# CHAPTER 4

# FUNCTIONAL SIGNIFICANCE OF THE OXYSTOMATOUS CONDITION:

## A comparative study of the respiratory structures and burying behaviour of *Calappa* and *Matuta*

## 4.1 INTRODUCTION

The term "oxystomatous" refers to a morphological characteristic of some brachyuran crabs in which the buccal frame or endostome is extended forward to form a triangle (*oxy* – Greek, "sharp"; *-stoma* – Latin, "mouth or opening"). Until recently, crabs possessing this morphological configuration were placed in the tribe Oxystomata as established by H. Milne Edwards in 1837. In this classification scheme, the Oxystomata included three groups of crabs, the pebble crabs (f. Leucosiidae), shield crabs (f. Dorippidae) and box crabs (f. Calappidae *sensu lato*).

As the name implies, the mouthparts of these crabs taper narrowly towards the front, giving the buccal area a pointed appearance. In these taxa, the exhalant openings of the respiratory channel are located terminally at the tip of the buccal frame, with the floor of the channel being extended by the elongate process of the 1st maxilliped. The functional significance of this condition is poorly understood, although it is widely assumed that it is enables the crabs to breathe whilst buried in the sediment (Garstang, 1897b, Warner, 1977). To date, however, no empirical evidence has been presented to support this assumption, apart from the inferences of Garstang (1897b ) which were based on a morphological study of preserved specimens.

In this chapter, I will investigate the functional basis of the oxystomatous condition and evaluate its role in burying. Phylogenetic evidence in Chapter 2 unequivocably demonstrates that the major taxa within the Calappidae *s.l.* do not share a common ancestry as previously thought. It appears that the oxystomatous condition has arisen independently on at least 3 occasions. This therefore, presents us with an interesting opportunity to investigate a case of evolutionary convergence in a functional system.

In this regard, this study will focus on the functional morphology of the respiratory structures of two distinct forms: *Calappa* and *Matuta*, which both show the oxystomatous condition. Despite their disparate ancestries, both genera share similar habitat and behavioural features and are common in soft-sediment communities in the tropics. Both genera are well-known for their burying behaviour. A key objective of this study is to examine the patterns of respiratory ventilation in *Calappa* and *Matuta* whilst buried. It has been shown that in non-burying crabs, the typical pattern of respiratory ventilation is the 'forward ventilatory mode' (Arudpragasam & Naylor, 1964, Warner, 1977), where water is drawn into the branchial chambers through the openings at the bases of the pereiopods and is exhaled anteriorly through paired openings between the antennae and the mouthparts. Burying crabs have also been reported to employ a reversal in the direction of ventilation whilst under the sediment (e.g. Garstang, 1896, Hartnoll, 1972, Caine, 1974, Taylor, 1984). Much of our knowledge of ventilatory currents in burying crabs, however, is based on non-oxystomatous groups. There is presently no detailed study of the functional morphology of respiratory structures and the patterns of respiratory ventilation of any oxystomatous taxa, apart from that of a temperate leucosiid, *Ebalia tuberosa* (Schembri, 1981a). The present study therefore, will broaden our understanding of the respiratory adaptations of oxystomatous crabs and will provide the focus for a broader investigation of the morphological constraints of burying in brachyurans as a whole.

This chapter contains two major sections: 1) a review of the occurrence of the burying behaviour within the Brachyura and 2) a comparative study of the morphology of the respiratory structures of oxystomatous crabs and an analysis of the burying behaviour and respiratory currents in *Calappa* (f. Calappidae *s.s.*) and *Matuta* (f. Matutidae).

## 4.2 SECTION 1 : A REVIEW OF THE OCCURRENCE OF THE BURYING BEHAVIOUR IN BRACHYURAN CRABS

Many crustaceans live in close association with the sediment, with some spending a large proportion of their life beneath the sediment surface. This mode of life is termed either burying or burrowing. Although these terms are often used interchangeably in the literature, there are clear distinctions between these two types of behaviour. This review will examine the nature of animal-sediment interactions, focussing on the burying habit of brachyuran crabs.

The "burrowing" habit of many brachyurans is well documented (see Vannini, 1980 Abele & Felgenhauer, 1982). In most cases, two types of "burrowing" are recognized. Warner (1977) describes "side-burrowing" in burrow-forming species and "back-burrowing" in species which temporarily cover themselves in sediment. Atkinson and Taylor (1988) distinguish the physiological challenges of living in a burrow from those associated with burying in soft sediment. They refer to "burying" as "burrowing for concealment but without burrow formation". Nye (1974) recognized the distinction between burrow excavation and rapid burial in the ocypode *Macrophthalmus hirtipes*, pointing out differences in movement and timing. Even the early classic work of Garstang (1897a) refers to two types of burrows, those which are "permanent subterranean tunnels" and those which are "temporary excavations, the [sediment] being in actual contact with the [animal] bodies when they are embedded". Despite this recognition of different "burrow" types, however, these distinctions are not always identified in the literature, with the terms *burrowing* and *burying* being often used interchangeably (e.g. Lucas, 1980, Schembri, 1981a, McLay & Osborne, 1985, Davidson & Taylor, 1995).

Based on the earlier studies of Garstang (1897a), Warner (1977) and Atkinson and Taylor (1988), the following definitions of burying vs burrowing will be used in this study. Burying - where the animal actively moves into the substratum with the sediment being in direct contact with, and encasing, the body. Burrowing - where the animal resides within a cavity or tunnel that it has excavated in the substratum. These two types of behaviour have very different physical and physiological consequences.

Despite the number of studies on burrowing in Crustacea, relatively little is known of burying, particularly in brachyurans. The mechanics of burrowing in crabs and the physiology of burrowing in decapods has been well-reviewed by Vannini (1980) and Atkinson & Taylor (1988), respectively. In contrast, references to the burying behaviour of crabs is dispersed with most reports contained in general taxonomic accounts and natural history studies of individual species. Although burying as a distinct behaviour has been recognized for over 100 years, there are few detailed studies on the mechanics and physiological implications of the process. Of the existing studies, most are focussed on the respiratory physiology of burial, particularly in temperate taxa. The primary aim of this review therefore, is to summarize our current knowledge of the burying behaviour in brachyurans, its taxonomic and evolutionary extent within the Brachyura, and the physiological and ecological consequences of the behaviour in this group. Recent advances in our understanding of crustacean evolution (Fig. 4.1) suggest that burying in brachyurans may have evolved independently in at least two lineages and that a range of structural modifications may be associated with the burying behaviour. This review will provide both a taxonomic survey of brachyuran families known to bury and an overview of the biological basis and consequences of the burying behaviour.

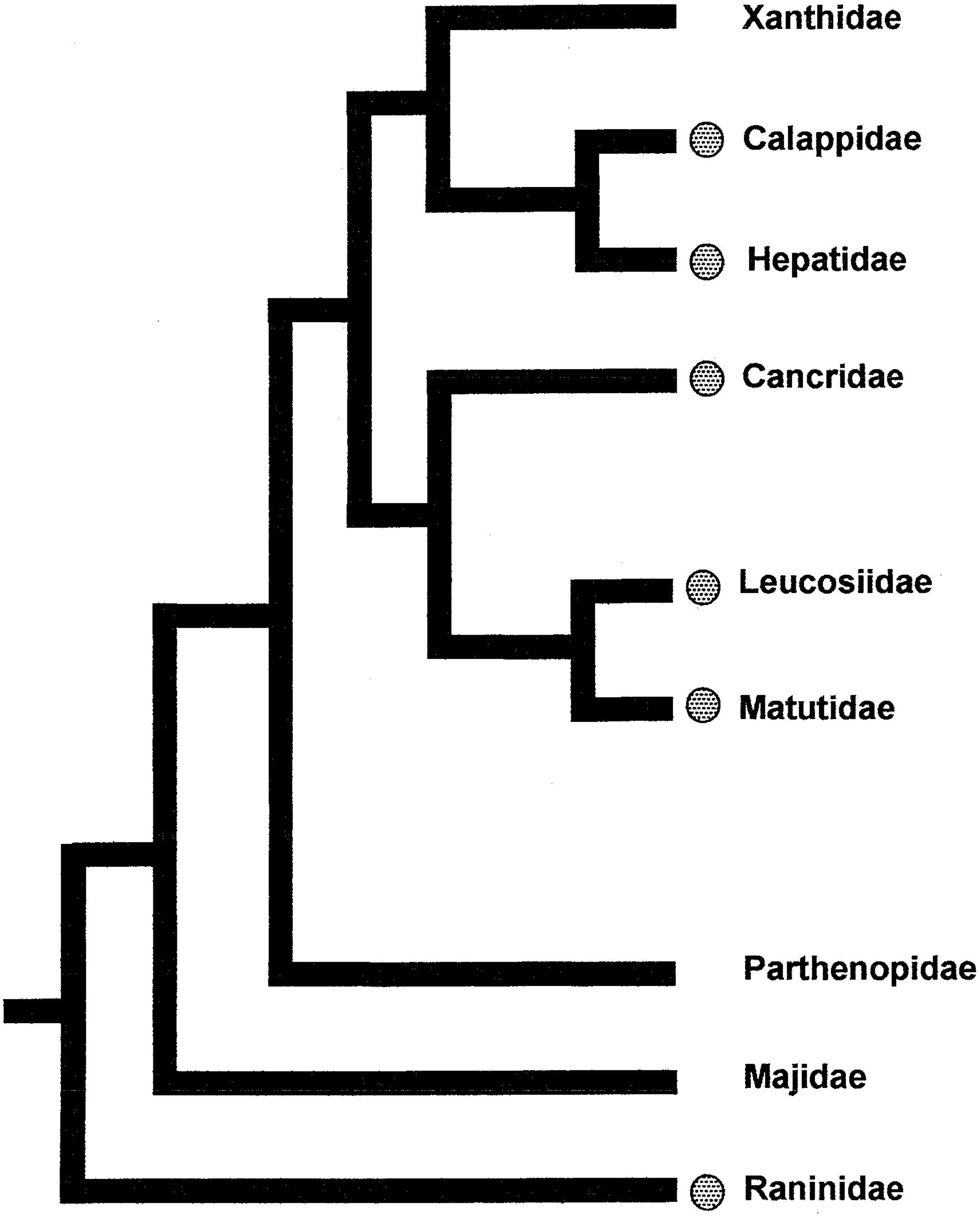


Figure 4.1 Putative brachyuran phylogeny, incorporating cladograms from Spears et al. (1992) and Bellwood (1996). ● = families where burying behaviour is reported.

### 4.2.1 A survey of families

For most of brachyuran families, our knowledge of the ecology of individual species is limited. Many studies are based on trawled specimens and the animals are rarely observed exhibiting normal behaviour in their natural habitat. This means that a large number of observations must be based on inferences from preserved material or observations of animals in captivity. While these provide a useful indication of the potential capabilities of the individual taxa there is clearly a need for more detailed observations in both the laboratory and field. Of the 50 or more families placed in the Brachyura (see Bowman & Abele 1982, Schram 1986), representatives of the following families are known to bury: Atelecyclidae, Calappidae, Cancridae, Corystidae, Hymenosomatidae, Leucosiidae, Matutidae, Orithyiidae, Portunidae, Raninidae. Those that form burrows are: Beliidae, Gecarcinidae, Goneplacidae, Grapsidae, Mictyridae, Ocypodidae, Portunidae, Potamoidea. It is interesting to note that whilst most of the families known to bury are found under subsection Heterotremata *sensu* Guinot (1979), a majority of the families known to form burrows are found under the subsection Thoracotremata. In the following section, each of the burying families will be considered separately. A summary of published information for each of these families is presented in Table 4.1.

**Raninidae** - Previously included within the section Oxystomata, this primitive group of crabs contains nine extant genera with the majority of species occurring in sandy substrata throughout the tropics (Abele & Felgenhauer, 1982). The burying habit of raninids is inferred by many authors based on their carapace shape and pereiopod structure (e.g. Hartnoll, 1971, Jones & Morgan, 1994). Raninids typically possess a smooth and elongate carapace, and flattened 2nd – 5th pereiopods. Several reports of

**Table 4.1** Summary of published information on aspects of burying in brachyuran crabs

| **Family** | **Reference** |
|---|---|
| Raninidae | None known (but see Hartnoll, 1971; Jones & Morgan, 1994; Steene, 1999) |
| Calappidae | Morphology (Garstang, 1897b; Schaefer, 1954)<br>Behaviour (Stevcic, 1983) |
| Matutidae | Behaviour (Seiler, 1976, Stevcic, 1983; Perez, 1986)<br>Morphology (Garstang, 1897b) |
| Orithyidae | None known |
| Leucosiidae | |
| *Ebalia tuberosa* | Behaviour (Schembri, 1981b)<br>Respiration (Schembri, 1979a, 1979b, 1981a) |
| *Philyra laevis* | Behaviour (Hale, 1926) |
| Portunidae | |
| *Ovalipes* spp | Biology (Caine, 1974)<br>Behaviour (McLay & Osborne, 1985)<br>Respiration (Davidson & Taylor, 1995) |
| *Callinectes sapidus* | Biology (Tagatz, 1968; Williams, 1974)<br>Ecology (Barshaw & Able, 1990) |
| *Carcinus maenas* | Respiration (Cumberlidge & Uglow, 1978) |
| *Macropipus puber* | Behaviour (Warner, 1977) |
| *Portunus pelagicus* | Behaviour (Sumpton and Smith, 1990) |
| *Scylla serrata* | Behaviour and ecology (Hill, 1975, 1982) |
| Corystidae | Respiration (Garstang, 1896; Bridges, 1979)<br>Biology (Hartnoll, 1972) |
| Atelecyclidae | Respiration (Garstang, 1896, Taylor, 1984) |
| Cancridae | Respiration (Arudpragasam & Naylor, 1966; Ansell, 1973; Cumberlidge & Uglow, 1979) |
| Hymenosomatidae | Behaviour (Barnard, 1950; Melrose, 1975; Lucas, 1980) |

burying (R. Steene and N. Coleman, pers. comm.) describe the crabs rapidly backing into the substratum leaving only the anterior portion of the carapace exposed (cf. photograph in Steene, 1999).

**Calappidae** - Commonly known as the "box crabs" or "shame-faced" crabs, the Calappidae s.s. is a distinctive group of crabs containing 8 genera with distributions extending throughout the tropical and subtropical regions of the world. Generally found in soft substrata ranging from muddy or fine sandy bottoms to sand/rubble aggregates, calappids have been reported to bury using their chelipeds (Schaefer, 1954). Much of their burying habit, however, is documented only in taxonomic descriptions (e.g. Rathbun, 1937, Sakai, 1965) and early natural history accounts (e.g. Henderson, 1893). Garstang (1897b) ascribed a number of morphological modifications in *Calappa* and *Mursia* to their burying habit. These included the existence of an "exostegal channel", denticulations on the carapace margins and "hairiness" of the pterygostomian regions (Garstang, 1897b). However, these functional assumptions were based on preserved specimens and were not confirmed using observations of live material. *Calappa* bury by pushing against the sediment with the chelipeds forcing the carapace backwards and under the sediment, either completely or with only the eyes exposed (see Section 2, this chapter.). The burying mode in other genera is unknown but the presence of exostegal channels and collections from sedimentary habitats point to a widespread burying habit. The results of the phylogenetic study of the Calappidae *sensu stricto* suggests that the group may have evolved in deep water, an environment characterized by fine sediment (cf Chapter 3).

