Life History of the Maritime Platygastrid

Echthrodesis lamorali Masner 1968

(Hymenoptera: Platygastridae: Scelioninae)

THESIS

Submitted in fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

At Rhodes University

By

Candice Ann Owen

December 2015

_____

Abstract

Echthrodesis lamorali Masner 1968 (Hymenoptera: Platygastridae, Scelioninae) is an intertidal parasitoid wasp that uses the eggs of the maritime spider, *Desis formidabilis* O.P. Cambridge 1890 (Araneae: Desidae), as a host. This species is one of only three known maritime parasitoids globally, and is the only known spider egg parasitoid that attacks its host within the intertidal region in southern Africa. Originally described from 'The Island', Kommetjie (Western Cape, South Africa), this shore was the only known locality of the species at the commencement of this thesis. Furthermore, the extent of the parasitism pressure the wasp exerts on *D. formidabilis* was largely unknown, along with its basic biology (drivers of its broad-scale and fine-scale distribution patterns; parasitism incidence; and sex ratios) and morphological and physiological adaptations for living within the frequently saltwater-inundated environment. This thesis unravelled these aspects, as well as experimentally provided data for many components of the life history of *E. lamorali* that had only been hypothesized by other authors, at a variety of scales, from the country-wide ecosystem, to single shores, and finally to the scale of the individual.

While the distribution of *E. lamorali* was found to be much wider than previously thought, it remained restricted to the shores of the Cape Peninsula (Western Cape, South Africa). The host spiders were located throughout a much wider distributional range than the wasp, from East London in the east to the Peninsula, but some behavioural and morphological differences were found between those within and outside of the range of the parasitoid, suggesting range-limitation imposed by the host on *E. lamorali*. This limitation may be strengthened by the general lack of suitable shore types within the close vicinity of the Peninsula. Modelling using macro-climatic conditions suggested that maximum temperatures and humidities were

_____

also largely limiting to *E. lamorali*, although these patterns were not observable in the micro-climates in which the species survives.

The wasp populations and spiders within the range of *E. lamorali* as identified in Chapter 2 were assessed to determine any preferences for local conditions, including location along the Peninsula, nesting sites and intertidal zones within single shores, using AICc modelling, which detected parasitism patterns in *D. formidabilis* and *E. lamorali* populations, as well as the sex ratios in the latter species. The models showed that the spider population size and distribution was more influenced by bottom-up factors such as abiotic components of the shore than by parasitism, which only showed density dependence with the host at certain scales. Both host and parasitoid populations illustrated a preference for the middle zone on single shores. Observed spider nest characteristics suggested opportunistic nesting behaviour in the species, although preference was shown for construction along an east-west orientation and in locations with low sun exposure. Along with larger population sizes on the west coast over the east coast (not reflected by *E. lamorali*), these observations suggest that *D. formidabilis* prefers cooler environments. Spider brood success was 50% in unparasitized egg-sacs, but this figure halved when *E. lamorali* gained access to the eggs (of which 100% within a single compartment were parasitized each time). Encapsulation of the embryo was found to be positively correlated with parasitism, indicating some form of host resistance. Resultant parasitoid broods illustrated a strong female bias in the species, as is common for this group of insects.

The life history of *E. lamorali* was then assessed at a smaller scale, that of the individual. Scanning electron microscopy of whole *E. lamorali* specimens and light microscopy of

sectioned specimens demonstrated no morphological adaptations in the trachea and spiracles to cope with saltwater inundation. On the other hand, respirometry experiments categorically demonstrated that the species copes with inundation through the formation of a physical gill over the full habitus, and the induction of a state of torpor to reduce metabolic needs, when submerged. This precludes the need for any further morphological adaptations. Determination of the wasp's critical thermal tolerance illustrated a wide temperature range with a particularly cold lower limit of -1.1°C ± 0.16, suggesting the presence of related genera or ancestors in much colder environments.

With a much broader distribution than previously thought, and the inclusion of the distributional range of *E. lamorali* within the Table Mountain National Park, this species is being effectively conserved and managed through the umbrella-conservation of the park. Links to the host proved to vary at differing scales, proving the need for scale considerations to be included in other similar biological and ecological studies. Finally, the species showed physiological adaptation to its intertidal existence. *Echthrodesis lamorali*, the only discovered maritime spider egg parasitoid in Southern Africa, displayed unusual characteristics at every scale of its life history and as such, warrants further investigation.

Table of Contents

_____

List of Tables

List of Figures

Publications Arising from this Thesis

OWEN, C. A., COETZEE, J. A. & VAN NOORT, S. 2014. Distributional range of the South African maritime spider-egg parasitoid wasp, *Echthrodesis lamorali* (Hymenoptera: Platygastridae: Scelioninae). *African Invertebrates* 55 (2): 323-332.

OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. In prep. Assessing the morphological and physiological adaptations of *Echthrodesis lamorali* Masner 1968 (Hymenoptera: Platygastridae, Scelioninae) for survival in the intertidal environment. *Journal of Hymenoptera Research.*

OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. In prep. The thermal physiology of *Echthrodesis lamorali* Masner 1968 (Hymenoptera: Platygastridae, Scelioninae), a maritime spider egg parasitoid. *Journal of Hymenoptera Research.*

Acknowledgements

I must thank the following people, without whom both myself and this thesis would not be where we are today.

First and foremost my brother, Michael. Out of everyone I think you were the one who most understood what was happening during the course of this thesis and the hordes of advice and strength, both academically and personally, that you poured my way are immeasurable. Mom and Dad, my personal heroes, despite the never-ending trials and tribulations you were going through on your side, you always sat on the other end of the phone listening to me rant or weep or excitedly ramble and always knew exactly what to say to put it all into perspective. I am proud to be able to call the three of you my family. To Craig, you always kept me looking forward to the next chapter of our lives, giving me the strength to deal with the current chapter of my thesis and for that, I thank you.

To my academic heroes, Martin Hill and Julie Coetzee for always maintaining your faith in me, which pushed me through more tough times than you will ever know. I am forever in your debt. To Simon van Noort, your constant advice and resolute faith in me and my work were beyond compare and solid nails in the construction of this thesis. To Conrad Matthee for the endless explanations and replies to my bizarre questions in a field I was completely lost in.

_____

I'm so glad we got to go through this together and I am beyond grateful to each and every one of you. If I were to go back and do it again, I would do it all the same. Thank you.

THIS THESIS IS DEDICATED TO MY FATHER

CHRISTOPHER JOHN FOXWELL OWEN

WHOSE UNDERSTANDING OF THE NATURAL WORLD I CAN

ONLY HOPE TO ACHIEVE ONE DAY

_____

Chapter 1: Introduction

1.1 The intertidal zone

The intertidal region forms a narrow band along the interface of all land mass and oceanic bodies (Lubke, 1998), stretching approximately 594 000km globally, a total of 8% of the earth's surface (Paetzold *et al.,* 2008). The combined effects of terrestrial and oceanic pressures on this zone result in a unique environment, producing a distinctive community structure (Tietz & Robinson, 1974; Lubke, 1998; Traut, 2005).

Organisms living here are generally immersed in seawater once or twice a day for varying periods of time (Sanford, 2002; Barber, 2009), although these high and low tide cycles may be shortened or prolonged at different spatial and temporal scales (Allen & Duffy, 1998). Thus they need to be able to cope with varying humidity, temperature, salinity and pH conditions (Teal, 1962; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002), as well as the physical impact of wave action (Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002). These issues are generally easier to overcome for marine forms such as certain fish, molluscs and crustaceans that are adapted to existence in saltwater (Lillywhite *et al.,* 2008), but for organisms entering the environment from a terrestrial habitat, respiration and osmoregulation are difficult to maintain at adequate levels for normal functioning (Cheng, 1976; Foster & Treherne, 1976; Hinton, 1976; Barber, 2009). This is particularly problematic for submerged historically terrestrial organisms or life stages, which either need to extract oxygen from the water; develop a breathing tube that can handle a rough, constantly-mobile water surface; or have a mechanism for keeping air trapped close to the breathing apparatus (Cheng, 1976;

_____

Foster & Treherne, 1976; Hinton, 1976; Barber, 2009). Furthermore, smaller and winged organisms need to deal with buoyancy and surface tension issues (Cheng, 1976).

These chemical and biological issues may, however, be beneficial to historically terrestrial organisms capable of overcoming the adverse conditions (Teal, 1962). The physical structure of a rocky shore may provide valuable shelter (Barber, 2009). Crevices present in rock surfaces serve as hiding spaces from predators; may trap air during flooding, providing an organism with its own air-source even when submerged; and generally have a regulating effect on the physical factors, such as temperature and humidity, which are known to vary greatly on the shore (Barber, 2009). Finally, the constant motion of the sea brings new resources into the intertidal region on a regular basis (Traut, 2005; Paetzold *et al.,* 2008; Barber, 2009).

1.2 Maritime arthropods

Although they are less complex than reef systems, which are known to host a high biodiversity of marine organisms, rocky shores can sustain similarly large communities of both historically marine and terrestrial forms with a great output from primary production (Ferreira *et al.,* 2001). Furthermore, they are important localities for collecting free-floating larval forms of many vertebrate and invertebrate organisms, providing them with relatively safer nursery grounds to develop in than the open ocean (Broitman *et al.,* 2008; Cheminee *et al.,* 2011). As a result of these factors, both micro- and macro-algae, fish, molluscs, barnacles (Sink *et al.,* 2005; Blanchette *et al.,* 2008; Cox *et al.,* 2013), anemones, annelids (Nakaoka *et al.,* 2006; Blanchette *et al.,* 2008), and crustaceans such as isopods, amphipods and decapods

_____

are common in rocky shore intertidal regions throughout the world (Ravinesh & Bijukumar, 2013; Abushaala *et al.,* 2014; Navarro-Barranco *et al.,* 2015). Despite this abundant vertebrate and invertebrate life, the intertidal zone is generally an unforgiving environment for aquatic-adapted terrestrial arthropods, where complex biological, physical and chemical factors greatly inhibit their colonisation of the region (Tietz & Robinson, 1974; Cheng, 1976; Lubke, 1998). Nonetheless, a variety of arthropods have successfully established in the zone (Cheng, 1976; Roth & Brown, 1976; Traut, 2005; Barber, 2009), invading it a number of times throughout evolutionary history (Barber, 2009).

Nutrients brought in from the sea, mainly in the form of floating wrack or kelp, and washed down from the land (Traut, 2005; Paetzold *et al.,* 2008; Barber, 2009) encourage algal growth, a valuable food source for herbivorous arthropods such as isopods, amphipods (Ravinesh & Bijukumar, 2013; Abushaala *et al.,* 2014; Navarro-Barranco *et al.,* 2015) collembolans, flies, and beetles, to name a few (Paetzold *et al.,* 2008; Barber, 2009). These herbivores then serve as an untapped food resource for predacious arthropods encouraging further invasion from both the terrestrial and marine environments (Paetzold *et al.,* 2008; Barber, 2009). Crustacea dominate the intertidal zone, both as herbivores and predators, the latter of which possibly inhibiting a wide variety of other arthropods from establishing there (Cheng, 1976). Furthermore, a variety of centipedes (Class Chilopoda); symphylids (Class Symphyla); pauropods (Class Pauropoda); millipedes (Class Diplopoda); scorpions (Order Scorpiones); whipscorpions (Order Palpigrada); pseudoscorpions (Order Pseudoscorpiones); mites and ticks (Order Acari); and spiders (Order Araneae) have been recorded from intertidal zones world-wide (Roth & Brown, 1976). Of particular interest to this thesis are the maritime spiders, of which eleven families occur globally (Roth & Brown, 1976).

1.3 Maritime spiders

Desis Walckenaer 1937 (Araneae: Desidae) represents the best known genus of maritime spiders (Roth & Brown, 1976). Living in intertidal zones from the south-western Pacific and Indian oceans and north to Japan, they are a cosmopolitan genus (Roth & Brown, 1976). In southern Africa, one species, *Desis formidabilis* O.P. Cambridge 1890 (Araneae: Desidae), has been found on rocky shores from Lüderitz (Namibia) in the west to East London (Eastern Cape, South Africa) in the east (Day, 1974; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997). South Africa also hosts a second fully nocturnal maritime spider, *Amaurobioides africanus* Hewitt 1917 (Araneae: Anyphaenidae), on rocky shores ranging from the Cape Peninsula to East London (Lamoral, 1968; Day, 1974; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997). Despite all its similarities with *D. formidabilis*, it is easily distinguished from the latter by a distinctive chevron pattern on the abdomen (Lamoral, 1968; Day, 1974; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997).

Depending on the wave action, both of these species nest in rock crevices, holes, polychaete tube masses or shells, sealing the entrance to the retreat off with silk at each high tide, thereby trapping air inside for respiration (Lamoral, 1968; Roth & Brown, 1976; Dippenaar-Schoeman & Jocqué, 1997; van Noort *et al.,* 2014). The silken 'door' acts as a semi-permeable membrane, allowing necessary gasses (such as carbon dioxide and oxygen) to exchange between the internal cavity and external seawater (Rovner, 1987). Nonetheless, should a spider be caught outside its nest or if the nest floods during submersion, a layer of hydrofuge hairs on the spider's exoskeleton traps air in a plastron, a mechanism known as an external physical gill, allowing for continuous respiration (Lamoral, 1968; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997).

Most spiders, *D. formidabilis* and *A. africanus* included, lay their relatively large eggs as clutches in one location (Austin, 1985). While clutch size differs vastly between species, spiders usually produce large batches of eggs at a time, as exemplified by *Neoscona oaxacensis* Keyserling 1864 (Araneae: Araneidae), which produces a mean of 230 eggs at a time; and *Latrodectus hesperus* Chamberlin & Ivie 1935 (Araneae: Theridiidae), producing a mean of 283 eggs per nest (Vetter & Carroll, 2013). Furthermore, eggs lack any form of cellular defence response and encapsulation of intruded material is unlikely (Strand, 1986a; Strand & Pech, 1995; Eslin & Prévost, 2000). These facts render spider eggs a valuable and abundant food resource for smaller organisms, particularly hymenopteran parasitoids (Austin, 1985).

1.4 Spider parasitoids

While a variety of features separate the Hymenoptera from other insect taxa (including wing, metasomal, ovipositor and mouthpart features), only the presence of more hamuli on the wings (where wings are present) than are seen in other taxa; ovipositors that articulate on the valvifer and are stored in internal sheaths; mandibulate mouthparts with labia that attach directly to the maxilla; and haplo-diploidy sex determination of offspring, can be used to truly distinguish this group (Mason & Huber, 1993). By 2013, approximately 153 000 species of Hymenoptera had been described (Aguiar *et al.,* 2013), placing them as the third largest order behind Coleoptera (400 000 described species) and Lepidoptera (174 250 described species) (Mason & Huber, 1993; Sharkey, 2007; Chapman, 2009; Huber, 2009). Thus this group comprises 10% of the described life on earth (Sharkey, 2007). It is hypothesized, however, that if all of the undescribed species of Hymenoptera are taken into account, this clade would be "the most species-rich insect order" (Mason & Huber, 1993; Austin *et al.,* 2005; Huber, 2009; Austin & Dowton, 2010).

The Platygastroidea is the fourth largest hymenopteran parasitoid superfamily, including 4460 described species that inhabit all regions of the globe other than the Arctic and Antarctic (Austin *et al.,* 2005). Murphy *et al.* (2007) contend that this huge number of species may be attributed to the early use (in evolutionary terms) of previously unutilized host eggs, of which there is a large spectrum that drives diversification. Classification within the group used here follows Sharkey (2007), who synonymised the family Scelionidae with Platygastridae based on the phylogenetic analysis of Murphy *et al.* (2007). As such, the Platygastroidea is taken to comprise the single family Platygastridae that contains five subfamilies: Platygastrinae, Sceliotrachelinae, Scelioninae, Teleasinae and Telenominae (Hymenoptera) (Johnson, 2015). The group is characterised by two unique morphological processes and structures, namely a hydrostatic ovipositor control mechanism and the presence of sensilla on the female antennae (Masner, 1993; Murphy *et al.*, 2007). The former of these two character systems involves either purely hydrostatic changes in the haemolymph of the metasoma that force the ovipositor out or retract it back into the tagma, or a combination of this process with various muscle groups (Murphy *et al.*, 2007). It is this control system, as well as the structure of the ovipositor itself, that have served as a good basis for inferring systematic relationships within the group (Murphy *et al.*, 2007). In the second adaptation, females developed multiporous basiconic sensillae in the apical segments of their antennae, which are hypothesized to perform host-recognition functions (Masner, 1993; Murphy *et al.*, 2007).

The scelionines are a group of parasitoids little investigated (Hickman, 1967; Austin *et al.,* 2005; Murphy *et al.*, 2007), but all of which attack the egg stage of the host, an uncommon observation for other similar taxa (Masner, 1993; Austin *et al.,* 2005; Murphy *et al.*, 2007). In 1993, Masner reported an approximate 3000 described Scelioninae species in 150 genera. By

2005, the number of known species was reported as 3308 (Austin *et al.,* 2005). Masner (1993) describes the group, which he identified as a family, not subfamily, as generally 1-2.5mm in length, mostly black and usually highly sculptured with 9-10 flagellomeres in the antennae (the third of which is highly modified in males) and diverse wing and metasomal structures, although the latter is usually dorso-ventrally depressed. In general, the displayed body structure can be attributed to the size and shape of the host egg in which the larval and pupal forms develop (Masner, 1993). As a result of the use of small, cryptic hosts that often produce eggs singularly and may be difficult to locate and collect, clear host records are generally lacking in the group (Murphy *et al.*, 2007; Johnson *et al.,* 2014).

Parasitoids of spider eggs aside, there are several records of parasitic wasps that attack submerged stages of their hosts in fresh water, such as *Agriotypus gracilis* Waterston 1930 (Hymenoptera: Ichneumonidae) that oviposits into submerged caddisfly cases (Aoyagi & Ishii, 1991), and *Caraphractus cinctus* Walker 1846 (Hymenoptera: Mymaridae) that attacks dytiscid eggs (Jackson, 1958). Within the Scelioninae there are eight genera that utilise the submerged forms belonging to the families Gerridae Leach 1815 (Hemiptera), Nepidae Latreille 1802 (Hemiptera) and Aeshnidae Rambur 1842 (Odonata) as hosts (Johnson & Masner, 2004). Wasps that attack marine or saltmarsh (a very similar, yet vegetated habitat) organisms are fewer in numbers and have been recorded in the hymenopteran superfamilies and families Chalcidoidea, Ichneumonidae, Braconidae and Platygastridae (van Achterberg *et al.,* 1990; O'Connor, 1996; Laegdsgaard *et al.,* 2006; Wu *et al.,* 2009; Veenstra *et al.,* 2011; Japoshvili & Russel, 2012; Dorchin *et al.,* 2014). Being a physically active environment, with constant wave action, the shore does not lend itself well to invasion by small, fragile, winged insects like hymenopteran egg parasitoids (Cheng, 1976; Barber, 2009) and only three species worldwide show true adaptation to intertidal conditions and can thus be considered strictly

maritime, including *Echthrodesis lamorali* Masner 1968 (Hymenoptera: Platygastridae, Scelioninae) (van Noort *et al.,* 2014), a spider egg parasitoid.

1.5 *Echthrodesis lamorali* Masner 1968

At "The Island" (Kommetjie, Cape Peninsula, South Africa), *D. formidabilis* eggs are parasitized by a small (less than 1mm in length) platygastrid, *E. lamorali* (Lamoral, 1968; Masner, 1968; Branch & Branch, 1981; van Noort, 2009; van Noort *et al.,* 2014). Despite only being found by Lamoral (1968) in and around *D. formidabilis* nests, one record of the wasp emerging from *A. africanus* eggs exists at the Ohio State University collection and is documented on the *Hymenoptera Online* website (Various Contributors, 2015). As such, this spider may serve as a second host for the wasp. The wasp is first mentioned in Masner (1968) as "the very first evidence of an intertidal maritime Scelionid ever known", as it is capable of full submersion and dispersal in saltwater, potentially living off air caught in a plastron by the dense setae that cover the body (Masner, 1968; van Noort *et al.,* 2014) in a similar manner to the host spiders (Lamoral, 1968; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997).

The appropriate phylogenetic placing of *E. lamorali* is still uncertain. Originally placed as close to *Embidobia* Ashmead 1896 (Hymenoptera: Platygastridae, Scelioninae), *Mirobaeus* Dodd 1914 (Hymenoptera: Platygastridae, Scelioninae) and *Mirobaeoides* Dodd 1914 (Hymenoptera: Platygastridae, Scelioninae) based on morphology (Masner, 1968), they were subsequently moved to be a sister group to an apomorphic terminal clade consisting of *Mirobaeoides*, *Baeus* Haliday 1833 (Hymenoptera: Platygastridae, Scelioninae), *Neobaeus* Austin 1988 (Hymenoptera: Platygastridae, Scelioninae) and *Apobaeus* Masner 1964

_____

(Hymenoptera: Platygastridae, Scelioninae), also based on several morphological traits (Iqbal & Austin, 2000). Subsequently, van Noort *et al.* (2014) assessed the phylogenetic affinities of *E. lamorali* from a molecular point of view. They found the species to be the basal sister taxon of *Neobaeus, Embidobia* and *Mirobaeoides* (van Noort *et al.,* 2014).

Females are apterous, while the wings on males have been reduced to small stubs, possibly as an adaptation to preclude unwanted, wind mediated dispersal away from their very narrow ecological niche in the intertidal zone (van Noort, 2009; van Noort *et al.,* 2014); to assist with movement through the web surrounding the eggs of *D. formidabilis* (van Noort, 2009); or for dealing with cramped conditions inside a parasitized egg (van Noort *et al.,* 2014). Such winglessness is common amongst the scelionines (Hickman, 1967; van Noort *et al.,* 2014) and a variety of other small parasitoid Hymenoptera (Austin 1988; Austin, 1995).

At Kommetjie, *D. formidabilis* compartmentalizes its nest, laying eggs in only some of the compartments, a behaviour hypothesized as being a response to parasitism (van Noort, 2009; van Noort *et al.,* 2014). Nonetheless, when *E. lamorali* does chew its way into an egg-laden compartment, parasitism nearly always reaches 100% of the eggs within (van Noort *et al.,* 2014). This parasitism frequency is recurrently seen in other small, wingless spider egg parasitoids (Eason *et al.,* 1967; Hickman, 1967). In turn, *E. lamorali* may be attacked by a mite, a *Veigeiia* sp. (Mesostigmata: Veigeiidae), found on site (Lamoral, 1968; Branch & Branch, 1981; van Noort *et al.,* 2014).

Oviposition behaviour has been recorded for a few other spider-egg parasitoid scelionines (Austin, 1985). Some pierce the silk wall of the nest and oviposit into the eggs using a long ovipositor (Austin, 1985), and others chew through the wall and oviposit straight into the eggs once inside using a relatively shorter ovipositor (Austin, 1985). *Echthrodesis lamorali* falls into the latter category, ovipositing a single egg in each spider egg once access is gained through a hole chewed in the silk using strong mandibles (van Noort, 2011; van Noort *et al.,* 2014). Despite the highly female-biased sex ratio, the few males in a nest will hatch first and battle for access to the later-hatching females, each of which will mate with only one male before exiting (van Noort *et al.,* 2014).

1.6 Thesis rationale

Approximately three percent of the insects already discovered are either fully aquatic or have at least one aquatic lifestage (Cheng, 1976). The proportion of these that can be considered marine or intertidal, however, is minute, with only a few hundred recorded species (Cheng, 1976; Gainey, Jr., 1984). Furthermore, the current knowledge on spider egg parasitoids has been assimilated from widely scattered and often brief or incomplete records (Eason *et al.,* 1967) and the Scelioninae are a generally neglected group (Austin *et al.,* 2005), as is illustrated by the now 39-year gap since the construction of a genus-level key has been attempted, the last completed by Masner (1976). *Echthrodesis lamorali* could stand as a representative for other small parasitoid Hymenoptera, just as Austin *et al.* (2005) suggest the Platygastroidea represent all parasitoids in a variety of areas of research, providing us with a window into maritime insects' and spider egg parasitoids' ecophysiology, two groups not yet adequately investigated.

The arthropods described in this thesis form a small feeding chain within a larger food web. This chain is poorly known and even less observable to the untrained or incurious eye (Figure 1.1). A *Veigeiia* sp. attacks *E. lamorali* (Lamoral, 1968; Branch & Branch, 1981; van Noort *et al.,* 2014), which in turn attacks one or both of the spider species discussed here (Lamoral, 1968; Masner, 1968; van Noort, 2009) (Figure 1.1). In turn, *D. formidabilis* and *A. africanus* feed on intertidal isopods (Day, 1974; Branch & Branch, 1981; Dippenaar-Schoeman & Jocqué, 1997, van Noort, 2009) and amphipods (Day, 1974), thereby potentially exhibiting an important control over their population sizes (Branch & Branch, 1981; Dobel *et al.,* 1990) (Figure 1.1). Maritime collembola, *Anurida maritima* Guérin 1836 (Poduromorpha: Neanuridae)*,* feed on the remains of the spider's prey items in their nests, and hence also rely on the spiders for survival (Branch & Branch, 1981) (Figure 1.1). In turn, the collembola also fall prey to the *Veigeiia* sp. described above (Branch & Branch, 1981) (Figure 1.1).

While seemingly small, the full potential impact that this feeding chain could be having on the intertidal and adjacent food web and thus, ecosystems is not yet clear. Previously conducted studies have illustrated entire rocky shore ecosystems fashioned by the interactions of only a few species (Sanford, 2002). In a terrestrial context, the same pattern was observable in lemon trees that were being attacked by a scale insect, *Aonidiella aurantii* Maskell 1879 (Hemiptera: Diaspididae) (DeBach & Rosen, 1991). Dichlorodiphenyltrichloroethane (DDT) sprays, a common form of chemical control at the time, were affecting the parasitoid of the scale more than the host itself, resulting in the decimation of sprayed trees by the pest, while unsprayed trees where the wasps survived remained healthy (DeBach & Rosen, 1991). Furthermore, the community-shaping processes occurring in the intertidal region could be affecting the surrounding terrestrial and marine dynamics both directly and indirectly (Paetzold *et al.,* 2008). According to Traut (2005),

"[u]nderstanding the transition between the components of a landscape can help increase effective management of these systems". As parasitoids are particularly sensitive to ecological perturbations (Sharkey, 2007), the location of this food web between residential development, some fynbos (the vegetation type typical of the area) on one side and the ocean on the other, "The Island" at Kommetjie may require such management. Further investigation into the food web structure of all the organisms concerned is required to provide an answer to this.
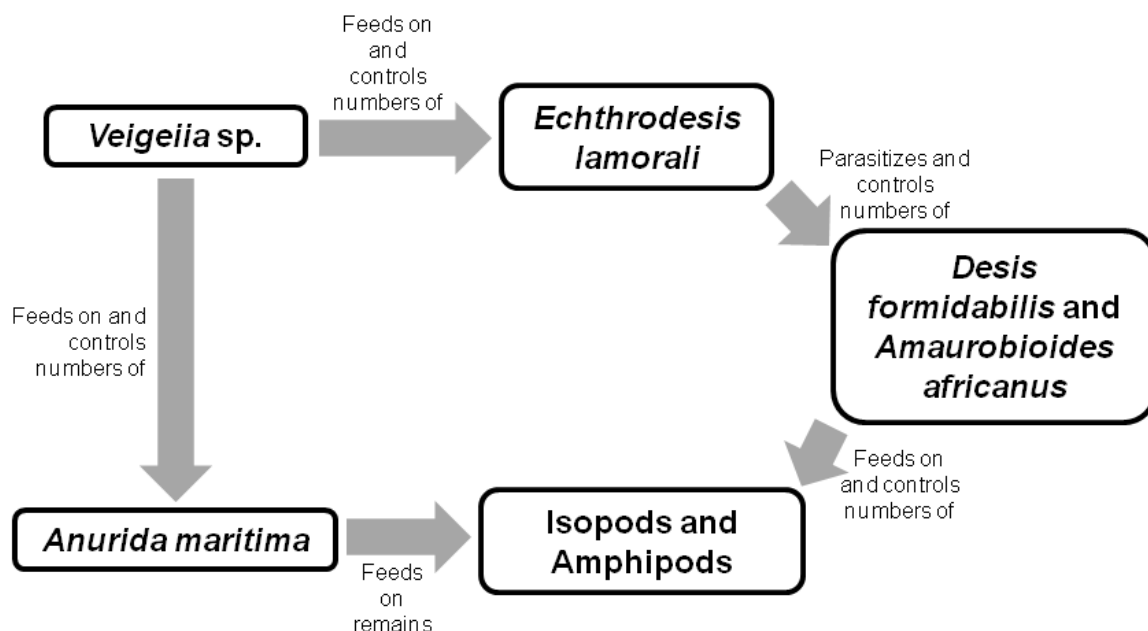
Figure 1.1: Arthropod food web structure in the intertidal region at Kommetjie (Western Cape, South Africa).

A few authors have hypothesized and some have even tested their theories on the distribution, physiology and behaviour of *E. lamorali* (see Masner, 1968; van Noort, 2009; van Noort *et al.,* 2014). However, thorough investigation has yet to be conducted on a variety of important aspects of the species' ecophysiology, all of which are linked (Figure 1.2) (Strand, 2010). An organism's distribution is determined by its behaviour (Carducci & Jakob, 2000) (Figure 1.2).

_____

Behaviour can in turn be linked to the animal's morphological and physiological adaptations, but also influences how these adaptations evolve (Carducci & Jakob, 2000; Strand, 2010) (Figure 1.2). Finally, the morphology and physiology of a species determine where it can survive, and thus, its distribution (Carducci & Jakob, 2000; Strand, 2010) (Figure 1.2). As such, it is necessary for all aspects to be comprehensively investigated before we can reach a more holistic understanding of the life history of a species as a whole.
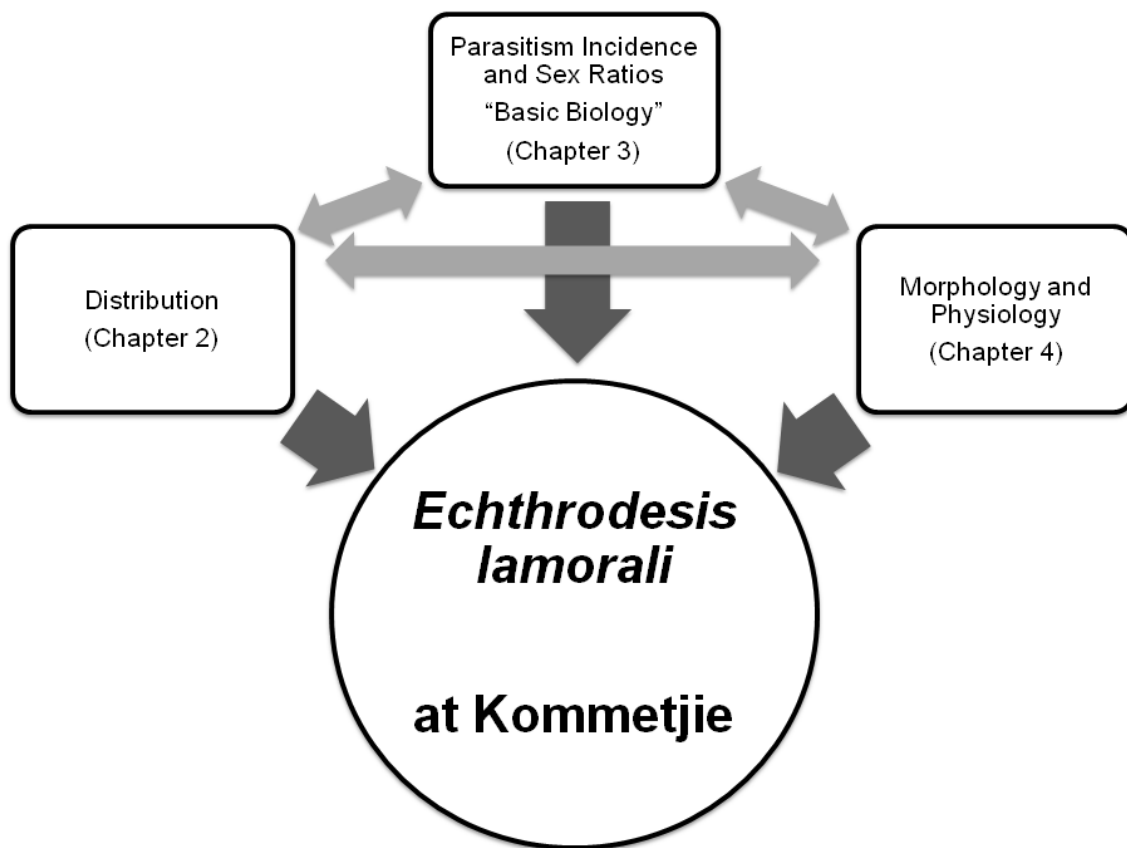
Figure 1.2: All aspects of the biology of *Echthrodesis lamorali* are linked.

1.7 Thesis aims

1.7.1 Chapter 2: Distribution

At the commencement of this thesis, *E. lamorali* had only been found at Kommetjie (van Noort *et al.,* 2014; van Noort, 2011). However, it is plausible that the wasp occurs throughout the full range of the spiders along a wide section of South Africa's coast (van Noort, 2009).

With the combination of the high variation in beach type along the South African coast (Teske *et al.,* 2011), the seeming inability of *E. lamorali* to disperse easily as a result of its apterous condition and small size (van Noort, 2009; van Noort et al., 2014) and the location of the insect's type locality on the border of two biogeographical zones (Teske *et al.,* 2011), it would be unsurprising if the distribution of *E. lamorali* is restricted to Kommetjie alone. However, zoochory (transport by birds and other animals), aerial transport by wind, anthropochory (transport by humans), rafting on floating matter (Barber, 2009) and phoresy (riding on host) (Strand, 1986a; Austin *et al.,* 2005) are all plausible methods of long-distance dispersal for small organisms such as *E. lamorali*, which may have used any method to reach other shores and new host pools.

Therefore, based on Emanuel *et al.*'s (1992) study, which used a variety of approaches (treating the coastline as a transect in particular) to determine hotspot areas in which marine nature reserves should be placed, a thorough survey of the South African coast was conducted for the presence of *D. formidabilis*, *A. africanus* and their parasitoid wasp to determine their full distributions as well as any patterns in nesting or breeding behaviours within this range. Surveys were conducted twice in 2012, each occurring in a different

season, to determine if any seasonal differences were observable in both the host and parasitoid populations. Furthermore, the exposure of the shore on which a nest was found, and the material/site in which the nest was constructed were recorded to test for differences in these factors within and outside of the wasp's distribution. The potential that climatic conditions may have a controlling effect on the distribution of *E. lamorali* was also assessed at multiple scales, i.e. macro-scale (with data obtained from the South African Weather Service) and micro-scale (from data collected by iButton temperature loggers [Fairbridge Technologies, DS1921G-F5] *in-situ*).

Knowledge of an organism's distribution and the links between different populations (if they exist) is important from a conservation perspective. On a broad scale, understanding the interactions and exchanges within ecotones between each population is a vital component of designing correct management plans for those stretches of coast or land (Traut, 2005). Furthermore, this chapter provided a large (country-wide) scale assessment of the role of *E. lamorali* in a multitude of ecosystems.

1.7.2 Chapter 3: Basic biology

To assess the smaller scale effects of *E. lamorali* on single shore ecosystems, their basic biology was assessed. As a result of the highly heterogeneous abiotic nature of the rocky shore (Tietz & Robinson, 1974; Lubke, 1998), species distributions within single beaches and between different localities can differ significantly (Tietz & Robinson, 1974; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002). These patterns are further influenced by biotic interactions within the region (Foster & Treherne, 1976; Sanford, 2002), resulting in marked

zonation within most shores (Tietz & Robinson, 1974; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002).

Desis formidabilis compartmentalises its egg sac, laying eggs only in some sections and leaving others barren (van Noort, 2009; van Noort *et al.*, 2014). This behaviour is hypothesized to have evolved as a response to parasitism by *E. lamorali*, which theoretically exerts all of its stored energy gaining access to a compartment that may then be empty, thereby protecting the eggs in neighbouring segments (van Noort, 2009; van Noort *et al.*, 2014). This hypothesis, as well as much of the basic biology of the parasitoid, was untested at the commencement of the thesis, as is common for the small, often cryptic Scelioninae (Eason *et al.,* 1967; Hickman, 1967; Austin, 1984; Austin *et al.,* 2005).

