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Behavior and Physiology of Hermit Crabs During Burial:
Shell Abandonment and Lactate Accumulation

by

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of the requirements for the degree of
Master of Science in Biology

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABBREVIATIONS

ppk	Parts per thousand
AR	Anaerobic respiration
mM	Millimolar
ATP	Adenosine triphosphate
LDH	Lactate dehydrogenase
NAD ⁺	Nicotinamide adenine dinucleotide
EDTA	Ethylenediaminetetraacetic acid

ABSTRACT OF THE THESIS
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Organisms living in the intertidal zone must adapt to environmental and physical stressors. One physical stressor that these organisms may face, and that may require specific behavioral and physiological responses, is burial by sediment. In this thesis I report the results of experiments in which I subjected the intertidal hermit crab, *Pagurus samuelis*, to burial and analyzed aspects of subsequent behavior and physiology. In the first set of experiments, hermit crabs were buried with the shell aperture facing either up or down, and at one of three depths (2, 4, or 6 cm). The factors hermit crab weight, shell weight, weight ratio, shell shape, aperture orientation, and depth of burial were analyzed by logistic regression to determine their influence on shell abandonment and survival. The results showed a significant number of hermit crabs abandoned their shells when compared with the control group. The only variable that influenced shell abandonment was aperture orientation. The aperture up position promoted shell abandonment, with 73.2 % of hermit crabs that abandoned their shells doing so from an aperture up position. A lighter shell weight, shell abandonment, aperture orientation, and shallower depth of burial were all found to be significant predictors of survival of *P. samuelis* when buried.

After being buried with sediment, hermit crabs were more likely to survive if they abandoned their shells. However, this would leave the soft abdomen vulnerable to predation or some other potential hazard. Additionally, I discuss that the behavior of

abandoning the shell may be one possible reason for the paucity of hermit crabs found *in situ* in the fossil record. I suggest that whether or not the hermit crab survives the burial event, if it abandons the shell, the body and shell are less likely to be found fossilized together.

In the second set of experiments *P. samuelis* was subjected to burial treatments to determine if hemolymph lactate concentration increased due to hypoxic conditions in this species over time. Hermit crabs were buried with 6 cm of sand and excavated at 2, 4, 6, 8, 10, or 12 h. Burial duration and state (alive or dead) of the crabs were analyzed for group differences with regard to the accumulation of lactate in the hemolymph. A significant interaction was found between the independent variables duration and state concerning lactate concentration. There was a trend for lactate to be low (8.8 ± 2.7 mM) for those hermit crabs surviving the short durations, but higher (19.7 ± 5.0 mM) in crabs that died. Surprisingly, in the long durations, lactate was found to be very high (40.1 ± 5.0 mM) in surviving crabs, but lower (22.5 ± 2.4 mM) among the dead. I suggest variations in energy reserves and expenditure determined the outcome of survival and lactate concentration within the survival category. Shell weight, crab weight, weight ratio, lactate, and burial duration were analyzed to determine their affect on survival of *P. samuelis*. Burial duration negatively influenced survival, while none of the other variables had a significant influence. These results imply that the longer *P. samuelis* is buried, the less likely it will survive an acute, shallow burial event. Anaerobic respiration can provide a short term solution, as long as a substrate for glycolysis is available to meet metabolic demands. Glycogen stored in the hepatopancreas, and muscles is suggested as the substrate for anaerobic glycolysis.

CHAPTER ONE

INTRODUCTION

In this Thesis I examine the shell abandonment behavior and anaerobic respiration responses of the hermit crab, *Pagurus samuelis*, when acutely buried with sand in the laboratory.

In this chapter, I begin with the research objectives tested. I then review the behavioral ecology of hermit crabs as a taxon. Next, the causes for burial events, the hermit crab fossil record, and anaerobic respiration (AR) in crustaceans are explored. Lastly, I discuss relevant aspects of ecology and physiology of *P. samuelis*.

Objectives

The four main objectives in this thesis are: 1) to determine factors involved in the abandonment of shells when hermit crabs are buried; 2) to investigate factors that promote survival of a burial event; 3) to determine the extent to which the hermit crab, *Pagurus samuelis*, undergoes anaerobic respiration while buried with sand; and 4) to explore the factors that increase survival of hermit crabs while in a hypoxic environment.

Five hypotheses are associated with the objective to determine factors involved in the abandonment of shells when hermit crabs are buried:

1. A significant number of buried hermit crabs will abandon their shells when compared to a control that will not be buried with sand.

2. The weight of the hermit crab and/or shell influences shell abandonment.
3. The shape of the shell inhabited by the hermit crab will affect shell abandonment.
4. The orientation of the shell aperture will influence shell abandonment.
5. Depth of burial will affect the number of crabs abandoning their shells.

Hypotheses associated with the objective to investigate factors that promote survival of a burial event are:

1. The weight of the hermit crab and/or shell influences survival during burial.
2. The shape of the shell inhabited by the hermit crab will affect survival of a burial event.
3. The orientation of the shell aperture will influence survival during burial.
4. Depth of burial will affect the number of crabs surviving a burial event.
5. Whether or not a crab abandons its shell will affect survival during burial.

Hypotheses associated with the objective to determine the extent to which the hermit crab, *Pagurus samuelis*, undergoes anaerobic respiration while buried with sand are:

1. Duration of burial will be a factor that influences hemolymph lactate concentration.
2. The state (alive or dead) a hermit crab is found in at excavation will be predictive of hemolymph lactate concentration.

Three hypotheses are associated with the objective to explore the factors that increase survival of the hermit crabs while in a hypoxic environment:

1. Hermit crab and/or shell weight will influence survival during hypoxia.
2. Hemolymph lactate concentration will affect survival of acute hypoxia.
3. Burial duration will be a factor that influences survival during hypoxia.

In Chapter 2, I present research on the probability of *P. samuelis* abandoning a gastropod shell during a burial event. I tested factors that may encourage shell abandonment and/or survival under burial conditions. I found that a significant number of hermit crabs abandoned the shell after burial and that this behavior is more likely if the hermit crabs were buried with the apertures facing upward. I also found that the shell abandonment behavior promoted survival after the simulated burial event, along with decreased depth of burial, upward aperture orientation, and decreased weight of shell.

In Chapter 3, I investigate the ability of *P. samuelis* to undergo anaerobic respiration in response to the lack of available oxygen after burial with sediment. Hemolymph lactate increased with increasing burial duration, and there was a significant interaction between state of the crab (alive or dead) and burial duration. In addition to affecting lactate, burial duration also had a significant negative effect on survival. Hemolymph lactate concentrations were found to vary significantly depending on the combination of state and duration of burial. I proposed an answer to the variation could be an increase in glycogen stored in the tissues prior to experimentation allowed for more anaerobic glycolysis and a greater hemolymph lactate concentration.

In Chapter 4, I conclude with a review of the behavioral and physiological responses of hermit crabs to burial, and discuss the implications of these findings on the

ecology of the hermit crab. I also provide five specific ideas for potential future work to build on what is presented in the current Thesis.

Behavioral Ecology of Hermit Crabs

Hermit crabs are decapod crustaceans that are within the Infra-order Anomura. Anomura is subdivided into the four superfamilies Galatheoidea, Hippoidea, Lomisoidea, and Paguroidea; Paguroidea being the superfamily to which hermit crabs belong. There are over 800 species of hermit crabs from 86 genera and 6 families recorded in the literature. With the exception of the family Coenobitidae, considered “semi-terrestrial” because they have adaptations for life on land, all other hermit crabs are marine. They are most often found in the intertidal zone, or shallow coastal waters, but can be found at depths of up to 4500 m (Selbie, 1921). They are usually absent from wave exposed coasts, especially with shifting rocks (Reese, 1969). Hermit crabs that live in the intertidal zone are frequently found associated with rock pools, and Eriksson *et al.* (1975) found that *Pagurus bernhardus* preferred a hard substrate to one of sand.

Hermit crabs possess an unsegmented, uncalcified abdomen that requires protection from predators and osmotic stress. Most hermit crabs do this by inhabiting empty gastropod shells, although some inhabit worm tubes (Kuris *et al.*, 2007). In the natural environment, hermit crabs do not appear to kill gastropods for their shells (for exceptions see Rutherford, 1977). The abdomens of most hermit crabs are coiled to the right, therefore they typically inhabit dextral, rather than sinistral shells (Lancaster, 1988). The telson and uropods, along with the reduced 4th and 5th pereopods, extend out against the shell wall to help maintain shell position. In addition, the 4th pair of pereopods

is used to clean the carapace, gills, and limbs. Hermit crabs also possess pleopods; abdominal appendages on the left side only, used for circulating water inside the shell to remove feces and increase respiratory flow over the gills. If the hermit crab is tightly adhering to the columella and contracts the abdomen, the body will be quickly retracted into the shell whenever the threat of danger is present. Hermit crabs have only two pairs of pereopods that are true walking legs and, unlike the brachyuran decapods who run sideways, hermit crabs can run in a straight forward line (Lancaster, 1988).

Hermit crabs are described as omnivorous detritivores (Hazlett, 1981), and remains of everything from algae, microscopic shells, polychaetes and crustaceans to sponge spicules, diatoms, and foraminifera are found in the guts. Seasonally, they may scavenge on larger prey, such as ophiuroids, bivalves, amphipods, and crangonid shrimp. Cannibalism is common in many species. Feeding involves taste more than sight and this is mainly done by the aesthetasc sense organs, which are branched hairs at the distal end of the 1st antennae. Other areas sensing taste are branched hairs of the mouthparts, the edges of the chelae, and the dactyls of the walking legs.

Hermit crabs cannot be sexed while inside the shell, but this can be done once they are removed from the shell. The most consistent way to determine the sex is to identify the gonopores. In females they are found at the bases (coxae) of the 3rd pereopods, and in males they are located at the coxae of the 5th pereopods. Mating has been observed and documented in many species, and generally follows the steps given by Hazlett (1981). The male will usually grasp the shell of a female and carry her around with him for several hours to days before copulation (MacGinitie and MacGinitie, 1968). When finally ready to mate, the female is turned toward the male and the male begins to

drum with his chelipeds (claws) on the chelipeds of the female who is withdrawn into her own shell. This may last for 15 – 20 min. When ready for copulation, both individuals come out of the shell far enough to position their ventral surfaces to face one another, and connect. They may remain in this position for 4 – 6 min before copulation and 10 minutes afterward. After that time, the female is driven away and eggs are usually in place on the pleopods within one hour (Coffin 1960). Hermit crab larvae are planktonic and undergo four zoeal stages and a glaucotheal form before they are considered to be classed in the “young hermit stage” and begin to settle out of the plankton to look for a microscopic shell. For the rest of their lives, the size of available shells will dictate how rapidly, and to what extent, they may grow.

Although the shell can restrict growth, it provides many advantages to the hermit crab. The shell protects the hermit crab from predators (Vance, 1972). It provides physical protection to the soft abdomen against rough substrates (Bollay, 1964), gives protection from extreme temperatures (Reese, 1969), water loss (Herreid, 1969), and salinity stress (Shumway, 1978; Davenport *et al.*, 1980). Having a shell also provides protection for developing eggs (Taylor, 1981).

Burial by Sediment

Burial by sand or other debris is a threat to organisms that live in near shore, high energy environments where sediment reworking is the most intense (Schaffner *et al.*, 2001). Because hermit crabs are common inhabitants of intertidal zones worldwide, the threat of burial is a hazard they must cope with. Organisms can be buried by numerous mechanisms. These can be anthropogenic; such as dredging (Poiner and Kennedy, 1984;

Lopez-Jamar and Mejuto, 1988; Somerfield et al., 1995; Essink, 1999; Schratzberger et al., 2000), bait collecting (Jackson and James, 1979), fishing (Hall *et al.*, 1990), and trampling (Chandrasekara and Frid, 1996), or natural; such as storms (McCall, 1978), tidal sand movements (Grant, 1983), deposition from rivers (McKnight, 1969), terrestrial runoff (Edgar and Barrett, 2000), and bioturbation (Thayer, 1983; François *et al.*, 2001).

Sediment disturbance may affect species differently, assuming that there is some variation of susceptibility to disturbance or recovery from disturbance based on species-specific properties. These properties could be tolerance to ecological changes and/or population growth rates. Schiel *et al.* (2006) found that even a small amount of sediment reduced attachment of newly settling algae by 70 %. Furthermore, substrate covered with sediment completely prevented attachment of settling algal larvae, and burial of algal germlings caused 100 % mortality within a few days (Schiel et al., 2006). Comparing a dredge disposal area to a non-disposal area in Liverpool Bay, UK it was found that, although abundance of nematodes was equal, species evenness, richness, diversity, and species abundance were all lower at the disposal site (Somerfield *et al.*, 1995). Copepods too, had a lower abundance and number of species at the disposal site (Somerfield *et al.*, 1995). In a study of vertical migration of four macrofaunal species, Bolam (2003) found that the polychaetes *Tharyx* sp.A. and *Streblospio shrubsolii* were unable to vertically migrate 6 cm, but that some individuals of the oligochaete *Tubificoides benedii* successfully migrated through 6 cm of sediment. He also found that the gastropod *Hydrobia ulvae* survived up to 16 cm of burial. These data suggest that certain taxa, or even species within a taxon, are better adapted to either survive a shallow burial event, or to recolonize after a deep one.