**Matutidae** - A widely distributed family of tropical-subtropical shallow-water crabs, the Matutidae are known for their ability to bury themselves very quickly into sandy substrata (Seiler, 1976, Warner, 1977; Perez, 1986). *Matuta* has been been observed to enter the substratum backwards using their flattened pereiopods for digging (Warner, 1977, also see Section 2, this chapter). Garstang (1897b) attributed a number of morphological features in *Matuta* to its burying habit, including the existence of an "exostegal channel" and the presence of large epibranchial spines.

**Orithyidae** - Very little is known of the biology of this monotypic family which is found only in shallow sandy or muddy habitats in the South China Sea (Dai & Yang 1991). Its burying habit is inferred based solely on the possession of flattened pereiopods with the 5th pair being paddle-shaped, similar to those of the Matutidae.

**Leucosiidae** - Commonly found in soft sediments, leucosiids often bury themselves rapidly by digging backwards into the sediment until half-covered (e.g. *Ebalia tuberosa* - Schembri, 1981b, *Philyra laevis* - Hale, 1926, *Leucosia granulata* – pers. obs.). Leucosiids possess a number of morphological features which support a burying habit, including a modified inhalant channel which runs underneath the outer edges of the 3rd maxillipeds and enters the branchial chambers through the respiratory openings at the bases of the maxillipeds (Warner, 1977).

**Portunidae** - Although members of this family are commonly called "swimming crabs" for their ability to swim using the flattened pair of 5th pereiopods, the burying habit of several portunid species is well documented (*Ovalipes* – Caine, 1974, McLay and Osborne, 1985, *Callinectes* - Tagatz, 1968, Williams, 1974, Barshaw & Able, 1990;

*Carcinus* - Cumberlidge & Uglow, 1978; *Macropipus* - Warner 1977; *Portunus* – Sumpton & Smith, 1990). During the day, portunids often bury in the substratum, emerging at night to feed. Morphological features which have been associated with swimming in portunids, including the possession of a streamlined carapace and flattened pereiopods, have also been identified as features which may facilitate digging into the substratum (Hartnoll, 1971, Warner, 1977). Portunids typically enter the substratum backwards, using the pereiopods to loosen the sediment followed either by a "body slam" or "sternal push" to force the crab deeper into the sediment and to cover the body with sediment (McLay & Osborne, 1985). Only one portunid genus is known to both bury and burrow (i.e. construct permanent burrows), *Scylla* (Hill, 1975; Hill *et al.*, 1982). The conditions which determine burying vs. burrowing behaviour in *Scylla*, however, remain unclear.

**Corystidae** - The burying habit of *Corystes cassivelaunus* is well documented (Garstang, 1896, Hartnoll 1972, Bridges, 1979). Found in sandy substrata in the North Sea, *C. cassivelaunus* is able to bury more deeply into the substratum than any of the above families, burying themselves completely with the only the tips of the antennae protruding. Members of this family possess a number of morphological and physiological features associated with their burying habit, most notably antennae which form a breathing tube of interlocking setae through which the inhalant current flows whilst the crab is buried.

**Atelecyclidae** - Burying in this family is reported in only one genus, *Atelecyclus* (Garstang, 1896, Taylor 1984). However, the common occurrence of other genera in soft sandy or muddy substrata (Sakai, 1976) suggests that burying may be more widespread within the family. Taylor (1984) described the burying behaviour and respiratory adaptations of *Atelecyclus rotundatus*. *A. rotundatus* enters the substratum backwards using the combined action of its pereiopods, including the chelipeds. It possesses a "breathing tube" formed by the interlocking setae of the 2nd antennae (as in *Corystes)*, through which the inhalant current flows when the crab is completely buried.

**Cancridae** - Although this family is primarily associated with rocky intertidal habitats, some species have been found in sandy substrata, e.g. *Cancer magister* (Abele & Felgenhauer, 1982) and *C. japonicus* (Sakai, 1976). The ability of the North Atlantic edible crab *C. pagurus* to bury in sand is well documented (e.g. Arudpragasam & Naylor, 1966; Ansell, 1973, Cumberlidge & Uglow, 1979) with several studies of their respiratory physiology. The ecological significance of burying in this species, however, is unclear.

**Hymenosomatidae** - Four species of hymenosomatids are known to bury in sandy to muddy substrates: *Halicarcinus varius*, *H. whitei*, *Hymenosoma orbiculare* and *Elamena producta* (Barnard, 1950; Melrose, 1975; Lucas, 1980). Burying is a gradual process which involves rocking of the carapace and thrusting movements of the pereiopods (Melrose, 1975). There appears to be no specific morphological adaptations associated with the burying habit.

### 4.2.2 Biological basis and consequences of burying

#### *Function*

The functions of burrows in crustaceans are well documented, particularly in terrestrial and semi-terrestrial taxa (Vannini, 1980, Powers & Bliss, 1983, Atkinson &

Taylor, 1988, Dunham & Gilchrist, 1988). These include refuge from predators and adverse environmental conditions, shelter, and as a site for moulting, reproduction and feeding. In contrast, burying behaviour is seen primarily as a means of predator avoidance (Nye, 1974; Barshaw & Able, 1990). Its role as an antifouling mechanism for restricting epibiont colonization on crab carapaces has also been suggested (Becker & Wahl, 1996). The ability to bury quickly may also be essential in maintaining position in an unstable substratum such as that on the surf zone, as observed in anomuran mole crabs (Trueman & Ansell, 1969) and in *Matuta lunaris* (Perez, 1986). For intertidal species, rapid burying also prevents desiccation and may provide a means of concealment for animals stranded by a receding tide (Warner, 1977; Perez, 1986).

### *Mechanics*

The excavating behaviour which results in the formation of a burrow has been referred to as "side-burrowing" (Warner, 1977). Basically, excavation is effected by using one set of walking legs as a scoop which the other set provides traction on the substratum. The chelipeds and 2nd and 3rd pereiopods on the leading side carry the excavated sediment out of the burrow. Such digging behaviour in crabs has been reviewed by Vannini (1980). In contrast, little is known of the mechanics of the burying behaviour in brachyurans. Crabs which bury themselves temporarily in the substratum have been termed "back-burrowers" by Warner (1977), referring to the manner by which the animal enters the substratum, i.e. backwards until half-covered by the sediment. The process of entering the substratum backwards often involves the use of the last pair of pereiopods to loosen the subsratum and to propel the individual backwards into the sediment as in *Ebalia* (Schembri, 1981b), *Matuta* (Perez, 1986) and *Ovalipes* (McClay & Osborne 1985) (Fig. 4.2b). In some taxa, the chelae are used to

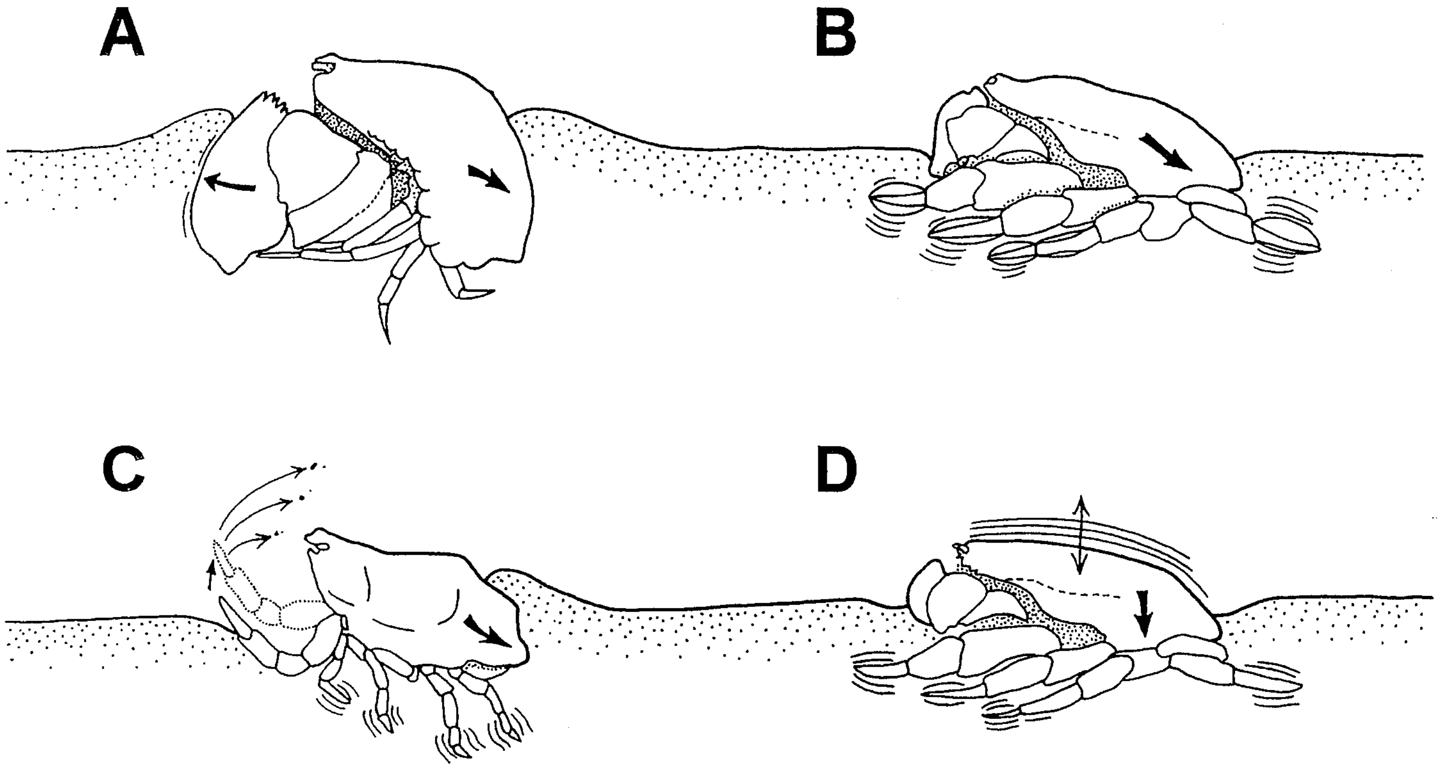


Figure 4.2 Variations in the mechanics of the burying behaviour of brachyurans. A – forward movement of the chelae against the substratum pushes the individual backwards into the sediment, as in *Calappa*; B – movement of pereiopods especially the last pair loosens the substratum and propels the individual backwards, as in *Matuta*; C – chelae used to gather sediment which is then either pushed backwards over the carapace or scooped and thrown over the carapace, as in *Ebalia*; D – body is vigorously slammed against the substratum, displacing sediment which then settles over the individual, pereiopods create a depression for the individual to move deeper into the sediment as in *Ovalipes*.

push the individual backwards as in *Calappa* (Schaefer, 1954), *Atelecyclus* (Taylor, 1984) and *Halicarcinus* (Melrose, 1975) (Fig. 4.2a).

Although a backward movement into the sediment appears to be the most common mechanism employed in burying, a variety of techniques may also be used to cover the body with sediment. In the leucosiid *Ebalia tuberosa*, techniques include: 1) the body-slam and 2) using the chelipeds as shovels (Schembri, 1981b). In the former, once the rear half of the body is buried, the body is vigorously slammed against the substratum displacing sediment that then settles back on the crab. In the latter, the chelae are used to gather sediment which is then either pushed backwards over the carapace or scooped and thrown over the carapace (Fig. 4.2c). In *Ovalipes guadelpensis*, however, the chelae are used to scoop and push sand forward from underneath the body creating a depression which enables the crab to move deeper into the substratum (Caine 1974). The "body-slam" method is then used to force waterborne sediment upwards which then settles on top of the crab (Fig. 4.2d). A variation of the "body-slam" method is the "sternal-push" method observed in *Ovalipes catharus* (McClay and Osborne 1985). This activity pushes the sand forwards and towards the side and creates the shallow depression into which the crab settles. The pereiopods are then used to push the crab backwards further into the sediment, this activity causing the sand to "spill" over the carapace thus covering the animal. The choice of burying method may be related to sediment type. Several studies have alluded to variation in burying technique in relation to sediment types. The body slam, for example, is hypothesised to be better in fine sediments than in coarse sediments (Schembri, 1981b). However, quantitative data on the relationship between sediment type and burying mode are lacking.

For most burying taxa, there is very little information on angle of entry and orientation when buried although there appears to be more variability than previously thought (Warner, 1977). In *Atelecyclus rotundatus*, the angle of burial was described as "moderately steep" (Taylor, 1984) whilst *Corystes cassivelaunus* has been observed to enter the substratum "obliquely backwards" (Garstang 1896). Barshaw & Able (1990) noted differences in the orientation of *Ovalipes ocellatus* and *Callinectes sapidus* when buried. *O. ocellatus* buries with the anterior part of the body closer to the surface of the substrate (i.e. oblique or diagonally), whereas *C. sapidus* buries horizontally with all parts of the body near the surface. McLay and Osborne (1985) also noted that the resting position of *Ovalipes catharus* under the sand was "always horizontal".

The depth of brachyuran burrows range from a few millimeters in soldier crabs, family Mictyridae, to more than a meter in the semiterrestrial *Ocypode* sp. (Vannini, 1980), where burrow depth appears to be correlated to the depth of the water table (Atkinson & Taylor, 1988). In contrast, the depth at which crabs are buried is invariably shallow, most species being recorded under only 10-50 mm of sediment (e.g. Taylor, 1984, Barshaw & Able, 1990). The whole animal is often completely covered by the sediment, the only visible evidence being the emergence of water from the exhalatory openings. In some cases, particularly in larger individuals, only the tips or margins of the carapace or the eyestalks are visible above the sediment. Burial depth appears to vary between taxa. Whilst burial depth may be function of size, there is evidence to suggest that burial depth may also be affected by substratum type. Barshaw & Able (1990) found substrate-specific behaviour in *Ovalipes catharus* which buried deeper and more readily in sand than in mud, whilst *Callinectes sapidus* tended to bury more readily into mud than sand.

Little information is available on the length of time crabs remained buried in the sediment. In most crabs, burying is associated with rapid, temporary concealment (Nye, 1974, Warner, 1977). A number of brachyuran taxa, however, are thought to spend much of their life buried in the substratum, e.g. *Corystes cassivelaunus* (Bridges, 1979) and *Ebalia tuberosa* (Schembri,1981a), emerging only when foraging for food. It appears that some taxa like *Ranina ranina*, *Portunus pelagicus*, *Ovalipes catharus* and *O. guadelpensis*, remain buried in the substratum during the day, emerging only at night to search for food (McLay & Osborne, 1985; Skinner & Hill, 1986; Sumpton & Smith, 1990).

### 4.2.3 Morphological adaptations to burying

In other crustacean groups, the mechanics of burying are often associated with special morphological adaptations particularly of certain appendages or in terms of body shape, as in the anomuran *Emerita* sp. (Pearse *et al.* 1942, Warner, 1977). In the Brachyura, however, few specific adaptations are found in burying taxa. Only in a few taxa have modified appendages and body shape been associated with the burying habit, namely the flattened pereiopods in the Matutidae and Portunidae and the elongated body of *Corystes cassivelaunus*. The mechanics of burial has been investigated in several portunid taxa (e.g. *Ovalipes* - Caine, 1974, McLay & Osborne, 1985, Barshaw & Able, 1990). In these taxa, the digging function of the flattened pereiopods is well documented. In *Matuta*, the distal segments of the pereiopods are typically flattened and broad, with those in the last pair being paddle-shaped. Although primarily attributed with a swimming function (Alcock, 1896, Hartnoll, 1971), the digging function of these appendages is often inferred (e.g. Seiler, 1976, Warner, 1977, Stevcic, 1983, Perez, 1986).