As such, this study aimed to assess the nest and population features of *D. formidabilis* in the field within the distribution of *E. lamorali* that was identified in Chapter 2. These data were used to determine if any preferences existed for certain materials, sites or zones within single shores and between different sites, which defined the local habitat selection of the species. Furthermore, the parasitism incidence and sex ratios of the resultant wasp broods was determined to indicate the effect that the parasitoid may be having on the host spider, while also testing whether *E. lamorali* is subject to the breeding patterns associated with most parasitic Hymenoptera.

_____

1.7.3 Chapter 4: Morphology and physiology

The causal reasons for the general lack in marine insect diversity and abundance are relatively unclear and broad, with postulations ranging around too large a crustacean predation pressure, a lack of calcium for cuticle development, buoyancy issues, salt and osmotic control, and respiratory difficulties (Usinger, 1957; Cheng, 1973; Cheng, 1976; Hinton, 1976; Treherne, 1976; Gainey, Jr., 1984; Andersen & Weir, 1994; Maddrell, 1998; Vermeij & Dudley, 2000; Ikawa *et al.,* 2012). Morphological and physiological adaptations are the only means to overcome the majority of these barriers to survival within the intertidal environment, and thus warrant further exploration (Cheng, 1976; Gainey, Jr., 1984). While the loss of wings in *E. lamorali* is hypothesized to be an adaptation to survive within the aqueous environment (Cheng, 1976; Austin *et al.,* 2005; van Noort, 2009), little is known about the other structures and processes employed by the species. Here the species is assessed at the scale of single individuals.

On examination of *E. lamorali* specimens, the dense hairy body covering is obvious (Masner, 1968; van Noort *et al.,* 2014). This layer is hypothesized to serve a similar function to the setae found on *D. formidabilis* and *A. africanus* (Lamoral, 1968; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997) - the formation of a plastron used as a physical gill during submersion (Masner, 1968; van Noort *et al.,* 2014). Respirometry (as used by Lamoral [1968] for the host spiders and in Köster *et al.* [2008]; Alton *et al.* [2012]; and Köster & Paffenhöfer [2012] on a copepod and tadpoles) and scanning electron microscopy (as used by Neumann & Woermann [2009] on *Clunio marinus* Haliday 1855 [Diptera: Chironomidae]) were used to determine the functionality and microscopic structure of the hairs on the wasp's body and

_____

establish if there are any particular spiracular and tracheal structures that assist with submerged breathing.

1.7.4 Chapter 5: General discussion

Finally, all the aspects discussed above were concluded in this chapter, detailing how they are all interconnected (Figure 1.2). This chapter illustrates where *E. lamorali* occurs, what it is doing there and how it is surviving on the rocky shore. Overall, this thesis strives to provide an overview of the life history of this unusual spider egg parasitoid and its ecological and physiological placement from the ecosystem to the individual level.

Parts of Chapter 2 have been published in *African Invertebrates*, while others are currently in preparation for a short communication. Chapter 4 has been divided into two papers, which are currently both in preparation for publication in the *Journal of Hymenopteran Research*. Consequently, there is some repetition in the introductions and discussions of all chapters, and tables and figures are consecutively numbered for each chapter and not the entire thesis. For convenience, all references are given as one section at the end of the thesis.

Chapter 2: The distribution *of Echthrodesis lamorali* **Masner 1968 (Hymenoptera: Platygastridae: Scelioninae) along the South African coast and its link to environmental characteristics**[1]

2.1 Introduction

To date, *Echthrodesis lamorali* Masner 1968 (Hymenoptera: Platygastridae: Scelioninae) has only been found on one stretch of rocky shore at "The Island" (Kommetjie, Cape Peninsula, South Africa [34° 8' 22.7034" S, 18° 19' 17.5794" E]) (Lamoral, 1968; van Noort, 2011; van Noort *et al.,* 2014). The wasp's host, *Desis formidabilis* O. P. Cambridge 1890 (Araneae: Desidae), however, has been recorded from Lüderitz in Namibia, down along the coast to East London (Eastern Cape, South Africa) (Day, 1974; Dippenaar-Schoeman & Jocqué, 1997). *Amaurobioides africanus* Hewitt 1917 (Araneae: Anyphaenidae), another possible host (van Noort et al., 2014), occupies a similar area to *D. formidabilis*, occurring from Namibia throughout much of the Western (Dippenaar-Schoeman & Jocqué, 1997) and Eastern Cape provinces as far as East London (Day, 1974; Filmer, 1995).

If *D. formidabilis* and *A. africanus* can be found throughout such a wide distribution in South Africa, it is plausible that their associated parasitoid could attack them outside of Kommetjie (van Noort et al., 2014). Previous studies focussing on the wasp (Lamoral, 1968; Masner, 1968; Branch & Branch, 1981; van Noort, 2009; van Noort et al., 2014) did not explore

---

[1]Chapter condensed and published in OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. 2014. Distributional range of the South African maritime spider-egg parasitoid wasp, Echthrodesis lamorali (Hymenoptera: Platygastridae: Scelioninae). *African Invertebrates* **55**(2): 323-332.

_____

beyond Kommetjie, thus this chapter aimed to determine the full range of *E. lamorali* throughout South Africa.

A species may be highly limited to a certain habitat due to its physiological abilities (Atkins, 1980a; Somero, 2005). Furthermore, local climatic conditions and habitat structure within an environment could have significant impacts on an organism's movement, inter- and intra-specific interactions and behaviour, which will in turn influence their distribution (Laing, 1937; Atkins, 1980a; Lubke, 1998). The combination of the high variation in shore type along the South African coast (Teske *et al.,* 2011), the seeming inability of *E. lamorali* to disperse easily as a result of their apterous condition and small size (van Noort, 2009; van Noort et al., 2014), and the location of the insect's type locality on the border of two biogeographical zones (Teske *et al.,* 2011), suggests that the species may have a very narrow distributional range and that it may even be endemic to the Kommetjie area.

Knowledge of an organism's distribution and the links between different populations (if they exist) is important from a conservation perspective. On a broad scale, understanding the interactions and exchanges within ecotones between each population is a vital component of designing correct management plans for those stretches of coast or land (Traut, 2005). At Kommetjie itself, *E. lamorali* falls into a food web (described in Chapter 1) not yet fully explored or understood (Lamoral, 1968; Masner, 1968; Day, 1974; Branch & Branch, 1981; Dippenaar-Schoeman & Jocqué, 1997; van Noort, 2009; van Noort et al., 2014). In many ecosystems, however, small parasitoids like *E. lamorali* can impose a significant stabilising or destabilising effect on other species, be it their own hosts or not, thereby maintaining a vital balance within the system (Combes, 1996; Morand & Gonzalez, 1997; Briggs &

Hoopes, 2004; Kremen, 2005). These effects on the ecosystem may extend as far as negatively or positively influencing processes like migration and speciation, which seem to be far removed from the basal interactions between the species and its host (Combes, 1996; Morand & Gonzalez, 1997; Briggs & Hoopes, 2004; Kremen, 2005). A good example of this is provided by Combes (1996) who describes a system where two fruit flies, *Drosophila melanogaster* Meigen 1830 (Diptera: Drosophilidae) and *D. simulans* Sturtevant 1919 (Diptera: Drosophilidae) are only capable of co-existing in the same habitat if the parasitoid wasp, *Leptopilina boulardi* Barbotin *et al.* 1976 (Hymenoptera: Figitidae), is introduced. With the existence of both *D. formidabilis* and *A. africanus* on the Kommetjie shore, the potential exists for the mechanism described above for flies, or any similar mechanism for that matter, to be occurring here. Furthermore, Combes (1996) points out that human perturbation may alter the effect that the parasitoid has on the ecosystem. If a population of *E. lamorali* is found at its type locality only, efforts may be required to ensure that this area is not further impacted upon by the surrounding residential area, holiday-goers or future coastal development.

2.2 Materials and methods

2.2.1 Surveys

Based on Emanuel *et al.*'s (1992) study, the South African section of the distribution of *D. formidabilis* and *A. africanus* (a region stretching from 33° 8' 51.4674" S, 27° 42' 10.5114" E to 32°57'53.61"S 17°53'07.11"E) was treated as a transect and rocky shores approximately every 100km were sampled in order to confirm the species' true distributional range. This resulted in a total of 14 sampling sites (Table 2.1). Sampling took place six months apart

during March and November, each respectively representing autumn and spring, the least climatically extreme seasons.

The second sampling session in November 2012, which had not been incorporated into the initial trip in March, included a finer scale search around Kommetjie. The coastline 100km above (up to Jacobsbaai, Western Cape, South Africa [32°57'53.61"S 17°53'07.11"E]) and below Kommetjie (down throughout the Table Mountain National Park to Pringle Bay, Western Cape, South Africa [34° 20' 52.188" S, 18° 49' 12.0354" E]) was divided into 10km stretches and suitable rocky shores were searched at each point. Where necessary, an even finer scale search was conducted by walking from the last known point of the wasp's presence. A considerable stretch of the coastline below Kommetjie, including much of the Table Mountain National Park, and the shore running alongside Grassy Park, Mitchells Plain, Khayelitsha and Macassar (Western Cape, South Africa), was excluded from exploration due to inaccessibility because of sheer cliff faces and lack of rocks, respectively.

_____

Table 2.1: GPS co-ordinates of the fourteen sites surveyed (sites numbered from east to west) for the presence of *Desis formidabilis, Amaurobioides africanus* and their parasitoid, *Echthrodesis lamorali.*

Site number	Site Name	Latitude	Longitude
1	Kidds Beach	33° 8' 51.4674" S	27° 42' 10.5114" E
2	Kenton-On-Sea	33° 41' 31.999" S	26° 40' 23.9982" E
3	Summerstrand	33° 58' 47.892" S	25° 39' 31.0674" E
4	Cape St. Francis	34° 10' 6.348" S	24° 49' 59.3394" E
5	Nature's Valley	33° 58' 56.928" S	23° 34' 34.1394" E
6	Brenton-On-Sea	34° 4' 28.308" S	23° 1' 11.136" E
7	Mossel Bay	34° 10' 27.4434" S	22° 8' 8.9874" E
8	Jongensfontein	34° 25' 37.38" S	21° 20' 30.4794" E
9	Cape Agulhas	34° 49' 29.1" S	20° 1' 38.3514" E
10	Pearly Beach	34° 40' 13.7274" S	19° 30' 1.4754" E
11	Kogelberg	34° 22' 17.1474" S	18° 52' 47.1" E
12	Simon's Town	34° 9' 43.7394" S	18° 25' 55.5234" E
13	Buffel's Bay (TMNP)	34° 19' 19.5594" S	18° 27' 44.028" E
14	Gifkommetjie (TMNP)	34° 19' 4.7994" S	18° 24' 56.7354" E
15	Olifantsbos Point (TMNP)	34° 15' 29.6274" S	18° 22' 54.0474" E
16	Kommetjie	34° 8' 22.7034" S	18° 19' 17.5794" E
17	Silwerstroomstrand	33° 35' 25.04" S	18° 21' 33.261" E
18	Jacobsbaai	32° 57' 53.61" S	17° 53' 7.11" E

Arrival on-site occurred at least an hour before low tide, as per Lubke & de Moor's (1998) suggestion, as the water was low enough to sample then and enough time was allowed before the area was re-immersed with seawater during high tide. Thereafter, the entire range of the shore, starting from the water/air interface, up to the inland edge of the rocks, was actively searched for the presence of spiders or their nests. Where necessary, encrusting material such as mussels or tubeworm conglomerates were dislodged using a paint scraper to check for underlying nests. Sites were surveyed twice daily (during the day for ease of navigation and

at night as both species are active nocturnal hunters [Lamoral, 1968], and if nests were yet to be found, foraging spiders could lead the observer to their nests) for a total of two days at each site in March. Due to time constraints, sites were only searched for a single day in November.

Date; GPS locality; photographic and video evidence of the prominent habitat type; material collected; species collected; exposure to wave activity of the shore, spiders, or nests (taken as exposed or sheltered); breeding state; and nesting material were all documented. At least two adults of each spider species and any egg masses were collected, if found, at each site. Egg sacs were retained; clipped open to allow spiderling escape post-hatching, and the species (spider or wasp) that emerged was recorded. All adult specimens were stored in 95% ethanol and sent to the Iziko South African Museum (Cape Town) and the Albany Museum (Grahamstown) for curation as vouchers and duplicates respectively. Distribution data were mapped using ArcCatalog and ArcMap (ArcGIS) 10 (ESRI, 2011).

2.2.2 Seasonality

Based on results obtained in the March 2012 survey, the spiders at Summerstrand and Kommetjie were monitored for the presence of egg sacs monthly from March 2012 to March 2013 to determine any seasonal effects in breeding status. Within this period, only the December and January 2013, and January and February 2013 surveys were omitted at Kommetjie and Summerstrand respectively.

2.2.3 Climatic data

The effect that climatic variables may have on the distribution of *E. lamorali* was assessed by determining if their patterns reflected the species' distributional pattern. This was achieved using Akaike Information Criterion (AICc) model building.

2.2.3.1 Macro-climatic conditions

Data on the maximum monthly temperature (°C), minimum monthly temperature (°C), total daily rainfall (mm), average monthly wind speed (m/s) and average monthly humidity (%) for the years 2003 – 2012 were obtained from the South African Weather Service for the coastal weather stations tabulated in Table 2.2.

Each component of the macro-climate data was analysed independently using sample size-corrected, Akaike Information Criterion (AICc) based, reverse model building processes in R version 3.1.3 (R Core Team, 2015). This technique made use of the packages MuMIn (Barton, 2015) and car (Fox & Weisberg, 2011). The most complex models using all possible predictor (independent) variables (site, month and year) were first constructed and co-linearity within them determined (GVIF>5), and removed where necessary. Akaike Information Criterion values (AICcs) were then calculated for all possible models that could be derived from the most complex one for each outcome variable as an individual model (maximum monthly temperature, minimum monthly temperature, total daily rainfall, average monthly wind speed and average monthly humidity). The models developed in this process that had a delta value of less than 2 were assessed as suggested by Symonds & Moussalli

_____

(2011), and the recurring predictors that made the most biological sense were used in construction of the final models (Zuur *et al.,* 2010; Symonds & Moussalli, 2011).

Table 2.2: Climate data were sourced from the South African Weather Service for the following coastal stations (Grey cells indicate stations within the distributional range of *Echthrodesis lamorali*).

Station Name	Latitude	Longitude
Cape Columbine	32° 29' 38.3994" S	17° 30' 43.56" E
Langebaanweg AWS	35° 34' 55.2" S	18° 55' 40.8" E
Dassen Island	33° 15' 20.5194" S	18° 31' 44.3994" E
SA Astronomical Observatory	33° 33' 57.6" S	18° 17' 2.0394" E
Cape Town WO	33° 35' 3.84" S	18° 21' 35.9994" E
Atlantis	33° 21' 46.08" S	18° 17' 9.24" E
Molteno Reservoir	33° 33' 41.4" S	18° 14' 38.04" E
Kirstenbosch	33° 35' 35.1594" S	18° 15' 20.88" E
Cape Town Slangkop	34° 51' 14.3994" S	18° 11' 56.4" E
Cape Point	34° 12' 40.68" S	18° 17' 32.28" E
Strand	34° 49' 40.8" S	18° 30' 20.16" E
Hermanus	34° 15' 20.5194" S	19° 7' 58.4394" E
Cape Agulhas	34° 29' 36.5994" S	20° 2' 49.2" E
Struisbaai	34° 28' 58.7994" S	20° 19' 55.2" E
George WO	34° 4' 12" S	22° 13' 48" E
Knysna	34° 15' 21.6" S	23° 27' 10.7994" E
Plettenbergbaai	34° 31' 22.8" S	23° 11' 35.88" E
Tsitsikamma	34° 7' 19.1994" S	23° 32' 7.7994" E
Cape St. Francis	34° 7' 27.84" S	24° 30' 32.4" E
Port Elizabeth WO	33° 35' 38.4" S	25° 21' 50.04" E
Port Alfred - Airport	33° 20' 0.2394" S	26° 31' 30.72" E
East London WO	33° 13' 47.9994" S	27° 29' 40.5594"

2.2.3.2 Micro-climate temperatures

Smit *et al.* (2013) gathered temperature data in the intertidal region at different scales and proved that a disparity existed between broad-scale and *in situ* readings. As such, iButton temperature data loggers (Fairbridge Technologies, DS1921G-F5) were glued underneath empty limpet shells to rocks within the intertidal region using Pattex Power Epoxy to

accurately capture the environment in which *E. lamorali* lives. These data loggers were capable of recording in a temperature range from -40°C to 85°C with a resolution of 1°C. As the orientation of temperature readers has been shown to have a significant influence on figures recorded in Coombes *et al.* (2003), one unit was placed facing south approximately halfway up the lower intertidal region, where *D. formidabilis* populations are well established, at Kommetjie (34° 08.421' S, 18° 19.273' E), Olifantsbos Point (34° 15.495' S, 18° 22.909' E), Cape of Good Hope (34° 21.441' S, 18° 28.393' E), Buffel's Bay (34° 19.308' S, 18° 27.716' E) and Simon's town (34° 09.717' S, 18° 25.894' E) (Table 2.1). Two loggers were also placed 10km apart from the last known location of *E. lamorali* on either side of the Peninsula, i.e. one was placed 10km above Simon's town (St. James Walkway [34° 06.794' S, 18° 27.980' E]), another 10km above that (Strandfontein [34° 05.294' S, 18° 33.379' E]), and the same above Kommetjie (Hout Bay [34° 02.950' S, 18° 21.711' E] and Llandudno [34° 00.336' S, 18° 20.449' E]). The devices were programmed using ColdChain Thermo Dynamics (Version 4.9.2010.01.08.100) to record the temperature every two hours and all data were downloaded every four months from all sites.

As for the South African Weather Service data, empirical temperature data, as well as the rates of temperature change, collected by the iButtons were analyzed using two independent sample size-corrected, Akaike Information Criterion (AICc) based, reverse model building processes in R. Site, year, month and time of day (morning [6am-12pm], day [12pm-6pm], evening [6pm-12am] and night [12am-6am]) were all used as predictor factors for these models.

_____

Whether significant differences existed between the mean monthly maximum and minimum temperatures collected in the micro-climate by the iButtons, or at a macro-climate by the South African Weather Service was assessed using a Student's *t*-test for independent samples, while correlation between the two datasets was determined using regression analysis in Statistica 10 (StatSoft Inc., 2011).

2.3 Results

2.3.1 Nest structure

Lamoral (1968), van Noort (2009) and van Noort *et al.* (2014) outline the general structure of a *D. formidabilis* nest, where shells or crevices are lined with white silk and eggs are laid in wedge-like compartments lining the edge of the nest. This structure was evident for all individuals living on the Cape Peninsula in this study, with some webbing exhibiting a pale pink colouration. A single nest found at Pearly Beach comprised only one compartment.

Amaurobioides africanus is described as constructing white web-lined nests in crevices or under shells (Lamoral, 1968; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997; Filmer & Larsen, in press). It was found in this study that the species only completely lined its nest when housing an egg sac, which took the form of a single white silk-covered sac containing all the eggs at the back of the nest (furthest from the entrance hole). Where no eggs were present, individuals only constructed a 'door' to the nest, sealing off the hole or crevice in which it had taken residence, as opposed to lining it completely.

2.3.2 March survey

Of the 14 sites surveyed, *D. formidabilis* was found at 10 (all except Kenton-on-Sea, Jongensfontein, Silwerstroomstrand and Jacobsbaai) and *A. africanus* at 11 (all except Cape St. Francis, Silwerstroomstrand and Jacobsbaai) (Figure 2.1.A). *Echthrodesis lamorali* was only recorded emerging from *D. formidabilis* egg sacs collected at Kommetjie (Figure 2.1.A). Thus, overall, both spider species were found along the coastline from Kommetjie, east to East London with the parasitoid occurring only on one shore within that range (Figure 2.1.A) and attacking only one host.
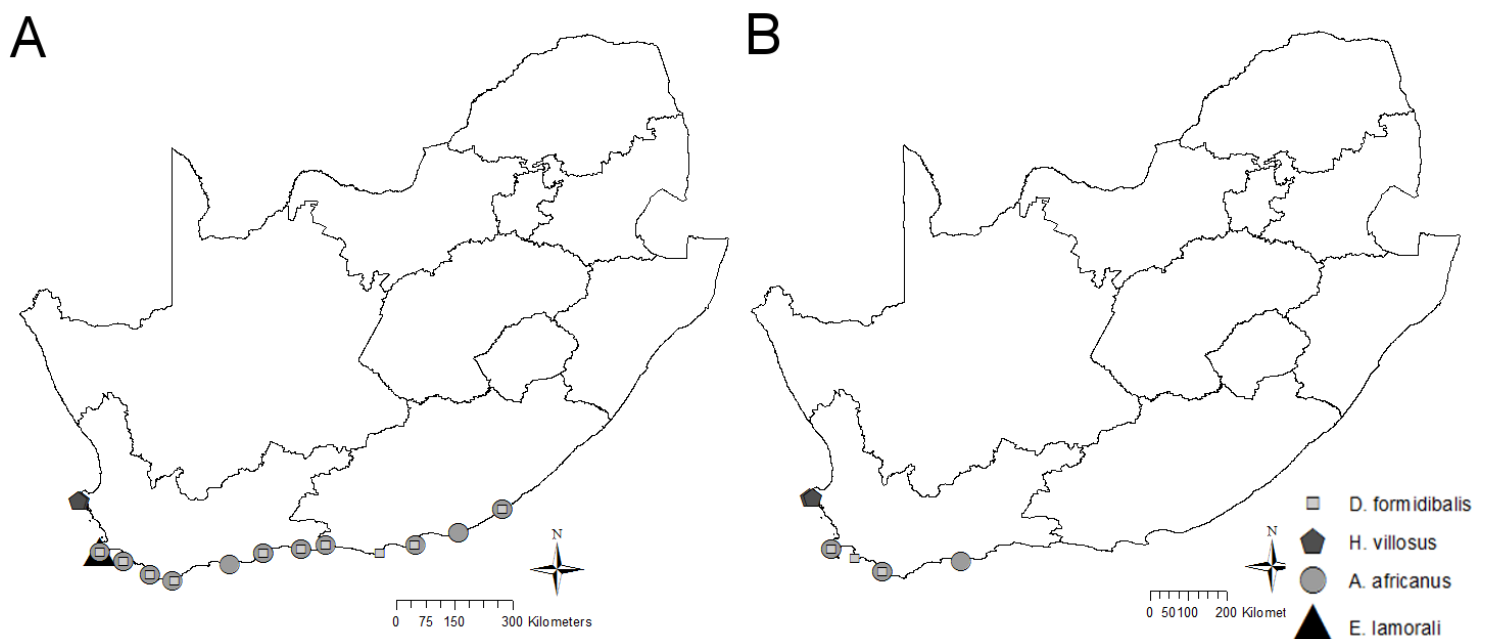
Figure 2.1: A: The distribution of *Desis formidabilis, Amaurobioides africanus, Heliophanus villosus* and *Echthrodesis lamorali* along the transect spanning from Jacobsbaai to Kidds Beach surveyed during this study in March 2012; B: The breeding distribution of *Desis formidabilis, Amaurobioides africanus* and *Heliophanus villosus* in March 2012.

The two sites north of Kommetjie, Silwerstroomstrand and Jacobsbaai, hosted only a previously unencountered species (in this study), a salticid (Jumping) spider (Figure 2.1.A). Nests resembled those of *A. africanus* on rock-faces, with a crack or crevice covered and lined in very stiff white silk just above the high tide mark. Most nests contained two spiders, a larger brown-green individual and a smaller black and white one, and some housed egg sacs, none of which gave rise to *E. lamorali* or any other parasitoid. Photographs and specimens of the unknown species were sent to Dr Charles Haddad (University of the Free State, South Africa) and Dr Wanda Wesolowska (Wroclaw University, Poland), and the spider was identified as *Heliophanus villosus* Wesolowska 1986 (Araneae: Salticidae), a species previously collected only on Signal Hill (part of Table Mountain, Cape Town, Western Cape, RSA) (Wesolowska, Wroclaw University, pers. comm.).

In March, *D. formidabilis* was found to be breeding only at sites west of Cape Agulhas, while *A. africanus* eggs were found and collected at only three sites (Jongensfontein, Pearly Beach and Kommetjie) (Figure 2.1.B). Localities where *D. formidabilis* is listed as 'not breeding' include shores where individual adult spiders were found, but no nests. *Heliophanus villosus* was found to be breeding in Jacobsbaai during the survey period (Figure 2.1.B).

Amaurobioides africanus nested in sheltered areas at all except two of the sites (Kidds Beach and Jongensfontein) (Figure 2.2). *Desis formidabilis* also appeared to favour sheltered shores, nesting on exposed rocks only at five sites (Kidds Beach, Summerstrand, Nature's Valley, Brenton-on-Sea and Mossel Bay) (Figure 2.2).
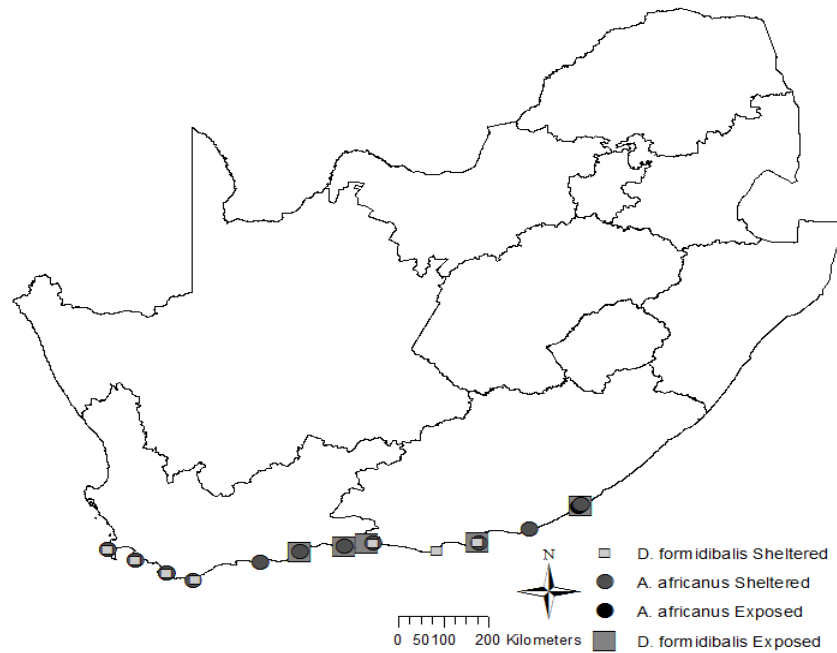
Figure 2.2: Relative exposure of different shores on which *Desis formidabilis* and *Amaurobioides africanus* were found nesting.

At Cape Agulhas, Pearly Beach and Kogelberg, *D. formidabilis* was found living behind tubeworm conglomerates, a *Gunnarea* sp. (Canalipalpata: Sabellariidae) (Figure 2.3.A), as reported by Tietz & Robinson (1974) for the Tsitsikamma shore, and by Lamoral (1968) in the more exposed areas of the Cape Peninsula. Other nesting sites used included bare rock-face at two of the exposed shores (Brenton-on-Sea and Mossel Bay); mussel beds at Summerstrand and Cape St. Francis (both sheltered beaches); live barnacles at Mossel Bay (exposed); and loose rocks at only two sheltered shores, Cape St. Francis and Kommetjie (Figures 2.2 and 2.3.A). *Amaurobioides africanus* displayed a much narrower range in nest location, favouring rocks at all sites except one, where it nested only under wedged *Donax serra* Dillwyn 1817 (Mollusca: Bivalvia) shells on a highly exposed shore (Jongensfontein) (Figure 2.3.B).
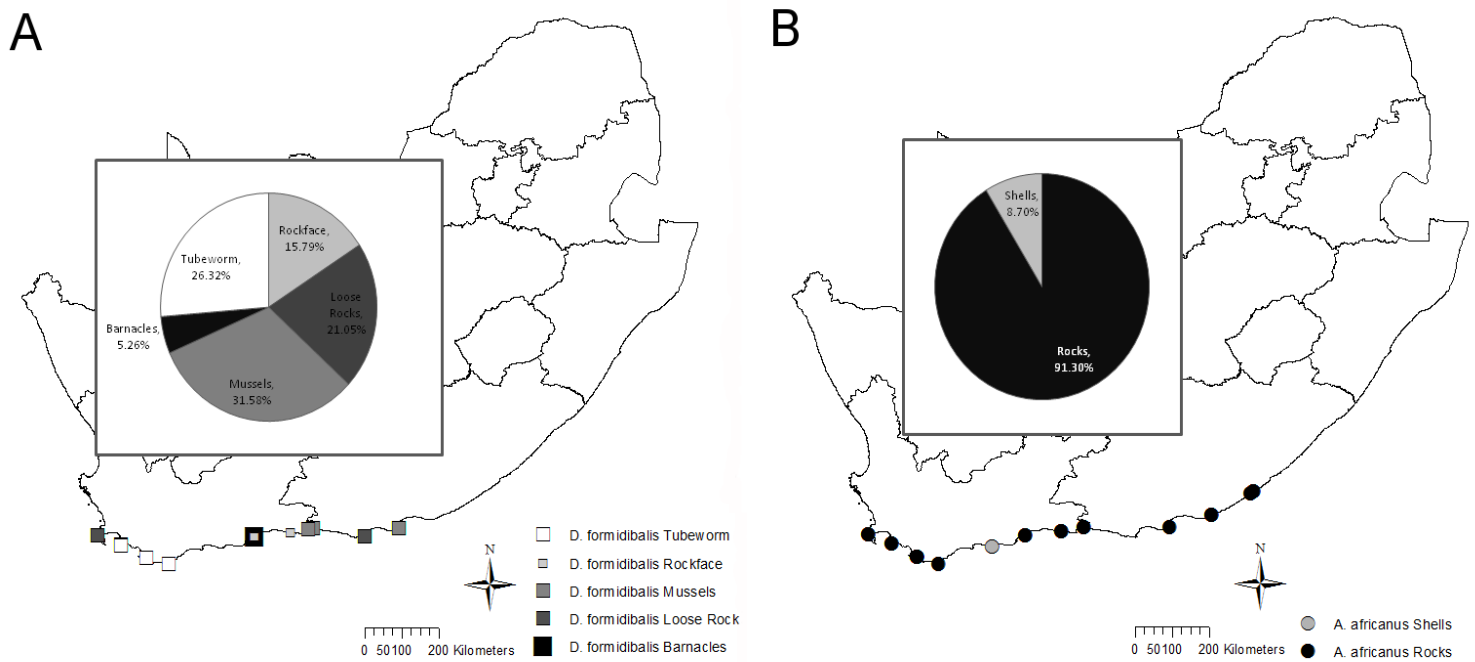
Figure 2.3: A: Nesting material/site choice displayed by *Desis formidabilis* in March 2012 (numbers in pie chart indicate percentage of individuals found nesting in that site/material); B: Nesting material/site choice displayed by *Amaurobioides africanus* in March 2012 (numbers in pie chart indicate percentage of individuals found nesting in that site/material).

Finally, although Filmer (1995) and Lamoral (1968) report both spider species to be mainly nocturnal, *D. formidabilis* specimens were observed to forage during the day and night at all sites from Summerstrand to Brenton-on-Sea. Nocturnal activity only (no diurnal foraging) was seen at all sites west of Mossel Bay.

2.3.3 November survey

Neither spider species was found at as many sites in November as in March, with *D. formidabilis* specimens located only along the Cape Peninsula and Kogelberg, and *A. africanus* not as prevalent in areas east of the Peninsula (Figure 2.4.A). The local scale survey

showed the distributional range of *D. formidabilis* to end only 200m north of 'The Point', Kommetjie (Figure 2.4.A). *Amaurobioides africanus* individuals were located 10km above that, but none were found any further (Figure 2.4.A).
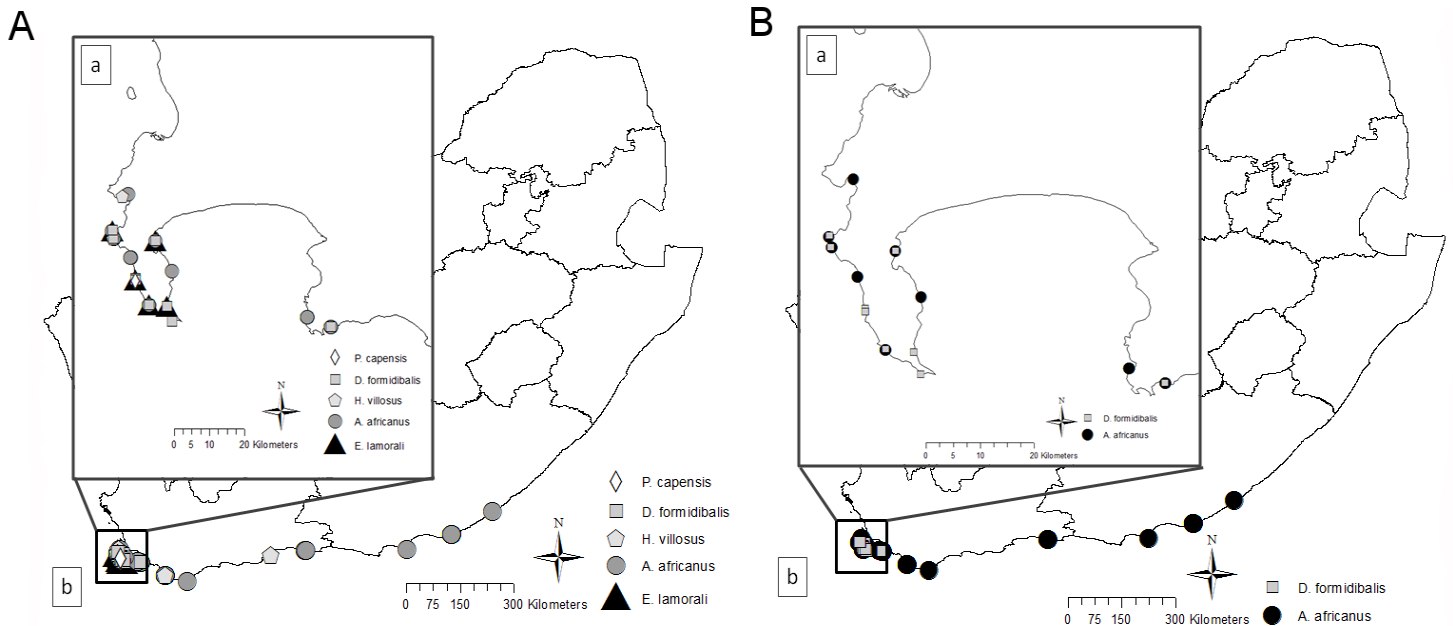
Figure 2.4: A: The distribution of *Palpimanus capensis*, *Desis formidabilis, Heliophanus villosus, Amaurobioides africanus* and *Echthrodesis lamorali* along both transects (a - Cape Peninsula and b – entire survey area) surveyed during this study in November 2012; B: The breeding distribution of *Desis formidabilis* and *Amaurobioides africanus* along both transects (a - Cape Peninsula and b – entire survey area) in November 2012.

The salticid previously only located north of Kommetjie was found in two other areas (Pearly Beach and Mossel Bay) during the second survey (Figure 2.4.A), both of which are new records. Furthermore, another new species, identified by Norman Larsen (Iziko Museums of Cape Town, South Africa) as *Palpimanus capensis* Simon 1893 (Araneae: Palpimanidae), was discovered and collected just below the high-water mark at Olifantsbos Point (Table

Mountain National Park, Cape Peninsula). Nests resembled those of *A. africanus*, taking the form of a white silk-lined crevice with a single individual and one round web-lined egg-case inside. This is also a new distribution record for the group. Finally, the distribution of *E. lamorali* was shown to be larger than originally found (Figure 2.4.A), occurring throughout much of the Peninsula south of Kommetjie as far as Simon's Town, barring a large inaccessible area along the eastern side of the Peninsula. No *E. lamorali* or any other parasitoids were reared from *A. africanus* eggs. Unlike in March (Figure 2.1.B), *D. formidabilis* and *A. africanus* were breeding at all sites where found in November (Figure 2.4.B).