Hermit Crabs in the Fossil Record

Not only are extant intertidal hermit crabs coping with sedimentation now, but hermit crabs have had to do so since their appearance in the Early Jurassic (Warner, 1977; Glaessner, 1969; Cunningham, 1992). During this same history, hermit crabs have been primarily restricted to living inside gastropod shells, although one specimen was found in an ammonite shell (Fraaije, 2003). Hermit crabs are underrepresented in the fossil record (Gordan, 1956). Hermit crab fossils that have been found are mostly fossilized chelipeds (Walker, 1988). Besides finding hermit crab body parts, other evidence of hermit crabs in the fossil record can be found on the gastropod shells they once inhabited. Evidence of hermit crab occupancy includes epibionts, such as bryozoans and hydrozoans, which would not normally live on a live snail (Walker, 1995). In fact, Walker (1995) found that out of all the fossilized *Tegula* shells in the San Diego County Museum, 29 % had epibionts that indicated hermit crab inhabitation. Another sign that a gastropod shell once had a hermit crab living in it is a worn down last whorl of the shell. This results from the hermit dragging it on the sea floor or shore (Boekschoten, 1967; Muller, 1979). Walker (1995) determined that 61 % of Recent hermitted shells had epibionts, therefore 39 % of the shells had no indication of hermit crab occupancy. This means that not all shells that belonged to hermit crabs would be recognized in the fossil record, and they therefore, may be underrepresented.

Especially rare are reports of fossil hermit crabs inside the gastropod shells they inhabited, with only a handful of *in situ* (as term is used by Fraaije (2003) and Jagt (2006)) specimens known worldwide (Hyden and Forest, 1980; Dunbar and Nyborg, 2003; Jagt et al., 2006). The oldest specimen was found in the Lower Jurassic strata (Jagt

et al., 2006). Other *in situ* specimens include a fine specimen found in the Lower Miocene of New Zealand (Hyden and Forest, 1980), one sample from the Upper Miocene of New Zealand (Feldmann and Keyes, 1992), and a poorly documented and preserved specimen from the Upper Cretaceous of Germany (Mertin, 1941). In addition to these, a concretion with a pair of chelipeds was found associated with, but not inside, an external mold of a gastropod shell from the Upper Cretaceous in Antarctica (Aguirre-Urreta and Olivero, 1992; Olivero and Aguirre-Urreta, 1994). This gastropod shell was encrusted with colonial bryozoans, indicating hermit crab inhabitation. There are also a few other described and undescribed (see Fraaije, 2003) *in situ* specimens from Taiwan, the Netherlands, Japan, the United Kingdom, Denmark, Portugal, Spain, and Costa Rica (Hu and Tao, 1996; McLaughlin and Forest, 1997; Jagt et al., 2000; Karasawa, 2002; Collins and Jakobsen, 2004; Todd and Collins, 2006). Thus, this represents a taphonomic problem: Why are so few hermit crabs fossilized inside their gastropod shells?

When alive, a hermit crab is more likely to let itself be torn in two before allowing something to remove it from its protective shell (MacGinitie and MacGinitie, 1968). Therefore, one answer to this taphonomic problem might be that once the hermit crab has died, it no longer has the same ability to adhere to the inside of its shell. Thus, it is likely that deceased crabs would dissociate from their shells because of the high-energy environments they inhabit.

The Oldest *In Situ* Hermit Crabs

In the collections of the Museum für Naturkunde, a body chamber of the ammonite *Pleuroceras solare* Phillips, 1829 from the Lower Spinitum Zone at Banz

(Southern Germany) was found to contain a right cheliped, lacking the dactylus, and shows an imprint of the other cheliped (Jagt *et al.*, 2006). In overall proportions and ornament, this cheliped is thought to be a paguroid of Middle Jurassic – Early Cretaceous age. However, details of ornament, handedness (right *vs.* left), length/width ratio and the fact that fingers appear to have been stout and short rather than long and slender, suggest it may be an undescribed genus, but was categorized as '*Palaeopagurus*' n. sp., (Jagt *et al.*, 2006).

An Early Cretaceous *in situ* hermit crab was discovered in Speeton, UK (Fraaije, 2003). The diagnosis was that it was a rectangular, granulated left cheliped much larger than the right, convex external surface, oblique carpo-propodial and dactylus articulations, and long and slender fingers. No other genus known from the Cretaceous shows the combination of these morphological characters, so the specimen was placed in the genus *Paleopagurus*. It was given the species name *vandenengeli* after its discoverer, Dr. Aad van den Engel.

One unusual characteristic of both of these *in situ* specimens is that the crabs were found inside ammonite shells, instead of the usual gastropod shells. Fraaije (2003) suggests that paleontologists may have concentrated on the wrong class of mollusks in their attempt to analyze the fossil record of hermit crabs. Hermit crab inhabitation of the ammonites might explain the absence of hermit crabs in gastropod shells of Jurassic and Early Cretaceous time periods. During the Cretaceous Period the Gastropoda rapidly diversified. There was a notable increase in shell sturdiness by shell ornamentation, increased thickness, narrow, elongate apertures, and apertural dentition (Vermeij, 1977). In addition to these, the conical shaped shell of the gastropod would be easier to drag

across the sea floor, than the planispiral shell of the ammonites. These adaptations would have made gastropod shells a more desirable home for hermit crabs and provide better protection against predators. Fraaije (2003) proposed this switch from ammonite to gastropod shells also caused a morphological change in hermit crab claws. In the specimen inhabiting the ammonite shell, the aperture-blocking hermit crab claws were more elongated to almost straight fingered chelae. However, with the gastropod aperture, the chelae became more rounded and curved to better fit the opening.

Anaerobic Respiration in Crustaceans

Burial by sediment is an appropriate condition in which to test the extent that hermit crabs undergo anaerobic respiration, because burial may cause the immediate environment to become hypoxic (Nichols *et al.*, 1978; Shives and Dunbar, 2010).

Anaerobiosis has been shown to occur in crustaceans in conditions such as exercise (Full and Herreid Jr, 1984; Briffa and Elwood, 2001), aerial exposure (Ridgway *et al.*, 2006), and hypoxia (Bridges and Brand, 1980; Hill *et al.*, 1991).

Many vertebrate and invertebrate species rely, at least partially, on anaerobic energy production during periods of exercise (Henry *et al.*, 1994). In crustaceans, reliance on anaerobic metabolism during strenuous bouts of exercise is usually characterized by the degree of lactate accumulation in the hemolymph (Henry *et al.*, 1994) (e.g. from resting values below 1 mM to post-exercise values up to 25 mM McDonald *et al.*, 1979; Smatresk *et al.*, 1979; Wood and Randall, 1981; Booth *et al.*, 1984; Forster *et al.*, 1989; Morris and Greenaway, 1989).

Burst contraction in crustacean muscles is similar to that in vertebrates, where intracellular phosphagen and glycogen are exhausted and lactate accumulates (Head and Baldwin, 1986; Milligan *et al.*, 1989; Hervant *et al.*, 1995; Johnson *et al.*, 2004). In crustaceans, there is preliminary dependence on the hydrolysis of the phosphagen, arginine phosphate (AP), after which anaerobic glycogenolysis is employed (Morris and Adamczewska, 2002). Lactate only accumulates during a succession of anaerobic contractions, which are thought to be a normal part of crustacean behavior in the environment (Booth and McMahon, 1992).

To our knowledge, the only previous investigations of anaerobic respiration in hermit crabs have been carried out by Briffa and Elwood (2001; 2005). They studied AR in aggressive contacts between *Pagurus bernhardus* individuals and analyzed the concentration of lactate and glucose in the hemolymph of individuals immediately following agonistic interactions over the ownership of a gastropod shell. They found lactate concentrations in attackers increased with the amount of shell rapping. Furthermore they found that attackers, but not defenders, gave up the fight when the concentration of lactate was high.

Hypoxia

The anaerobic capacity of crustaceans has been described with respect to hypoxia. Hypoxia causes hemolymph lactate levels to significantly increase (Bridges and Brand, 1980). An increase in lactate levels can also be seen in the tissue during hypoxia and in the initial stages of recovery (Hill *et al.*, 1991). Although lactate accumulates in the hemolymph during both functional and environmental hypoxia in decapod crustaceans,

the extent of accumulation is much greater during long-term environmental hypoxia when lactate concentrations may exceed 40 mM in the crayfish, *Orconectes limosus* (Gade 1984) and the stone crab, *Menippe mercenaria* (Gade, 1984; Albert and Ellington, 1985). During a 12 h period of hypoxia, lactate was found to be the major end-product of anaerobic respiration in *Carcinus maenas*, although fumarate and alanine also increased (Hill *et al.*, 1991). However, the amounts detected indicate that alternative metabolic pathways are of very little significance in *Carcinus maenas* (Hill *et al.*, 1991). These results point to lactate as the indicator of the rate of anaerobic respiration occurring within crustaceans.

We are unaware of studies testing hypoxia-induced AR in crustaceans during burial by sediment, or studies demonstrating that hermit crabs may compensate for environmental hypoxia by undergoing AR. It is reasonable to investigate if hermit crabs undergo AR during hypoxia, since it has been well documented in other crustaceans.

Pagurus samuelis

Of the 32 genera in the family Paguridae (right-handed hermit crabs), *Pagurus* is the representative genus with over 150 species (Garcia-Gomez, 1982). *Pagurus samuelis* Stimpson, 1857 (the blueband hermit) is a common species found along the coasts of the Eastern Pacific Ocean, from British Columbia to Punta Eugenia, Baja California (Ricketts *et al.*, 1985). Its habitat is limited to high rocky intertidal areas on the outer coast (Nyblade, 1974; Jensen, 1995), which, except in a few areas, separates the habitat of *P. samuelis* from 2 sympatric species *P. granosimanus* and *P. hirsutiussculus* (Abrams, 1987). Because it lives in the high intertidal, *P. samuelis* must be able to tolerate

fluctuations in temperature, pH, salinity, and dissolved oxygen (Coffin, 1958; Reese, 1963). Defining characteristics of *P. samuelis* include bright blue dactyls and bands around the 2nd and 3rd propodi, along with red, unbanded antennae (Kuris *et al.*, 2007). After mating and gestation, *P. samuelis* larvae are released in the spring and summer (Nyblade, 1974). The percentages of gravid females in the summer is extremely high, as many as 98% in some pools (Coffin, 1960). Instead of blue bands, juvenile *P. samuelis* have white bands around the 2nd and 3rd propodus (Kuris *et al.*, 2007).

When compared with the two sympatric species, *P. samuelis* was two times faster at finding and occupying shells (Abrams, 1987), and was able to evict *P. hirsutiusculus* from its shell more often than were evicted by *P. hirsutiusculus* (Abrams, 1987). When given the choice, mature *P. samuelis* individuals prefer black turban snail (*Tegula funebris*) shells to all other species of gastropod shells (Abrams, 1987; Hahn, 1998). Shell selection usually occurs after a lengthy period of investigation, and it is based on numerous factors such as, shell characteristics (weight and height), internal space, shell species, shell availability, and experience (Côté *et al.*, 1998). Hermit crabs must choose between a smaller shell that would be lighter to carry, and a larger, heavier shell that would aid in predator avoidance, allows for growth and reproduction, and delays the need to find a new shell (Côté *et al.*, 1998).

Despite having the protective shell, hermit crabs are preyed upon by a number of taxa. These include other crustaceans such as the rock crab, *Cancer irroratus* Say, 1817 (Grant and Pontier, 1973), the green crab, *Carcinus maenas* Linnaeus, 1758 (Rotjan *et al.*, 2004), the stone and calico crabs, *Menippe mercenaria* Say, 1818 and *Hepatus epheliticus* Linnaeus, 1763, respectively (Brooks and Mariscal, 1985), and the American

lobster, *Homarus americanus* H. Milne Edwards, 1837 (Weissberger, 1995). Other predators of hermit crabs include the Atlantic pygmy octopus, *Octopus joubini* Robson, 1929 (Brooks and Mariscal, 1986; Brooks, 1991), the common octopus, *Octopus vulgaris* Cuvier, 1797, (Ross, 1971; 1984) and the toadfish *Opsanus tau* Linnaeus, 1766 (McDermott, 1964). Predators of *P. samuelis* include the pile perch *Rhacochilus vacca* Girard, 1855, the sheepshead, *Semicossyphus pulcher* Ayres, 1854, and the spotted kelpfish *Gibbonsia elegans* Cooper, 1864 (Cowles, 2005).

Since *P. samuelis* lives in a high energy environment that likely experiences irregular sedimentation, there may be adaptive advantages to having behavioral and metabolic mechanisms for surviving acute burial. Hermit crabs carry the extra burden of gastropod shells to protect their vulnerable abdomens. However, in a burial event, it may be advantageous to abandon the shell for survival. This would facilitate escape and make it improbable for the hermit crab body and the shell to be articulated, should fossilization occur.

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CHAPTER TWO

BEHAVIORAL RESPONSES TO BURIAL IN THE HERMIT CRAB, *PAGURUS* *SAMUELIS*: IMPLICATIONS FOR THE FOSSIL RECORD

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Abstract

The intertidal hermit crab, *Pagurus samuelis*, was subjected to various treatments analyzed to determine behavioral responses to the ecological stress of burial. Hermit crabs were buried at varying depths (2, 4, and 6 cm), and in two orientations (shell aperture up and aperture down). Hermit crab weight, shell weight, shell shape, aperture orientation, and depth of burial were analyzed to determine their influence on shell abandonment and survival. We found a significant number of hermit crabs abandoned their shells when compared with the control group. Aperture orientation strongly influenced shell abandonment, with 73.2 % of hermit crabs that abandoned their shells doing so from an aperture up position. None of the other variables significantly affected shell abandonment behavior. However, shell weight, shell abandonment, aperture orientation, and depth of burial were all found to be significant factors in the survival of *P. samuelis* when buried. Although abandoning the shell significantly increases the chances of surviving a sedimentation event, such as flooding, this behavior likely puts the crab at increased risk of both predation and being buried in a subsequent event if flooding persists in the short term. Hermit crabs are underrepresented in the fossil record. Especially rare are *in situ* specimens. We suggest one possible reason for this paucity is that, whether the hermit crab survives the burial event or not, if it abandons the shell, the body and shell are less likely to be found fossilized together.

