition

The subunit composition of thalassinidean haemocyanins was determined using sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). Pooled, previously frozen haemolymph samples were diluted to a final concentration of 1.0, 0.5 or 0.1% with SDS sample buffer (containing 2% SDS, 59% 2-mercaptoethanol, 10% glycerol, 0.0625M Tris-HCL (pH 6.8) and 0.002% bromophenol blue as reference front) and heated to 100°C for 2-3 minutes. Aliquots of 30µl were loaded onto a 2.5% (w/v N,N' methylenebisacrylamide) stacking gel and resolved in a 7.5% gel. Electrophoresis was conducted at a constant current of 15 mA per gel

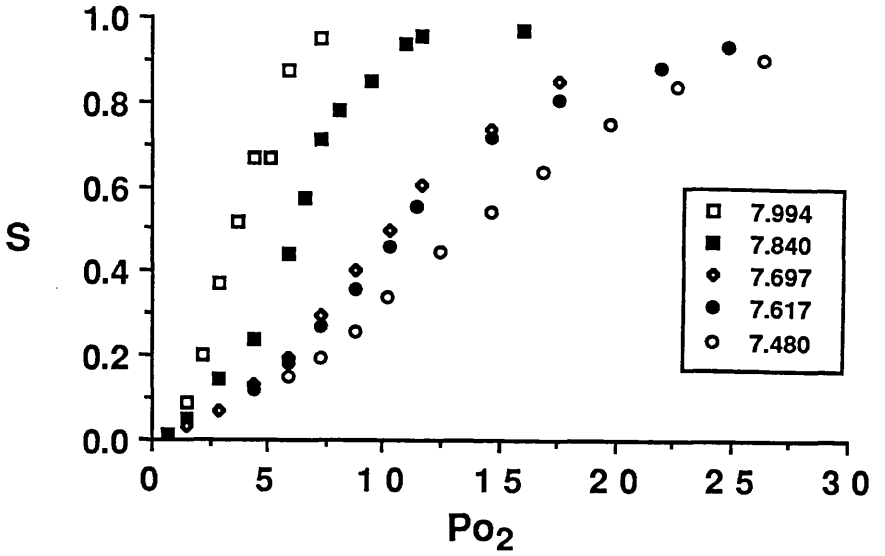


Fig. 6.1. Series of oxygen equilibriation curves showing the saturation of haemocyanin (S) as a function of PO_2 at various pH's (shown in legend) for *Upogebia deltaura* at 10°C.

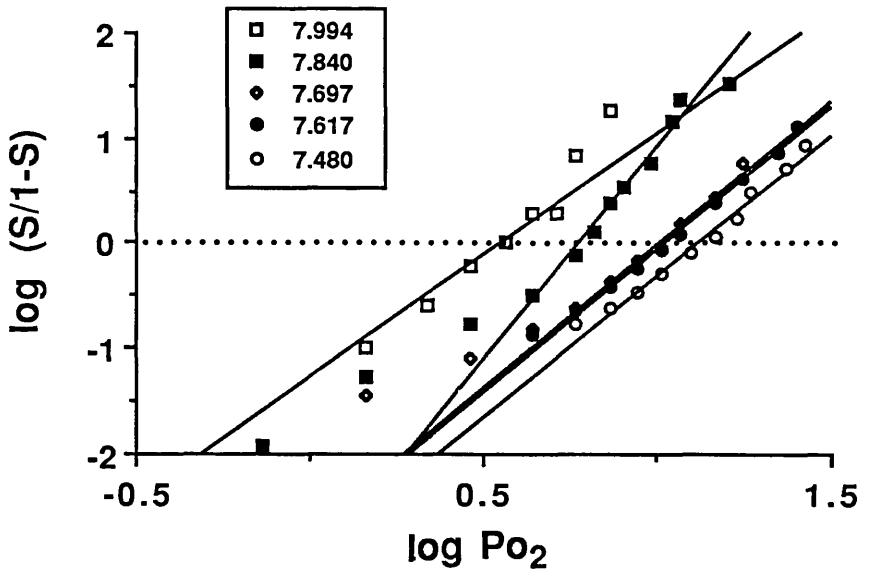


Fig. 6.2. Hill plots of the oxygen binding properties of *Upogebia deltaura* haemocyanin. The slope of the regression line, calculated between 25 and 75% saturation and shown for each pH, quantifies the level of cooperativity (n_{50}). The value for the half-saturation of oxygen partial pressure (P_{50}) is given by the intercept at log (S/1-S) = 0.

(BioRad mini-gel System) until the reference front reached *ca.* lcm from the edge of the gel plates (*ca.* 45 mins). After electrophoresis, the gels were stained for 1-2h with 0.25% Coomassie Brilliant Blue R and then destained in 7% glacial acetic acid.

The molecular weights of the haemocyanin subunits were determined by comparing their electrophoretic mobility with known protein markers. Each gel was calibrated with SDS molecular weight markers in the weight range 29,000-205,000 (MW-SDS-200, Sigma Chemical Co.). A scanning densitometer (GS 300, Hoefer Scientific Instruments, San Fransisco) was used to determine the number of bands and their relative mobility (Rm), the ratio between the migration distance of the protein band and the reference front from the top of the resolving gel. The Rm values were plotted against the known molecular weights and the molecular weight of the unknown protein band was estimated from the calibration curve.

Association state of the haemocyanins

The association states of the haemocyanins were assessed using fast protein liquid chromotography (FPLC). A 100µl aliquot of fresh, not frozen, haemolymph from individuals or pooled samples, previously diluted with thalassinidean Ringer's solution was applied to a Superose 6 gel filtration column (HR10/30, Pharmacia). The column (30cm in length) was packed with Superose 6 prep grade and connected to an FPLC system (Pharmacia, Uppsala, Sweden). The column was eluted with the Ringer's solution (previously filtered through a 0.22µm filter) at a low rate of 0.2ml min^{-1} and 30, 1ml fractions were collected.

The activity of naturally occurring protease enzymes in the haemolymph may lead to degredation of the haemocyanin aggregates (Mangum *et al.*, 1987; Terwilliger *et al.*, 1979). The association state of the haemocyanin was also assessed for haemolymph treated with a serine protease inhibitor (phenylmethansulphonyl flouride (PMSF)) at 1 $mmol.l^{-1}$ final concentration) and a trypsin-like serine and cysteine protease inhibitor (leupeptin at 100$\mu mol.l^{-1}$ final concentration).

The column was calibrated with proteins of known molecular weight; blue dextran (mol. wt. 2,000,000), thyroglobulin (669,000), apoferritin (443,000), alcohol dehydrogenase (150,000) and albumin (66,000) (Sigma Chemical Co.).

Statistical analysis

Data were plotted and appeared normally distributed. Statistical analyses were carried out using analysis of variance, two-way analysis of variance, and Students *t*-test where appropiate (Sokal & Rohlf, 1981). Where the mean of a number of observations is given the deviation from the mean is expressed as standard deviation (SD). However, where the mean of a number of mean

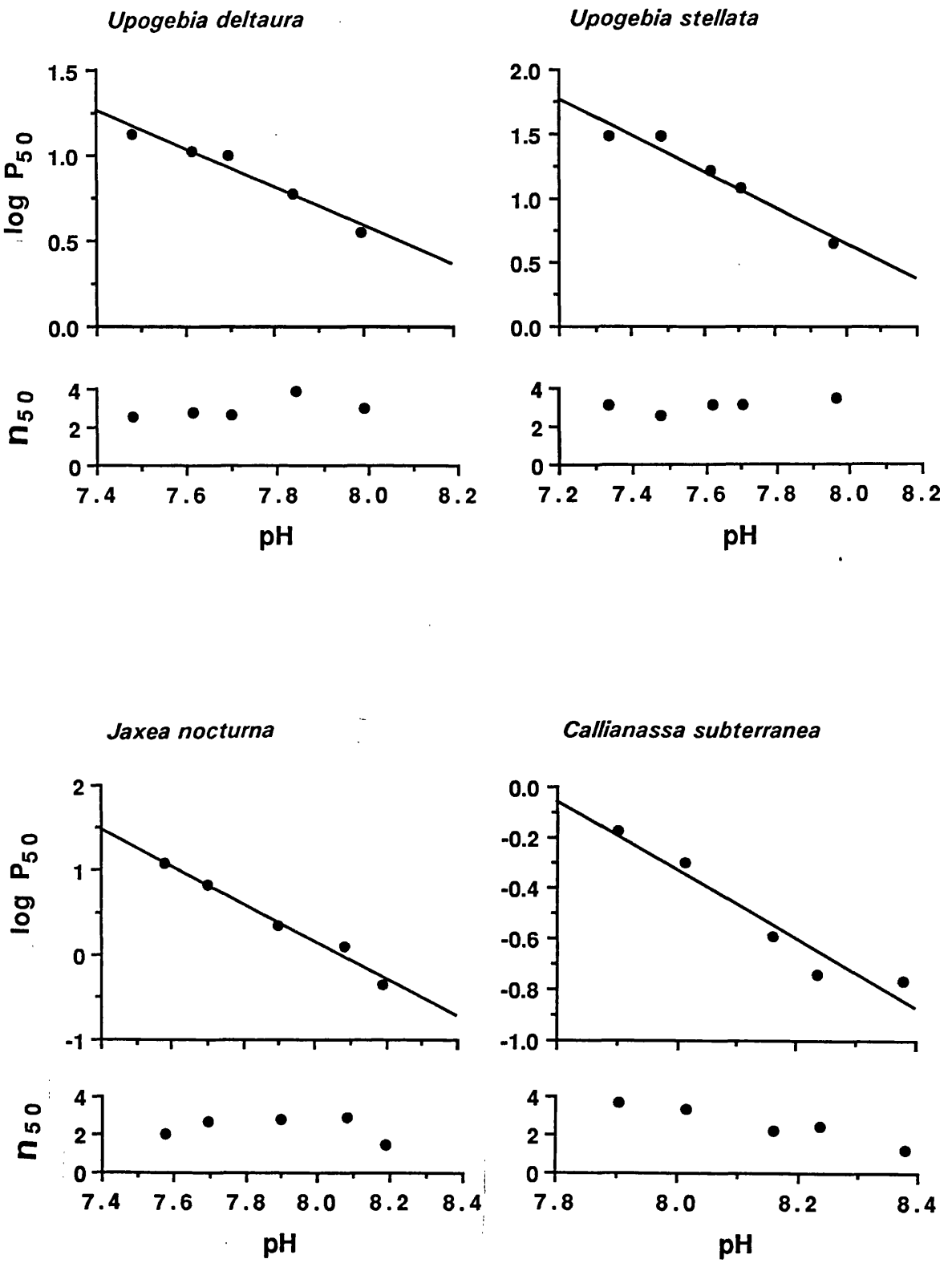


Fig. 6.3. The relationship between pH and the oxygen binding by the haemocyanin at 10°C, characterized by the oxygen affinity (P_{50}) and the cooperativity (n_{50}), are shown for *Upogebia deltaura, U. stellata, Jaxea nocturna* and *Callianassa subterranea*. The equations of the regression lines describing the data are given in Table 6.3.

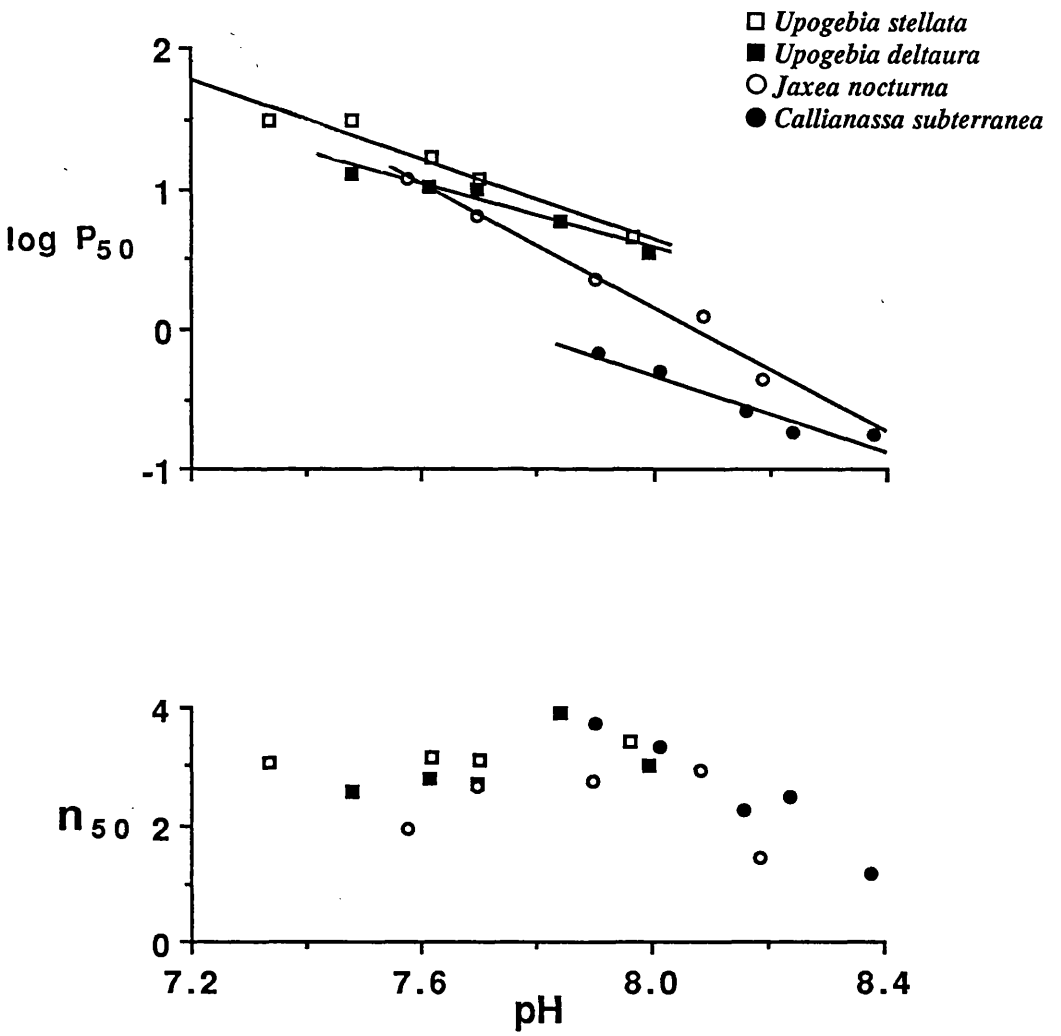


Fig. 6.4. Summary of the relationship between pH and the oxygen affinity (P_{50}) and cooperativity (n_{50}) of the haemocyanin for four species of mud-shrimp at 10°C.

values are determined, the deviation is expressed as standard error (SE).

Results

Haemolymph ionic composition

The concentration of major ions present in the pooled haemolymph samples of four species of thalassinidean mud-shrimp are given in Table 6.1.

Table 6.1. The concentration of major ions present in the haemolymph and the calcium: magnesium ratio are given for four species of thalassinidean shrimp. All measurements were made from pooled, frozen haemolymph samples.

Species	Concentration ($mmol.l^{-1}$)					
	Na^{2+}	Cl^{-}	K^{+}	Mg^{2+}	Ca^{2+}	Ca^{2+}:Mg^{2+}
Ubogebia stellata	401.1	457.2	17.1	40.2	7.8	0.19
Ubogebia deltaura	394.9	446.8	14.8	43.8	10.7	0.24
Jaxea nocturna	358.4	440.0	19.8	34.4	6.7	0.19
Callianassa subterranea	348.7	407.0	22.5	39.9	8.1	0.20
Seawater	440.5	501.1	9.3	52.4	10.2	0.19

All of the species examined had high concentrations of magnesium ions (34.4-43.8$mmol.l^{-1}$) and low calcium to magnesium ratios (0.24). The concentration of L-lactate in pooled haemolymph taken from quiescent individuals was low (0.17-0.58$mmol.l^{-1}$) for all four species of mud-shrimp (Table 6.2).

Table 6.2. Total haemolymph oxygen-carrying capacity (Co_2) and haemocyanin (bound) oxygen-carrying capacity ($C_{HCY}O_2$) measured at 10°C for four species of thalassinidean shrimp. Concentration of L-lactate are shown for pooled haemolymph samples. All values are reported as means ±1S.E.. The number of individual observations are shown in parenthesis.

Species	Co_2 ($mmol.l^{-1}$)	$C_{HCY}O_2$ ($mmol.l^{-1}$)	L-Lactate ($mmol.l^{-1}$)
Upogebia deltaura	0.70 ± 0.04 (7)	0.42 ± 0.04 (7)	0.41 ± 0.03 (2)
Upogebia stellata	0.51 ± 0.08 (2)	0.22 ± 0.01 (2)	0.17 ± 0.05 (2)
Jaxea nocturna	0.55 ± 0.02 (5)	0.27 ± 0.03 (5)	0.33 ± 0.01 (2)
Callianassa subterranea	1.12 ± 0.05 (5)	0.83 ± 0.05 (5)	0.58 ± 0.09 (2)

Sufficient blood was removed from one individual *Jaxea nocturna* and one *Upogebia deltaura* to

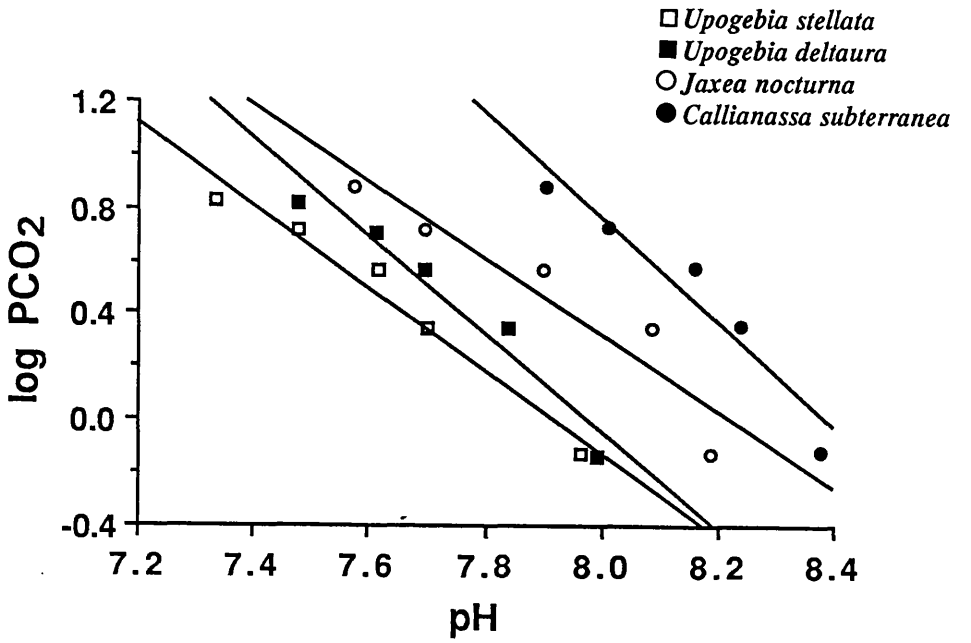


Fig. 6.5. The haemolymph buffering capacity, the relationship between PCO_2 and pH, is shown for four species of mud-shrimp at 10°C. The equations of the regression lines describing the data are given in Table 6.4.

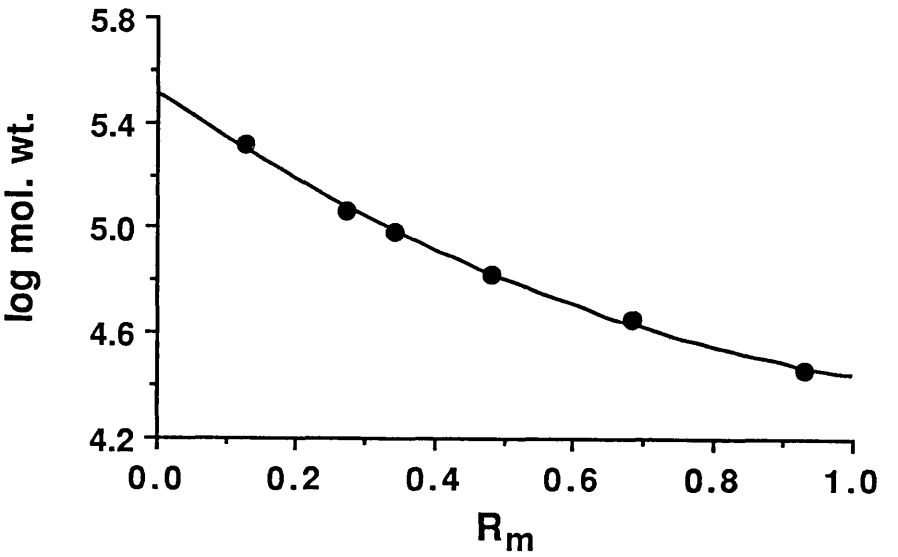


Fig. 6.6. Calibration curve for the molecular weight determination of haemocyanin subunits, using 7.5% SDS-PAGE. The molecular weight (expressed as Daltons) and Rm is shown for each standard.

determine their *in vivo* blood pH, which was 7.68 and 7.83 respectively. These values should only be used as indicators of the *in vivo* pH, as only one measurement was taken from each individual and the effect of stress was not quantified.

Oxygen carrying capacity

The total oxygen (soluble and bound) carrying capacity of the haemolymph (Co_2) and the oxygen carrying capacity of the haemocyanin ($C_{HCY}O_2$) are given in Table 6.2. *Callianassa subterranea* had a significantly higher ($P<0.05$) Co_2 and $C_{HCY}O_2$ than the other mud-shrimps examined. There was no significant difference ($P>0.05$) in the Co_2 and $C_{HCY}O_2$ between *Upogebia stellata* and *Jaxea nocturna*, although the values for *Upogebia deltaura* were significantly higher values ($P<0.05$) than both of these shrimps.

Oxygen dissociation curves

A series of oxygen dissociation curves was constructed from pooled frozen haemolymph samples for each of the mud-shrimps at 10°C. The concentrations of lactate in the pooled samples are shown in Table 6.2. The saturation of haemocyanin was expressed as a function of PO_2 at each of the pH values used (Fig.6.1).

Changes in the oxygen affinity of the haemocyanin with pH were determined using a Hill plot in which the slope of the regression line, calculated between 25 and 75% saturation indicated the level of cooperativity (n_{50}) and the value of the half saturation oxygen partial pressure (P_{50}) is given by the intercept at log(S/l-S)=0 (Fig.6.2). The decrease in the saturation of the haemocyanin with a reduction in the pH of the haemolymph (Fig.6.1), or Bohr coefficient (ϕ), can be quantified by examining the change in P_{50} that occured with pH. The relationship between pH and the log P_{50} and n_{50} of the haemocyanin for each of the four species is shown in Fig.6.3, and summarized in Fig.6.4 and Table 6.3.

Table 6.3. Relationship between pH and oxygen-binding by the haemocyanin, shown here as P_{50} for four species of thalassinidean shrimp (Fig.6.3). Regression equations are given in the form of log P_{50} (Torr) = a + b(pH), the correlation coefficient r is also given. All relationships were significant at $P<0.05$. The Bohr coefficient (ϕ) is quantifiecl by the the b value.

Species	*a*	*b*	*r*
Upogebia deltaura	9.77	-1.15	0.99
Upogebia stellata	13.9	-1.67	0.97
Jaxea nocturna	17.8	-2.26	0.99
Callianassa subterranea	10.2	-1.32	0.98

Fig. 6.7. Scanning densitometer traces of thalassinidean haemolymph run under denaturing conditions using 7.5% SDS-PAGE; *Upogebia deltaura*, A; *U. stellata*, B; *Calocaris macandreae*, C; *Callianassa subterranea*, D; *Jaxea nocturna*, E. For each species of mud-shrimp the bands (haemocyanin sub-units), shown here as peaks, have been numbered arbitarily but have been aligned according to their Rm values. Details of the molecular weights and Rm are given in Table 6.5.

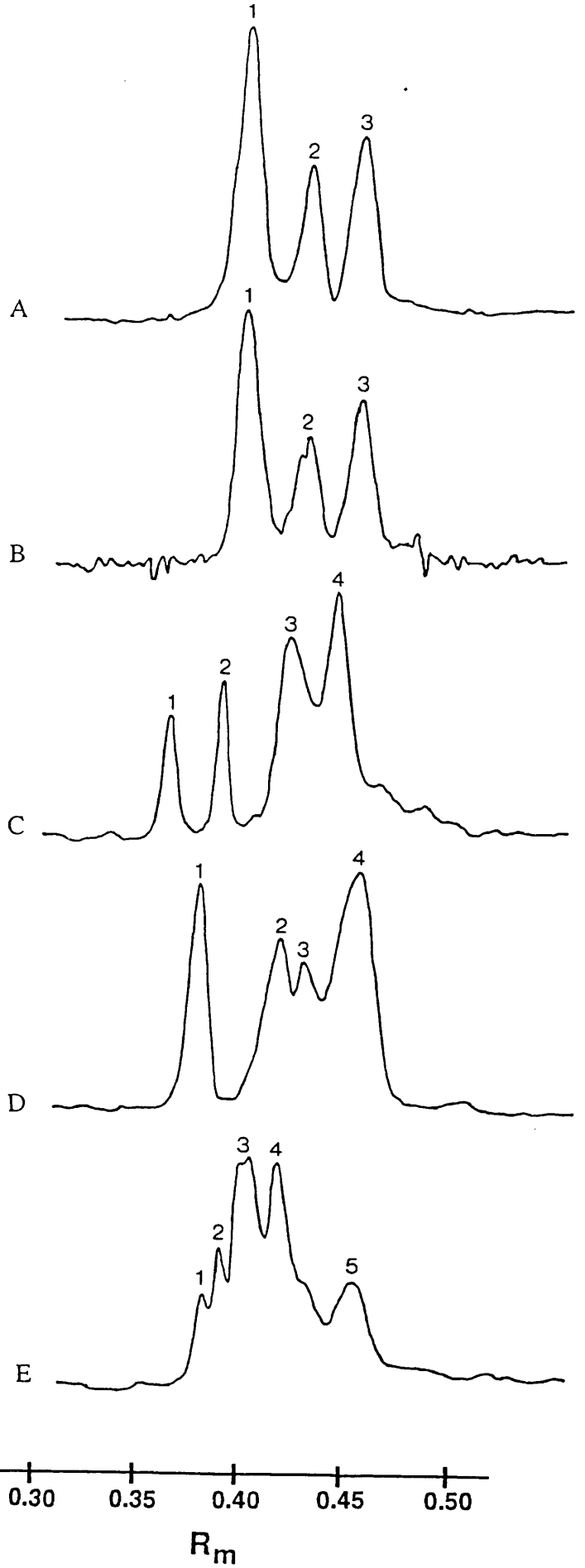
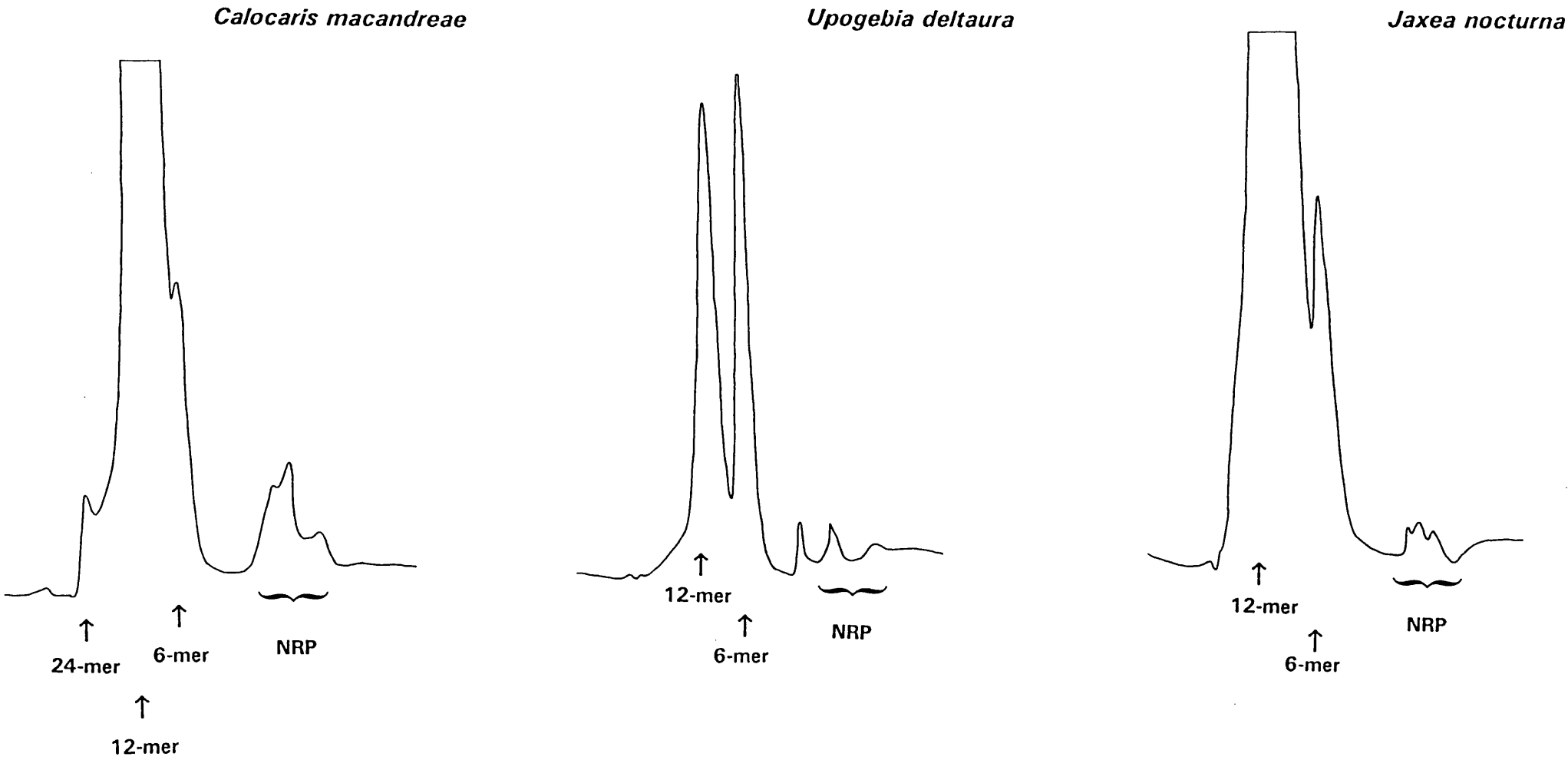

A
1
2
3
B
1
2
3
C
1
2
3
4
D
1
2
3
4
E
1
2
3
4
5
0.30
0.35
0.40
0.45
0.50
Rm

Fig. 6.8. Haemocyanin association state of pooled haemolymph samples, treated with protease inhibitors, from three species of thalassinidean shrimp determined using FPLC. A superose 6 gel filtration column was eluted at 0.2ml.min^{-1} with thalassinidean Ringers solution (pH 7.8). The eikositetramer (24-mer), dodecamer (12-mer), hexamer (6-mer) and non-respiratory proteins (NRP) are indicated for each species.