Burying taxa cover a wide range of body shapes ranging from the streamlined portunids to the globular leucosiids. There appears to be no simple functional correlation between body shape and the burying habit, although in *C. cassivelaunus* the elongated body shape presumably enables the individual to bury into the substratum rapidly (Hartnoll 1972). A similar morphology in the Raninidae and in anomuran mole crabs has likewise been associated with rapid burial (Warner, 1977). McLay and Osborne (1985) attributes the ability of *O. catharus* to bury in seconds to its streamlined shape, whilst Schembri (1981) raises the possibility that the dome-shaped abdomen of female *Ebalia tuberosa* may affect its speed of burrowing. Past studies on the biomechanics of brachyuran carapaces have largely been focussed on its hydrodynamic properties (e.g. Blake, 1985), however, its role in the burying process remains to be determined.

### 4.2.4 Physiological limitations of burying

Although burrows essentially provide refuge from adverse environmental conditions, a number of physiological problems have been associated with burrow-dwelling, especially in aquatic species. Burrow-dwelling brachyurans face the problem of coping with low oxygen concentrations (hypoxia) and high $CO_2$ concentrations (hypercapnia) in their burrows. Several studies have identified various respiratory adaptations of burrowing decapods to cope with these problems (reviewed by Atkinson & Taylor, 1988).

Whilst hypoxia is the main problem of burrowing taxa, this appears to be of little significance to burying crabs. In most cases, buried individuals continue to have access to oxygenated water above the sediment. The challenge, however, is to maintain a flow of water through the branchial chambers in the face of mechanical problems resulting from direct contact with the sediment, i.e. clogging of respiratory openings (Garstang, 1896, Atkinson & Taylor, 1988). A number of behavioural and morphological adaptations have been associated with the burying habit, particularly in relation to the problem of respiration encountered by various taxa. These fall into two broad categories: 1) accessory respiratory channels, and 2) altered respiratory rhythms.

### *Accessory respiratory channels*

Apart from the early work of Garstang (1896, 1897b) on the respiratory adaptations of sand-burrowing crabs, there is very little information on the form and function of the respiratory channels in burying taxa. Garstang noted the existence of an accessory "exostegal" channel formed when the chelipeds are flexed and held close to the body (Garstang, 1896, 1897b). He suggested that, in buried individuals, water is drawn through these channels into the branchial openings located at the base of the chelipeds, as in *Calappa* and *Matuta*. The use of exostegal channels in respiration has since been suggested for some members of the Portunidae (Ansell, 1973, Cumberlidge & Uglow 1978, Davidson & Taylor, 1995). The function of these channels, however, has yet to be demonstrated.

In the Leucosiidae, an accessory channel is formed without involving the chelae. Water is drawn through the orbits and flows through a channel formed by a broad and deep gutter on the pterygostome and the exopods of the third maxillipeds. Water then enters the branchial chamber through the openings at the bases of the 3rd maxillipeds (Warner, 1977, Schembri, 1981a).

In *Corystes cassivelaunus* which buries deeper than any of the above families, water is drawn through an "antennal tube" formed by the interlocking setae of the long and stiff antennae. The 3rd maxillipeds are elongate and cover the bases of the antenna.

In combination with reverse ventilation, water flows down the tube to the antennal bases and into the branchial chamber (Bridges, 1979). A similar mode of respiration has also been observed in *Atelecyclus rotundatus* (Taylor, 1984).

### *Reversal of ventilatory currents*

In aquatic brachyurans, the typical ventilatory current has a postero-anterior flow, with water flowing in posteriorly through the openings at the bases of the legs into the branchial chambers and anteriorly out through exhalant openings just above the buccal area. The current is maintained by the forward pumping action of the scaphognathites of the maxillae. Regular reversals in the ventilatory current, however, are a common feature in brachyuran respiration, the frequency of which varies between taxa (Warner, 1977). The function of reversed ventilation is unclear, although it has been primarily associated with a cleaning function (Hughes, *et al.* 1969, Warner, 1977, Taylor 1984) and in low oxygen conditions, as a mechanism for irrigating dead spaces in the branchial chambers (Arudpragasam & Naylor, 1966) . Variations in the frequency of reversal has been associated with ventilation volume and environmental conditions.

Prolonged reversals of the ventilatory current, however, have been recorded in buried crabs such as *Ovalipes guadulpensis*, *O. catharus*, *Carcinus maenas*, *Corystes cassivelaunus* and *Atelecyclus rotundatus*. In *Ovalipes* prolonged reversal in ventilatory currents enables individuals to maintain a supply of water to the branchial chambers even whilst buried (Caine, 1974). In *C. cassivelaunus* and *A. rotundatus*, reversal of the ventilatory current allows water to be drawn down the antennal tube into the branchial chambers through the buccal area, thus enabling individuals to bury much deeper with only the tips of the antennae protruding. In addition to reversed ventilation, *C. cassivelaunus* is also reported to withstand brief periods of apnoea (Bridges 1979). In all these taxa, brief periods of the normal postero-anterior pattern has also been recorded whilst buried presumably to clear the anterior openings of accumulated particles. Reversals in the ventilation current during burial therefore not only maintains continuous access to oxygenated water in these crabs but also prevents the respiratory channels from being blocked by flushing the gills and dislodging accumulated particles from the filtering setae and the respiratory openings.

Despite the volume of literature on the respiratory physiology of brachyurans, there are relatively few studies on the respiratory patterns of burying taxa (notably Cumberlidge & Uglow, 1978, Schembri, 1979a,b, Davidson & Taylor 1995, and reviewed by Atkinson & Taylor, 1988). From these studies, it appears that in addition to reversals in ventilatory currents, a number of changes to the normal respiratory pattern occur in buried individuals. These include reduced ventilation volumes, extended periods of apnoea and the possible use of alternative respiratory gas exchange sites such as the branchiostegites (Davidson & Taylor, 1995). In some taxa, the occurrence of respiratory pigments with high oxygen affinity has been reported (Bridges, 1979, Taylor *et al.* 1985). Whether this is a widespread characteristic of burying taxa, however, remains to be established.

### 4.2.5 Synthesis

This review highlights the fundamental differences between burrowing and burying in brachyuran crabs. There is a clear distinction between the two types of behaviour, both in terms of the ecological, mechanical and physiological implications (Table 4.2). Burrowing results in a cavity within which the animal lives, whilst burying brings the animal's body into direct contact with the surrounding sediment. Burrowing involves gross excavation of the sediment using the appendages in a coordinated manner

**Table 4.2** Summary of characteristics associated with burying vs. burrowing

| | BURROWING | BURYING |
|---|---|---|
| No. of taxa reported | 7 marine, 8 freshwater | 9 marine |
| Habitat type/depth | Terrestrial (up to 8 km inland); Intertidal;<br>Subtidal (down to 160 m) | Intertidal; Subtidal (down to abyssal depths) |
| Substratum type | Mud, sand (coarse - fine) | Sand (coarse – fine), sand/rubble mixture |
| Depth in substratum | 2 cm – 2 m | 2 –10 cm |
| Reasons | Predator avoidance; shelter from harsh environment; site for moulting, reproduction and feeding | Predator avoidance; temporary shelter from harsh environment |
| Time spent | > 24 hrs | 10 sec – 7 days |
| Problems associated with | Reduced oxygen pressure; Increased $CO_2$ levels; | Clogging of respiratory openings; reduced water flow |
| Mechanics | Enters substratum sidewards; using either chelipeds or pereiopods on one side only | Enters substratum backwards; primarily using 5th pair of pereiopods |
| Morphological modifications (mechanics) | Scooped dactylus | Non-specific; flattened dactylus |
| Morphological modifications (respiration) | Modified gill structures | Accessory respiratory channels |
| Fossil record | Eocene - Recent | Late Cretaceous - Recent |
| Biological correlates | Detritivores, microphagous, filter feeders, scavengers, herbivores | Mollusc predators, generalist carnivores |

with the aim of moving sediment away from the immediate vicinity of the animal. Burying involves the initial displacement of sediment but not away from body, rather to cover the body. As with other burying invertebrates, burying occurs in stages including loosening of sediment, bracing or anchoring the body, pushing the animal downwards into the sediment. In all cases, the body enters the sediment backwards. Within burying taxa, there is considerable diversity in the mechanics of sediment displacement, from the involvement of the pereiopods to specific body or carapace movements. In most species, the pereiopods are not necessarily modified for digging, although it appears that the efficiency of burial in the Matutidae, Portunidae and Raninidae may be enhanced by their possession of flattened terminal segments.

The physiological challenges experienced by burrowing vs. burying crustaceans are also different. Whilst burrowing groups are faced with problems associated with oxygen levels in a burrow environment, i.e. hypoxia and hypercapnia, burying groups do not have to deal with changing oxygen levels. For burying species, the challenge is to ensure constant access to oxygenated water above the sediment and to deal with the threat of the respiratory channels being clogged by sediment. To this extent, the range of respiratory adaptations in burying species is more conservative than those found in burrowing groups, comprising mainly of accessory respiratory channels and altered respiratory rhythms.

The benefits arising from burrowing vs burying are quite distinct. Burrowing and burrow construction have been associated with a range of functions other than the provision of shelter and protection, including reproduction, feeding and moulting. The advantages of burying lies mainly in the ability of an individual to rapidly disappear under the sediment, providing visual protection from predators. To date, this is the only function of the burying behaviour that has been demonstrated experimentally (Barshaw

& Able, 1990). Other benefits such as those associated with the energetics of maintaining position in unstable and high energy environments have also been proposed although these have not been examined experimentally.

Habitats associated with the burrowing and burying habits are also distinct. Burrowing is common amongst semi-terrestrial and terrestrial groups whereas burying is only reported in sub-littoral taxa. Burrowing is not particularly common in sublittoral brachyurans. It appears that for most taxa, burrowing and burying are mutually exclusive. There are however, two reported exceptions, *Scylla serrata* (Hill, 1975, 1982) and *Macrophthalmus hirtipes* (Nye, 1974). The ecological conditions associated with burrowing vs. burying in these taxa remain unclear.

The burying behaviour has been reported in at least 9 of the 50 families of brachyuran crabs and appears to have independently arisen in 3 occasions. Whilst this appears to be a small proportion, most of these families are amongst the most speciose and numerically dominant brachyurans in marine systems both in tropical and temperate areas. There is a suggestion that the burying habit may have arisen as an adaptation to life in soft sediment. Indeed, most of the burying families reach their greatest diversity in sandy or muddy substrata. Although the fossil record of the Brachyura is sparse and the phylogeny of higher taxa are still not resolved, it is possible that burying may have been an ancestral way of life with many of the taxa evolving in habitats characterized by soft sediment (e.g. deep water as in the Calappidae, Chapter 3). In this regard, the occurrence of the burying habit in brachyurans may be more widespread than previously thought. Little is known of the biology of many crab families especially those found in deep water habitats. Given the nature of the substratum in these habitats, the likelihood of a burying habit in many of these families is relatively high.

## 4.3 <u>SECTION 2</u>: A MORPHOLOGICAL STUDY OF RESPIRATORY STRUCTURES IN OXYSTOMATOUS CRABS AND ANALYSIS OF THE BURYING BEHAVIOUR AND RESPIRATORY CURRENTS IN *CALAPPA HEPATICA* AND *MATUTA LUNARIS*

From Section 1, it can be seen that one of the key attributes of burying taxa is the modification of the respiratory system. As with many other taxa, however, details of the respiratory system of burying crabs are limited. Although many invertebrate textbooks often incorporate descriptions of crustacean respiratory structures, most of what we know for marine brachyurans are based on anatomical studies of a few temperate groups, particulary *Carcinus maenas* and *Cancer sp.* (e.g. Arudpragasam & Naylor, 1964, Warner, 1977). While these descriptions are broadly applicable to other taxa in terms of the gross anatomy of the branchial chamber, very little attention is given to variations in the structures associated with ventilation, i.e. the inhalant and exhalant channels and their openings. Studies of brachyuran respiratory physiology may also include morphological descriptions of respiratory structures but these mainly focus on the branchiae (e.g. Bridges, 1969, Taylor, 1984). Of all the brachyuran groups, the Oxystomata possess what is arguably the most unusual respiratory system. Yet respiratory structures have only been studied in the temperate leucosiid, *Ebalia tuberosa* (Schembri, 1981) whilst, to date, there have been no detailed morphological study of these structures in any other oxystomatous group. As a key to our understanding of the role of the oxystomatous condition in the biology of burying crabs, in this section, the morphology of the respiratory system of two putative oxystomatous taxa, *Calappa hepatica* and *Matuta lunaris,* will be examined.

This section will have three components. A) The morphology of the respiratory system of *Atergatis*, a non-oxystomatous taxon and the two oxystomatous taxa, *Calappa* and *Matuta* will be provided. B) The morphology of the two oxystomatous taxa will be evaluated from a functional perspective and functional predictions presented. C) The functional predictions will be examined based on direct experimental observations of the function of the respiratory system in the laboratory. Finally, the results will be discussed in relation to the form and function of the oxystomatous condition in these two disparate taxa.

### 4.3.1 Materials and methods

Specimens were collected using a seine net and by hand from Pallarenda Beach in Townsville, Cockle Bay on Magnetic Island and Pioneer Bay on Orpheus Island, all in North Queensland, Australia. Only adult specimens were used in the analyses. Crabs were maintained in the laboratory in 50L holding tanks with a 10 cm layer of sand for burying. The tanks were supplied with circulating seawater at 26°C and crabs were fed regularly on a mixed of diet of fish and prawn flesh.

Morphological analyses were based on the examination of freshly killed and preserved material of *Calappa hepatica*, *Matuta lunaris* and *Atergatis floridus*. Anatomical illustrations were prepared primarily from drawings made using a camera-lucida mounted on a dissecting microscope. Details of the ventilatory channels were based on digital recordings using Sony TRV 900E and Nikon Coolpix 995 digital cameras. Setal structure was examined using a Philips XL 20 scanning electron microscope. The setal terminology used in this study follows that of Factor (1978) and Watling (1989).

Observations of the burying process were carried out either directly or recorded using a Sony TRV900E digital video camera. Crabs were placed in aquaria containing a sand/mud mixture and observed for 30 minutes. Complete digital video (DV) sequences of burying behaviour were recorded for 5 individuals. Burying sequences were filmed from above in 1L experimental aquaria to record speed and mode of burial. For consistency, the sediment used in all experiments was collected from a single source, Pallarenda Beach (*Matuta lunaris* habitat). The particle size composition of the sediment was determined by dry-sieving four 250 ml subsamples (Appendix IIA). For comparison, the particle profile of sediments from the inner reef flat of Pioneer Bay, Orpheus Island (*Calappa hepatica* habitat) is also provided in Appendix IIB.

To measure the depth and angle of burial, a 12cm long, 0.9mm-diameter stainless steel wire was attached to the dorsal surface of the carapace using cyanoacrylate glue (Fig. 4.3). The crab was removed from water, the surface of the carapace blotted dry using highly absorbent paper and then a steel wire was attached perpendicular to the surface using a piece of fabric with a drop of glue (Fig. 4.3). Selleys 'Supa glue' was the preferred glue because of its bonding speed (10 seconds) and its waterproof nature. The glue enabled the wire to attach directly on the carapace and did not affect the total length of the wire. The procedure lasted no longer than 4 minutes. Individual crabs were identified by a colour code using different coloured paint marks on the end of the steel wire. The wire was inflected at the tip to indicate the anterior orientation. This procedure was approved by the JCU Ethics Committee (Ethics Approval No. A447). Burial depth and angle data were measured separately.