Key Words and Phrases – Rapid sedimentation, Flooding, Anaerobic condition, Intertidal zone, *In situ* fossilization, Survival.

Introduction

Burial of Extant Marine Invertebrates

Hermit crabs are common intertidal, lower trophic invertebrates. Because of their physiological responses to fluctuating conditions in the intertidal zone they can be used as ecological indicators of freshwater inundation (Dunbar *et al.*, 2003). Flooding is a common event in the intertidal zone, and a substantial amount of sediment is often carried by this freshwater. The term ‘sediment’ includes a broad range of materials such as silt, sand, and gravel from both terrestrial and marine sources (Schiel *et al.*, 2006). Burial with sediment occurs by both natural and anthropogenic means, and is a periodic stress that near shore animals must face. Sediments are physically displaced by a wide variety of mechanisms such as storms (McCall, 1978), tidal sand movements (Grant, 1983), deposition from rivers (McKnight, 1969), terrestrial runoff (Edgar and Barrett, 2000), dredging (Messieh *et al.*, 1991; Essink, 1999; Schratzberger *et al.*, 2000), bait collecting (Jackson and James, 1979), fishing (Hall *et al.*, 1990), bioturbation (Thayer, 1983; François *et al.*, 2001) and trampling (Chandrasekara and Frid, 1996). Disturbed sediment can vary in depth from 1 mm (Niedoroda *et al.*, 1989) to 5 m (Maurer *et al.*, 1981) depending on the strength of the disrupting force. Thus, even in one location there can be high spatial and temporal variability in the amount of sediment movement.

Other studies have investigated marine invertebrates after burial with sediment both *in situ* and in the laboratory. Nichols *et al.* (1978) carried out an *in situ* study involving the burial of a pelecypod – polychaete assemblage. They found that within 4 h of burial with 10 cm of sediment, individuals of large size (>0.42 mm) had moved upward at least 5 cm, whereas smaller individuals (0.30 – 0.42 mm) had not. However,

when the experiment was repeated with a 24 h duration the results showed both sizes being distributed equally throughout the 10 cm of sediment. They concluded that *in situ* burial with 10 cm or less did not significantly affect the survival of the pelecypod – polychaete infaunal community as a whole, even though many individuals remained buried.

However, other taxa may have a more difficult time surviving a burial event. Chandrasekara and Frid (1998) assessed the survival of two epibenthic gastropod species, *Hydrobia ulvae* and *Littorina littorea*, after burial with sediment, and in different temperatures. They found the number of *H. ulvae* surviving burial with 5 cm of sediment decreased with increased burial duration and temperature. *Littorina littorea* did not survive the 5 cm burial up to 24 h at any temperature. In addition, increasing burial depth in 1cm increments up to 5 cm significantly reduced the ability of *L. littorea* to escape out of the sediment.

Studies have investigated the responses of hermit crabs to life in the often disrupted intertidal zone with respect to salinity (Dunbar *et al.*, 2003), temperature (Burggren and McMahon, 1981; Dunbar, 2001) and industrial and agricultural runoff (Lyla *et al.*, 1998). Although hermit crabs are very common along the sediment-water interface, we are aware of no previous studies that have investigated the effects of burial on this taxon.

Hermit Crabs and the Fossil Record

When an intertidal organism is buried immediately *in situ*, that organism has a higher likelihood of becoming part of the lithosphere (fossilization), instead of being

recycled into the biosphere (decomposition, scavenging), than those organisms remaining exposed, or transported at death (Behrensmeyer *et al.*, 2000). Although hermit crabs live in habitats that are conducive to fossilization, they are nevertheless underrepresented in the fossil record (Gordan, 1956; Dunbar and Nyborg, 2003; Jagt *et al.*, 2006). Of the hermit crab fossils that have been found, disarticulated chelipeds are most common (Walker, 1988). Records of fossil hermit crabs articulated with the gastropod shells they once inhabited are especially rare (Hyden and Forest, 1980). Only a handful of *in situ* specimens have been described worldwide (Dunbar and Nyborg, 2003; Fraaije, 2003; Jagt *et al.*, 2006). Fossilized, unoccupied gastropod shells that hermit crabs once inhabited also provide indirect evidence of hermit crabs in the fossil record (Boekschoten, 1967; Muller, 1979; Walker and Carlton, 1995). Thus, both the hermit crab body and the shell are fossilizable. This begs the question why hermit crabs are not found more often fossilized within their gastropod shells after burial.

Hermit crabs provide an appropriate model for testing behavioral strategies to escape burial since they require gastropod shells to protect their soft abdomens but, unlike gastropods, are able to abandon the shell when necessary. The purpose of this study was to analyze the behavioral responses of the intertidal hermit crab, *Pagurus samuelis*, to the environmental stress of burial, and to investigate factors influencing shell abandonment and survival of buried hermit crabs.

Materials and Methods

Collection and Care of Hermit Crabs

Individuals of the hermit crab, *Pagurus samuelis* (Stimpson 1857) were collected by hand from tide pools at Shaw's Cove (33° 32' 43" N, 117° 47' 57" W) and Little Corona del Mar (33° 35' 21" N, 117° 52' 05" W) in Southern California from February 2005 – February 2006. We selected individuals of all sizes, with hermit crab body weights from 0.018 – 0.629 g. Animals were not sexed because other studies have found no effect of sex on behavioral response of hermit crabs (Bertness, 1980; Hazlett, 1996; Briffa et al., 2008). No gravid females were used in treatments or controls. Hermit crabs were transported to the laboratory within 2 h of collection, and subsequently kept in aquaria. Salinity was maintained at 36 ± 3 ppK and temperature at 24 ± 2 °C with ambient light. Diet consisted of frozen, commercial salad shrimp once a week, and water was changed every three weeks.

Pre-burial Methods

To test the hypotheses that animal size and shell size affects survivability and shell abandonment, morphometric measurements were recorded for each *P. samuelis* before burial. Hermit crabs were randomly selected from the aquarium for experimentation, shaken gently and blotted with paper towel to remove excess water. The shape of the shell was categorized and recorded as either “round” (i.e. *Tegula funebris*) or “elongate” (i.e. *Acanthina spirata*). Length and width for both the shell and the aperture were measured using Vernier calipers (± 0.25 mm). Total wet weight of each crab inside the gastropod shell was measured and recorded to ± 0.001 g.

Burial Methods

Hermit crabs were placed into plastic containers previously filled with 3 cm of sand of preselected grain size (> 0.3 mm – < 0.5 mm) in one of two aperture orientations. This sediment size range is considered “medium” (Wentworth, 1922; Alexander *et al.*, 1993). In order to test the effect of aperture orientation on shell abandonment and survivability, 45 hermit crabs were placed with apertures up and 45 down. Aperture orientation of the replicates was decided ahead of time, but hermit crabs were selected randomly from the aquarium. Individuals were held in place while they were slowly buried with water saturated sand, until the desired depth of sand covered the crab, and there was 1 cm of standing water. We tested hermit crabs at three burial depths: 2, 4, and 6 cm of sand. At 8 h intervals hermit crabs were checked and, if applicable, we recorded escape time and shell association. Treatments were ended after 24 h, at which time hermit crabs that had not escaped were excavated. Depths at which the crab and shell were found during excavation were recorded. A control group ($n = 10$) underwent the same pre-burial and burial methods, short of being buried.

Post-burial Methods

After each treatment, forceps were used to gently pull dead hermit crabs from their shells. If a live crab remained in its shell, the crab was separated from its shell by placing the animal into a labeled paper bag and cracking the shell open with a bench vise. The paper bag enabled all shell fragments to be weighed. Once

disarticulated from the shell, wet weights were obtained for the crab and the shell separately.

These methods were repeated until 30 data sets were obtained for each of the 3 sand depths. After each treatment, water inside each burial container was changed, and the sand rinsed to reoxygenate the sediment and removed any metabolic wastes, or traces of previous hermit crabs.

Statistical Analyses

To determine the relationship between the weight of the hermit crab and the weight and size of the shell, a 2-tailed Pearson correlation was done for each pair of variables. To test the hypothesis that more hermit crabs abandoned their shells among the treatment group than the control group, a one-tailed Fisher exact test was used because the data violated assumptions for a chi-square test (Wheater and Cook, 2000). The independent variables shell weight, crab weight, weight ratio, shell shape, aperture orientation, and burial depth were analyzed using two step-wise logistic regressions ($\alpha = 0.05$) to test their affect on the dependant variables; shell abandonment and survival. All statistical tests were performed with the program Statistical Package for the Social Sciences (SPSS) 14.0.

Results

Preliminary Tests

Preliminary tests with a TPS 90 - D oxygen meter and Clark-type oxygen electrode were used to determine the extent to which conditions within the sediment

became hypoxic. Results of the oxygen saturation tests showed a decline in the percent saturation with time. Oxygen saturation dropped to a mean of 26.6 ± 4.4 % within 15 minutes of burial. An oxygen concentration of less than 10 % was reached within 7.5 h after burial and continued to decline to a mean of 1.8 ± 0.7 % at 24 h.

We found that hermit crab weight correlated significantly with both shell weight and shell aperture width (shell weight: $r = 0.855$, $p < 0.001$; shell aperture width: $r = 0.852$, $p < 0.001$).

Factors Affecting Shell Abandonment

The total number of hermit crabs that abandoned their shells after burial in the treatment group was found to be significantly higher than individuals that abandoned the shells in the control group (treatment = 46%, $n = 90$; control = 0%, $n = 10$; Fisher exact test, $p = 0.004$).

A forward, step-wise logistic regression was conducted to determine which independent variables (shell weight, crab weight, shell shape, aperture orientation, and burial depth) were predictors of shell abandonment. Regression results indicated the overall model of one predictor (aperture orientation) was statistically reliable in distinguishing between hermit crabs abandoning their shells or remaining inside (2 Log Likelihood = 107.340, $\chi^2_{(1)} = 16.715$, $p < 0.001$). The model correctly classified 71.1 % of the cases. Regression coefficients were $B = -1.822$ SE = 0.469, Wald = 15.061, $df = 1$, $p < 0.001$, Odds ratio = 0.162. Aperture orientation strongly influenced shell abandonment so that 66.7 % of crabs buried aperture up abandoned the shell, while only 24.4 % of crabs that were buried aperture down abandoned (Figure 1). Of

the total number of hermit crabs that abandoned the shell 73.2 % did so from an aperture up orientation. None of the other variables significantly affected shell abandonment behavior.

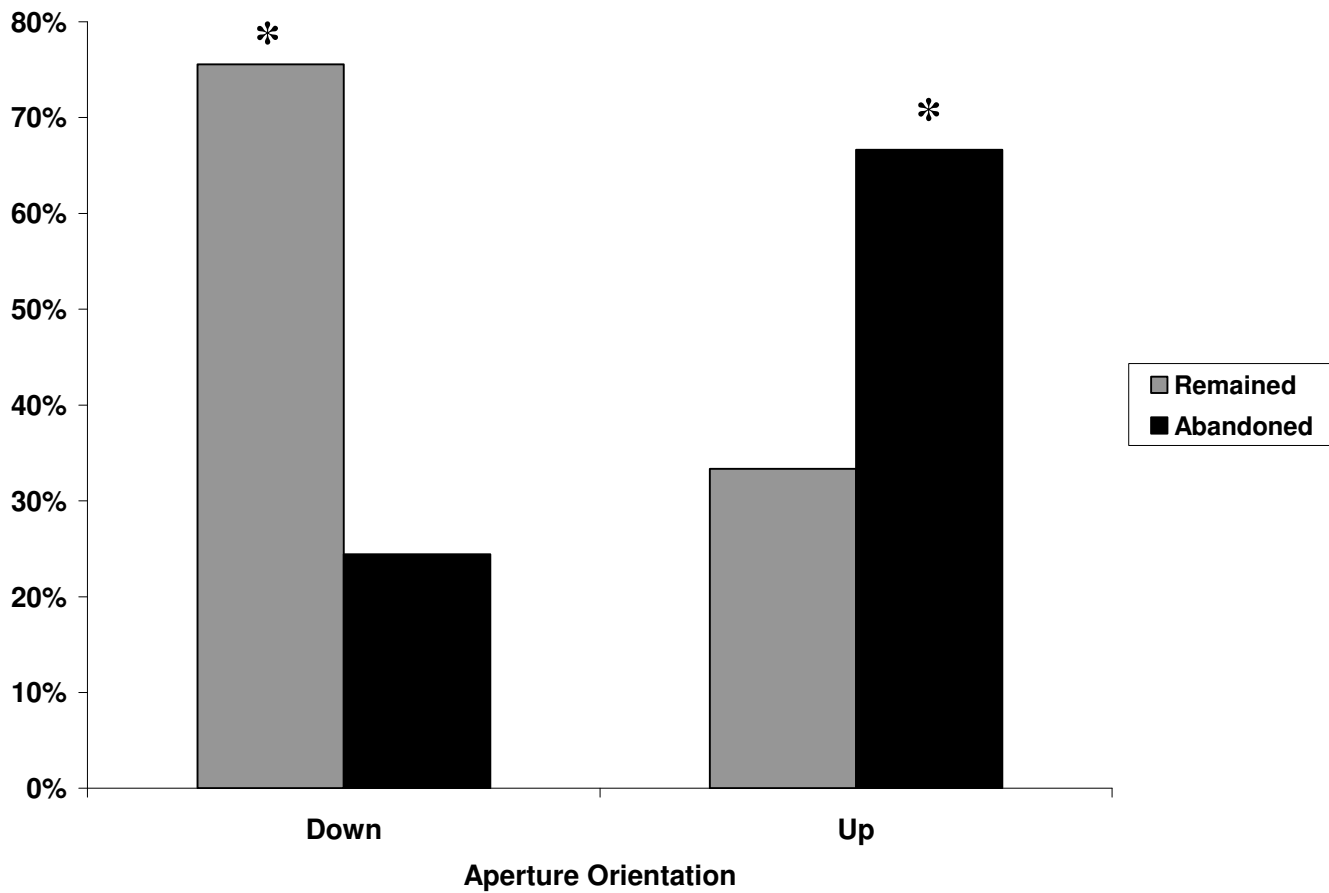


Fig. 1. The percent of individuals that remained in or abandoned the shell at each shell aperture orientation after burial with sediment. Logistic regression $B = -1.822$ S.E. = 0.469, Wald = 15.061, $df = 1$, $p < 0.001$, Odds ratio = 0.162. An asterisk represents $p < 0.05$.