In November, the vast majority of the eastern sites were distinctively visually different from when they were surveyed in March, particularly with a marked reduction in invertebrate covering of the rocks (algae, mollusc and other encrusting intertidal organisms) (Figure 2.5). Both spider species displayed a clear preference for sheltered shores as *D. formidabilis* was not found in any exposed areas and *A. africanus* was located at only one exposed site (Kenton-On-Sea) (Figure 2.6).
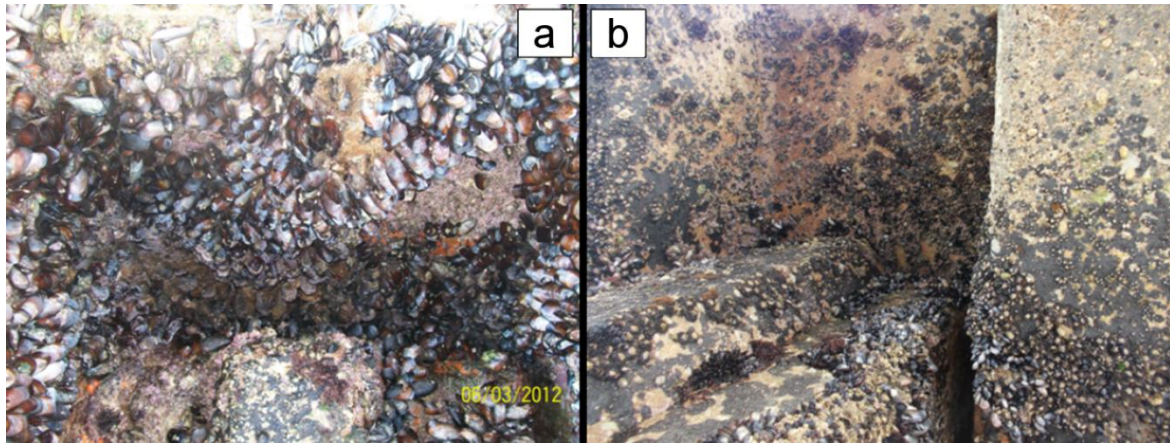
Figure 2.5: The same locality in Summerstrand (33° 58' 47.892" S, 25° 39' 31.0674" E) in March 2012 (A) and November 2012 (B) showed marked visual differences, with a great reduction in invertebrate covering of the intertidal rocks.
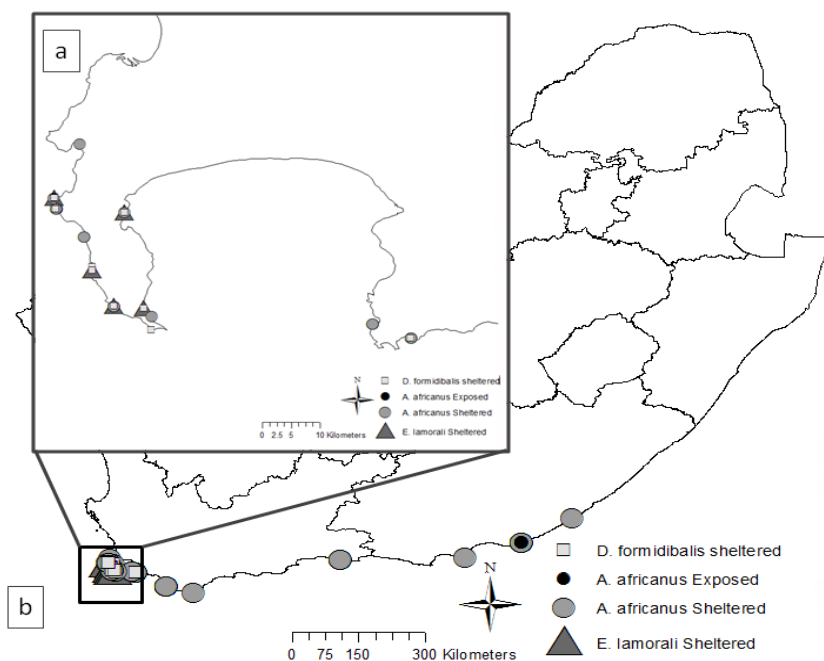
Figure 2.6: Relative exposure of different shores on which *Desis formidabilis, Amaurobioides africanus* and *Echthrodesis lamorali* were located in November 2012 (a - Cape Peninsula and b – entire survey area).

_____

Finally, unlike in March, no *D. formidabilis* nests were found amongst mussels, barnacles or in crevices in rockfaces in November, while some were found in snail shells (Figure 2.7.A). Furthermore, and again not following the results collected in March, no *A. africanus* individuals were found under shells in November (Figure 2.7.B), with all restricted only to cracks and crevices between loose rocks and in rock-faces (Figure 2.7.B). *Echthrodesis lamorali* was found in most nesting material choices (Figure 2.7.C), suggesting no preference for a certain nest site/type.

2.3.4 Spider species recorded

A total of four spider species were found living in the intertidal zone in this study (Table 2.3). Two of these, *D. formidabilis* and *A. africanus* are included in Haddad & Dippenaar-Schoeman's (2009) list as living in this zone (Table 2.3). *Heliophanus villosus* may have been included in their study (Table 2.3), but a species identification is not provided, so the records here may be novel. While Haddad & Dippenaar-Schoeman (2009) do not mention *P. capensis*, Larsen (2012) provides a record of the species feeding in the intertidal region at Kommetjie. Thus, the locality recorded in this study for Olifantsbos Point (34° 15' 30.888" S, 18° 22' 52.284" E) is a new distribution record (Table 2.3).
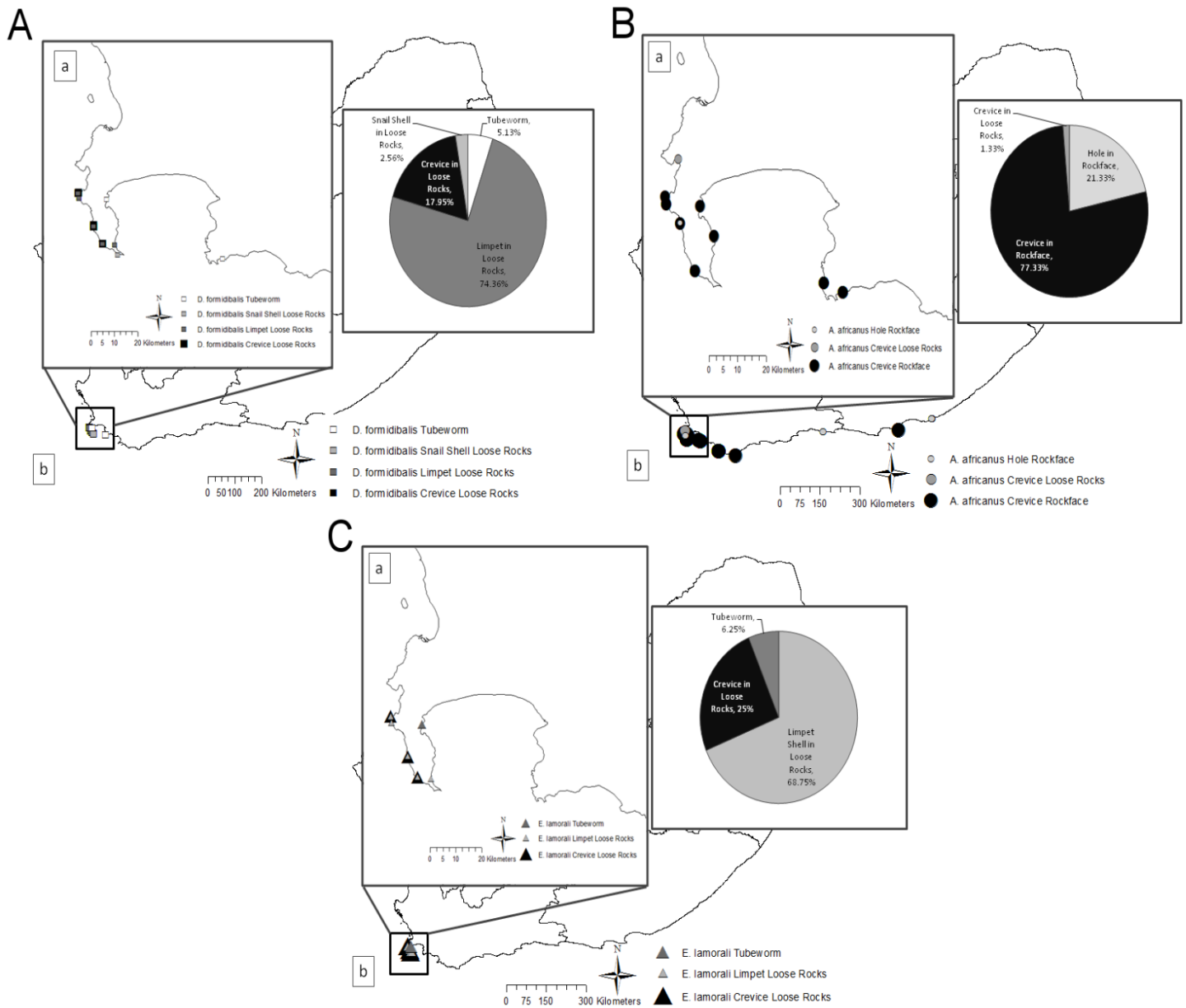
Figure 2.7: A: Nesting material/site choice displayed by *Desis formidabilis* in November 2012; B: Nesting material/site choice displayed by *Amaurobioides africanus* in November 2012; C: Nesting material/site choice displayed by *Echthrodesis lamorali* in November 2012 (a - Cape Peninsula and b – entire survey area; numbers in pie chart indicate percentage of individuals found nesting in that site/material).

_____

Table 2.3: All spider species recorded in the intertidal region in southern Africa by Haddad & Dippenaar-Schoeman (2009) and Larsen (2012) (white cells) and additional records found in this study (grey cells).

Recorded by	Family	Species
Haddad & Dippenaar-Schoeman (2009) and present study	Anyphaenidae	*Amaurobioides africanus* Hewitt 1917
Haddad & Dippenaar-Schoeman (2009)	Araneidae	*Larinia natalensis* Grasshoff 1971
Haddad & Dippenaar-Schoeman (2009) and present study	Desidae	*Desis formidabilis* O. P. Cambridge 1890
Haddad & Dippenaar-Schoeman (2009)	Salticidae	*Heliophanus* sp. 4
Present study	Salticidae	*Heliophanus villosus* Wesolowska 1986
Haddad & Dippenaar-Schoeman (2009)	Salticidae	*Massagris regina* Wesolowska 1993
Haddad & Dippenaar-Schoeman (2009)	Theridiidae	*Steatoda capensis* Hann 1990
Haddad & Dippenaar-Schoeman (2009)	Zoropsidae	*Griswoldia robusta* Simon 1898
Haddad & Dippenaar-Schoeman (2009)	Atemnidae	*Cyclatemnus* sp.
Haddad & Dippenaar-Schoeman (2009)	Geogarypidae	*Geogarypus purcelli* Ellingsen 1912
Larsen (2012) and present study	Palpimanidae	*Palpimanus capensis* Simon 1893

2.3.5 Seasonality

Throughout the entire survey year, *D. formidabilis* was found to be breeding at Kommetjie. Due to the difficulty in finding nests hidden in the holdfasts of the mussel beds at Summerstrand, as they were easily destroyed during mussel removal and beds changed frequently with time and stochastic oceanographic events, *A. africanus* was used as a proxy for seasonality behaviour at this site (Table 2.4). The spider's breeding season appeared to begin in August 2012 and end in February 2013, from early Spring to late Summer in South Africa (Table 2.4).

_____

Table 2.4: Breeding state of the Kommetjie and Summerstrand populations from March 2012 to March 2013 (Black – Breeding; Grey – Not Breeding; White – Not Surveyed).

Shore	Kommetjie														
	Summerstrand														
Month		Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Year		2012										2013		

2.3.6 Climate data

Macro-climatic conditions were different between sites within the wasp's distribution and those outside of it for certain components, while no temperature patterns were evident concerning the distributional range in the microclimate data. The weak correlation between the two datasets (micro- and macro-climates) and statistically significant differences between temperatures recorded at the two scales provide supporting reasons for this disparity in results.

2.3.6.1 Macro-climatic conditions

All climatic components analysed (temperature, rainfall, wind speed and humidity) showed much variation within the years assessed, across them and between different sites, although seasonality is obvious in temperature, rainfall and wind patterns across the months. Sites falling within the distributional range of *E. lamorali* proved to boast significantly different mean monthly maximum temperatures and mean monthly humidities, but no differences existed in the patterns of the amount of rainfall and wind strength.

_____

2.3.6.1.1 Temperature

The Akaike Information Criterion assessment (AICc) of monthly maximum and minimum temperatures recorded by the 22 weather stations resulted in only one usable model (delta<2) for each outcome variable, although these models included all predictor variables (site, year and month) (Table 2.5). For monthly maximum temperatures, significant differences were detected between most months, except for between January and February; April and November; and June and July (Figure 2.8.A). A similar pattern is evident in the monthly minimum temperatures, with figures recorded for January and February; March and December; and July and August being statistically similar and all other months different (Figure 2.8.A). Both maximum and minimum temperatures declined in the middle of the year and increased towards the end of it, indicating the winter months (Figure 2.8.A). No clear patterns were evident for the yearly changes in monthly maximum and minimum temperatures with 2003 and 2009; and 2008 and 2010 experiencing statistically similar maximum temperatures, and 2005, 2006 and 2008 experiencing statistically similar minimum temperatures (Figure 2.8.B). Finally, the most important observation from the comparisons between maximum monthly temperatures at different sites was the statistical similarity and dissimilarity between sites within the distribution of *E. lamorali* (Slangkop and Cape Point) and outside of it respectively (Figure 2.8.C). These sites reached significantly cooler maximum temperatures, with only Cape Columbine experienced statistically similar results (Figure 2.8.C). Similarities were also evident in monthly maximum temperatures between Dassen Island and Kirstenbosch; the South African Astronomical Observatory, Cape Town, Molteno Reservoir, Strand, George, Knysna, Tsitsikamma and Port Elizabeth; Atlantis and East London; and between Hermanus, Cape Agulhas, Struisbaai, Plettenberg Bay and Cape St. Francis (Figure 2.8.C). The distinction between sites within the wasp's distribution and outside of it does not hold for the minimum monthly temperatures (Figure 2.8.C). For this

temperature range, statistical similarities were detected between Cape Columbine and Cape Town; the South African Astronomical Observatory, Cape Point, Struisbaai and Knysna; Molteno Reservoir and East London; Kirstenbosch, Port Elizabeth and Port Alfred; and Strand, Hermanus and Cape St. Francis (Figure 2.8.C).

Table 2.5: Contingency table of the models using month, site and year as predictors of monthly maximum and minimum temperatures (Temp.).

	Estimate		Standard Error		t-Value		P-Value	
	Monthly Maximum Temp.	Monthly Minimum Temp.	Monthly Maximum Temp.	Monthly Minimum Temp.	Monthly Maximum Temp.	Monthly Minimum Temp.	Monthly Maximum Temp.	Monthly Minimum Temp.
Intercept (Month_April; Site_Atlantis; 0)	68.503	65.197	16.644	14.784	4.116	4.410	0.000	0.000
Month_August	-4.360	-4.289	0.117	0.103	-37.393	-41.544	0.000	0.000
Month_December	1.410	1.991	0.117	0.103	12.078	19.283	0.000	0.000
Month_February	2.974	3.405	0.117	0.103	25.479	32.982	0.000	0.000
Month_January	2.792	3.288	0.117	0.103	23.914	31.845	0.000	0.000
Month_July	-3.969	-4.377	0.117	0.103	-34.042	-42.395	0.000	0.000
Month_June	-3.763	-3.585	0.117	0.103	-32.235	-34.681	0.000	0.000
Month_March	2.108	2.067	0.117	0.103	18.061	20.022	0.000	0.000
Month_May	-2.069	-1.698	0.117	0.103	-17.703	-16.423	0.000	0.000
Month_November	-0.171	0.323	0.117	0.103	-1.465	3.136	0.143	0.002
Month_October	-1.661	-1.125	0.117	0.103	-14.228	-10.892	0.000	0.000
Month_September	-3.264	-2.938	0.117	0.103	-27.962	-28.491	0.000	0.000
Site_Cape Agulhas	-2.604	4.120	0.156	0.138	-16.666	29.952	0.000	0.000
Site_Cape Columbine	-3.964	1.695	0.157	0.138	-25.316	12.296	0.000	0.000
Site_Cape Point	-4.021	2.790	0.156	0.138	-25.732	20.283	0.000	0.000
Site_Cape St. Francis	-3.402	3.413	0.163	0.157	-20.911	21.772	0.000	0.000
Site_Cape Town	-0.983	1.396	0.156	0.138	-6.288	10.148	0.000	0.000

_____

Site_Dassen Island	-4.541	2.650	0.175	0.154	-25.993	17.229	0.000	0.000
Site_East London	-0.144	3.723	0.156	0.138	-0.923	27.063	0.356	0.000
Site_George	-1.638	1.100	0.156	0.138	-10.479	7.997	0.000	0.000
Site_Hermanus	-2.677	3.308	0.156	0.138	-17.130	24.052	0.000	0.000
Site_Kirstenbosch	-1.681	1.939	0.156	0.138	-10.757	14.098	0.000	0.000
Site_Knysna	-0.703	2.543	0.156	0.138	-4.501	18.484	0.000	0.000
Site_Langebaan	0.343	0.591	0.156	0.138	2.192	4.305	0.028	0.000
Site_Molteno Reservoir	-1.244	3.948	0.156	0.138	-7.962	28.698	0.000	0.000
Site_Plettenberg Bay	-2.787	2.961	0.156	0.138	-17.834	21.525	0.000	0.000
Site_Port Alfred	-0.371	2.125	0.156	0.138	-2.373	15.449	0.018	0.000
Site_Port Elizabeth	-0.803	2.233	0.156	0.138	-5.136	16.230	0.000	0.000
Site_SA Astronomical Observatory	-0.673	3.277	0.156	0.138	-4.304	23.821	0.000	0.000
Site_Slangkop	-3.728	2.379	0.156	0.138	-23.860	17.297	0.000	0.000
Site_Strand	-1.043	3.106	0.156	0.138	-6.677	22.579	0.000	0.000
Site_Struisbaai	-3.089	2.863	0.156	0.138	-19.770	20.810	0.000	0.000
Site_Tsitsikamma	-1.238	3.671	0.156	0.138	-7.920	26.687	0.000	0.000
Year	-0.022	-0.027	0.008	0.007	-2.654	-3.651	0.008	0.000

Figure 2.8: Statistically significant differences (as indicated by letters above points or bars) in macro-climate mean monthly temperatures supplied by the South African Weather Service for different A: months; B: years; and C: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*) illustrated seasonality and a distinction in maximum temperatures between within the distributional range of *Echthrodesis lamorali* and outside of it.

2.3.6.1.2 Rainfall

Two models were outlined by the Akaike Information Criterion test (AICc) as appropriate (delta<2) for explaining the monthly rainfall patterns, with the final model using all possible predictors (site, year and month) (Table 2.6). As with temperature, monthly macro-climate rainfall patterns illustrated seasonality, with an increase in rain in the mid-year period (Figure 2.9.A). A statistically similar amount of rain was recorded for January and February; March and December; April, September, October and November; and June, July and August (Figure 2.9.A). Rainfall appears to increase along a west to east gradient along the coastline, but with similar readings for Cape Columbine and Langebaan; Dassen Island, Cape Town, Atlantis, Slangkop, Cape Point, Hermanus, Cape Agulhas and Struisbaai; the South African Astronomical Observatory, Molteno Reservoir, Strand, George, Plettenberg Bay, Cape St. Francis, Port Elizabeth and Port Alfred; and Knysna and Tsitsikamma (Figure 2.9.B). Sites within the range of *E. lamorali* were not distinct from those outside of its distribution.

Table 2.6: Contingency table of the model using month, site and year as predictors of macro-climate monthly rainfall.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Month_April; Site_Atlantis; 0)	-133.316	638.453	-0.209	0.835
Month_August	29.413	4.439	6.626	0.000
Month_December	-15.837	4.444	-3.563	0.000
Month_February	-23.529	4.450	-5.288	0.000
Month_January	-24.080	4.444	-5.418	0.000
Month_July	26.895	4.444	6.052	0.000
Month_June	28.792	4.450	6.471	0.000
Month_March	-13.815	4.455	-3.101	0.002

_____

Month_May	17.565	4.450	3.947	0.000
Month_November	-2.317	4.444	-0.521	0.602
Month_October	3.747	4.444	0.843	0.399
Month_September	-3.988	4.439	-0.898	0.369
Site_Cape Agulhas	-1.135	5.907	-0.192	0.848
Site_Cape Columbine	-16.736	5.907	-2.833	0.005
Site_Cape Point	-8.218	5.907	-1.391	0.164
Site_Cape St. Francis	18.512	6.705	2.761	0.006
Site_Cape Town	4.162	5.907	0.705	0.481
Site_Dassen Island	-10.202	6.533	-1.562	0.118
Site_East London	32.210	5.907	5.453	0.000
Site_George	23.072	5.907	3.906	0.000
Site_Hermanus	5.088	5.907	0.861	0.389
Site_Kirstenbosch	74.413	5.907	12.597	0.000
Site_Knysna	41.928	5.907	7.098	0.000
Site_Langebaan	-15.979	6.165	-2.592	0.010
Site_Molteno Reservoir	20.191	5.907	3.418	0.001
Site_Plettenberg Bay	19.599	5.907	3.318	0.001
Site_Port Alfred	19.083	5.920	3.224	0.001
Site_Port Elizabeth	14.781	5.907	2.502	0.012
Site_SA Astronomical Observatory	13.653	5.907	2.311	0.021
Site_Slangkop	7.973	5.907	1.350	0.177
Site_Strand	11.614	5.907	1.966	0.049
Site_Struisbaai	-3.721	5.907	-0.630	0.529
Site_Tsitsikamma	41.649	5.907	7.051	0.000
Year	0.084	0.318	0.263	0.792

Figure 2.9: Statistically significant differences (as indicated by letters above points or bars) in macro-climate mean monthly rainfall supplied by the South African Weather Service for different A: months; and B: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*) illustrated some seasonality but no distinction in rainfall between within the distributional range of *Echthrodesis lamorali* and outside of it.

2.3.6.1.3 Wind

As for rainfall, the Akaike Information Criterion analysis (AICc) identified two appropriate models (delta<2) for outlining the effect of site, year and month on wind strength. The final model results are illustrated in Table 2.7. Seasonal difference were visible in macro-climate wind speeds with generally slower winds in the mid-year period, coinciding with the increase in rain and decline in temperatures (Figures 2.8, 2.9 and 2.10). Winds experienced in January, October, November and December; March, April, May and July; and August and September were all statistically similar (Figure 2.10). While Cape Point (located within the distribution of *E. lamorali*) experienced significantly higher winds than any other site, this trend was not

carried over to the other site within the wasp's range, Slangkop. Statistically similar speeds were recorded for Langebaan, George and Plettenberg Bay; Dassen Island, Struisbaai, Port Elizabeth and East London; the South African Astronomical Observatory, Atlantis and Tsitsikamma and between Cape Town and Cape Agulhas; Strand, Hermanus and Port Alfred (Figure 2.10).

Table 2.7: Contingency table of the model using month, site and year as predictors of macro-climate monthly wind speed.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Month_April; Site_Atlantis; 0)	-12.328	10.902	-1.131	0.258
Month_August	0.325	0.074	4.372	0.000
Month_December	0.753	0.074	10.146	0.000
Month_February	0.299	0.074	4.021	0.000
Month_January	0.583	0.075	7.828	0.000
Month_July	0.133	0.074	1.794	0.073
Month_June	0.205	0.074	2.754	0.006
Month_March	0.045	0.074	0.610	0.542
Month_May	0.063	0.074	0.841	0.401
Month_November	0.696	0.074	9.386	0.000
Month_October	0.622	0.074	8.364	0.000
Month_September	0.310	0.074	4.170	0.000
Site_Cape Agulhas	1.835	0.143	12.846	0.000
Site_Cape Columbine	2.950	0.143	20.659	0.000
Site_Cape Point	6.240	0.143	43.697	0.000
Site_Cape St. Francis	3.520	0.151	23.378	0.000
Site_Cape Town	1.777	0.143	12.444	0.000
Site_Dassen Island	2.140	0.150	14.291	0.000
Site_East London	2.050	0.143	14.358	0.000

Site_George	0.520	0.143	3.644	0.000
Site_Hermanus	1.132	0.143	7.927	0.000
Site_Kirstenbosch	-0.476	0.175	-2.715	0.007
Site_Knysna	-1.055	0.143	-7.384	0.000
Site_Langebaan	0.604	0.143	4.228	0.000
Site_Molteno Reservoir	-0.542	0.143	-3.796	0.000
Site_Plettenberg Bay	0.633	0.143	4.432	0.000
Site_Port Alfred	1.165	0.143	8.161	0.000
Site_Port Elizabeth	2.144	0.143	15.011	0.000
Site_SA Astronomical Observatory	-0.178	0.172	-1.036	0.300
Site_Slangkop	2.589	0.143	18.127	0.000
Site_Strand	1.173	0.143	8.213	0.000
Site_Struisbaai	2.166	0.143	15.169	0.000
Site_Tsitsikamma	0.176	0.143	1.234	0.217
Year	0.007	0.005	1.293	0.196
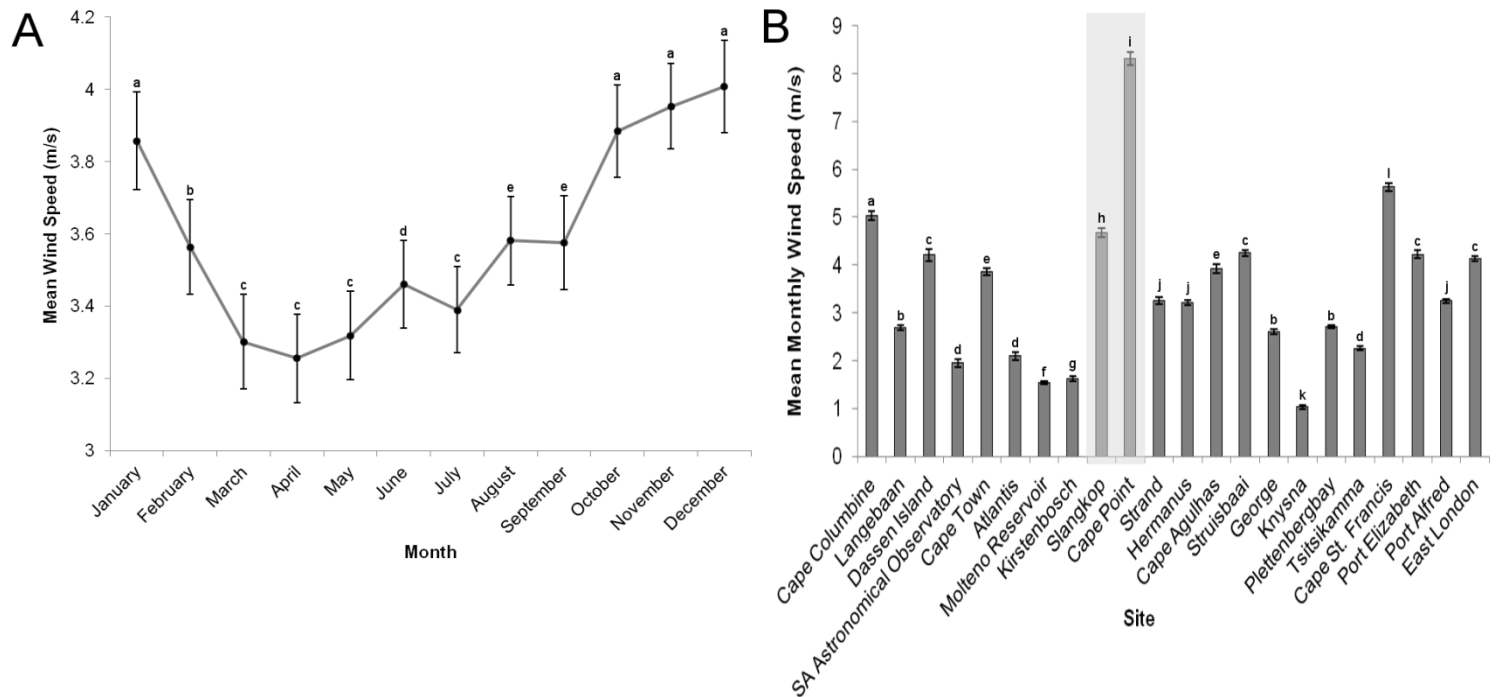
Figure 2.10: Statistically significant differences (as indicated by letters above points or bars) in macro-climate mean monthly wind speed supplied by the South African Weather Service for different A: months; and B: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*) illustrated some seasonality but no distinction in rainfall between within the distributional range of *Echthrodesis lamorali* and outside of it.

2.3.6.1.4 Humidity

The Akaike Information Criterion analysis (AICc) identified only one model that was needed to adequately describe the effect of all predictors (site, year and month) on the macro-climate mean monthly humidity (Table 2.8). Seasonality was not as distinct for mean monthly humidity as it was for the other variables, with statistically similar readings only in March, April and May (Figure 2.11.A). Across the years, however, a decline is evident, with 2003 and 2004; 2005 and 2006; and 2007 to 2012 all forming individual groups with statistically similar humidities (Figure 2.11.B). The sites falling within the distributional range of *E.*

_____

lamorali were statistically distinct from all other sites, despite not being the largest or smallest readings (Figure 2.11.C). The South African Astronomical Observatory, Atlantis, Kirstenbosch, Hermanus, Cape Agulhas, George, Tsitsikamma, Port Elizabeth and Port Alfred all experienced statistically similar humidities, with all other sites ranging around them (Figure 2.11.C).

Table 2.8: Contingency table of the model using month, site and year as predictors of macro-climate monthly humidity.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Month_April; Site_Atlantis; 0)	958.223	104.046	9.210	0.000
Month_August	-1.922	0.696	-2.760	0.006
Month_December	-8.396	0.697	-12.040	0.000
Month_February	-1.964	0.695	-2.825	0.005
Month_January	-4.929	0.695	-7.090	0.000
Month_July	-3.876	0.696	-5.568	0.000
Month_June	-1.737	0.695	-2.499	0.013
Month_March	-0.900	0.693	-1.298	0.194
Month_May	0.482	0.696	0.693	0.489
Month_November	-7.727	0.696	-11.098	0.000
Month_October	-5.090	0.698	-7.288	0.000
Month_September	-3.057	0.697	-4.384	0.000
Site_Cape Agulhas	-0.501	1.314	-0.381	0.703
Site_Cape Columbine	5.545	1.341	4.134	0.000
Site_Cape Point	4.264	1.325	3.217	0.001
Site_Cape St. Francis	4.730	1.282	3.691	0.000
Site_Cape Town	3.102	1.264	2.454	0.014
Site_Dassen Island	8.298	1.442	5.755	0.000
Site_East London	-4.081	1.264	-3.229	0.001

_____

Site_George	1.102	1.264	0.872	0.383
Site_Hermanus	2.219	1.264	1.755	0.079
Site_Kirstenbosch	-1.333	1.550	-0.860	0.390
Site_Knysna	4.904	1.290	3.802	0.000
Site_Langebaan	3.202	1.264	2.533	0.011
Site_Molteno Reservoir	-8.057	1.271	-6.341	0.000
Site_Plettenberg Bay	-3.130	1.314	-2.381	0.017
Site_Port Alfred	0.535	1.264	0.423	0.672
Site_Port Elizabeth	0.910	1.264	0.720	0.472
Site_SA Astronomical Observatory	-1.772	1.520	-1.166	0.244
Site_Slangkop	3.430	1.321	2.597	0.009
Site_Strand	-3.431	1.264	-2.715	0.007
Site_Struisbaai	6.969	1.272	5.478	0.000
Site_Tsitsikamma	2.258	1.290	1.750	0.080
Year	-0.437	0.052	-8.449	0.000
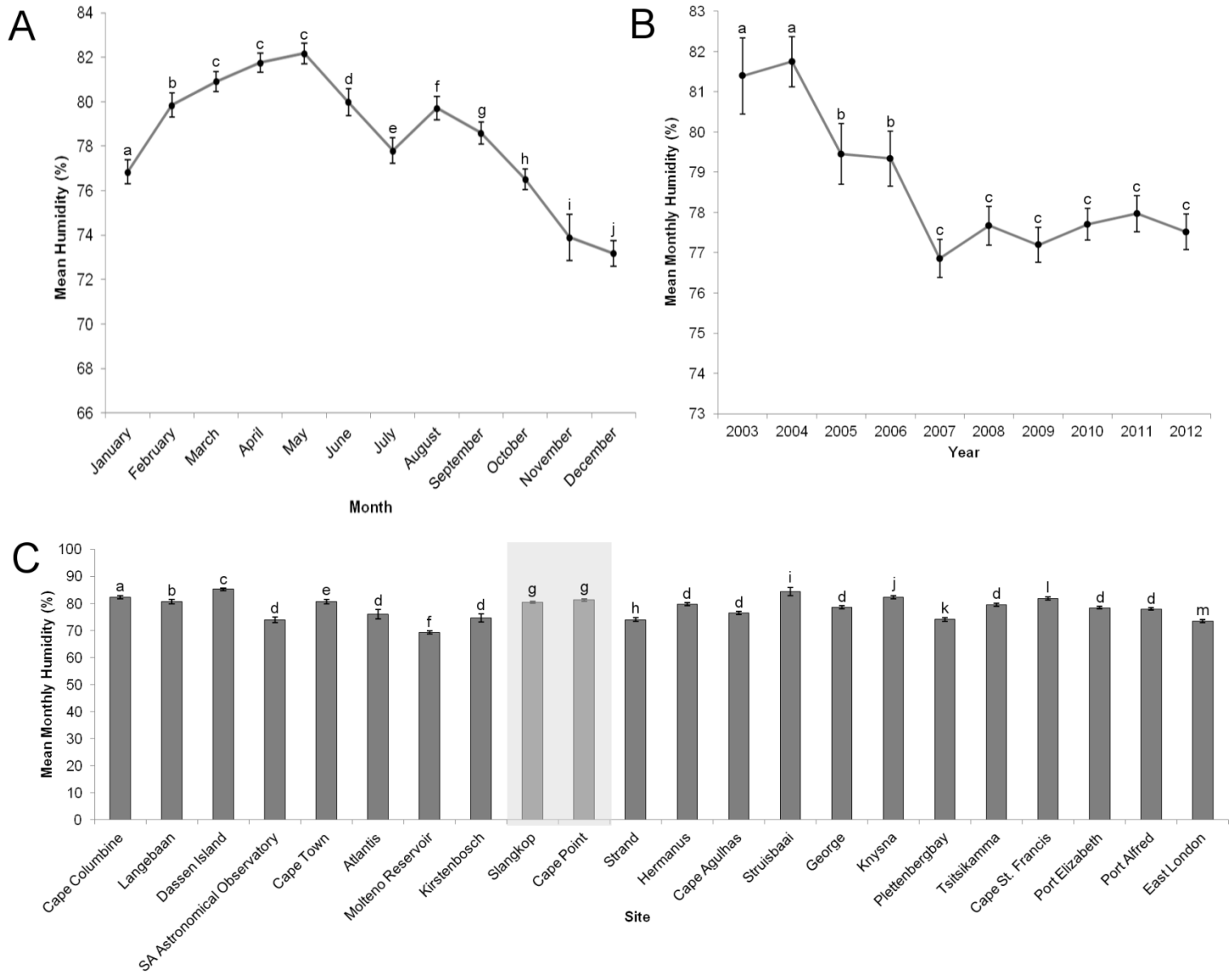
Figure 2.11: Statistically significant differences (as indicated by letters above points or bars) in macro-climate mean monthly humidity supplied by the South African Weather Service for different A: months; B: years; and c: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*) illustrated no seasonality, a general decline in humidity over the years distinct figures within the distributional range of *Echthrodesis lamorali*.

2.3.6.2 Microclimate conditions

No patterns were evident concerning both the empirical temperature and temperature ramp data differences between sites within the distribution of _E. lamorali_ and those outside of it, with consistent statistically significant differences between all sites. Temperatures recorded by the iButtons range from a minimum of 7ºC up to a maximum of 51.5ºC, with a mean value of 16.31ºC ±0.03 SE, and a mean ramp rate of 0.73ºC/h ±0.01 SE. Temperature ramps ranged from a minimum of no change to a maximum of 14ºC/h.

2.3.6.2.1 Temperature

Year and month were found to be co-linear for these data and as such could not be used in model building processes. Only one model, combining site and time of day as predictor variables, was identified by Akaike Information Criterion analysis (AICc) as significantly impacting the daily temperatures recorded by the iButtons (Table 2.9).