The angle of burial was measured first. The wire was attached perpendicular to the horizontal axis of the crabs. Burial angle is based on the angle of the exposed wire (degrees from the substratum to the wire in lateral aspect; there was little evidence of

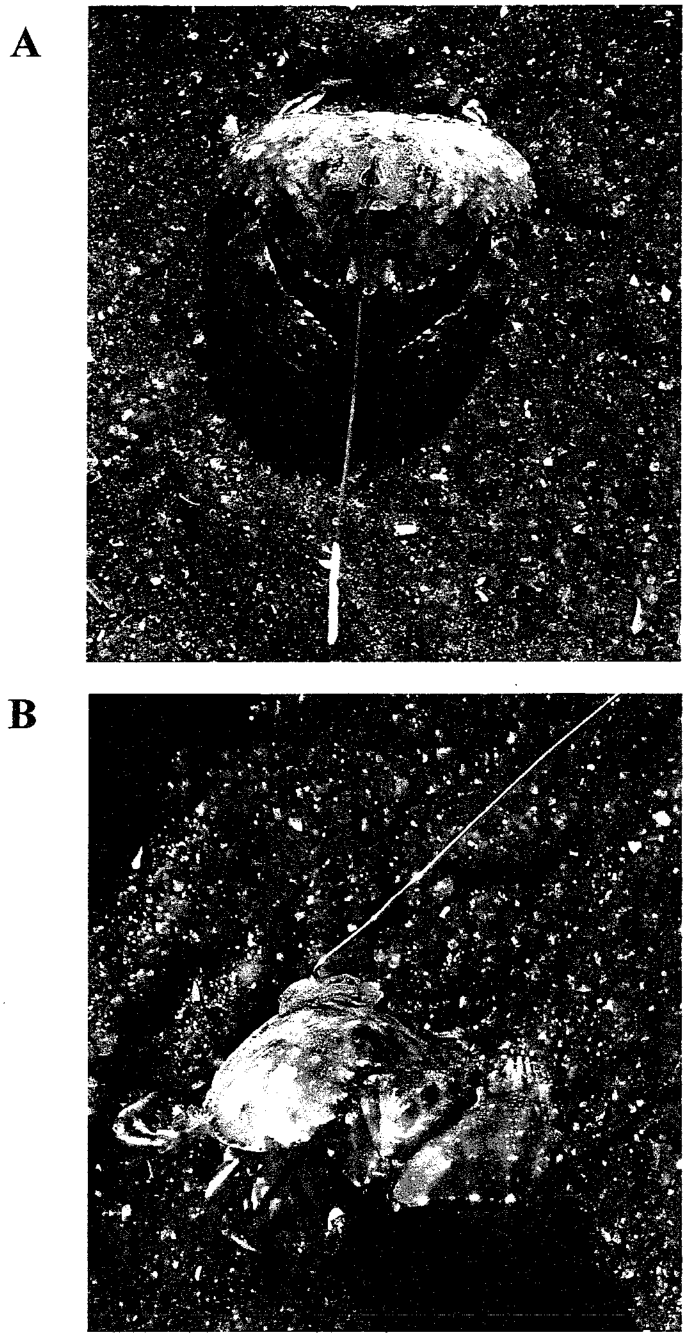


**Figure 4.3** Methodology used to measure burial depth

A. Dorsal view showing wire attached to the carapace.
B. Lateral view showing the wire bent at an angle so that the wire was vertical to the substratum when the crab is buried.

uneven burrowing marked by a leaning to the right or left). For depth measurements, the wire was bent at the base to the mean angle recorded for each species so that the wire was vertical to the substratum when the crab is buried (Fig. 4.3B). Burial depth was calculated as the difference between the total length of wire on the crab and the length of wire exposed above the surface of the sediment. The maximum depth at which crabs buried whilst remaining in contact with the surface of the sediment was also noted. Evidence for surface contact was taken as any of the anterior body parts being visible at the surface of the sediment. Depths were standardized to *effective depths* for each individual to indicate the depth to which the inhalant channels were covered by sediment (= depth recorded – maximum depth with surface contact). Measurements were made on a sample of 12 adult crabs each of *Calappa hepatica* and *Matuta lunaris* over a 5-day period, where depth and angle measurements were taken every two hours, four times per day.

Respiratory currents were visualized using India ink in seawater introduced near the inhalant and exhalant openings in exposed crabs. To study respiration in buried crabs, a 30-cm long 25-gauge scalp vein catheter (hypodermic needle removed) was attached to various regions of the carapace, and near the pereiopods, using cyanoacrylate glue. This enabled India ink to be introduced via a syringe within 1-2mm of the putative inhalant openings of buried crabs (Fig. 4.4).

To visualize branchial ventilation, methylene blue, a vital stain, was used instead of India ink. Dye was introduced into the inhalant and exhalant openings in the same manner described above. As water moved through the branchial cavity, methylene blue invariably stained the gill laminae before being expelled out of the cavity. Crabs once stained were immediately sacrificed and dissected.

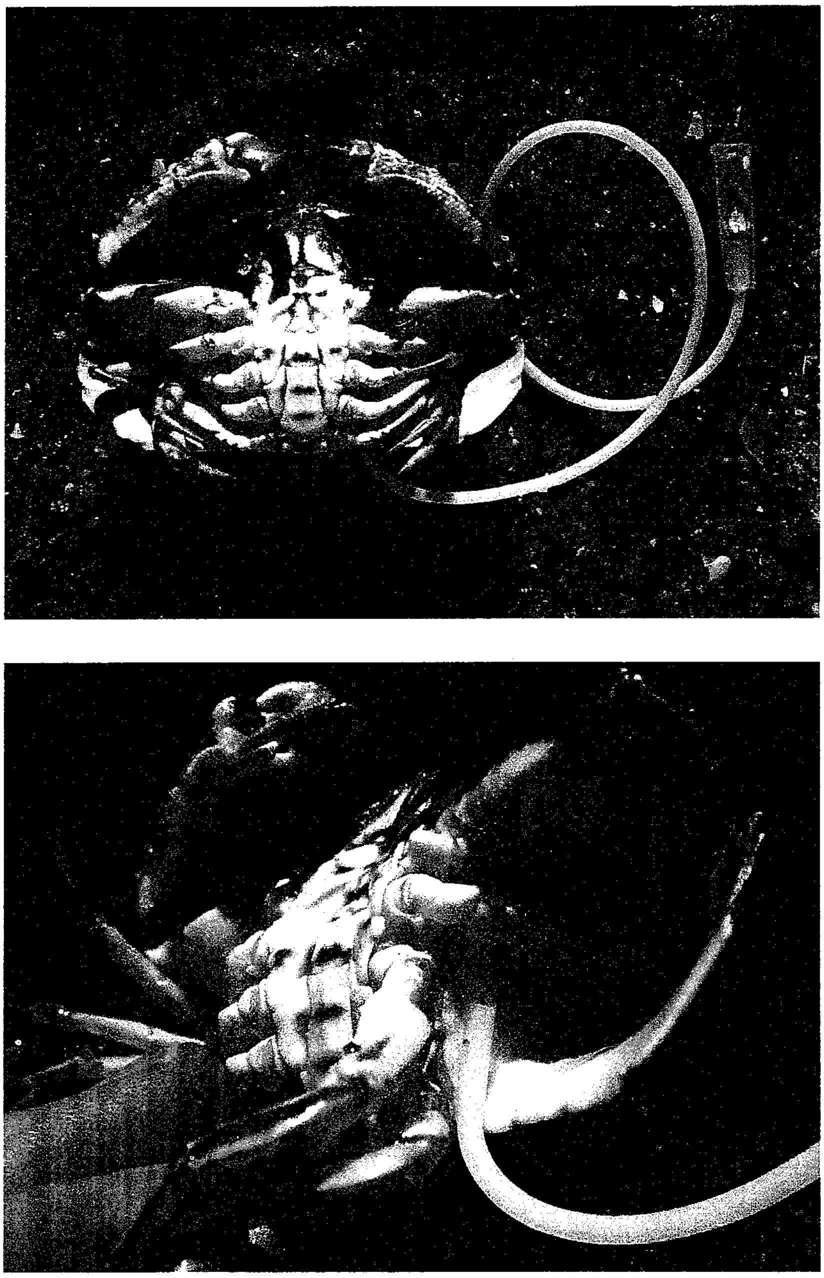


**Figure 4.4** Methodology used to visualise ventilation patterns in buried crabs. *Calappa hepatica*, ventral view, showing a 25-gauge scalp vein catheter attached close to the pereiopods to enable ink to be introduced via a syringe within 1-2 mm of the openings in buried crabs.

## 4.3.2 Results

### 4.3.2.1 Comparative anatomy

*Atergatis floridus* (f. Xanthidae), a non-oxystomatous taxon (Fig. 4.5A)

As with most xanthids, *Atergatis* possesses a typical brachyuran respiratory system (cf. Warner, 1977, McLaughlin, 1980). The branchial chambers are contained anterolaterally under the carapace, bounded on the outside by the branchiostegites and by the body wall on the inside. Each chamber is roofed by a thin cuticular membrane which separates it from the hepatopancreas which lies anteriorly above the branchiae. Oxygen-bearing seawater enters the branchial chambers directly through the various openings between the appendages and the lower edges of the branchiostegites, primarily through the largest of these openings located at the base of the chelipeds, the Milne-Edwards openings (M-E). Other openings are found near the bases of the other four pereiopods, between p2 and p3, p3 and p4, and p4 and p5. There are no distinct inhalant channels leading to any of these openings.

Within the chamber, water passes between the gill bases, into the hypobranchial space, and up between the branchiae into the epibranchial space. As in most crabs, *Atergatis* possesses 9 phyllobranchiate branchiae in each chamber: 1 podobranch and 1 arthrobranch (GI, GII) arising from the 2nd maxilliped, another podobranch and 2 arthrobranchs (GIII, GIV & GV) from the 3rd maxilliped, 2 arthrobranchs (GVI, GVII) from the 1st pereiopod or the cheliped and 1 pleurobranch each (GVIII, GIX) arising from the 2nd and 3rd pereiopods.

The branchial structure and arrangement in *Atergatis* also follows the typical brachyuran pattern: Gill I (mx2 podobranch) is small and lies horizontally along the bases of GIV, V and VI; Gill II (mx2 arthrobranch) lies vertically and parallel to GIV

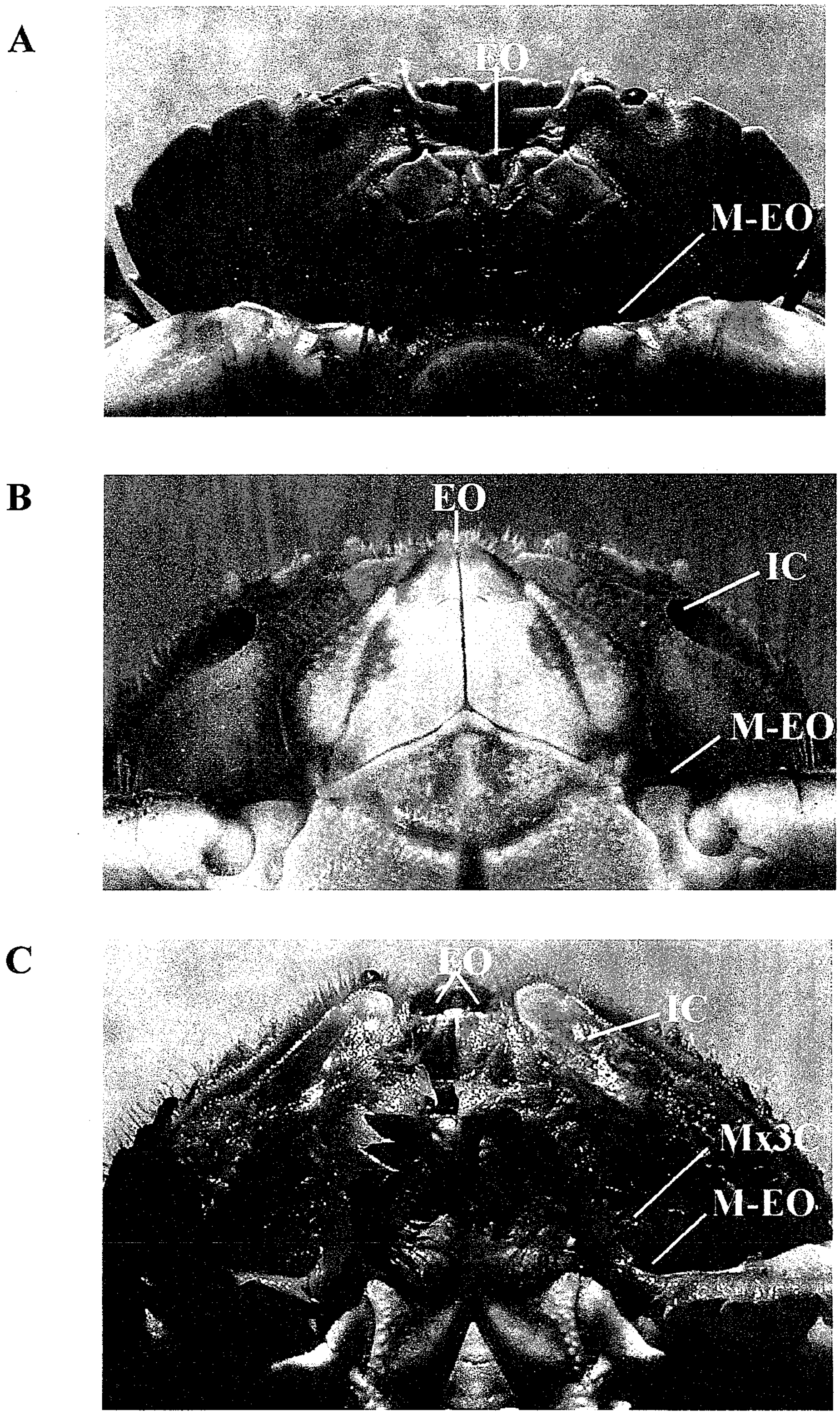


**Figure 4.5** Anteroventral view of A. *Atergatis floridus*, B. *Matuta lunaris* and C. *Calappa hepatica*, showing the external respiratory morphology. EO, exhalant opening; M-EO, Milne-Edwards opening; IC, inhalant channel; Mx3C, coxa of the 3rd maxilliped

(or a bit under); Gill III (mx1 podobranch) is small and truncated, lies under GI, at the base of GV; Gills IV to IX are large and prominent, curving vertically inwards to converge medially just beneath the roof of the branchial chamber at the level of the 2nd pereiopod.

Also found within the branchial chamber are the epipodites of the 3 maxillipeds. The location of each epipodite within the chamber varies. Mx3 epipodite lies in the hypobranchial space between the bases of GIV and V, the mastigobranch being narrow and flimsy with a few long setae on the edges. Mx2 epipodite also lies ventrally in the hypobranchial space across the bases of GIV and V, the mastigobranch is wider and longer that mx3 but also flimsy and with long setae on the edges. The mx1 epipodite, however, lies in the epibranchial region, the mastigobranch being well developed and also possessing long setae on the edges. The basal section of the mx1 epipodite is greatly expanded and forms the posterior ventral floor of the pumping chamber.

Anteriorly, the branchial chamber narrows to become the pump chamber which houses the scaphognathite. Dorsally the roof of the pump chamber is formed by the cuticular membrane, ventrally the floor of the chamber is formed by the expanded basal region of the mx1 epipodite. The exopods of mx1 and mx2 appear to seal the chamber floor anteriorly. The flange on the mx2 exopod fits under the edge of the buccal frame to seal the chamber. The pump chamber opens directly into the anterior region of the buccal cavity. Water flows anteriorly from the epibranchial space into the pump chamber and is pumped out through the exhalant openings found on either side of the epistome just above the mouth. In *Atergatis*, as in most brachyurans, these openings are broad, wide and confluent.