Factors Affecting Survival

Over all treatment depths 76.7 % of the hermit crabs escaped the sediment (Table 1). Hermit crabs that escaped the sediment (n = 69) remained alive until the trial was ended. Conversely, all crabs (except one) that remained buried in the sediment (n = 21), were dead at the end of the trials (Table 1). When comparing the amount of time hermit crabs took to escape, most crabs (91.3 %) did so within the first 8 h of the trial, and only 5.6 % escaped the sediment at the 24 h point (Figure 2).

Table 1. The number of hermit crabs that remained in the shell or abandoned the shell for each state

	Number of Individuals	In Shell	Abandoned
Escaped (Survived)	69	30	39*
Remained Buried (Died)	21	19 ^a	2
N	90	49	41

* Logistic regression B = 4.223, S.E. = 1.109, Wald = 14.505, df = 1, p = < 0.001, odds ratio = 68.257. a = One individual remained buried, but was alive.

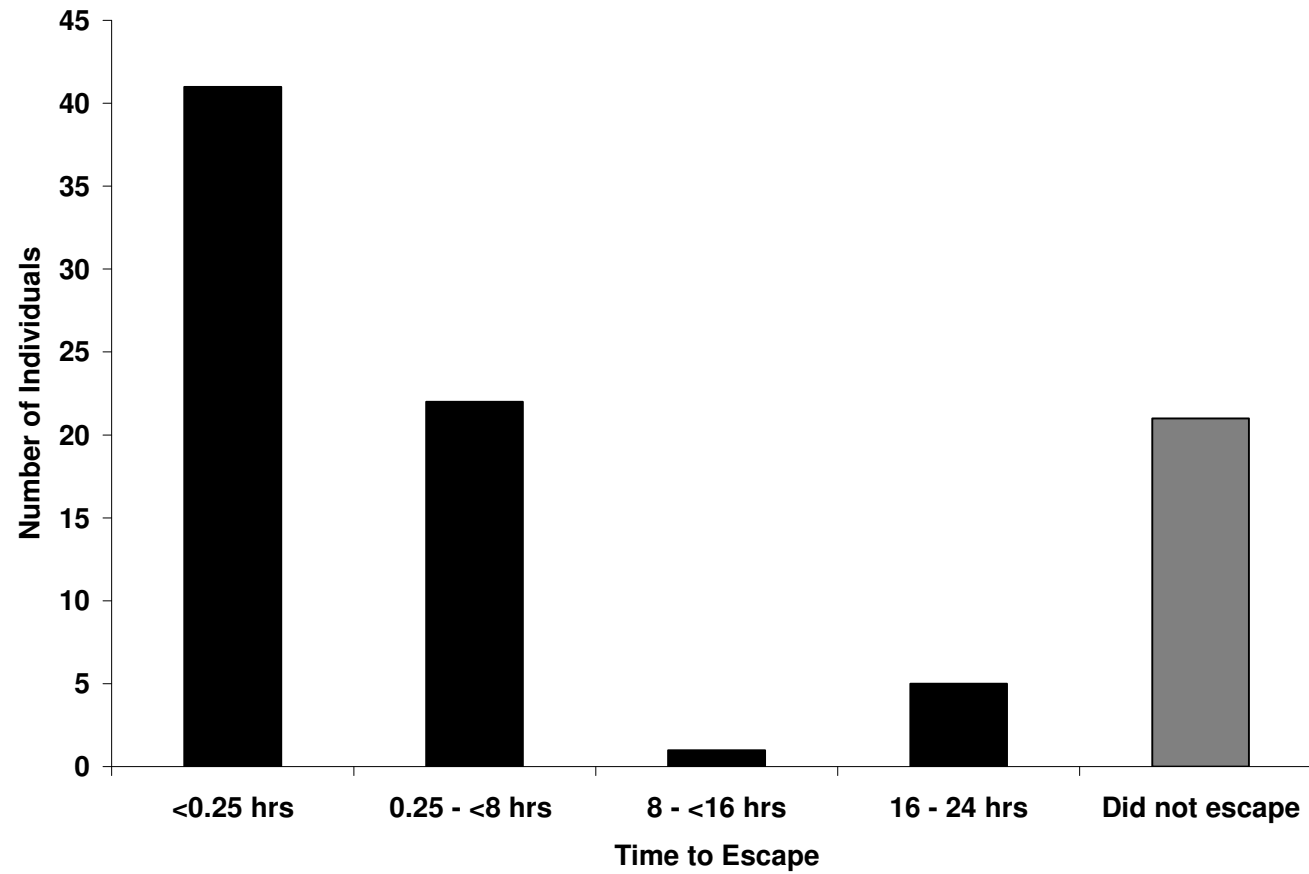


Fig. 2. The number of hermit crabs that escaped the sediment for each time interval.

Regression results indicated the overall model of four predictors (shell weight, shell abandonment, aperture orientation, and burial depth) was statistically reliable in distinguishing between survival and death of the hermit crabs when buried (-2 Log Likelihood = 50.344; $\chi^2_{(5)} = 45.003$, $p < 0.001$). The model correctly classified 87.8 % of the cases. Shell weight was a significant predictor of survival (logistic regression $B = 1.239$, S.E. = 0.632, Wald = 3.840, $df = 1$, $p = 0.05$). Hermit crabs that survived the burials irrespective of aperture orientation had a smaller mean shell weight (Figure 3).

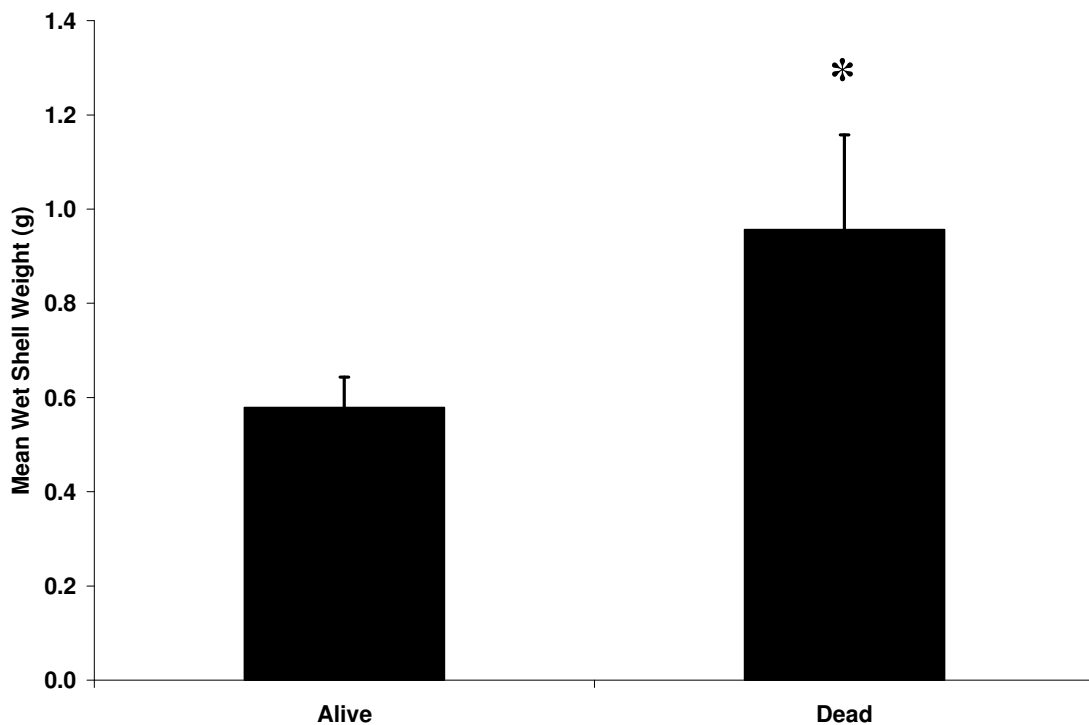


Fig. 3. The mean shell wet weights of hermit crabs that survived the burial and those that died. Error bars represent ± 1 S.E. Logistic regression: $B = 1.239$, S.E. = 0.632, Wald = 3.840, $df = 1$, $p = 0.05$. An asterisk represents significance at the $\alpha = 0.05$ level.

There was an obvious interaction between the factors shell abandonment (logistic regression $B = 4.223$, $S.E. = 1.109$, $Wald = 14.505$, $df = 1$, $p < 0.001$, odds ratio = 68.257) and aperture orientation (logistic regression $B = -1.968$, $S.E. = 0.897$, $Wald = 4.817$, $df = 1$, $p = 0.028$, odds ratio = 0.140). While survival rate for crabs that remained in the shell was 63.3%, survival for those that abandoned their shells was significantly higher (95.1%) (Table 1). This becomes more evident when taking aperture orientation into account. Figure 4 shows the percentage of crabs that survived from the two different aperture orientations, and whether or not they used shell abandonment as the primary method of survival. Hermit crabs buried with the aperture down primarily survived by remaining in their shells (69.4%), and secondarily survived by abandoning. Conversely, those crabs buried with the aperture up primarily survived by abandoning shells (82.4%) and secondarily survived by remaining in shells.

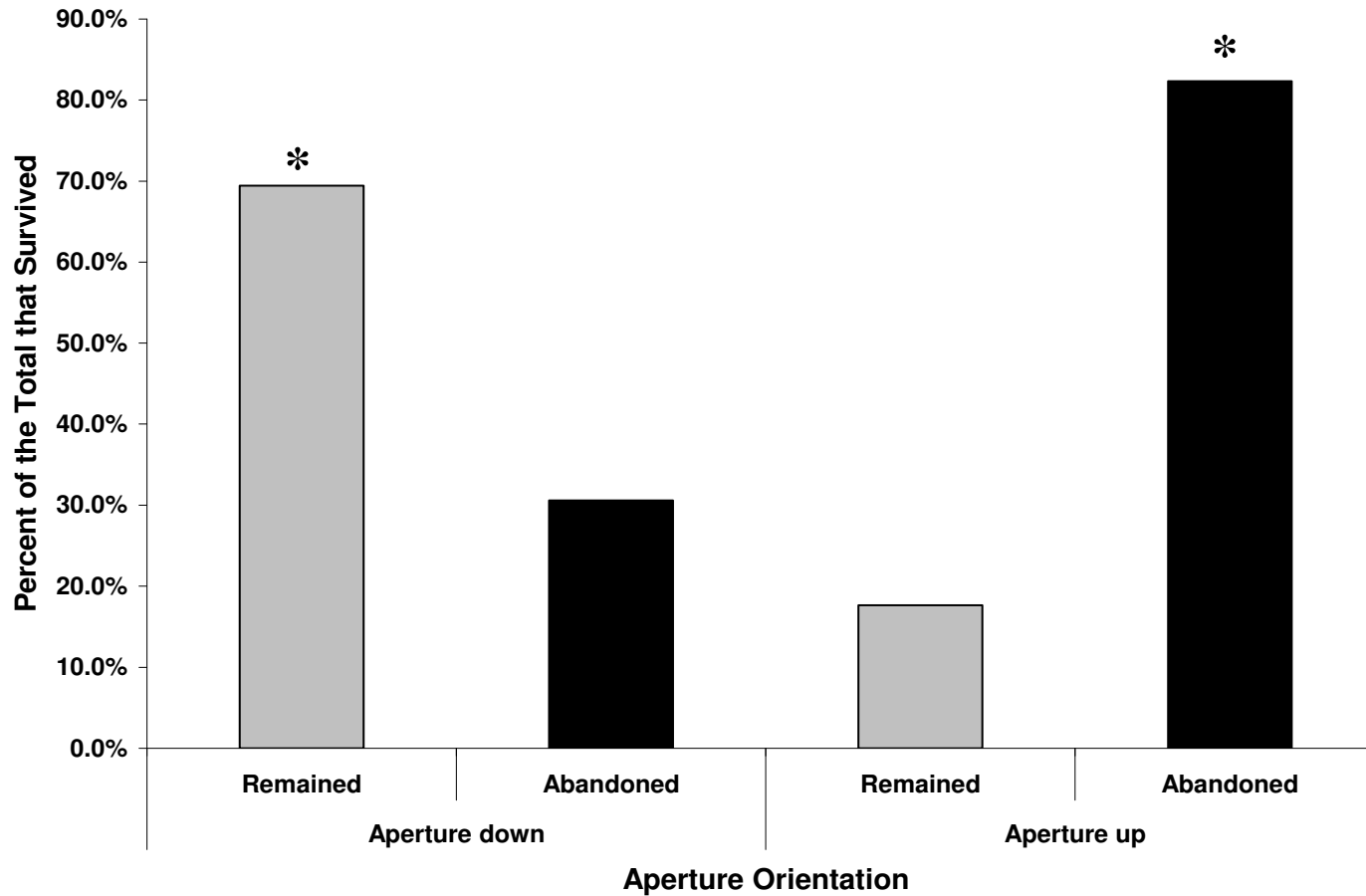


Fig. 4. The percentage of crabs that survived the experiment by either remaining in the shell (gray bars) or abandoning the shell (black bars) and in which aperture orientation they were buried. Logistic regression $B = -1.968$, $S.E. = 0.897$, $Wald = 4.817$, $df = 1$, $p = 0.028$, odds ratio = 0.140. An asterisk represents $p < 0.05$.