_____

Table 2.9: Contingency table of the model using site and time of day as predictors of micro-climate daily temperatures.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Site_10km Above Kommetjie; Time_Day)	21.395	0.091	234.569	0.000
Site_10km Above Simon's Town	-1.166	0.142	-8.199	0.000
Site_20km Above Kommetjie	-2.515	0.142	-17.663	0.000
Site_Buffels Bay	-3.399	0.110	-31.009	0.000
Site_Kommetjie	-2.404	0.093	-25.956	0.000
Site_Olifantsbos	-4.515	0.101	-44.545	0.000
Site_Simon's Town	-0.155	0.102	-1.525	0.127
Time_Evening	-3.347	0.077	-43.508	0.000
Time_Morning	-3.512	0.077	-45.668	0.000
Time_Night	-4.827	0.077	-62.743	0.000

No patterns in temperatures between sites within or outside of the distribution of *E. lamorali* were clear, with significantly similar temperatures at the sites 10km above Kommetjie and Simon's Town; and 20km above Kommetjie and Kommetjie, while all other sites were statistically different (Figure 2.12.A). Time of day proved to be a large controlling factor in temperatures, with statistically significant differences between all time periods. Maximums were reached during the 'day' (12pm-6pm) and minimums during the 'night' (12am-6am) (Figure 2.12.B).
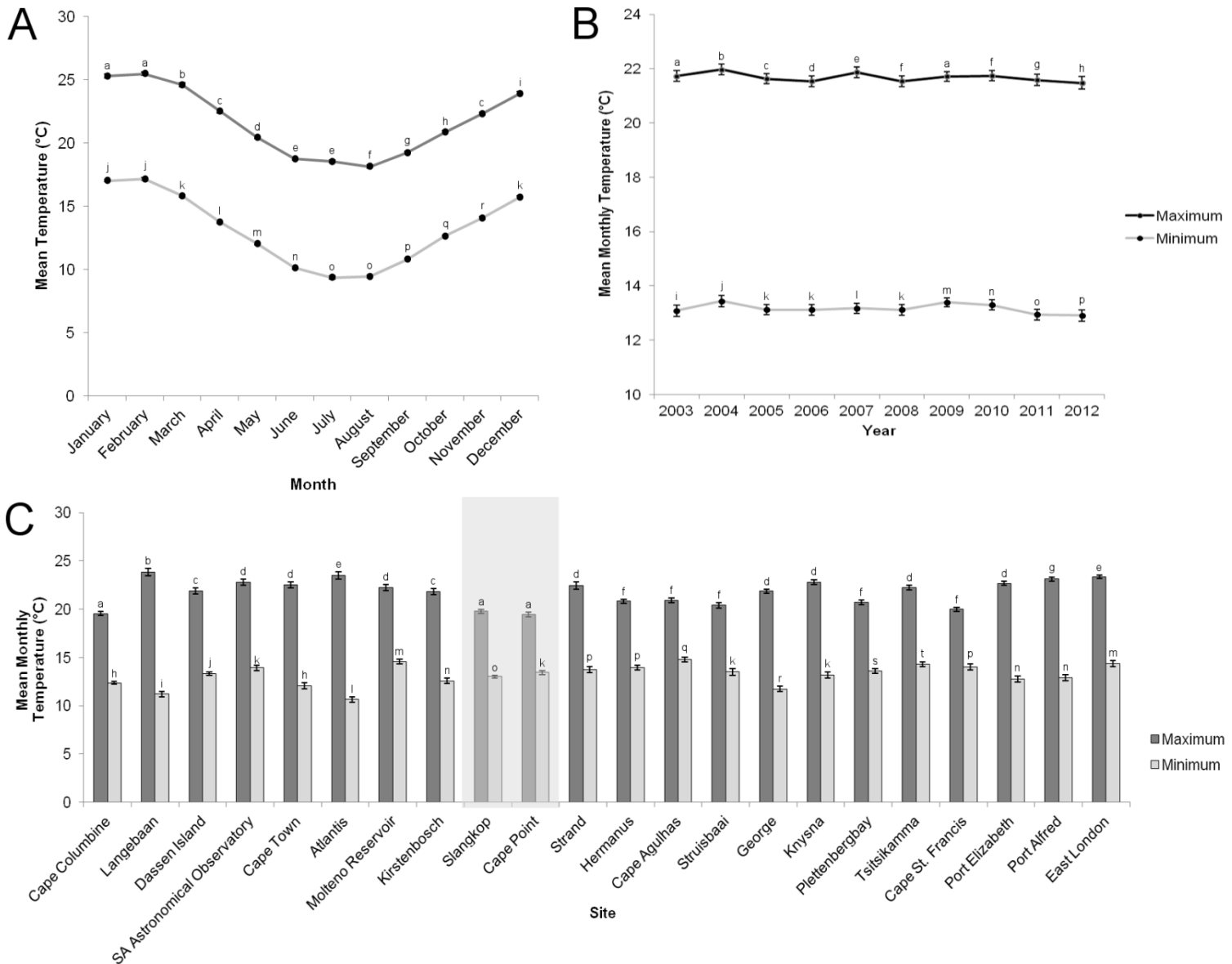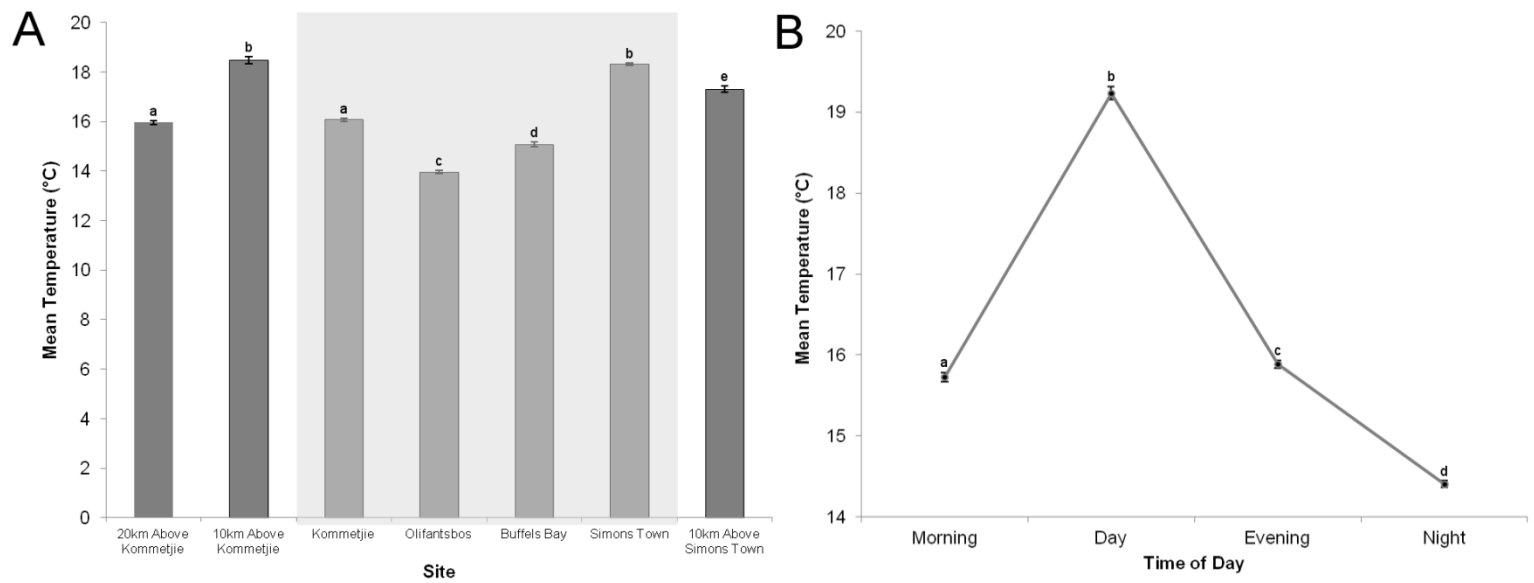
Figure 2.12: Statistically significant differences (as indicated by letters above points or bars) in micro-climate mean temperatures for different A: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*); and B: times of day illustrated no patterns concerning the distributional range of *Echthrodesis lamorali*, although temperatures did change significantly throughout the course of the day.

2.3.6.2.2 Temperature ramps

As for empirical temperatures, only one model was identified by Akaike Information Criterion tests (AICc) that explained the rate at which temperatures changed (Table 2.10). Year and month were again found to be co-linear, leaving site, time of day and empirical temperature as the only predictive factors used in the final model (Table 2.10).

Table 2.10: Contingency table of the model using site, time of day and empirical temperature as predictors of micro-climate daily temperature ramps.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Site_10km Above Kommetjie; 0, Time_Day)	-0.275	0.048	-5.732	0.000
Site_10km Above Simon's Town	-0.449	0.035	-12.711	0.000
Site_20km Above Kommetjie	-0.630	0.036	-17.683	0.000
Site_Buffels Bay	-0.154	0.028	-5.505	0.000
Site_Kommetjie	-0.524	0.023	-22.367	0.000
Site_Olifantsbos	-0.240	0.027	-9.001	0.000
Site_Simon's Town	-0.644	0.025	-25.532	0.000
Temperature	0.103	0.002	52.331	0.000
Time_Evening	-0.434	0.020	-21.499	0.000
Time_Morning	-0.221	0.020	-10.890	0.000
Time_Night	-0.520	0.021	-24.396	0.000

Statistically significant differences were detected between all sites, time of day and empirical temperatures (Figures 2.13.A, 2.13.B and 2.13.C). Sites within the distribution of *E. lamorali* lay between the fastest and slowest changing sites outside of this range, so no patterns were detectable between the two areas (Figure 2.13.A). As for empirical temperatures, the rate of change between them was highest during the 'day' (12pm-6pm) and slowest during the 'night' (12am-6am) (Figure 2.13.B). The correlation between empirical temperatures and how quickly they changed showed a positive relationship, although this was not strongly supported (R^2=0.249) (Figure 2.13.C).
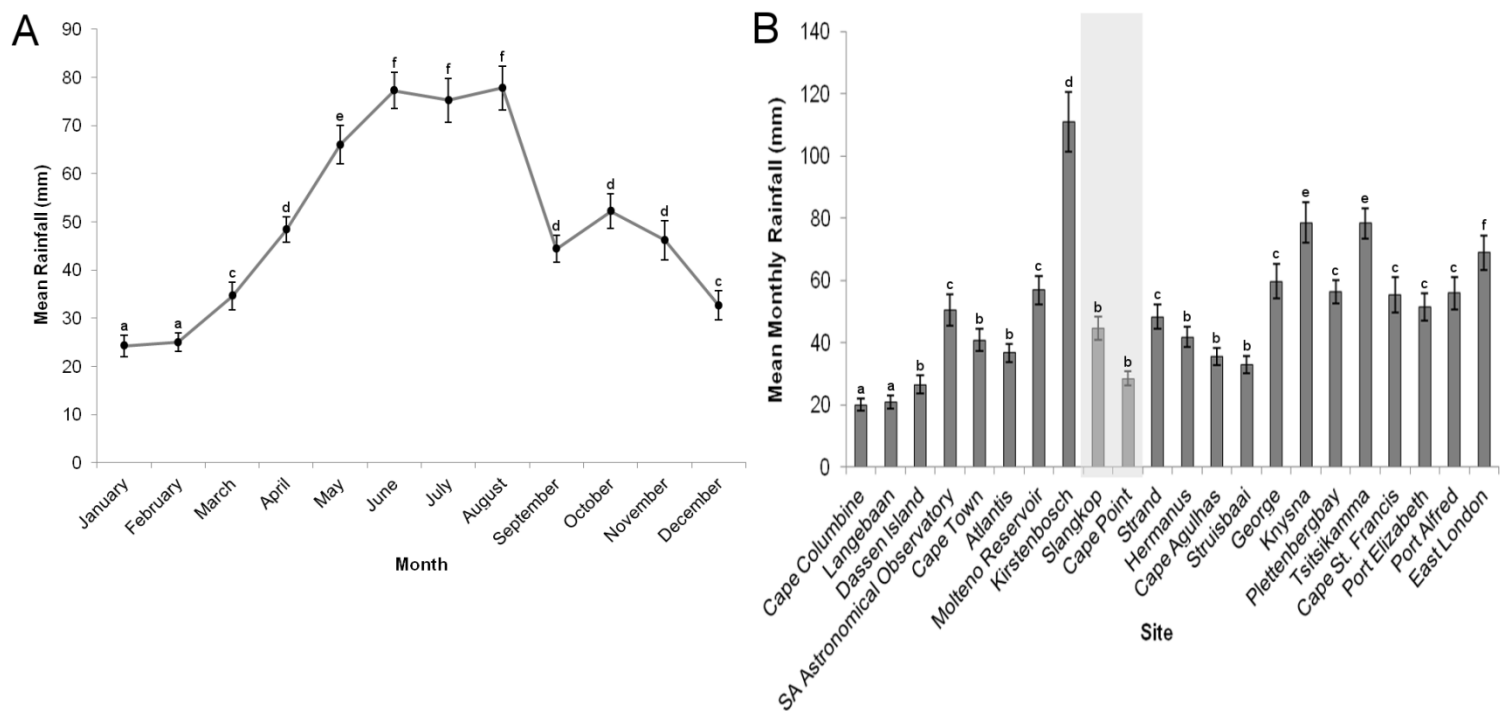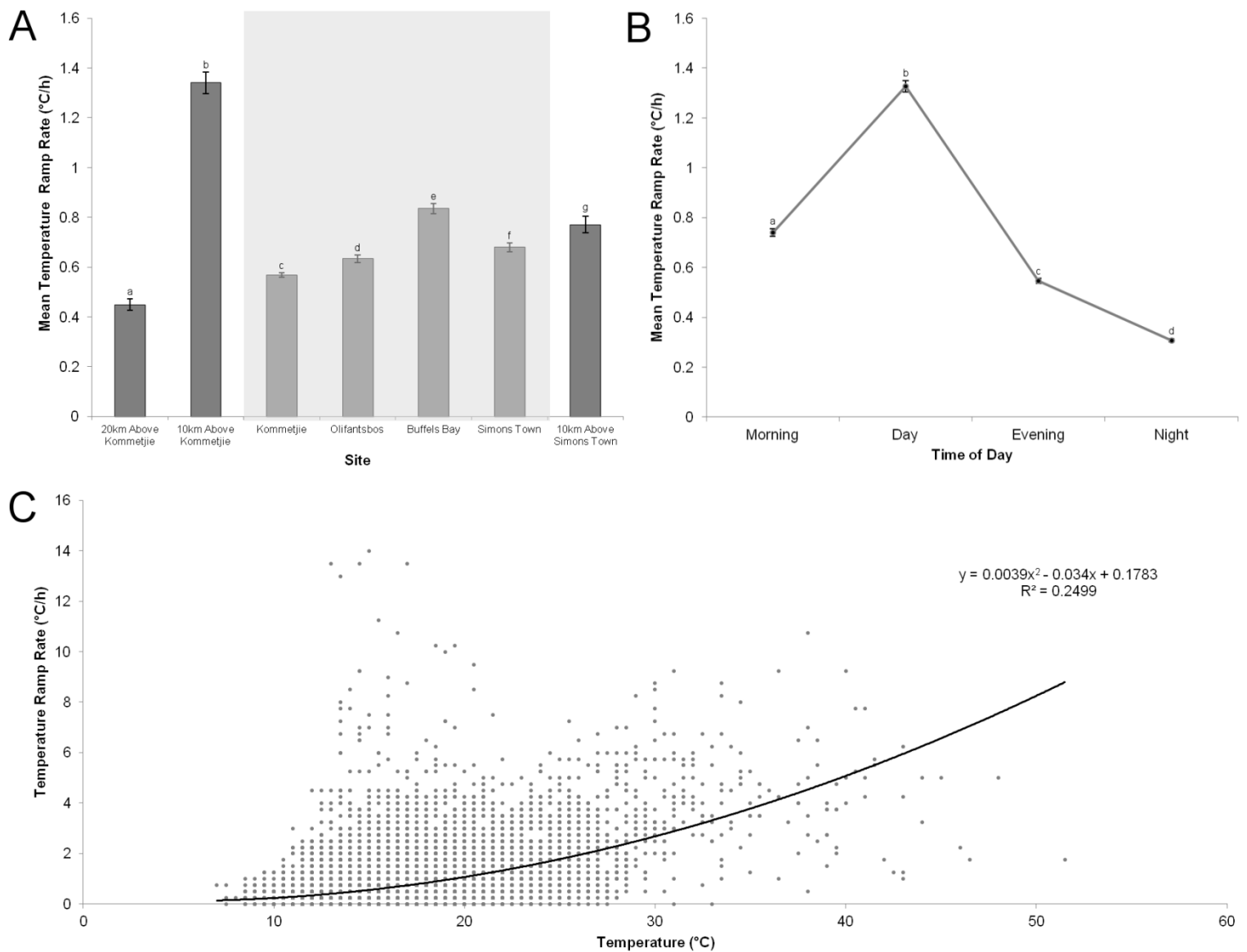
Figure 2.13: Statistically significant differences (as indicated by letters above points or bars) in micro-climate mean ramp rates between temperatures for different A: sites (grey background bar indicates sites within the distribution of *Echthrodesis lamorali*); and B: times of day illustrated no patterns concerning the distributional range of *Echthrodesis lamorali*, although ramp rates were significantly different at all sites and throughout the course of the day. A weak, yet positive correlation was evident between the empirical temperatures and the rate of change between them (C).

2.3.6.3 Micro- versus macro-climate temperatures

Although positively correlated (Maximum: R^2=0.74; Minimum: R^2=0.75) (Figures 2.14.A and 2.14.C) for both monthly maximum and minimum temperatures, statistically significant differences (t_{22}=3.103, *P*=0.005; t_{22}=-3.730, *P*=0.001 respectively) were found between the figures recorded at a micro- and macro-climates (Figures 2.14.B and 2.14.D). Maximum temperatures recorded at the micro-climate level were consistently higher than those recorded for the macro-climate (Figure 2.14.B), while the pattern was reverse for minimum temperatures, where iButtons recording micro-climate conditions reported statistically lower mean temperatures (Figure 2.14.D).
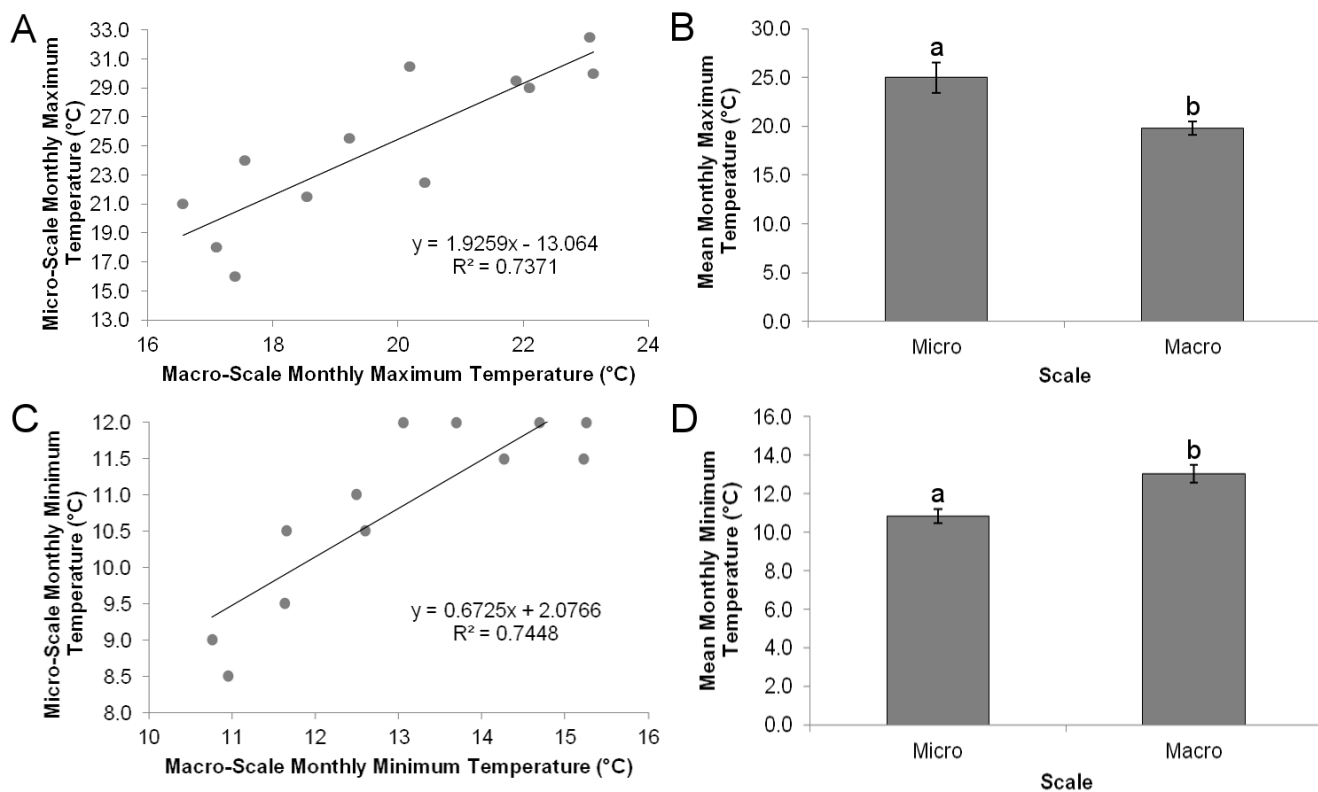
Figure 2.14: Monthly maximum (A) and minimum (C) temperatures recorded for both macro- and micro-climates were positively correlated, but were statistically significantly different (B; *P*=0.005, and D; *P*=0.001 respectively) (means given with ±SE).

2.4 Discussion

These results show that the distributional range of *E. lamorali* is much broader than previously recorded, although still restricted to a small area within the host's full distribution. Differences in host characteristics and macro-climatic conditions within and outside the range of the wasp may provide some clues as to the observed patterns.

Desis formidabilis produced compartmentalized egg sacs at Kommetjie, only some of which contained eggs (van Noort, 2009; van Noort et al., 2014). This behaviour is hypothesized by van Noort (2009) and van Noort *et al.* (2014) as a possible decoy to reduce the parasitism rates. Small parasitic wasps, such as *E. lamorali*, utilize all their energy burrowing into an empty compartment and then are not able to parasitize eggs in neighbouring compartments (Austin, 1985; van Noort et al., 2014). *Amaurobioides africanus* nests do not display this adaptation, nor did a single *D. formidabilis* nest found at any area other than the Peninsula. Two pressures are outlined by Austin (1985) as affecting spider nest structure: environmental factors and parasitism. Where only environmental effects are present, it follows that all nests in the same habitat will exhibit the same structure (Austin, 1985). The difference in structure between *D. formidabilis* and *A. africanus* nests on the same shore suggests an effect other than environmental characteristics acting on the nest structure (Austin, 1985). If compartmentalization is indeed a parasitism response, it is possible that *A. africanus* and the non-Peninsula *D. formidabilis* individuals are not being attacked by the wasp and thus conserve their web by producing only a single compartment. Only one record exists of *E. lamorali* emerging from the eggs of *A. africanus* (Ohio State University collection, *Hymenoptera Online* website, Various Contributors, 2015). The fact that not a single collected *A. africanus* nest (n=74) bore any parasitoids in this study suggests this may be an

anomaly. The distributions of *D. formidabilis* and *A. africanus* on a single shore are known to overlap (Lamoral, 1968) and individuals of both species may even nest together within a single retreat. As such, it is possible that confusion about the identification of the nest, or a mixing of samples led to this error.

While the results from this study indicate both spider species to be found where expected based on previous records, *A. africanus* and *D. formidabilis* are reported by Day (1974) and Dippenaar-Schoeman & Jocqué (1997) as occurring north of Kommetjie into Namibia, yet no individuals were found at any of the sites north of Hout Bay. While the lack of sightings during a survey does not prove that the species is not there, the current distribution of *D. formidabilis* and *A. africanus* may require revision.

The lack of establishment of *D. formidabilis* at Kenton-on-Sea and Jongensfontein could be explained by the absence of appropriate nesting areas at those two sites. In both areas, the lower shore region that *D. formidabilis* would usually inhabit was exposed to high wave action, a characteristic that appears to have driven the spider to nest behind tubeworm conglomerates, mussel beds or barnacles at other sites. At these two shores, however, this covering was not present, leaving *D. formidabilis* with nowhere to nest. Similarly, *A. africanus* was not found at Cape St. Francis, where all rocks are covered by water at every tide, leaving no suitably dry areas for the spider to nest in.

While *H. villosus* was collected in this study within the intertidal zone, it had previously only been recorded in a mountainous habitat in Cape Town (Haddad, University of the Free State,

pers. comm.). Being one of the most abundant and diverse groups of spiders globally; having a known ease of adaptation to adverse conditions (Haddad, 2003; Haddad & Louw, 2006); and displaying a commonly generalist diet (Haddad, 2003), the presence of a jumping spider (Family Salticidae) in the intertidal zone is not surprising and the group have previously been reported living in the upper intertidal zone in New Zealand, Singapore, California (Roth & Brown, 1976) and South Africa (Haddad & Dippenaar-Schoeman, 2009). Haddad (2003) reports similar nesting behaviour to those described here for *H. villosus* in *Heliophanus pistaciae* Wesolowska (Araneae: Salticidae), a salticid found in pistachio (*Pistacia vera* L.) orchards in the Northern Cape (South Africa) (Haddad, 2003; Haddad & Louw, 2006), although these studies documented it only for individuals that have yet to reach maturity, while *H. villosus* here was found on several occasions with a male and female in each nest along with an egg-sac or spiderlings. *Heliophanus pistaciae* is attacked by two hymenopteran parasitoids, one (Scelioninae) egg parasitoid and one ectoparasitoid, an Ichneumonidae species (Hymenoptera) (Haddad, 2003). The egg parasitoid, a species of *Odontacolus* Kieffer 1910 (Hymenoptera: Scelioninae)*,* belongs to the tribe Baeini, the same tribe to which *E. lamorali* belongs. This group is polyphyletic, however, with *Echthrodesis* belonging to a different smaller clade than the one Odontacolus is in, and thus is likely to not be closely related to this genus (van Noort et al., 2014.). No *E. lamorali* individuals, nor any other parasitoids, were recorded emerging from collected salticid nests in this study. Thus, the wasp does not appear to attack *H. villosus*, despite it living within the same biogeographical habitat. The palpimanids are widely known as spider predators (Pekár *et al.,* 2012) and the specimen found in the present study could be feeding on *A. africanus* individuals. Further investigation into the find is necessary.

_____

Desis formidabilis alters its nest site choice depending on the wave action at a site (Lamoral, 1968; Dippenaar-Schoeman & Jocqué, 1997). Generally this spider species shows a preference for nesting between rocks and in cracks in rocks on shores with low wave action, while shells, and specifically tubeworm conglomerates (Lamoral, 1968), are used on shores with high wave action (Lamoral, 1968; Dippenaar-Schoeman & Jocqué, 1997). Throughout the range of *E. lamorali*, *D. formidabilis*, and to some extent *A. africanus*, make use of empty shells caught between rocks to nest in (Lamoral, 1968). At Kommetjie and Olifantsbos Point, *D. formidabilis* most commonly uses empty limpet shells that are fastened with silk to the undersides of small boulders in the lower intertidal zone, while in higher regions, shells caught in rock crevices are mostly used. On other, more exposed shores surveyed in this study, such shells were largely absent, having been washed away by wave action due to the lack of sufficiently sized cracks or crevices in the rock face (as recorded by Lamoral, 1968 on the Cape Peninsula). Thus, the spider was forced to nest in alternative ways, namely underneath tubeworm conglomerates or mussel beds. Lamoral (1968) also reports *A. africanus* nesting in crevices on larger, more irregular rocks further up shore at Kommetjie. This nesting behaviour was widespread for *A. africanus* throughout the surveyed area. Alternatively, *A. africanus* individuals were found to nest under shells on one of the two shores (Jongensfontein) where they were exposed to high wave activity. Lack of this nesting material choice at the other shore (Kidds Beach) could be related to the absence of adequate shells.

To continue this discussion it is important to outline how the oceans and coastal areas bordering South Africa can be divided into three biogeographical regions based on differing species assemblages and hydrological characteristics in each area (Figure 2.15) (Baird, 2006;

_____

Teske *et al.,* 2006; Teske *et al.,* 2011; Turpie *et al.,* 2000; Smit *et al.,* 2013). These areas include a 'cool temperate South-West coast' province; a 'warm temperate South coast' province; and a 'subtropical (which may include a 'tropical East coast' province (Teske *et al.,* 2011)) East coast' province (Figure 2.15) (Baird, 2006; Teske *et al.,* 2006; Teske *et al.,* 2011; Turpie *et al.,* 2000; Smit *et al.,* 2013). The two latter regions are affected by the warmer Agulhas current, while the former is kept cool through action of the cold Benguela current (Baird, 2006). Although the biogeographical regions identified by Teske *et al.* (2011) are based on marine fauna and flora, and intertidal organisms with free-floating larval forms that rely on currents for dispersal, of which neither species assessed here are, it is important to note that other aspects that are used to define biogeographical regions have large effects on intertidal ones (Helmuth *et al.,* 2006; Blanchette *et al.,* 2008). Water temperatures, which are a factor of a variety of processes, such as up- or down-welling, can significantly influence temperatures experienced within the intertidal region for example (Helmuth *et al.,* 2006; Blanchette *et al.,* 2008). Furthermore, Teske *et al.* (2011) take freshwater discharges from terrestrial systems into account when defining their biogeographic zones. This surely has implications for salinities experienced within the intertidal regions of these differing areas. The effects that these may have on organisms appear to be species-specific (Teske *et al.,* 2006). For this study, the location of the boundary between the first two zones appears to be the most important in keeping *E. lamorali* within the Western region of the distribution of *D. formidabilis* only. Although the exact positioning of the boundary line is disputed and appears to change temporally and spatially (Baird, 2006), it is generally found to lie somewhere between Kommetjie and Cape Agulhas (Teske *et al.,* 2006; Teske *et al.,* 2011; Turpie *et al.,* 2000).

_____
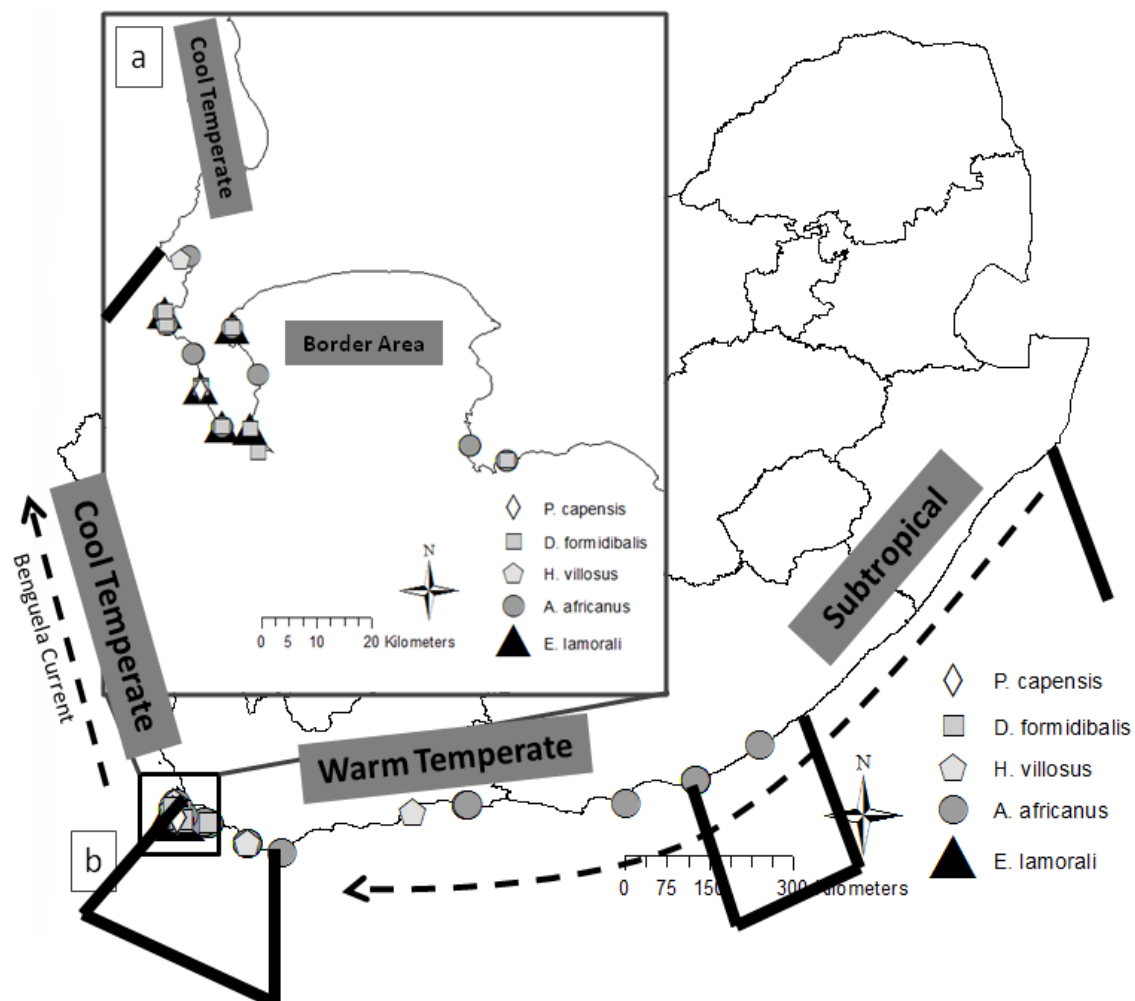
Figure 2.15: The main biogeographical zones bordering the South African coast [brackets: regions in which the border between zones could fall; dotted arrows: current direction and name; grey textboxes: zone name] (After: Teske *et al.* 2011).

Interestingly, spider egg-sacs were only found close to and within the boundaries of the cool temperate South-West biogeographical region in March. Not finding eggs during a survey is no guarantee that the spiders were not breeding at the time. This is especially true for sites where the spiders nested behind structurally complex shells, such as tubeworm conglomerates and mussel beds, where nests may be destroyed during removal of the covering material. As such, it may be presumptuous to assume that *D. formidabilis* was not breeding at sites were no nest was found. Silk is, however, energetically expensive to produce (Bodasing *et al.,*

2002). Thus, where no nests were found, the spider may eat its silk (Whitehouse *et al.,* 2002) at low tide to assimilate energy for foraging. It would not be able to do this should there be eggs in the nest, resulting in the conclusion that no nest was indicative of no breeding. This is supported by the marked seasonality in *A. africanus* breeding at Summerstrand. Furthermore, the relative cost of silk production could also be applied to *A. africanus*, which used less silk by not fully lining the nest when not breeding in the period between March and August. Additionally, the lack of diurnal activity in *D. formidabilis* at all sites east of Mossel Bay could be linked to these breeding patterns. Lamoral (1968) reported the species to stay in the nest with the young for a period after hatching. This implies some form of adult protection. Prior to breeding, spiders may forage at both low tides in order to assimilate enough energy for silk and gamete production, but remain in the nest at day-time low tide post-laying to protect the eggs, leaving only once a day, at night, to feed. During this time there may be less predators, such as birds, who rely on visual means to source their prey, protecting the spiders from predation to some extent, as is seen in many Orb Web spider species (Blackledge & Wenzel, 2000; Herberstein *et al.,* 2000; Li *et al.,* 2003). Year-round egg-sacs and nocturnal behaviour were always recorded during previous sampling and the surveys conducted as part of this study at Kommetjie by van Noort (Iziko South African Museum, pers. comm.) and Lamoral (1968), suggesting no seasonality in breeding on the Peninsula, but clear seasonality east of it. Whether *E. lamorali* is capable of 'overwintering' during non-breeding seasons is unknown. As further evidence of the potential for seasonality to exist, grouping of climatic conditions from closely-situated weather stations in data obtained from the South African Weather Service corresponds relatively well to areas where the spiders are believed to display seasonal versus non-seasonal breeding. Perhaps the presence of seasonality in spider breeding cycles outside of the Peninsula prevents the wasp from establishing there.