Oxystomatous taxa: 1. *Matuta lunaris* (f. Matutidae) (Fig. 4.5B, Fig. 4.6)

The structure of the respiratory system of *Matuta lunaris* follows the typical brachyuran pattern in terms of the branchiae but the structures leading to and from the branchial chamber are highly modified. In *Matuta*, there is a distinct inhalant channel leading from each orbit to the pterygostomian region. It appears that water flows into these channels via the ovoid orbit which is characterised by raised edges and lined with multidenticulate serrate setae on the inner surface. Water then flows through a deep gutter on the orbital floor, formed by the incomplete fusion of the upper and lower orbital margins and in to a deep groove in the suborbital region (the inhalant channel, see Fig 4.5B). The suborbital groove is bordered by dense interlocking pappose setae which cover the channel anteriorly forming a closed tube (Fig. 4.6a, b). There are no setae within the groove, although a number of pit organs can be found on the surface. In the upper pterygostomian region, the groove becomes less well defined and the channels widen considerably.

Although the suborbital inhalant channel is no longer distinct in the pterygostomian region, an auxiliary (exostegal) channel is formed by the close apposition of the carpus and propodus of the cheliped to the pterygostomian surface. The inner surface of the propodus is slightly concave; the upper margins lined with thick pappose setae. The upper margin of the carpus is also lined with pappose setae which, when the cheliped is flexed, corresponds with a row of pappose setae running parallel to the lateral edge of the carapace. In addition there is a distinct row of serrate setae running vertically between the upper and lower margins of the carpus, near the carpo-propodal junction. The pterygostomian region is convex and is covered in setae of various lengths: short, tufted setae anteriorly and along the margins of the buccal cavity,

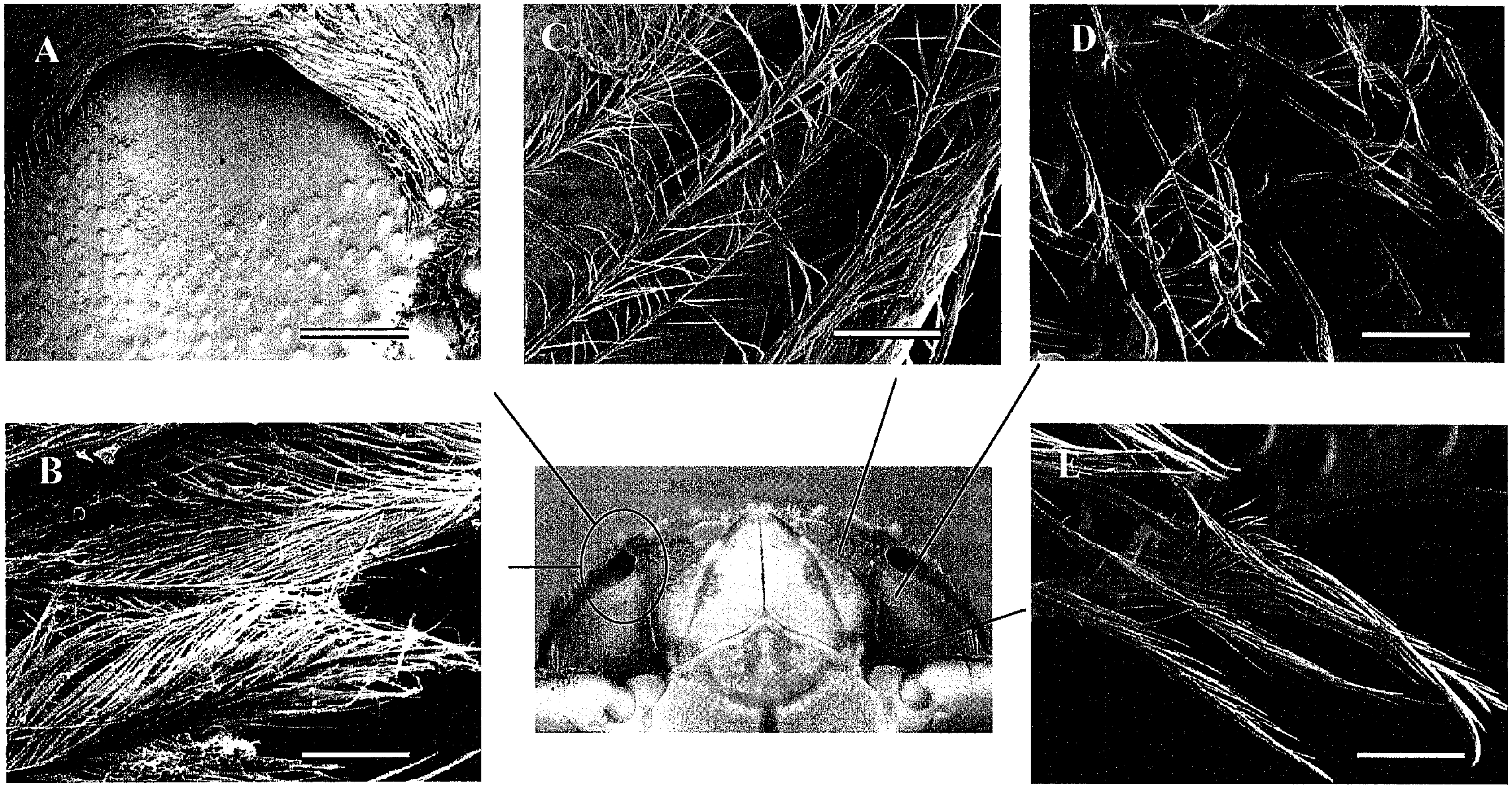


**Figure 4.6** *Matuta lunaris* antero-ventral aspect (central photo). Scanning electron micrographs of setae along the inhalant pathway.

A. Sub-orbital groove (x50); scale = 500µm

B. Dense interlocking pappose setae along the sub-orbital groove (x1000), scale = 25µm

C. Plumose setae on the anterior section of the pterygostome (x500), scale = 100µm

D. Plumose setae on the medial section of the pterygostome (x100), scale = 250µm

E. Pappose setae on the brachiostegite margin of M-E opening (x200), scale= 125µm short, stiff setae medially, and long and brushlike posteriorly close to the margins of the inhalant openings. Detailed SEM analysis shows that both the anterior and medial setae are plumose, with the setules of the medial setae being concentrated only on the distal half of the shaft (Fig. 4.6c, d). The posterior setae are pappose with radially arranged setules covering more than half of the shaft. The morphology of the channel suggests that water flows through this auxiliary channel into the inhalant (Milne-Edwards) openings located at the base of the chelipeds and then into the branchial chamber. The Milne-Edwards openings in *Matuta lunaris* are C-shaped and bounded dorsally by pappose setae on the edge of the branchiostegite (Fig. 4.6e), and by setae on the coxa and epipod of the 3rd maxilliped. Ventrally, the M-E openings are bounded by a thick row of pappose setae on the dorsal margin of the coxa, basi-ischium and merus of the cheliped. Like *Atergatis*, up to three other inhalant openings are found near the bases of the other pereiopods, however, unlike *Atergatis*, these openings are bounded dorsally by thick pappose setae parallel to the branchiostegite margin.

The exhalant channels in *Matuta lunaris* are elongate and the opening narrow, in contrast to that in *Atergatis* where the openings are broad and confluent (Fig. 4.7). In *Matuta lunaris*, the exhalant openings are at the end of a narrow channel formed by the elongate merus of the endopod of the 1st maxilliped which fits closely over the wall of the buccal frame. The roof of the channel is formed by the endostome which is also elongate and it is sealed posteriorly by a medial perpendicular flange on the mx1 endopod which fits closely to the surface of the mandible. These channels direct the exhalant flow forward through a common exhalant tube with a narrow opening located medially under the epistome.

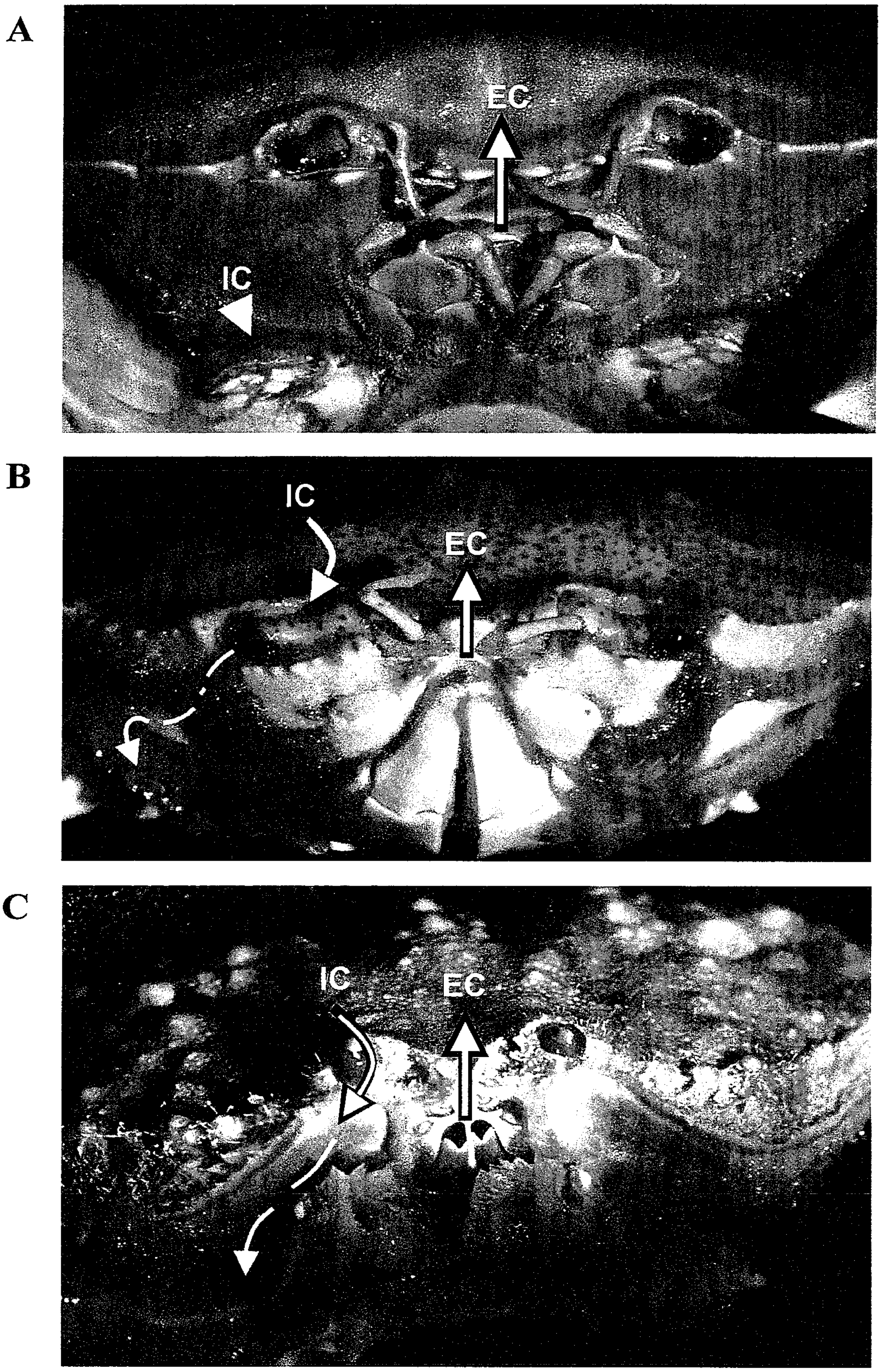


**Figure 4.7** Frontal view showing the exhalant current (EC) arising from the exhalant openings and their position relative to the inhalant current (IC) in A. *Atergatis floridus*, B. *Matuta lunaris* and C. *Calappa hepatica*

Oxystomatous taxa: 2. *Calappa hepatica* (f. Calappidae) (Fig. 4.5C, Fig. 4.8)

As in *Matuta lunaris*, the branchial structure of *Calappa hepatica* follows the typical brachyuran pattern but with a number of significant modifications to the inhalant and exhalant openings. In *Calappa hepatica*, the inhalant openings are located at the bases of the chelipeds and the other pereiopods. The entrance of the Milne-Edwards openings, however, are 'guarded' by the enlarged coxa of the 3rd maxilliped which sit outside the openings. The coxa is expanded dorsally and is characterized by two types setae: long plumodenticulate setae along the margins and short multidenticulate combed setae (*sensu* Johnston, 1995) on the surface (Fig. 4.8A,B). The M-E opening itself is lined by dense pappose setae along the branchiostegite margins as well as on the dorsal margin of the basi-ischium and merus of the cheliped (Fig. 4.8E). In this regard, *Calappa* appears to have the ability to regulate, and filter, the water entering the branchial chamber. The openings between the bases of the other pereiopods are bounded by rows of pappose setae on the brachiostegite margins.

Like *Matuta*, *Calappa* also possesses distinct inhalant channels leading to the Milne-Edwards openings. The morphology of these channels, however, are different. Each channel consists of a wide, shallow groove on either side of the buccal frame. This groove runs downward from the suborbital region, across the concave pterygostomian region to the base of the cheliped. A channel is formed by the apposition of the propodous of the cheliped over this groove. Medially, the groove is bounded by a dense row of pappose setae on the surface of the robust exopod of mx3 (Fig. 4.8C). The lateral margin of the groove is also bounded by pappose setae which covers the subhepatic region (Fig. 4.8D). No setae are found within the groove. Fine plumose setae cover the pterygostome surface.

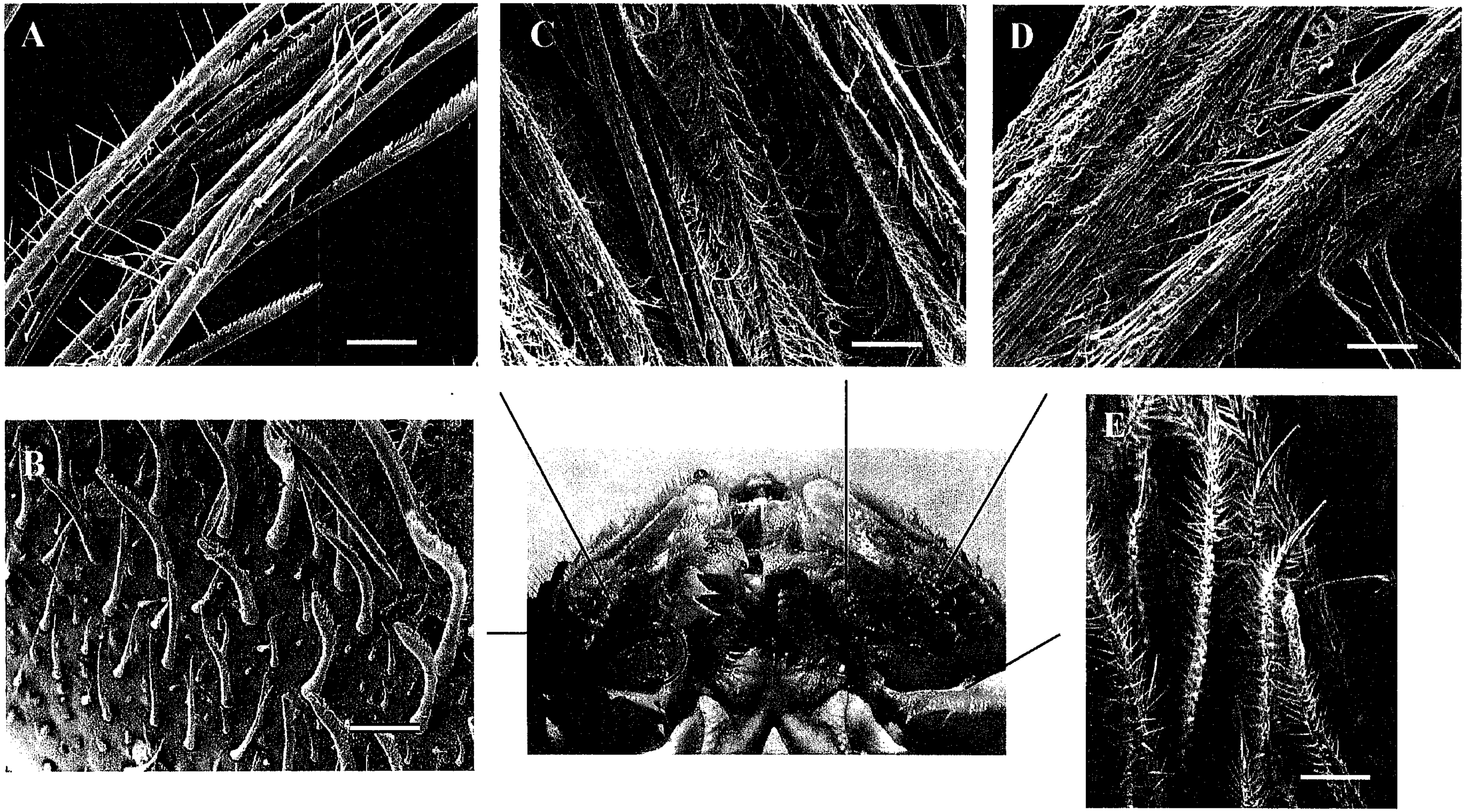


**Figure 4.8** *Calappa hepatica* anteroventral aspect (central photo). Scanning electron micrographs of setae along the inhalant pathway.