Calocaris macandreae
24-mer
12-mer
6-mer
NRP
Upogebia deltaura
12-mer
6-mer
NRP
Jaxea nocturna
12-mer
6-mer
NRP

Each of the mud-shrimps exhibited a highly significant Bohr coefficient (Table 6.3). The haemocyanin of *U. deltaura*, *U. stellata* and *C. subterranea* showed a similar Bohr coefficient (ANCOVA, $P<0.05$), whereas *J. nocturna* showed a significantly larger Bohr coefficient than the haemocyanin of other shrimps. The oxygen affinity of the haemocyanin of *Callianassa subterranea* was significantly higher than that of the upogebiids at 10°C (ANCOVA, $P<0.05$), although *U. stellata* haemocyanin had a lower oxygen affinity than that of *U. deltaura* ($P<0.05$, Fig.6.4). The oxygen affinity of the haemocyanin from *J. nocturna* and *U. deltaura* at the *in vivo* haemolymph pH values of 7.68 and 7.83 was quite low (P_{50}=2.8 and 5.8 Torr, respectively).

Although the pH was measured in at least some species, the *in vivo* haemolymph pH of *U. stellata* and *C. subterranea* could not, unfortunately, be determined because of an insufficient number of individuals .For this reason *in vivo* values of pH for some species have been taken from the literature. Assuming that the haemolymph of *U. stellata* has a similar *in vivo* pH to that of *U. deltaura*, the oxygen affinity of the haemocyanin at a pH of 7.83 would be P_{50}=6.7 Torr. Similarly, assuming the *in vivo* blood pH of *C. subterranea* is similar to that determined by Miller & van Holde (1981) for the haemolymph of *Neoptrypaea* (as *Callianassa*) *californiensis* at 10°C (pH=8.20), the oxygen affinity of the haemocyanin from *C. subterranea* would be P_{50}=0.24 Torr.

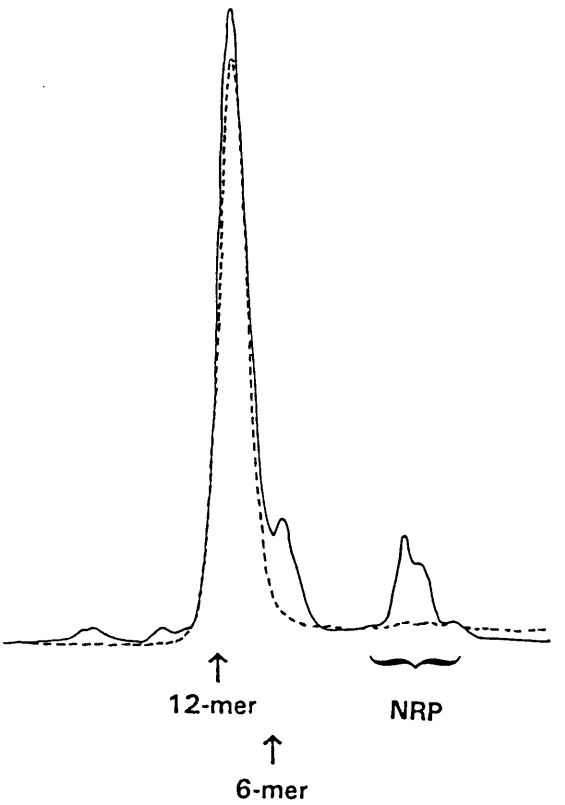
Table 6.4. Relationship between $\log PCO_2$ and pH, haemolymph buffering capacity, for the haemocyanin of four species of thalassinidean shrimp (Fig.6.5). Regression equations are given in the form of Log PCO_2 (Torr) = $a + b$(pH), the correlation coefficient r is also given. All relationships were significant at $P<0.05$.

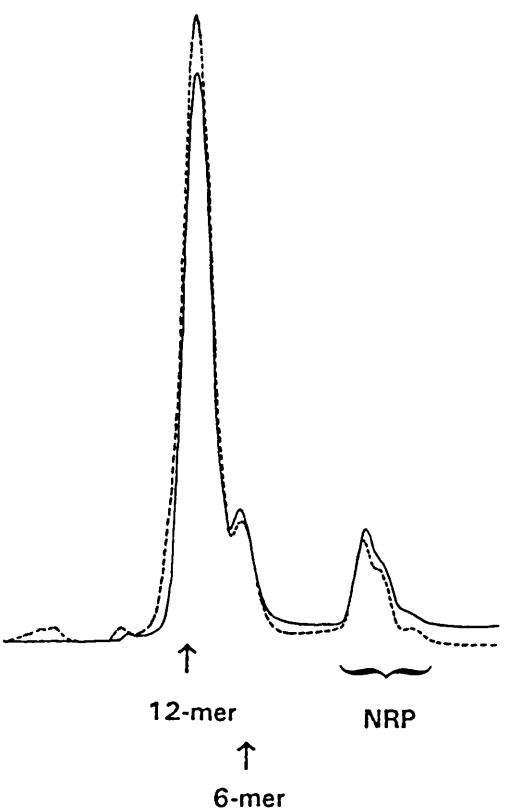
Species	*a*	*b*	*r*
Upogebia deltaura	14.7	-1.84	0.97
Upogebia stellata	12.3	-1.56	0.98
Jaxea nocturna	11.8	-1.44	0.94
Callianassa subterranea	16.7	-1.99	0.96

The cooperativity (n_{50}) remained independent of pH for *U. deltaura*, *U. stellata* and *J. nocturna*, however, a decrease in n_{50} associated with an increase in pH was observed for *C. subterranea* at pH values >8.1 (Fig.6.4). The mean n_{50} was slightly higher in *U. stellata* (3.05 ± 0.1) and *U. deltaura* (2.99 ± 0.2) than either *J. nocturna* (2.32 ± 0.28) or *C. subterranea* (2.59 ± 0.9), although there was no significant difference between the mean n_{50} values for any of these species examined (Two sample-t, $P>0.05$).

Fig. 6.9. *Calocaris macandreae.* Haemocyanin association state of pooled untreated haemolymph taken from two different groups of individuals (dashed line and solid line) determined using FPLC. A superose 6 gel filtration column was was eluted at 0.2ml.min^{-1} with thalassinidean Ringers solution (pH 7.8). The dodecamer (12-mer), hexamer (6-mer) and non-respiratory proteins (NRP) are indicated for each species

Fig. 6.10. *Calocaris macandreae.* The effect of one freeze-thaw cycle (solid line) compared to fresh haemolymph (dashed line) on the association state of the haemocyanin, determined using FPLC.


12-mer
NRP
6-mer


12-mer
NRP
6-mer

Haemolymph buffering capacity

The PCO_2 of the haemolymph could not be measured using *in vivo* Astrup titration because of the small amounts of haemolymph obtained from each shrimp.

However, an indication of the buffering capacity can be gained by examining the change in pH with PCO_2, determined previously during the construction of oxygen equilibrium curves (Fig.6.5, Table 6.4). There was no significant difference in the buffering capacity of the haemolymph from the four species of thalassinidean shrimp (ANCOVA, $P<0.05$).

Haemocyanin sub-unit composition

Scanning densitometer traces of thalassinidean haemolymph run under denaturing conditions using polyacrylamide gel electrophoresis revealed between three and five sub-units (Fig.6.7). Both the upogebiid shrimps showed three distinguishable sub-units, the molecular weights of which were similar for each species (Table 6.5). The haemolymphs of *Calocaris macandreae* and *Callianassa subterranea* were characterized by four sub-units, whereas five sub-units were revealed in the haemolymph from *Jaxea nocturna* (Fig.6.7). The molecular weights for each of the numbered sub-units range from 70,700 to 93,700 Daltons, and are summarized in Table 6.5.

Table 6.5. The subunit composition of the haemocyanin, as detrmined by SDS-PAGE, is shown for five species of mud-shrimp. The relative mobility (Rm) was determined for each band (Fig.6.7) and the molecular weight calculated from a calibration graph (Fig.6.6).

Species	Band	Rm	mol.wt
Upogebia stellata	1	0.393	84700
	2	0.428	76900
	3	0.459	70900
Upogebia deltaura	1	0.393	84700
	2	0.425	77400
	3	0.455	71500
Calocaris macandreae	1	0.357	93700
	2	0.386	86200
	3	0.427	77000
	4	0.454	71700
Callianassa subterranea	1	0.370	90300
	2	0.416	79400
	3	0.430	76500
	4	0.460	70700
Jaxea nocturna	1	0.368	90900
	2	0.381	87500
	3	0.400	83000
	4	0.418	79000
	5	0.415	71600

Association state of the haemocyanins

The association states of pooled, fresh haemocyanin treated with protease inhibitors was measured for the mud-shrimps *Calocaris macandreae*, *Jaxea nocturna* and *Upogebia deltaura* using fast-protein liquid chromatography (Fig.6.8).

In *Upogebia deltaura*, haemocyanin was present in equal portions as both a dodecameric (approx. Mr 960,000) and hexameric (Mr 420,000) form. The haemocyanin in pooled haemolymph of *Jaxea nocturna* was mainly in the dodecameric form (Mr 1,020,000) with some hexameric (Mr 480,000) haemocyanin. In both of these shrimps the eikosotetramer (24-subunit aggregation state) was not present. However, pooled haemolymph from *Calocaris macandreae* showed approximately equal amounts of eikosotetramers (Mr 1,900,000) and hexamers (Mr 940,000), although the majority of the haemocyanin was present as dodecamers (Mr 940,000).

The fraction corresponding to the elution of the eikosotetramer was confirmed to be a copper containing respiratory protein, as the absorbance peak at 335nm was greatly reduced when the fraction was treated with sodium sulphite. The haemocyanin of pooled haemolymph not previously treated with a proteolytic inhibitors was present only in the dodecameric form for *U. deltaura* and *J. nocturna*, although in *C. macandreae* some hexamers were also recorded. The eikosotetramer form of the haemocyanin was not found in untreated haemolymph of any of the mud-shrimps. There was little difference in the haemocyanin aggregation states of pooled haemolymph samples, either treated or untreated, from groups of different individuals (Fig.6.9).

Therefore, the variation in haemocyanin aggregation as a result of adding protease inhibitors to the haemolymph was not considered to be an experimental artifact because pooled haemolymph samples were used to determine haemocyanin association states.

The effect of one freeze-thaw cycle at -20°C on the haemocyanin association of haemolymph from *Calocaris macandreae* that had not been treated with protease inhibitors is shown in Fig.6.10. There was little difference in the haemocyanin associations following thawing; the proportion of hexamers and non-respiratory proteins increased slightly, with a decrease in the proportion of the dodecameric form.

Discussion

Haemocyanin aggregation states

The haemocyanins from *Upogebia deltaura* and *Jaxea nocturna* exist in the haemolymph as multiples of either 6 monomers (forming a hexamer of about 450,000 daltons) or 12 monomers (forming a dodecamer of about 900,000 daltons). *Calocaris macandreae* also showed a larger

aggregation state of 24 monomers (an eikositetramer of about 1,900,000 daltons). The dodecamer was the predominant polymer in the haemolymph of *J. nocturna* and *C. macandreae*, whereas in *U. deltaura* equal fractions of the hexamer and dodecamer were recorded.

The native molecule in many decapods exists as either hexamers or dodecamers with either form being predominant and the levels of native aggregates being species-specific (Markl, 1986). The hexamer is the typical aggregate in caridean, penaeid, pagurid and palinuran decapods (Mangum, 1983). In many other decapod species the dodecamer is the main component, although in some groups both hexamers and dodecamers may co-exist in various proportions (Markl *et al*, 1979).

The formation of eikositetramers (24-mers) has previously been recorded for a number of thalassinidean shrimps; *Calocaris macandreae* (Svedberg, 1933), *Neotrypaea* (as *Callianassa*) *californiensis* (Roxby *et al.*, 1974), *N.* (as *Callianassa*) *gigas* (Miller *et al.*, 1977) and *Upogebia pugettensis* (Miller *et al.*, 1977). The haemocyanins from these thalassinidean shrimps have been shown to exist in at least three different stable states of aggregation depending on the temperature, pH and ionic composition of the solution (Roxby *et al.*, 1974; Miller *et al.*, 1977; Miller & Van Holde, 1981a; b).

In the filter feeding shrimp *U. pugettensis*, Miller *et al.* (1977) found evidence for a stable dodecamer at room temperature but in *N.* (as *Callianassa*) *gigas*, the dodecamer component was largely absent above 20°C. At room temperature the haemocyanin of *Callianassa major* was composed primarily of eikositetramers (some hexamer and dodecamers were also present) whereas a reduction in room temperature favoured the dissociation of the eikositetramer (Miller & Van Holde, 1981).

The association of hexamers and dodecamers requires divalent cations, either Mg^{2+} or Ca^{2+}, as well as competent monomers (Mangum, 1983). Van Holde *et al* (1977) found that about 3 Mg^{2+} were required per hexamer of *Neotrypaea* (as *Callianassa*) *californiensis* haemocyanin. Since dodecamers must precede the formation of higher multiples, Mg^{2+} is critical to the assembly of thalassinidean eikositetramers. The level of Mg^{2+} in the haemolymph was quite high (34.4-43.8mM) in the thalassinideans *Upogebia stellata*, *U. deltaura*, *J. nocturna* and *C. subterranea*. Anderson (1989) also recorded levels of 45.4-52.9mmol.l^{-1} Mg^{2+} in the haemolymph of *Calocaris macandreae* and values of 48 mmol.l^{-1} were measured in the haemolymph of *N. californiensis*, 54.5 mmol.l^{-1} in *C. gigas* and 60.0 mmol.l^{-1} in the haemolymph of *U. pugettensis* (Miller *et al.*, 1977). These Mg^{2+} levels are much higher than recorded for nearly all other decapods (Robertson, 1960; Mantel & Farmer, 1983). Although Mg^{2+} ions are required for the formation of different aggregation states, high levels of these cations are regarded as potentially disadvantageous because of the possibility of narcotizing effects (Robertson, 1960; Mangum, 1983).

The stability of the 24-mer will also depend, amongst other variables, on the hydrogen ion

concentration. A low haemolymph pH induces dissociation in *N. californiensis* haemocyanin (Anasaka & Van Holde, 1979) within the physiological pH range 8.2-7.3. In this study, the absence of 24-mers in the haemolymph of *U. deltaura* and *J. nocturna* could be a result of the conditions used during HPLC analysis. The lower Mg^{2+} levels (50 $mmol.l^{-1}$) and pH (7.8) could favour dissociation of any 24-mers at room temperature (18-20°C). As this was only an initial examination of the association states of thalassinidean haemocyanins, it is clear that the effect of divalent cation concentration, pH and temperature should be assessed in far more detail. The presence of some 24-mers in the haemolymph of *C. macandreae* confirmed that the HPLC column used was able to detect such large aggregation states. In addition, other workers have used large volumes (5ml) of purified (concentrated) haemocyanins, compared with whole haemolymph used in this study, in the analysis of *N. californiensis, C. gigas* and *U. pugettensis* haemocyanins (e.g. Roxby *et al.*, 1974; Miller *et al.*, 1977; Miller & Van Holde, 1981a; b; Anasaka & Van Holde, 1979).

What is the physiological significance of the different association states? The current evidence suggests that 85% of the haemocyanin of thalassinideans is present as eikositetramer form under *in vitro* conditions (high Mg^{2+} levels, pH 7.8 and 10°C) and 15% in the dodecamer form (although there is distinct variability between species in the predominant association state) (Miller *et al.*, 1977). Extreme changes in temperature, pH and ion concentration (mediated though salinity changes) may, however, affect the association states. The association state has significant physiological importance since it has been shown to influence the binding of oxygen, Mangum *et al.*, (1991) found isolated dodecamers of the crab *Callinectes sapidus* had lower oxygen affinity and greater cooperativity than isolated hexamers, and even the physical properties of the haemolymph such as viscosity and colloidal osmotic pressure (Snyder & Mangum, 1982; Mangum, 1983; Mangum *et al.*, 1991).

Haemocyanin subunit structural composition

The haemocyanins of *Upogebia deltaura* and *U. stellata* consist of three major mobility groups with a large part of the haemocyanin present in a single slow moving band weighing 84,700 daltons. Interestingly, Miller *et al* .(1977) observed a similar banding pattern for *U. pugettensis*. The scans of the SDS-PAGE gels of *Calocaris macandreae, Jaxea nocturna* and *Callianassa subterranea* haemolymph, although not identical, exhibit a number of common components. Similarly, as many as 6 subunits were resolved for *Neotrypaea* (as *Callianassa*) *californiensis* and 4 subunits for *C. gigas* (Miller *et al.*, 1977). Roxby *et al.* (1974) found that the 24-mer of *N. californiensis* (termed haemocyanin C) when treated with SDS gel electrophoresis consisted of only one major subunit of 70-75,000 daltons. The incompetent hexamer of *N. californiensis* (termed haemocyanin I), as well as showing a strong band at 70-75,000, also gave 3 distinct

smaller subunits (Roxby *et al.*, 1974).

Although Miller *et al.* (1977) did not find any clear correlation between the subunit composition and the capacity to form 24-mers for *U. pugettensis, N. californiensis* and *C. gigas*, the authors did suggest that the stability of both the eikositetramer and dodecamers probably results from differences in the subunit composition. Similarly, in this study there appeared to be little correlation between the range of subunits and the ability to form aggregated structures, especially since the haemocyanin from all five species formed similar aggregation states.

In other crustacean species, between one and eight different haemocyanin subunits have been observed (with average molecular weights ranging from 70-90,000 daltons) with as many as 3 smaller additional non-respiratory proteins (e.g. Markl *et al.*, 1979; Mangum, 1983; Chan & Weeks, 1992).

A correlation between the aggregation state and subunit heterogeneity has been discovered in crustaceans, with the heterogeneity of the haemocyanin subunit in dodecamers being greater than that of the hexamers (Markl *et al.*, 1979). Indeed, haemocyanin subunit heterogeneity is thought to influence haemocyanin aggregate assembly (Chan & Weeks, 1992) resulting in the concept of competence. A single type of subunit may be adequate for the reassembly of the basic hexamer form but, to produce larger assemblies certain bridging subunits are required. The absence of these bridging units may explain why only certain aggregations are found in the haemolymph (Chan & Weeks, 1992).

Although the functional significance of subunit heterogeneity is unclear (see Mangum, 1983), subunit variability has been shown to influence haemocyanin oxygen affinity (Mangum, 1983; Mangum & Rainer, 1988; Mangum, 1990). The native haemocyanin polymers of the blue crab *Callinectes sapidus* are made up of 6 subunits. Differences in three of the subunits were found between estuarine and seaside populations of the crab, and it was found that these differences were correlated with haemocyanin oxygen affinity (Mangum & Rainer, 1988). This suggests that the various subunits have not only a structural role but a (probable) concomitant functional role as well.

Carrying capacity

The total haemolymph oxygen carrying capacities determined during the present study ranged from 0.51 mmol.l^{-1} for *Upogebia stellata* to 1.12 mmol.l^{-1} for *Callianassa subterranea* (Table 2), reflecting differences in haemocyanin concentration. These values are within the range recorded for other thalassinidean species, 0.52 mmol.l^{-1} for *Calocaris macandreae* (Anderson, 1989) and 0.73 mmol.l^{-1} for *Neotrypaea* (as *Callianassa*) *californiensis* (Miller *et al.*, 1976), and fall within the range recorded for aquatic crabs (Mangum, 1983). Among decapods, terrestrial and semi- terrestrial crabs have the highest total oxygen carrying capacities (e.g.

Mangum, 1983; Taylor and Spencer Davies, 1981), with the haemolymph of the majority of aquatic decapods having relatively low oxygen carrying capacities (e.g. Magnum, 1983; Bridges, 1986). Taylor & Spicer (1989) also noted that the haemolymph of natant decapods is typically characterized by having higher oxygen carrying capacities than that of many reptant species.

Previously, variations in oxygen carrying capacity have been attributed to haemocyanin concentration, however, the method of calculating the carrying capacity should also be taken into account. Miller *et al.* (1976) found significant differences when the oxygen carrying capacity was calculated using the protein concentration and molecular weight of haemocyanin subunits compared with gasometric methods.

To understand haemocyanin function *in vivo*, it is essential to obtain postbranchial (arterial, P_aO_2) and prebranchial (venous, P_VO_2) oxygen tensions. Unfortunately, because of the paucity of specimens and small volumes of haemolymph obtained from each individual it was not possible to determine values of P_aO_2 or P_VO_2 for any of the thalassinidean species during the present study. Typical P_aO_2/P_VO_2 values from crustaceans vary within a wide range from 112-8.4 Torr (Mangum, 1983).

Interpolation of P_aO_2/P_VO_2values from oxygen dissociation curves will indicate the degree of haemolymph saturation *in vivo*. A fairly high value of P_VO_2 would show that the haemocyanin gives up a limited amount of oxygen to the tissues and that a significant quantity of oxygen comes from physical solution. Thus, a high P_VO_2 would suggest that the haemocyanin acts as a venous reserve to be used during exercise or exposure to hypoxia (e.g. Taylor, 1976; Zainal *et al.*, 1991). Because P_aO_2 /P_VO_2 was not determined, haemocyanin function can only be hypothesised.

The high affinity of the haemocyanin indicates that the pigment is unlikely to unload oxygen at the tissue level unless tissue PO_2 is extremely low. Although values for P_VO_2 were not determined for any of the thalassinidean shrimp, nor are there data from other studies, it is unlikely that the P_VO_2 would reach such low levels except during severe hypoxia when both P_aO_2 and P_VO_2 probably decline (as seen for other decapod species, see Mangum, 1983).

For two burrowing crustaceans, *Corystes cassivelaunus* (crab) and *Nephrops norvegicus* (lobster), Bridges (1986) found that the physically dissolved oxygen in the haemolymph accounted for 40% and 77% of the total oxygen transported in the haemolymph. Interestingly, the two burrowing species had a venous oxygen reserve approximately three times greater than that of the two non-burrowing species examined. Similarly, Taylor *et al.* (1985) also found that under normoxic conditions the haemolymph accounted for between 66% and 88% of the total oxygen transported in the haemolymph of three species of burrowing crabs, and Bradford & Taylor (1982) found that the respiratory pigment of the crab *Cancer pagurus* provided only 9% of the oxygen supplied to the tissues under normoxic conditions.

Therefore, there is some evidence to suggest that, under normoxic conditions, the

haemocyanin gives up only a limited amount of oxygen to the tissues and that the majority of oxygen supplied to the tissues is derived from oxygen in physical solution in the haemolymph. (e.g. McMahon & Wilkens, 1975; Taylor, 1976; McMahon *et al.*, 1979; Bradford & Taylor, 1982; Mangum, 1983; Zainal *et al.*, 1992).

During periods of activity or when exposed to hypoxia it is likely that the haemocyanin derived venous oxygen reserve will be used to meet tissue oxygen demands. Indeed, for a number of decapods the increase in the total amount of oxygen delivered to the tissues at low oxygen tensions was due to the greater release of oxygen combined with the respiratory pigment (e.g. McMahon & Wilkens, 1975; Taylor, 1976; Bradford & Taylor, 1982).

Haemocyanin oxygen affinity

The haemocyanins of all four thalassinidean species examined during the present study are characterised by having a high oxygen affinity (low P_{50}). At 10°C, the P_{50} values at the appropriate *in vivo* pH were 0.24, 2.3, 5.8 and 6.7 Torr for *Callianassa subterranea*, *Jaxea nocturna*, *Upogebia deltaura* and *U. stellata* respectively. The differences in P_{50} between these species cannot be attributed to differences in the concentration of L-lactate or other ions in the haemolymph, as these did not differ significantly between samples. These oxygen affinities are similar to those previously reported for *Neotrypaea* (as *Callianassa*) *californiensis* (P_{50} = 3.5 at pH 8.3, 10°C) (Miller & Van Holde, 1976); *N. californiensis* (P_{50}=4, pH 8.0, 10°C) (Miller *et al.*, 1977); *N. californiensis* (P_{50}=3.7, pH 8.0, 15°C) (Sanders & Childress, 1992); *C. gigas* (P_{50}=4, pH 8.0, 10°C) (Miller *et al.*, 1977); *Upogebia pugettensis* (P_{50}=11.5, pH 7.85, 10°C) (Miller *et al.*, 1977); *Calocaris macandreae* (P_{50}=1.6, pH 7.69, 10°C) (Anderson, 1989).

The haemocyanin oxygen affinities of those species such as burrowing crustaceans that regularly experience hypoxia appears to be consistently higher than those of decapods from normoxic habitats (McMahon, 1984; Bridges, 1986; Taylor *et al.*, 1985, Atkinson & Taylor, 1988). The possession of a respiratory pigment having a high oxygen affinity is an adaptation shown by many crustaceans which are regularly exposed to hypoxia, for example rock pool species (Taylor 1988), vertically migrating deep sea shrimps (Sanders & Childress, 1990a), bathypelagic mysids inhabiting deep sea oxygen minimum layers (Sanders & Childress, 1990b) and hydrothermal vent crabs (Sanders *et al.*, 1988).