It is clear to ecologists that a wide variety of external and internal factors affect the movements and behaviour of organisms, parasitoids included (Laing, 1937; Atkins, 1980a; Lubke, 1998). These factors may include characteristics of the species' immediate surroundings, climatic conditions and the host organism's population dynamics (Laing, 1937; Atkins, 1980a; Lubke, 1998). The data obtained from the South African Weather Service showed sites within the distribution of *E. lamorali* to differ from those outside of its range in the maximum temperatures and humidities experienced. The maximum temperatures reached were significantly lower within the wasp's distribution than at other sites, except at Cape Columbine. This result can be possibly directly linked to the thermal tolerance range of *E. lamorali*, which is discussed in Chapter 4. Upper thermal limits have been shown to directly impact the distribution of some *Drosophila* species (Kellermann *et al.,* 2012). The upper critical thermal limit of a species is usually more constraining and limited for insects species than the lower limit (Chown *et al.,* 2015), resulting in a weak, yet positive correlation between temperatures and distribution for many species (Hoffmann *et al.,* 2013; Chown *et al.,* 2015). Denaturation of proteins and enzymes in the body occur at both high and low temperatures outside of an organism's critical thermal range, although the process is generally irreversible at the upper limit, as opposed to the reversible, ordered unfolding of the structures at lower temperatures (Pörtner, 2002; Somero, 2002; Somero, 2005; Iyer & Ananthanarayan, 2008). With this in mind, the disparity in distribution controlling processes between minimum and maximum temperatures, with the latter exerting much stronger distributional range limitations, is understandable. Although Cape Columbine remained within the wasp's physiologically tolerable temperature range according to these models, the lack of host spiders in the areas surrounding the site would prevent *E. lamorali* from establishing there. The distinction in humidity experienced between sites within the wasp's range and those outside of it is interesting, but unlikely to be structuring *E. lamorali*, as

_____

humidities are logically vastly different between the micro-environment in which the wasp lives and the broader scale at which weather stations take readings. As a side-note, in light of recent devastating fire events experienced by Cape Town (News24, 2015; Phakathi, 2015; Sapa, 2015; SAPeople, 2015; Thaw & Malgas, 2015), the decline in humidity across the years is of concern, as less moisture in the air can conversely significantly increase the chance of fires occurring (Bergeron & Flannigan, 1995). Temperatures, rainfall and wind demonstrated clear seasonal patterns within the years sampled with lower temperatures and winds, and higher rainfall in the winter period (May to August), although beyond the maximum temperatures and humidities, the macro-climatic data showed no other patterns concerning the distribution of the parasitoid.

The apparent controlling effect of macro-climate maximum temperature was not reflected in micro-climatic conditions detected by iButtons in the environment in which *E. lamorali* lives. This disparity is explained in the statistical difference and low correlation between macro- and micro-climate readings, as was found by Smit *et al.* (2013) in their study along the South African coast. As the iButtons were placed within limpet shells where *E. lamorali* theoretically spends much of its time, the disparity in temperatures between this and macro-climatic conditions indicates the use of microcosms to mediate abiotic conditions (Gripenberg & Roslin, 2007).

The physical and environmental conditions at a particular rocky shore are determined by the action and interaction of a variety of factors, the most important of which are the prevalent tidal and current conditions and local geology (Stephenson & Stephenson, 1949; Lubke, 1998). Much of the South African coast can be considered exposed coastline, where the tides

and current make for high wave activity, with few sheltered bay areas (von der Meden *et al.,* 2008). One of the major results of the combination of wave activity and geology is the sort and arrangement of substrate type (Stephenson & Stephenson, 1949; Lubke, 1998). High wave activity will erode the coastline, removing or degrading material, while gentle waves deposit sediments and may roll any loose stones into smooth pebbles (Stive, 1988; Lubke, 1998). Dobel *et al.* (1990) showed spider assemblages to differ significantly between two different plant architectures within a single saltmarsh, and the same could be applied to the community assemblages between different rock types. Highly fractured rock-faces or cliff-like rocks with lots of grooves for water to pool will provide a highly heterogeneous thermal environment, with hot rocks heated by the sun interspaced with cooler, high salinity (as a result of evaporation by the sun) pools (Lubke, 1998; Blanchard & Bourget, 1999), while smooth pebbly shores may drain very quickly, leaving very little or no water and therefore rising to very high temperatures. Furthermore, the slope of the shore may be affected by the substrate type, with cliff-like rock-faces making a much steeper gradient than smoother, piled boulders and pebbles (Stephenson & Stephenson, 1949). As such, tidal and wave movements may be felt more drastically on cliff-like shores as compared to pebble beaches. The latter shores, where smooth stones and boulders dominate, are rare along the South African coastline (Lubke, 1998). In fact, only two (Kommetjie and Cape St. Francis) of the 14 sites and all the shores in the Table Mountain National Park surveyed in this study were dominated by loose, smooth rocks (pers. obs.; Lamoral, 1968 (for Kommetjie)). Kommetjie and the western shore of the National Park provide particularly well sheltered shores due to their gentle slopes; loose, smooth rocks and a prominent band of fringing kelp (Lamoral, 1968). Organisms living in the intertidal zone may only be able to cope with specific mechanical disturbance, exposure times and desiccation rates (Teal, 1962; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002), all of which are affected by the tidal and wave movements

_____

(Stephenson & Stephenson, 1949; Lubke, 1998), and may thus only be able to live on certain shores. This may serve as an insurmountable barrier to *E. lamorali* individuals attempting to disperse from the Peninsula.

As a result of a parasitoid's species-specific physical capabilities, they may be linked to only certain characteristics of their host's living conditions, not necessarily the host itself (Laing, 1937; Atkins, 1980a). Although dated, Laing (1937) showed that some parasitoids search for the particular environment in which their host is found before searching for the host itself (Atkins, 1980a). This search for a particular stimulus, which is in this case of an environmental nature, is known as orthokinesis (Atkins, 1980a). As a result, the parasitoid may not be exploring the entirety of its host's range (Laing, 1937). While many scenarios exist to explain the distribution of *E. lamorali*, if the spiders living outside of the Peninsula have taken up occupancy in areas with different habitat conditions, which they appear to have done by settling on small cliff-like rock-faces or under other marine organisms on highly exposed shores, *E. lamorali* may not be driven to search for their host there and thus have remained within the area they evolved in. Congregation in one locality due to the inhibition of movement by the lack of stimuli in another area is a common outcome of orthokinesis (Atkins, 1980a).

To further this discussion it is important to explore how *E. lamorali* may disperse from one area to another, a factor which could take a variety of forms, all of which may not be mutually exclusive (Barber, 2009). Biologically, transport by birds or other animals (zoochory), humans (anthropochory) (Barber, 2009) or the host itself (phoresy) (Strand, 1986a; Austin *et al.,* 2005) could all be employed as transportation. *Echthrodesis lamorali*

could also be easily blown around by the strong Cape Peninsula coastal winds (Barber, 2009; van Noort, 2009; van Noort et al., 2014), although their lack of wings suggests this not to be an important dispersal mechanism (van Noort, 2009; van Noort et al., 2014). Finally, rafting on plant or other material floating on the oceanic currents could transport individuals, small populations of wasps or even *D. formidabilis* nests containing parasitized eggs or adults far from their original location, only to be settled on another shore (Barber, 2009). Floating material carrying live insects has previously been found up to 16km offshore (Barber, 2009).

Upwelling (characteristic of the system in which the Peninsula is located (Baird, 2006)) may prevent dispersal of *E. lamorali* from Kommetjie up the west coast from Africa due to differing environmental conditions along the coast (Teske *et al.,* 2011). Furthermore, specifically if the wasp is using rafting for dispersal, upwelling cells may cause eddies that then remain within the vicinity of their type locality, thereby hindering dispersal to other areas (Teske *et al.,* 2011). At a larger scale, the Benguela currents runs from the Cape Peninsula, upwards towards central Africa, diverging from the land mass as it proceeds (Baird, 2006). Rafting wasps would therefore be swept out to sea and could only possibly have established north of Kommetjie, a stretch where neither *D. formidabilis* nor *A. africanus* was found in this study. Mixing of the Benguela and Agulhas currents also occurs offshore from the Peninsula (Teske *et al.,* 2011). With the Benguela flowing north (Baird, 2006), it is thus unlikely that any individuals enter the Agulhas and disperse east. Finally, lack of rocks and therefore adequate nesting sites on coastal dunefields may prevent establishment of the spiders and therefore the wasps at a site. With the highly varied nature of the South African coast where dunefields are highly interspaced with rocky shores (Teske *et al.,* 2011),

_____

dispersal by such a small organism could be limited to rocky shores spaced close together (Barber, 2009; Emanuel *et al.,* 1992; Teske *et al.,* 2011), such as is found in the Peninsula.

In conclusion, although the exact cause is difficult to establish, possibly due to a variety of physiological aspects and its host's ecology in other areas, *E. lamorali* is limited to the cool temperate South-West biogeographical region and, specifically, the Cape Peninsula itself. This distribution pattern is positive in that the full distribution of the species is included in the Table Mountain National Park, which encompasses the entire Peninsula, ensuring its effective conservation. This country-wide broad scale assessment of the interaction between the wasp and host indicated a much smaller effect of the former on the latter than expected as a result of the limited distribution of *E. lamorali.* The interaction is investigated at the smaller scale of single shores in the following chapter.

Chapter 3: Investigations into the biology of _Echthrodesis lamorali_ Masner 1968 (Hymenoptera: Platygastridae: Scelioninae) on the Cape Peninsula (Western Cape, South Africa)

3.1 Introduction

The rocky shore is an area of high productivity, supplying large amounts of resources to the terrestrial and marine ecosystems on either side of it (Paetzold _et al.,_ 2008; Barber, 2009; van de Koppel _et al.,_ 2015). For example, Oakes & Eyre (2015) demonstrated that rocky shore communities absorbed much nitrogen and trace metals from waste water released from the bordering terrestrial systems, thereby reducing the proportions of these in the water finally reaching the ocean. Algal growth and detritus deposited by wave action in the intertidal area provide a large source of food for a variety of organisms, thus increasing the carrying capacity and diversity of the communities that establish there (Paetzold _et al.,_ 2008; Barber, 2009). Nonetheless, the environment is a harsh one to first colonise (Foster & Treherne, 1976; Lubke, 1998), with each individual shore boasting different characteristics depending on the activity of the tides and currents (Tietz & Robinson, 1974; Lubke, 1998). These factors have far-reaching effects on both the biotic and abiotic composition of the shore (Tietz & Robinson, 1974; Lubke, 1998).

As a result of varying tidal and wave movements, a single shore alone is a highly heterogeneous environment from a variety of abiotic aspects, such as substrate composition and stability, salinity, temperature, pH and humidity (Lubke, 1998). These abiotic factors in turn act as 'stressors' on biotic ones, leading to community structuring (Foster & Treherne,

1976; Sanford, 2002). Stressors may be physical, i.e. through direct mechanical forces acting on the animals, or physiological, which affect an organism's bodily functioning (Sanford, 2002). In intertidal organisms, the temperature changes associated with tidal movements are considered one of the main stress factors from a physiological viewpoint (Sanford, 2002). These stress factors can be countered by a species through behavioural means to some extent, and spatial or temporal refuges may be available to them at some point in their life cycles to escape them, but they ultimately play a strong role in community structuring (Foster & Treherne, 1976; Sanford, 2002).

As a result of the interactions between abiotic (mainly wave and tidal action) and biotic (competition, parasitism and predation) stressors, zonation on rocky shores is usually very marked in the communities that settle in each region (Tietz & Robinson, 1974; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002). Frequently-recognised zones include high water spring to high water neap (littoral fringe); high water neap to low water neap (eulittoral region); and low water neap to low water spring (sublittoral fringe) (Tietz & Robinson, 1974). No true maritime animals reside above the spring high tide mark (Tietz & Robinson, 1974). For example, in the Tsitsikamma, a South African shoreline just east of the area investigated in this study, the upper zone is characterised by the presence of small snails, *Afrolittorina knysnaensis* Philippi 1847 (Mesogastropoda : Littorinidae); the mid-shore hosts more encrusting material like tube worms, *Balanus* spp. (Sessilia: Balanidae) barnacles and mussels; while the lowest region comprises mostly pink *Lithothamnion* spp. (Corallinales: Hapalidiaceae) algae and *Scutellastra cochlear* Born 1778 (Docoglossa: Patellidae), the pear-shaped limpet (Tietz & Robinson, 1974). The rocky shores along the Cape Peninsula (Western Cape, South Africa), the habitats in which *Echthrodesis lamorali* Masner 1968

(Hymenoptera: Platygastridae, Scelioninae) lives, also exhibit this species assemblage zonation, but to a lesser extent (Lubke, 1998). The zones in this region are better demarcated by different algal growth (Lamoral, 1968).

Although environmental stability and the strength of biotic and abiotic stressors in an area are an important factor for community assemblages (Foster & Treherne, 1976; Sanford, 2002), organisms living in a more complex and extreme environment are hypothesized to enjoy enemy release over those in more stable habitats, which allows for accumulation of a diversity of species, including predators and parasitoids (Foster & Treherne, 1976). Apart from the fish, seaweeds, microorganisms, molluscs, fungi and algae found on the shore, a variety of arthropods have flourished (Cheng, 1976; Barber, 2009), invading the shore a number of times throughout evolutionary history (Barber, 2009). The material deposited through wave action and the algal blooms that dominate the intertidal region provide a good resource for scavenging species, which in turn are predated upon and parasitized by higher-order insects and other arthropods (Paetzold *et al.,* 2008; Barber, 2009).

Of particular interest here are the parasitoid wasps. Species belonging to the Chalcidoidea (Hymenoptera), Ichneumonidae (Hymenoptera), Braconidae (Hymenoptera) and Platygastridae (Hymenoptera) have all been previously recorded as parasitizing other insects in salt marshes, an environment similar to the intertidal zone (van Achterberg *et al.,* 1990; O'Connor, 1996; Laegdsgaard *et al.,* 2006; Wu *et al.,* 2009; Veenstra *et al.,* 2011; Japoshvili & Russel, 2012; Dorchin *et al.,* 2014). In contrast, only three species of wasps worldwide show proper adaptation to true intertidal conditions and can thus be considered truly maritime (van Noort *et al.,* 2014). This broader range of parasitoids in salt marshes than in the

intertidal region indicates a causal factor other than purely physiological reasons for the exclusion of species from the latter zone. Cheng (1976) and Barber (2009) point towards the physically active environment in the form of constant wave action as a major impediment to invasion by small, fragile, winged insects such as hymenopteran egg parasitoids.

Eggs serve as a suitable host resource for attack by parasitoids because they lack the cellular defence responses to parasitism that are present in older life stages (Strand, 1986a; Pennacchio & Strand, 2006). This means that the host is less likely to reject and therefore kill the parasitoid (Strand, 1986a; Pennacchio & Strand, 2006). Embryo development and the change of hormonal levels during the process, however, serve as difficult hurdles for egg parasitoids to overcome (Austin, 1984; Strand, 1986a; Strand *et al.,* 1986). Timing of oviposition is often vital (Austin, 1984; Strand, 1986a; Strand *et al.,* 1986; Pizzol *et al.,* 2012), as a too advanced host embryo may be able to reject the parasitoid (Strand, 1986a); the hormones it is releasing might negatively impact wasp development (Strand, 1986a; Strand *et al.,* 1986); or the host may have depleted too much yolk in the egg for the parasitoid larvae to survive off (Austin, 1984). This is exemplified in the case of *Trichogramma cacoeciae* Marchal 1927 (Hymenoptera: Trichogrammatidae), which showed clear preferences for freshly oviposited *Lobesia botrana* Denis and Schiffermüller 1775 (Lepidoptera: Torticidae) eggs (Pizzol *et al.,* 2012). Once degradation of the host tissue begins, the release of hormones is generally accepted to be halted, thereby not affecting the parasitoid larvae further (Strand, 1986a; Strand *et al.,* 1986; Pennacchio & Strand, 2006). Most parasitoids appear to be able to detect the developmental progress of an egg using olfactory and tactile cues from both external and internal structures and cues (Strand, 1986a; Mattiacci *et al.,* 1993; Pennacchio & Strand, 2006). Despite the need for most parasitoids to gain access to the eggs in a specific time period, for spiders this life stage is often surprisingly not well protected from attack

_____

(Strand, 1986a; Pennacchio & Strand, 2006). 'Safety in numbers' in species with large egg masses may serve some protection, but other than this, concealment of the clutch (Strand, 1986a; Pennacchio & Strand, 2006) and parental guarding (Austin, 1985), not many adaptations exist in spider hosts to protect their eggs (Strand, 1986a; Pennacchio & Strand, 2006).

One of the intertidally living spider species in South Africa, *Desis formidabilis* O.P. Cambridge 1890 (Araneae: Desidae) appears to have developed a defence strategy in its egg sacs to reduce the incidence of parasitism (van Noort, 2009; van Noort *et al.*, 2014). Along the Peninsula, the spider compartmentalises its nest into several purse-like wedged structures that fit together within the shell (van Noort, 2009; van Noort *et al.*, 2014). Not all compartments will contain eggs, with some left barren (van Noort, 2009; van Noort *et al.*, 2014). These were proposed to act as 'red herrings' for the species' parasitoid, *E. lamorali*, potentially reducing the impact on the broods in neighbouring compartments as a result of the wasps exerting all of their energy on gaining access to the empty ones (van Noort, 2009; van Noort *et al.*, 2014). Such an interaction suggests a long history of association between the host and parasitoid to allow for evolution of this response (Austin, 1985).

Very little information is available on basic aspects of most platygastrid wasps' biology and life histories (Eason *et al.,* 1967; Hickman, 1967; Austin, 1984; Austin *et al.,* 2005), particularly in the case of egg parasitoids, which are usually very small and therefore difficult to observe (Strand, 1986a; Pennacchio & Strand, 2006). Where information is present, it is mainly for species used in biological control of insect pests (Austin *et al.,* 2005). Nonetheless, platygastrids and their interactions with their hosts are often used as model

systems in a variety of investigations, including sex ratio allocations (Austin *et al.,* 2005). Due to the transient nature of their resource (i.e. the host), the huge diversity of hosts available and the seemingly infinite possibilities of adaptation in both parasitoids and their hosts, there is vast potential for unique adaptation in parasitoid morphology and behaviour (Strand, 1986a).

As such, this study aimed to assess the parasitoid host spider, *D. formidabilis*', nest site and material choices, nest compositions and population sizes to determine if any preferences existed for certain materials, sites or zones within single shores and between different sites, which would explain its distribution patterns. Furthermore, the parasitism incidence and sex ratios of the resultant wasp broods was determined. These dynamics indicated the effect that the parasitoid may be having on the host spider, while also testing whether *E. lamorali* is subject to the breeding patterns associated with most parasitic Hymenoptera. These intra-community dynamics have never before been assessed in this system. As shown in Chapter 2 and Owen *et al.* (2014), the distribution of *E. lamorali* falls within an ecotone between biogeographical regions. Ecotones are areas of high variability through both space and time that are dependent on their interactions with the biogeographical zones around them (Traut, 2005; Paetzold *et al.,* 2008). Community interactions within this habitat can have large effects on the functioning of the ecosystem, and therefore its outputs, which have knock-on effects on the surrounding habitats (Paetzold *et al.,* 2008). Therefore, it is important that the interactions within this region are understood, as changes here could imply changes in surrounding ecosystems (Paetzold *et al.,* 2008). The parasitism of *D. formidabilis* potentially provides a strong regulatory function, thereby maintaining the spider population sizes and contributing to the balance of the intertidal community as a whole. Balance within this region may then help regulate community dynamics in surrounding regions (Paetzold *et al.,* 2008).

_____

In comparison to the general small number of cases for field studies, laboratory-based investigations into a variety of biological aspects of other parasitoid wasps abound as a result of the ease in observing the small organisms that can often be reared in large numbers (Heimpel & Casas, 2008). However, because many behavioural reactions that impact biological decisions are context-dependent, laboratory studies can often not be extrapolated to the natural field interactions where realised behaviour (versus the fundamental behaviour often observed in the laboratory trials) is observed (Atkins, 1980b; Southwood & Henderson, 2000; Heimpel & Casas, 2008). This is particularly true for sex ratio patterns as a result of differing larval mortalities and adult behaviour (Waage, 1986). As such, only field-collected samples were used in this study in order to gain insight into the natural dynamics between and within *E. lamorali* and *D. formidabilis* populations in terms of spider nest sites, nest composition and population sizes of both species. Furthermore, the sex ratios in resultant parasitoid broods were determined and all aspects assessed to determine within- and between site dynamics.

3.2 Materials and methods

3.2.1 Sites

Spider nests were collected from four sites located on either side of the Cape Peninsula (Western Cape, South Africa) that were known to host populations of *E. lamorali* (Chapter 2; and Owen *et al.,* 2014), namely Kommetjie (34° 8' 22.7034" S, 18° 19' 17.5794" E) and Olifantsbos Point (Table Mountain National Park [TMNP]; 34° 15' 29.6274" S, 18° 22' 54.0474" E) on the western perimeter; and Buffels Bay (TMNP; 34° 19' 19.5594" S, 18° 27' 44.028" E) and Simon's Town (34° 9' 43.7394" S, 18° 25' 55.5234" E) on the eastern side. Nest collection took place during the lowest tides every two weeks between 9 September and

22 November 2014, for a total of six collection events. This places all collections within the season of spring.

The rocky shore at each site was separated into three zones: the sublittoral fringe, eulittoral range and littoral fringe, named zones A, B and C respectively for the remainder of the study. The sublittoral fringe was closest to the water and located between the spring low tide mark and neap low tide level; the eulittoral range covered the area between neap low and neap high tides; and the littoral fringe was the furthest away from the water, encompassing the region between neap high tide and the spring high marks. Following Lamoral (1968), all zones were demarcated using visual assessment of the coastline during the changing tides, in combination with patterns in encrusting materials on the shore, such as algal clusters. For example, the low water neap line at Kommetjie is demarcated by the presence of green algae and seaweed deposits, while the high water neap mark is strongly demarcated by a band of brown algae (Lamoral, 1968).

3.2.2 Nest collection

A transect was randomly placed from the upper limit to the lower end of each zone at each site for each collection event. The area surrounding this line and occurring not more than 2m from it was then actively searched and all *D. formidabilis* nests that were located within a period of 30 minutes per zone were collected. The total number of nests assimilated in this time was divided by 30 (#nests/minute) to give a comparable figure for the spider population size in each zone. The location of the transect line changed during each collecting trip to account for natural variation in the spider population and the heterogeneous environment

typical of a rocky shore. A variety of parameters were recorded for each nest, namely the material in which the nest was constructed; the shell opening area as a proxy for nest size where shells were used in nest construction; the location of the nest relative to the rock it was attached to (between the rock and substratum or between adjacent rocks); the main alignment of the nest (along a north-south or east-west orientation); the nest's orientation relative to the ocean and land; and how exposed it was to the sun. Where the dependent variable was numerical, logit-normal generalized linear models (GLZs) were conducted in Statistica 10 (StatSoft Inc., 2011) to test for statistical differences in parameters between sites; zones; zones nested within sites; and different collecting trips. For categorical dependent variables, log-linear analyses of contingency tables were completed in R version 3.1.3 (R Core Team, 2015).

3.2.3 Nest contents

In the laboratory, each nest was separated into its constituent compartments, the number, contents and state of which were recorded. Unhatched eggs were isolated within their compartment (which maintain adequate humidity levels for the eggs) in individual rearing chambers, such that each egg clutch could be directly associated with a certain nest, yet a single nest was investigated in its smallest unit - that of the single compartment. These chambers were kept at room temperature and flushed with seawater daily to simulate natural conditions. To gauge parasitism incidence, any species of emerged specimens was recorded, while failed eggs (those that did not hatch) were assessed under a light microscope to determine the contents. All material was stored in 98% ethanol, catalogued and accessioned into the entomology wet collection at the Iziko South African Museum (Cape Town, Western Cape, South Africa).

_____

A log-linear model determined if any significant differences in the number of compartments per nest existed between different sites and zones, and regression analyses were used to assess the relationship between nest size (the number of compartments) and clutch size. A GLZ determined if the number of failed eggs differed between sites and zones for all eggs, as well as unparasitized and parasitized ones independently, while Pearson Chi-Square tests determined if egg failure and colour could be used to indicate parasitism.

3.2.4 Sex ratios

Wasps that emerged from the collected material were stored in 98% ethanol and their sex determined under a light microscope. Sex ratios were calculated using Equation 1:

Equation 1: % Males in brood = # Males/(# Males + # Females)

The relationship between spider brood size (continuous regressor) and wasp sex ratio (dependent variable) was analysed using regression analysis for both males and females together, as well as with the sexes isolated. This allowed for assessment of factors affecting the sex ratios of a population as a whole, as well as the isolation of differing processes in each sex.

3.2.5 Parasitism incidence, spider population size and sex ratio model construction

In order to determine which factors imposed structuring effects on the spider and wasp populations along the Cape Peninsula, three models were independently constructed using a

sample size-corrected, Akaike Information Criterion (AICc) based, reverse model building process with the spider population size, parasitism incidence and sex ratio of resultant wasp broods as the outcomes for each model (dependent variables). All analyses were completed using R and the packages MuMIn (Barton, 2015) and car (Fox & Weisberg, 2011). The most complex model using all possible predictor (independent) variables (those recorded for each nest during sample collection) was first constructed and co-linearity within them determined. Where co-linearity was detected, the variable indicated (GVIF>5) was removed. For the parasitism incidence model, the outcome variable was modelled as a binomial data family (parasitism was either present [1] or not [0]), while both sex ratio and spider population size were Gaussian in nature as outcome variables. The presence of a binomial outcome for the parasitism model, as well as the mainly categorical nature of the predictor variables in all models precluded the need for dispersion and zero inflation tests and data manipulations.

Akaike Information Criterion values (AICcs) were then calculated for all possible models using the variables from the initial one. All models developed in this process with a delta value of less than 2 were considered and the variables used in the construction of these assessed as per the suggestion of Symonds & Moussalli (2011). The most commonly occurring predictors that made the most biological sense were used in construction of the final model that outlined the effect that the remaining predictor variables had on the outcome (spider population size, parasitism incidence or *E. lamorali* sex ratio) (Zuur *et al.,* 2010; Symonds & Moussalli, 2011).

3.3 Results

3.3.1 Nest collection

A total of 127 nests were collected for all sites, zones and over all collecting trips. These nests comprised a total of 566 individual compartments. For all sites, zones and collection trips, nests ranged from 1-23 compartments, with the average nest containing 4 ± 0.34 (SE) compartments (Figure 3.1.A). Significant differences in nest sizes were detected only between different sites ($F_{(3, 121)}=3.156$, $P=0.027$), and not zones ($F_{(2, 121)}=2.667$, $P=0.073$), with significantly larger nests at Olifantsbos than Kommetjie, but no differences between these two and the other two sites (Figure 3.1.A).

As with the mean number of compartments in a nest, significant differences were only detected between shell sizes used at different sites ($F_{(3, 85)}=3.671$, $P=0.015$), not between zones ($F_{(2, 85)}=0.675$, $P=0.512$) (Figure 3.1.B). Spiders at Buffels Bay used significantly smaller shells than at Kommetjie and Olifantsbos, while shell sizes at Simon's Town were not different from any other sites (Figure 3.1.B).
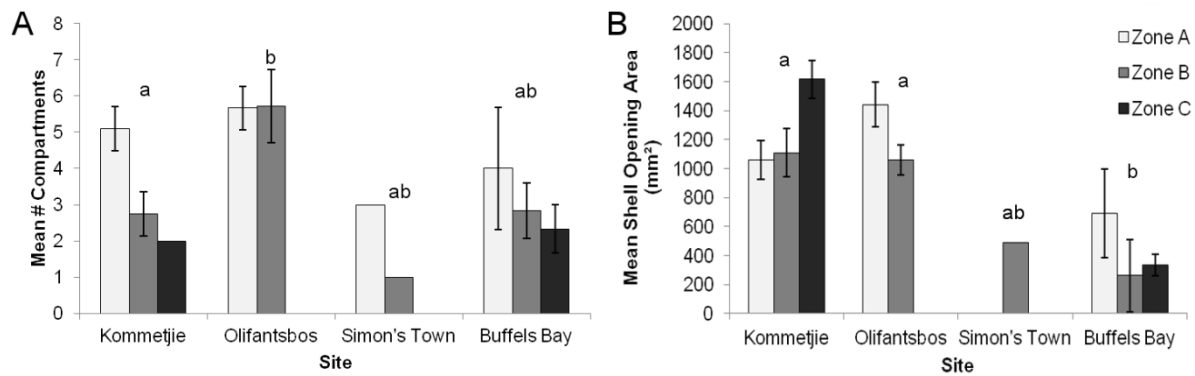
Figure 3.1: Mean number of compartments per *Desis formidabilis* nest (±SE) (A), and mean shell opening area (±SE) (B) for each site and intertidal zone averaged over all collection trips (letters above bars indicate significant differences between localities).

Desis formidabilis showed a significant preference for constructing nests aligned along the east-west line ($X^2_{(17, N=24)}$=37.942, *P*=0.003) (Figure 3.2.A), as well as for limpet shells, then crevices, followed by snail shells and finally mussel shells as materials in which to build its nest ($X^2_{(39, N=48)}$=54.924, *P*=0.047) (Figure 3.2.C). No differences were detected, however, in the frequency of nests between different orientations and locations (orientation: $X^2_{(17, N=24)}$=19.639, *P*=293; location: $X^2_{(17, N=24)}$=24.777, *P*=0.100) (Figure 3.2.B and D) (differences in population sizes between sites and zones are discussed in the results of the models below). The spiders also favoured nesting in low exposure areas as opposed to medium exposure areas and locations with high sun exposure ($X^2_{(36, N=28)}$=55.842, *P*=0.001) (Figure 3.2.E).
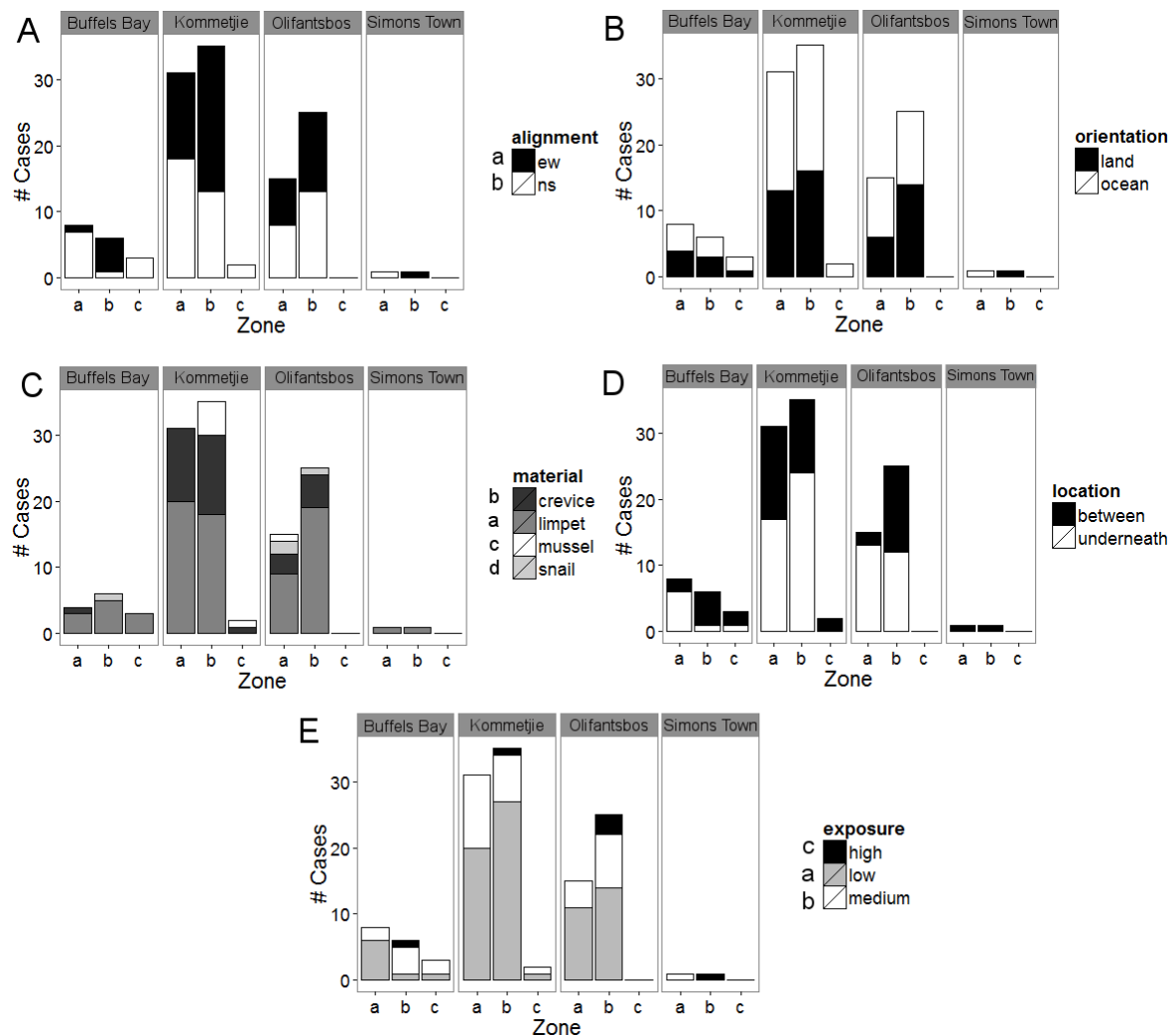
Figure 3.2: Categorical parameters of collected spider nests for each site and zone that were totalled for all collecting events: A: alignment of the nest along a north/south or east/west line; B: orientation of the nest on either the land-, or ocean-facing side of the rock; C: the material used in nest construction; D: whether the nest was located between rocks, or between a rock and the substrate (underneath); E: the exposure of the nest to sunlight.

3.3.2 Nest contents

The nest contents for all sites, zones and collecting trips were pooled, isolating four major categories of contents (Figure 3.3). Just under half of all collected nests comprised empty compartments (Figure 3.3.A). Most of these were complete, but 9% showed evidence of *E.*

lamorali adults entering and exiting the compartment in the form of small holes chewed through the web (Figure 3.3.B). A small proportion of compartments (4%) contained adult wasps, most of which were alive (88%) (Figure 3.3.D). Twenty-one percent of compartments contained spiders (Figure 3.3.E). The majority of these were alive (98%) and ranged in age with 60% older and 38% freshly hatched (Figure 3.3.E). The remainder were adult spiders infected by an unidentified fungus (Figure 3.3.E).