Depth of burial was also a significant factor in determining the survival of *P. samuelis* (logistic regression Wald = 10.734, df = 2, p = 0.005). We found that depth and survival were inversely related. At depths of 2, 4, and 6 cm, 96.7 %, 73.3 %, and 63.3 % of the crabs survived the trials, respectively (Figure 5).

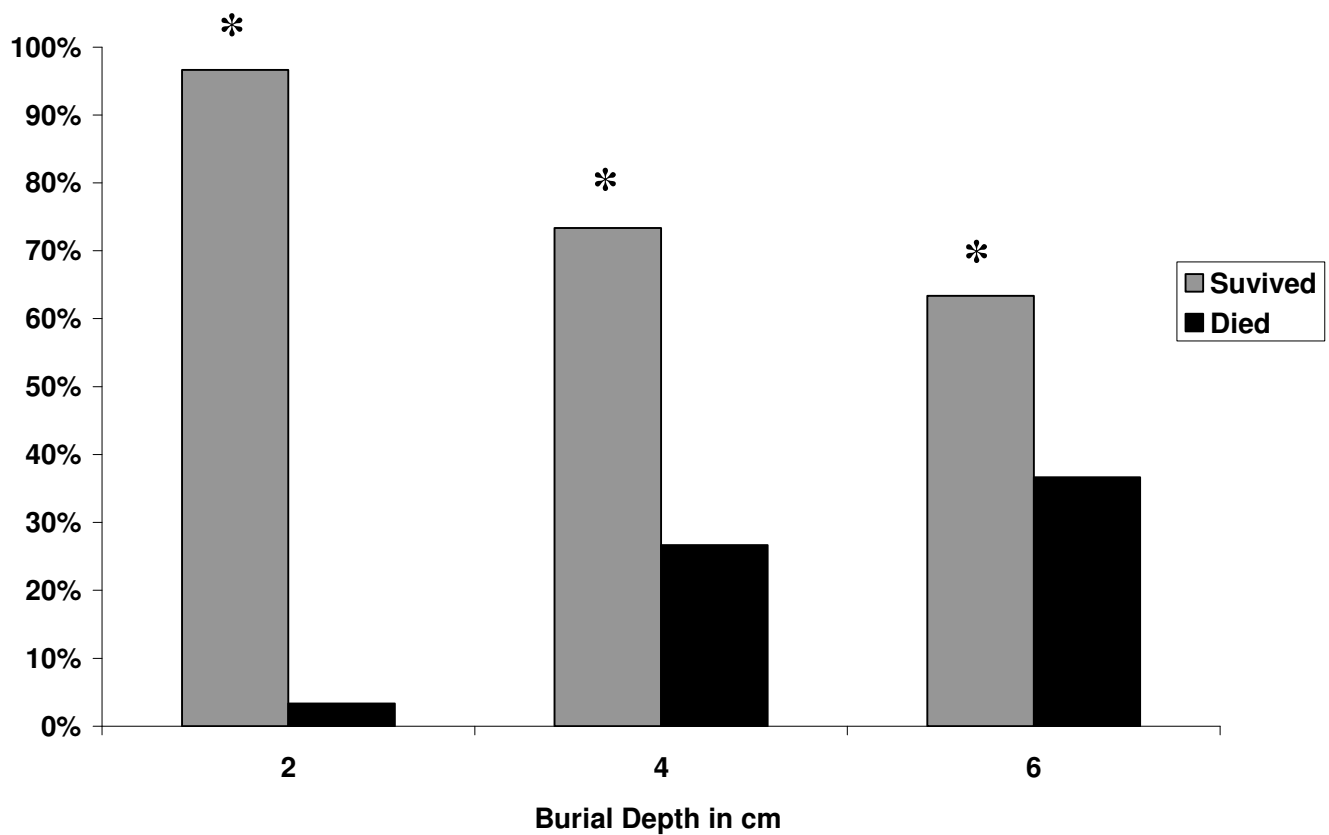


Fig. 5. The proportion of hermit crabs that survived the burial based on burial depth. Logistic regression B = -1.968, S.E. = 0.897, Wald = 4.817, df = 1, p = 0.028, odds ratio = 0.140. An asterisk represents p < 0.05.

Discussion

We found that a significant number of hermit crabs abandoned their shells when buried with sediment, and that the only factor influencing this behavior was the orientation of the shell aperture at the time of burial. Hermit crabs that were buried with shell apertures facing upwards abandoned the shells significantly more often than hermit crabs buried with shell apertures downward. We suggest two reasons for this. First, it was more difficult for hermit crabs to pull the shell upwards through the sediment compared to the aperture down orientation in which crabs could push their shells from underneath towards the surface. This may be because when the shell is in an aperture down position, its external architecture may provide a substrate over which sediments above can flow (McNair *et al.*, 1981; Holomuzki and Biggs, 2006). Secondly, as hermit crabs buried aperture up emerged from shells to pull themselves and their shells to the surface, sediment likely began to fill the shell. The further out of the shell the hermit crab emerged, the more the shell would fill with sand. This would, in all likelihood, cause the hermit crab to leave the sediment filled shell.

Although gastropod shell type has been shown to influence hermit crab fitness (Bertness, 1981), and probability of being oviparous (Bach *et al.*, 1976; Fotheringham, 1980), we did not find it to be a predictor of shell abandonment or survival. Bertness (1981) found that shell species with a higher internal volume and a lower weight were optimal for increasing hermit crab growth and clutch size. Not only does shell species affect hermit crab fitness, but so also does weight variations within a given shell species. Childress (1972) calculated weight ratios for hermit crab shell weight to body weight and discovered optimum ratios for growth and fecundity. Côté *et al.* (1998) found that when

faced with the stress of hypoxia hermit crabs will choose to abandon larger, heavier shells in favor of smaller, lighter ones. Therefore, it is likely that a hermit crab in a shell that is either lighter or heavier than preferred, and faced with the additional stress of a shallow burial event, such as those presented here, would abandon that shell. However, we found no effect of weight ratios on shell abandonment or survival. This may be because vacant shells in a wide range of sizes were available in the aquaria, so it is unlikely hermit crabs inhabited inadequate shells. The highly significant correlation between hermit crab weight and shell aperture size supports this conclusion.

We found shell weight, shell abandonment, aperture orientation, and depth of burial were all significant factors in survival of buried hermit crabs. Shell weight, among other factors, significantly influences hermit crab shell choice (Reese, 1963). Studies have found that a moderately large (and likely also moderately heavy) shell is beneficial for protection from predation (Vance, 1972; Vermeij, 1974), growth rate (Markham, 1968), and fecundity (Childress, 1972). However, an increase in shell weight beyond optimum can cause hermit crab growth rate (Bertness, 1981) and clutch size (Childress, 1972) to decrease. Moreover, our study suggests that crabs with heavier shells are less likely to survive a burial event, independent of orientation or depth. One possible explanation for this is that a heavier shell may be more difficult to maneuver through the sand. Fotheringham (1976) concluded that an increase in shell weight would increase the energy a hermit crab must expend carrying it, and could reach a point at which the weight may impede mobility. In addition we found that shell weight and aperture size correlated directly with hermit crab weight, so crabs having large shells are themselves large.

Another hypothesis is that the larger crabs were more likely to remain tucked inside the shell to wait out the stress. We observed instances in which some large crabs made no effort to escape the burial (J.S., personal observation). Fink (1941) found that larger *Pagurus longicarpus* once startled into retraction, took longer to reemerge from the shell than smaller hermit crabs. However, a more recent study found no correlation between startle response and weight of *Pagurus bernhardus* (Briffa *et al.*, 2008). In the current study, individual hermit crabs were prevented from crawling up the sediment until the determined burial depth was reached, but Hinchey *et al.* (2006) observed some amphipod individuals swimming upward through the sediment as it was being deposited. They concluded that, among species, motility was the most important survival factor, rather than whether the species was naturally infaunal or epibenthic. We suggest that this is true within *P. samuelis* as well, and those individuals that exhibited greater motility were more likely to survive our treatments.

Shell abandonment behavior also played a significant role in whether or not crabs survived burial. In both groups (crabs that escaped and those that remained buried) some individuals abandoned their shells. However, the majority of hermit crabs that abandoned their shells escaped out of the sediment and survived the burial event. This was verified by the high odds ratio (68.3) for shell abandonment indicating that if the hermit crab abandoned the shell instead of remaining inside, it was very likely to switch survival categories as well. Herreid and Full (1986) showed that it was energetically costly for hermit crabs to carry shells while running on a treadmill when compared to hermit crabs without shells. To expend less energy is most likely an

important motivation for hermit crabs in the present study to abandon shells and therefore survive the burial event.

Our data suggest that abandoning the shell may facilitate easier mobility and maneuvering through the sediment to emerge at the surface, however not all crabs did so. When faced with multiple stressors, hermit crabs may actually evaluate each situation and choose to fulfill the most urgent need, whether metabolic or behavioral. Côté *et al* (1998) demonstrated that hermit crabs facing hypoxia will choose shells in which they must expend less energy to carry. Additionally, Billock and Dunbar (2008) found that hermit crabs can prioritize information depending on what they decide is their most immediate need, and that they are more motivated to find a protective shell than feed when both needs are present. Although abandoning the shell may help the crab survive a burial event, it may also increase susceptibility to other threats upon reaching the surface. Without the shell, the soft abdomen of the hermit crab is exposed and vulnerable to predation (Reese, 1969; Vance, 1972; Angel, 2000), desiccation (Reese, 1968) and osmotic stress (Shumway, 1978). The overarching need to have the protection of the gastropod shell may be a reason some hermit crabs in the current study remained in the shell even in hypoxic conditions.

Aperture orientation was also a significant indicator of survival. We demonstrated that crabs buried with shell apertures down were more likely to survive while remaining in their shells. In contrast, crabs buried in the aperture up position were more likely to survive by abandoning their shells. Thus, we suggest that being buried aperture up may increase the potential for surviving a shallow burial event such as those simulated by our experiments.

With respect to burial depth, our results demonstrated that depth was strongly and negatively correlated with survival. Therefore, if a hermit crab is buried during a sedimentation event in the intertidal zone, the deeper the sedimentation the more likely the crab will be unable to escape, making it a candidate for *in situ* fossilization. The converse is also true; that the more shallow the hermit crab is buried, the more likely it will survive the sedimentation event, and will not be fossilized at that time.

However, different burial depths affect various taxa in diverse ways. A study involving polychaetes found that survival of both juvenile and adult *Streblospio benedicti* exponentially declined with deeper burial depth (Hinchey *et al.*, 2006). The same study also investigated burial in the clam, *Macoma balthica*, and juveniles of the oyster, *Crassostrea virginica*. Depths used in treatments were considered “high” based on the greatest sedimentation amount found by the authors in the natural environment of each species. *Macoma balthica*, which is a natural burrower, showed no decrease in survival or growth with an increase in burial depth. *Crassostrea virginica*, which is typically anchored to a substrate, was also able to survive six days of the highest burial depth used in the study. However, the highest mean depth of 0.5 cm of sediment for *C. virginica* was not at all high when compared to the highest depth used for *M. balthica* (24.6 cm). Moreover, one could hypothesize that *C. virginica* would not survive any burial in which the sediment was not removed by the researcher, water, wind, or some other force. Additionally, Hinchey *et al.* (2006) investigated the amphipod, *Leptocheirus plumulosus* and found survival of this species declined exponentially with increasing burial depth, but not nearly as sharply as the polychaete, *Streblospio benedicti*. Despite this decline, the authors still demonstrated that even at high depths (5.9 – 20.2 cm) *L. plumulosus* and *M.*

balthica had greater survival than other species studied, and that one reason for this was the greater motility exhibited by amphipods and clams. Nichols *et al.* (1978) found that with a burial depth of 30 cm, no polychaetes or pelecypods moved upward at all in the sediment column. They suggested that 30 cm was a critical burial depth in which some event, such as compaction, inhibited any movement. In our study, the deepest burial depth of 6 cm was not a critical depth, because some individuals escaped.

We suggest that shell abandonment behavior is a possible reason for the paucity of *in situ* fossilized hermit crabs. Although shell abandonment occurred in either aperture orientation, it is more likely that hermit crabs will leave their shells behind if buried aperture up. This empty, buried shell, with evidence of hermit crab inhabitation, could then be fossilized. If the hermit crab abandons the shell but is unable to reach the surface (as two crabs did in this study) the crab may also become disarticulated and fossilized away from its shell. Therefore, *in situ* fossilization may be less likely for crabs buried with the shell aperture upward.

Finding possible reasons for the lack of *in situ* fossilized hermit crabs has implications for geology, biology, and ecology. Understanding a possible taphonomic bias can improve interpretations of paleoenvironments. Successful escape from burial decreases the probability of being fossilized and biases the resulting fossil assemblage (Nichols *et al.*, 1978).