A. Plumodenticulate setae on the margin of the expanded coxa of the 3rd maxilliped (x200), scale = 100μm

B. Serrulate setae on the surface of the coxa of the 3rd maxilliped (x200), scale = 100μm

C. Dense pappose setae on the exopod of the 3rd maxilliped (x350), scale = 57μm

D. Pappose setae on the subhepatic region (x750), scale = 27μm

E. Pappose setae on the basi-ischium of the cheliped (x500), scale = 40μm

The volume of the pump chamber in *C. hepatica* appears to be much deeper and larger than either *Matuta* or *Atergatis*.

As in *Matuta*, the exhalant channel in *C. hepatica* is elongate and narrows towards the front. Formed by the elongate endopods of the 1st maxilliped and a narrow endostome, the exhalant channel delivers the exhalant flow forward to a narrow opening under the epistome. In *C. hepatica*, however, unlike *Matuta*, a septum is formed by the endostome dividing the channel into two tubes with separate openings.

#### 4.3.2.2 Functional predictions

The morphology of the respiratory structures of *Matuta* and *Calappa* suggest that a number of ventilation pathways are available to the two taxa. Each of these respiratory modes are considered separately below:

1. Forward ventilation (*sensu* Garstang, 1897a, Warner 1977) - Fig 4.9A,B,C

As in most crabs, water may be simply drawn directly into the branchial cavity of *Matuta* and *Calappa* through the M-E openings and the other pereiopod openings. Once inside the cavity, water may then move from the hypobranchial space upwards into the branchial space passing between the gill laminae where oxygen extraction occurs. In the epibranchial space, water may then be pumped forwards into the pump chamber and through the exhalant channel where it is expelled out of the exhalant openings. This pattern is most likely to be effective when the crab is on the surface of the sediment. This is the most widespread pattern in Brachyura.

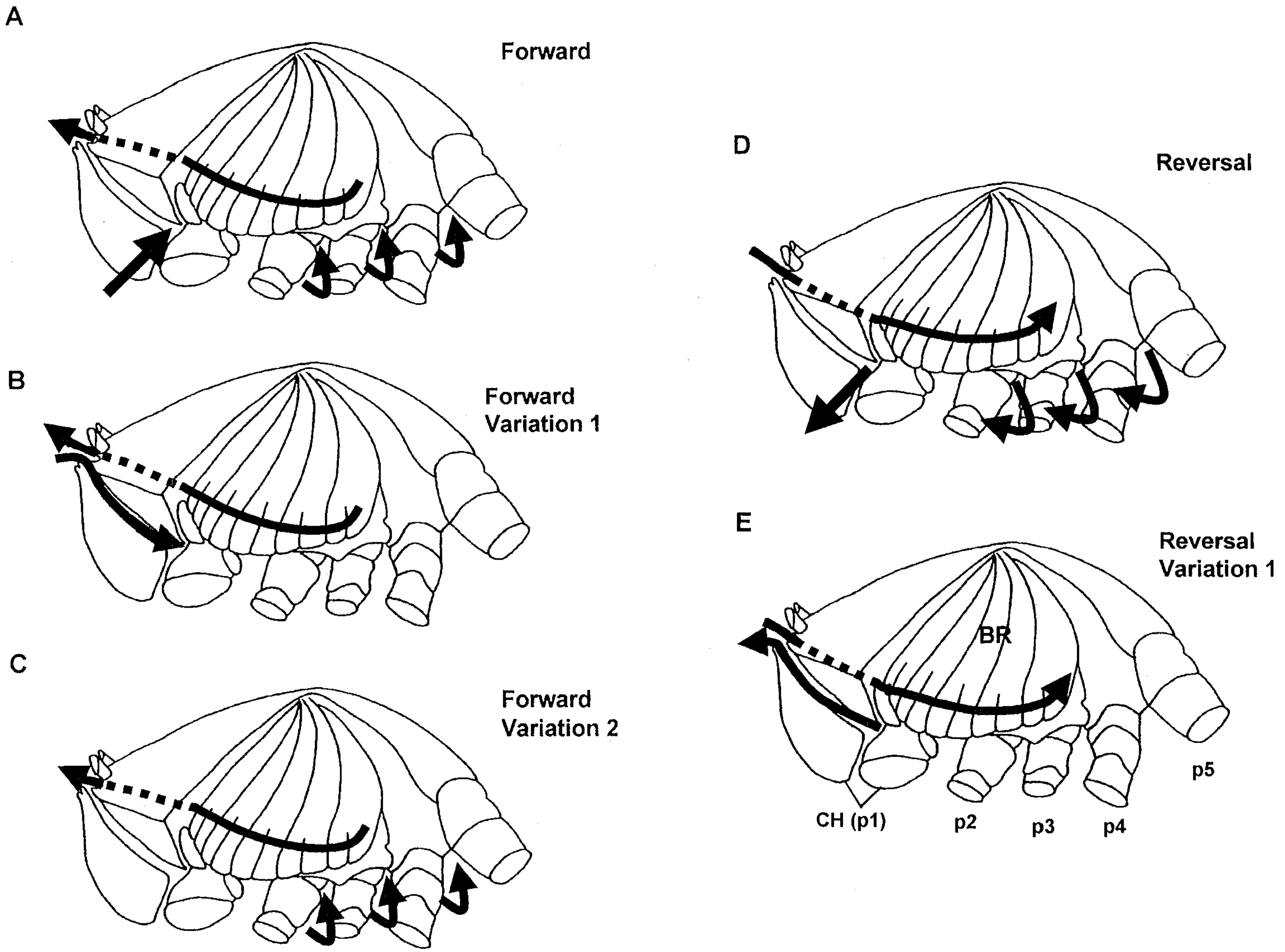


**Figure 4.9** A schematic diagram of a dissected crab with the lateral carapace section removed to expose the branchiae, showing the 5 potential ventilation patterns in oxystomatous crabs. CH(p1) - cheliped/pereiopod 1; p2, p3, p4, p5 – pereiopods 2 to 5; BR - branchiae

Forward, Variation 1 (Fig. 4.9B)

A variation to this pattern of ventilation appears to be possible in *Matuta* if the inhalant current is drawn through the orbit, into the suborbital canal and through the exostegal channel into the inhalant M-E openings. In this scenario, once inside the cavity, water flows into the hypobranchial space, and then upwards into the epibranchial space passing between the gill laminae. In the epibranchial space, water then flows anteriorly into the pump chamber and is pumped out through the exhalant channel.

In *Calappa*, a comparable modification appears possible where the water is drawn into the inhalant channels, which lie beneath the orbital region, through the exostegal channel and into the M-E openings. Inside the branchial cavity, the current direction follows the same forward route as *Matuta*, to be expelled through the anterior exhalant openings.

This pattern is most likely to be effective whilst the crabs are buried, but with the inhalant channel in contact with water about the sediment. In this position, the presence of sand around the animal, especially around the bases of the chelipeds could prevent water being drawn directly into the branchial chamber through the M-E openings, as a result of reduced water flow or through clogging.

Forward, Variation 2 (Fig. 4.9C)

In both *Matuta* and *Calappa*, water may be drawn in through the pereiopod openings and enter the branchial chamber directly. Inside the chamber, it once again would be predicted to flow from the hypobranchial space upwards into the epibranchial and then forwards through the pump chamber, where it is expelled through the exhalant channel and out of the exhalant openings.

This pattern is also likely to be effective whilst the crab is buried, particularly if the inhalant channels are covered in sediment or if the M-E openings are blocked. The role of this variation, however, may be limited if the composition of the sand surrounding the appendages impedes water movement. Unlike Forward Variation 1, however, this pattern does not need contact with water above the substratum.

2. Reversed ventilation (*sensu* Garstang, 1897a, Arudpragasam & Naylor, 1964, Bridges, 1976, Taylor, 1984) - Fig 4.9D, E

During reversal, the exhalant opening may become the inhalant opening with water being drawn through the opening, along the exhalant channel inside the buccal cavity and from here, into the pump chamber and the branchial cavity. Inside the branchial cavity, water may move through the epibranchial down to the hypobranchial and out through the M-E openings and/or the other openings near the bases of the other pereiopods. This is a reversal of the normal forward flow. In most taxa where reversals are reported, respiratory currents are usually simply reversed in this manner. The advantage of this mode in the study taxa is unclear.

Reversal, Variation - Fig 4.9E

A variation to this pattern in both *Matuta* and *Calappa* would be for water to be expelled solely through M-E openings and exits at the dorsal end of the exostegal channel. This reversed ventilation pattern is most likely effective during burial when the anterior openings are in contact with water above the sediment.

All of the above functional predictions may work in concert, none are necessarily mutually exclusive, although forward and reverse ventilation would have to be temporally distinct.

### 4.3.2.3 Experimental evaluation of the burying behaviour and respiratory currents in *Calappa* and *Matuta*

The preceding morphological and functional analyses of respiratory structures in *Calappa* and *Matuta* present 5 possible ventilation patterns in these taxa. The aim of this section is to evaluate the burying behaviour and realized ventilation patterns used by *Calappa* and *Matuta* by direct observations of captive specimens.

#### A. Burying behaviour

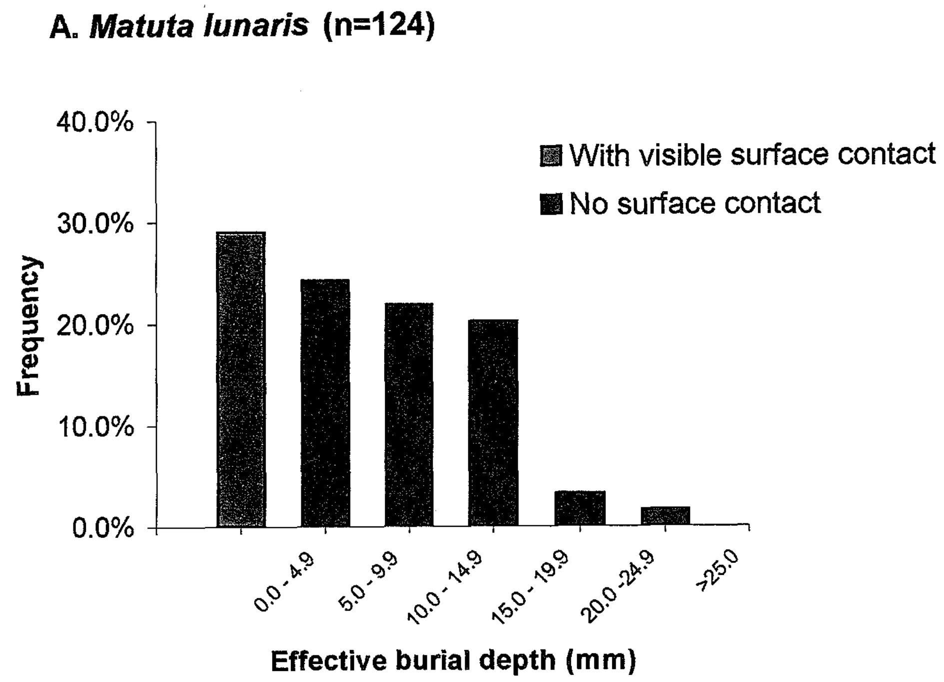
*Matuta lunaris*

When placed on a sandy substratum, *Matuta lunaris* immediately uses its pereiopods to bury its body into the sediment. Initially, the substratum is probed using the dactylus of the 3rd, 4th and 5th pereiopods. Excavation commences with the body tilting upwards and the 5th pereiopods rotating along the merus so that the distal segments are perpendicular to the body axis. Rapid movements of the distal segments then push sand away from the posterior region of the body. This action effectively draws the crab backwards into the sediment. The 2nd pereiopods are then alternately used to rapidly move sand outward and away from the body as the body "rocks" from one side to another. This action creates a temporary depression in the substratum causing the sand to "spill" over the carapace on the opposite side of the moving pereiopod. Movement of the other pereiopods appears to loosen the sediment under the body and enables the crab to sink further into the substratum. This process is repeated until the crab is completely covered by sediment. Excavation time is relatively quick with crabs completely disappearing under the sediment within three seconds (2.6 sec ± 0.30 S.E., n=5). During excavation, the chelipeds remain flexed and are held close to the pterygostomian region. *Matuta* enters the substratum, at approximately 40° (38° ±

5.4 S.E.), resulting in the posterior half of the body being buried deeper than the anterior.

When buried while maintaining contact with the surface, the eyestalks are often raised and the antennules are extended above the sediment. The exhalant opening may also be visible. Once below the surface, the eyestalks and antenules are no longer visible and crabs are assumed to have no direct contact with the surface of the sediment. The maximum effective depth (i.e. depth of sediment above the dorsalmost portion of the body) that *Matuta* has been observed to bury in experimental aquaria is 20 mm (Fig. 4.10A). At this depth, the inhalant channels would be covered by 20mm of sand. As the crabs bury at an angle with the inhalant channels remaining at the highest position, the remainder of the body is considerably deeper, sloping away at an angle of 40°. On several occasions when a predatory fish of the family Labridae was added to tanks containing *Matuta*, crabs were observed to bury down to an effective depth of 50mm. With a mean body depth of 14.5mm for adult crabs, this is almost 4.5 times their body depth.

Although the effective depth to which *Matuta* buries appears to be highly variable, two types of burial depth are apparent: shallow burial and deep burial. Shallow burial occurs when the crab buries itself under the sediment with the inhalant channels remaining in contact with the surface. Deep burial occurs when the crab is completely covered by sediment with no contact to the surface. In this study, *Matuta* was observed to spend a large proportion of its time (approximately 65%) deeply buried in sediment (Fig. 4.11A).