To be an effective oxygen transporter, the respiratory protein must be able to become fully saturated when at the gills and unload a large fraction of the oxygen at the tissues. The high affinity haemocyanin of the thalassinids appears well adapted for this purpose, being able to bind oxygen from the low PO_2 water found within the burrow. To unload oxygen, however, the P_VO_2 must be very low otherwise the pigment would not function properly. Alternatively, if the P_VO_2 is not very low haemocyanin is likely to unload oxygen only during periods of severe hypoxia

when P_VO_2 declines. As discussed earlier, it is likely that *in vivo* the haemocyanin will act as a venous reserve, however, oxygen unloading at the tissues will also be facilitated by a large Bohr coefficient (see below).

Among the thalassinideans there is growing evidence to suggest that functional differences in oxygen binding characteristics are correlated with the degree of hypoxic stress experienced. The higher oxygen affinity of the haemocyanin of the deposit feeding shrimps *Callianassa* spp, *Jaxea nocturna* and *Calocaris macandreae* (Miller *et al.*, 1977; Anderson, 1989) compared with that of the haemocyanin of the filter feeding upogebiids indicates that the haemocyanin function of the deposit feeders is particularly suited for the more severe hypoxic conditions found within their burrows. During this study the effect of pH on oxygen binding by the haemocyanin was investigated. The haemocyanins of all four thalassinidean shrimp species show pH sensitivity with the affinity of the haemocyanin being markedly increased with only small changes in $[H^+]$. The Bohr coefficient is the change in the oxygen affinity of the haemocyanin associated with a change in the pH of the haemolymph. A 'normal' Bohr effect is a decrease in oxygen affinity (increase in P_{50}) associated with an decrease in pH. For each of the thalassinidean species, the Bohr coefficient was quite large (ϕ=- 1.15 to -2.26, Table 6.3), although within the range given for a number of other burrowing and non-burrowing decapod crustaceans (Mangum, 1983; Taylor *et al.*, 1985; Bridges, 1986). Anderson (1989) recorded a Bohr value of -0.58 for *Calocaris macandreae* and values of -1.15 and -0.63 were determined for *Neotrypaea* (as *Callianassa*) *californiensis* and *Upogebia pugettensis*, respectively (Miller *et al.*, 1979). A Bohr value of -1.12 has also been recorded for *N. californiensis* by Sanders & Childress (1992).

A positive Bohr effect may be beneficial in hypoxic or hypercapnic conditions since oxygen unloading at the tissues will be facilitated via a reduction in haemolymph pH. However, the Bohr effect may be maladaptive because oxygen loading at the gills may be reduced by metabolic acidosis (Mangum, 1983). Again, without knowledge of pre and postbranchial pH the functional significance of the Bohr effect in these species remains unclear.

The high cooperativity of thalassinidean haemocyanins has the functional effect of increasing the PO_2 gradient, enabling the haemocyanin molecule to release most of the bound oxygen with only a small change in the PO_2 (5-6 Torr). A haemocyanin with a high cooperativity also means that a greater volume of oxygen will be released from a given amount of haemocyanin. Cooperativity of crustacean haemocyanin varies between 2.1 and 4.5 (Mangum, 1983). Although conservative, the cooperativity of some decapod crustaceans has been shown to be affected by freezing, although the oxygen affinity was not changed (Mangum, 1983; Morris, 1988; Spicer & McMahon, 1991).

The haemocyanins of the thalassinideans show a high oxygen affinity which should enable the haemocyanin to bind oxygen from the low PO_2 water of the burrow and encourage the uptake

of oxygen at the gills. The pH sensitivity of the haemocyanins would be expected to assist the unloading of oxygen at the tissues. In response to hypoxia the shrimps are likely to increase the supply of oxygen to the gills via hyperventilation (see Chapter 5). This hyperventilation response is initially associated with enhanced CO_2 transport across the gills, with a reduction in PCO_2 and hydrogen ion levels resulting in a respiratory alkalosis (McMahon *et al.*, 1978; Butler *et al.*, 1978; Burnett, 1979). The increased pH will, via the Bohr effect, facilitate the efficiency of oxygen uptake at the gills by increasing the oxygen affinity of the haemocyanin.

Apart from the pH effects, the oxygen affinity of decapod crustacean haemocyanins can be modulated by various factors such as temperature, inorganic ions, CO_2 concentration and organic modulators (see review by Mangum, 1983; Morris 1990). Considering the burrow habit of thalassinideans, the most important modulator is likely to be L-lactate. If exposure to hypoxia is severe enough, lactate production, an end product of anaerobic metabolism, will occur in response to anoxia at the tissue level (Bridges & Brand, 1980; Hill *et al.*, 1991).

L-lactate has been shown to act as a modulator of haemocyanin oxygen affinity for many, but not all, crustaceans. An increase in lactate concentration in the haemolymph results in an increase in the haemocyanin oxygen affinity (Truchot, 1980; Mangum, 1983; Taylor, 1988; Morris, 1990). Anderson (1989) showed that L-lactate accumulated in the haemolymph of *Calocaris macandreae* below a PO_2 of 5 Torr and Hawkins (1970) found that *Neotrypaea* (as *Callianassa*) *californiensis*, when deprived of oxygen, accumulated lactate at a steady rate. Similarly, Pritchard & Eddy (1970) showed that both *N. californiensis* and *Upogebia pugettensis* accumulated L-lactate in the haemolymph as a result of anoxic stress. The accumulation of lactic acid (and build up of CO_2) results in a respiratory acidosis in the haemolymph. A lactate sensitive haemocyanin would counter the maladaptive effects of the Bohr shift, and prevents a reduction in oxygenation at the gills, during acidosis by increasing the haemocyanin oxygen affinity.

No specific lactate effect could be demonstrated for *Calocaris macandreae* (Anderson, 1989) nor *Neotrypaea* (as *Callianassa*) *californiensis* (Mangum, 1983). Thus in *C. macandreae*, at least, the metabolic acidosis resulting from exposure to severe hypoxia would be unopposed by a lactate effect and under these conditions oxygen unloading at the tissues would be faciltated by utilising the venous oxygen reserve (i.e haemocyanin bound oxygen). Although the haemocyanin of thalassinideans appears lactate insensitive, many decapod species when exposed to severe hypoxia show a positive lactate effect, resulting in a significant increase in oxygen loading at the gills (Booth *et al.*, 1982; Bridges *et al.*, 1984; Morris, 1990).

In addition to L-lactate, urate and the catecholamine, dopamine, have been shown to have significant potentiating effects on some crustacean haemocyanins (Morris *et al.*, 1985; Lallier & Truchot, 1989; Morris, 1990). Urate has been shown to be regulated with environmental PO_2 especially above the Pc whereas the production of L-lactate appears to be of greater significance

below the Pc (Lallier & Truchot, 1989). From this work these authors suggested that urate may act as a true modulator of haemocyanin oxygen affinity.

Changes in haemocyanin oxygen affinity will also affect CO_2 binding and release via Haldane effects as has been demonstrated for many crustaceans (e.g. Truchot, 1976; Booth, 1982; Taylor *et al.*, 1985; Anderson, 1989; Zainal *et al.*, 1991). Thus, modulators which affect the function of the haemocyanin oxygen release and uptake may also have some effect on CO_2 transport. Although CO_2 transport and acid-base balance was beyond the scope of this study, Anderson (1989) showed a Haldane effect for the mud-shrimp *Calocaris macandreae* comparable to that of other non-burrowing decapods.

To summarise, the haemolymph of *Jaxea nocturna*, *Callianassa subterranea*, *Upogebia stellata* and *U. deltaura* are characterised by having a moderately high oxygen carrying capacity. The oxygen affinity of the respiratory pigment, haemocyanin, is very high for each of the species and exhibits a large Bohr coefficient.

The haemocyanin is thought to act as a venous oxygen store which may be utilised during periods of hypoxia. Possession of a high affinity respiratory pigment is considered to be an adaptation to the severe conditions found within the burrow habitat. Indeed, differences between the oxygen affinity of thalassinidean haemocyanins may reflect adaptations to the different feeding lifestyles and burrow environments.

Chapter 7

Sulphide tolerance and detoxification in the mud-shrimp *Calocaris macandreae*

Introduction

Below a superficial oxidised zone (how superficial will depend on bioturbation rates, sediment granulometry and organic loadings), marine sediments are anaerobic, highly reducing and are rich with toxic compounds such as hydrogen sulphide (Theede *et al.*, 1969; Fenchel & Reidel, 1970). Hydrogen sulphide can reversibly inhibit aerobic respiration at nanomolar concentrations by binding closely with the heme of cytochrome c oxidase and thus interfering with mitochondrial electron transport (National Research Council, 1979). As hydrogen sulphide is such an integral component of many marine sediments many epibenthic animals are likely to be exposed to sulphide. Sulphide rapidly oxidises in the presence of oxygen without the need for biological catalysts and can, therefore, accumulate only in anoxic environments (Jorgenson, 1982). Thus, those animals that construct burrows or live within sediments are likely to be exposed to high concentrations of hydrogen sulphide.

At present, evidence shows that animals inhabiting sulphide-rich environments tend to have an increased tolerance of this toxic compound; for example Bagarinao and Vetter (1989) have shown that tolerance to sulphide was quite variable in a number of fish from a shallow-water marine habitat, the tidal marsh killifish (*Fundulus parvipinnis*) was very tolerant (up to 1.4mmol.l^{-1}), but the speckled sand-dab (*Citharichthys stimaeus*) from the open coast was intolerant of sulphide. Vetter *et al.* (1987) found the hydrothermal vent crab *Bythograea thermydron* was significantly more tolerant of sulphide than other non-vent crustaceans. Similarly, tolerance of sulphide has also been shown to influence the distribution of marine invertebrates (Vetter *et al.*, 1987; Bagarinao & Vetter, 1989; Vismann, 1990; Llanso, 1991; Levitt & Arp, 1991; Volkel & Grieshaber, 1992).

The detoxification of sulphide via enzymatic oxidation is an important aspect of sulphide tolerance. Such enzymatic oxidation has been demonstrated in meiofauna (Powell *et al.*, 1979), crustaceans (Vetter *et al.*, 1987), polychaetes (Oeschager & Schmaljohann, 1988; Vismann, 1990), molluscs and echiurans (Hand & Somero, 1983; Powell & Arp, 1989) and a variety of animals which harbour sulphur-oxidising bacteria either as ecto- or endo-symbionts (e.g. Childress *et al.*, 1991).

Other strategies used to avoid poisoning of aerobic respiration by sulphide have also been described for invertebrates living in sulphide-rich environments. The vascular haemolymph and coelomic fluid of the hydrothermal vent pogonophoran, *Riftia pachyptila*, contains extracellular

haemoglobins capable of binding free sulphide (Arp & Childress, 1983; Arp *et al.*, 1987). In this case, the sulphide-binding proteins function both in protection of aerobic respiration and in the transport of sulphide to the symbiotic sulphide-oxidising bacteria located in the trophosome of the pognophoran (Arp & Childress, 1983). More recently, the potential of anaerobic energy metabolism as a physiological strategy for sulphide tolerance has been examined in the bivalves *Macoma nasuta* and *M. secta* (Levitt & Arp, 1991), the priapulid *Halycryptus spinulosus* (Oeschger & Storey, 1990), the peanut worm *Sipunculus nudus* and the lugworm *Arenicola marina* (Volkel & Grieshaber, 1992).

A common infaunal species of the muddy sediments around the Scottish west coast is the thalassinid mud-shrimp *Calocaris macandreae* Bell. This species constructs a two-tiered burrow, with the upper primary level (10-15cm depth) consisting of a series of complex U-shaped tunnels (see Chapter 2). These tunnels connect, via vertical shafts, to a deeper secondary level (20-25cm) which, in the more developed system, contain circular galleries (Nash *et al.*, 1984). The burrow environment is often severely hypoxic since oxygen tensions as low as 15 Torr have been recorded from burrows constructed within laboratory mud-tanks (Anderson *et al.*, 1991). Mud-shrimps show a number of physiological traits that are probably adaptive responses to these conditions. The rate of oxygen consumption of *C. macandreae* is quite low compared with other decapods, respiratory independence is maintained down to very low oxygen tensions (Pc of 10 Torr) and the haemocyanin has a high oxygen affinity (Anderson, 1989).

Species exhibiting tolerance of hypoxia may also be tolerant of sulphide. This chapter, therefore, sets out to examine a number of questions, namely (1) what is the sulphide tolerance of the mud-shrimp *C. macandreae* and how does it compare to that of a non-burrowing (but occasionally burying) decapod from a sulphide-free habitat (*Liocarcinus depurator*) ? (2) which tissues have the capacity to oxidise sulphide and is this capacity similar amongst various decapods likely to be exposed to different levels of sulphide (e.g. burrowing and burying species) ? (3) what are the products of sulphide detoxification? (4) how do the concentrations of hydrogen sulphide and detoxification products vary *in vivo*, in different tissues, under normoxic and hypoxic conditions during (a) exposure to hydrogen sulphide and (b) following recovery in sulphide-free water.

The decapod species examined were divided into two comparable groups. The first consisted of those species that constructed burrows (the mud shrimp *Calocaris macandreae*, the Norway lobster *Nephrops norvegicus* and the crab *Goneplax rhomboides*). Accordingly, the depth and form of the burrows will probably influence the degree of exposure to sulphide each of these species will receive. The second group were non-burrow dwelling (i.e. they did not construct a permanent burrow), but may bury themselves for concealment. This distinction between burrowing and burying is important since behavioural strategies are likely to differ and different adaptations may be involved (see review by Atkinson & Taylor, 1988 and Taylor & Atkinson,

1989). Species considered from this second category were used that were *Cancer pagurus, Atelecyclus rotundatus* and *Liocarcinus depurator.* Non-burrow dwelling species which may periodically bury for concealment are unlikely to be exposed to sulphide to the same extent as burrow dwelling species.

Materials and methods

Measurement of hydrogen sulphide

Potentiometric analysis

Direct measurements of sulphide in sea water were made using a silver/sulphide electrode (94-4169, Russell) and a double junction reference electrode (90-0029, Russell) connected to a pH meter (PHM 83 Radiometer, Denmark). When the electrode is placed in a solution containing sulphide ions, the potential developed across the silver/sulphide membrane is measured against a constant reference potential. When a constant high background ionic strength is maintained, relative to the measured ion concentration, ionic activity is proportional to concentration. To achieve this, a sulphide anti-oxidant buffer (SAOB) was used. The SAOB buffer consisted of 0.2 $mol.l^{-1}$ ascorbic acid, 0.18 $mol.l^{-1}$ disodium EDTA and 2 $mol.l^{-1}$ sodium hydroxide made up in deoxygenated deionised water.

The anti-oxidant buffer also converts hydogen sulphide (H_2S) and hydrosulphide (HS^-) ions into divalent sulphide (S^{2-}) ions, which is the ion species detectable by the silver/sulphide electrode, as well as preventing 'poisoning' of the redox potential by oxygen diffusion (Green & Schmitker, 1974).

The electrodes were calibrated daily using serial dilutions of a stock solution of sodium sulphide (100g $Na_2S.9H_20$ in 100ml deoxygenated deionised water). The exact concentration of the sodium sulphide solution was determined by potentiometric titration with $0.1 mol.l^{-1}$ lead perchlorate (or a solution at least 10-20 times more concentrated than the sulphide solution) using the electrode pair as end point indicators.

The concentration of sulphide, C, was calculated from the equation (Russell)

$$C = 3206 \left(Vt / Vs \right)$$

where; C is the concentration of sulphide (ppm), Vt is the volume of titrant at the end point (ml), Vs is the volume of sulphide standard (10ml).

All standards and samples were diluted in proportion 1:1 with SAOB prior to measurement (20°C).

Spectrophotometric analysis

Gilboa-Garber (1971) described a basic method for detecting sulphide by measuring the formation of methylene blue (3,7-bis(dimethylamino)phenothiazin-5-iumchloride) following the reaction between sulphide and acidic diamine (p-phenylene diamine) with ferric chloride as catalyst, and using zinc acetate for in situ separation and fixation of sulphide. The method described here is basically that of Gilboa-Garber (1971) with the modifications of A. Schneider (pers. comm.).

To 2ml of 0.2 mol.l^{-1} zinc acetate and 0.5ml 1.5 mol.l^{-1} sodium hydroxide either a) 30µl of sample and 970µl of deionised water (sample H_2S concentration in the range 0.8-3 mmol.l^{-1}) or b) 300µl sample and 700µl deionised water (sample H_2S concentration 0-0.8mmol.l^{-1}) was added. The resulting mixture was shaken and then 0.5ml of 0.3% N,Ndimethyl-1,4-phenlyen-diamin in 5.5 mol.l^{-1} HCl and 0.5ml of 11.5mmol.l^{-1} $FeCl_3$ in 0.6 mol.l^{-1} HCl was added. The solution was allowed to react in the dark for 45min at 20°C and the absorbance was then measured at 670nm (PU8720 spectrophotometer, Philips). The diamine solution is stable for several months at 4°C (A. Schneider pers. comm.), therefore each time a new batch was made it was calibrated with sulphide stock solution for sample volumes of 30 and 300µl. The concentration ot each calibration standard was determined using iodometric titration.

Iodometric titration

Iodometric titration was used to standardize both the sulphide working and stock solutions (Fonselius, 1983). The basis of the reaction involved the reduction of iodine, by sulphide, to iodide which was subsequently titrated with sodium thiosulphate.

To 200ml conical flasks, 10ml of deionised water and 1-2g potassium iodide were added. This was followed by the addition of 10ml 16.67. 10^{-4} mol.l^{-1} potassium iodate (having an oxidation concentration of 0.01 mol.l^{-1} electrons) and 1 ml sulphuric acid (1 + lv/v). To this solution either 50ml of sulphide working solution or 50ml of deionised water was added. The flasks were stoppered and left for 10min, after which time their contents were titrated using 0.02 mol.l^{-1} sodium thiosulphate and 1ml starch (1+100 w/v) as an indicator. The thiosulphate solution was standardized prior to use (Fonselius, 1983). The concentration of sulphide was calculated using equation (2).

$$C = 10.f.(A-B)/50 \text{ mol.l}^{-1} \quad \text{.....(2)}$$

where; A is the mean titre of 3 solutions without sulphide (ml), B is the mean titre of 3 solutions containing sulphide (ml) and f is the factor of thiosulphate solution (Fonselius, 1983).

Collection and maintenance of specimens

The nephropid lobster *Nephrops norvegicus* and the crabs *Goneplax rhomboides, Liocarcinus depurator, Atelecyclus rotundatus* and *Cancer pagurus* were all obtained from the Specimen Supply Department, U.M.B.S. Millport. Individuals were brought to the University of Glasgow Zoology Department where they were immediately sacrificed for the appropiate experiment. Specimens of *Calocaris macandreae* were collected at depths of >50m, with an Agassiz trawl in the Firth of Clyde, west coast of Scotland. Once captured, these shrimps were transported back to the Zoology Department where they were kept in small plastic containers in a recirculating sea water system, maintained at a constant temperature (10°C) and salinity (33‰).

Measurement of sulphide oxidising capacity

Tissue preparation

Samples of haemolymph, gill, hepatopancreas and abdominal or chelar muscle were taken from each individual and pooled. Each pooled tissue sample was homogenized (Ultra Turrax, T25) on ice in 20mmol.l^{-1} Tris/HCI buffer containing 0.1% Triton X-100 (pH adjusted to 7.95). The pooled samples were then centrifuged at 10,000x g for 10min (0°C) (MSE Prepspin centrifuge) to compact any cellular debris.

Sulphide oxidation activity

The sulphide oxidising capacity of the different tissues were measured using the method developed by Powell and Somero (1985). This spectrophotometric assay follows the reduction of the artificial electron acceptor benzyl viologen (BV), which is colourless when oxidised and purple when reduced. BV is rapidly oxidised, however, in the presence of oxygen and assays must therefore be performed under anoxic conditions (Peck, 1968). The reaction mixture, consisting of 800µl of 40mmol.l^{-1} glycine buffer (pH 9.0), 100µl of 2mmol.l^{-1} BV and 100µl of 0-5mmol.l^{-1} sodium sulphide, and 150µl of the tissue homogenate was kept separate in a Thunberg anaerobic cuvette. Each cuvette was then placed under vacuum and the reaction was initiated by gently shaking the cuvette combining the reaction mixture and tissue homogenate.

The rate of reduction of BV (at 20°C) was followed at 578nm using a spectrophotometer (PU8720, Philips). The concentrations of sulphide used were 5mmol.l^{-1} or less, since higher levels lead to an increase in the rate of non-enzymatic reduction (Powell & Somero, 1985). Blank (control) assays were performed by replacing the tissue extract with Tris/HCI buffer. Sulphide

concentrations were measured with a silver/sulphide electrode (94-4169, Russell) and a double junction reference electrode (90-0029, Russell) connected to a pH meter (PHM 83 Radiometer, Denmark) as described earlier. The oxidation activity at each sulphide concentration was calculated using an extinction coefficient of 8.65 absorbance units $mmol.l^{-1}$ cm^{-1} (McKeller & Sprott, 1979) and expressed as μmoles of substrate converted to product per minute per gram fresh weight of material at 20°C. Michaelis-Menton kinetics, in particular the maximal enzymatic activity (Vmax) for each tissue sample were calculated from Lineweaver-Burke plots (Fig.7.1).

Analysis of reduced sulphur compounds

Sample preparation

Specimens of *Calocaris macandreae* were dried thoroughly before sampling. Haemolymph samples were taken from the pericardium using a 100μl syringe (Hamilton) as described in Chapter 6. The carapace was removed, the hepatopancreas excised and samples of muscle tissue were taken from the abdominal region. Hepatopancreas and abdominal tissue was placed in 1.5ml Eppendorf tubes containing 100μl buffer. The buffer consisted of $200mmol.l^{-1}$ HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid) and $mmol.l^{-1}$ EDTA. The pH was adjusted to 8.0 since a high pH is required for the derivatization of reduced sulphur compounds (Vetter *et al.*, 1984).

The samples were then homogenized rapidly at 5°C. Fifty microlitres of buffer, 10μl of $55mmol.l^{-1}$ mBBR (monobromobimane; CalBiochem) and 50μl of the homogenate was added to an Eppendorf tube. The haemolymph did not require homogenization, therefore 50μl was added directly to 50μl buffer and 10μl $55mmol.l^{-1}$ mBBR. The resulting mixture was left to derivatize in darkness for 15min. Once derivatization was complete, 100μl acetonitrile was added and the sample heated to 60°C tor 10min to precipitate proteins. Three hundred microlitres of methanesulphonic acid ($25mmol.l^{-1}$) was added so that the initial aqueous phase of the HPLC gradient was not affected by the organic solvent.

The samples were centrifuged at 13,000x g for 10 min to compact any precipitated protein and then stored at -20°C prior to analysis. Storage at -4, -20 and -70°C for up to 20 months has been shown to have no affect on the sample flourescence (Fahey & Newton, 1987). Deionised water was substituted for the tissue sample to test for background fluorescence.

High-performance liquid chromotography (HPLC)

Reduced sulphur species are derivatized with monobromobimane (mBBr) to form flourescent

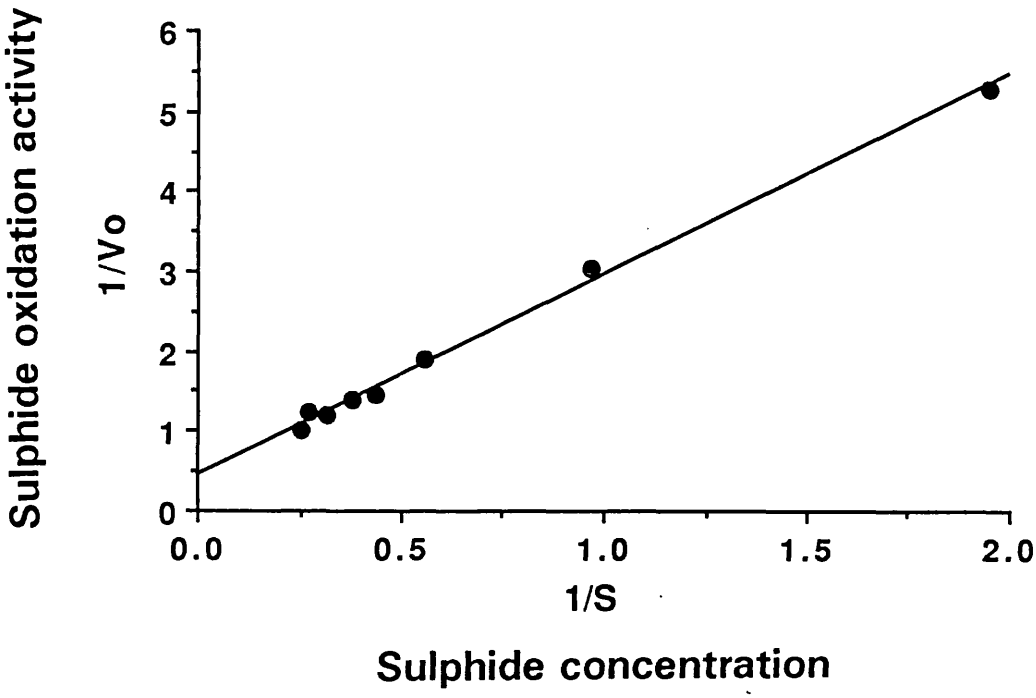


Fig. 7.1. *Goneplax rhomboides.* Lineweaver-Burke double reciprical plot of the sulphide oxidation activity (1/Vo) $\mu mol.min^{-1}.g^{-1}$) of the hepatopancreas as a function of sulphide concentration (1/S$mmol.l^{-1}.min^{-1}$). The maximal velocity (Vmax) calculated from the intercept 1/Vo = 0 was 2.18 $\mu mol.min^{-1}.g^{-1}$.

adducts which are then separated by reverse-phase HPLC (Newton *et al.*, 1981; Vetter *et al.*, 1989). Separation and analysis of the thiol adducts was performed using a LiChroCART (250-4, Merck, F.R.G.) 5μm HPLC column (250 x 4mm i.d.). The column was connected to a Gilson dual pump (302, Gilson) HPLC system with an in-line flourescence detector (121, Gilson). The fluorimeter had an excitation wavelength ot 305-395nm and a narrow band emission filter of 430-470nm. Samples, injected onto the column via a 20μl loop, were separated with an increasing hydrophobic gradient profile at a constant flow rate of lml min^{-1}.

The two buffers used were; Buffer A, 0.25% acetic acid made up in HPLC grade deionised water (pH 4.0); Buffer B, 100% methanol. Both buffers were filtered (0.22μm), deoxygenated and maintained under helium. The elution profile (Fig.7.2) was as follows: 0 to 5min: 8% Buffer B (isocratic); 5-15min: 40% Buffer B (linear gradient); 15-20min: 40% Buffer B (isocratic); 20-25min: 100% Buffer B (linear gradient); 25-30min: 100% Buffer B (isocratic); 30-35min: 8% Buffer B (linear gradient); 35-40min: 8% Buffer B (isocratic).

The following standards were run in order to establish retention times and to calibrate the column; reduced glutathione (GSH), sodium sulphite, sodium thiosulphate and sodium sulphide. HPLC system control, peak detection, intergration and analysis were performed using 715 HPLC System Controller Software (Gilson).

Sulphide tolerance

The sulphide tolerance of the mud-shrimps, *Calocaris macandreae*, and of the swimming crab, *Liocarcinus depurator* could not be measured using a conventional LC_{50} assay because of insufficient numbers of shrimp. Therefore, the tolerance was determined by exposing crustaceans to increasing sulphide concentrations over time (the stepwise increase method). The main problem inherent within this method is that it compounds both concentration and exposure time nevertheless it does provide a useful indicator of sulphide tolerance. Both species were exposed to increasing sulphide concentrations over time using a flow through system.

A single 10 litre plastic aquarium containg 9 litres aerated sea water (33‰; 10°C) was divided by a perforated plastic partition and 10 *L. depurator* were placed in one half ot the aquarium. Ten *Calocaris macandreae*, placed individually into perforated plastic tubes (60x25mm i.d.) and sealed with plankton gauze, were placed in the other half of the aquarium. The surface of the aquarium was covered with small plastic spheres (to reduce sulphide oxidation) and the water was mixed by the action of a stirrer bar and the aerator.