Ecology and biology are impacted by determining the adaptations of marine invertebrates to rapid sedimentation and physiological responses. A catastrophic burial may selectively kill certain organisms that cannot crawl up through the sediment, thus creating vacant patches (Hall, 1994) in which other organisms can become established.

Several factors determine which organisms survive a burial event including mobility (Hinchey *et al.*, 2006; current study), being a naturally burrowing species (Hinchey *et al.*, 2006), and ability to provide for the body's energy needs by undergoing anaerobic respiration (Henry *et al.*, 1994). While further investigations into the physiological responses of buried hermit crabs are needed, the current study has shown that some behaviors demonstrated by hermit crabs during shallow burial events may increase their chances of survival.

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CHAPTER THREE

LACTATE ACCUMULATION IN THE INTERTIDAL HERMIT CRAB, *PAGURUS* *SAMUELIS*, IN RESPONSE TO BURIAL-INDUCED HYPOXIA

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Abstract

We subjected the intertidal hermit crab, *Pagurus samuelis* (Stimpson 1857), to various treatments to determine the physiological responses of this species to the environmental stress of burial. Hermit crabs were buried with 6 cm of sediment and excavated at 2 h intervals up to a maximum of 12 h. Duration of burial and state (alive or dead) of the crab were analyzed for effects on the accumulation of lactate in the hemolymph. Hermit crab weight, shell weight, weight ratio, lactate, and burial duration were analyzed to determine their relation to survival. As expected, lactate levels rose with duration of burial. A significant interaction, however, was found between burial duration, crab state and lactate concentration. During short burial durations, there was a trend for lactate concentration to be low for surviving crabs, but higher for dead crabs. Conversely, during long burial durations, lactate concentration was very high in surviving crabs. Duration had a negative impact on survival, indicating that the longer hermit crabs are buried and constrained to anaerobiosis, the less likely they will survive shallow burial events. Since some crabs were able to survive with much higher lactate levels than others over the same treatment period we suggested that they benefitted from higher glycogen reserve capacity for anaerobiosis, and that prior experience with burial events may lead to adaptive behaviors during burial.

Key Words and Phrases – Rapid sedimentation, Flooding, Anaerobic conditions, Intertidal zone, *L*-lactate, Survival, Hypoxia, Anoxia.

Introduction

Hermit crabs can be found inhabiting intertidal zones panglobally. Because of their physiological sensitivity to freshwater they can be used as ecological indicators of hyposaline conditions (Hall et al., 1990; Dunbar et al., 2003). Flooding is a common threat to animals living near the sediment-water interface and can include with it a significant amount of sediment (McCall, 1978; Maurer et al., 1981; Niedoroda et al., 1989). Organisms can also become buried by sediment from river deposits (McKnight, 1969), tides (Grant, 1983), bioturbation (Thayer, 1983), terrestrial runoff (Edgar and Barrett, 2000) dredging (Messieh et al., 1991), fishing (Jackson and James, 1979; Hall et al., 1990), wind erosion, or trampling (Chandrasekara and Frid, 1996).

Burial by sediment may cause the immediate environment to become hypoxic (Nichols *et al.*, 1978; Shives and Dunbar, 2010), but some decapods exhibit various physiological adaptations to short-term hypoxia, including increased scaphognathite (Anderson *et al.*, 1991; Paterson and Thorne, 1995) or pleopod (Torres et al., 1977; Felder, 1979) beat frequency, and increased cardiac output (Thompson and Pritchard, 1969). If hypoxia persists, concentrations of urate (Dyken, 1991) and the respiratory pigment, hemocyanin (deFur *et al.*, 1990) will also increase. Urate allosterically binds to hemocyanin causing a greater affinity for oxygen and increased delivery to the tissues (Menze *et al.*, 2005). If ambient oxygen concentration drops below a critical level, decapods compensate by decreasing oxygen consumption (Grieshaber *et al.*, 1994) and energy expenditure (Hand, 1998; Hochachka and Lutz, 2001), including decreasing pleopod beat frequency (Torres et al., 1977; Felder, 1979), followed by a shift to anaerobic respiration (AR) to meet energetic demands (Grieshaber *et al.*, 1994).

Anaerobiosis has been shown to occur in crustaceans in conditions such as exercise (McDonald et al., 1979; Smatresk et al., 1979; Full and Herreid Jr, 1984; Henry et al., 1994; Briffa and Elwood, 2001), aerial exposure (Ridgway et al., 2006), and hypoxia (Butler *et al.*, 1978; Hervant *et al.*, 1995; Holman and Hand, 2009). The crustacean physiological response to functional and environmental hypoxia is similar to that in vertebrates. When the intracellular phosphagen buffer (arginine phosphate in crustaceans) is exhausted, anaerobic glycogenolysis is employed, and lactic acid fermentation occurs (England and Baldwin, 1983; Booth and McMahon, 1985; Morris and Adamczewska, 2002; Johnson et al., 2004; Gornik et al., 2008). Conversion of pyruvate to lactic acid enables the restoration of NAD^+ , which is essential for continued glycolysis under hypoxic conditions. Anaerobic glycolysis appears to be the only source of ATP in the absence of oxygen in Crustacea (Albert and Ellington, 1985; Grieshaber *et al.*, 1994) evidenced by a significant increase in hemolymph lactate levels (Bridges and Brand, 1980; Sneddon et al., 1999). Fumarate, alanine, aspartate, glutamate, succinate, and malate also increased during hypoxia, however, amounts detected indicate that alternative metabolic pathways are of very little significance in decapods (Pritchard and Eddy, 1979; Zebe, 1982; Hill et al., 1991). These results point to lactate as the indicator of the rate of anaerobic respiration occurring within crustaceans.

Investigations of anaerobic respiration in hermit crabs have been carried out by Briffa and Elwood (2001; 2005). They studied AR in aggressive contacts in specimens of *Pagurus bernhardus* by analyzing the concentration of lactate and glucose in the hemolymph of individuals immediately following agonistic interactions over the

ownership of gastropod shells. Still, no studies investigating hypoxia-induced AR during burial in Paguroidea have previously been reported.

The aim of the current investigation was to determine the extent to which the hermit crab, *Pagurus samuelis*, undergoes anaerobic respiration while experiencing burial with sediment. We hypothesized that lactate concentrations in the hemolymph would increase in relation to burial duration in crabs found alive at excavation.

Secondly, we expected to find a critical level of lactate, after which hermit crabs could no longer survive.

Materials and Methods

Collection and Care of Hermit Crabs

Pagurus samuelis of body weight ranging from 0.61 - 1.94 g were collected from tide pools at Shaw's Cove (33 32' 43" N, 117 47' 57" W) and Little Corona del Mar (33 35' 21" N, 117 52' 05" W) in Southern California from March to May, 2007. Gravid females were excluded from collection. Hermit crabs were kept in aquaria in the laboratory where salinity was maintained at 36 ± 3 ppK and temperature at $24 \pm 2^\circ\text{C}$ with ambient light. Hermit crabs were fed frozen commercial salad shrimp once a week, and water was changed every three weeks.

Burial Methods for Lactate Experiments

We randomly selected hermit crabs for treatments from the aquarium, shook them gently, and blotted them with paper towel to remove excess water. The total wet weight of each crab inside the gastropod shell was measured and recorded to \pm

0.001 g. Hermit crabs were then placed into plastic containers previously filled with 3 cm of sand, one crab per container. In each treatment, a plastic mesh was placed above the crab to allow sand grains to pass through to bury the crab, but ensured the crab could not crawl upward through the sand. Crabs, with their shells in the aperture down position, were slowly buried with water saturated sand, until 6 cm of sediment covered them, and there was 1 cm of standing water.

Hermit crabs were buried at the deepest of the three depths used in a previous investigation (6 cm), because this depth resulted in the greatest proportion of mortality (Shives and Dunbar, 2010). In treatments for time, we excavated crabs at 2 h increments until the maximum burial time of 12 h. Whether the crab was alive or dead was recorded and hemolymph was collected.

Lactate Assays of Hermit Crab Hemolymph

After each treatment, hermit crabs were removed from their shells using a bench vise, and a 50 μ l sample of hemolymph was taken from each crab. This was done by piercing the arthroal membrane at the base of the third pereopod with a 21 gauge hypodermic needle attached to a 1 ml disposable syringe. We recognized that the procedure took approximately 5 - 15 min for each crab, but complete recovery from high lactate levels normally takes up to 24 h in crustaceans (Hervant *et al.*, 1999). Moreover, comparative studies of lactate levels in other crustaceans have shown that lactate declined during recovery from hypoxia more rapidly in burrowing crustaceans compared to non-burrowing species (Bridges and Brand, 1980). Since *Pagurus samuelis* is not a burrowing species, its lactate recovery period would not be expected to be rapid,

although this was not tested. During this 5 - 15 minute period it was possible for some of the lactate to be metabolized, or that in living hermit crabs more lactate might be produced due to stress. A control group was used to standardize for these stresses. The control group was not buried, but subjected to the stresses of cracking the shell open and collecting the hemolymph sample. All hemolymph samples were immediately deproteinized with 100 μ l of cold 0.3 M perchloric acid and centrifuged. A 50 μ l sample of the supernatant was stored at -20°C until the assay for lactate was conducted within 24 h.

Samples were thawed and neutralized by the drop-wise addition of potassium bicarbonate. Methyl orange was used as the indicator to identify samples with a pH of less than 4.4. The assay followed the procedure of Gutmann and Wahlefeld (1974), and Engel and Jones (1978). Samples (25 μ l) were added to Eppendorf tubes containing 500 μ l of hydrazine-glycine buffer, 25 μ l of NAD⁺, and 1.25 μ l of lactate dehydrogenase (LDH), and incubated for 1 h at 37 °C. EDTA was added to the hydrazine-glycine buffer to prevent interference by Cu²⁺ ions associated with the hemocyanin, as recommended by Engel and Jones (1978). Diluted samples (1/10), and a series of standards were also prepared for analysis in the same way. After incubation, 300 μ l from each tube were pipetted into a 96 well microtiter plate (Nunc, Fisher Scientific, Pittsburgh, PA, USA). Standards were pipetted into the top row of wells, the neat samples in the second row, and the diluted samples in the third row. The absorbance of the standards and samples were measured at 340 nm in a plate reading spectrophotometer (μ Quant, Bio-Tek Instruments, Inc., Winooski, VT, USA) and converted to lactate concentrations using a calibration curve constructed with the standards of known lactic acid concentrations.

Statistical Analyses

Each data set was statistically analyzed together and separately using the program Statistical Package for the Social Sciences (SPSS) 14.0. After preliminary data screening, lactate values were \log_{10} transformed to conform to the assumptions of parametric statistics, and 3 outliers were removed. Due to small sample sizes, duration categories were merged, 2 with 4 h, 6 with 8 h, and 10 with 12 h, leaving 3 treatment duration categories and the control. A two-way ANOVA was done to determine if the factors state (alive or dead) and time (0 h, 2 – 4 h, 6 – 8 h, 10 – 12 h) had an effect on the dependant variable, log lactate concentration. Additionally, a one-way ANOVA was done to determine if the factor time (0 h, 2 – 4 h, 6 – 8 h, 10 – 12 h) had an effect on the dependant variable, log lactate concentration, in living crabs only. To determine which variables (shell weight, crab weight, weight ratio, lactate, and burial duration) had a significant effect on state of the crab, a forward, stepwise logistic regression was conducted. All significance levels were set at $\alpha = 0.05$.

Results

Oxygen Saturation

In a previous study, Shives and Dunbar (2010) confirmed that percent oxygen saturation of the sediment measured with a Clark-type oxygen electrode, dropped to a mean of $26.6 \pm 4.4\%$ within 15 minutes of burial. Percent saturation was less than 10% after 7.5 h of burial and continued to decline to a mean of $1.8 \pm 0.75\%$ after 24 h.

Factors Affecting Lactate Concentration

Before logarithmic transformation of data, we obtained lactate values of 1.4 – 10.5 mM for control individuals and 3.2 – 58.4 mM for treatment individuals. The extreme values for living crabs were 1.4 and 58.4 mM and for dead crabs were 6.6 and 48.2 mM. The ranges of values over time and by state are shown in Table 2.

Table 2. The minimum and maximum values of lactate (in mM) found in each time category after burial with sediment (N = 71).

	<u>0 hrs</u>		<u>2 – 4 hrs</u>		<u>6 – 8 hrs</u>		<u>10 – 12 hrs</u>	
Alive	1.4	10.4	3.2	27.9	3.6	34.4	26.2	58.4
Dead	-		6.6	44.8	11.7	37.9	5.7	48.2

We found that duration of burial had a significant effect on hemolymph lactate concentration ($F_{(3,64)} = 15.032$, $p < 0.001$, partial $\eta^2 = 0.413$). Interaction between the factors state and duration was significant ($F_{(2,64)} = 5.619$, $p < 0.006$, partial $\eta^2 = 0.149$) (Figure 6). However, the calculated effect size indicated more of the lactate variance was accounted for by duration alone.