One third of the collected compartments contained eggs (Figure 3.3). These fell into five states, with roughly half being fresh and of a normal colour; 23% already hatched; 20% dead (16% fungus and 4% putrefied); and 15% unhatched, but brown in colour (Figure 3.3). The mean number of eggs (brood size) in compartments that contained eggs was 19 ± 0.81 (SE), with a minimum of one egg and a maximum of 54. Brood size differed significantly between sites ($F_{(3, 79)}$=4.280, P=0.007), but not zones ($F_{(2, 79)}$=1.343, P=0.267), with spiders at Olifantsbos producing more eggs than at Buffels Bay (Figure 3.4). The number of eggs produced did not correlate with nest size (Wald Chi=0.007, P=0.932).
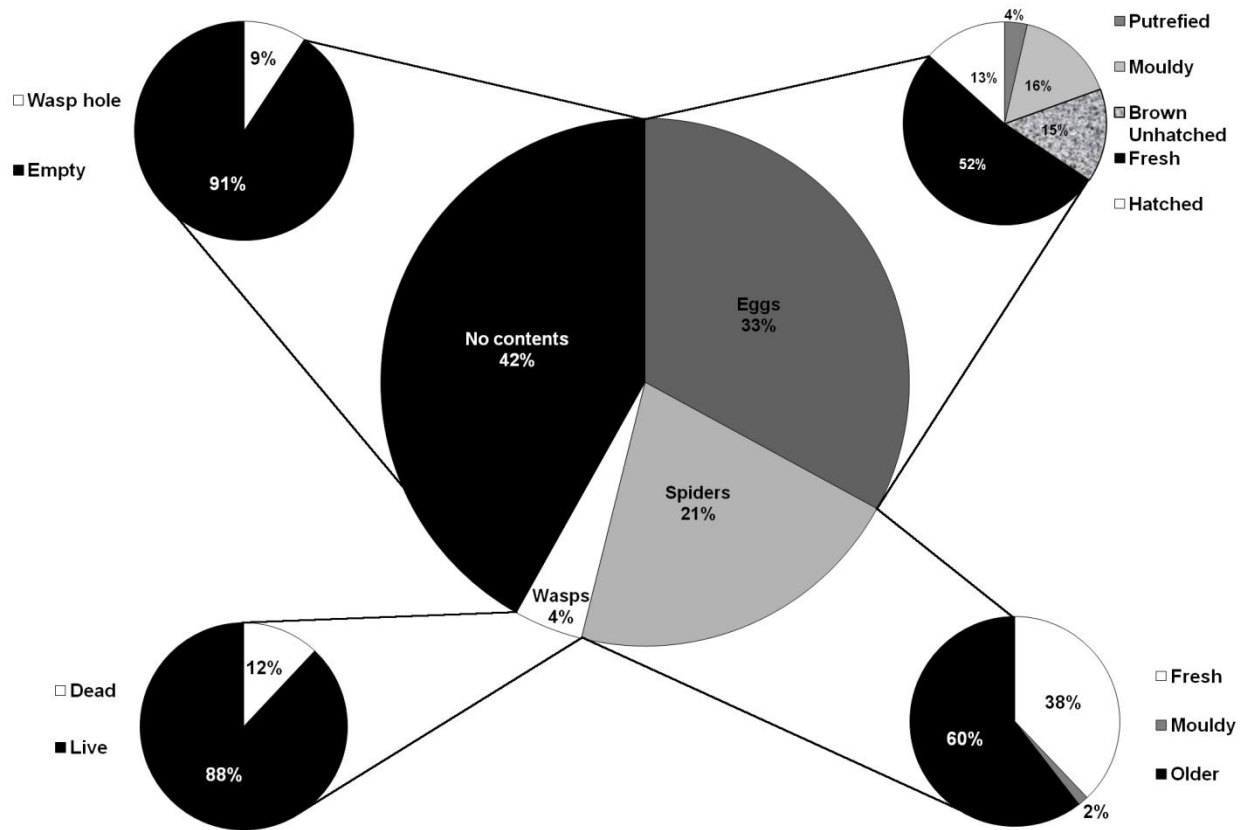
Figure 3.3: Contents of all *Desis formidabilis* nests collected in all zones of all sites for all collection trips. "No contents" compartments were either whole, or had holes chewed by *Echthrodesis lamorali*, but no specimens; "Eggs" found in compartments fell into five categories; "Wasps" were either dead or alive; and "Spiders" inside compartments fell into three categories.
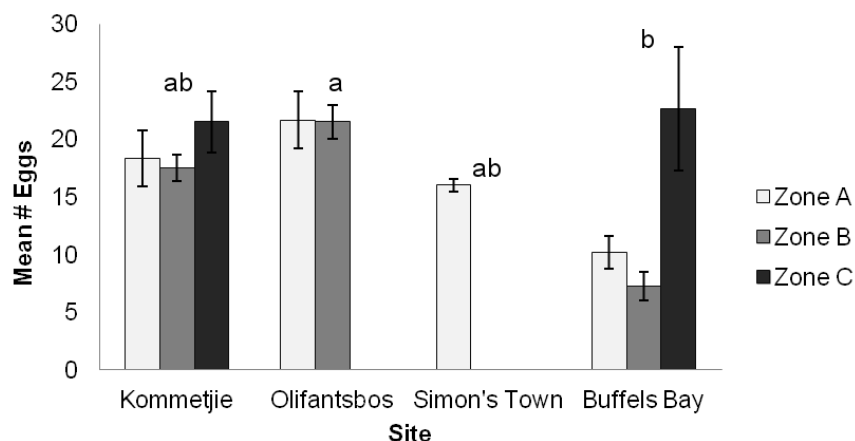
Figure 3.4: Mean number of *Desis formidabilis* eggs (±SE) in compartments that contained them for each site and zone and averaged across all collecting events (letters above bars indicate significant differences between localities).

Egg success here is defined as the full development and hatching of an organism from the egg. For all eggs pooled together, no statistical differences were detected between sites ($F_{(3, 155)}$=1.330, *P*=0.267) and zones ($F_{(2, 155)}$=1.380, *P*=0.255) (Figure 3.5.A). Mean success rate across all sites and zones for all eggs was 36.19% ± 7.83 (SE) with a range of zero to 100%. Parasitized eggs demonstrated an approximately 50% less success rate to those that had not been attacked, with an average rate of 24.13% ± 8.61 (SE) for the former and 47.99% ± 10.40 (SE) for the latter. No significant differences were detected between either of these success rates at all sites (not parasitized: $F_{(3, 62)}$=0.291, *P*=0.832; parasitized $F_{(2, 45)}$=0.444, *P*=0.644) and zones (not parasitized $F_{(2, 62)}$=2.108, *P*=0.130; parasitized $F_{(2, 45)}$=1.138, *P*=0.330). In most cases where parasitized eggs failed, the wasp embryo reached full development, but never eclosed.
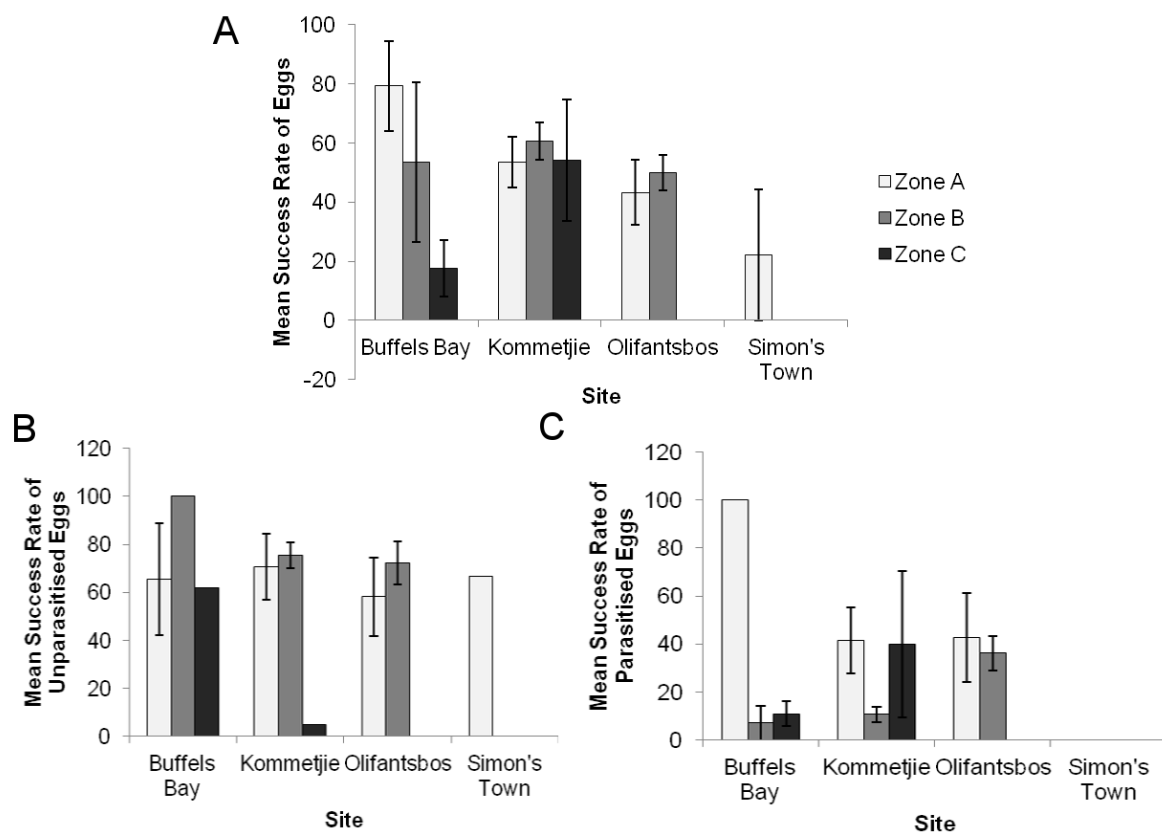
Figure 3.5: Mean developmental success rate of: A: all; B: unparasitized; and C: parasitized *Desis formidabilis* eggs (±SE) for all sites and zones and averaged across all collection trips.

Just over half of all unsuccessful eggs were unidentifiable due to putrefication or encapsulation of the contents (Figure 3.6). Twenty-seven percent contained fully formed but dead wasps, very few (2%) contained spiders, and a total of 17% were already hatched, but their contents were not accounted for in the adult specimens (Figure 3.6).
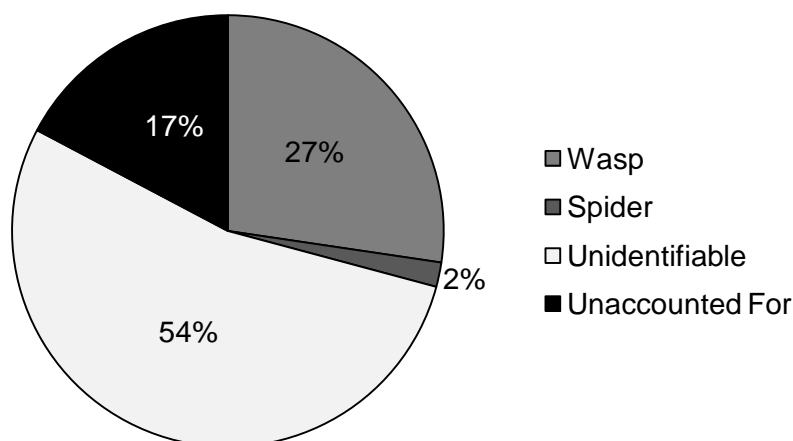
Figure 3.6: Contents of unsuccessful *Desis formidabilis* eggs for all sites, zones and collecting events.

Encapsulation of material within an egg that lead to its unsuccessful maturation was correlated with parasitism ($X^2_{(1, N=84)}$=10.715, *P*=0.001). In other words, where an egg failed because of encapsulation material within it, it was more than likely parasitized. Furthermore, egg colour was also an appropriate indicator of whether it had been parasitized ($X^2_{(1, N=105)}$=37.690, *P*=0.000), as eggs that had turned brown were significantly more often parasitized than not.

3.3.3 Sex ratios

Pooled data for all sites, zones and collecting events showed a strong female bias in resultant wasps with 83% female and only 17% male. Regression models to check for correlation between brood size and sex ratios showed different patterns depending on the whether the sexes were tested as a ratio or individually. Both males and females together and females alone indicated a polynomial relationship, although the relationship was not significant ($R^2_{(15)}$=0.096, *P*=0.514; and $R^2_{(16)}$=-0.056, *P*=0.754 respectively). Brood size and the number

_____

of males present were significantly correlated ($R^2_{(16)}$=0.161, P=0.055) in cases where both sexes of offspring were produced, however, with more male eggs oviposited in larger brood sizes (Figure 3.7).
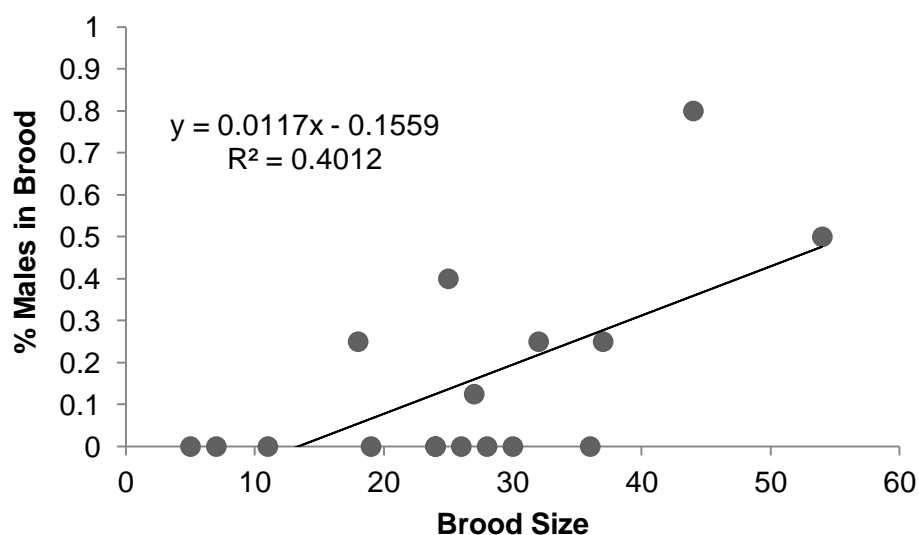
Figure 3.7: Relationship between the number of *Echthrodesis lamorali* males in a brood relative to the total number of *Desis formidabilis* eggs (brood size) (P=0.055).

3.3.4 Modelling

3.3.4.1 Spider population size

A GLZ conducted for all sites and zones illustrated that the number of nests collected for each site, zone and zone nested within site did not differ significantly between different collecting trips ($F_{(1, 11)}$=1.259, P=0.262). As such, it was appropriate to use each survey as a replicate despite the temporal differences between them.

_____

The Akaike Information Criterion assessment (AICc) for spider population size as a resultant parameter returned a total of eight plausible models with deltas of less than two. Recurring predictive factors used for the final model were site, zone and presence of parasitism, none of which contained any co-linear data (Table 3.1).

Spider population sizes differed significantly between the zones and sites (Table 3.1). Populations were largest in zone B (the middle region on a shore), followed by A (closest to the water) and finally zone C (furthest from the water) (Figure 3.8). The western shore of the Peninsula hosted the most spiders, with the highest population density at Kommetjie, then Olifantsbos, followed by Buffels Bay and finally Simon's Town (Figure 3.8).

Table 3.1: Contingency table of model using zone, site and parasitism rate as predictors of spider population size.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Zone A; Site_Buffels Bay)	0.122	0.010	12.200	0.000
Zone B	0.017	0.005	3.156	0.002
Zone C	-0.066	0.018	-3.734	0.000
Site_Kommetjie	0.087	0.009	9.613	0.000
Site_Olifantsbos	0.025	0.009	2.704	0.007
Site_Simon's Town	-0.082	0.034	-2.428	0.016
Parasitism	0.006	0.007	0.872	0.384

_____
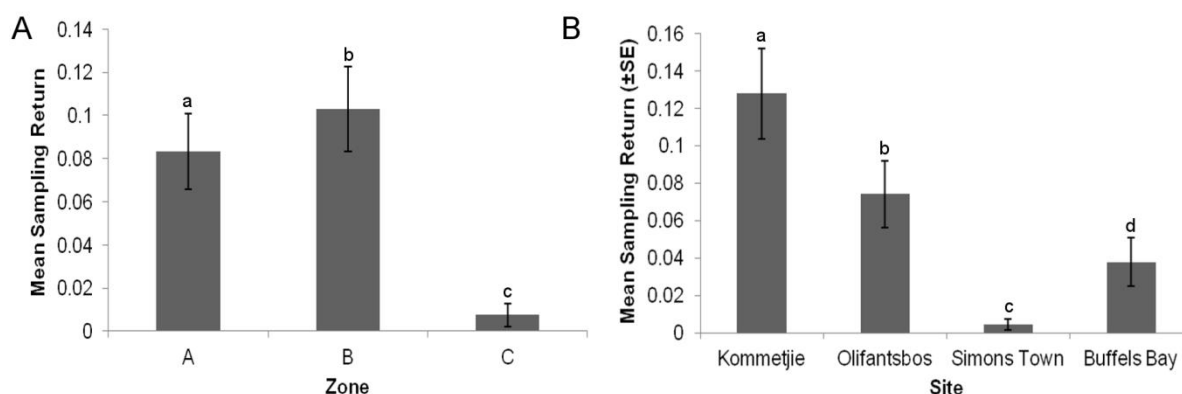
Figure 3.8: Mean sampling return (±SE) (a proxy for spider population size) plotted against: A: zone and B: site, averaged over all other parameters (letters above bars indicate significant differences).

3.3.4.2 Parasitism rates

Prior to modelling, the parasitism incidence within a single compartment was calculated for all cases of wasp attack. Parasitism of eggs in a single compartment ranged from 11.36% to 100%, with a mean of 60% ± 5.45 (SE).

The parasitism rate model Akaike Information Criterion assessment (AICc) returned zone, nest orientation, spider population size and number of compartments per nest as recurring predictive factors in 11 models (delta <2). None of these proved to be co-linear so could all be used for the final model. The final model returned zone and orientation as the only significant predictors as to whether a compartment was parasitized or not (Table 3.2).

Following a similar trend to the spider population size modelled previously, most parasitism took place in the eulittoral zone (B), followed by the sublittoral fringe (B) and lastly the littoral fringe (A) (Figure 3.9). Nests on the ocean-facing side of the rock were also significantly more commonly attacked than those facing the land (Figure 3.9).

_____

Table 3.2: Contingency table of model using zone, orientation of nest, spider population size and number of compartments in nest as predictors of parasitism rates by *E. lamorali* in *D. formidabilis* populations.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Zone A; Orientation_Land; 0)	-2.595	0.518	-5.010	0.000
Zone B	0.015	0.260	0.057	0.955
Zone C	-14.858	685.967	-0.022	0.983
Orientation_Ocean	0.823	0.277	2.965	0.003
Spider Population Size	2.066	1.978	1.044	0.296
# of Compartments	0.026	0.025	1.047	0.295
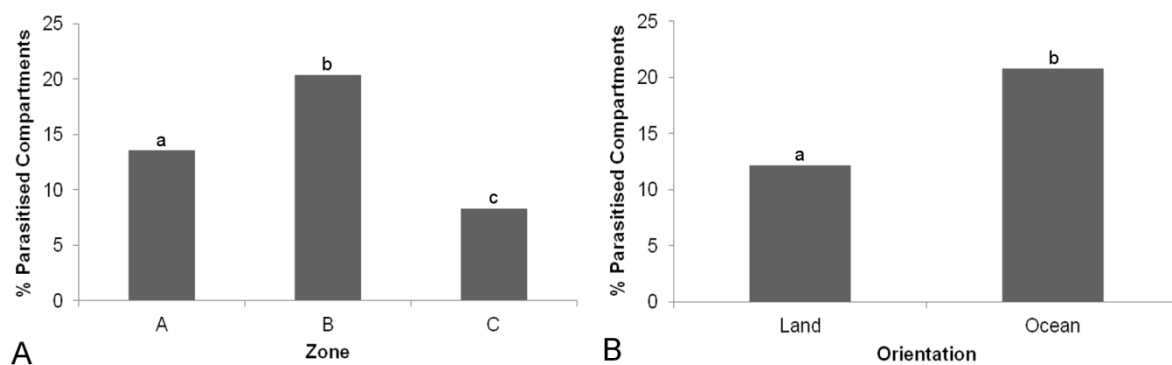
Figure 3.9: The percentage of parasitized compartments plotted against: A: zone; and B: nest orientation; totalled for all other parameters (letters above bars indicate significant differences).

3.3.4.3 Sex ratios

Exposure, location and orientation of nests were identified as important factors for three models with no co-linearity, as constructed by AICcs conducted on models predicting sex ratios of resultant wasp broods (Table 3.3). The location and orientation of the nest were the only factors that showed significant differences. Broods were significantly less female biased in nests found between rocks than those below them (Figure 3.10A). The same pattern was

observed for nests facing the ocean, while those facing the land gave rise to significantly more females (Figure 3.10B).

Table 3.3: Contingency table of model using location and alignment of spider nests as predictors of sex ratios in resultant *Echthrodesis lamorali* broods.

	Estimate	Standard Error	t-Value	*P*-Value
Intercept (Exposure_Low; Exposure_High; Location_Between; Orientation_Land)	0.275	0.111	2.481	0.028
Exposure_Medium	-0.243	0.118	-2.057	0.060
Location_Underneath	-0.351	0.108	-3.237	0.006
Orientation_Ocean	0.328	0.096	3.400	0.005
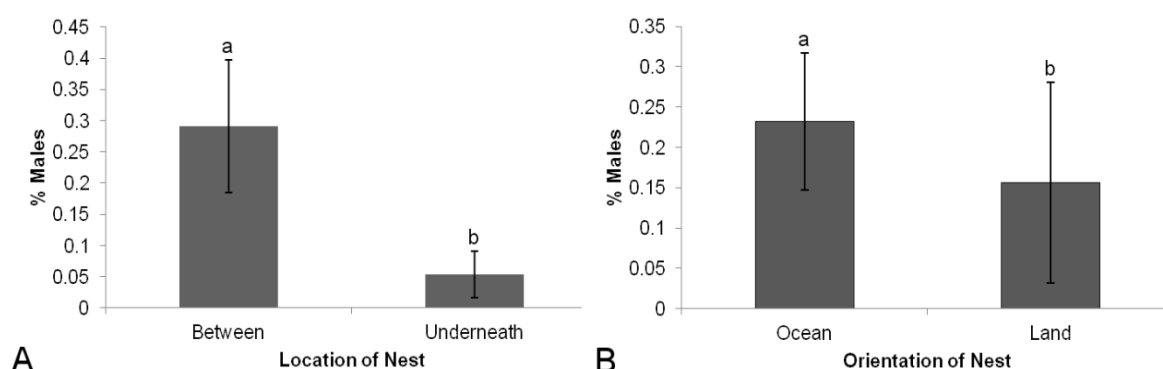
Figure 3.10: Sex ratios (±SE) of resultant *Echthrodesis lamorali* broods reared from *Desis formidabilis* nests located (A) and orientated (B) in different positions and pooled for all sites, zones and collection trips (letters above bars indicate significant differences).

_____

3.4 Discussion

Intra-community dynamics can have large controlling effects on an ecosystem's functioning (Paetzold *et al.,* 2008). For an ecotonal environment such as the intertidal zone (Traut, 2005; Paetzold *et al.,* 2008), these interactions could have knock-on effects for both terrestrial and marine ecosystems that border the region (Paetzold *et al.,* 2008). It was originally hypothesised in this study that *E. lamorali* may be influencing *D. formidabilis* populations through top down control, which could have wider reaching effects on the spider's prey and the intertidal ecosystem, as well as neighbouring communities. The data here suggest, however, that the spider is more influenced by bottom-up factors, while the parasitoid shows density dependence (a bottom-up dynamic) at the scale of a single shore. As they impose such a controlling effect over an ecosystem, the recognition of the direction of community controlling factors, i.e. either top-down or bottom-up, is an important component in the process of habitat management (Munkittrick & McCarty, 1995; Landis *et al.,* 2000; Castella *et al.,* 2007; Gripenberg & Roslin, 2007).

3.4.1 Spider population size and nest characteristics

Van Noort (2009) reported that the spider populations are "localized, but occur in reasonably high density". The data collected here support this notion, but also illustrate significant differences in population sizes between intertidal zones, sites and collecting events. The eulittoral zone (B) hosted the largest spider population, followed by the sublittoral fringe (A) and finally the littoral fringe (C). Interestingly, Lamoral (1968) only recorded *D. formidabilis* in the eulittoral region (B), declaring that "The delimitation, especially near H.W.N. [high water neap, the upper demarcation of zone B], is sharp". The pattern observable in this study is easily explained through the influence exerted by environmental conditions on a shore. As

one proceeds up the intertidal region, the conditions become drier and hotter for longer periods of time due to the lack of wave action during low tide (Sanford, 2002). Lower down, however, the physical force of wave action may be too stressful for certain species and individuals (Sanford, 2002). These changes have large effects on the in-shore distributions of a huge variety of intertidal organisms and appear to be influencing *D. formidabilis* too (Sanford, 2002).

The populations on the west coast were significantly bigger than those on the eastern side of the peninsula. A large variety of environmental and community-based parameters have an effect on a population's ability to maintain a large size (Foster & Treherne, 1976; Sanford, 2002). This may explain the recurring result found for the nest characteristics where Buffels Bay showed repeated deviance from the mean values. The population at Simon's Town was relatively small, a possible result of the unusual beach type that was surveyed for *D. formidabilis* nests at this location. The Simon's Town site was much steeper and more cliff-like when compared to the flatter boulder-strewn beaches at the other sampled shores, making comparisons of the eastern and western sides of the peninsula difficult. Buffels Bay is more comparable from a shore-composition point of view, and appears to indicate large differences in conditions between the two regions. This is common for intertidal regions, where the combination of varying biotic and abiotic conditions on different shores lead to unique patterns (Tietz & Robinson, 1974; Lubke, 1998). As discussed in Chapter 2, these populations fall within the ecotone between the warm temperate biogeographical region to the east and the cool temperate region to the west (Owen *et al.,* 2014). The differences between these two biogeographical zones could have strong influences on the conditions and therefore, community structures on the two shores (Tietz & Robinson, 1974; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002).

A significant outcome of the model on spider population sizes is that parasitism was not identified as a controlling factor. In many other systems, parasitoids exert a top-down controlling effect on spider populations, such as in spider populations affected by pompilid wasp abundances on islands in the Gulf of California (Polis *et al.,* 1998), and web building spiders attacked by *Trypoxylon figulus* L. (Hymenoptera: Crabronidae) on the Swiss Plateau between the cities of Bern, Solothurn and Fribourg (Switzerland) (Coudrain *et al.,* 2013). In this study, the spiders appear to be controlled by bottom-up dynamics, which incorporate both biotic and abiotic factors (Cheng & Wise, 1999; Denno *et al.,* 2002; Menge *et al.,* 2015). Such bottom-up control has previously been recorded in detritus-driven ecosystems such as the intertidal zone (Chen & Wise, 1999; Menge *et al.,* 2015) and in other arachnid species (Denno *et al.,* 2002). Bottom-up population control is highly likely in this case as it explains all of the patterns in spider population sizes discussed above.

No significant differences were detected between frequencies of nests placed with different orientations and in different locations. The spiders did, however, make use of more limpet shells over crevices, snail shells and mussels. All three of these results point towards the environment that the spiders are exposed to and an 'opportunistic' nest building behaviour. Due to the highly heterogeneous nature of the rocky shore (Lubke, 1998), many potential nest-building sites with different orientations and locations will be available to them. The lack of significant differences between these two nest characteristics point towards the spiders either nesting at random wherever they can, or another factor controlling the nest site choice. The nest material choice directly reflects the abundances of various shells or crevices available to the spiders. Limpets were by far the most abundant shell at all sites, while empty snail and mussel shells were much rarer to encounter, explaining their reduced use and the more common crevice nesting. Furthermore, limpets on the western coast grow larger than

_____

those on other South African coasts as a result of high productivity in the area (Bustamante *et al.*, 1995; Branch & Steffani, 2004). The availability of larger shells is reflected in the spiders' larger shell choices on this coastline. *Desis formidabilis* also showed a preference for nest sites lying along the east-west line. Nests aligned this way are theoretically less exposed to sunlight and heat as they never have one side of the nest being heated by the sun at some point during the day, as a north-south lying nest will (the eastern-facing side in the morning and western-facing side in the afternoon). As such, the nest should be kept cooler and experience a more consistent temperature throughout the day, resulting in less chance of desiccation of the eggs. This ties in well with their preference for nest sites that are less exposed to the sun, indicating a preference for cooler, wetter nest sites.

Van Noort *et al.* (2014) placed the number of compartments within the average *D. formidabilis* nest at between five or six. The data presented here support this, with the average number of compartments for all nests at around four, although there was high variability between the sites with no clear patterns. In many cases, larger spider nest sizes can indicate better resource availability and living conditions (Marshall & Gittleman, 1994; Ward & Lubin, 1993). As such, the spiders in areas with larger nests may be living in environments better suited to their needs. Following the theory that the spiders produce multiple compartments in an attempt to escape parasitism (van Noort, 2009; van Noort *et al.*, 2014), it could also be hypothesised that the spiders in areas with more compartments per nest are more heavily parasitized than the other zones or sites. The model on parasitism does not support this, however, suggesting that the spiders in this region have more resources available to them and are thus able to expend more on web production (Marshall & Gittleman, 1994; Ward & Lubin, 1993).

Almost half of all compartments in the spider nests were empty, with 9% of these exhibiting signs of *E. lamorali* activity (chewed holes), supporting the "red herring" hypothesis postulated by van Noort (2009) and van Noort *et al.* (2014). This theory suggests that wasps will not have enough resources left to enter a second compartment once having chewed into a first that contains no eggs (van Noort, 2009; van Noort *et al.,* 2014). Austin (1984) showed that *Ceratobaeus masneri* Austin 1983 (Hymenoptera: Platygastridae, Scelioninae) females usually die shortly after ovipositing their first brood because dead individuals were often found on or in the host's nests, although this may be the result of starvation during brood guarding that is seen in some platygastrids following oviposition (Matsuo *et al.,* 2014), rather than a depletion of resources during oviposition. None of the 9% of compartments assessed in the present study, however, contained dead adult wasps. This therefore casts doubt on the 'red herring' theory (van Noort 2009; van Noort *et al.,* 2014). It would appear that *E. lamorali* is capable of entering more than one compartment before losing energy. This makes sense, as a female must have enough resources to inspect and oviposit into all the eggs available in a compartment that does contain eggs once access is gained. Should the first compartment accessed not contain eggs, the foundress will theoretically still have energy left. This can then be used to access a second part of the nest. The question of how many compartments the wasps are capable of accessing is further complicated by the observation that 4% of compartments contained wasps, but only 12% of these were dead. Whether *E. lamorali* foundresses guard their parasitized broods is unknown, but if females die shortly after exhausting their egg supply by ovipositing into all eggs available, or die during guarding behaviour, at least one dead individual should be present in all parasitized compartments. A large portion of mortality in this species may thus take place during dispersal between nests. The highly heterogeneous environment that is the intertidal habitat can be a particularly hostile one (Lubke, 1998), so this is a likely conclusion.

Van Noort *et al.* (2014) reported that each compartment of the average *D. formidabilis* nest collected at Kommetjie contained 8-19 eggs. Data here place the average at 19 ± 0.81 (SE), with the total number reaching as high as 54 eggs in a single compartment. These values contain those found by van Noort *et al.* (2014) but indicate a generally larger brood than they encountered. This mean number of eggs was consistent for all zones, although Buffels Bay had lower brood sizes than Olifantsbos. Larger broods indicate better resource availability and fewer environmental stressors for the spiders in that region (Marshall & Gittleman, 1994; Ward & Lubin, 1993) and reflect the preference of *D. formidabilis* for the cooler west coast. As discussed previously, the larger nest sizes, and now also smaller broods, may be indicative of increased parasitism, but the parasitism model does not support this hypothesis.

Despite both complete failures and complete successes, the average spider nest experienced a successful reproduction rate of approximately 36%, which is relatively low. This value held for all sites and zones with no statistically significant differences between them. When separated into parasitized or unparasitized eggs, this figure is more understandable. Most sites experienced a 50% success rate of juvenile spider emergence from eggs that had not been parasitized. This did not include fungal infections or putrefication. As discussed above, lower success rates of eggs is common for species with high reproductive outputs (Deevey, Jr., 1947; Murdoch, 1966; Gadgil & Bossert, 1970), such as *D. formidabilis*. When eggs are parasitized, the number of successful cases is halved to 24%. Of all the unsuccessful contents, 54% were unidentifiable due to encapsulation of the embryo, while 27% contained wasps close to full development, 2% spiders close to full development and 17% of eggs were those that had already hatched prior to collection. The high proportion of encapsulated embryos, i.e. those eclosed with abnormal tissue growth such that the embryo could no longer be

identified, which was shown to correlate with parasitism, suggests that the host egg may possess the ability to fight back against parasitism, thereby killing the parasitoid larvae. Ill-timing of oviposition into a host egg and the development of a variety of hormones already present in the egg as a result of this are thought to be the most common cause of host-destruction of the parasitoid (Austin, 1984; Strand, 1986a). However, in this case, only certain eggs in a brood failed, with failure of an entire clutch rare. While van Noort (2011) and van Noort *et al.* (2014) detail the oviposition behaviour of *E. lamorali* females, no indication of the time taken to attack an entire brood is given. It is unlikely, however, that enough time elapses between the first and last oviposition for the host to develop beyond the time frame for safe oviposition by the wasp. As such, the encapsulation of only certain embryos is puzzling. Furthermore, most parasitoids reached maturity within the egg but then never eclosed. It is unknown what results in the failure of such a large proportion of parasitized eggs. The best possible explanation could be unfavourable environmental characteristics in the laboratory where the eggs were reared, which may stop egg development (Germano & Picollo, 2015; van Asch & Visser, 2007), although this also does not explain the failure of only some eggs in a brood.

Egg colour was correlated with the presence of parasitism, with colour changing from yellow to brown once parasitized. These findings are in line with the work of van Noort *et al.* (2014), who found that spider eggs change colour, shape and texture once parasitized. Austin *et al.* (2005) confirm this as a common reaction amongst the platygastrid hosts, and it is seen in a variety of other egg-parasitism cases (Takada *et al.,* 2000; Lobdell *et al.,* 2005). This significant relationship could serve as an appropriate quick, in-field test to determine if a brood has been parasitized in future studies.

3.4.2 Parasitism rates

Parasitism rates within a single compartment ranged between 11.36% and 100%, with a mean of 60% ± 5.45 (SE). Van Noort *et al.* (2014), however, consistently recorded a parasitism rate of 100% within a single compartment. The disparity between these results is puzzling, but explainable. Of the 566 compartments assessed in this study, only one contained both spiders (either as hatched spiderlings or failed embryos) and wasps. The lowered parasitism rates are probably the result of the inability to confirm the species of encapsulated or putrefied embryos. As such, it would appear that parasitism is almost always 100% within a host egg batch. This parasitism frequency is not common for the Scelioninae (van Noort *et al.,* 2014).

The zone in which a nest was built and its orientation either towards or away from the sea were the only factors identified as significantly impacting parasitism, with the highest values recorded in the eulittoral zone (B), followed by the sublittoral fringe (A), and finally the littoral fringe (C). Parasitism incidence is largely affected by an organism's ability to find and recognise the host nest, which is a factor of a variety of environmental components (Fox *et al.,* 1990; Austin *et al.,* 2005). In this case, the parasitism rate followed density patterns displayed by the host spider and may indicate a density-dependence between *E. lamorali* and *D. formidabilis* at this scale.

Ocean-facing nests experienced higher parasitism rates than those facing the land despite an approximate 50/50 split in spider nests between the two. This pattern points towards preferable abiotic conditions in nests facing sea-wards for *E. lamorali* (Foster & Treherne, 1976; Sanford, 2002). The intertidal region is a highly heterogeneous one (Lubke, 1998), and

abiotic conditions are likely to differ from one side to the other of the same rock. Wave exposure was shown to alter other abiotic parameters in different zones on a variety of shores in South Africa (Bustamante *et al.,* 1997). At the smaller scale of a single rock, wave action must also have an influence on the other abiotic elements in that habitat, with different forces acting on either side of it. Theoretically, the ocean-facing side might take more force from crashing waves, but there may be less turbulence that could rip nests or individuals away. Furthermore, the ocean-facing side should be relatively cooler and wetter (through wave or water action [Bustamante *et al.,* 1997]), factors that *E. lamorali* may prefer. Many parasitoid species appear to be initially attracted to an environment that suits them and that they are used to finding their host in, than to the actual presence of the host itself (Laing, 1937; Atkins, 1980c). This may explain the increased parasitism in the ocean-facing nests, which may have more favourable conditions for the wasp.

The independent (predictor) factors that did not significantly influence parasitism indicate some strong hypotheses about the interaction between *D. formidabilis* and *E. lamorali.* Regression of parasitism incidence against population size should indicate the presence of any density dependences (Heimpel & Casas, 2008). Return, a proxy for host population size, was not a significant predictor for parasitism incidence. Furthermore, the parasitism rate was not significantly different between sites, which did host significantly different spider population sizes. Differences in parasitism between zones did reflect spider population sizes, however, indicating a positive density dependence (Heimpel & Casas, 2008). This disparity in density dependence patterns could be an example of the influence of assessments at different scales on community patterns (Gripenberg & Roslin, 2007; Donadi *et al.,* 2015), but is more likely indicative of another factor influencing the wasp's distribution on the shore other than density dependence (Heimpel & Casas, 2008). This lack of a statistically

significant density dependent pattern, despite seeming density dependence at face value, could be the result of two processes (Heimpel & Casas, 2008). Firstly, increased superparasitism at high wasp densities could counter density dependence patterns (Heimpel & Casas, 2008). Austin (1984) reported 20-30% of parasitized egg masses in his study to have been attacked by multiple females, although only 13% of the eggs in these masses showed evidence of superparasitism. As yet, as a result of relying solely on field-collected nests to test hypotheses on parasitism incidence and sex ratios (Heimpel & Casas, 2008), it is unknown whether *E. lamorali* suffers from superparasitism. Van Noort *et al.* (2014) recorded up to three females ovipositing in the same compartment in some nests, with each making their own individual entrance hole, but whether superparasitism took place is unknown. The only way to test hypotheses on superparasitism in the field would be to track individuals using a camera, as was done for *Drosophila* Fallén 1823 (Diptera: Drosophilidae) parasitoids by Janssen (1989), but the relative size of *E. lamorali* precludes this as an appropriate sampling technique in the heterogeneous intertidal environment. Alternatively, where more wasps occupy the same area, they may spend more time engaging in behaviour that is not oviposition, such as handling of eggs to check for parasitism by other females, or interacting with other wasps (Heimpel & Casas, 2008). In this case, density-dependence is being displayed, but based on *E. lamorali* itself, not the patterns of the host, *D. formidabilis.*

3.4.3 Sex ratios

Wasps showed strongly female-biased broods (83% female) here, echoing the findings of van Noort *et al.* (2014) who recorded 87.29% females. In fact, foundress females of this species did not appear to be able to produce more than four males per brood. More females than males in a brood is common for platygastrids (Austin, 1984; Strand, 1988; Fox *et al.,* 1990;

Austin *et al.,* 2005). In many scelionines, the males eclose first and wait for their siblings to emerge to mate with (Strand, 1986b; Strand, 1988). This has possibly developed in response to the often limited pool of conspecifics, both from a temporal as well as spatial time scale (Strand, 1986b). Furthermore, in such a turbulent environment as the intertidal region, males may remain in their natal compartment as they are more guaranteed a mate than if they were to exit the compartment to find another nest, during which the chances of mortality are high. Mating between siblings in this way also theoretically allows for the retention of the advantageous characters that assisted the foundress to successfully reproduce in future generations.