The sulphide concentration was raised at two hourly intervals from 50μmol.l^{-1} to 3.0mmol.l^{-1} over a 16h period. This was achieved by increasing the concentration of the sulphide stock solution ($Na_2S.9H_20$) flowing into the aquarium (ca. 10ml.min^{-1} sea water and 1ml.min^{-1} sulphide stock solution). The response of the mouthparts and antennae to tactile stimulation was assessed

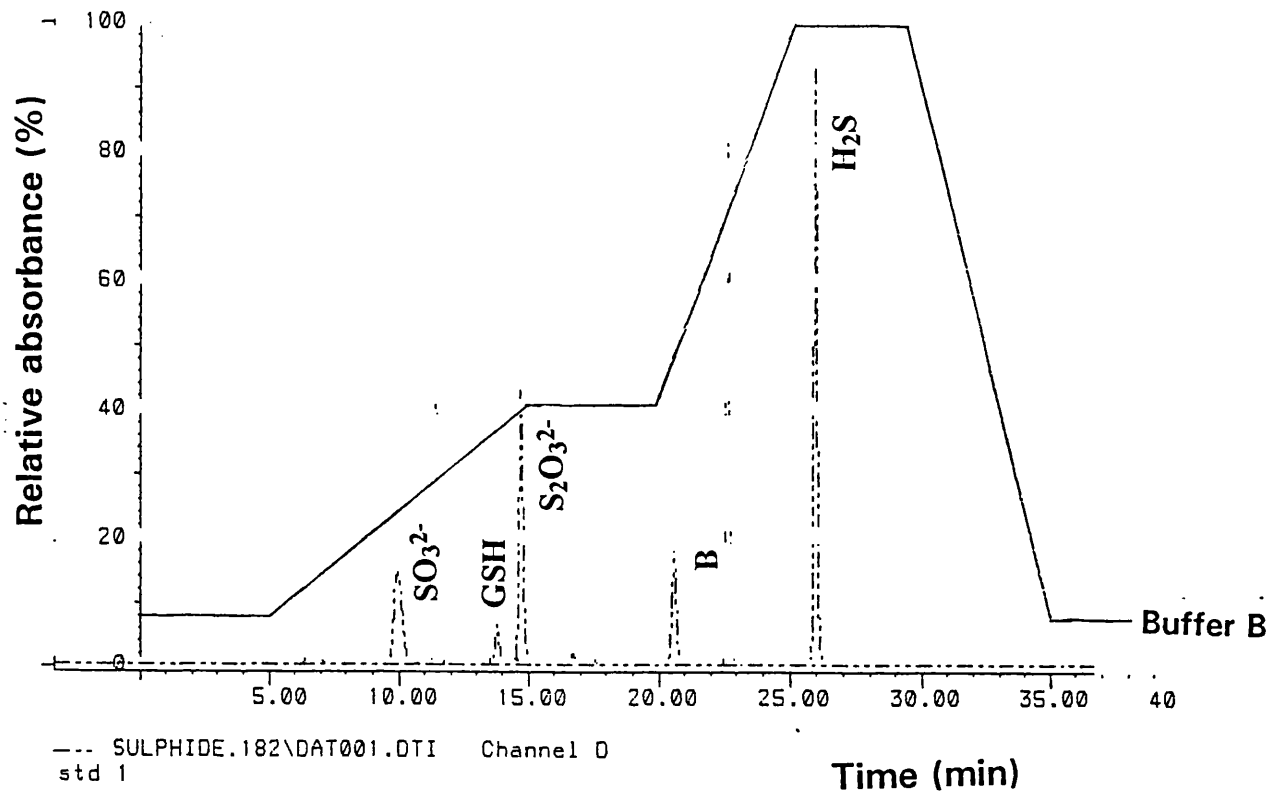


Fig. 7.2. Chromatograms of sulphur standards derivitized with monobromobimane. The flouresence adducts formed were separated by reverse-phase HPLC using a 250x4mm i.d., 5μm column eluted at a flow rate of 1.0ml.min^{-1}. The elution profile of the buffer system, 0.25% acetic acid (Buffer A) and 100% methanol (Buffer B), is also shown on the chromatogram. The standards were: hydrogen suphide (H_2S), bimane (B), Thiosulphate ($S_2O_3^{2-}$), reduced glutathione (GSH) and sulphite ($S_2O_3^{2-}$).

for both species every hour. The criterion of death used was cessation of movement of the mouthparts and antennae following tactile stimulation.

A control experiment (sea water only) was run at the same time as the treatment (receiving sulphide and sea water). The sulphide concentration was measured every 30min using a silver/sulphide electrode. In addition, 50µl water samples were derivitised with monobromobimane and the sulphide concentration determined using reversed-phase HPLC.

Long term survival

The response of *Calocaris macandreae* to long term exposure to a constant sulphide concentration under hypoxic conditions was assessed using a static system. Shrimps were placed individually into artificial burrows namely, perforated plastic tubes (60mm x 25mm i.d.) sealed with plankton gauze. The artificial burrows were placed into a plastic aquarium which contained 9 litres of hypoxic (Po_2 <30 Torr; 20% air saturation) sea water . A sulphide concentration of 1mol.l^{-1} was maintained by constantly adding sulphide stock solution into the tank using a peristaltic pump. The surface of the tank was covered with small plastic spheres to help reduce oxygen ditfusion from the air. The oxygen tension of the water was maintained below 30 Torr by bubbling N_2 through the water.

Three shrimp were removed from the aquarium at 0, 1, 3, 6 and 12h intervals and haemolymph, hepatopancreas and abdominal tissue samples were taken and the sulphur compounds derivatized with monobromobimane as described earlier. The time taken between removal of an individual and complete fixation of the tissues, with bimane, was less than 3min. Triplicate 30µl water samples were taken at each of these sampling times and the concentration of sulphide measured using the methylene blue method (Gilson-Garboa, 1971).

The sulphide concentration in the sea water declined slightly over the duration of the experiment as a result of oxidation. Even so, the method used was quite good at maintaining the sulphide concentration: the mean (±S.D.) sulphide concentration over the 12h period was 0.93 ± 0.13mol.l^{-1} (n=4). At each sample time the oxygen tension of the sea water was monitored using a polarographic oxygen electrode (1302, Strathkelvin Instruments) connected to an oxygen meter (781, Strathkelvin Instruments). To prevent the effects of electrode 'poisoning', the length of time that the electrode was exposed to solutions containing sulphide was kept to a minimum.

Exposure to sulphide under hypoxic and normoxic conditions

Specimens of the mud-shrimp, *Calocaris macandreae* were contained individually within artificial burrows, as described previously. A number of these burrows were distributed between four different treatments: a) normoxia (Po_2>140 Torr); b) hypoxia (Po_2<30Torr); c) normoxia +

lmmol.l^{-1} sulphide; d) hypoxia + 1mmol.l^{-1} sulphide.

Plastic aquaria (10 litre) were filled with sea water (33‰;10°C) and the surface was covered with small plastic spheres to reduce oxygen diffusion. For the hypoxic treatments, N_2 was bubbled through the sea water via a diffuser until the Po_2 reached < 30 Torr. The water in the normoxic treatments was aerated using an air pump and diffuser stone. Sulphide was added to the appropriate treatments via a 10 litre stock solution of sodium sulphide. With the aid of a peristaltic pump, a water flow of lml.min^{-1} was passed through the tanks producing a relatively constant sulphide concentration (0.73 ±0.09mmol.l^{-1}; n=5).

The sulphide concentration of the sea water was monitored every 2-3h using the methylene blue method (Gilson-Garboa, 1971). The oxygen tension was monitored as described previously. Shrimps placed into the treatments were exposed for 12h. After this period haemolymph, hepatopancreas and abdominal tissue samples were taken and the reduced sulphur compounds were derivatized with monobromobimane as described previously.

Recovery following exposure to sulphide

Following 12h exposure to a constant sulphide concentration (lmmol.l^{-1}) under hypoxic conditions, the removal (or oxidation) of sulphide from the tissues of *Calocaris macandreae* was examined in individuals recovering in either normoxic or hypoxic sea water.

Mud shrimps were placed individually into artificial burrows as described previously and exposed for 12h to hypoxic (Po_2<30 Torr; 20% air saturation) sea water (33‰; 10°C) containing 0.93mmol.l^{-1} sulphide (S.D. ±0.13; n=4). At the end of the 12h exposure, 10 individuals were transferred to a 9 litre plastic aquarium containing aerated (normoxic) sea water, and 10 individuals were placed into deoxygenated (hypoxic) sea water (Po_2<30 Torr; 20% air saturation). Two individuals were removed from each treatment (normoxic and hypoxic recovery) at 0, 1, 3, 6 and 9h intervals.

At each sampling time the concentration of reduced sulphur compounds in the haemolymph, hepatopancreas and abdominal muscle was measured. The oxygen tension in each of the treatments was monitored throughout the experiment.

Statistical analysis

Data were plotted and appeared normally distributed. Statistical analyses were carried out using analysis of variance, two-way analysis of variance, and Students *t*-test where appropiate (Sokal & Rohlf, 1981). Where the mean of a number of observations is given the deviation from the mean is expressed as standard deviation (SD). However, where the mean of a number of mean values are determined, the deviation is expressed as standard error (SE).

Results

Measurement of hydrogen sulphide

The accuracy of the potentiometric method of sulphide analysis was examined by calibrating the sulphide electrodes against known sulphide standards and then measuring the concentration of a series of working solutions; the sulphide levels of which had been previously calculated using iodometric titration. An example of the calibration graph obtained with the sulphide electrode is shown in Figure 7.3. The sulphide concentration of the working solutions, as determined from the calibration graph, was not significantly ($P<0.05$) different from the actual concentrations measured by iodometric titration (Fig.7.3). In addition there was good agreement ($P<0.05$) between the methylene blue method (using both 30µl and 300µl sample volumes) and the potentiometric means of detecting sulphide.

Sulphide oxidation activity

The sulphide oxidation activity of the tissues of burrowing and non burrow dwelling Crustacea showed Michaelis-Menton kinetics (Fig.7.1), suggesting that the sulphide oxidising capacity appears to be due to a sulphide oxidase enzyme. The sulphide oxidation activity of the tissues was calculated from the maximal catalytic rate (Vmax; Fig.7.1) and expressed as µmol sulphide oxidised min^{-1} g^{-1} fresh weight (Table 7.1).

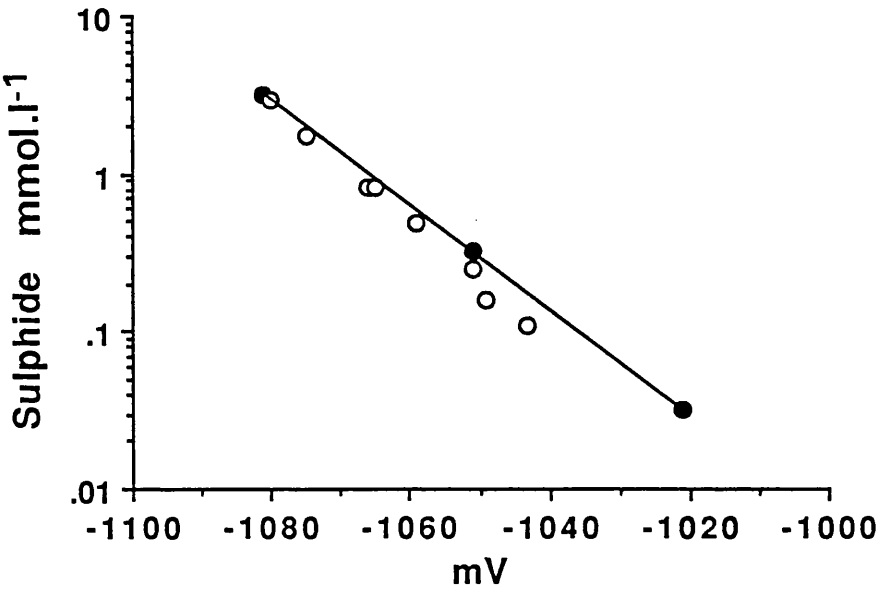
Sulphide-oxidising activity in the hepatopancreas tissue was significantly higher ($P< 0.05$) than in the other tissues tested. Background oxidation rates (basal levels), representing non-specific catalysis, of approximately 0.25 µmol min^{-1} g^{-1} have previously been recorded in invertebrate tissue (Powell & Somero, 1985; Vismann, 1990). Tissues, other than the hepatopancreas, did not exhibit sulphide oxidation rates that exceeded that of non-specific oxidation activity. Therefore, in those species in which a range of tissues were examined the sulphide oxidation activities appear to be restricted to the hepatopancreas. The oxidation activity ot the hepatopancreas was highest in *Cancer pagurus* (3.77µmol min^{-1} g^{-1}.) and *Nephrops norvegicus* (3.44µmol min^{-1} g^{-1}). The active swimming crab, *Liocarcinus depurator*, had the lowest hepatopancreas oxidation activity (0.62µmol min^{-1} g^{-1}) of all the species.

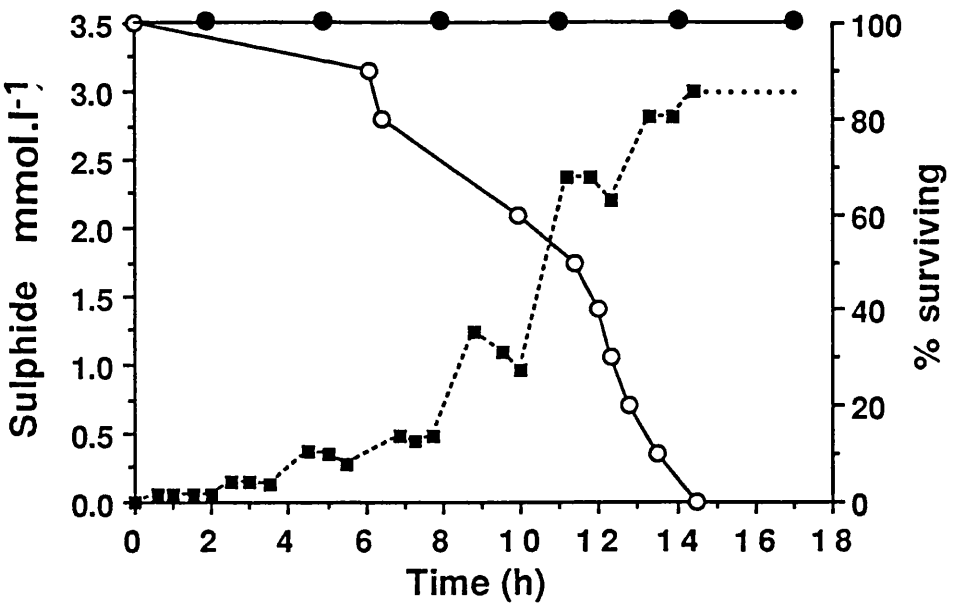
Sulphide tolerance

The results of the sulphide tolerance experiments are shown in Fig.7.4. More than 50% of individuals of the swimming crab, *Liocarcinus depurator*, survived for < 11h at a total sulphide concentration of 2.3mmol.l^{-1}, after which the crabs succumbed very rapidly to any further

Fig. 7.3. Potentiometric measurement of sulphide in sea water. The elctrodes were calibrated against three sulphide standards of known concentration (•). The mV readings of a number of sea water working solutions containing sulphide were measured and plotted against the sulphide concentration measured using iodometric titration (o). The accuracy of the sulphide electrode can be assessed from the degree of deviation of these measurements from the calibration line.

Fig. 7.4. Sulphide tolerance shown by *Liocarcinus depurator* (o) and *Calocaris macandreae* (•) measured using the stepwise-increase method. The concentration of sulphide in the sea water (■), measured using HPLC and sulphide electrodes, was raised at 2h intervals. The percentage of individuals surviving (from an original sample of 10) during the exposure period are shown.


Sulphide mmol.l-1
10
1
.1
.01
-1100
-1080
-1060
-1040
-1020
-1000
mV


Sulphide mmol.l-1
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
% surviving
0
2
4
6
8
10
12
14
16
18
Time (h)

increase in sulphide concentration. The mud-shrimp, *Calocaris macandreae*, survived for >16h at a final total sulphide concentration of 2.9mmol.l^{-1}.

At low sulphide concentrations (< 100μmol.l^{-1}) *L. depurator* exhibited a strange behaviour; individuals became very agitated at first and then grouped together in the centre of the aquarium to form a stacked pyramid. At concentrations >400μmol.l^{-1}, the crabs appeared very stressed and the muscles appeared to be locked in rigour, however, the mouthparts and antennae did respond to tactile stimulation.

Table 7.1. Sulphide oxidising activity in various tissues of burrowing and non burrow dwelling decapod Crustacea. Values are means ±SD for 3 assays. n; number of individuals used for pooled tissue samples.

Species (n)	Tissue	Sulphide oxidising activity (μmol.sulphide oxidised.min^{-1}.g^{-1}. fresh weight)
Burrowing crustaceans		
Nephrops norvegicus (6)	hepatopancreas	3.44 ± 1.46
	gill	0.04 ± 0.08
	muscle	0.12 ± 0.08
	blood	0.12 ± 0.02
Goneplax rhomboides (5)	hepatopancreas	2.01 ± 0.47
Calocaris macandreae (15)	hepatopancreas	1.75 ± 0.30
Non burrow dwelling crustaceans		
Cancer pagurus (5)	hepatopancreas	3.77 ± 2.78
	gill	0.09 ± 0.07
	blood	0.09 ± 0.01
Atelecyclus rotundatus (5)	hepatopancreas	1.94 ± 0.04
Liocarcinus depurator (7)	hepatopancreas	0.62 ± 0.61
	gill	0.25 ± 0.44
	muscle	0.23 ± 0.27
	blood	0.00

After removing the crabs exposed to the low sulphide concentrations and placing them into fresh, sulphide free sea water, they recovered within 10min. The torpor response shown by *L. depurator* made the assessment of death very difficult. However, once the mouthparts or antennae failed to respond to stimuli, observations showed that the individual was unlikely to recover once replaced in fresh sea water. This criterion was therefore used to assess the point of death.

Calocaris macandreae showed little response to sulphide at the lower concentrations, although at levels exceeding 1.0 mmol.l^{-1} individuals started to irrigate their Perspex 'burrows'

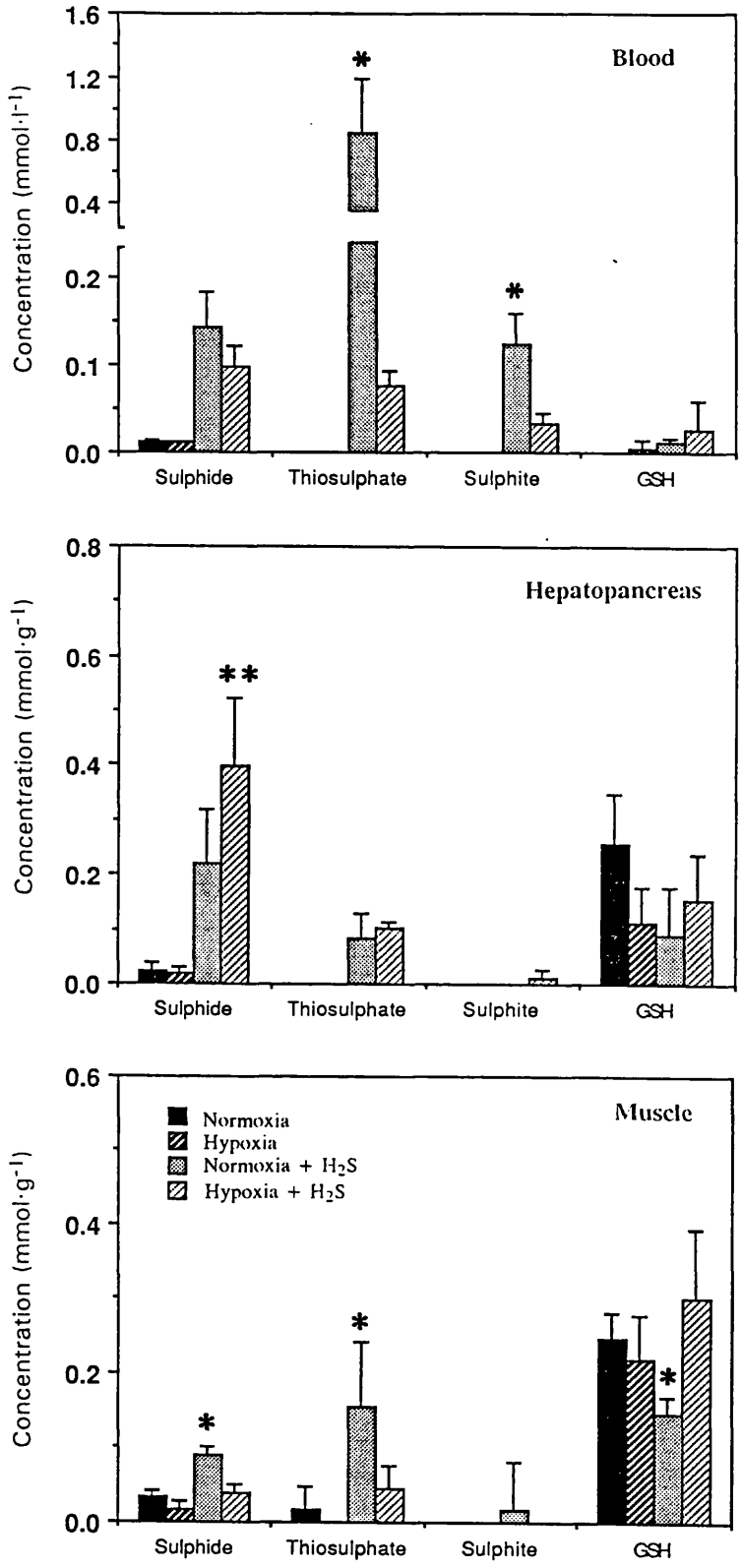


Fig. 7.5. *Calocaris macandreae.* Tissue concentrations of sulphide, thiosulphate, sulphite and GSH of shrimps exposed to normoxia (PO_2>140 Torr) (n=3), hypoxia (PO_2<20 Torr) (n=3), normoxia + 0.8mmol.l^{-1} H_2S (n=5) and hypoxia + 0.8 mmol.l^{-1} H_2S (n=5) at 10°C. Mean values and standard deviation bars are shown. The reduced thiols were analysed using reverse-phase HPLC. * significant difference from hypoxia + H_2S, ** significant difference from normoxia + H_2S.

more frequently. At higher sulphide concentrations the pleopod activity was maintained throughout the duration of the experiment.

Oxygen tension and sulphide exposure

The sulphide concentration in the aquarium during 12h hypoxic exposure (0.85 ±0.03mmol.l^{-1}; n=5) did not vary significantly from that recorded during the 12h period of exposure under normoxic conditions (0.73 ±0.09mmol.l^{-1}; n=5; $P<0.01$).

The concentrations of reduced sulphur species in the tissues of *Calocaris macandreae* following 12h exposure to sulphide in either normoxic+ H_2S or hypoxic+ H_2S conditions, are shown in Fig.7.5. The limit of thiol detection for this series of experiments was approximately 20μmol.g^{-1} fresh weight, therefore the natural levels of sulphide, thiosulphate and sulphite, following exposure to hypoxia or normoxia, in each of the tissues examined was negligible.

Levels of reduced glutathione (GSH) in the hepatopancreas and muscle did not vary significantly following normoxic or hypoxic exposure ($P>0.05$), but were higher than haemolymph GSH levels, which were negligible.

The concentration of sulphide in all the tissues of individuals exposed to normoxic + H_2S or hypoxic + H_2S was significantly higher ($P<0.05$) than in those specimens exposed only to normoxic or hypoxic conditions. There were no significant differences ($P>0.05$) in the mean haemolymph sulphide concentrations of individuals exposed to either hypoxia+ H_2S or normoxia+ H_2S. The sulphide levels in the muscle increased to a significantly higher level ($P<0.01$) in those specimens exposed to hypoxia+ H_2S, whereas the sulphide concentration in the hepatopancreas was higher in the hypoxic + H_2S treatment compared with the normoxic + H_2S group.

Thiosulphate accumulated in the hepatopancreas, haemolymph and muscle during exposure to sulphide. The highest concentrations were recorded in the haemolymph and abdomen during normoxic + H_2S exposure ($P<0.01$; Fig.5). Sulphite was recorded in trace amounts only in the hepatopancreas of individuals exposed to hypoxia + H_2S. The concentration of sulphite in the haemolymph was much greater, however, in normoxia + H_2S compared to hypoxia + H_2S exposed individuals ($P<0.01$). Sulphite was recorded only in the muscle of individuals exposed to normoxia + H_2S.

No significant differences ($P>0.05$) in the concentrations of GSH in the hepatopancreas or haemolymph were found between individuals in normoxia or hypoxia, with or without H_2S. GSH concentration in the muscle of the normoxic + H_2S group were significantly lower ($P<0.05$) than for the other treatments.

Those individuals exposed to sulphide in either hypoxic or normoxic situations, showed evidence of black deposits on the gills, around the mouthparts and at the base of the walking

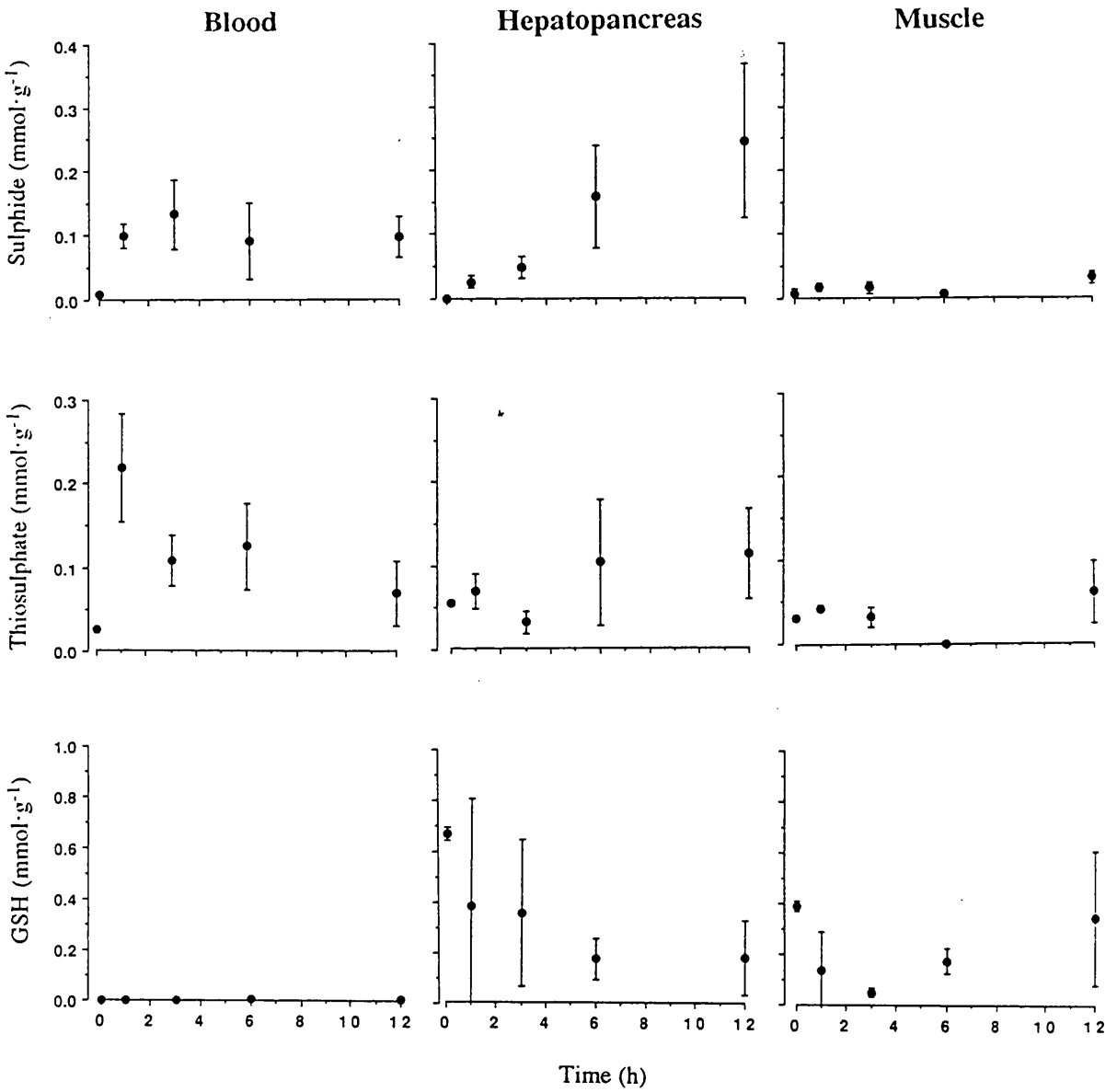


Fig. 7.6. *Calocaris macandreae.* Changes in tissue concentrations of reduced thiols of shrimps exposed to a constant sulphide concentration (0.93 ±0.07mmol.l^{-1}) under conditions of hypoxia (PO_2<20 Torr) at 10°C over a 12h period. Individuals were removed at successive time intervals and concentrations of reduced sulphur compounds in the tissues were determined using reverse-phase HPLC. Values given are means ±1SD of 3 individuals, except for the control (n=2) and 12h (n=5).

legs. In one extreme case (hypoxia + H_2S), the complete surface of the primary and secondary pereiopods, the majority of gills and the mouthparts were covered with black deposits. These deposits were found in greater amounts on those individuals exposed to sulphide under conditions of hypoxia and it was in these specimens that the hepatopancreas appeared light grey in colour.