Among hermit crabs that were found alive at the end of the treatments ANOVA results showed a significant increase in lactate with increasing duration

($F_{(3,33)} = 14.993$, $p < 0.001$, partial $\eta^2 = 0.577$). Tukey HSD post hoc tests were run to determine which duration categories were significantly different from the others (Figure 6). Results revealed that the control (0 h; mean = 0.563 ± 0.087) was significantly different from all treatment groups for mean lactate concentration of the hemolymph in surviving crabs (control vs. 2-4 h alive, mean = 0.869 ± 0.066 , $p = 0.045$; control vs. 6-8 h alive, mean = 1.063 ± 0.108 , $p = 0.002$; control vs. 10-12 h alive, mean = 1.584 ± 0.075 , $p < 0.001$). Comparing the treatment groups of living crabs, the lactate levels in crabs buried for the interval of 2 – 4 h (mean = 0.869 ± 0.066) were not significantly different from those buried for 6 – 8 h (mean = 1.063 ± 0.108), but were significantly lower than from those buried for 10 – 12 h (mean = 1.584 ± 0.075 , $p < 0.001$). The lactate levels for the category 6 – 8 h (mean = 1.063 ± 0.108) were also significantly lower than for 10 – 12 h (mean = 1.584 ± 0.075 , $p = 0.014$).

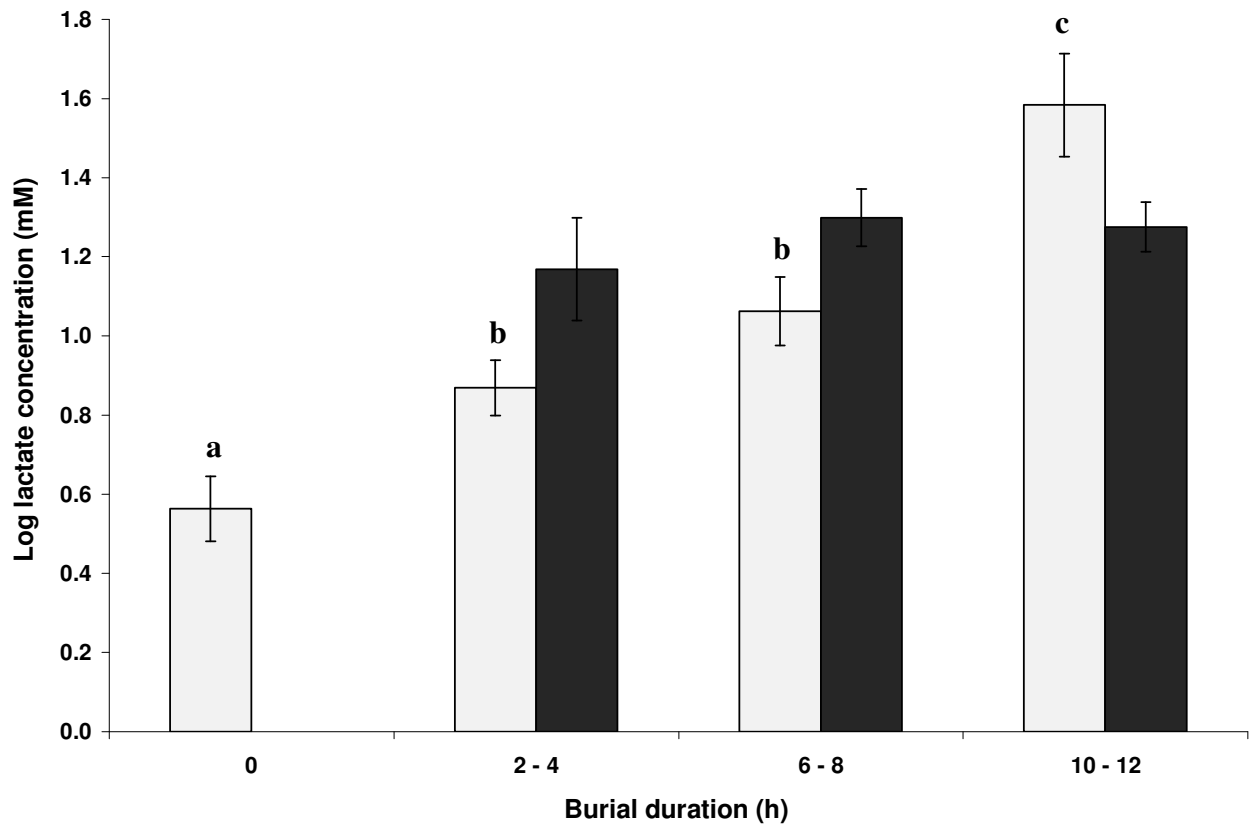


Fig. 6. The effects of state and burial duration on hermit crab lactate accumulation. Light bars represent hermit crabs found alive after excavation while dark bars represent crabs found dead. Significant differences among surviving groups are indicated with different letters ($p < 0.05$ one-way ANOVA and Tukey's HSD). Error bars are ± 1 s.e.m. $N = 71$.

Factors Affecting Survival

A forward, step-wise logistic regression was conducted to determine which independent variables (shell weight, crab weight, weight ratio, lactate, and burial duration) were predictors of survival. Regression results indicated the overall model of one predictor (burial duration) was statistically reliable in distinguishing between the hermit crab surviving the treatment or not (2 Log Likelihood = 69.287, $\chi^2_{(1)} = 29.013$, $p = 0.008$). The model correctly classified 76.1% of the cases.

The shorter the burial duration, the more likely *P. samuelis* was to survive the treatment (Figure 7). Among the crabs that were buried for 2 – 4 h ($n = 18$) only 4 were found dead. For those crabs buried for 6 – 8 h ($n = 22$) 13 died. For the crabs that were buried for 10 – 12 h ($n = 21$) 17 were found dead.

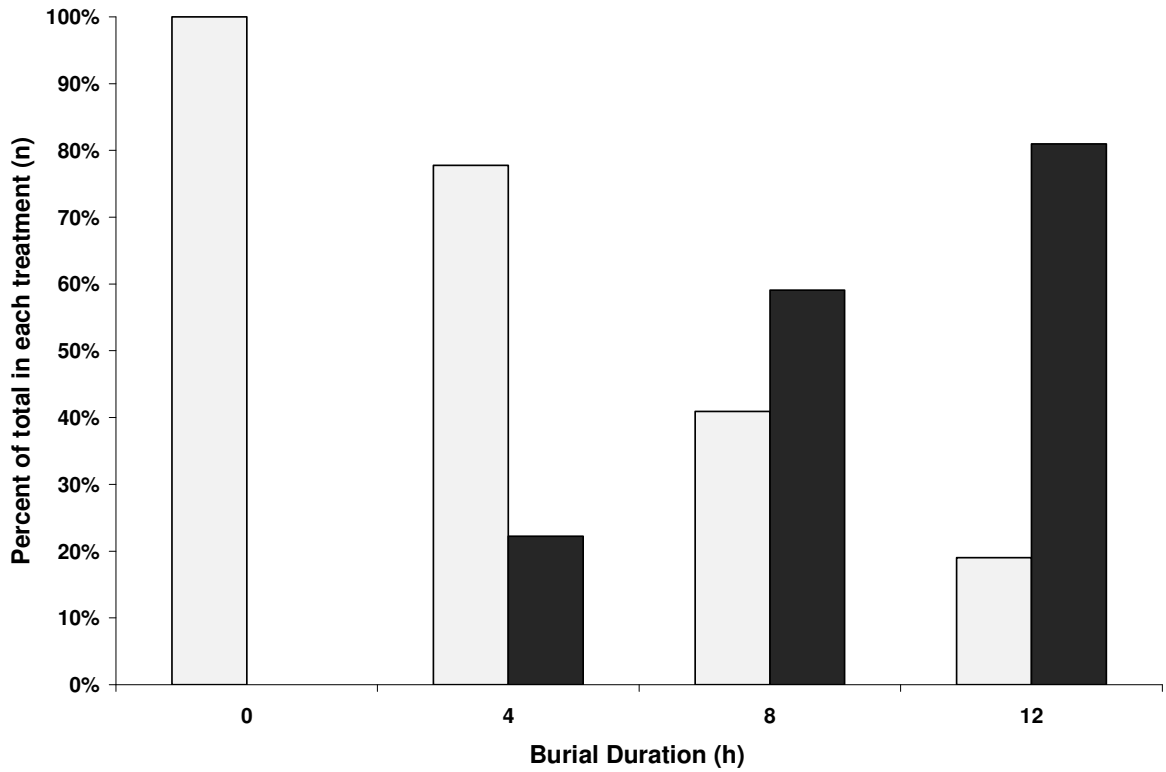


Fig. 7. The percent of hermit crabs found alive (Light bars) or dead (Dark bars) at each time interval. Burial duration was a significant factor for survival (Logistic regression, 2 Log Likelihood = 69.287, $\chi^2_{(1)} = 29.013$, $p = 0.008$). $n_0 = 10$, $n_4 = 18$, $n_8 = 22$, $n_{12} = 21$, $N = 71$.

Discussion

As the duration of burial increased, lactate accumulation in hemolymph from surviving hermit crabs also increased. The longer hermit crabs were buried the more dissolved oxygen was consumed, causing hypoxia. When available oxygen was insufficient to support aerobic respiration, crabs relied on anaerobic fermentation, which was indicated by increasing lactate levels. Several studies have also demonstrated an elevation in lactate with increasing duration of hypoxia in crustaceans (Albert and Ellington, 1985; Lallier et al., 1987; deFur et al., 1990; Hill et al., 1991; Spicer et al., 2002; Gornik et al., 2008). This report, however, demonstrates for the first time that increasing lactate levels in a hermit crab species are directly attributable to hypoxia. Moreover, lactate levels we found during hypoxia are greater than those found by Briffa and Elwood (2001) investigating hermit crab agonistic interactions leading to functional hypoxia. Although lactate accumulates in the hemolymph of decapod crustaceans during both functional and environmental hypoxia, the extent of accumulation is much greater during long-term environmental hypoxia when lactate concentrations may exceed 40 mM in the crayfish, *Orconectes limosus* (Gade 1984) and the stone crab, *Menippe mercenaria* (Gade, 1984; Albert and Ellington, 1985). Our results suggest that lactate levels may rise higher in *P. samuelis* than in either of these species, although in the former reports the organisms were likely less stressed.

The statistical variables designating crab state and duration of burial affected lactate levels in a biphasic manner. After short burial time intervals, there was a trend for lactate to be lower (8.8 ± 2.7 mM) in individuals that survived when

compared with those that died (19.7 ± 5.0 mM). However, there was a trend for hemolymph lactate concentration to be higher (40.1 ± 5.0 mM) in surviving crabs than in dead crabs (22.5 ± 2.4 mM) after long burial time intervals. We found 2 hermit crabs that were dead after only 2 hours of burial, with final lactate levels of approximately 7 mM. This was no different than the control mean of 4.4 ± 3.1 mM.

Based on the observed ranges in hemolymph lactate levels in surviving and dead crabs we concluded that death most likely was not due to lactate toxicity. Instead, we suggest a different cause of death. Hermit crabs have two internal sources of energy, circulating glucose and stored glycogen (Briffa and Elwood, 2004). Once circulating glucose has been utilized, glycogen stored in muscles and hepatopancreas is likely the most rapidly accessible during severe acute demands for energy. Zou *et al.* (1996) found that during severe hypoxia, the Chinese freshwater crab, *Eriocheir sinensis*, acclimated by hemolymph glucose rising quickly, with lactate rising a few hours afterward. They concluded that glucose was mobilized from glycogen in preparation for the switch to anaerobiosis. Inadequate glycogen stores would likely lead to a delayed, partial, or ineffective shift to anaerobic metabolism. Other studies have confirmed that glycogen stores decrease with increasing lactate concentration in the hemolymph (Taylor and Spicer, 1987; Hill et al., 1991; Sneddon et al., 1999), indicating glycogenolysis, rather than gluconeogenesis, as the main source of glucose for glycolysis. Moreover, diet was found to directly affect crab hemolymph glucose levels as well as hepatopancreas and muscle glycogen concentrations (Vinagre and Da Silva, 1992). Therefore, some individual *P. samuelis* crabs may have exhibited ineffective anaerobic metabolism due to insufficient glycogen stores,

and subsequently died quickly with potentially lower hemolymph lactate levels. Conversely, crabs with adequate glycogen stores were able to sustain their metabolic processes and survive 10-12 h of hypoxia while ATP was supplied largely by glycolysis and lactic acid fermentation.

Gastropod shells aid in survival and are known to provide protection from desiccation (Reese, 1968) and osmotic stress (Shumway, 1978). Childress (1972) calculated weight ratios for hermit crab shell weight to body weight, and found optimum ratios for growth and reproduction. Therefore, it is likely that a hermit crab in a shell that is lighter (smaller) than preferred and faced with the stress of a shallow burial event, such as those presented here, would have less capacity for water storage inside the shell (Bertness, 1981). Without water and its dissolved oxygen stored in the shell, the crabs may be more susceptible to death from anoxia. However, we found no effect of weight ratios on survival. This may be because an abundance of vacant shells were available in the aquaria, so it is unlikely hermit crabs inhabited smaller than optimal shells.

We hypothesized that a critical concentration of lactate would be found, above which hermit crabs would no longer survive. This was not the case. The individual with the highest lactate concentration found in the current study (58.4 mM) was found alive after 12 h of burial. This suggests that *P. samuelis* can indeed undergo a significant amount of AR to survive even severe, acute hypoxia, and that it is unlikely that a moderately high concentration of lactate caused increased mortality in hermit crabs found dead in our treatments. In contrast, studies have shown that a rise in hemolymph lactate causes crustacean hemocyanin to increase its oxygen

affinity, and therefore binds the less available oxygen better than under normoxic conditions (Truchot, 1980; Lallier and Truchot, 1989; Bridges, 2001).

In addition to anaerobic physiology, hermit crab behavior may be an important additional factor in survival of acute burial events. Although not tested, we surmise that prior experience with burial may affect a hermit crab's coping effectiveness with the burial associated stresses. Crabs which are burdened by ineffective or dysfunctional coping mechanisms, and associated higher metabolic energy demands, would be more likely to succumb.