Most Hymenoptera give rise to male eggs when unfertilized (Atkins, 1980b; Green *et al.,* 1982; Austin, 1984; Waage, 1986; King, 1987; Strand, 1988). This implies that all unmated females will produce only male eggs (Atkins, 1980b; Green *et al.,* 1982; Austin, 1984; Strand, 1986a; Waage, 1986; King, 1987; Strand, 1988). Mated females, on the other hand, are capable of withholding sperm from certain eggs to ensure a mix of both sexes in the eggs (Atkins, 1980b; Strand, 1986a; Waage, 1986; King, 1987). The presence of females in most broods collected here indicates that the majority of the foundress females are mating, as was found for field collected *Ceratobaeus masneri* Austin 1983 (Hymenoptera: Platygastridae, Scelioninae) and *Ceratobaeus clubionus* Austin 1983 (Hymenoptera: Platygastridae, Scelioninae) in Austin's (1984) work. Theoretically, the sex ratio produced by an organism should be precise, rather than random, as is evident for many organisms (Green *et al.,* 1982; Waage, 1986). Mortality rates in developing embryos could differ between males and females (Green *et al.,* 1982; Waage, 1986). As such, where not all *E. lamorali* individuals eclosed, the sex ratios recorded in those that did could be incorrect (Green *et al.,* 1982).

As overall clutch size increases, Green *et al.* (1982) showed that the number of males follows the same trend for other parasitoid wasps, as demonstrated here with *E. lamorali*. This theory is known as the local mate competition model and while the general principles behind it are logical and well understood, the mechanisms that influence the female's decision to lay male or female eggs is relatively unknown (Waage, 1986; Hamilton, 1987; King, 1987; Strand, 1988). Two main processes are thought to influence these decisions in solitary parasitoids like *E. lamorali*, namely the number of females encountered while ovipositing and the distribution of the host (King, 1987; Strand, 1988). The theory predicts more male offspring where many foundress females enter a single egg sac, and environments with more nests in a small area will also host more males (Hamilton, 1987; King, 1987; Strand, 1988). Up to three females ovipositing in a single compartment in some nests was recorded by van Noort *et al.* (2014), but whether superparasitism took place is unknown. Furthermore, it was not possible to ascertain whether superparasitism was present in any of the field-collected nests used in the present study. Nonetheless, both of these ideas centre on the fact that if more females from differing foundresses are present, males will have a greater chance of passing their genetic material along into a non-sibling (King, 1987; Strand, 1988).

A large variety of factors influence the sex ratio of hymenopteran broods (King, 1987; Fox *et al.,* 1990). Where the sex ratio of a brood is not 'decided' on (i.e. is not a conscious decision) by a foundress, egg order; maternal size; maternal diet; genetics; polyembryony; the delay between emergence and insemination; the number of times the foundress has mated; the maternal and paternal age at insemination; host size; and the photoperiod, temperature and relative humidity of the surrounding environment have all been hypothesized as affecting sex ratios (King, 1987; Fox *et al.,* 1990). Clearly, various factors affect each species differently and individual species have great potential to be unique. A permanent sex-converting factor

has been reported for some parasitoid species, where males carry an extra chromosomal factor that disallows fusion of the male and female gametes in the fertilized eggs (Strand, 1986b). As such, all oviposited eggs effectively remain unfertilized and thus give rise to males (Strand, 1986b). As no broods collected here contained all males, it is unlikely that this is in effect in *E. lamorali* populations. A paper produced in 1982, however, reported a female version of this in the same species, *Nasonia vitripennis* Walker 1836 (Hymenoptera: Pteromalidae), where all eggs produced are female (Skinner, 1982; Strand, 1986b). This could account for the all-female broods in some cases.

The only environmental factors that significantly influenced the sex ratios in *E. lamorali* were location and orientation of the host nest. Nests placed underneath and on the land side of rocks produced more female offspring than those in-between and on the ocean side of them. Nests underneath and on the ocean side of rocks should be kept colder than those between them because they are less exposed to sunlight and are generally wetter from water pooling at the base of the rock and moisture coming from the ocean. Temperature-linked sex ratios are commonly known for many reptile species (Berec *et al.,* 2001; Janzen & Morjan, 2001), but have only been detected in a few insect species (King, 1987b; Wermelinger & Seifert, 1999). In other parasitoid wasps that exhibit temperature-linked sex ratios, extreme high or low temperatures lead to higher sex ratios (more females) (King, 1987b). Temperature can affect these species during all life stages through any of the following mechanisms: sterilizing males; increasing or decreasing movement so that mating is hindered; incapacitating sperm; increasing the rate of oviposition beyond the rate of sperm release from the spermatheca; differential mortality between the sexes; and foundress decisions based on perceived environmental cues as a result of temperature differences (King, 1987b). King (1987b) goes on to include humidity in sex ratio determination in some parasitoids, with a varied result

depending on the species. It would appear that *E. lamorali* is a species whose sex ratio is correlated with temperature and humidity, an interesting phenomenon that warrants further investigation.

3.4.4 Conclusion

In conclusion, *D. formidabilis* made use of four nesting materials on the Cape Peninsula, the shells of which were larger on the west coast as a result of the opportunistic nest-building behaviour of the spider. The species did not show any preference for the orientation or location of its nests, but did prefer the cooler, more consistently temperature-regulated nesting areas along an east-west line and that were not directly exposed to sunlight (Coombes *et al.,* 2013). Significant differences were found in spider population sizes between different sites and zones, with the east coast of the Peninsula hosting fewer spiders than the west and the largest populations located in the mid-shore region. All of these patterns could be directly related to differing and changing abiotic and biotic components of their host environments. Of particular interest was the evidence for bottom-up control of *D. formidabilis* as opposed to top-down control through the action of the parasitoid. Nests averaged four compartments, half of which were empty with only a few displaying evidence of *E. lamorali* entrance. Dead wasps were not common in these, casting doubt on the 'red herring' theory and suggesting that, contrary to previous hypotheses, females were capable of accessing more than one compartment. Mortality in this species appeared to be highest during dispersal between nests. Where *D. formidabilis* eggs were present in compartments, they averaged 19 per segment, all of which were parasitized when wasps had gained access to them. Unparasitized eggs, which remained yellow throughout their development, experienced a 50% failure rate, a figure not uncommon for spiders that lay multiple eggs in a brood. This figure was halved when

parasitism had occurred (brown eggs), although the reasons behind this were unclear, as many wasp embryos reached full maturity but did not eclose. Parasitism followed host density patterns at the in-shore scale, but this pattern changed at the larger scale of the shores compared to one another. This reinforced the need for multi-scale analyses in both parasitoid/host and intertidal investigations in future work. Furthermore, the wasps showed an affinity for ocean-facing nests, a possible result of more favourable abiotic conditions. *Echthrodesis lamorali* broods were strongly female biased, following the general patterns exhibited by the Platygastridae. A higher sex ratio in nests placed underneath and on the ocean side of rocks suggested temperature- and humidity-linked sex allocations in this species. The assessment of the wasp's physiological and morphological adaptations in the following chapter sheds some light on the patterns evident in its in- and between-shore distributions and sex ratios at the scale of the individual.

Chapter 4: The physiological and morphological adaptations of _Echthrodesis lamorali_ Masner 1968 (Hymenoptera: Platygastridae: Scelioninae) to living within the intertidal environment[1]

4.1 Introduction

Despite comprising over 75% of described animal taxa, insects have not readily colonised the marine environment, with only several hundred species living in intertidal or marine habitats at some point in their life cycles (Cheng, 1976; Gainey, Jr., 1984). The lack of calcium necessary to construct exoskeletons in seawater has been postulated as the main reason for this, while some believe that the domination of Crustacea in the ecosystem presents too large a predation pressure (Cheng, 1976; Maddrell, 1998). Others contest that there may also exist much more profound physical and physiological explanations (Usinger, 1957; Cheng, 1973; Cheng, 1976; Andersen & Weir, 1994; Maddrell, 1998; Vermeij & Dudley, 2000; Ikawa _et al.,_ 2012). Buoyancy in saltwater and overcoming surface tension are difficult to adapt to, particularly as most flying insects exhibit a reduced body weight, increased surface area and have large wings that become heavy and rigid when wet (Cheng, 1976). Furthermore, control of salt and water content in the body (osmoregulation) and respiration in the aqueous medium need to be overcome (Cheng, 1976; Treherne, 1976; Gainey, Jr., 1984; Maddrell, 1998). Being an air-breathing organism that has to respire in water is a major feat to overcome, as water (H_2O) molecules are smaller than oxygen (O_2) molecules (Hinton, 1976). As a result of this, spiracles will tend to allow water to leak through them over oxygen (Hinton, 1976).

---

[1]Chapter condensed and manuscripts are currently in preparation as:

OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. In prep. Assessing the morphological and physiological adaptations of _Echthrodesis lamorali_ Masner 1968 (Hymenoptera: Platygastridae, Scelioninae) for survival in the intertidal environment. _Journal of Hymenoptera Research._

OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. In prep. The thermal physiology of _Echthrodesis lamorali_ Masner 1968 (Hymenoptera: Platygastridae, Scelioninae), a maritime spider egg parasitoid. _Journal of Hymenoptera Research_

Cheng (1976) and Gainey, Jr. (1984) state that any adaptations to counter these obstacles are generally unknown for most maritime species, and warrant further exploration and investigation.

Echthrodesis lamorali Masner 1968 (Hymenoptera: Platygastridae, Scelioninae) lives and survives within the intertidal region and is fully immersed in seawater at each high tide (Masner, 1968). Masner (1968) therefore postulated that the species must exhibit a variety of adaptations for coping with inundation. The most obvious is that the species has lost its wings, a common response in other maritime species (Cheng, 1976). As a result of a higher body weight to surface area ratio, winglessness also aids in remaining in one area during strong coastal winds (Cheng, 1976; Austin *et al.,* 2005; van Noort, 2009). It is important to note, however, that wing reduction is common amongst the Scelioninae (Austin *et al.,* 2005; Carey *et al.,* 2006), and may rather be a plesiomorphic character than a maritime adaptation. Little more is known about their adaptations to saltwater inundation, and it is thus hypothesized that *E. lamorali* exhibits some morphological and physiological adaptations to surmount this.

There are eight scelionine genera that are known to parasitize the eggs of freshwater insects (Johnson & Masner, 2004). *Echthrodesis lamorali*, however, is the first species documented to have a maritime lifestyle (Masner, 1968; van Noort *et al.,* 2014). While more than half of the recorded intertidal insects are documented as evolving from terrestrial forms, all adaptations evident in marine forms can be found in their freshwater counterparts (Hinton, 1976). Consequently, drawing a comparison between the two is credible.

To cope with water inundation, many freshwater aquatic insects exhibit the ability for quiescence during submersion (Foster & Treherne, 1976). This involves a coma-like state with reduced respiratory requirements that allows the insects to remain underwater for a period (Foster & Treherne, 1976), as has been recorded in the four beetle species (*Bledius spectabilis* Kratz, 1857 [Coleoptera: Staphylinidae], *Heterocerus fenestratus Thunberg 1784* [Coleoptera: Heteroceridae]*, Dichirotrichus pubescens* Paykull 1790 [Coleoptera: Carabidae] and *Cillenus lateralis* Samouelle 1819 [Coleoptera: Carabidae]) inhabiting the littoral zone in Scolt Head Island (Norfolk, England) (Evans *et al.,* 1971); and the Eastern subterranean termite, *Reticulitermes flavipes* Kollar 1837 (Rhinotermitidae: Isoptera) (Collins, 2013). Furthermore, some insects (e.g. *Pogonomyrmex californicus* Buckley 1867 [Hymenoptera: Formicidae] [Lighton *et al.,* 2004]) are capable of closing their spiracles (Wigglesworth, 1953; Hinton, 1976), much like the stomata of trees in dry conditions (Fernández *et al.,* 1997), but this limits the amount of time that they can remain submerged. In a turbulent environment like the intertidal region, an individual may also not be able to close its spiracles in time under unpredictable water level conditions as a result of wave action.

Beyond the capability to control spiracle aperture size, a variety of behavioural and morphological mechanisms are exhibited by freshwater aquatic insects (Hinton, 1976). Many species, most notably the backswimmers (Hemiptera: Notonectidae) (Matthews & Seymour, 2006), are able to swim and repeatedly return to the surface to renew air bubbles collected there (Hinton, 1976; Foster & Treherne, 1976; Pedersen & Colmer, 2012). Many aquatic fly larvae (Diptera) and other freshwater insects also have breathing tubes that break the surface of the water body when the individual is submerged, allowing them to continue breathing atmospheric air (Cheng, 1976; Hinton, 1976; Dobson, 2013), or sharp-ended spiracular extensions to pierce air-filled submerged plants (Hinton, 1976; Pedersen & Colmer, 2012), as

in the larvae of the aquatic fly *Erioptera squalida* Loew 1871 (Diptera: Tipuloidea) (Przhiboro, 2000). These have not been recorded in any intertidal-living species (Hinton, 1976), and are not displayed by *E. lamorali.* Finally, loss of the waxy lining to the cuticle in certain sections of the body allows for gas-permeable sections and has been shown as the primary means of dealing with water immersion in both freshwater and maritime species (Hinton, 1976; Buchwalter *et al.,* 2003).

Lamoral (1968) illustrated how the host spider of *E. lamorali*, *Desis formidabilis* O.P. Cambridge 1890 (Araneae: Desidae), possesses dense, short, chitinous setae spaced at regular intervals on the edges of the openings to the booklungs. These are hypothesized to avert water entering and mechanically prevent the 'leaves' of the booklungs from closing due to pressure when underwater, allowing for continued respiration (Lamoral, 1968). It is unknown whether *E. lamorali* possesses similar structures in its trachea and this chapter thus aimed to determine if the spiracles and trachea of this species display any such adaptations.

Furthermore, *D. formidabilis* exhibits a physical gill when submerged (Lamoral, 1968; Branch and Branch, 1981). This mechanism employs the use of setae over the spider's body, which trap a film of air that is capable of exchanging gasses with the surrounding environment without collapsing (Lamoral, 1968; Hinton, 1976; Branch and Branch, 1981). A variety of insect species possess this adaptation too (Cheng, 1976; Foster & Treherne, 1976; Neumann & Woermann, 2009), including the intertidal midge genus, *Clunio* Haliday 1855 (Diptera: Chironomidae) (Neumann & Woermann, 2009). Studies on these have shown that the angle between the setae or micro-cuticular structures and the insect body are of particular importance, with angles less than 90° resulting in the disruption of bubble formation

(Neumann & Woermann, 2009). Furthermore, the distance between setae may be critical (Lamoral, 1968). Due to the relative differences in solubility of oxygen and nitrogen, the two gases making up the majority of a physical gill bubble, these structures are not permanently self-sustaining, but they may last for significant periods of time (Lamoral, 1968; Hinton, 1976; Branch & Branch, 1981), for example, a total of more than 12 hours for *D. formidabilis*, which is longer than the duration of the average high tide (Lamoral, 1968). Some freshwater species also display this adaptation (Hinton, 1976) and *E. lamorali* could be employing the strategy, as is suggested by the dense coating of setae all over its body (Masner, 1968; van Noort *et al.,* 2014). Thus, another aim of this chapter was to determine whether the species is capable of forming a physical gill.

Further investigation into the respiratory dynamics of *E. lamorali* requires an understanding of some basic physiological information. The most crucial data to determine are the critical thermal limits of a species, which is the temperature bracket in which the insect can maintain normal bodily functionality (Huey *et al.,* 1992; Hazell *et al.,* 2008). This is important for establishing a working temperature bracket in other experiments, such that the physiological capabilities of the species are not violated. Following exposure beyond the maximum and minimum threshold temperatures, the calcium current across membranes in cells in an individual are disrupted, resulting in a loss of motor control and an inability to self-right (Huey *et al.,* 1992; Terblanche *et al.,* 2007; Hazell *et al.,* 2008). Not only is this information important for experimentation, but also to fully understand the "ecology, evolution and physiology" (Hazell *et al.,* 2008) of a species, as its distribution and physiological adaptations are influenced by its thermal tolerance (Terblanche *et al.,* 2007; Hazell *et al.,* 2008).

In light of the extreme habitat in which the species persists, this study aimed to determine if there are any outer or internal structures associated with the spiracles and tracheae of *E. lamorali* that allow it to withstand saltwater inundation, and to determine if the insect forms a physical gill in a similar manner to its host spider. Based on the morphology and physiology of other freshwater and marine insects, as well as the dense pilosity of the body of *E. lamorali*, it is hypothesized that the spiracles and trachea of this species may show high modification. Furthermore, a physical gill is expected to form around the habitus of the species when submerged.

4.2 Materials and methods

A variety of methods were used to investigate the morphological and physiological adaptations of *E. lamorali* for coping with respiratory requirements when submerged. These included scanning electron microscopy for external features, sectioning for internal adaptations and respirometry experiments to determine differences in respiratory rates when submerged or not. These methods are discussed in detail in the following sections.

4.2.1 Morphological adaptations

Independent dissections of *E. lamorali* by O. Popovici revealed that the species lacks the posterior metasomal spiracles common in other Hymenoptera (O. Popovici, Facultatea de Biologie, Universitatea Alexandru Ioan Cuza IASI, Romania, pers. comm.). As such, attention was paid entirely to the mesosomal spiracles in this study.

4.2.1.1 External spiracular structures

In order to examine the outer spiracular structure for evidence of modification for water inundation, individual specimens of *E. lamorali* were prepared for scanning electron microscopy using standard methods (Cross & Pinchuck, 1987). Each specimen was coated with gold and images of the ventral mesosoma were captured at various magnifications and angles using a Tescan Vega LMU Scanning Electron Microscope (20kV current; AnalySIS photo capture and measurement software) housed in the Rhodes University Electron Microscopy Unit.

4.2.1.2. Internal tracheal structures

To prepare *E. lamorali* for sectioning into habitus segments that could be viewed and examined under a light microscope, live insects were placed into a 2.5% Glutaraldehyde ($CH_2(CH_2CHO)_2$) in 0.1M Sodium phosphate (Na_3PO_4) buffer solution overnight for fixation of the tissues. Thereafter the liquid was removed and the specimens rinsed twice in 0.1M sodium phosphate buffer for ten minutes each time.

The process of sealing in Avaldite/TAAB 812 resin (Cross and Pinchuck, 1987) is detailed in Table 4.1, where the wasps were exposed to a variety of dehydrating agents and resins to ensure full fixation. Once left in pure resin overnight, the solution was poured into capsule moulds with one specimen at the tip of each and baked at 60°C for 36 hours in an oven.

_____

Table 4.1: Embedding protocol followed to fix specimens in resin for sectioning.

Solution specimens exposed to	Period of time exposed to solution (minutes)
1% Osmidium tetroxide (OsO_4)	90
0.1M Sodium phosphate	10
0.1M Sodium phosphate	10
30% Ethanol (CH_3CH_2OH)	10
50% Ethanol	10
70% Ethanol	10
80% Ethanol	10
90% Ethanol	10
Absolute Ethanol	10
Absolute Ethanol	10
Propylene oxide (CH_3CHCH_2O)	20
Propylene oxide	20
75/25 Pro-Resin	60
50/50 Pro-Resin	60
25/75 Pro-Resin	60
Pure Resin	Overnight

Due to liquefying of the resin prior to hardening, the wasps drifted in the capsules. Therefore, prior to any sectioning, a small block of resin containing the insect was cut off and re-glued at the required angle to ensure that sections were cut straight.

All sectioning was performed using a glass blade (prepared by a LKB Knife Maker Type 780 1B at 80°) in a RMC MT-7 Microtome, set to cut 120 micron thick sections. Both longitudinal and transverse sections were prepared. These were then placed on glass slides; stained with 1% Toluidine Blue Stain in a Sodium Carbonate solution; rinsed with 30%, 70%

and then 100% Ethanol; and images of the sections were captured using an Olympus BX50 Microscope and the associated AnalySIS software for identification of internal structures that may assist with submerged respiration.

4.2.2 Physiological adaptations

In order to test whether *E. lamorali* is capable of maintaining normal respiratory functioning when submerged, the thermal tolerance of the species was investigated. This set the thermal parameters in which the respirometry experiments were conducted.

4.2.2.1 Critical thermal tolerance

To determine the thermal range of *E. lamorali*, a Grant GP200 R4 programmable waterbath was used and filled with 70% ethanol for ramp-down trials and distilled water in ramp-up trials. Three trials comprising ten field-collected insects (collected from Kommetjie, Western Cape, South Africa [34° 8' 22.7034" S, 18° 19' 17.5794" E]) in each were run for each temperature ramp, resulting in a total of 30 individuals in each experiment.

Freshly eclosed wasps were placed individually into Eppendorf® tubes containing filter paper moistened with seawater to maintain the moisture content inside the enclosure. An operative thermometer placed inside an empty Eppendorf® confirmed close correlation between internal chamber conditions and water temperatures. The insects were allowed to acclimatise at 18°C for ten minutes, where after the temperature was ramped up or down at a rate of 0.25°C/minute. Specimens were checked for motor function every four minutes (every

change of 1°C) by tapping the Eppendorf® and checking for falling and self-righting behaviour. Temperatures at which insects lost motor control were recorded and a mean was calculated for all 30 individuals as the upper (ramp-up) and lower (ramp-down) critical thermal limits. When specimens lost their self-righting response, they were removed from the waterbath and maintained at room temperature. Recovery to full motor function was determined 1 and 24 hours after losing motor function.

4.2.2.2 Respirometry

To determine whether *E. lamorali* is capable of forming a physical gill, the respiratory rate of 131 adult *E. lamorali* individuals, collected from in-field populations at Kommetjie, was determined in 12 separate trials using a 25°C calibrated PreSens GmbH respirometer in both air (62 wasps) and seawater (69 wasps). All trials were conducted at the Aquatic Ecophysiology Research Platform (Rhodes University, Eastern Cape, South Africa) in a temperature-controlled laboratory set to the working temperature of the experiment and with a standardized 12 hour day-night light cycling. Because seawater filtered using 0.7µm pore size filter paper still exhibited a high amount of oxygen consumption in pre-experimental test control chambers, all water used was filtered and autoclaved in a Sturdy SA300-VL autoclave to remove all biological organisms that may consume oxygen other than the test wasps.

Wasps were first acclimatized in a single, large, sealed chamber in a flow-through unit attached to a PolyScience PP20R waterbath at 18°C for ten minutes. As temperature can have significant effects on respiration (Verberk *et al.,* 2015), thereafter the temperature was ramped down to 17°C (the environmental mean as calculated in Chapter 2 at the time of

completion of these experiments) at a rate of 0.25°C/minute. The *E. lamorali* individuals were kept at this temperature for five minutes before being transferred into the individual test chambers that either contained atmospheric air or filtered, autoclaved seawater. During the course of this acclimatisation, the chambers that were to contain seawater were soaked in water that was replaced just prior to the addition of the wasps. This allowed sufficient wetting of the sensors so that readings were accurate. This was not necessary for the air treatment, the chambers of which were permanently exposed to atmospheric air.

Specimens were placed into randomly-selected Loligo 1425 200µl chambers filled with either seawater (sealed to prevent air-bubble formation) or air, allowing for both control and experimental chambers of both treatments in each trial for comparison of same-trial consumption rates. The chamber tray was then submerged in the flow-through unit attached to the waterbath and placed on top of the SDR-436 respirometer sensor reader.

Twelve separate trials taking readings in 190 chambers (39 wasps in air, 65 air controls, 31 submerged wasps and 55 water controls) were run for a total of 24 hours with oxygen readings (cO_2: mg/L) in each chamber recorded and assimilated using PreSens SDR version 38 software every minute. Data collected in chambers containing wasps that did not gain full motor control within 24 hours after the end of the study were removed from the final analysis. Those wasps that were still alive and active after the 24 hours were weighed while still alive using a Mettler Toledo XP205 balance (with anti-static attachment), whereafter they were euthanized in 96% ethanol for further assessment of their volumes. These were determined by treating the head and body of each individual as two ellipsoids, the volumes of which were calculated by determining the length, breadth and depth (Equation 1) of the

specimen using an Olympus SZX16 stacker microscope (Olympus SDF Plapo 1XPF camera) with the associated Steam Motion 1.9 software. The volumes of the head and body were summed to determine the total volume for the entire organism. These values were crucial for per-unit-volume calculations of oxygen consumption.

Equation 1: volume = (4/3)*PI*(1/2length)*(1/2breadth)*(1/2depth)

The first two hours of data were removed from the dataset to allow for oxygen drift common at the start of such experiments (Köster *et al.*, 2012). Thereafter the remaining data were analysed using regression analysis in Statistica 10 (StatSoft Inc., 2011). As experimental error is frequent in such investigations as a result of the small scale of readings (P. Patrick, South African Institute for Aquatic Biodiversity, pers. comm.), the slopes of these lines were compared for all trials within each treatment using a Thompson Tau test, which removes statistical outliers. Oxygen consumption in each chamber was then calculated using equation 2, whereafter values for wasps in air could be compared to those submerged in water using a Student's *t*-test for independent samples in Statistica.

Equation 2: $VO_2 = (\frac{(mw - mc)}{100})$ x $(V_c - V_w)$ x $\text{ß}O_2$

where VO_2 is the consumption rate per volume of wasp per unit of time (O_2.ml.min^{-1}); mw is the slope of the line of the change in oxygen during the experiment for each test wasp chamber (cO_2.min^{-1}); mc is the mean slope of the lines of the change in oxygen during the experiment for all controls in the same trial (cO_2.min^{-1}); V_c is the volume of the chamber; V_w

_____

the volume of each wasp; and βO_2 is the oxygen capacitance of each medium (Alton *et al.,* 2012).

At 20°C, atmospheric air is cited as having a 54.73μmol.L^{-1}.Torr^{-1} βO_2 (Truchot, 1987). No reference could be found for the βO_2 at 17°C or any other temperature. As such, working backwards from 54.73μmol.L^{-1}.Torr^{-1} at 20°C, and using the ideal gas law (Equation 3), the βO_2 of atmospheric air at 17°C was calculated in Equations 4 and 5.

Equation 3: $PV = nRT$

where P is pressure (in Pa); V is volume (m^3); n is the number of mols of the medium; R is the universal gas constant (8.31451 J/K.mol); and T is the temperature (K).

In order to achieve a final figure in a unit of μmol.L^{-1}.Torr^{-1}, as is frequently cited in the literature (Alton *et al.,* 2012), the following conversions were necessary:

Conversion 1 (atmosphere to Pascal to torr assuming normal atmospheric pressure in chambers): $P = 1atm = 101325Pa = 760torr$

Conversion 2 (m^3 to L): $V = 1m^3 \times 1000$

Conversion 3 (mol to μmol): $n = n \times 10^6$

Conversion 4 (Kelvin to Celsius): $T = 17°C + 273.15 = 290.15$

Equation 4: $\quad \dfrac{n}{V} = \dfrac{P}{RT}$

$$\frac{n}{V} = \frac{101325Pa}{(8.31451 \text{ x } 290.15°C)} = 42.001 \text{mol/m}^3$$

$$\frac{n}{V} = \frac{(42.001 \text{mol/m}^3) \text{ x } 10^6}{1000} = 42\,000.7821 \mu \text{mol/L}$$

Equation 5: $\quad$ ßO2 of atmospheric air (ßO$_2$) at 17°C

$$\frac{n}{VP} = \frac{42\,000.7821 \mu \text{mol/L}}{760 \text{torr}} = 55.261 \mu \text{mol.L}^{-1}.\text{Torr}^{-1}$$

The ßO$_2$ of seawater was cited as $1.618 \mu \text{mol.L}^{-1}.\text{Torr}^{-1}$ at 15°C (Ragg & Taylor, 2006) and $1.76 \mu \text{mol.L}^{-1}.\text{Torr}^{-1}$ at 12°C (Maxime *et al.,* 1990). Working with these figures and assuming linear changes in ßO2 with temperature changes, the ßO2 of seawater declines at a rate of $0.047 \ \mu \text{mol.L}^{-1}.\text{Torr}^{-1}$ per °C (Equation 6).

Equation 6: $\quad \dfrac{\text{ßO2 at 12°C} - \text{ßO2 at 15°C}}{15°C - 12°C}$

$$= \frac{1.76 - 1.618}{3}$$

$$= 0.047 \ \mu \text{mol.L}^{-1}.\text{Torr}^{-1}.°\text{C}^{-1}$$

Therefore, at 17°C, the ßO2 of seawater is calculated as $1.523 \ \mu \text{mol.L}^{-1}.\text{Torr}^{-1}$ (Equation 7).

Equation 7:   ßO2 at 15°C - ((17-15) x 0.047 µmol.L^{-1}.Torr^{-1})

= 1.618 - 0.0947

= 1.523 µmol.L^{-1}.Torr^{-1}

4.3 Results

4.3.1 Morphological adaptations

Echthrodesis lamorali exhibited little spiracular and tracheal adaptation to cope with saltwater inundation. It did, however, show morphological modifications for improved nest access through body streamlining and increased reproductive output. Furthermore, unusual structures located in female specimens may serve a body orientation, oxygen supply, salt control or oviposition assistance function.

4.3.1.1 External spiracular structure

No unique adaptations, such as hairs, sutures or plates, were evident on the spiracles when viewed externally (Figure 4.1).
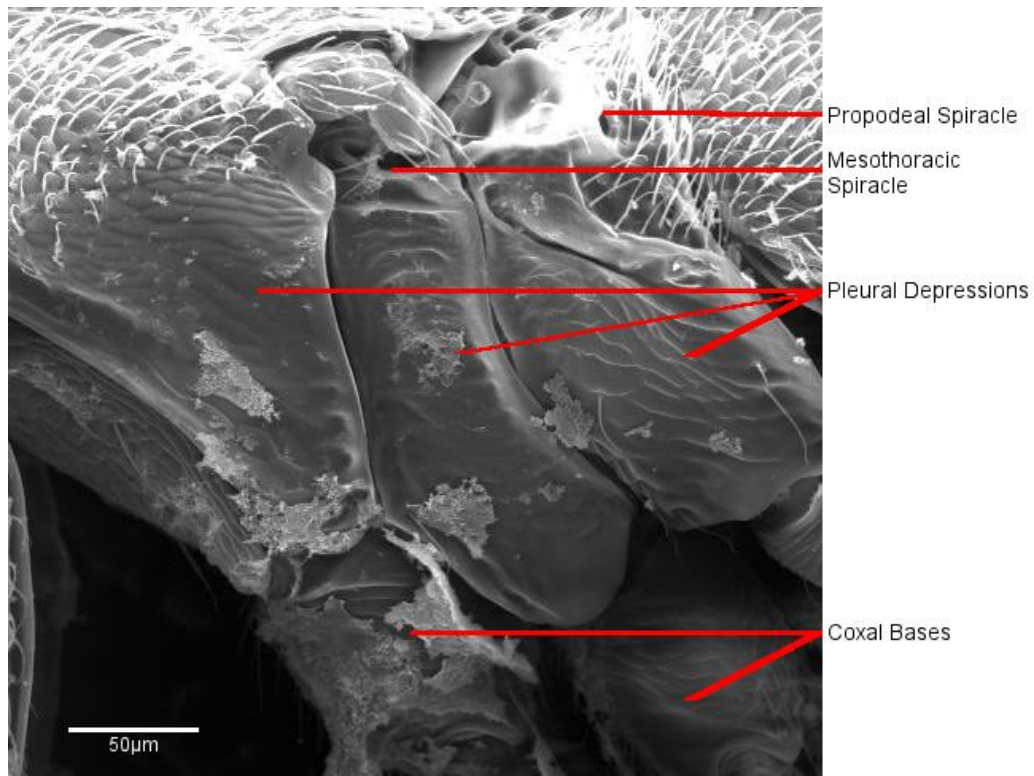
Figure 4.1: Lateral view of the external features of the spiracle on the mesosoma of *Echthrodesis lamorali*.

4.3.1.2 Internal tracheal structure

Longitudinal sections through various specimens of *E. lamorali* also displayed no hair-like or other structures in the trachea when viewed under a light microscope (Figure 4.2.A). Furthermore, transverse sections through the mesosomas of other specimens confirmed no structures further into the trachea (Figure 4.2.B). The trachea did, however, exhibit taenidia, or thickened rings of cuticle along the length of the tube (Figure 4.2.B).
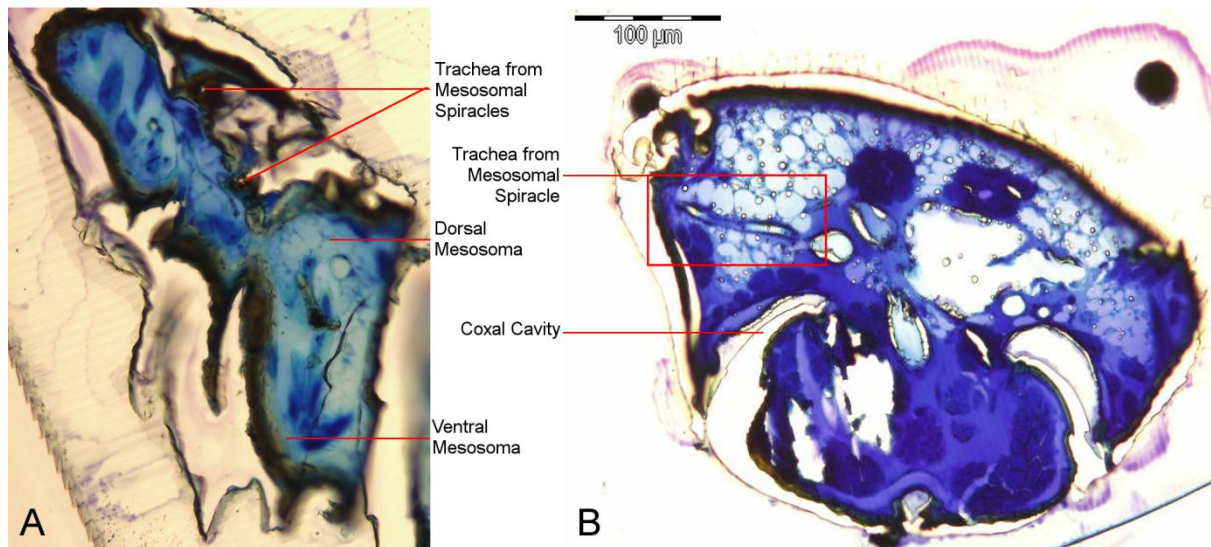
Figure 4.2: A: Longitudinal sections through the mesosoma of *Echthrodesis lamorali* specimens revealed no internal structure in the tracheae; B: Transverse section through the mesosoma of *Echthrodesis lamorali* revealed taenidia (in red box), but no other internal structure in trachea.