Long term exposure to sulphide

Changes in the concentration of sulphide, thiosulphate, sulphite and GSH in the tissues of *Calocaris macandreae* exposed to a constant sulphide concentration of 0.93 ±0.06mmol.l^{-1} ;n=4) under conditions of hypoxia (Po_2<20 Torr) for a 12h period are shown in Fig.7.6. There was a significant increase (P<0.01) in the concentration of sulphide in the haemolymph of the shrimp during the first hour, thereafter there was no significant change (P>0.05) and a value of 0.10 ± 0.03 mmol.l^{-1} was recorded after 12h. The haemolymph thiosulphate concentration reached a maximum of 0.22 ±0.06mmol.l^{-1} after 1h, then fell significantly (P<0.05) to 0.11 ±0.03mmol.l^{-1} after 3h. Thereafter, there was no significant change (P>0.05) in the concentration of thiosulphate in haemolymph with time. Haemolymph sulphite and GSH concentrations remained at <40 µmol.l^{-1} throughout the experiment.

The concentration of sulphide in the hepatopancreas of *Calocaris macandreae* increased steadily over 12h to a maximum value of 0.25 ±0.12mmol.g^{-1}. Although there was little change in the thiosulphate concentrations in the hepatopancreas over the first 3h, the concentration of thiosulphate increased at 6h and thereafter was maintained at a final concentration of 0.11 ± 0.05mmol.g^{-1}. There was a trend for a decline in the hepatopancreas GSH concentration over the first 6h after which levels remained constant and a concentration of 0.18 ±0.14mmol.g^{-1} was recorded after 12h exposure. Hepatopancreas sulphite levels were < 40 µmol.g^{-1} after 1 2h exposure.

The levels of sulphide and thiosulphate in the muscle showed no significant changes (P<0.05) over the 12h period, however; the concentrations were <40 µmol.g^{-1}. The muscle GSH concentration decreased significantly (P<0.05) over the first 3h, thereafter the level of GSH increased steadily to a final value of 0.34 ±0.26mmol.g^{-1} after 12h exposure to sulphide. Muscle sulphite concentrations were <40 µmol.g^{-1} after 12h exposure.

Recovery following exposure to sulphide

Normoxic recovery

The tissue concentrations of thiols in shrimps exposed to sulphide under hypoxic conditions for 12h and then allowed to recover in normoxic sulphide free sea water are shown in Table 7.2.

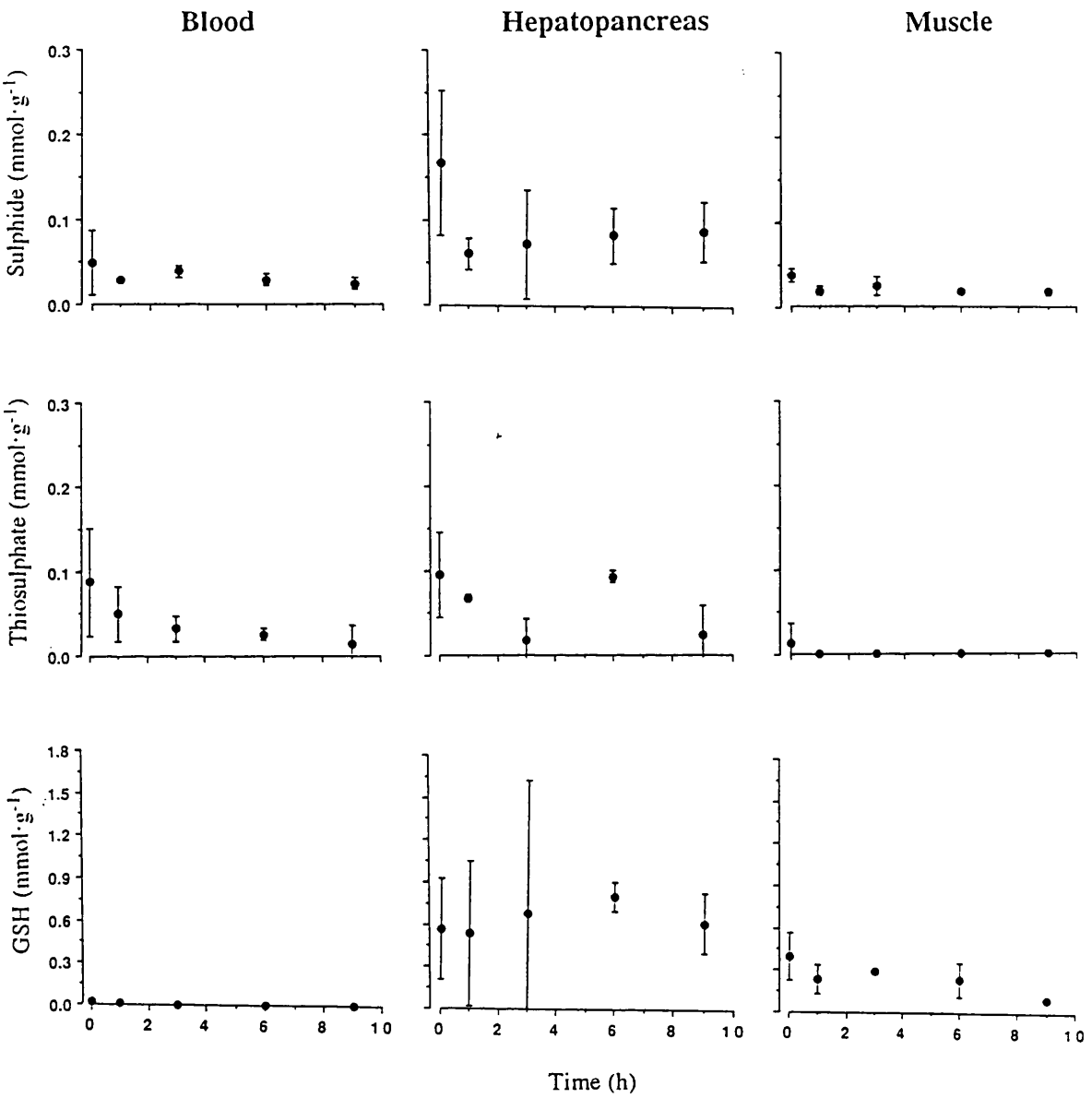


Fig. 7.7. *Calocaris macandreae.* Changes in tissue concentrations of reduced thiols of shrimps exposed to a constant sulphide concentration (0.73 ±0.06mmol.l^{-1}) under conditions of hypoxia (PO_2<20 Torr) at 10°C for 12h, and then subsequently placed into sulphide-free hypoxic (PO_2<20 Torr) sea water to recover. Individuals were removed at successive time intervals and concentrations of reduced sulphur compounds in the tissues were determined using reverse-phase HPLC. Values given are means ±1SD of 2 individuals, except for 12h exposure to sulphide (time 0) where n=3.

However, during the experiment three individuals died. The results should be treated with some caution. They are therefore presented in tabular form only. The concentration of sulphide in the haemolymph decreased from 0.05 ±0.04mmol.l^{-1} following 12h exposure to sulphide to 0.018mmol.l^{-1} after 9h recovery. Haemolymph thiosulphate levels decreased steadily during recovery in normoxic sea water. Sulphite was not detected in the haemolymph following 12h exposure and GSH was maintained at <20μmol.l^{-1} during both exposure and recovery.

The concentration of sulphide in the hepatopancreas initially decreased after lh from 0.17 ± 0.09 to 0.04 ±0.0l mmol.g^{-1} but this was followed by a transient increase over the following 5h. However, because of the large standard deviations, and the individual observation after 9h recovery, the results are difficult to interpret. The presence of thiosulphate and sulphite in the hepatopancreas was recorded only in individuals sampled after 6h recovery, and GSH levels in both the muscle and hepatopancreas did not differ significantly from the values obtained after 12h exposure to sulphide. Similarly, the concentration of sulphite in the haemolymph changed little throughout the experiment. In contrast, the thiosulphate levels in the muscle increased during recovery and a final value of 0.22 mmol.g^{-1} was recorded in the individual sampled at 9h recovery.

Hypoxic recovery

The tissue concentrations of reduced sulphur compounds in *Calocaris macandreae* exposed to a constant sulphide concentration (0.73 ±0.06mmol.l^{-1}) in hypoxic sea water for 12h, and then subsequently placed into hypoxic sulphide-free sea water to recover, are shown in Fig.7.7.

It is interesting to note that the concentrations of sulphide and thiosulphate found in the haemolymph and hepatopancreas following 12h exposure to sulphide under hypoxic conditions (Fig.7.6) were not significantly different ($P<0.05$) from those values recorded under similar conditions in a previous experiment where the sea water sulphide concentration was 0.93 ±0.07 mmol.l^{-1} and the Po_2<20 Torr.

Following 12h exposure to sulphide the haemolymph sulphide levels did not change significantly($P<0.05$) during the recovery period, and a concentration of 0.02 ±0.0lmmol.l^{-1} was recorded after 9h recovery.Although there was an apparent decrease in the concentration of thiosulphate in the haemolymph of the shrimps during the 9h recovery period, these changes were not statistically significant. The haemolymph GSH levels remained below 15 μmol.l^{-1} both following 12h exposure to sulphide and during the recovery period.

The concentration of sulphite in the hepatopancreas after lh recovery decreased significantly ($P<0.05$) from 0.10 ±0.04 to 0.07 ±0.01mmol.g^{-1} and thereafter did not change significantly during the rest of the recovery period. The levels of thiosulphate in the hepatopancreas decreased following 3h recovery in hypoxic sulphide-free sea water, but then increased significantly

($P<0.05$) after 6h from 0.02 ±0.02 to 0.10 ±0.01mmol.g^{-1} and then decreased again to the previous 3h concentration. There was no significant change ($P>0.05$) in the GSH concentration of the hepatopancreas tissue during recovery and a concentration of 0.62 ±0.21mmol.g^{-1} was recorded after 9h.

Muscle sulphide and thiosulphate concentrations were <20 μmol.g^{-1} following 12h exposure to sulphide, following 9h recovery in hypoxic sulphide-free sea water the level of GSH was not significantly different from these initial concentrations. The concentration of GSH in the muscle decreased significantly ($P<0.05$) during the recovery period from an initial value of 0.34 ±0.17 to 0.09 ±0.01mmol.g^{-1} after 9h. Sulphite was detectable only in the haemolymph and the hepatopancreas (<30 μmol.g^{-1}) following 12h exposure to sulphide under hypoxic conditions.

Table 7.2. Calocaris macandreae. Tissue thiol concentrations of shrimps exposed to a constant sulphide concentration (0.93 ±0.07mmol.l^{-1}, n=4) under hypoxia (PO_2<20 Torr) for 12h and subsequently placed into sulphide-free normoxic sea water (PO_2>140 Torr) at 10°C. Individuals were then removed at successive time intervals and the tissue thiol (sulphide, thiosulphate, sulphite and GSH) concentrations were determined using reversed phase HPLC. All values are expressed as means and, where appropriate, standard deviations are shown in parenthesis; n, number of observations; ND, not detectable.

	Concentration (mmol.l^{-1})			
	Sulphite	GSH	Thiosulphate	Sulphide
12h exposure (n=3)				
Abdomen	ND	0.339 (0.173)	0.013 (0.023)	0.036 (0.008)
Haemolymph	0.022 (0.010)	0.015 (0.013)	0.088 (0.064)	0.049 (0.038)
Hepatopancreas	0.029 (0.008)	0.565 (0.361)	0.095 (0.051)	0.167 (0.085)
1h recovery (n=2)				
Abdomen	ND	0.277 (0.144)	ND	0.016 (0.004)
Haemolymph	ND	0.013 (0.018)	0.018 (0.016)	0.010 (0.000)
Hepatopancreas	ND	0.469 (0.409)	ND	0.036 (0.013)
3h recovery (n=2)				
Abdomen	ND	0.266 (0.047)	ND	0.016 (0.001)
Haemolymph	ND	0.011 (0.002)	0.090 (0.054)	0.027 (0.006)
Hepatopancreas	ND	0.353 (0.277)	ND	0.322 (0.256)
6h recovery (n=2)				
Abdomen	ND	0.323 (0.038)	0.037 (0.001)	0.028 (0.007)
Haemolymph	ND	0.017 (0.009	0.053 (0.033)	0.022 (0.009)
Hepatopancreas	0.051 (0.041)	1.314 (1.485)	0.056 (0.003)	0.120 (0.040)
9h recovery (n=1)				
Abdomen	ND	0.556	0.223	0.071
Haemolymph	ND	ND	0.020	0.018
Hepatopancreas	ND	0.202	ND	0.184

Discussion

The measurement of sulphide and reduced compounds

A number of methods were employed to measure the concentration of sulphide and its oxidation products (a) spectrophotometric analysis where the formation of methylene blue was measured; (b) potentiometric measurement of hydrogen sulphide via an Ag/Ag^{2+} electrode - using the electrode, changes in the potential were measured during titration with $HgCl_2$; (c) iodometric titration was used to standardise the concentration of the stock and working sulphide solutions and (d) the bimane-HPLC method for detecting and measuring sulphide and its reduced compounds (thiols).

There was little difference in the compatability and reproducibility between the spectrophotometric and potentiometric analysis of sulphide over a range of 0.02 to 3 $mmol.l^{-1}$ sulphide. Each method of sulphide analysis has its own merits and disadvantages. The main disadvantage of the spectrophotometric method was that it was very time consuming and a linear relationship between concentration and optical density existed only between 0-0.6 O.D. The potentiometric method is relatively quick to use and may also be used to measure polysulphides, thiols, sulphite and thiolosulphate. However, there is no method of sample preservation and to measure sulphide the pH must be adjusted to 13 (Vetter *et al.*, 1989). The bimane-HPLC method may not be as sensitive but is highly reproducible and, following derivitization, the samples may be stored for longer than one year with no loss of stability (Vetter *et al.*, 1989). In addition, once derivitized it is simple to determine the concentration of a range of reduced sulphur compounds. The merits of a number of methods used to measure sulphide and its reduced compounds have been reviewed by Vetter *et al.*(1989).

Sulphide tolerance

The mud-shrimp, *Calocaris macandreae* survived sulphide concentrations in excess of $2.9mmol.l^{-1}$ when concentrations were increased from zero over 16h. In contrast, only 50% of individuals of the swimming crab *Liocarcinus depurator*, survived for less than 11h at a total sulphide concentration of $2.3mmol.l^{-1}$. At sulphide concentrations of less than $100\mu mol.l^{-1}$ *L. depurator* became agitated and exhibited an escape response. The muscle rigour shown by this species at concentrations greater than $400\mu mol.l^{-1}$ was very similar to the response of the sulphide intolerant crab *Cancer antennarius*, typically found in sulphide-free environments, where morbidity occurred at sulphide concentrations of $300\mu mol.l^{-1}$ (Vetter *et al.*, 1987).

The difference in sulphide tolerance shown by these two species appears to be in accord with the concentration of sulphide likely to be encountered in their environment; data from the

Clyde River Purification Board (unpubl. obs.) have shown that in the Clyde Estuary sediment sulphide concentrations of 40 mg.S.Kg^{-1} (12.4μmol.l^{-1}) 19km seaward of the tidal limit and values of 8790 mg.S.Kg^{-1} (2.7mmol.l^{-1}) at 7km from the tidal limit were recorded. Similarly, an increased tolerance to sulphide, consistent with the higher concentration experienced in the habitat, has been shown for crustaceans (Vetter *et al.*, 1987), shallow-water marine fish (Bagarinao & Vetter, 1989) and polychaetes (Vismann, 1990) and Levitt & Arp, 1991). Since sulphide can influence the ecological distribution of species (e.g. Vismann, 1990; Levitt & Arp, 1991; Vismann, 1991; Vogel & Grieshaber, 1992), differences in sulphide tolerance between species and the ability to exploit a potential toxic sulphide-rich environment will depend on the development of defensive or protective mechanisms to sulphide.

Defense strategies against sulphide toxicity

In their review, Vetter *et al.* (1991) proposed a number of passive and active mechanisms to prevent sulphide poisoning:

1. Exclusion of sulphide from the body either by impermeability or protection by mucus and/or sulphide oxidising bacteria.
2. Symbiotic association with chemoautotrophic bacteria capable of sulphide oxidation.
3. Possession of sulphide insensitive cytochrome-c-oxidase systems.
4. Reliance on anaerobic metabolism.
5. Oxidation to a less toxic form, which may be enzymatic or non-specific detoxification.
6. Possession of sulphide-binding proteins with subsequent unloading on return to low sulphide levels or at sites of detoxification.

Some of these strategies have been recorded, others remain hypothetical and the authors noted that more than one adaptation may occur in an individual. There is no current evidence to suggest that animal body surfaces are, or may be, impermeable to sulphide (Vetter *et al.*, 1991). In crustaceans at least, the exoskeleton covering the body surface may prevent the entry of sulphide into the tissues, but sulphide does diffuse freely through the areas where the chitin layer is reduced, for example over the gills or at the arthrodial membranes.

The results from this study suggest that sulphide could not be excluded from the body regions of the burrowing shrimp *C. macandreae*. When exposed to 800μmol.l^{-1} sulphide under both hypoxic and normoxic conditions, *C. macandreae* could not exclude sulphide from any of the body regions examined (Fig.7.5). However, the sulphide concentrations within the haemolymph, hepatopancreas and muscle remained far below external concentrations.

Similarly, other Crustacea have been found to be permeable to sulphide, for example at low concentrations of sulphide Vismann (1991) found an increase in external sulphide levels was

instantaneously paralleled in the haemolymph of the isopod *Saduria entomon*. Even so, Vetter *et al.* (1987) suggest that the thick exoskeleton of the Crustacea may reduce the rate at which sulphide is taken up, and it could also explain the difference in the distribution of potential sulphide-oxidising activity in the tissues of crustaceans compared to other invertebrates which lack an exoskeletal covering.

In other marine invertebrates the exclusion of sulphide may be achieved by a mucus layer or by the presence of ectosymbiotic bacteria (e.g. *Halicryptus spinulosus*, Oeschger & Schmaljohann, 1988). The nematode *Eibostrichys dianeie* has mucus containing sulphide-oxidising bacteria covering the body surface (Powell *et al.*, 1979). An epicuticular layer of sulphide-oxidising bacteria has also been described for polychaetes, for example *Alvinella pompiejana*, an inhabitant of hydrothermal vents (Laubier *et al.*, 1983), and in the oligochaetes *Phallodrilus leukdermatus* and *P. planus* the sulphide-oxidising bacteria are internal, located beneath the cuticle, but not intracellular (Giere, 1981; Felbeck *et al.*, 1983). Bacterial symbionts are important to some marine invertebrates living in sulphide-rich environments as they protect the respiratory capacity of the host, as in *Riftia pachyptila*, and they can also provide a substantial proportion of the host's requirements for reduced carbon compounds (see review by Vetter *et al.*, 1991).

Although levels of cytochrome c oxidase were not examined in this study, it is unlikely that *C. macandreae* has a sulphide insensitive cytochrome oxidase system. Bagarinao and Vetter (1989) examined both the cytochrome c oxidase activity and 50% inhibition constant for sulphide (Ki) for six different estuarine fish species. These authors found that those species which differed in sulphide tolerance did not differ in cytochrome c oxidase activity and the Ki's were in the range 31-514nmol.l^{-1} sulphide. Similarly, cytochrome c oxidase activities recorded for a range of invertebrate species from sulphide-rich hydrothermal vent habitats were qualitatively and quantitatively similar to related species from sulphide-free habitats (Hand & Somero, 1983). The Ki values for sulphide of the cytochrome c oxidase for all of these species were in the nanomolar to micromolar range, similar to values reported for mammalian cytochrome-c-oxidases (National Research Council, 1979). This suggests that the capacity for aerobic metabolism in species from sulphide-rich and sulphide-free habitats are similar, and in the absence of a sulphide insensitive cytochrome c oxidase system, low levels of sulphide are likely to poison aerobic respiration.

Few species are, however, known to utilize anaerobic metabolism in defense against exposure to sulphide (Vetter *et al.*, 1991). Glycolytic metabolic rate depression in response to sulphide exposure has been demonstrated in the priapulid *Halicryptus spinulosus* (Oeschger & Storey, 1990), and the greater sulphide tolerance of the bivalve *Macoma nasuta* compared with *M. secta* may be due to regulation of basal metabolic rate (Levitt & Arp, 1991).

For motile species, exposure to sulphide is unlikely to be a problem as they can simply

move from the area. The swimming crab, *Liocarcinus depurator*, showed this response when exposed to low concentration of sulphide in a small aquarium. Because the crabs could not escape from the sulphide they formed a pyrimidal stack with the more aggressive crabs remaining near the apex. This unusual response is probably indicative of a form of behavioural escape response expressed under 'artificial' conditions. Many species, though are not motile or are restricted by their habitat, e.g. burrowing forms.

Although *C. macandreae* is able to survive up to 43h of anoxia (Anderson, 1989), it is unlikely that reliance on anaerobic metabolism is the single most important protective mechanism against sulphide exposure. Anaerobic glycolysis in response to sulphide stress may be a viable short term option but since any sulphide that enters the body of *C. macandreae* is rapidly oxidised it is more likely that this species, like many other sulphide-tolerant species, utilizes some active mechanism of sulphide detoxification.

Sulphide oxidation activity

Of the various tissues examined in each of the crabs only the hepatopancreas showed a capacity for sulphide oxidation that was higher than background (basal) levels (< 0.25mmol.l^{-1}mol.min^{-1}.g^{-1}; Powell & Somero, 1985). The sulphide oxidising activities of the hepatopancreas differed among species and may reflect the different burrowing or burying stratagies and hence ambient sulphide concentrations that species are likely to be exposed to.

Although the rates of sulphide oxidation measured using the benzylviologen method are quantitatively similar to those recorded for hydrothermal vent crustaceans (Vetter *et al.*, 1987), they are much lower than that recorded for the isopod *Saduria entomon* in which hepatopancreas sulphide oxidation activity was found to be *ca.* 104μmol.min^{-1}.g^{-1} (Vismann, 1991). Even though such high oxidation rates were recorded for *S. entomon*, Vismann (1991) measured a sulphide oxidation rate of 0.78 μmol.min^{-1}.g^{-1} in the hepatopancreas of *Carcinus maenas* from a sulphide-free habitat in the same study, which corresponds well with the value for *Liocarcinus depurator* (0.62μmol.min^{-1}.g^{-1}) and activities measured for other brachyurans from similar, sulphide-free habitats (Vetter *et al.*, 1987).

Sulphide oxidation activity has also been measured in a number of fish species, the highest activity was correlated with tissue having a high haemoglobin content (Bagarinao & Vetter, 1989). Vismann (1990) found greater sulphide oxidising activity in the haemolymph of the polychaetes *Hediste (as Nereis) diversicolor* (2.2μmol.min^{-1}.g^{-1}) than the less sulphide tolerant species *Neanthus (as Nereis) virens* (0.62μmol.min^{-1}.g^{-1}), and sulphide oxidation rates of 3μmol.min^{-1}.g^{-1} have been recorded in the haemolymph and stomach of the echiuran worm *Urechis caupo* (Powell & Arp, 1989). In the bivalve *Solemya reidi*, a gutless clam from sulphide-rich habitats, the gill sulphide oxidation activity has been found to be *ca.* 6μmol.min^{-}

$^{1}.g^{-1}$ (Powell & Somero, 1985).

Despite the clear differences in sulphide oxidising activity measured between species from sulphidic compared to sulphide-free habitats, the determination of sulphide oxidation rate using the *in vitro* benzylviologen method has been criticised a number of times (Vetter *et al.*, 1987; Bagarinao & Vetter, 1990; Vismann, 1991). The benzylviologen method is considered to have little *in vivo* physiological significance (Bagarinao & Vetter, 1990; Vismann, 1991) since (a) the assay is performed under anoxic conditions, making the production of oxidation compounds impossible; (b) unrealistically high concentrations of sulphide are used in the *in vitro* assay (5mmol.l^{-1}) which are known to inhibit mitochondrial sulphide oxidation and (c) the presence of inhibitors in the crude tissue homogenates used may affect the determination of Km. However, even though the sulphide oxidation rates estimated using this *in vitro* assay have been criticised they still provide a useful qualitative indicator of the relative sulphide oxidising potential and tissue localization of detoxification processess (Vetter *et al.*, 1991). Even so, to confirm any trends, more work is required on a wider range and number of species from different habitats.

Detoxification of sulphide

Calocaris macandreae exposed to hydrogen sulphide under hypoxic or normoxic conditions survives by detoxifying H_2S in the hepatopancreas. Analysis of sulphide oxidation products using HPLC showed that most of the H_2S removed by the hepatopancreas was oxidised to thiosulphate ($S_2O_3^{-2}$) and sulphite (SO_3^{-2}). Thiosulphate subsequently appeared at appreciable concentrations in the haemolymph and also in the muscle (Fig.7.5), whereas SO_3^{-2} was found primarily in the haemolymph.

The accumulation of $S_2O_3^{-2}$ as the major metabolic end product of H_2S oxidation has been shown for a variety of marine animals including crustaceans (Vetter *et al.*, 1987; Vismann, 1991), fish (Bagarinao & Vetter, 1989), bivalves (O'Brien & Vetter, 1990), sipunculids and polychaetes (Volkel & Grieshaber, 1992). It is probable that $S_2O_3^{-2}$ is the initial and major product of H_2S detoxification, because in terms of efficiency more H_2S can be removed per molecule of oxygen compared to the production of SO_3^{2-}- or SO_4^{-2}. This is particularly important if the oxygen comes from molecular oxygen rather than water as the detoxification of sulphide will place additional demands on aerobic metabolism; especially since areas rich in sulphide are by definition oxygen deficient (Vetter *et al.*, 1991).

Thiosulphate is also non-toxic, soluble and not strongly acidic (Bagarinao & Vetter, 1989) and is therefore advantageous as an end product of sulphide oxidation, since it is unlikely to interfere with other metabolic/physiological processes (e.g. thiosulphate has been shown to have no effect on the oxygen binding properties of the haemocyanin of the thalassinidean *Callianassa californiensis* (Sanders & Childress, 1992)). Although $S_2O_3^{-2}$ production is more efficient, its

limited ability to cross membranes (Holmes & Donaldson, 1969) means that the gills are not freely permeable with respect to $S_2O_3^{-2}$ and that to remove $S_2O_3^{-2}$ it would have to be oxidised further or slowly excreted, perhaps via the antennal gland (Vetter *et al.*, 1991) or digestive system (Vismann, 1991).

The rate of oxidation of H_2S was much greater in individuals that had been incubated under normoxic conditions. The levels of H_2S in the haemolymph remained similar, but the concentration of $S_2O_3^{-2}$ and SO_3^{-2} was significantly higher than under hypoxic conditions (Fig.5). Coupled with the lower amounts of H_2S in the hepatopancreas of *C. macandreae* exposed to H_2S under normoxia, the results suggest that the majority of H_2S is removed via an oxygen-dependent mechanism of sulphide detoxification.