Duration strongly influenced survival of a shallow burial event, with death positively correlated with burial duration. Other studies have likewise found that the chances of an organisms' survival of a shallow burial event decrease with increasing duration (Chandrasekara and Frid, 1996; Schiel et al., 2006). As demonstrated in previous studies, mobility can decrease burial duration if a taxon is able to crawl up through the sediment (Hinchey *et al.*, 2006; Shives and Dunbar, 2010). Indeed, Shives and Dunbar (2010) demonstrated that hermit crabs may survive shallow burial events either by carrying the shell to the surface, or by abandoning the shell and reaching the surface without it. Hermit crabs are very mobile, and many of them would likely have maneuvered through the sediment had we not restricted them in the current study.

Hermit crabs that face the environmental stress of burial may have the ability to cope with decreasing oxygen saturation by using AR to meet acute energy demands, and prolong the functioning of metabolic processes. In order for hermit crabs to undergo glycolysis and lactic acid fermentation, there must be a supply of

glucose. Previous work indicates that, in decapods, glycogen serves as the glucose source once hemolymph glucose has been depleted (Hill et al., 1991; Sneddon et al., 1999; Briffa and Elwood, 2004). Although aerobic respiration yields significantly more ATP molecules, anerobiosis may be utilized in *P. samuelis* to survive acute and relatively short-term environmental hypoxia. This adaptation would prove beneficial to hermit crabs that may experience hypoxia in the dynamic environment of the rocky intertidal zone. Further studies are needed to investigate if glycogen stores are the limiting factor for survival of acute, extreme hypoxia in hermit crabs.

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CHAPTER FOUR

CONCLUSIONS ON HERMIT CRAB BEHAVIOR AND PHYSIOLOGY

Conclusions

This thesis has described the influence of behavior and physiology on survival of the hermit crab, *Pagurus samuelis*, after burial with sediment. I found that hermit crabs will abandon their shells when faced with the stress of burial. Additionally, I showed that the direction of shell aperture orientation after being covered with sand makes a difference in whether or not hermit crabs will abandon the shell. The behavior of shell abandonment was suggested as a possible reason for the paucity of *in situ* fossilized hermit crabs (in which crab body and gastropod shell are found articulated). Results presented in this thesis also suggested that hermit crabs, while buried, may be able to undergo anaerobic respiration to supply a portion of their energy needs during acute hypoxia.

Because hermit crabs likely encounter burial with sediment in their natural habitat, I aimed to record behaviors, and discover factors involved in surviving burial under realistic environmental conditions simulated in the laboratory. I hypothesized that there would be a significant difference in the number of hermit crabs abandoning the shell when crabs buried with sediment were compared to a control group that were treated under the same conditions with the exception of actually being buried. The results supported this hypothesis, which indicated that burial with sediment either directly or

indirectly caused *P. samuelis* to abandon its shell (Shives and Dunbar, 2010). Shell abandonment has also been documented as occurring with extreme thermal stress (Bertness, 1982), direct sunlight (Taylor, 1981), during dredging (Young, 1979), and when the hermit crab is being chased (Harvey in: Greenaway 2003). It has been suggested that during environmental stresses, such as aerial exposure and increased temperature, abandoning the shell might allow the crab to seek small crevices in which to hide (Bertness, 1982), or that temperature may be higher within the shell than outside of it (Taylor, 1981). Hermit crabs in our burial experiments did not face an increase in temperature, so it is unlikely that temperature was a factor in the responses of hermit crabs exiting their shells in the current study. However, hermit crabs in our study did experience a reduction in available oxygen, and I suggest that this environmental stress may have led to the observed abandonment of the shells. As in the case of hermit crabs that abandoned the shells while being dredged (Young, 1979), hermit crabs in my experiments were physically distressed. Results for 'time to escape' in the current study suggest that as sediment accumulated over the hermit crabs, they vacated the shells quickly, with 46.2 % of the crabs that abandoned the shell before escaping the sediment doing so within 0.25 h of burial.

Furthermore, I suggest that another factor influencing crabs to abandon the shell is the amount of metabolic energy required to move the shell through the sediment overburden. Herreid and Full (1986) found that a hermit crab required twice the energy to carry a shell in air as opposed to walking in air without a shell. This was suggested as the reason that hermit crabs would leave the shell behind when being pursued (Harvey (pers. comm.) in: Greenaway 2003) and may serve as an explanation for abandoning the shell

after burial, as well. Under conditions of burial, moving the shell through the sediment would require a higher energy demand when compared with moving the body of the crab without the mass of the shell.

Although shell abandonment occurred among hermit crabs buried in either shell aperture orientation, crabs buried with apertures facing upward were more likely to abandon their shells and subsequently survive the burial by crawling out of the sediment. I suggested that crabs buried aperture up may be more likely to abandon the shell for two reasons. Firstly, hermit crabs may have an extremely difficult time pulling shells that are aperture up through the sediment. The second reason is that when the hermit crab emerges from the shell, gravity will likely cause sediment to fill the shell. As the shell fills with sediment, it not only becomes heavier for the hermit crab to pull upward, but also may eventually leave no room for the hermit crab body, forcing the crab out of the shell.

In either aperture orientation, survival was high over all burial treatments (76.6%), suggesting that hermit crabs may be able to survive shallow, short-term burial events in the natural environment. I then demonstrated that shell weight, shell abandonment, aperture orientation, and burial depth had either significantly positive or negative effects on survival. Greater shell weight had a negative effect on survival. I suggested that a heavier shell was more difficult, and therefore energetically more costly, to maneuver through the sediment. Côté *et al.* (1998) came to the same conclusion when examining why hermit crabs chose lighter shells while in hypoxic conditions. Hermit crabs in my experiments also experienced hypoxia. Studies have found hypoxia causes fatigue (Smith and Taylor, 1993; Thorpe *et al.*, 1995; Briffa and Elwood, 2000), which

makes a heavier shell even more difficult to carry out of the sediment than when in normoxic conditions.

Abandoning the shell increased the likelihood that a hermit crab crawled out of the sediment, and therefore survived, from 61% to 93%. However, once at the surface without a shell, hermit crabs may become more susceptible to predation (Kuhlmann, 1992; Angel, 2000), desiccation (Taylor, 1981; Brodie, 1999), and osmotic stress (Shumway, 1978; Pechenik *et al.*, 2001). Many species of fish and crabs prey upon *P. samuelis*. Without the protection of the shell, the hermit crabs' soft, uncalcified abdomens are exposed and at greater risk of predation.

Intertidal organisms need to be capable of adapting to fluctuating tides and salinities, and a shell aids in both. The shell provides a reservoir of seawater during periods of low tide. Hermit crabs that have abandoned their shells would therefore be susceptible to desiccation if they crawled out of the sediment in a location where water was not present. A hermit crab's shell also acts as a protection against salinity changes. When individuals of the hermit crab *P. bernhardus* were placed in hypotonic seawater, the hemolymph osmolarity declined less rapidly when they were inside shells than when they were without shells (Shumway, 1978).

If hermit crabs experience a burial event in the natural environment, they may be rolled in any direction. I discovered, in the laboratory, that the primary method of survival in hermit crabs buried aperture down was to remain in the shell and push the shell upward to the surface. In contrast, shell abandonment is the best survival strategy when hermit crabs are buried aperture up. This "up-side down" posture promotes shell abandonment which leads to greater survival of the burial event. In contrast, the

significant interaction between shell abandonment and aperture orientation implies that if the hermit crabs come to rest “right-side up” after being tumbled with sediment deposition, those crabs are more likely to unbury themselves while remaining in the shells.

Additionally, the deeper specimens of *P. samuelis* were buried in either aperture position, the less likely they were to survive. It is feasible for hermit crabs living in the intertidal zone to survive a shallow burial event. However, as sedimentation increases, the weight of overburden increases and makes crawling out of the sediment less likely, leading to increased mortality with increasing burial depth. This result agreed with previous studies done on other marine invertebrates buried with increasing depths (Nichols *et al.*, 1978; Hinchey *et al.*, 2006).

Taken together, these results indicate that once buried, a significant number of hermit crabs do abandon their shells and therefore, if fossilization were to occur, it is unlikely that both hermit crab and shell would be found articulated. Since there are few *in situ* fossil hermit crabs recorded, this indicates that burial depths are generally shallow enough to allow for hermit crab movement, whether abandonment or escape.

Although I demonstrated that hermit crabs are able to escape some conditions of sedimentary burial depending on shell orientation, I was also interested in discovering if hermit crabs were accumulating lactate in hypoxic conditions during burial. I tested the proposition that during burial-induced hypoxia, metabolic pathways in *P. samuelis* change to meet a proportion of the energy requirements in the absence of oxygen. Studies have shown that the end result of anaerobic respiration in crustaceans is the accumulation of lactate in the hemolymph (Morris and Greenaway, 1989; Spicer *et al.*, 2002).

We found that hermit crab survival was significantly lower in the second set of experiments (44.3%) than in the first (76.6%) ($\chi^2 = 17.778$, $df = 1$, $p < 0.001$), presumably because the crabs in the second treatments were forced to stay buried for up to 12 h. This demonstrated that the hermit crabs ability to physically unbury themselves is important for survival. Hinchey *et al.* (2006) made similar conclusions, after burying marine invertebrates with sediment.

Assays for lactate confirmed that hermit crabs were undergoing anaerobic respiration, and that in living crabs, lactate increased with increasing burial duration. Other studies have likewise found lactate to increase with increasing duration of hypoxia in crustaceans (Lallier *et al.*, 1987; deFur *et al.*, 1990; Hill *et al.*, 1991; Spicer *et al.*, 2002; Gornik *et al.*, 2008). When I tested the factors involved in lactate concentration, there was a significant interaction between state (living or dead) that the crab was found in and duration of burial. For surviving hermit crabs in the shorter durations lactate tended to be low, but when the crabs were found dead lactate levels were high, at all durations. During the short durations, if hermit crabs struggled to escape and expended more energy, used up the available oxygen faster, and produced more lactate, these crabs were likely found dead at the short durations. Conversely, if hermit crabs that struggled less, or not at all, had a slower rate of oxygen consumption, could wait longer to switch to anaerobiosis, and accumulate less lactate in the hemolymph, these crabs were likely found alive at the short durations.

In contrast, those hermit crabs found alive at the longest duration had the greatest lactate concentrations. Struggling may still play a role for the longer durations, but it is likely that energy reserves are the more important factor. Hermit crabs have two internal

sources of energy, circulating glucose and stored glycogen (Briffa and Elwood, 2004). If hermit crabs had high glycogen stores in the hepatopancreas and muscle tissue, it is possible that glycogenolysis may provide glucose to power anaerobic fermentation, even when circulating glucose has been depleted. The more glucose is fermented, the more ATP and lactate are synthesized. Therefore, hermit crabs with higher glycogen stores before the burial event may have the potential to produce greater hemolymph lactate concentrations, and remain alive. Although glycogen concentrations were not measured, our data that individual crabs can live longer, with elevated lactate when compared to dead crabs, with lower lactate, at any duration, lend themselves well to this explanation.

Neither the weight of the crab, shell, nor the ratio of shell weight to crab weight had any effect on survival of *P. samuelis* in hypoxic conditions during burial. However, burial duration was a significant predictor of survival. The longer hermit crabs remained buried, the less likely they were to survive.

Hermit crabs are faced with many acute environmental changes that affect their behavior and physiology. These include salinity changes, desiccation, temperature, and available oxygen. Because of the close proximity to land, intertidal organisms are prone to flooding and burial. Results of this thesis have shown that *P. samuelis* is able to survive shallow burial events by two key mechanisms. The first, and more important mechanism for surviving a burial event, is the ability to crawl through the sediment to the surface. The second mechanism is by using anaerobic respiration to compensate for reduced available oxygen caused by burial. The second survival strategy may merely prolong the lives of the hermit crabs until some other force (such as wind or water) unburies them.

Further Research

Although this thesis intended to answer questions about the behavior and physiology of *P. samuelis*, it also raised many questions. In chapter 2, a critical burial depth in which all crabs died was not found. Since burial events may result in accumulation of greater than 6 cm of sediment, it would be of interest to investigate the critical burial depth for *P. samuelis*. More studies could be done with varying factors, such as salinity, temperature, and sediment diameter to determine the effects on shell abandonment and survival.

In chapter 3 a toxic level of lactate in the hemolymph of *P. samuelis* was not discovered. Further research is needed to find the lactate concentration threshold. Additionally, more research should be conducted to find out if elevated glycogen stores increase the length of time hermit crabs can anaerobically ferment glucose, and prolong survival in hypoxic conditions.

Another study might employ a sound or vibration meter to detect whether the hermit crabs are struggling, or not, while buried. It could then be determined what role struggle plays in either lactate accumulation, survival, or both. In addition, the behavior of hermit crabs that have prior exposure to burial could be compared with crabs that have never been buried.

It would be interesting to know the destination of hemolymph lactate. Does *P. samuelis* excrete or metabolize lactate? It has been demonstrated that some crustacean species are able to excrete lactate into the surrounding medium (Hervant *et al.*, 1995), but that others are unable (Spicer *et al.*, 2002).

In this thesis I examined the behavior and physiology of a Southern California population of *P. samuelis*, but it has been shown that *P. hirsutiunculus* and *P. granosimanus* abandon their shells more frequently than *P. samuelis* when facing exposure (Taylor, 1981). Other studies could test whether similar results might be obtained in other populations of *P. samuelis*, and other species of intertidal hermit crabs.

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