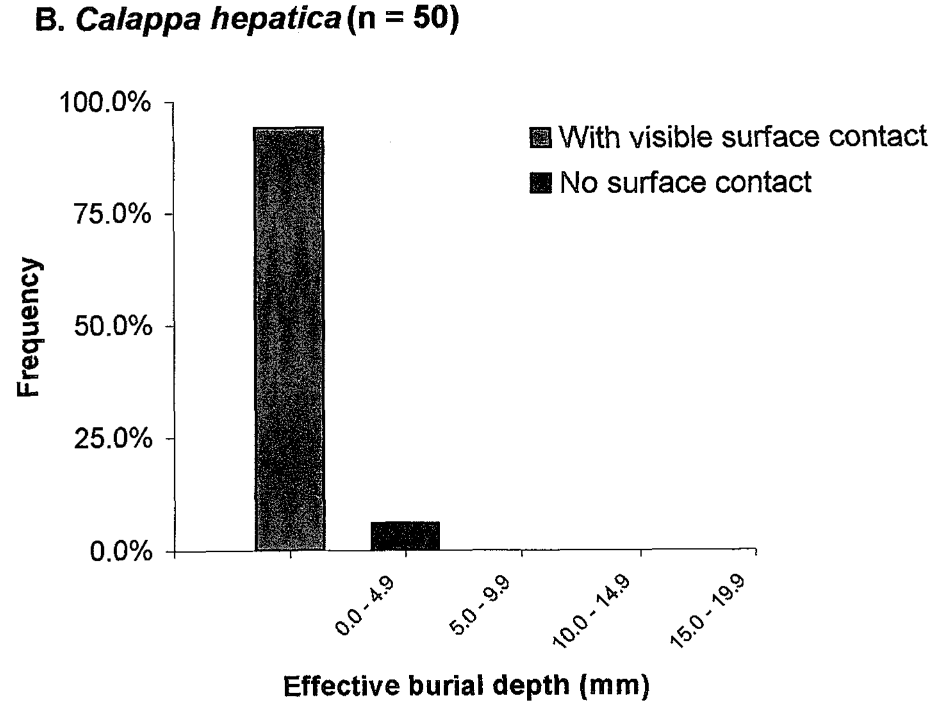
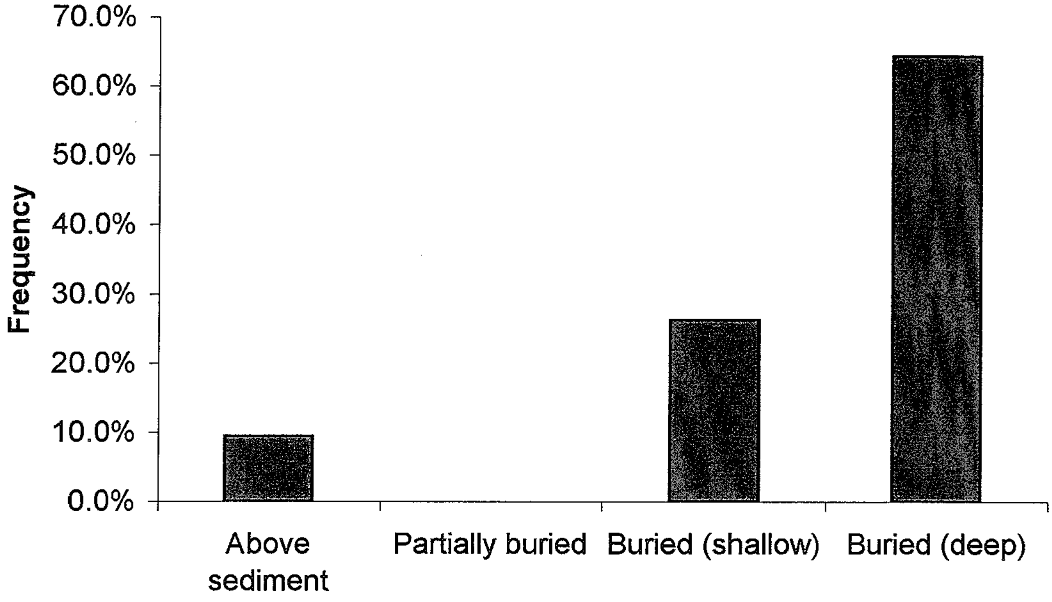


**Figure 4.10** Effective burial depth (depth of sediment above the dorsalmost portion of the body) of *Matuta lunaris* and *Calappa hepatica* (n = number of observations on 12 individuals each of *M. lunaris* and *C. hepatica* over a 5-day period)

**A. *Matuta lunaris* (n=137)**



**B. *Calappa hepatica* (n=94)**

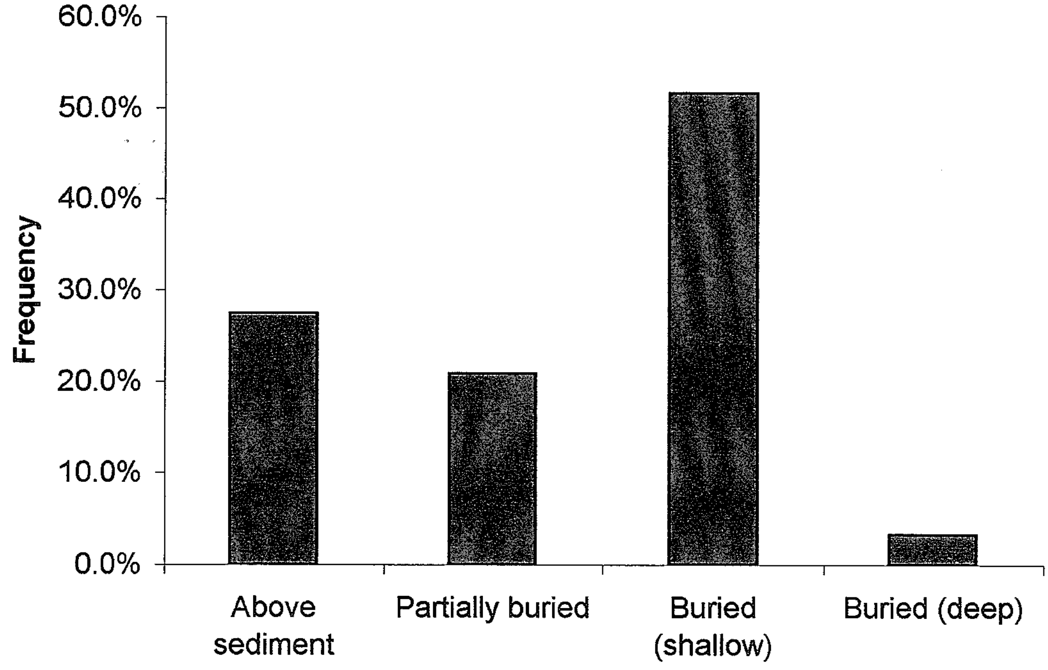


**Figure 4.11** Patterns of burial in *Matuta lunaris* and *Calappa hepatica* (n = number of observations on 12 individuals each of *M. lunaris* and *C. hepatica* over a 5-day period)

*Calappa hepatica*

Like *Matuta*, *Calappa* also buries quickly into the substratum using its pereiopods. The dactylus of p2, p3, p4 and p5 are initially used for probing the sediment, followed by rapid movements to loosen sand from under the body. Unlike *Matuta*, however, *Calappa* employs its massive chelae in the excavation process, using the anterior surface of the propodus to push sediment forward and away from the body. This action also pushes the crab backwards and downwards into the sediment as it enters the substratum at a moderately steep angle, approximately 50-60° (mean 63° ± 10.8 S.E.). The movement of the pereiopods are similar to those of *Matuta*, although excavation time in *Calappa* is comparatively longer, with most crabs completely burying within 15 seconds (12 sec ± 2.5 S.E., n=5).

In contrast to *Matuta*, *Calappa* was observed spend a large proportion of time exposed, either completely above the sediment or buried partially with the anterior half of the carapace above the sediment (fig 4.11B). When buried, *Calappa* is usually still in contact with the surface of the sediment with its orbit, exhalant opening and the margins of the chelae being visible at the surface (Fig. 4.10B). When attempts were made to completely cover *Calappa* with sediment, crabs usually adjusted their position to ensure that the exhalant openings remain exposed to the surface (or within 1-2mm of the surface). Throughout this study, *Calappa* was rarely observed to completely bury under the sediment, the few exceptional instances usually following extensive handling (e.g. immediately after application of wire, catheters etc.). Exposure to predatory fish (f. Labridae) did not elicit deeper burial as observed in *Matuta*.

## B. Ventilatory behaviour

### *Matuta lunaris*

Dye tracer studies confirmed the use of three of the five possible inhalant patterns in *Matuta*: 1) forward ventilation (normal) directly through the M-E openings 2) forward ventilation (Variation 1) via the orbit and suborbital channel and 2) forward ventilation (Variation 2) through the pereiopodal openings, between p2-p3, p3-p4 and p4-p5 (Table 4.3). Above the sediment, dye tracer studies show that *Matuta* ventilates its branchial chambers by forward ventilation, drawing water through both inhalant pathways (M-E and pereiopodal openings). Dye applied near the orbit or the pereiopodal openings was drawn into the branchial chamber and expelled through the anterior exhalant opening. However, the relative contribution of the two pathways varied, with the M-E being the primary pathway.

The role of the orbit and the suborbital channel in respiration is unequivocal. These structures form the dominant pathway by which water enters the branchial chambers even when the crab is above the sediment. Water is drawn through the orbit and flows down the suborbital channel before it enters the M-E openings. Dye introduced near the orbit was rapidly taken in and expelled in a distinct cohesive pulse in the exhalant current shortly thereafter (in less than a second). The force of the exhalant current expels the dye in a distinct plume to a distance of 40 to 60mm in the water column. In contrast, dye introduced around the periopods was taken in slowly and was expelled as a diffuse stream over a period of several seconds. Dye applied ventrally near the base of the chelae, adjacent to the M-E openings was also drawn in and expelled in a manner similar to the periopodal openings.

During shallow burial, *Matuta* continues to employ forward ventilation. Dye applied onto the surface of the sand overlying buried crabs was rapidly taken in through

**Table 4.3** Realized ventilation patterns in *Matuta lunaris* and *Calappa hepatica.*

| | | **Forward Ventilation** | | | **Reversed Ventilation** | |
|---|---|---|---|---|---|---|
| | | Normal | Variation 1 | Variation 2 | Normal | Variation 1 |
| *Matuta lunaris* | Above Sediment | • | ⬤ | • | | |
| | Partially buried | | | | | |
| | Buried (shallow) | • | ⬤ | • | | ◌ |
| | Buried (deep) | | ⬤ | • | | |
| *Calappa hepatica* | Above Sediment | • | ⬤ | • | | |
| | Partially buried | | ⬤ | | | |
| | Buried (shallow) | | ⬤ | | | |
| | Buried (deep) | | | | | |

**Key:**

⬤ Dominant ventilation pathway, marked by discreet plume in the exhalant current

• Contributing ventilation pathway, marked by slow and diffuse stream in the exhalant current

◌ Alternative short-term ventilation pathway exhibited in very fine sediment (see Appendix IIB).

the orbit, followed by a strong jet of dye being expelled through the anterior exhalant opening. It appears that most of the inhalant flow enters through the M-E openings. Passage of the dye down the orbital channels and through the M-E openings was confirmed by the discolouration of setae lining the channels and the dye residue on the branchiae. Dye applied near the pereiopods was also expelled through the exhalant opening, although as in crabs above the sediment, the flow was weak and diffused. Dye applied ventrally using intravenous tubes at the base of the chelae also appeared to be drawn into the branchial chambers, as with the periopodal openings, and expelled slowly.

During deep burial, *Matuta* still employs forward ventilation with water being drawn through the orbit and the pereiopodal openings. Dye applied around the orbit reappears through the sand in a plume corresponding to the position of the anterior exhalant opening. Likewise, dye applied near the pereiopodal openings could be seen percolating slowly through the sand in the same location. However, dye applied ventrally at the base of the chelae did not appear to be inhaled, the dye merely percolated through the sand from the same location as it was applied.

Dye tracer studies using vital stains reveal that during forward ventilation water enters the branchial chambers through the M-E and pereiopodal openings. Water entering through the M-E openings flow directly into the ventral hypobranchial space, staining the anterior gills as it flowed between their lamellae through to the dorsal epibranchial space. Water entering through the pereiopodal openings most likely flows forwards into the hypobranchial space, as it stains the posterior gills as it passes through to the epibranchial space.

In very fine sediment (66% 0.125-0.25mm grain size, Appendix IIB – Orpheus Island sand), however, *Matuta* was observed to shift to reverse ventilation shortly after burial. Dye pipetted onto the surface of the sand is drawn through the exhalant channel and into the branchial chambers. Water exited the branchial chambers primarily via the M-E openings and through the exostegal channel, but not through the suborbital channel. Exhalant ventilatory streams could be seen as sand plumes welling up from the sediment on either side of the carapace corresponding to the position of the exostegal channel and the M-E openings below. Dye could be seen percolating up through these sand plumes. No other sand plumes could be detected around the buried crab suggesting that during reverse ventilation the exhalant flow is mainly through the M-E openings. In this type of sediment, crabs were observed to continue with reverse ventilation for several minutes before either emerging from the sediment or readjusting its position and resumes forward ventilation. Reverse ventilation was not observed in sediment used in the experimental tanks (Appendix IIA – Pallarenda sand).

### *Calappa hepatica*

As with *Matuta*, dye tracer studies shows that *Calappa* also ventilates its branchial chamber primarily by forward ventilation when above the sediment (Table 4.3). Dye applied near the anterior margin of the carapace confirms the dominant role of the exostegal channel as an inhalant pathway into the branchial chambers. Dye may be seen drawn into the narrow openings of the inhalant channels and is expelled through the anterior exhalant openings in a distinct plume in a strong jet of water 60-70mm into the water column. The orbit is not a point of entry for the inhalant current. Dye applied around the orbital region was not drawn through the orbit, instead was observed to enter the inhalant channels just in front of the carapace margin near the orbit. Dye tracer studies reveal that within the branchial chamber, vital stains entering through the M-E openings stained gills I to VIII.

The use of pereiopodal openings, particularly towards the posterior region, was also confirmed. Dye introduced between p4-5 was drawn in and expelled through the anterior exhalant openings as a weak diffuse plume in the exhalant current. However, dye introduced between the other pereiopodal openings could not be detected in the exhalant current. Vital stains introduced through the posterior pereiopodal opening stained the posteriormost gills VIII and IX.

When buried, *Calappa* continued forward ventilation exclusively through the anterior inhalant channels, i.e. the exostegal channels. Dye introduced at the posterior and other pereiopodal openings were not taken in and percolated through the sand approximately at the region of application. As in *Matuta*, dye introduced near the inhalant openings of buried crabs was rapidly taken in and expelled through the exhalant openings in a strong cohesive well-defined plume.

### 4.3.3 DISCUSSION

This work represents the first detailed study of the functional morphology of respiratory structures and ventilation patterns in tropical burying crabs. Most respiratory studies in the past have been of temperate and subtemperate groups (e.g. *Carcinus maenas*, *Corystes cassivelaunus*, *Atelecyclus*, *Ebalia* – see Section 1, this chapter). Furthermore, this study assesses the respiratory role of a unique morphological configuration, the oxystomatous condition, which is shared by these crabs and which has long been associated with their burying habit.

Morphologically, the respiratory structures of *Matuta* and *Calappa* follow the typical brachyuran pattern (*sensu* Warner 1977). The internal branchial configuration of 9 is highly conserved, suggesting that for these taxa variations in lifestyle and habitat and their physiological consequences, does not necessarily require significant modifications to the gas exchange structures. Although the location of the openings to the branchial chambers are also essentially similar to that reported in most other crabs, *Matuta* and *Calappa* possess two unusual morphological modifications, 1) the elaborate accessory inhalant channels and 2) narrowed and elongate exhalant channels. These characteristics are shared by only one other family, the Leucosiidae, although the morphology of the channels differ greatly reflecting their disparate phylogenetic affiliations. In all cases, however, these modifications appear to be associated with their burying habit.