The haemolymph of *Liocarcinus depurator*, *Nephrops norvegicus* and *Cancer pagurus* did not show any appreciable sulphide oxidation activity (Table 5.1). Similarly H_2S was found not to interact with the haemolymph of the vent crustacean *Bythogreae thermydron* (Childress *et al.*, 1987) and Vetter *et al.* (1987) found that in the same species the haemolymph showed no sulphide oxidation activity. Although it was not possible to collect sufficient haemolymph from *C. macandreae* to examine the sulphide oxidation activity, it is a reasonable assumption that the haemolymph of this species, like that of other crustaceans, would exhibit little oxidation activity. Therefore H_2S is likely to be transported in free form within the haemolymph and not to interact with the haemocyanin (Vismann, 1991), unlike haemoglobin or hematin which reacts with H_2S (Patel & Spencer, 1963; Powell & Arp, 1989). Since the high oxygen affinity haemocyanin of *C. macandreae* (Anderson, 1989) will not be affected by the H_2S, it is suggested that oxygen transported in the haemolymph (both physically dissolved and bound to the pigment) will serve as a primary oxidative defense mechanism.

The role of the haemolymph as an oxidising system has probably been under-estimated for many species as often those species that are sulphide tolerant are also hypoxia tolerant and typically have high affinity respiratory pigments. Indeed, the use of oxygen bound to the respiratory pigments for immediate sulphide detoxification was clearly illustrated for the sipunculid *Sipunculus nudus* and the polychaete *Arenicola marina* (Volkel & Grieshaber, 1992).

Specific sulphide detoxification has been demonstrated in the liver mitochondria of the sulphide tolerant killifish, *Fundulus parvipinnis*, (Bagarinao & Vetter, 1989) and isolated mitochondria of the bivalve *Solemya reidi* (O'Brien & Vetter, 1990). Interestingly, the mitochondrial oxidation of H_2S to $S_2O_3^{-2}$ was found to be obligately linked to mitochondrial electron transport and the electrons liberated in the oxidation of sulphide are used to generate ATP (O'Brien & Vetter, 1990).

So far, no mitochondrial sulphide detoxification scheme has been observed in crustaceans, although Vismann (1991) suggests such that a mechanism may occur in the muscles of *Saduria entomon* since $S_2O_3^{-2}$ accumulated *in vivo* in the muscles of these isopods exposed to 1.5mmol.l^{-}

[1] H_2S. In the present study, $S_2O_3^{-2}$ was recorded in the muscles of *C. macandreae* exposed to 12h H_2S under hypoxic conditions (Fig7.5) and $S_2O_3^{-2}$ was also seen to accumulate in the muscle (Fig.7.6). Although this may provide some evidence for *in vivo* mitochondrial sulphide detoxification, it is unlikely since $S_2O_3^{-2}$ occurring in the muscle may represent the export of $S_2O_3^{-2}$ from the hepatopancreas or haemolymph. Since sulphide oxidation by mitochondria only works below a concentration of 15μmol.l^{-1} H_2S and is inhibited at higher concentrations (Bagarinao & Vetter, 1991), it is unlikely that this detoxification process operates *in vivo* at the H_2S levels used in this study. Only further work on isolated mitochondria of *C. macandreae* will clarify the importance of the mitochondrial detoxification system as a mechanism against sulphide toxicity.H_2S

When exposed to H_2S in hypoxic seawater, *C macandreae* exhibited a rapid equilibration between the concentration of H_2S in the haemolymph and the seawater (Fig.6). The maintenance of a constant H_2S concentration in the haemolymph was probably due to immediate oxygen-dependent detoxification, this may account for the high $S_2O_3^{-2}$ concentration after 1h exposure prior to the lower equilibrium level and accumulation of H_2S in the hepatopancreas. Continuous exposure to sulphide may modify metabolic adaptation and probably haemolymph oxygen levels until a state of equilibrium is reached. The basis for this accumulation could be due to a number of processes such as sulphide-binding proteins, metallo-protein complexes, formation of disulphide bonds or binding to metallic ions (Vismann, 1991).

Sulphide binding proteins may act as a mechanism for temporarily removing sulphide from the haemolymph, thus preventing poisoning of aerobic respiration, until it can be delivered to a site which has a high sulphide oxidising capacity, e.g. mitochondria, or until oxygen levels are sufficient for oxygen-dependent detoxification. Sulphide-binding proteins in the haemolymph are best known in the pognophoran *Riftia pachyptila* and the hydrothermal vent clam *Calyptogena magnifica* (Arp & Childress, 1983; Arp *et al.*, 1987).

Vismann (1991) found that 40% of the H_2S removed from the hepatopancreas of *Saduria entomon* was achieved by oxygen-independent mechanisms and, in particular, he suggested that a metallic ion rechargeable buffer system was present in the hepatopancreas. The use of metallo-sulphide precipitation has previously been recorded in the priapulid *Halycryptus spinilosus* (Oeschger & Storey, 1990), in echinoderms (Buchanan *et al.*, 1980) and in bivalves (Levitt & Arp, 1991). Typically, a dark, usually black metallic-sulphide precipitate was observed in the tissue or on external surfaces following sulphide exposure. In the present study, black deposits were generally found around the mouth and associated appendages and also over the surface of the gills. This suggests that some form of precipitation may be used for excreting sulphide in *C. macandreae* or it may be just external accumulation as occurs in penaeids occuring in acid sulphate soil ponds.

In mice, oxidised glutathione (GSSG) has been shown to react with H_2S forming the

glutathione-protein mixed disulphides GSSH and GSH, so protecting mice from acute sulphide poisoning (Smith & Abbanat 1966). In normal tissue, GSH is the predominant form of the glutathione pool. It is likely, however, that on exposure to H_2S, GSSG will be reduced and this will appear as an increase in the GSH levels (Bagarinao & Vetter, 1989). In *C. macandreae* GSH levels did not change significantly following exposure to sulphide. Furthermore, hypoxia itself has been shown to reduce GSSG formation, which is seen as an increase in the levels of GSH. Although levels of GSH in *C. macandreae* were similar in muscles of individuals exposed to hypoxia and normoxia, Vismann (1991) showed an increase in GSH in the muscle of *Saduria entomon* following exposure to hypoxia but not in isopods exposed to hypoxia and sulphide. As GSH levels may also increase with tissue damage, it is very difficult to assess the importance of glutathione-protein mixed disulphides as a mechanism of sulphide detoxification.

Calocaris macandreae exposed to sulphide and hypoxia for 12h and then subsequently placed in normoxic or hypoxic sulphide-free water, showed rapid recovery and clearence of the products of sulphide detoxification (Fig.7.7). It would be interesting to examine in more detail the removal of sulphide and oxidation products following exposure and to attempt to determine the complete pathway of sulphide detoxification during recovery in this shrimp.

To summarise, the burrowing mud-shrimp *Calocaris macandreae* had a higher tolerance to sulphide (>16h at a final concentration of 2.9mmol.l^{-1}) than the swimming crab *Liocarcinus depurator* (50% survived < 11h at final concentration 2.3mmol.l^{-1}). In addition, *L. depurator* held at concentrations exceeding 400μmol.l^{-1} were extremly stressed and exhibited signs or rigour.

The sulphide oxidising capacity of a number of tissues was examined for a range of decapods. Only the hepatopancreas exhibited sulphide oxidising activity, which differed among species probably reflecting the different burrowing or burying strategies and ambient sulphide concentrations.

Using HPLC, the physiology of sulphide detoxification was examined in more detail for *C. macandreae*. Following exposure, it is assumed that sulphide primarily enters the haemolymph of *C. macandreae* via the gills. Detoxification of sulphide at this stage may occur in a variety of ways. Oxygen available in the haemolymph, both physically dissolved and that bound to haemocyanin, probably acts as the primary oxidative defence strategy which results in initial, rapid thiosulphate production.

The haemolymph then transports sulphide to the hepatopancreas prior to entering other body regions. Sulphide passing into the hepatopancreas may be further detoxified by specific or non-specific enzymes, or bound to metallic ions in an oxygen-independent reaction. The role of the specific mitochondrial oxidation system is, at present, unclear. The primary oxidation product, thiosulphate, accumulated in the haemolymph and it is suggested that it is excreted (as sulphite?) prehaps via the antenal gland or digestive system. Evidence of metallo-precipitation,

probably as a ferrous-sulphide, was possibly indicated by the black deposits covering the gills and mouthparts.

These results suggest that *C. macandreae* is adapted to a potentially sulphide-rich habitat, as seen by the high tolerance and capacity to oxidize sulphide to a less toxic form. Since thalassinideans typically construct burrows that extend deep into the sediment and have a deposit feeding lifestyle, it would be interesting to examine if the ability to tolerate and detoxify sulphide are important factors in the distribution and burrowing strategy of these mud-shrimps. One important facet of sulphide tolerance is to determine the concentrations and fluxes of sulphide that the species is likely to be exposed to. A thorough study of the measurement of sulphide (and reduced compounds) in the porewater, sediment and burrow water needs to be undertaken to provide a more solid basis for interpolating the degree of tolerance to sulphide shown by the burrowing shrimp *Calocaris macandreae*.

Chapter 8

General discussion and conclusions

General comments

The burrow morphology of the upogebiids *U. stellata* and *U. deltaura* was described in chapter 2. It was concluded that the burrows typically had a basic structure consisting of a U and a descending blind-ending shaft. The burrow shape provides for an efficient water flow path suitable for filter/suspension feeding purposes. Burrow irrigation profiles were described for both *U. stellata* and *U. deltaura* and the results of this study indicate that both species exhibit a degree of regular periodic irrigation activity and spend similar amounts of time irrigating the burrow. The volume of water moved through the burrow will, however, depend on the irrigation rate and duration of irrigation as well as the size of the shrimp (Dworschak, 1987).

Although oxygen availability within the burrow of these filter feeders was limited, the degree of hypoxia experienced was not as serious as that within the burrow of other deposit feeding thalassinideans, e.g. *Callianassa subterranea*, *Jaxea nocturna* and *Calocaris macandreae*. A pronounced gradient of oxygen tension was always present through the burrows of *U. stellata* and *U. deltaura*, as found for other thalassinideans; however, differences in the severity of the burrow environment (in terms of oxygen availability) were thought to reflect the feeding strategy of the occupant. Those thalassinideans that are deposit feeders have burrows that are typically complex structures and, coupled with their continual mining activities through the anoxic sediments, the burrow water tends to be more hypoxic. Temporal fluxes in oxygen tension within the burrows of *U. stellata* and *U. deltaura* (and presumably other thalassinideans) are dependent on a number of factors such as burrow irrigation, movement of the individual within the burrow, respiration of the occupant and of the meiofauna and microflora of the burrow wall and the diffusion of oxygen from the overlying sea water.

Clearly, as a consequence of their fossorial habit, thalassinideans will be exposed to varying degrees of oxygen stress. The next series of chapters examined a number of mechanisms (or respiratory adaptations) for maintaining oxygen supply, uptake and delivery, particularly during periods of low oxygen stress.

Since the gill is the primary site of gas exchange, the results presented in Chapter 3 showed some aspects of the comparative branchial morphology, gill area and ultrastructure for a number of thalassinidean shrimps. There were distinct differences in both the number and shape of the gills of different thalassinideans, with a trend towards simplification of the gill formulae in the 'higher', more advanced (taxonomically) families (Upogebidal and Callianassidal). The gill area of the deposit feeders (*C. subterranea* and *J. nocturna*), which are regularly exposed to

Haemocyanin oxygen affinity may also be influenced by haemocyanin subunit composition and association states. The results from this study showed that the haemocyanin of thalassinideans exist in a number of forms (hexamers, dodecamers and eikositetramers), although no attempt was made to determine the functional role of the association states. The high affinity respiratory pigments of the thalassinideans appears to reflect the respiratory conditions associated with burrowing. Functional differences in oxygen binding and regulatory ability are thought to be a result of the comparative differences between the oxygen affinity of thalassinidean haemocyanin (i.e. the deposit feeders had lower P_{50} values as compared to filter feeders) and hence burrowing habit. However, any interpretation of these results as possible respiratory 'adaptations' to environmental conditions must be limited because of the lack of specific *in vivo* data.

Although a burrowing habit confers a number of advantages, the majority of aquatic species are faced with the problem of coping with lowered oxygen tensions often prevalent within the burrow. The greater part of this thesis has examined a number of responses and possible respiratory adaptations to one parameter, low oxygen tension. One problem of living in sediments is that H_2S concentrations are often high. Hydrogen sulphide is an intergral component of reduced sediments and many infaunal species living in hypoxic environments are likely to be exposed to high concentrations. Sulphide is a potent (reversible) inhibitor of aerobic respiration and has been shown to influence the ecological distribution of a number of species, In Chapter 7, the tolerance and ability to detoxify sulphide in the burrowing shrimp *Calocaris macandreae* (a species whose distribution and physiology is likely to be affected by sulphide) and the epifaunal swimming crab *Liocarcinus depurator* (a species less likely to come into contact with high sulphide concentrations) was determined.

Although *C. macandreae* can tolerate much higher levels of sulphide than *L. depurator* (a non-burrowing crab although it may bury in superficial sediment) there appeared to be little difference in the rate of accumulation of sulphide in the blood. *Calocaris macandreae* was able to detoxify sulphide to the less toxic oxidation products thiosulphate and sulphite. A detoxification strategy was suggested for *C. macandreae*, mediated primarily via the oxygen store of the blood pigment and dissolved oxygen present in the haemolymph (although it is not clear if the oxidation of sulphide is specific or non-specific). Although these results suggest that this burrowing shrimp is adapted to a potentially sulphide rich habitat (a function of a deposit feeding lifestyle ?) this chapter provides the basis for further study of the tolerance of burrowing species to sulphide and detoxification strategies.

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Adaptational response and the role of physiological ecology

In the present study it was discovered that the burrow morphology and hence the degree of hypoxia within the burrow depends upon the feeding strategy of the occupant. A number of adaptations to the low oxygen levels found within burrows have been described for the thalassinideans *Upogebia stellata*, *U. deltaura*, *Callianassa subterranea* and *Jaxea nocturna*. These adaptations, which are both morphological and physiological, served to improve or facilitate oxygen uptake. At the morphological level, adaptations to low environmental oxygen included an increase in the branchial surface are available for gas exchange and a decrease in the diffusion barrier. All the species of thalassinidean shrimp exhibited a number of physiological mechanisms to facilitate oxygen uptake during hypoxia, although the magnitude of the response differed between species depending on the degree of hypoxic exposure regularly experienced. For one thalassinidean, *U. deltaura*, physiological adaptations to hypoxia included both a ventilatory and circulatory compensation. All the mud-shrimps examined possessed a respiratory pigment with a high oxygen affinity, which may be viewed as an adaptation to low oxygen availability.

Anderson *et al.* (1991) suggested that for the thalassinidean *Calocaris macandreae*, the major adaptive response to a hypoxic burrow environment was the adoption of a sedentary behavioural regime which then allowed a series of secondary physiological and morphological responses (adaptations) to evolve. A similar argument could be applied to the species examined in this study, however, it is not possible to conclude which was the primary causal factor that resulted in the series of adaptational responses (e.g. sedentary lifestyle, oxygen availabilty).

Physiological ecology is still a relatively newly forged discipline which emerged during the 1950s from the blending of ecology with comparative physiology (Watt, 1991). More recently, however, physiological ecology has taken numerous new directions encompassing aspects of molecular biology, evolutionary physiology and environmental biology (see reviews by Feder & Block, 1991; Watt, 1991; Burggren, 1991). One of the major problems faced by physiological ecologists is the ability to maintain a complete overview ('holocoenotic view') of the environment (Vernberg, 1985) so the ecological significance of the results can be assessed. Typically, organisms function in a multi-factorial complex environment and, when examining the physiological ecology of one or more species, there is usually a tendency to examine the influence of only a single factor at a time. Even so, this approach (as used during this thesis) is valuable because it provides information on how specific processes respond, or have adapted, to environmental stresses.

Finally, one of the most important elements in physiological ecology is the need for comparison, simply because adaptation is a comparative concept (Bradshaw, 1987). A comparative approach was maintained throughout this thesis so as to provide the basis for

adaptational interpretations of thalassinidean physiological ecology. Indeed, Anderson (1989) argued that it is becoming increasingly difficult to produce consistent adaptational interpretations of some [physiological] characteristics and many problems may be overcome by using a comparative approach.

References

Abele, L.G. (1982). Biogeography. In *The biology of Crustacea.* Vol 1. (ed L.G. Abele) pp. 241-304. Academic Press, New York.

Abu-Hilal, A., Badran, M. and Vaugelas, J. de., (1988). Distribution of trace elements in *Callichirus laurae* burrows and nearby sediments in the Gulf of Aqaba, Jordan (Red Sea). *Mar. environ. Res.* **25**: 233-248.

Adema, J.P.H., Creutzberg, F. and Van Noort, G.J., (1982). Notes on the occurence of some poorly known Decapoda (Crustacea) in the southern North Sea. *Zoologische Bijdragen.* **28**: 9-32.

Allanson, B.R., Skinner, D. and Imberger, J., (1992). Flow in prawn burrows. *Est. Coast. Shelf Sci.* **35**: 253-266.

Allen, J.A., (1966). Notes on the relationship of the bopyrid parasite *Hemiarthus abdominalis* (Kroyer) with its hosts. *Crustaceana.* **10**: 1-6.

Allen, J.A., (1967). *The fauna of the Clyde Sea area. Crustacea: Euphausida and Decapoda.* S.M.B.A. Millport. 116pp.

Aller, R.C., (1983). The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. *J. Mar. Res.* **41**: 299-322.

Aller, R.C., Yingst, J.Y. and Ullman, W.J., (1983). Comparative biogeochemistry of water in intertidal *Onuphis* (polychaeta) and *Upogebia* (crustacea) burrows: temporal patterns and causes. *J. mar. Sci.* **41**: 571-604.

Aller, R.C. & Yingst, R.E. (1985). Effects of the marine deposit feeder *Heteromastus filiformis* (Polychaeta), *Macoma baltica* (Bivalvia) and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport, reaction rates and microbial distributions. *J. mar. Res.* **43**: 615-645.

Altman, P.L. and Dittmer, D.J., (1971). *Respiration and circulation.* Federation of American Society of Experimental Biologists, Bethesda. (Biological Handbook, vol.3).

Anasaka, F. and Van Holde, K.E., (1979). Allosteric properties and the association equilibria of haemocyanin from *Callianassa californiensis. J. Mol. Biol.* **134**: 41-73.

Anderson, S.J., (1989). Physiological ecology of the mud-burrowing shrimp *Calocaris macandreae* Bell. Ph.D. Thesis, University of Glasgow, Scotland, U.K.

Anderson, S.J., Atkinson, R.J.A. and Taylor, A.C., (1991). Behavioural and respiratory adaptations of the mud-burrowing shrimp *Calocaris macandreae* Bell (Thalassinidea: Crustacea) to the burrow environment. *Ophelia.* **34**(2): 143-156.

Arp, A.J. and Childress, J.J., (1983). Sulphide binding by the blood of the hydrothermal vent tube-worm *Riftia pachyptila. Science.* **219**: 295-297.

Arp, A.J., Childress, J.J. and Vetter, R.D., (1987). Sulphide binding protein in the blood of *Riftia pachyptila* is the extracellular haemoglobin. *J. Exp. Zool.* **128**: 139-158.

Atkinson, R.J.A., (1986). Mud-burrowing megafauna of the Clyde Sea Area. *Proc. R. Soc. Edinb.* **90B**:

351-361.

Atkinson, R.J.A. and Schembri, P.J., (1981). The marine fauna of Lundy. Crustacea: Euphausiacea and Decapoda. *Rep. Lundy Field Soc.* **31**: 25-51.

Atkinson, R.J.A. and Chapman, C.J., (1984). Resin casting: A technique for investigating burrows in sublittoral sediments. *Prog. Underwater. Sci.* **10**: 109-115.

Atkinson, R.J.A., Pelsler, B., Bridges, C.R., Taylor, A.C. and Morris, S., (1987). Behavioural and physiological adaptations to a burrowing lifestyle in the snake blenny *Lumpenus lampretaeformis* and the red band fish *Cepola rubescens. J. Fish. Biol.* **31**: 639-659.

Atkinson, R.J.A. and Taylor, A.C., (1988). Physiological ecology of burrowing decapods. *Symp. Zool. Soc. Lond.* **59**: 201-226.

Atkinson, R.J.A. and Nash, R.D.M., (1990). Some preliminary observations on the burrows of *Callianassa subterranea* (Montagu) (Decapoda: Thalassinidea) from the west coast of Scotland. *J. Nat. Hist.* **24**: 403-413.

Balss, J., (1957). Decapoda. In. *Klassen und Ordnungen des Tierreichs.* (ed. Bronns, H.G.). Leipzig Akd. Verlangsgesellschaft, Fufter Band., Buch. 7: 1505-1672.

Bagarino, T. and Vetter, R.D., (1989). Sulphide tolerance and detoxification in shallow water fishes. *Mar. Biol.* **103**: 291-302.

Bagarino, T. and Vetter, R.d., (1990). Oxidative detoxification of sulphide by mitochondria of the California killifish *Fundulus parvipinnis* and the speckled sanddab *Citharichthys stigmaeus. J. Comp. Physiol* **160B**. 579-527.

Barra, J.A., Pequex, A. and Humbart, W., (1983). A morphological study on gills of a crab acclimated to fresh water. *Tissue Cell.* **15**: 583-596.

Bate, C.S., (1888). Report on the Crustacea Macrura collected by HMS Challenger during the years 1873-1876. *Challenger Rept. Zool.* **24**: 1-942.

Batterton, C.V. and Cameron, J.N., (1978). Characterisics of resting ventilation and response to hypoxia, hypercapnia and emersion in the blue crab *Callinectes sapidus* (Rathburn). *J. Exp. Zool.* **203**: 403-418.

Bayne, B.L., (1971). Oxygen consumption by three species of lamellibranch mollusc in declining ambient oxygen tension. *Comp. Biochem. Physiol.* **40A**: 955-970.

Bell, T., (1853). *A listing of the British stalk-eyed Crustacea.* London. pp 306.

Belman, B.W. and Childress, J.J., (1976). Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens. Biol. Bull. mar. Biol.Lab., Woods Hole.* **150**: 15-37.

Biffar, T.A., (1971). The genus *Callianassa* (Crustacea: Decapoda: Thalassinidea) in south Florida, with keys to the western Atlantic species. *Bull. Mar. Sci.* **21**(3): 637- 715.

Blair, D.P. and Van Holde, K.E., (1976). Sedimentation equilibrium studies of a complex association reaction. *Biophys. Chem.* **5**: 165-170.

Bonnier, J., (1900). Contribution a l'etude des Epicarides les Bopyridae. *Trav. Stat. Zool. Wimereux.* **7**:

1-475.

Booth, C.E., McMahon, B.R. and Pinder, A.W., (1982). Oxygen uptake and the potentiating effects of increasing haemolymph lactate on oxygen transport during excercise in the blue crab, *Callinectes sapidus*. *J. Comp. Physiol.* **148**: 111-121.

Borradaile, L.A., (1903). On the classification of the Thalassinidea. *Ann Mag. nat. Hist.* 7(12): 534-551.

Borradaile, L.A., (1907). On the classification of the decapod Crustacea. *Ann. Mag. nat. Hist. London.* **19**(7): 457-486.

Bourdon, R., (1963). Epicarides et Rhizochepales de Roscoff. *Cah. Biol. Mar.* **4**: 415- 434.

Bourdon, R. (1967). Donnees complimentaires sur les epicarides et les rhizochepales de Roscoff. *Bull. Acad. Soc. Lorr. Sci.* 6(4): 287-292.

Bourdon, R., (1968). Les Bopyridae des mers Europeennes. *Mem. Mus. natn. Hist. nat. Paris. Series A, Zoologie*. **50**(2): 75-424.

Bourdon, R., (1981a). Bopyriens nouveaux pour la faune Europeenne de l'Atlantique (Isopoda, Epicaridea). *Bull. Mus. natn. Hist. nat. Paris. Zool.* **3**(2): 615-634.

Bourdon, R., (1981b). Sur cinq Bopyrides parasites de Thalassinides ouest-africans. *Bull. IFAN.* **43**, ser A. 1-2: 111-134.

Bouvier, E.L., (1940). Decapodes marcheurs. *Faune. Fr.* **37**: 1-399.

Bradford, S.M. and Taylor, A.C., (1982). The respiration of *Cancer pagurus* under normoxic and hypoxic conditions. *J. exp. Biol.* **97**: 273-288.

Bradshaw, A.D., (1987). Functional ecology = comparative ecology?. Forum. *Functional Ecology.* **1**: 71-72.

Braithwaite, C.J.R. and Talbot, M.R., (1972). Crustacean burrows in the Seychelles, Indian Ocean. *Paleogeogr. Paleoclimatol. Paleo. Ecol.* **11**(4): 265-285.

Branch, G.M. and Pringle, A., (1987). The impact of the sand prawn *Callianassa kraussi* Stebbing on sediment turnover and on bacteria, meiofauna and benthic microflora. *J. exp. mar. Biol. Ecol.* **107**: 219-235.

Bridges, C.R., (1986). A comparative study of the respiratory properties and physiological function of haemocyanin in two burrowing and two non-burrowing crustaceans. *Comp. Biochem. Physiol.* **83A**: 261-270.

Bridges, C.R. and Brand, A.R., (1980b). Oxygen consumption and oxygen independence in marine crustaceans. *Mar. Ecol. Prog. Ser.* **2**: 133-144.

Bridges, C.R. and Brand, A.R., (1980a). The effect of hypoxia on oxygen consumption and blood lactate levels of some marine crustacea. *Comp. Biochem. Physiol.* **65A**: 399- 409.

Bridges, C.R. and Morris, S., (1986). Modulation of haemocyanin oxygen affinity by L-lactate- a role for other co-factors. In *Invertebrate oxygen carriers* (ed. Linzen, B.). pp 341-351. Springer-Verlag, Berlin

Bridges, CR., Bicudo, J.E.P.W., and Lykkoboe, G., (1979). Oxygen content in blood containing haemocyanin. *Comp. Biochem. Physiol.* **83A**: 457-462.

Bridges, C.R., Morris, S. and Grieshaber, M.K., (1984). Modulation of haemocyanin oxygen affinity in the intertidal prawn, *Palaemon elegans* (Rathke). *Respir. Physiol.* **57**: 189-200.

Bromley, R.G., (1990). *Trace fossils: Biology and taphonomy.* Special topics in Palaeontology: 3. Unwin Hyman Inc.

Bruce, J.R., Coleman, J.S. and Jones, N.S., (1963). *Marine fauna of the Isle of Man.* (2nd edition). Liverpool University Press. pp 307.

Buchanan, J.B., (1963). The biology of *Calocaris macandreae* (Crustacea, Thalassinoidea). *J. mar. Biol. Ass. U.K.* **43**: 729-747.

Buchanan, J.B., Brown, B.E., Coombs, T.L., Pine, B.J.S. and Allen, J.A., (1980). The accumulation of ferric ion in the gut of some spatangoid echinoderms. *J. mar. Biol. Ass. U.K.* **60**: 631-640.

Burd, B.J., (1987). Comparative gill characteristics of *Munida quadrispina* (Decapoda, Galatheidae) from different habitat oxygen conditions. *Can. J.* Zool. **66**: 2320-2323.

Burrgren, W.W., (1991). Does comparative respiratory physiology have a role in evolutionary biology (and vice versa)? In *Physiological Strategies for gas exchange and metabolism* (eds. Woakes, A.J., Grieshaber, M.K. and Bridges, C.R.). *SEB Seminar Series.* **41**.

Burggren, W.W., McMahon, B.R. and Costerton, J.W., (1974). Branchial water- and blood-flow patterns and the structure of the gill of the crayfish *Procambarus clarkii. Can. J. Zool.* **52**: 1511-1518.

Burkenroad, M.D., (1981). The higher taxonomy and evolution of Decapoda (Crustacea). *Trans. San Diego Soc. nat. Hist.* **19**: 251-268.