Video observations show that both *Matuta* and *Calappa* are both back burrowers (*sensu* Warner 1977) and are consistent with published reports of both species in the field (e.g. Stevcic, 1983, Perez, 1986). Burial time for both taxa is relatively quick (average of 2.6 sec in *Matuta*, 12 sec in *Calappa*, 4 and 15 sec maximum, respectively). While there are little comparative quantitative data of burial times in other crabs, both taxa bury much faster than *Ebalia* (5-10 min; Schembri, 1981b) and is comparable with records of *Ovalipes catharus* (4-24 sec; McLay & Osborne, 1985). Differences in burial time, however, may be related to the substratum type. *Matuta, Calappa* and *Ovalipe*s live in soft, sandy substrata, whereas *Ebalia* is found in coarse gravelly substrata. In burrowing taxa (see Section 1, this chapter), burrow shape and stability have been correlated with the nature of the sediment (e.g. Hartnoll, 1973, Vannini, 1976). It is likely that sediment characteristics may also affect burying time and depth in burying taxa.

The angle at which *Matuta* and *Calappa* enter the substratum is relatively steep, resulting in complete burial under the sediment, with only the orbits and the anterior margin of the carapace exposed. Amongst burying taxa, there appears to be variation in the angle of entry and orientation when buried. A number of groups are reported to enter the substratum obliquely, i.e. in an angle, whilst a number bury horizontally (see Section 1, this chapter). Barshaw & Able (1990) suggest that differences in the burial orientation of *Ovalipes* and *Callinectes* may be related to their modes of respiration whilst buried. The ability of *Ovalipes* to reverse its ventilation current has been suggested to be a probable factor influencing both deep and oblique burial, although the link between these factors is unclear. For *Matuta* and *Calappa*, the advantages of entering the substratum at an oblique angle may be related to predation. Oblique burial ensures that at least half of the body is buried deeply whilst keeping the respiratory openings in contact with the surface. This may decrease detectability of the crabs to electro-sensitive predators such as benthic elasmobranchs.

In the present study, 3 types of burial depth were observed: **partial** (Type 1), **shallow** (Type 2) and **deep** (Type 3) burial. In partial burial, the crab is only half-buried, whilst in shallow and deep burial the crab is completely covered by the sediment. In shallow burial, however, the crab is located only just below the surface of the sediment, with the anteriormost parts of the body such as the orbital region and anterior margins of the carapace and chelae often visible at the surface. Both the inhalant and exhalant openings have contact with water at the surface of the sediment. In contrast, in deep burial, the crab is buried with no body parts visible or in contact with the surface. In *Matuta*, for example, this represents burial in sediment twice the body depth.

Several differences were found between *Matuta* and *Calappa* in terms of their depth of burial. Both *Matuta* and *Calappa* were observed to bury just below the surface (Type 2 burial) for extended lengths of time, with only the orbit and exhalant openings visible. Partial burial (Type 1), however, was only observed in *Calappa*, while deep burial (Type 3) was only observed in *Matuta*.

The respiratory implications of burial depths are clear. In partial and shallow burial, the respiratory openings remain in contact with the overlying water column either directly or through accessory channels. Continuous respiration is therefore possible either by forward or reverse ventilation. Complete deep burial, however, presents a significant problem. The volume of sediment surrounding the animal would preclude the free flow of oxygenated water through the ventral respiratory openings.

For most crabs, shallow burial is the most common type of burial, e.g. *Ovalipes*, *Carcinus*, *Ebalia*. Most species have been recorded to bury under only 10-20 mm of sediment (e.g. Taylor, 1984, Barshaw and Able, 1990), with only the tips or margins of the carapace or the eyestalks being visible above the sediment. In only a few crabs has complete deep burial been reported and even in these taxa, contact with the surface water has been maintained through extra long antennal tubes in combination with prolonged reverse ventilation (*Corystes* and *Atelecyclus*, see Section 1, this chapter). The ability of *Matuta* to maintain forward ventilation whilst deeply buried is a previously unrecorded ventilation mode.

*Matuta* is unusual in its ability to bury deeply with no contact to the surface of the sediment. In the field, *Matuta* has been observed to remain deeply buried up to 4 hours in moist sand in the intertidal region during low tide (pers.obs.). The results of this study show that *Matuta* continues to respire when deeply buried through forward ventilation. It is highly probable that *Matuta* is able to do this owing to the permeability of the sediment. The circulation of water through the pore spaces in sediment is greatly influenced by grain size and grain size distribution. Coarse to medium beach sand allows a considerable amount of surface water to penetrate the sediment (Davis and Cornwell, 1991). It is therefore likely that *Matuta* still has access to oxygenated surface water percolating through the sediment even when completely buried. It is also highly probable that *Matuta* has physiological modifications to permit prolonged hypoxia in conditions where surface water penetration is limited, as in the intertidal region during low tide.

It is interesting to note that *Matuta* switches to reverse ventilation when in very fine sediment (>50% fine sand). It is likely that forward ventilation may not be possible in fine sediment as water circulation is much reduced (Davis & Cornwell, 1991) or the grain size is so small that it reduces the efficiency of the brush-like setae lining the inhalant channels.

The ventilation pattern observed in both *Matuta* and *Calappa* highlight the role of setae in respiration. In both *Matuta* and *Calappa*, plumose setae line the inhalant channels, and the M-E and periopodal openings. These setae interlock and form a matted structure, trapping sediments between the setules and appear to play a role in preventing sand particles from entering the branchial cavity. Interlocking setae along the suborbital gutter and on the anterior margins of the chelae in *Matuta* and on the lateral edge of the 3rd maxilliped in *Calappa* may also serve a gasket-like function of sealing the channels and directing the inhalant flow only through these channels. Plumose and pappose setae have been suggested to have filtering or particle trapping function (e.g. Farmer, 1974) in other crustacean taxa. In addition, plumose setae on the margin of scaphognathites have long been attributed to sealing the space between the scaphognathite and the branchial cavity wall thereby preventing backflow during the pumping process (Warner, 1977, Factor, 1978). Similar setae have been found to line the lateral edge of the 3rd maxilliped in *Ebalia* and have been suggested to form a screen preventing sediment from fouling the underlying structures (Schembri, 1981a).

The reliance of *Calappa* on the M-E openings as the sole inhalant opening when buried may be facilitated by the flanged coxa of the 3rd maxilliped. It is possible that this flange enables *Calappa* to regulate both the volume and direction of water entering the branchial chambers, while the extensive setation along the margins may act as a secondary filter to prevent sand particles entering the branchial chamber. By opening fully, it may be possible to ventilate even the posteriormost gills through the M-E openings alone. A survey of brachyuran families (including those used the phylogenetic analysis earlier in this study) suggests that this flanged condition of the 3rd maxilliped coxa is unique to the Calappidae.

The continued use of forward ventilation by *Matuta* and *Calappa* when buried (Table 4.3) has not been recorded previously in burying taxa with the exception of the Leucosiidae. It is possible that reverse ventilation is not advantageous in these oxystomatous taxa. Reverse ventilation is an energetically less efficient means of respiration, as oxygen extraction is low because of the absence of a countercurrent between oxygenated water and blood within the gills. As a general rule, parallel current exchangers are not as effective as countercurrent exchangers (Dejours, 1981). In reverse ventilation, water flow through the gills is passive and may also affect the rate of extraction. All three taxa (Calappidae *s.s.*, Matutidae and Leucosiidae) share two morphological characteristics: well developed inhalant channels which maintain access to oxygenated water above the surface of the sediment when buried, and narrow exhalant openings which ensure effective dispersal of exhaled water. Together these features represent the basis of the oxystomatous condition. These characteristics are in most likelihood all that is required to maintain a burying way of life without having to compromise an already efficient forward ventilatory pattern. It is interesting to note that these three families are the most speciose burying taxa found in soft-sediment habitats.

The role of reversed ventilation varies among brachyuran groups. In the portunids it has been suggested that its role is to irrigate dead spaces in the branchial chambers (Arudpragasam & Naylor, 1984), whereas in *Corystes*, it has been associated with both gill cleaning and respiration. In oxystomatous groups, the results of this study suggests that reverse ventilation does not form a significant part of the respiratory pattern whilst buried. In these groups, it is most likely associated with normal cleaning and maintenance of the gills and not as an alternative direction for ventilation, with the possible exception of deep burial in fine sediments.

The results of the present study point to the clear role of the oxystomatous condition in respiration. As both the inhalant and exhalant openings of oxystomatous crabs are located anteriorly, and often in close proximity to each other it is imperative that the two flows are kept separate. A narrowed exhalant opening would ensure that the exhalant stream is dispersed as a jet with sufficient force away from the crab to prevent oxygen-depleted water from re-entering in the exhalant current. Similar narrowing of the excurrent openings in order to increase flow speed has been noted in suspension feeders such as ascidians and bivalve molluscs (Vogel, 1994). In addition, based on Bernoulli's principle, the jet would create suction, drawing oxygenated water towards the base of the jet, i.e. in the vicinity of the inhalant openings. This oxystomatous condition appears to have arisen independently in a number of brachyuran taxa, most of which bury in soft sediment. It appears that in addition to significant modifications to the inhalant channels, for these crabs, a narrowing of the exhalant opening may also be a functional requirement for respiration when buried.

# CHAPTER 5

# GENERAL DISCUSSION

The primary aim of this study was to review the systematics of a distinct group of tropical and subtropical crabs, the Calappidae, commonly known as the box crabs. Traditionally placed under the section Oxystomata, calappids possess the characteristic triangular buccal frame and elongate mouthparts, i.e. the oxystomatous condition. Contrary to 150 years of taxonomic utility, however, the results of this study show that the oxystomatous condition is not a phylogenetically derived character state, rather it has independently arisen in at least three lineages of burying crabs. The cladistic analysis of the Calappidae and its component subfamilies clearly demonstrate that the traditional characters used to define the family are not unique and that the family as it stands is not monophyletic. The value of the oxystomatous condition as a unifying feature for a number of brachyuran families is therefore questionable. Whilst there remains a suggestion that a much reduced 'oxystomatoid' lineage does exist, unreversed synapomorphies supporting the grouping does not include the oxystomatous condition.

What then is the basis of the oxystomatoid condition? Available evidence suggests that the Oxystomata is effectively a group united by a functional condition. In the majority of taxa it appears to be associated with the ability to bury in soft substrata with the body completely covered in sediment, whilst maintaining continued access to overlaying oxygenated water. This study demonstrates the respiratory adaptations in two of these taxa and provides us with a morphological and functional description of the role of the oxystomatous condition in respiration. In order to maintain access to oxygenated water while buried, crabs are basically constrained by their morphology. With the normal respiratory openings located at the bases of the chelae and pereiopods, when buried, the volume of sediment surrounding the crab would make it difficult for water to enter the branchial chambers. Burying crabs must deal with the dual challenges of maintaining a flow of water through these openings while avoiding clogging of the openings and branchiae with sediment. Most oxystomatous crabs possess inhalant channels which although varying in morphology all achieve the same function of directing water from above the substratum directly into the branchial chambers. In all cases, the openings of these channels are located anteriorly, either medially or near the orbit. This position, however, brings the inhalant openings close to the exhalant openings which are also located antero-medially under the epistome. This presents a significant potential for mixing of the inhalant and exhalant water streams. The oxystomatous condition appears to minimize this problem. The narrow exhalant channel and opening ensures a forceful expulsion of exhaled water away from the inhalant openings thereby reducing the opportunity for mixing of the exhalant water with the inhalant current. It may also facilitate the movement of fresh oxygenated water into the vicinity of the inhalant opening via the Bernoulli effect.

It appears therefore, that the oxystomatous condition enhances the function of inhalant channels in some burying crabs. Whilst inhalant channels are not unique to oxystomatoid crabs, it is in these groups that these channels are morphologically distinct and frequenty highly modified. Amongst the oxystomatoid crabs, inhalant channels are found in all members of the Calappidae *sensu stricto*, the Matutidae, the Leucosiidae and in one genus of the Hepatidae (*Hepatus*). Only one oxystomatoid group do not have inhalant channels, the Dorippidae. Interestingly, they are one of the few taxa which is not known to bury in soft substrata. Dorippids are known to carry objects such as bivalve shells and sea anemones above their carapace with their posterior pereiopods, using these objects as shield from potential predators (Guinot *et al.*, 1995). Their respiratory system is unusual in that the inhalant openings are oblique, pocket-like slits on the pterygostomian region and are covered by long, stiff setae. The basal position of dorippids in brachyuran evolution is well documented (sensu Guinot, 1979, Rice, 1980) and the group was previously associated with such primitive forms as the deepwater Tymolidae (Abele & Felgenhauer, 1982, Tavares, 1993). The presence of the oxystomatous condition in such a basal group without inhalant channels suggests that this condition may predate the occurrence of these channels.

The evolution of the oxystomatous condition in *Matuta* is unclear. However, for the Calappidae, the evidence suggests that it may have first appeared in deepwater taxa, possibly in association with life in a sedimentary environment where the substratum is semi-fluid. It would appear that the oxystomatous condition was initially associated with the generation of strong respiratory currents which may help reduce sediment load on the branchiae. From the cladogram of the Calappidae *sensu stricto*, both the oxystomatous condition and the inhalant channels are present in the outgroup *Hepatus* and in the deep water, and presumably basal, calappids (*Acanthocarpus*, *Mursia*, *Platymera*). The inhalant channels, however, are less setose and broader than the shallow water *Calappa* and *Cycloes*. These features reflect the widespread potential for burying within the family. Burial in deep water (>200m deep) may be inevitable, as the fine sediment often exist in a semi-fluid condition. The presence of the oxystomatous condition followed by the channels may originally have been associated with maintaining respiratory currents in this semi-fluid environment. Once established, however, these features permitted full burying and may have been a preadaptation (exaptation, *sensu* Gould & Vrba, 1982) which permitted their successful colonization of shallow open sediment areas where burial may act as an effective predation avoidance mechanism.

The function of burying may influence respiratory adaptations of burying taxa. Those that use it primarily for an escape mechanism such as most portunids (*Ovalipes* spp., *Carcinus* spp) appear to trade off temporary protection with an inefficient ventilation pattern (prolonged reversals) over a short period. Those taxa that spend most of their time buried (i.e. those that live in soft-sediment, e.g. *Calappa*, *Matuta*, *Leucosia*), all display morphological adaptations that enable them to maintain normal ventilation patterns. The possession of the oxystomatous condition within the Calappidae *sensu lato* is clearly a case of evolutionary convergence associated with burying or living in fine sediments. For *Matuta* and *Calappa*, burying is not merely a short-term predator escape mechanism. The crabs spend most of their time buried in the substratum, emerging only to feed. In common with other burying taxa, *Matuta* and *Calappa* must deal with the problem of respiration whilst buried. Both taxa have solved the problem through the possession of well developed inhalant channels which maintain access to oxygenated water above the surface of the sediment and narrow exhalant openings which ensure effective dispersal of exhaled water. This enables both taxa to bury under the sediment for prolonged periods of time, with *Matuta* also being capable of complete deep burial. Most significantly, it also enables both taxa to maintain the normal forward ventilation pattern even under the substratum, a unique condition amongst burying taxa.

In summary, the oxystomatous condition refers to a structural appearance which reflects a common functional characteristic of the respiratory system. It is not a unifying character state, rather an example of convergent evolution, an independently derived feature. In all cases, it appears to be associated with burial in fine sediments. Initially arising in deepwater, it may have underpinned the ecological and evolutionary success of the Matutidae, Calappidae and Leucosiidae in shallow waters.