Burnett, L.E., (1979). The respiratory function of haemocyanin in the spider crab *Libinia emarginata*, and the ghost crab, *Ocypode quadrata* in normoxia and hypoxia. *J. exp.* Zool. 210: 289-300.

Burnett, L.E. and Bridges, C.R., (1981). The physiological properties and function of ventilatory pauses in the crab *Cancer pagurus. J. Comp. Physiol.* **145**: 81-88.

Butler, P.J., Taylor, E.W. and McMahon, B.R. (1978). Respiratory and circulatory changes in the lobster (*Homarus vulagaris*) during long term exposure to moderate hypoxia. *J. exp. Biol.* **73**: 131-146.

Calderon-Perez, J.A. (1981). *Some aspects of the biology of Calocaris macandreae Bell (Crustacea: Decapoda, Thalassinoidea) in Isle of Man waters.* Ph.D. Thesis, University of Liverpool.

Calman, W. T., (1909). Crustaceana. In, *A Treatise on Zoology.* (ed. Lankaster, R.), part 7, Fasc. 3. Ray Society, London. pp 1-346.

Caroli, E., (1945). Un Bopyride parasite di un altro Bopyridae. *Pubb. Staz. zool. Napoli.* **20**: 61-65.

Catalano, N. and Restivo, F., (1965). Ulteriori notizie sulla Pseudione euxinica parassita di *Upogebia litoralis*, a *Napoli. Pubb. Staz. zool. Napoli.* **34**: 203-210.

Chan, H.M. and Weeks, J.M., (1992). The subunit structural composition of amphipod haemocyanin

(Crustacea: Amphipoda: Taltridae). *Comp. Biochem. Physiol.* **101**(4): 567- 572.

Childress, J.J., (1975). The respiratory rates of mid-water crustaceans as a function of the oxygen minimum layer off southern California. *Comp. Biochem. Physiol.* **50A**: 787-799.

Childress, J.J., Felbeck, H. and Somero, G.N., (1987). Symbiosis in the deep-sea. *Scient. Am.* **256**(5): 106-112.

Childress, J.J., Fisher, C.R., Favuzzi, J.A., Kochevar, R.E., Sanders, N.K. and Alayse, A.M., (1991). Sulphide-driven autotrophic balance in the bacterial symbiont-containg hydrothermal vent tubeworm, *Riftia pachyptila* Jones. *Biol. Bull. mar. Biol. Lab., Woods Hole.* **180**: 135-153.

Christiansen, M. and Grere, L., (1982). First record of the thalassinid *Callianassa subterranea* (Montagu) (Crustacea Decapoda) from the west coast of Norway. *Sarsia.* 67(3): 143-214.

Cioffi, M., (1984). Comparative ultrastructure of arthropd transporting epithelia. *Am. Zoologist.* **24**: 139: 156.

Clark, E., (1936). The freshwater and land crayfishes of Australia. *Mem. Nat. mus. Vict.* **10**: 5-58.

Compere, P., Wanson, S., Pequex, A., Gilles, R. and Goffinet, G., (1989). Ultrastructural changes in the gill epithelium of the green crab *Carcinus maenas* in relation to the external salinity. *Tissue Cell.* **21**: 299-318.

Copeland, D.E. and Fitjarrel, A.T., (1968). The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus*, Rathburn) with notes on modified mitochondria. *Z. Zellforsch. mikrosk. Anat.* **92**: 1-22.

Coyer, P.E., (1977). Responses of heart and scaphognathite rates in *Cancer borealis* and *C. irroratus* to hypoxia. *Comp. Biochem. Physiol.* **56A**: 165-167.

DeFur, P.L. and Mangum, C.P., (1979). The effects of environmental variables on the heart rates of invertebrates. *Comp. Biochem. Physiol.* **62A**: 283-294.

DeMann, J.G., (1925). The Decapoda of the Siboga-expedition. Part IV. The Axiidae collected by the Siboga-expedition. *Siboga-expedite.* **39**a5 (102): 1-127.

Depledge, M.H. (1977). Cardiac activity of the shore crab *Carcinus maenas* (L.). *Comp Biochem Physiol.* **60A**: 65-67.

Devine, C.E., (1966). Ecology of *Callianassa filholi* Milne-Edwards 1878. (Crustacea: Thalassinidea). *Trans. R. Soc.N.Z., Zool.* **8**: 93-110.

Dickson, J.S., Dillaman, R.M., Roer, R.D. and Roye, D.B., (1991). Distribution and characterisation of ion transporting and respiratory filaments in the gills of *Procambarus clarkii. Biol. Bull. Mar. Biol. Lab., Woods Hole.* **180**: 154-166.

Drach, P., (1930). Etude sue le systeme branchial des Crustacea Decapodes. *Mem. Fac. Sci. Univ. Paris.* **401**: 83-133.

Dworschak, P.C., (1981). The pumping rates of the burrowing shrimp *Upogebia pusilla* (Petagna)(Decapoda: Thalassinidea). J. *Exp. Mar. Biol. Ecol.* **52**(1); 25-35.

Dworschak, P.C. (1983). The biology of *Upogebia pusilla* (Petagna) (Decapoda: Thalassinidea) I. The

burrows. *P.S.Z.N.I. Mar. Ecol.* **4**(1): 19-43.

Dworschak, P.C., (1987). Burrows of Mediterranean Decapoda. *Investigacion pesq.* **51** (supl. I): 264

Dworschak, P.C., (1988). The biology of *Upogebia pusilla* (Petagna) (Decapoda, Thalassinidea) III. Growth and production. *P.S.Z.N.I. Mar. Ecol.* **9**(1): 51-77.

Dworschak, P.C. and Pervesler, P., (1988). Burrows of *Callianassa bouvieri Nobili* 1904 from Safaga (Egypt, Red Sea) with some remarks on the biology of the species. *Senckenberg. maritima.* **20**: 1-17.

Dyer, M.F. and Uglow, R.F., (1977). On a technique for monitoring heart and scaphognathite activity in natantia. J. *exp. mar. Biol. Ecol.* **27**: 117-124.

Ellis, E.A. and Baker, R.E., (1972). Some observations on the rare sand burrowing crustacean *Axius stirhynchus* (Leach). *Trans. Norfolk Norwich. nat. Soc.* **23**(4): 250-253.

Engel, P. and Jones, J.B., (1978). Causes and elimination of erratic blanks in enzymatic metabolic assays involving the use of NAD^+ in alkaline hydrazine buffers: improved conditions of the assay of L-glutamate, L-Lactate and other metabolites. *Analyt. Biochem.* **88**: 475-484.

Erdman, R.B., Blake, N.J. & Torres, J.J. (1991). Oxygen consumption of the deep-sea crabs *Chaecon fenneri* and *C. quinquedens* (Brachyura:Geryonidae). *Comp. Biochem. Physiol.* **99A** (3): 383-385.

Fahey, R.C., Donan, R.D., Newton, G.L. and Uttley, J., (1983). Determination of intracellular thiol levels using bromobimane flourescent labeling applications involving radioprotective drugs. In. *Radioprotectors and anticarcinogens.* (eds. Nygaard, O.F. and Simic, M.G.). Academic Press, New York. pp 103-120.

Farley, R.D. and Case, J.F., (1968). Perception of external oxygen by the burrowing shrimp, *Callianassa californiensis* Dana and *C. affinis* Dana. *Biol. Bull. mar. Biol. Lab. Woods Hole.* **134**: 261-265.

Felbeck, H. Leubezeit, G. Dawson, R. and Giere, O., (1983). CO_2 fixation in tissues of marine oligochaetes (*Phallodrilus leukodermatus* and *P. planus*) containing symbiotic, chaemoautotrophic bacteria. *Mar Biol.* **75**: 187-191.

Feder, M.E. and Block, B.A., (1991). On the furure of animal physiological ecology. *Functional Ecology.* **5**: 136-144.

Felder, D.L., (1979). Respiratory adaptations of the estuarine mud shrimp, *Callianassa jamaicense* (Schmitt, 1935) (Crustacea, Decapoda, Thalassinidea). *Biol. Bull. mar. Biol. Lab. Woods Hole.* **157**: 125-138.

Felder, D.L. and Lovett, D.L., (1989). Relative growth and sexual maturation int he estuarine ghost shrimp *Callianassa louisianensis* Schmitt, 1935. J. Crust. *Biol.* 9(4): 540-553.

Felgenhauer, B.E. and Abele, L.G., (1983). Phylogenetic relationships among shrimp-like Decapods. *Crust. Issues.* **1**: 291-311.

Fenchel, T.M. and Reidl, R.J., (1970). The sulphide system: A new biotic community underneath the oxidised layer of marine sand bottoms. *Mar. Biol.* &: 255-268.

Finol, H.J. and Croghan, P.C., (1983). Ultrastructure of the branchial epithelium of an amphibious brackish-water crab. *Tissue Cell.* **15**: 63-75.

Fisher, J.M., (1972). Fine structural observations on the gill filaments of the freshwater crayfish, *Astacus pallipes* Lereboullet. *Tissue Cell.* **4**: 287-299

Forbes, A.T., (1973). An unusual abbrieviated larval life in the estuarine burrowing prawn *Callianassa kraussi* (Crustacea: Deacpoda: Thalassinidea). *Mar. Biol.* **22**: 361-365.

Forbes, A.T., (1977). Breeding and growth of the burrowing prawn *Callianassa Kraussi* Stebbing (Crustacea: Decapoda: Thalassinidea). *Zoologica Africana.* **12**(1): 149-161.

Foster, A.C. and Howse, H.D., (1978). A morphological study of the gills of the brown shrimp, *Penaeus aztecus. Tissue Cell.* **10**: 72-92.

Frey, R.W. and Howard, J.D., (1969). A profile of biogenic sedimentary structures in a Holocene barrier island-salt marsh complex, Georgia. *Trans. Gulf-Coast Ass. geol. Socs.* **19**: 427-444.

Frey, R.W. and Howard, J.D., (1975). Endobenthic adaptations of juvenile thalassinidean shrimp. *Bull. Geol. Soc. Den.* **24**: 283-297.

Frey, R.W, Howard, J.D. and Pryor, W.A., (1978). Ophiomorpha: its morphologic, taxonomic and environmental significance. *Palaeogeogr. Palaeoclimatol. Palaeocol.* **23**: 199-229.

Fry, F.E.J. (1957). The aquatic respiration of fish. In *The Physiology of Fishes* Vol 1. (ed M.E. Brown), pp 1-63. New York, Academic Press.

Giere, O., (1981). The gutless marine oligochaete *Phallodrilus leukodermatus.* Structural studies in an abberant tubificid associated with bacteria. *Mar. Ecol. Prog. Ser.* **5**:353-357.

Gilboa-Garber, N., (1971). Direct spectrophotometric determination of inorganic sulphide in biological materials and in other complex mixtures. *Ana. Biochem.* **43**: 129- 133.

Goodman, S.H. and Cavey, M.J., (1992). Organisation of phyllobranch gill from the green shore crab *Carcinus maenas* (Crustacea: Decapoda). *Cell Tissue Res.* **260**: 495- 505.

Gray, I.E., (1957). A comparative study of the gill area of crabs. *Biol. Bull. Mar. Biol. Lab., Woods Hole.* **112**: 34-42.

Gravitz, N. and Gleye, L., (1975). A photochemical side reaction that interferes with the phenylhypochlorite assay for ammonia. *Limnol. Oceanogr.* **20**: 1015-1017.

Greenaway, P., (1984). The relative importance of the gills and lungs in the gas exchange of amphibious crabs of the genus *Holothusina. Aust. J. Zool.* **32**: 1-6.

Greenaway, P., Bonaventura, J. & Taylor, H.H. (1983). Aquatic gas exchange in the freshwater/land crab *Holothusiana transversa. J. Exp. Biol.* **103**: 225-236.

Griffis, R.B. and Chavez, F.L., (1988). Effects of sediment type on burrows of *Callianassa californiensis* Dana and *C. gigas* Dana. J. *exp. mar. Biol. Ecol.* **117**: 239-253.

Griffis, R.B. and Suchanek, T.H., (1991), A model of burrow architecture and trophic modes in thalassinidean shrimp (Decapoda: Thalassinidea). *Mar Ecol. Prog. Ser.* **79**: 171-183.

Gurney, R., (1942). *Larva of Decapod Crustacea.* **129.** Ray Society, London.

Gust, G. and Harrison, J.T., (1981). Biological pumps at the sediment-water interface: mechanistic evaluation of the Alpheid shrimp *Alpheus mackayi* and its irrigation pattern. *Mar Biol.* **64**: 71-78.

Gustafsen, G., (1934). On the Thalassinidea of the Swedish west coast. *Ark. For. Zool.* **28A**(1): 1-19.

Gutmann, I. and Wahlfield, A.W., (1974). L-$^{(+)}$lactate. Determination with lactate dehydrogenase and NAD. In *Methods of enzymatic analysis*: 2nd Ed (eds. Bermeyer, H.V.). Academic Press, New York.

Hailstone, T.S. and Stephenson, W., (1961). The biology of *Callianassa* (Trypaea) *australiensis* Dana 1852 (Crustacea, Thalassinidae). *Univ. Queensland papers Dept. Zool.* **1**(12): 259-285.

Hagerman, L. and Uglow, R.F., (1985). Effects of hypoxia on the respiratory and circulatory regulation of *Nephrops norvegicus*. *Mar. Biol. Bull.* **87**: 273-278.

Hand, C.H and Somero, G.N., (1983). Energy metabolism pathways of hydrothermal vent animals: adaptations to a food rich and sulphide rich deep-sea environment. *Biol. Bull. Mar. Biol.* Lab., *Woods Hole*. **165**: 167-181.

Hanekom, N.M. and Baird, D., (1987). Oxygen consumption of *Callianassa kraussi* stebbing (Thalassinidea, decapoda, crustacea) in relation to various environmental conditions. *S. Afr.* J. *Zool.* **22**(3): 183-189.

Hanekom, N.M. and Erasmus, T., (1988). A qualitative study to assess standing biomasses of macrobenthos in soft substrata of the Swartkops estuary and possible influencing factors. *S. Afr. J. Zool.* **23**(4): 259-265.

Hartnoll, R.G., (1964). Reduction of the gill number in Spider Crabs. *Crustaceana*, **7**: 145-148.

Hawkins, D.L., (1970). Metabolic responses of the burrowing shrimp *Callianassa californiensis* to anoxic conditions. *M.S. Thesis, Oregon State University.*

Hawkins, A.J.S. and Jones, M.B., (1982). Gill area and ventilation in two mud crabs, *Helice crassa* Dana (Grapsidae) and *Macrophthalmus hirtipes* (Jacquinot) (Ocypodidae), in relation to habitat. *J. exp. Mar. Biol. Ecol.* **60**: 103-118.

Haywood, PJ. and Ryland, J.S. (eds) (1990). *The marine fauna of the British Isles and the north west Europe*. Vol 1. Introduction and protozoans to arthropods. Clarendon Press. Oxford.

Henry, R.P., Perry, H.M., Trigg, C.B., Handley, H.L. and Krarup, A., (1990a). Physiology of two species of deep-water crabs *Chaceon fenneri* and *C. quinquedens*: gill morphology and haemolymph and ionic and nitrogen concentrations. *J. Crust. Biol.* **10**(3). 375-381.

Henry, R.P., Handley, H.L, Krarip, A. and Perry, H.M., (1990b). Respiratory and cardivascular responses of two species of deep-sea crabs, *Chaecon fenneri* and *C. quinquedens* in normoxia and hypoxia. *J. Crust. Biol.* **10**(3): 413-422.

Herreid, C.F., (1980). Hypoxia in invertebrates. *Comp. Biochem. Physiol.* **67A**: 311-320.

Hill, B.J., (1971). Osmoregulation by an estuarine and an a marine species of *Upogebia* (Anomura: Crustacea). *Zool. Afr.* **6**(2): 229-236.

Hill, B.J. and Allinson, R.R., (1971). Temperature tolerance of the estuarine prawn *Upogebia africana*

(Anomura: Crustacea). *Mar. Biol.* **11**: 337-343.

Hill, B., (1981). Respiratory adaptations of three species of *Upogebia* (Thalassinidea, Crustacea) with special reference to low tide periods. *Biol. Bull. Mar. Biol. Lab. Woods Hole.* **160**: 272-279.

Hoggarth, K.R. and Trueman, E.R., (1967). Techniques for recording the activity of aquatic invertebrates. *Nature, Lond.* **213**: 1050-1051.

Hohenegger, J. and Pervesler, P., (1985). Orientation of crustacean burrows. *Lethaia.* **18**: 323-339.

Holmes, W.N. and Donaldson, E.M., (1969). The body composition and distribution of electrolytes. In. *Fish Physiology*, (eds. Hoar, W.S. and Randell, D.J.). vol 1. Academic Press, New York.

Hong, S.Y., (1988). Development of epipods and gills in some pagurids and brachyurnas. *J. nat. Hist.* **22**: 1005-1040.

Hughes, G.M., (1982). An introduction to the study of gills. In *Gills.* SEB Seminar Series No 15. C.U.P.

Hughes G.M., (1983). Allometry of gill dimensions in some British and American decapod crustacea. *J. Zool., Lond.* **200**: 83-97.

Hughes, G.M., Knights, B. and Scammell, C.A., (1969). The distribution of PO_2 and hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab *Carcinus maenas*. *J. Exp. Biol.* **51**: 203-220.

Huxley, T.H., (1878). On the classification and distribution of the crayfishes. *Proc. Zool. Soc. London.* **1878**: 752-788.

Innes, A.J., (1985). Aerobic scope for activity of the burrowing mantis shrimp *Heterosquilla tricarinata* at low environmental oxygen tensions. *Comp. Biochem. Physiol.* **81A**(4): 827-832.

Jenner, C.E., (1978). Sex identification, sex ratio and social organisation in the thalassinid shrimp *Upogebia affinis* (Crustacea: Decapoda). *ABS Bulletin.* **25**(2): 71.

Johanson, K., Lenfant, C & Mecklenburg, T.A. (1970). Respiration in the crab, *Cancer magister. Z. vergl. Physiol.* **70**: 1-19.

Johnson, L. and Rees, C.J.C., (1988) Oxygen consumption and gill surface area in relation to habitat and lifestyle of four crab species. *Comp. Biochem. Physiol.* **89A**: 243- 246.

Jorgenson, B.B., (1982). Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. *Phil. Trans. Roy. Soc. Lond. Series B.* **298**: 543-560.

Jorgenson, D., Bourne, G., Burnett, L. deFur, P. and McMahon, B., (1982). Circulatory function during hypoxia in the Dungeness crab *Cancer magister. Am. Zool.* **4**: 958A.

Kamler, E., (1969). A comparison of the closed bottle and flowing water methods for measurement of respiration in aquatic invertebrates. *Pol. Arch. Hydro. Biol.* **29**: 31-49.

Kaufmann, R., Forstner, H. and Weiser, W., (1989). Respirometry methods and approaches. In. *Techniques in comparative respiratory physiology. An experimental approach.* (eds. Bridges, C.R. and Butler, P.J.). Cambridge University Press.

Kensley, B. (1989). Systematics of the thalassinidea. In *Biology of the Thalassinidea*. (ed Felder, D.L.)

Boston, Dec 1984.

Kleemann, K.K., (1984). Lebensspuren von *Upogebia operculata* (Crustacea: Decapoda) in karibischen Steinkorallen (Madreporana: Anthozoa). *Beitr. Palaontol. Osterr.* **11**: 35- 57.

Koike I. and Mukai H., (1983). Oxygen and inorganic nitrogen contents and fluxes in burrows of the shrimps *Callianassa japonica* and *Upogebia major*. *Mar. Ecol. Prog. Ser.* **12**: 185-190.

Krogh, A., (1919). The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. *J. Physiol (London).* **52**: 391-408.

Lallier, F. and Truchot, J.P., (1989). Modulation of haemocyanin oxygen affinity by L- lactate and urate in the prawn *Penaeus japonicus*. *J. exp. Biol.* **147**: 133-146.

Laubier, L., Desbruyeres, D. and Chassard-Bouchard, C., (1983). Microanalytical evidence of sulphur accumulation in a polychaete from deep-sea hydrothermal vents. *Mar. Biol. Letters.* **4**: 113-116.

Laurent, P. and Perry, S.F., (1991). Environmental effects on fish gill morphology. *Physiological Zoology.* **64**(1): 4-25.

Leach, W.E., (1813). Crustaceology. In. *The Edingburgh encyclopedia.* (ed. Brewster, D.). Edingburgh. 7: 383-437.

Leach, W.E., (1815). *Trans. Linn. Soc. London.* VII: 335-342.

Levitt, J.M. and Arp, A.J., (1991). The effects of sulphide on the anerobic metabolism of two congeneric species of mudflat clams. *Comp. Biochem. Physiol.* **98B**: 339-347.

Liddicoat, M.I., Tibbitts, S. and Butler, E.I., (1975). The determination of ammonia in seawater. *Limnol. Oceanogr.* **20**: 131-132.

LLanso, R.J., (1991). Tolerance of low dissolved oxygen and hydrogen sulphide by the polychaete *Streblospio benedicti* (Webster). *J. Exp. Mar. Biol. Ecol.* **153**: 165-178.

Lutz, R.J., (1938). Uber systematik Entwicklung und Oekologie von *Callianassa. Helgolander. Wiss. Meeres.* **1**(2): 162-199.

MacGinitie, G.E., (1930). The natural history of the mud shrimp *Upogebia pugettensis* (Dana). *Ann. Mag. nat. Hist.* **6**: 36-44.

MacGinitie, G.E., (1934). The natural history of *Callianassa californiensis* Dana. *Am. midl. Nat.* **15**: 166-177.

MacGinitie, G.E. and MacGinitie, N., (1968). Arthropoda. In. *Natural history of marine animals.* (eds. MacGinitie, G.E. and MacGinitie, N.). McGraw Hill, New York.

Maina, J.N., (1990). The morphology of the gills of the freshwater African crab *Potomon niloticus* (Crustacea: Brachyura: Potamonidae): A scanning and transmission electron microscopic study. *J. Zool. Lond.* **211**: 499-515.

Mangum, C.P., (1983). Oxygen transport in the blood. In *'Biology of the Crustacea'*. Vol 5. (ed. Mantel, L.H.). pp 373-429. Academic Press, New York, London.

Mangum, C.P., (1990). Inducible O_2 carriers in the Crustaceans. In *Animal nutrition and transport processes* (eds. Truchot, J.P. and Lahlou, B.). pp 92-103. Karger, Basel

.Mangum, C.P. and Van Winkle, W., (1973). Responses of aquatic invertebrates to declining oxygen conditions. *Amer. Zool.* **13**: 513-528.

Mangum, C.P. and Rainer, J.S., (1988). The relationship between subunit composition and O_2 binding of blue crab haemocyanins. *Biol Bull. Mar. Biol.*Lab., *Woods Hole.* **174**: 77-82

Mangum, C.P., Greaves,J. and Rainer, J.S., (1991). Oligomer composition and oxygen binding of the haemocyanin of the blue crab *Callinectes sapidus. Biol. Bull. Mar. Biol.*Lab., *Woods Hole.* **181**: 453-458.

Mangum, C.P., Miller, K.I., Scott, J.L., Van Holde, K.E. and Morse, M.P., (1987). Bivalve haemocyanin structural, functional and phylogenetic relationships. *Biol. Bull. Mar. Biol.* Lab., *Woods Hole.* **173**: 205-221.

Manning, R.B. and Felder, D.L., (1991). Revision of the American Callianassidae (Crustacea: Decapoda: Thalassinidea). *Proc. Biol. Soc. Wash.* **104**(4): 764-792.

Marine Biological Association, (1957). *Plymouth Marine Fauna.* 3rd edition, Plymouth.

Markl, J., Hofer, G. Bauer, G., Markl, A., Kempler, M., Brenzinger, M and Linzen, B., (1979). Subunit heterogeneity in arthropod haemocyanins. II, Crustacea. *J. Comp. Physiol.* **133**: 167-175.

Markl, J., (1986). Evolution and function of structurally diverse subunits in the respiratory protein haemocyanin from arthropods. *Biol. Bull. Mar. Biol.* Lab. *Woods Hole.* **171**: 733-742.

Martello, M. J., Zanders, I. P., (1986). Modifications of gill ultrastructure and ionic composition in the crab *Goniopsis cruentata* acclimated to various salinities. *Comp. Biochem. Physiol.* **84A**: 383-389.

Massabuau, J-C., (1983). The gas exchanger of the crayfish: A crosscurrent system. *J. Physiol.* (London). **345**: 170p.

McGrath, D. and Atkins, P., (1979). Some parasitic isopoda (Epicaridea) from the Galway Bay area, west coast of Ireland. *Ir. Nat. J.* **19**(12): 437-439.

McKeller, R.C. and Sprott, G.D., (1979). Solubilization and properties of a particulate hydrogenase from Methanobacterium strain G2R. *J. Bacteriol.* **139**: 231-238.

McLauglin, P.A., (1980). A comparative morphology of recent Crustacea. *Freeman*, San Fransisco, California.

McLaughlin P.A., (1983). Internal Anatomy. In: *The biology of Crustacea Vol. 5*: Internal Anatomy and Physiological Regulation. Ed Mantel L.H. New York and London.: Academic Press.

McMahon, B.R., (1988). Physiological responses to oxygen depletion in intertidal animals. *Amer. Zool.* **28**: 39-53.

McMahon, B.R. and Wilkens, J.L., (1972). Simultaneous apnoea and bradycardia in the lobster *Homarus americanus. Can. J. Zool.* **50**: 165-170.

McMahon, B.R. and Wilkens, J.L., (1975). Respiratory and circulatory responses to hypoxia in the lobster *Homarus americanus. J. exp. Biol.* **79**: 265-281.

McMahon, B.R. & Wilkens, J.L. (1983). Ventilation, perfusion and oxygen uptake. In '*The biology of Crustacea*' (ed. Mantel, L.H.). Vol 5, pp 289-372. Academic Press, New York, London.

McMahon, B.R., Buggren, W.W. and Wilkens, J.L., (1974). Respiratory responses to long term hypoxia stress in the crayfish *Orconectes virilis*. *J. Exp. Biol.* **60**: 195-206.

McMahon, B.R., Butler, P.J. and Taylor, E.W., (1978). Acid-base changes during recovery from disturbance and during long-term hypoxic exposure in the lobster *Homarus vulagaris*. *J. exp. Zool.* **205**: 361-370

McMahon, B.R., McDonald, D.G. and Wood, C.M., (1979). Ventilation, oxygen uptake and haemolymph oxygen transport following enforced exhausting activity in the Dungeness crab *Cancer magister*. J. *exp. Biol.* **80**: 271-285.

Mickle, T.J. and Childress, J.J., (1982). Effects of temperature, pressure and oxygen concentration on the oxygen consumption rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). *Physiol. Zool.* **55**: 199-207.

Miller, K.I. and Van Holde, K.E., (1974). Oxygen binding by *Callianassa californiensis* haemocyanin. *Biochemistry*. **13**: 1668-1674.

Miller, K.I. and Van Holde, K.E., (1981a). The effect of environmental variables on the structure and function of haemocyanin from *Callianassa californiensis* I. Oxygen binding. *J. Comp. Physiol.* **143**: 252-260.

Miller, K.I. and Van Holde, K.E., (1981b). The effect of environmental variables on the structure and function of haemocyanin from *Callianassa californiensis*. II. Subunit association-dissociation equilibrium. *J. Comp. Physiol.* **143**: 261-267.

Miller, K.I., Pritchard, A.W. and Rutledge, P.S.,(1976). Respiratory regulation and the role of the blood in the burrowing shrimp *Callianassa californiensis* (Decapoda: Thalassinidea). *Mar. Biol., Berlin.* **36**: 233-242.

Miller, K.I., Eldred, N.W., Anasaka, F. and Van Holde, K.E., (1977). Structure and function of haemocyanin from thalassinid shrimp. *J. Comp. Physiol.* **115**: 171-184.

Montagu, G., (1808). Description of several marine animals found on the south coast of Devonshire II. *Trans. Linn. Soc. Lond. (Zool).* **9**: 81-114.

Morgan, W. de., (1910). On the species *Upogebia stellata* and *Gebia deltaura*. *Mar Biol. Ass. U.K.* **8**: 475-478.

Morris, S., (1988). Effects of freezing on the function and association state of crustacean haemocyanins. *J. exp. Biol.* **138**: 535-539.

Morris, S., (1990). Organic ions as modulators of respiratory pigment function during stress. *Physiol. Zool.* **62**: 253-287.

Morris, S., (1991). Respiratory gas exchange and transport in crustaceans: ecological determinants. *Memoirs of the Queensland Museum*, **31**: 241-261.

Morris, S. and Taylor, A.C., (1985). Oxygen consumption of *Palaemon elegans* (Rathke) in response to temperature change: A determinant of distribution. *Exp. Biol.* **44**: 255-268.

Morris, S., Bridges, C.R. and Grieshaber, M.K., (1985). A new role for uric acid: modulator of

haemocyanin oxygen affinity in crustaceans. *J.exp.* Zool. **235**: 135-139.

Morse, H.C., Harris, P.J. and Dornfield, E.J., (1970). *Pacifastacus leniusculus*: fine structure of arthobranch with reference to active ion uptake. *Trans. Am. Microsc. Soc.* **89**: 12-27.

Mukai, H. and Koike, I., (1984). Behaviour and respiration of the burrowing shrimps *Upogebia major* (deHaan) and *Callianassa japonica* (deHaan). *J. Crust. Biol.* **4**: 191-200.

Mukai, H., Koike., Nishihira, M. and Nojima, S., (1989). Oxygen consumption and ammonium excretion of mega-sized benthic invertebrates in a tropical seagrass bed. *J. exp. Mar. Biol. Ecol.* **134**: 101-115.

Nakao, T., (1974). Electron microscopic study of the open circulatory system of the shrimp *Cardinia japonica* I. Gill capillaries. *J. morph.* **144**: 361-380.

Nash, R.D.M., Chapman, C.J., Atkinson, R.J.A. and Morgan, P.J., (1984). Observations on the burrows and burrowing behaviour of *Calocaris macandreae* (Crustacea: Decapoda: Thalassinoidea). *J. Zool. Lond.* **202**: 425-439.

National Research Council (Division of Medical Science, Subcommittee on Hydrogen Sulphide), (1979). Hydrogen sulphide. University Press, Baltimore.

Newton, G.L., Donain, R.D. and Fahey, R.C., (1981). Analysis of biological thiols: derivitization with monobromobimane and seperation by reversed-phae high-performance liquid chromotography. *Anal. Chem.* **114**: 383-387.

Nichols, F.H. (1975). Dynamics and energetics of three deposit feeding benthic invertebrate populations in Puget Sound, Washington. *Ecol. Monogr.* **45**: 57-82.

Nickell, L.A., (1992). Deep bioturbation in organically enriched marine sediments. *Ph.D. Thesis. University of London, U.K.*

Ngoc-Ho, N., (1990). Nine Indo-Pacific species of *Upogebia* Leach (Crustacea, Thalassinidea, Upogebiidae). *J. nat. Hist.* **24**: 965-985.

Normon, A.M., (1907). Notes on the Crustacea of the Channel Islands. *Ann. Mag. nat. Hist.* **20**(7): 356-371.

O'Brien, J.V. and Vetter, R.D., (1990). Production of thiosulphate during sulphide oxidation by mitochondria of the symbiont containing bivalve *Solemya reidi. J. Exp. Biol.* **149**: 133-143.

Oeschager, R. and Schmaljohann, R., (1988). Association of various types of epibacteria with *Halicryptus spinulosus* (Priapulida). *Mar. Ecol. Prog. Ser.* **48**: 285-293.

Oeschager, R. and Storey, K.B., (1990). Regulation of glycolytic enzymes in marine invertebrates *Halicryptus spinulosus* (Priapulida) during environmental anoxia and exposure to hydrogen sulphide. *Mar. Biol.* **106**: 261-266.

Ohshima, K., (1967). Burrows of japanese Thalassinidea. *Chiku Kagatu.* **21**(1): 11-18.

Ott, A., Fuchs, B., Fuchs, R. and Malasek, A., (1976). Observations on the biology of *Callianassa stebbingi* Borradaille and *Upogebia litoralis* Risso. *Senckenberiana maritima.* **8**(1/3): 61-79.

Patel, S. and Spencer, C.P., (1963). The oxidation of sulphide by the haem compounds from the blood

of *Arenicola marina*. *J. Mar. Biol. Ass. U.K.* **43**: 167-175.

Patterson, M.R. (1992). A mass transfer explanation of metabolic scaling relations in some aquatic invertebrates and algae. *Science* **5**: 1421-1423.

Pearse, A.S., (1945). Ecology of *Upogebia affinis* (Say). *Ecology*. **26**: 303-305.

Pemberton, F.S., Risk, M.G. and Buckley, D.E., (1976). Supershrimp: deep bioturbation in the strait of Canso Nova Scotia. *Science*. **192**: 790-791.

Pervesler, P. and Dworschak, P.C., (1985). Burrows of *Jaxea nocturna* NARDO in the Gulf of Trieste. *Senckenberg. maritima.* **17**: 33-53.

Pike, R.B., (1953). The bopyrid parasites of the anomura from British and Irish waters. *J. Linn. Soc. Lond. Zool.* **42**(285): 219-237.

Pohl, M.E., (1946). Ecological observations on *Callianassa major* Say at Beaufort, North Carolina. *Ecology*. **27**: 71-80.

Popov, B.K., (1929). Rhizochephala and Bopyridae of the Bay of Sevastopol. *Trav. stat. Biol. Sebastopol.* **1**: 1-126.

Posey, M.H., (1986). Changes in benthic community associated with dense beds of a burrowing deposit feeder *Callianassa californiensis*. *Mar Ecol. Prog. Ser.* **31**: 15-22.

Posey, M.H., Dumbald, B.R. and Armstrong, D.A., (1991). Effects of a burrowing mud-shrimp, *Upogebia puggetensis* (Dana), on abundancea of macro-infauna. *J. Exp. Mar. Biol. Ecol.* **148**: 283-284.

Powell, E.N., Crenshaw, M.A. and Rieger, R.M., (1979). Adaptations to sulphide in the meiofauna of the sulphide system. I 35S-sulphide accumulation and the presence of a sulphide detoxification system. *J. exp. Mar. Biol. Ecol.* **37**: 57-76.

Powell, M.A. and Arp, A.J., (1984). Hydrogen sulphide oxidation by abundant nonhaemoglobin heme compounds in marine inverebrates from sulphide-rich habitats. *J. exp.* Zool. **249**: 121-132.

Powell, M.A. and Somero, G.N., (1985). Sulphide oxidation occurs in the animal tissue of the gutless clam, Solemya reidi. *Biol. Bull. Mar. Biol. Lab., Woods Hole*. **169**: 164- 181.

Poulson, E.M. (1940). On the occurance of the Thalassinidea in Danish waters. *Vidensk. Meddr dansk naturh. Faren.* **104**: 207-239.

Pritchard A.W. and Eddy S., (1979). Lactate formation in *Callianassa californiensis* and *Upogebia puttensis*. (Crustacea: Thalassinidea). *Mar. Biol., Berlin*. **50**: 249-253.

Pullin, R.S.V., Morris, S., Bridges, V.R. and Atkinson, R.J.A., (1980). Aspects of the respiratory physiology of the burrowing fish *Cepola rubescens L. Comp. Biochem. Physiol.* **66A**: 35-42.

Redmond, J.R. (1968). The respiratory function of haemocyanin. In *Physiology and biochemistry of haemocyanins* (ed Ghiretti, F.). pp 5-25, Academic Press, New York, London.

Robertson, J.D., (1960). Osmotic and ionic regulation. In *Physiology of Crustacea* (ed. Waterman, T.H.). Vol 1. pp 317-339. Academic Press, New York.

Rodrigues, S. de A., (1971). Mud-shrimps of the genus *Callianassa* Leach from the Brazilian coast

(Crustacea: Decapoda). *Archos. Zool. Est. S. Paulo.* **20**(3): 191-223.

Rodrigues, S. de A., (1976). Sobre a reproducao, embriologie e de senvolvimento larval de *Callichirus major* Say 1818 (Crustacea, Decapoda, Thalassinidea). *Bolm. Zool. Univ., S. Paulo.* **1**: 85-104.

Rodrigues S. de A., (1978). Mud shrimps of the genus *Callianassa* Leach from the Brazilian coast (Crustacea: Decapoda). *Archos Zool. Est. S. Paulo.* **20**(3): 191-223.

Rodrigues, S. de A., (1985). Sobre o crescimento relativo de *Callichirus major* (Say 1818) (Crustacea, Decapoda, Thalassinidea). *Bolm. Zool., Univ. S. Paulo.* **9**: 195-211.

Rodrigues, S. de A. and Hodl, W., (1990). Burrowing behaviour of *Callichirus major* and *C. mirim. Wiss. Film Nr.* **41**: 48-58.

Rosas, C., Sanchez, A., Escobar, E., Soto, L. & Bolongero-Grevenna, A. (1992). Daily variations of oxygen consumption and glucose haemolymph level related to morphophysiological and ecological adaptations of crustacea. *Comp. Biochem. Physiol.* **101(2)**: 323-328.

Roxby, R., Miller, K.I., Blair, D.P. and Van Holde, K.E., (1974). Subunits and association equilibrium of *Callianassa californiensis* haemocyanin. *Biochemistry.* **13**: 1662-1668.

Saint Laurent, M. de, (1972). Un Thalassinide nouveau du Golfe de Goscogne, *Calastacus laevis* sp. nov. Remarques sur la genre Calastacus Faxon (Crustacea Decapoda Axiidae). *Bull. Mus. natn. Hist. nat., Paris.* **29**: 347-356.

Saint Laurent M. de, (1973). Sur la systematique et la phylogenie des Thalassinidea: definition des familles des *Callianassidae* et des *Upogebiidae* et diagnose de cinq genres nouveaux (Crustacea: Decapoda). *C.r. hebd. Seanc. Acad. Sci., Paris.* **277**: 513- 516.

Saint Laurent, M. de and Le Loeuff, P., (1979). Crustaces Decapodes Thalassinidea. I. Upogebiidae et Callianassidae, Campagnes de la Calypso au larges de cotes Atlantiques Africanes (1956 et 1959). 22. *Ann. Inst. Oceanogr. Monaco,* (n. ser). **55** (suppl.): 29-101.

Sakai, K. and Saint Laurent, M. de., (1989). A check list of Axiidae (Decapoda, Crustacea, Thalassinidea, Anomura), with remarks and in addition descriptions of one new subfamily, eleven new genera and two new species. *Naturalists* **3**. Tokushima Biological Laboratory, Shikoku Womens University, Japan. pp104.

Samuelson, T.J., (1974). New records of *Upogebia delataura* and *U. stellata* (Crustacea: Decapoda) from western Norway. *Sarsia.* **56**: 131-134.

Sanders, N.K., Arp, A.J. and Childress, J.J., (1988). Oxygen binding characteristics of the haemocyanins of two deep-sea hydrothermal vent crustaceans. *Respir. Physiol.* **71**: 57-68.

Sanders, N.K. and Childress, J.J., (1990a). A comparison of the respiratory function of the haemocyanins of vertically migrating and non-migrating pelagic, deep-sea ophophorid shrimps. *J. exp. Biol.* **152**: 167-187.

Sanders, N.K. and Childress, J.J., (1990b). Effects of freezing on the function and association state of crustcean haemocyanins. *J. exp. Biol.* **138**: 535-539.

Sanders, N.K. and Childress, J.J., (1992). Specific effects of thiosulphate and L-lactate in haemocyanin-

O_2 affinity in a brachyuran hydrothermal vent crab. *Mar. Biol.* **113**: 175-180.

Scammell, C.A. and Hughes, G.M., (1982). Comparative study of the functional anatomy of the gills and ventilatory currents in some British decapod crustaceans. *Bull. Biol., India.* **4**: 1-23.

Schembri, P.J., (1979). Oxygen consumption and the respiratory responses to declining oxygen tension in the crab *Ebalia tuberosa* (Pennant) (Crustacea: Decapoda: Leucosiidae). *J. exp. Mar. Biol. Ecol.* **41**: 133-142.

Schram F.R., (1983). Method and madness in phylogeny. In: *Crustacean Issues* I. Crustacean phylogeny. Ed Schram, F.R. Rotterdam: Balkema.

Scott, P.J.B., Reising, H.M. and Marcotte, B.M., (1988). Ecology, functional morphology, behaviour and feeding in coral- and sponge-boring species of *Upogebia* (Crustacea: Decapoda: Thalassinidea) *Can. J. Zool.* **66**: 483-495.

Selbie, C.M., (1914). The Decapoda Reptantia of the coasts of Ireland. *Dept. Agric. Tech. Instr. Ireland Fisheries Branch Sci. Invest.* pp 116.

Shiino, S.M., (1965). Phylogeny of the genera within the family Bopyridae. *Bull.* Mus. *Natn. Hist. nat. 2e Ser.* **37**(3): 462-465.

Shinn, E.A., (1968). Burrowing in recent lime sediments off Florida and the Bahamas. *Jour. Paleont.* **42**: 879-894.

Sick, H. and Gersonde, K., (1969). Method for the continuous registration of O_2-binding curves of haemoproteins by means of a diffusion chamber. *Analyt. Biochem.* **32**: 362-376.

Smith, R.P. and Abbanat, R.A., (1966). Protective effect of oxidised glutathione in acute sulphide poisoning. *Toxic appl. Parmac.* **9**: 209-217.

Smith, K.L., (1967). Callianassid crustacean burrows as a possible palaeoenvironmental indicators. *M.Sc., University of Delaware.* 51pp.

Snyder, G.K. and Mangum, C.P., (1982). The relationship between the capacity for oxygen transport, size, shape and aggregation state of an extracellular oxygen carrier. In *Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals* (eds. Bonaventura, J., Bonaventura, C. and tesh, S.). pp 173-188. Allan Liss Co., New York.

Solorzano, L., 1969). Determination of ammonia in natural waters by the phenol hypochlorite method. *Linol. Oceanogr.* **14**: 799-801.

Spicer, J.I. and McMahon, B.R. (1991). Haemocyanin oxygen binding and the physiological ecology of a range of talitroidean amphipods (Crustcea) II. Effect of freezing, inorganic ions and urate on O_2 binding *in vitro*. *J. Comp. Physiol.* **161B**: 217-223.

Spicer, J.I. and Taylor, A.C., (1986). A comparative study of the gill area relationships in some talitrid amphipods. *J. nat. Hist.* **20**: 935-947.

Spoek, G.L., (1974). The relationship between blood haemocyanin levels, oxygen uptake and heart beat and scaphognathite beat frequencies in the lobster *Homarus gammarus. Neth. J. Sea. Res.* **8**: 1-26.

Stebbing, T.R.R., (1900). *South African Crustacea.* Pt I. Marine Investigations in S. Africa.

Steffensen, J.F., (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiol. Biochem.* **6**(1): 49-59.

Stephenson, K., (1939). *Crustacea Decapoda. The zoology of Iceland.* Vol III (35). Copenhagen and Reykjavik.

Strangeways-Dixon, J. and Smith, D.S., (1970). The fine structure of gill 'podocytes' in *Panulirus argus* (Crustacea). *Tissue Cell.* **2**: 611-624.

Suchanek, T.H., (1983). Control of seagrass community and sediment distribution by *Callianassa* (Crustacea: Thalassinidea) bioturbation. *J. Mar.* Res. **41**: 281-298.

Suchanek, T.H., (1985). Thalassinid shrimp burrows: ecological significance of species-specific architecture. In *Proc. Vth Int. Coral Reef Congress, Papeete, Tahiti. Anteene Museum-Ephe, Moorea, French Polynesia.* **5**: 205-210.

Suchanek, T.H., Colin, P.L., McMurtry, G. M. and Suchanek, C.S., (1986). Bioturbation and redistribution of sediment radionuclides in Enewetak Atoll lagoon by callianassid shrimp: biological aspects. *Bull. Mar. Sci.* **38**(1): 144-154.

Svedberg, T., (1933). Sedimentation constants, molecular weights and isoelectric points of the respiratory proteins. *J. Biol. Chem.* **103**: 311-325.

Swain, R. Marker, P.F. and Richardson, A.M.M., (1987). Comparison of the gill morphology and branchial chambers in two fresh-water crayfishes from Tasmania: *Astacopsis franklinii* and *Parastacoides tasmanicus. J. Crust. Biol.* **8**(3): 355-363.

Swinbanks, D.D. and Murray, J.W., (1981). Biosedimentological zonation of Boundary Bay tidal flats, Fraser river delta, British Columbia. *Sedimentology.* **28**: 210-237.

Swinbanks, D.D. and Luternauer, J.L., (1987). Burrow distributions of thalassinidean shrimp on a Fraser delta tidal flat, British Columbia. *J. Paleont.* **61**(2): 315-332.

Talbot, P., Clark, W.H. and Lawrence, A.L., (1972). Light and electron microscopic studies in osmoregulatory tissue in the developing brown shrimp *Penaeus aztecus. Tissue Cell.* **4**: 271-286.

Tang, P.S., (1933). Oxygen consumption as a function of oxygen pressure. *Q. Rev. Biol.* **8**: 260-274.

Taylor, A.C., (1976). The respiratory responses of *Carcinus maenas* to declining oxygen tension. *J. exp. Biol.* **65:** 309-322.

Taylor, A.C., (1984a). Branchial ventilation in the burrowing crab *Atelecylus rotudatus. J. Mar. Biol.* Ass. *U.K.* **64**: 7-20.

Taylor, A.C., (1984b). The ecophysiology of decapods in the rock pool environment. *Symp. Zool. Soc. Lond.* **59**: 226-261.

Taylor, A.C. and Spencer-Davies, P., (1981). Respiration in the land crab *Geocarcinus lateralis. J. exp Biol.* **93**: 197-208.

Taylor, A.C and Spicer, J.I., (1989). Interspecific comparison and the respiratory response to declining oxygen tensions and the oxygen transporting properties of the blood of some Palaemonid prawns (Crustacea: Palaomonidae). *Mar. Behav. Physiol.* **14**: 81-91.

Taylor, A.C. and Atkinson, R.J.A., (1991). Respiratory adaptations of aquatic decapod crustaceans and fish to a burrowing mode of life. In. *Physiological strategies for gas exchange and metabolism.* (eds. Woakes, A.J., Grieshaber, M.K. and Bridges, C.R.). Cambridge University Press. Cambridge (*Semin. Ser. Soc. exp. Biol.* **41**):

Taylor, A.C., Morris, S. and Bridges, C.R., (1985). Oxygen and carbon dioxide tranporting properties of the blood of three sublittoral species of burrowing crab. *J. Comp. Physiol.* **155**: 733-742.

Taylor, E.W. and Butler, P.J., (1978). Aquatic and aerial respiration in the shore crab, *Carcinus menas* (L.) acclimated to 15°C. *J. Comp. Physiol.* **127**: 315-323.

Taylor, E.W., Butler, P.J. & Al-Wassia, A. (1977). Some responses of the shore crab, *Carcinus maenas* (L.) to progressive hypoxia at different acclimation temperatures and salinities. *J. Comp. Physiol.* **122**: 391-402.

Taylor, H.H & Taylor, E.W., (1992). Gills and lungs: The exchange of gases and ions. In *Microscopic Anatomy of Invertebrates.* Volume 10: Decapod Crustacea. 203-293. Wiley-Liss, Inc.

Terwilliger, N.B., Terwilliger, R.C., Applestein, M., Bonaventura, C. and Bonaventura, J., (1979). Subunit structure and oxygen binding by haemocyanin of the isopod *Ligia exotica.* Biochemistry. **18** (1): 101-108.

Theede, H., Ponat, A., Hiroki, K and Schlieper, C., (1969). Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. *Mar. Biol.* **2**: 325-337.

Thomas, H. (1954). The oxygen uptake of the lobster *Homarus vulgaris. J. exp. Biol.* **31**: 228-251.

Thompson, R.K., (1972). Functional morphology of the hind gut gland of *Upogebia pugettensis* (Crustacea: Thalassinidea) and its role in burrow construction. *Ph.D. dissertation.* Univ. of California, Berkeley.

Thompson, R.K. and Pritchard, A.W., (1969). Respiratory adaptations of two burrowing crustaceans, *Callianassa californiensis* and *Upogebia pugettensis* (Decapoda, Thalassinidea). *Biol. Bull. Mar. Biol. Lab. Woods Hole.* **136**: 274-287.

Titulaer, W.A. (1991). A respirometer of flexible design. *Comp. Biochem. Physiol.* **99A** (3): 347-350.

Torres, J.J., Gluck, D.L. and, Childress J.J., (1977). Activity and physiological significance of the pleopods in the respiration of *Callianassa californiensis* (Dana) (Crustacea: Thalassinidea). *Biol. Bull. Mar. Biol. Lab. Woods Hole.* **152**: 134-146.

Truchot, J.P., (1976). Carbon dioxide combining properties of the blood of the shore crab *Carcinus maenas* (L). CO_2-dissociation curves and Haldane effect. *J. Comp. Physiol.* **112**: 283-293.

Truchot, J.P., (1980). Lactate increases the oxygen affinity of crab haemocyanins. *J. exp. Zool.* **214**: 205-208.

Trueman, E.R. (1967). Activity and heart rate of bivalve molluscs in their natural habitat. *Nature. Lond.* **214**: 832-833.

Tucker, V.W., (1930). On the effects of an epicaridean parasite *Gyge branchialis*, on *Upogebia littoralis. Q. J. Microsc. Sc.* **74**: 1-118.

Tunberg, B., (1986). Studies on the population ecology of *Upogebia deltaura* (Leach) (Crustacea: Thalassinidea). *Est. Coast. Shelf. Sci:* **22** (6): 753-766.

Vallejo, Salazar-, S.I. and Tristan, Leija-, A., (1989). *Progebiophilus bruscai* n.sp., a new bopyrid isopod parasitic on the mud shrimp, *Upogebia dawsoni* Williams (Thalassinoidea), from the Gulf of California. *Cah. Biol. Mar.* **30**: 423-432.

Van Holde, K.E., Blair, D., Eldred, N and Anasaka, F., (1977). Association equilibria of *Callianassa* haemocyanin. In *Structure and function of haemocyanin* (ed Bannister, J.V.). pp 22-30. Springer-Verlag, Berlin and New York.

Vaugelas, J. de., (1984). Preliminary observations on two types of Callianassid (Crustacea: Thalassinidea) burrows, Gulf of Aquaba, Red Sea. *Proc. Symp. Coral Reef Environ. Red Sea, Jeddah*, Jan. 1984: 520-539.

Vaugelas, J. de., (1990). Ecologie des callianasses (Crustacea, Decapoda, Thalassinidea) en milieu recital Indo-Pacifique, Consequences du remaniement sedimentaire sur la distribution des matieres humiques, des metaux traces et des radionucleides. *Doctoral d'Habilition a Diriger des Recherches,* University de Nice. pp226.

Vaugelas, J. de and Jaubert, J., (1989). An experimental closed circuit limarium for investigating the behaviour and ecophysiology of burrowing crustaceans. *Bull. Ins. Oceanogr. Monaco.*, no special. **5**: 71-77.

Vaugelas, J. de. and Buscaile, R., (1990). Organic matter distruibution in burrows of the thalassinid crustacean *Callichirus laurae*, Gulf of Aqaba (Red Sea). *Hydrobiologia.* **207**: 269-277.

Vaugelas, J. de, Delsalle, B. and Monier, C., (1986). Aspects of the biology of *Callichirus armatus* (A.Milne Edwards, 1870) (Decapoda, Thalassinidea) from French Polynesia. *Crustaceana:* **50** (2): 204-216.

Vernberg, J.F., (1985). Envoronmental physiology. In. *Physiological adaptations of marine animals.* (ed. Laverack, M.S.). Symposia of the Society for Experimental Biology No 39. The Company of Biologists Ltd.

Vetter, R.D., Wells, ME., Kurtsman, A.L. and Somero, G.N., (1987). Sulphide detoxification by the hydrothermal vent crab *Bythograea thermydron* and other decapod crustaceans. *Physiol. Zool.* **60** (1): 121-137.

Vetter, R.D., Matrai, P.A., Javour, B. and O'Brien, J., (1989). Reduced sulphur compounds in the marine environment. Analysis by high-performance liquid chromotography. In. *Biogenic sulphur in the environment.* (eds. Slatman, E. and Cooper, W.). American Chemical Society Symposia. Series 393: 243-261.

Vismann, B., (1991). Physiology of sulphide detoxification in the isopod *Saduria (Mesidotea) entemon. Mar. Ecol. Prog. Ser.* **76**: 283-293.

Vogel, S. and Bretz, W.L., (1971). Interfacial organisms: Passive ventilation in the velocity gradients near surfaces. *Science.* **175**: 210-211.

Vokel, S. and Grieshaber, M.K., (1992). Mechanisms of sulphide tolerance in the peanut worm, *Sipunculus nudus* (Sipunculidae) and in the lugworm, *Arenicola marina* (Polychaeta). *J. Comp. Physiol.* **162B**: 149-477.

Von Oertzen, J-A., (1984). Open and closed systems-their suitability for functional respiration measurements. *Limnologica (Berlin).* **15** (2): 541-545.

Waslenchuk, D.G., Matson, E.A., Zajac, R.N., Dobbs, F.C. and Tramontano, J.M., (1983). Geochemistry of burrow waters vented by a bioturbating shrimp in Bermudian sediments. *Mar. Biol., Berlin.* **72**: 219-225.

Watt, W.B., (1991). Biochemistry, physiological ecology and population genetics-the mechanistic tools of evolutionary biology. *Functional Ecology.* **5**: 145-154.

Wear, R.G. and Yaldwyn, J.C., (1966). Studies on thalassinid Crustacea (Decapoda: Macrura: Reptantia) with a description of a new *Jaxea* from New Zealand and an account of its larval development. *Zoology Publs Vict. Univ. Wellington.* **4**.

Webb, G.E., (1919). The development of the species of *Upogebia* from Plymouth Sound. *J. Mar. Biol.* Ass. *U.K.* **12**: 81-135.

Wells, R.M.G. and Weber, R.E., (1991). The measurement of oxygen affinity in the blood and haemoglobin solutions. In *Physiological strategies for gas exchange and metabolism.* (eds. Woakes, A.J., Grieshaber, M.K. & Bridges, C.R.). Cambridge University Press. Cambridge (Semin. *Ser. Soc. exp. Biol.* **41**).

Wheatly, M.G. (1989). Standard rate of oxygen uptake and body size in the crayfish *Pacifastacus leniusculus* (Dana, 1852) (Decapoda: Astacidae): Intra- versus Inter-specific relations in crustaceans. *J. Crustacean Biol.* **9**: 212-216.

Wheatly, M.G. and Taylor, E.W., (1981). The effect of progressive hypoxia on heart-rate, ventilation, respiratory gas-exchange and acid-base status in the crayfish *Austropotamobius pallipes. J. Exp. Biol.* **98**: 119-137.

Witbaard, R. and Duineveld, G.C.A.,(1989). Some aspects of the biology and ecology of the burrowing shrimp *Callianassa subterranea* (Montagu) (Thalssinidea) from the southern North Sea. *Sarsia.* **74**: 209-219.

Wycliffe, J.M. and Job, S.V., (1977). Standard, routine and active oxygen consumption of a freshwater shrimp. *Hydrobiologia.* **54** (1): 33-39.

Yeager, D.P. and Ultsch, G.R., (1989). Physiological regulation and conformation. A BASIC programme for the determination of critical points. *Physiological Zoology.* **40** (4): 888-907.

Zainal, K.A.Y., (1990). Aspects of the biology of the squat lobster, Munida rugosa (Fabricius, 1775). *PhD Thesis, University of Glasgow.*

Zainal, K.A.Y., Taylor, A.C. and Atkinson, R.J.A., (1992). The effect of temperature and hypoxia on the respiratory physiology of the squat lobsters, *Munida rugosa and Munida sarsi* (Anomura, Galatheidae). *Comp. Biochem. Physiol.* **101A** (3): 557-567.

Zebe, E., (1982). Anaerobic metabolism in *Upogebia* pugettensis and *Callianassa californiensis* (Crustacea: Thalassinidea). *Comp. Biochem. Physiol.* 72B: 613-617.

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