4.3.1.3 Other structures

Three bare, dorso-ventrally running grooves (pleural depressions) corresponding to where the legs fold into position were evident on the mesosoma of *E. lamorali* (Figure 4.3). Reproductive and other tissues were evident in prepared sections. Eggs can be seen in Figures 4.4.A and 4.4.C. Figure 4.4.B shows part of a malpighian tubule as well as the segment of the hindgut. All female specimens exhibited bifurcation of the ventral metasoma into an upper fat- and egg-filled region, and a lower cuticle-lined sac-like section filled with an unknown substance (Figures 4.4.C and 4.4.D). Internal sclerotized tubules (possibly tracheae) joining the two sections, and within the lower area, are clearly visible (Fig 4.4.D). The divide disappears closer to the centre of the insect, where the dorsal fatty area assumes the full extent of the metasoma. Unfortunately all attempts to cut transverse sections through female metasomas led to collapse of the tissues as a result of structural weakness because of the two

_____

sac-like segments on either side of the tagma. Male specimens did not illustrate this

bifurcation (Figure 4.5).
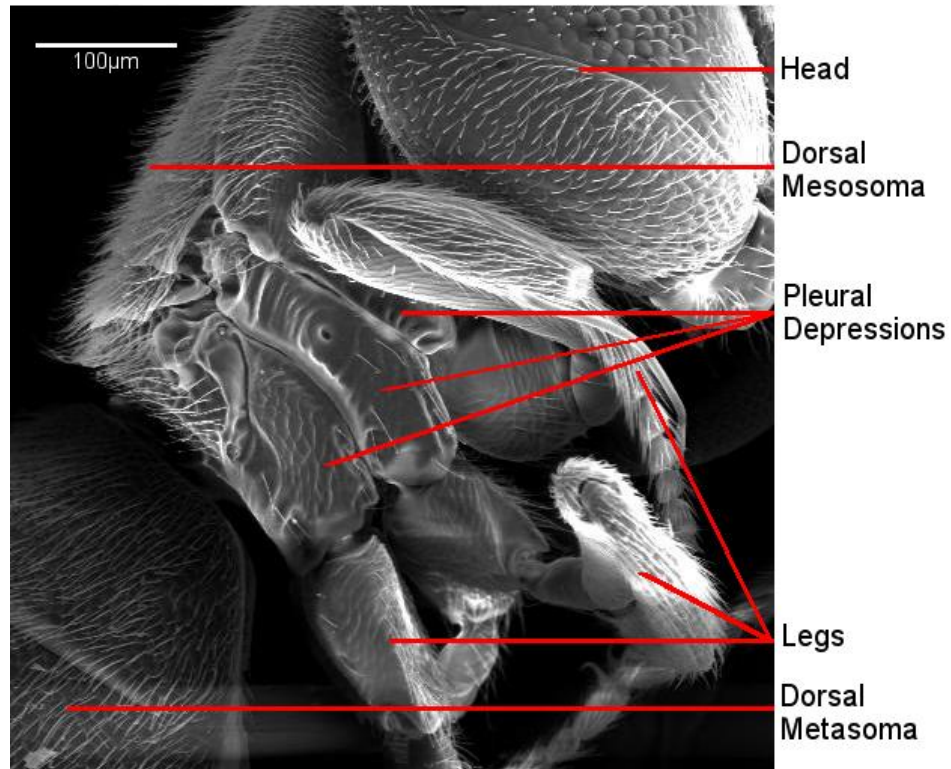
Figure 4.3: *Echthrodesis lamorali* exhibits bare dorso-ventrally orientated grooves (pleural depressions) on the

mesosoma.
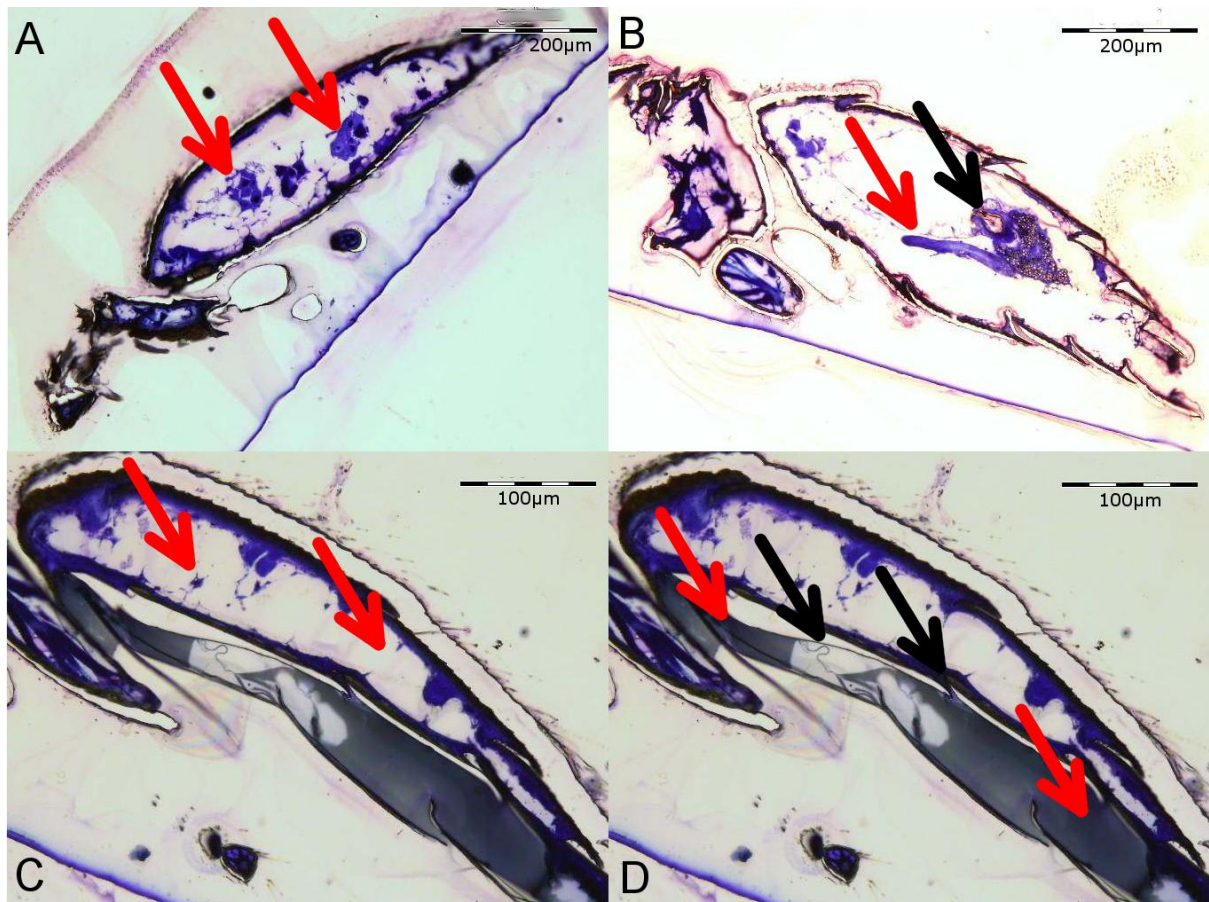
Figure 4.4: A: Eggs (red arrows) are clearly visible in a longitudinal section through the full body of a female *Echthrodesis lamorali* specimen; B: A malpighian tubule (red arrow) with a portion of the hind gut (black arrow) can be seen in this section of a female *Echthrodesis lamorali* specimen; C: The dorsal fat- and egg-filled segment (red arrows) along the ventral regions of metasomas of female *Echthrodesis lamorali* specimens; D: The ventral section in the metasomas (red arrows) of female *Echthrodesis lamorali* specimens is connected to the dorsal region with sclerotized tubes (black arrows), which are possibly tracheae.
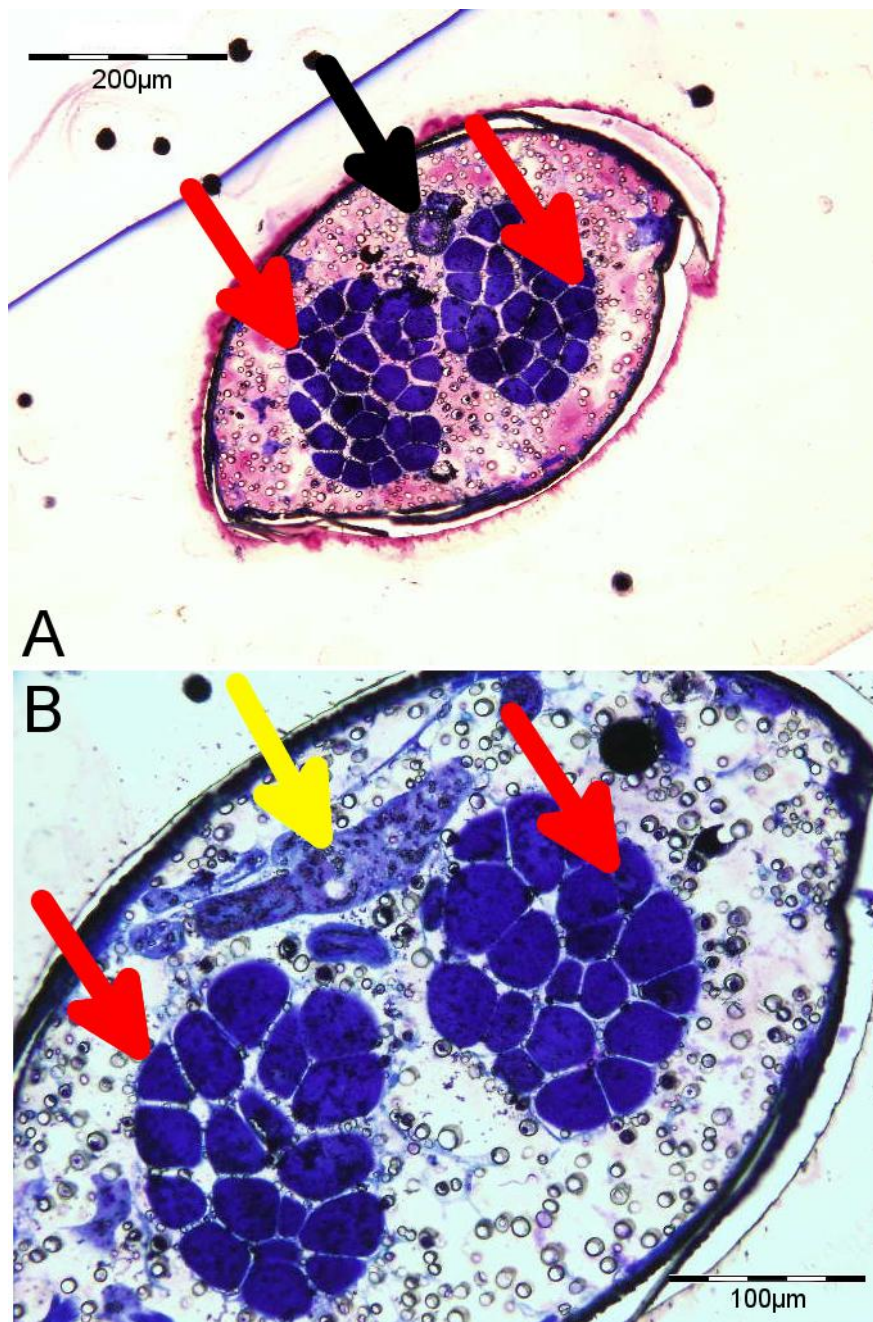
Figure 4.5: The testes (red arrows in A and B), alimentary canal (black arrow in A) and a malpighian tubule (yellow arrow in B) surrounded by fat are evident in transverse sections through the metasomas of male *Echthrodesis lamorali* specimens.

4.3.2 Physiological adaptations

Echthrodesis lamorali was found to be physiologically adapted for life within the intertidal zone with a wide range of temperatures tolerated by the species. Furthermore, it entered a quiescent state when submerged to assist with the maintenance of a physical gill.

4.3.2.1 Critical thermal tolerance

A wide thermal tolerance range was exhibited by the species, with minimum and maximum temperatures at which individuals lost motor control as low as -3°C and as high as 48°C respectively. The mean minimum and maximum temperatures (±SE) at which individual specimens of *E. lamorali* lost motor control were -1.1°C ± 0.16 and 45.7°C ± 0.26 respectively. In ramp down experiments, all specimens recovered full motor functionality within an hour, while it took 24 hours for them to recover in the ramp-up.

4.3.2.2 Respirometry

Once all outliers had been removed by the Thompson Tau test, a total of 27 wasps in air; 53 air controls; 23 wasps in water; and 15 water controls were left for analysis. Freshly submerged wasps were observed rapidly expanding and contracting their limbs for a brief period followed by the cessation of movement. On removal from the seawater chambers, no individuals were immediately active, with movement being regained within 24 hours following removal.

Echthrodesis lamorali individuals sealed in the chambers in air exhibited significantly higher oxygen consumption rates than those isolated in seawater (t=17.422, P<0.0001) (Figure 4.6). As oxygen was removed from the water, it can be concluded that the wasp formed a physical gill when submerged, although their metabolic requirements were lowered by entering a quiescent state (supported by the lack of movement and greatly reduced oxygen consumption in this treatment).
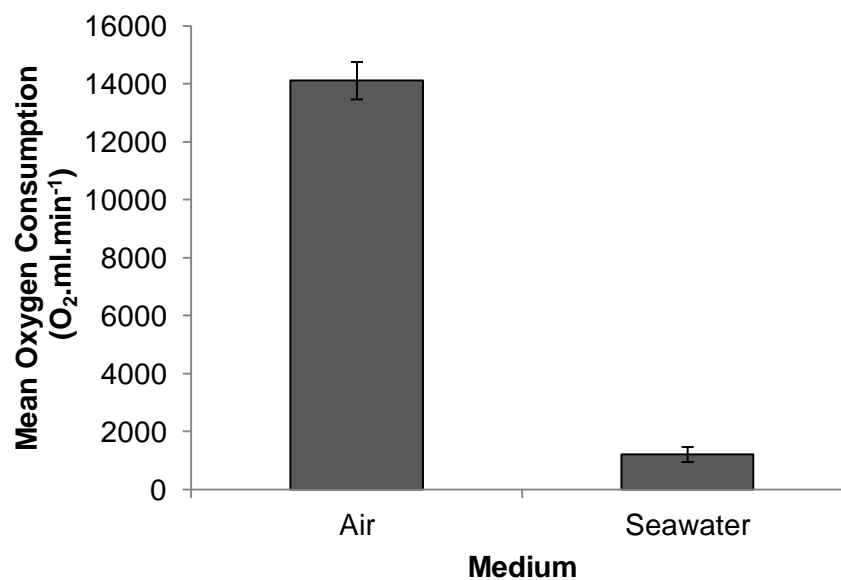
Figure 4.6: Mean oxygen consumption rates ± SE (O_2.ml.min^{-1}) of _Echthrodesis lamorali_ individuals immersed in air or seawater.

4.4 Discussion

Tracheal taenidia were the only structures found within the spiracles and tracheal tubes of _E. lamorali_ that could assist with submersion. These may retain the integrity of the trachea when under pressure from water inundation (Lamoral, 1968), but the lack of any other adaptations

suggests that the wasp can only remain submerged for short periods of time. Many spiders use the silk in their nests to retain an air bubble for continued breathing when immersed in water (Rovner, 1987). This bubble has been shown to retain oxygen significantly longer than those formed around the body of the spider when outside of the nest in some species (Lamoral, 1968; Rovner, 1987). The nest of *D. formidabilis* is no different (Lamoral, 1968) and it should theoretically also be available to the wasp at high tide, allowing them to behaviourally counter submersion (van Noort *et al.,* 2014).

To gain access to the host spiders' eggs, *E. lamorali* has to crawl through a hole chewed into the silken wall of the nest (van Noort *et al.,* 2014). Entry through silk is a difficult process and requires streamlining of the insect's body (Edgerly *et al.,* 2007). It is believed that this may have contributed to the loss of wings in the species (van Noort, 2009), as hypothesised for other spider egg parasitoid wasps (Austin *et al.,* 2005). Bare grooves on the external mesosoma are clear from the scanning electron microscope photographs of *E. lamorali* and it is hypothesized that during entry into the nest, the legs are folded into these spaces to improve smoothness of the outer area and allow for easier passage through the web.

Two sacs filled with either air or an unknown substance run along the entire length of the ventral, lateral metasoma of all female specimens, tapering off to an end towards the centre of the tagma. These are linked to the dorsal, fat- and egg-filled portion of the metasoma by small tubes, suggesting the need for exchange of contents between the two regions. The function of the sclerotized sac-like areas is unknown. It is likely that as a result of the loss of metasomal spiracles, the wasps use these structures as air sacs that are filled through the use of the mesosomal spiracles and tracheae. Oxygen could be released from these to supply the

metasoma. This would make the connecting tubes that are visible tracheae. Alternatively, they could form a buoyancy function for when the insect is submerged, or used to maintain the correct orientation of the body when in water, like the ballast of a ship. They may also serve a physiological function, providing the wasps with antifreeze necessary to maintain bodily functioning at subzero temperatures (discussed below) (Sinclair *et al.,* 2003), or assisting with salt- and water-balance as a result of frequent salt water inundation. In marine teleost fish, this latter function is controlled through the use of extracellular fluids (Grosell *et al.,* 2007), while in invertebrates the excretory system and some epithelial tissues maintain internal conditions (Pierce, 1982). All of these functions are unlikely as the feature is only observed in female specimens. Thus, these structures may be performing a sexually-linked role.

Oviposition and other sex pheromones are produced by glands (Pompanon *et al.,* 1997), which display a much more organised cellular structure than presenting a homogenously-filled sac when viewed as sections under a light microscope (Raina *et al.,* 2000). The structures seen here could, however, be storage sacs for sex pheromones. These pheromones could play a role in mate attraction, as the males hatch first from clutches of eggs and wait for females to emerge from eggs that they identify using the pheromone traces, whereafter they mate immediately (van Noort *et al.,* 2014). They may also be important as oviposition markers to prevent superparasitism (Bragg, 1974). Another possible explanation for the large sacs may be that they are filled with oxygen-rich air. This may aid the females during dispersal in wet conditions from the nest they eclosed in to the one they will oviposit in. Furthermore, studies on oxygen consumption by female insects with developing eggs are relatively rare (Clifford and Woodring, 1986), but Clifford and Woodring (1986) showed that the fertilized eggs of *Acheta domesticus* L. 1758 (Orthoptera: Gryllidae) consume 0.26ml

_____

O_2/g wet wt/h. Furthermore, they found female crickets displayed increased oxygen consumption during oviposition (Clifford and Woodring, 1986). *Echthrodesis lamorali* females may store air to aid them in maintaining fertilized eggs.

The most likely explanation for the presence of these sacs is, however, supplied by Austin (1983) Masner (1993), Field & Austin (1994), Austin *et al.* (2005) and Murphy *et al.* (2007). The Platygastroidea can be divided into two groups, each with a differing ovipositor control mechanism (Austin *et al.,* 2005). These are known as the '*Scelio* Latreille 1810 (Hymenoptera: Platygastridae, Scelioninae) type' and the '*Ceratobaeus* Ashmead (Hymenoptera: Platygastridae, Scelioninae) type' (Austin *et al.,* 2005). Field & Austin (1994) detailed the oviposition mechanics in the genus *Scelio* and demonstrated that ovipositor control is achieved through solely hydrostatic pressure changes in the metasoma (Masner, 1993; Field and Austin, 1994; Austin *et al.,* 2005; Murphy *et al.*, 2007). In the *Ceratobaeus* type, this control achieved through partial muscle movement and partial hydrostatic pressure (Austin, 1983; Austin *et al.,* 2005). Based on the muscle structure and the presence of the large sacs in the mesosoma, *E. lamorali* appears to exhibit the *Ceratobaeus* type ovipositor control.

In order to determine if any of these physiological adaptations assist in continued underwater respiration, it was necessary to determine the thermal tolerance displayed by the species such that temperatures used in the respirometry experiments did not violate the wasps' tolerances. *Echthrodesis lamorali* was found to exhibit a wide thermal tolerance. Based on the environment in which it lives, however, where turbulent wave action reduces the temperature at regular intervals during both high and low tides, and the rocks comprising the habitat in

which this species is found are heated by the sun at low tides (Teal, 1962; Foster & Treherne, 1976; Lubke, 1998; Sanford, 2002), a wide thermal tolerance would be expected.

Although the upper critical thermal limit is generally accepted as being more controlling of a species' life history (Chown *et al.,* 2015), a lower critical thermal tolerance threshold of sub-zero is significant. Sinclair *et al.* (2003) state tolerance of temperatures around zero to be particularly important, as this is the freezing point of water. Solidification of liquid within an insect can be fatal, with cellular dehydration and disruption of chemical reactions across the cellular membranes inhibiting normal functioning (Sinclair *et al.,* 2003). To counter this, there are generally two strategies displayed by other insects (Sinclair *et al.,* 2003). Firstly, some are capable of freeze avoidance, such as in *Eurosta solidaginis* Fitch 1855 (Diptera: Tephritidae) (Levis et al. 2012, Gantz & Lee, Jr 2015), where fluids in the individual prevent it from freezing (Sinclair *et al.,* 2003). In some cases, structures known as ice-nucleators, which act as starting points for ice formation, are removed from the body (Sinclair *et al.,* 2003). Some other species contain antifreeze, which is sometimes a unique compound or a collection of sugars and polyols in the body fluids (Sinclair *et al.,* 2003). The second is known as freeze tolerance, where the insect is capable of surviving the freezing of liquids within itself (Sinclair *et al.,* 2003). *Epiblema scudderiana* Clemens 1860 (Lepidoptera: Tortricidae) is commonly known for its freeze avoidance mechanisms (Holden & Storey 2011, Lyons et al. 2015). These species usually restrict the formation of ice to certain parts of the body, such as the haemolymph or gut, by concentrating ice nucleators there (Sinclair *et al.,* 2003). This limits damage to more vital regions (Sinclair *et al.,* 2003). Certain antifreeze molecules may also be present to prevent the growth of crystals from forming sizes too large for the insect to cope with (Sinclair *et al.,* 2003). In this strategy, sugars and polyols are focused along cell membranes, thereby protecting the cells from dehydration (Sinclair *et al.,*

2003). Further experimentation should outline which strategy *E. lamorali* utilizes, but Sinclair *et al.* (2003) do illustrate a general trend of tolerance amongst the higher taxa, while avoidance is seen mainly in the basal groups. As such, it is likely that this species is capable of freeze tolerance. It is important to note that while *E. lamorali* is able to physiologically withstand these temperatures in the laboratory, it does not mean they will be exposed to them in their natural habitat. Foster & Treherne (1976), Sinclair *et al.* (2003) and Terblanche *et al.* (2007) point out that many insects mediate their environmental conditions through behaviour and this species may well do the same by remaining in the spiders' nests during adverse conditions.

In 2015, the mean temperature, as recorded underneath limpet shells to capture the accurate environment that nests would be exposed to, at Kommetjie was 15.53°C ± 0.06 (SE) (Chapter 2). No temperatures below 7.5°C were recorded. With this in mind, tolerance of temperatures as low as those illustrated in this study was unexpected. Although it is a debated topic (Araújo *et al.,* 2013), Pörtner (2001) points out that physiological acclimation to temperature can drive genetic differentiation and thus, evolution. With this in mind, the very cold lower critical thermal tolerance limit may be indicative of the evolutionary distributional history of *E. lamorali*, although this is difficult to determine. There are two plausible options for the evolution of the species, namely an ancestor based on the colder southern Ocean Islands (while *D. formidabilis* is not present there, some relatives are [Pugh, 2004]); or a mainland African ancestor, which could have evolved during a historically colder period (Pörtner, 2001). Nondula *et al.* (2004) suggest the intertidal regions of the southern Ocean Islands, while relatively untouched by research investigation (Chown *et al.,* 2008), to be one of the habitat types on the islands with the highest arthropod diversity and abundance. This

precludes that this area also exhibits the highest number of interspecific interactions within the group, including parasitism (Nondula *et al.,* 2004). The few studies that have been conducted on the intertidal regions of these island groups tend to focus on the largest organisms found there (Mercer *et al.,* 2000). This means they may miss some of the smaller, cryptic species (Mercer *et al.,* 2000), which include small hymenopteran parasitoids. Nonetheless, Mercer *et al.* (2000) report six insects species in one bay on Marion Island, including one midge, one fly, two weevils, one beetle and one wasp (*Kleidotoma icarus* Quinlan 1964 (Hymenoptera: Eucoilidae) (Crafford *et al.,* 1986; Mercer *et al.,* 2000), which was only found in the lower regions on the shore. While Chown *et al.* (2008) state that parasitism on the islands is generally relatively low, *E. lamorali* or close relatives may once have, or currently do, exist on these islands. Following the mainland Africa theory, historical repeated changes in global temperatures are well documented (Pörtner, 2001; Hansen *et al.,* 2010; Marcott *et al.,* 2013). If *E. lamorali* speciated during one of the colder periods, their very cold lower thermal tolerance limit may be explained. An African origin has been determined for many insect species around the globe, particularly amongst the fruit flies (Diptera: Tephritidae), as is exemplified by *Ceratitis* populations found in Europe, Latin America, Hawaii, Australia (Gasperi *et al.,* 2002), South Africa, Reunion and Mauritius (Baliraine *et al.,* 2004).

There are a variety of methodological dangers associated with performing critical thermal threshold experiments, particularly with small insects (Huey *et al.,* 1992; Chown *et al.,* 2009). Firstly, removal of specimens from the waterbath and the knocking of the structure used to retain them to check for the righting response may significantly stress the insects, affecting their behaviour (Hazell *et al.,* 2008). Hazell *et al.* (2008) thus recommend using a converted condensing tube and recording when insects fall to the bottom, but this method is

suitable only for flying insects. Furthermore, some studies have shown that the temperatures experienced during development may affect an individual's thermal tolerance (Huey *et al.,* 1992). Experiments run here, however, were conducted on field-collected individuals in the hopes of illustrating a more natural critical thermal range. During the experiment itself, the rate of temperature change could affect the results, with ramps too slow allowing acclimatisation and too fast not allowing time for compensation by the individual (Terblanche *et al.,* 2007; Chown *et al.,* 2009). Furthermore the amount of time specimens are exposed to each temperature is vital (Terblanche *et al.,* 2007). As such, a rate of a 0.25°C adjustment per minute was chosen as this is the standard for these sorts of experiments in order to make them comparable. In reality, the natural temperature change in the field may actually be as fast at 4°C per minute in extreme cases (Stillman and Somero, 1996), although the maximum ramp rate recorded in Chapter 2 was only 0.23ºC per minute. This slope was also kept constant throughout the study, with no plateaus, to ensure that insects were not retained at a certain temperature for too long. Chown *et al.* (2009) and Terblanche *et al.* (2007) illustrate that the starting temperature has a significant impact on the outcomes of such experiments. A starting temperature of 18°C was selected to mimic the mean daily temperature on rocky shores along the Cape Peninsula (as established from data acquired from the South African Weather Bureau at the time of experimentation). Because of these confounding factors, researchers are divided on whether a standardized or custom-built approach relative to the organism's natural conditions should be followed (Chown *et al.,* 2009). This study chose to copy the natural environmental conditions experienced by *E. lamorali* in the field as far as possible in the hopes of illustrating natural responses, while still maintaining standard procedures for comparability of the results.

The respirometry experiments led to two conclusions concerning physiological adaptation in *E. lamorali*. First, the presence of oxygen consumption, albeit at a low rate, in the seawater indicates that a physical gill capable of exchanging gasses with the surrounding medium is formed around the wasp when submerged, as occurs in *D. formidabilis* (Lamoral, 1968). This result was supported by the dense pilosity of the setae that cover the species' whole body (Masner, 1968; van Noort *et al.,* 2014), and their silvery appearance when submerged. Secondly, the vast, statistically significant difference in oxygen consumption between wasps under normal air exposure and those immersed in seawater points towards another inundation-coping mechanism in the organism. These data, along with the observations that freshly-immersed insects quickly stopped moving and were immobile on removal from the medium, suggest the ability for quiescence in *E. lamorali*. While in a quiescent state, most bodily functions are reduced to such an extent that the entire animal's metabolism slows to a significantly smaller rate in comparison to its usual functioning (Foster & Treherne, 1976). This allows it to survive through adverse conditions where the demand for the substances it would usually need to survive, such as oxygen, is greatly reduced (Foster & Treherne, 1976). *Echthrodesis lamorali* appears to quiesce when submerged, which is associated with an accompanying oxygen consumption rate decline, allowing survival off the gas-exchange between the plastron in the physical gill and the surrounding water, as was demonstrated by Lamoral (1968) for *D. formidabilis*.

Very few insects have been able to colonise the intertidal environment (Cheng, 1976). In comparison, 75% of recorded species can be found in marine saltmarshes at some point in their life cycles (Foster & Treherne, 1976). All of these must possess some ability for coping with saltwater, both from a respiratory and osmotic perspective (Foster & Treherne, 1976). Thus it is clear that this is not the main factor preventing establishment in the intertidal

environment. Foster & Treherne (1976) outline tidal patterns (mechanical disturbance), substrate type (mechanical disturbance of rolling rocks, sand inundation or lack of appropriate material for nesting) and rapid temperature changes as having significantly negative impacts on colonisation of the region by insects. All of these factors also make dispersal more difficult and risky (Foster & Treherne, 1976). Furthermore, the reduction in abundance and distribution of available food sources when switching from a terrestrial environment to an intertidal one is noticeable, with the latter bearing much more ephemeral characteristics (Foster & Treherne, 1976). Thus, while *E. lamorali* displays some physiological and morphological adaptations to cope with saltwater, there must be other adaptations, particularly in its behaviour, still to discover.

In conclusion, investigations into the thermal tolerance and morphological adaptations of *E. lamorali* demonstrated that the species is capable of surviving submersion in seawater for extended periods of time through the use of a physical gill. The maintenance and longevity of this structure is aided by quiescent behaviour in the wasp when submerged, such that the metabolic requirements for oxygen are reduced. Should the plastron be disrupted, however, *E. lamorali* is not further adapted for longer immersion and individuals will most likely drown within a short period of time. Furthermore, the species exhibits a very large thermal tolerance range that is not fully explained, or required, by the contemporary environmental conditions encountered within its known distributional range. This suggests that the species, or ancestral related taxa, may have historically been exposed to, and hence adapted to, more adverse weather conditions.

Chapter 5: General discussion

5.1 *Echthrodesis lamorali* life history pre-2011

At the commencement of this study in 2011, relatively little was known about *Echthrodesis lamorali* Masner 1968 (Hymenoptera: Platygastridae, Scelioninae). The species was first described by Masner (1968) after being discovered during a study on the host spider, *Desis formidabilis* O.P. Cambridge 1890 (Araneae: Desidae), conducted by Lamoral (1968), who remarked on the hairiness of the specimens and their lack of wings, as well as an apparent host-specificity. Beyond a morphological description, Masner (1968) also commented on the dense pilosity of the body of *E. lamorali* and its possible function in the formation of a physical gill. Furthermore, the study placed the new genus and species as a sister to the *Embidobia* Ashmead 1896, *Mirobaeus* Dodd 1914 and *Mirobaeoides* Dodd 1914 clade (Masner, 1968).

The next reference to the wasp came almost 20 years later, when Austin (1985) included it in a list of spider-attacking wasps, with a host record belonging to the *Amaurobiidae* Thorell 1870 (Araneae). At the time Austin (1985) made no more reference to *E. lamorali*, but returned to the species in 2005, when the hairy body and related physical gill functions were commented on (Austin *et al.,* 2005). This study was the first to suggest the use of air trapped within the host nests during high tides as physical refuges from submersion (Austin *et al.,* 2005).

_____

Up until 2009, when van Noort published a popular article (van Noort, 2009) stating that data were being collected for a publication soon to be submitted for review (later published as van Noort, *et al.,* 2014), the information available on this species remained limited to a description, host record, the apterous condition and hypotheses centered around the formation of a physical gill when submerged. Van Noort (2009) added the theories of dispersal during low tides and an apparent restriction to the insect's type locality, 'The Island', Kommetjie (Cape Peninsula, Western Cape, South Africa). Although this study did state that other populations may exist, all of these remained untested hypotheses. This popular article was followed by another in 2011, where the wasp's oviposition behaviour was detailed (van Noort, 2011).

The study by van Noort *et al.* (2014) is the most concise collection of information on *E. lamorali* published to date. Once again, the presence of setae covering the body and lack of wings was detailed, yet the function of these remained hypothetical with no testing or data collection to support their hypotheses. The use of air trapped in *D. formidabilis* nests as physical refuge during submersion and the theory that dispersal occurs during low tide were also discussed (van Noort *et al.,* 2014). The study aimed mostly at describing and determining the wasp's phylogenetic affinities using previous literature published on the clade, as well as genetic analytical interpretation, and a revised species description. Using a variety of specimens collected in specimens collected in New Zealand, Australia, Yemen, Costa Rica, French Guiana, the United States of America and Peru, van Noort *et al.* (2014) found *E. lamorali* to be a sister to *Mirobaeoides* and *Embidobia*. Only *E. lamorali* represented the South African fauna. Furthermore, the study detailed the nest structures and egg clutch size of *D. formidabilis*, followed by parasitism incidences within single nests, post-parasitism changes in egg morphology, wasp eclosion characteristics, sex ratios and

mating and oviposition behaviour (van Noort *et al.,* 2014). These were novel observations for the species.

5.2 An expansion on the knowledge base of *Echthrodesis lamorali*

The general lack of information on a species that was described 47 years ago and is considered to be "the very first evidence of an intertidal maritime scelionid ever known" (Masner, 1968) warranted further investigation. This thesis adds much novel knowledge about *E. lamorali* on a distributional, basic biology, morphological and physiological basis. Attempts were also made to resolve its phylogenetic ties to other African genera using genetic methods, although these were unsuccessful. While some aspects of the topics overlap with the work conducted by van Noort *et al.* (2014), they serve to support theories postulated in the paper, as well as provide additional support for some of the data collected. The contribution of information to each category is discussed below.

5.2.1 Distribution

Echthrodesis lamorali was originally described from one locality only, namely Kommetjie (Lamoral, 1968; van Noort, 2011). Until this study commenced, no surveys to determine its distribution had been conducted, despite the presence of the host, *D. formidabilis*, from Lüderitz (Namibia), down and around the coast to East London (Eastern Cape, South Africa) (Day, 1974; Filmer, 1995; Dippenaar-Schoeman & Jocqué, 1997). Both broad- and fine-scale active searching surveys illustrated the true distribution of the wasp to be broader than just Kommetjie, but limited to the Cape Peninsula (Western Cape, South Africa). The restriction of *E. lamorali* to the Cape Peninsula can be explained by a variety of hypotheses, all of which

may be acting in conjunction with one another (Laing, 1937; Atkins, 1980a; Lubke, 1998). Patterns in the host distribution may restrict the parasitoid to a limited area, while physiological limits, which are linked to abiotic factors at a shore, are also a major controlling factor (Laing, 1937; Atkins, 1980a; Lubke, 1998). Ultimately, the expansion in known range of *E. lamorali* is a positive one. Species distributions that encompass more than one locality indicate the possibility for gene flow between regions (Nadeau *et al.,* 2013), as well as a reduced possible effect of stochasticity at a single shore on the entire population (Lacy, 1993). In the case of this parasitoid, its full range is encompassed within the Table Mountain National Park, ensuring its effective conservation for the foreseeable future, barring major natural or anthropogenic catastrophes.

5.2.2 Parasitism incidence and sex ratios

Truly maritime insects that spend their entire life-cycles within the intertidal zone are relatively rare, with only three wasp species (excluding *E. lamorali*) recorded worldwide (van Noort *et al.,* 2014). The result of this small pool of intertidal insect species is a lack in basic biological information about them, particularly in the case of parasitoid wasps (Eason *et al.,* 1967; Hickman, 1967; Austin, 1984; Austin *et al.,* 2005). Chapter 3 determined the parasitism incidences and sex ratios of wasps reared from field-collected nests, taking both between-shore and in-shore components into account. Furthermore, aspects of the nests of *D. formidabilis* were revisited for the first time since Lamoral's study in 1968. The data assimilated suggested that the spider population sizes are controlled by bottom-up factors, rather than top-down through the actions of the parasitoids. Spider populations on the west coast were also larger than on the eastern side, an observation mirrored to some extent in the brood sizes.

Female wasps always attacked 100% of the brood in a compartment, but survival of the eggs to emergence of spiderlings was halved when compared to an unparasitized egg sac. Furthermore, parasitism was found to follow spider population patterns at the in-shore scale, but not at larger between-shore levels. This stands as a good example for the need to realise the effect of scale on ecological studies (Donadi *et al.,* 2015) and demonstrates a much larger bottom-up effect on wasp population control than just their host resource. As is common for scelionines and many parasitoid wasps (Austin, 1984; Strand, 1988; Fox *et al.,* 1990; Austin *et al.,* 2005), *E. lamorali* populations on the Cape Peninsula all displayed a strongly female-biased sex ratio.

5.2.3 Physiology and morphology

One of the many main theories for the reduced number of insect species found living within the intertidal zone is that morphological and physiological reasons, such as the presence of cumbersome wings or an inability to withstand the high salt concentrations, led to their absence amongst the maritime taxa (Usinger, 1957; Cheng, 1973; Cheng, 1976; Andersen & Weir, 1994; Maddrell, 1998; Vermeij & Dudley, 2000; Ikawa *et al.,* 2012). In the case of *E. lamorali*, which may counter submersion through the use of behavioural avoidance as a first defence, the loss of wings is immediately identifiable as an adaptation to water inundation (Cheng, 1976; van Noort, 2009), although this could also be a plesiomorphic character as it is widespread throughout the Scelioninae (Austin *et al.,* 2005; Carey *et al.,* 2006). Based on a similar observation for the host, Masner (1968) and van Noort *et al.* (2014) also hypothesised that the dense pilosity of the body serves a physical gill function, although this was never tested. Furthermore, *D. formidabilis* shows adaptation to living in a wet environment in their booklungs (Lamoral, 1968), which were thought to possibly be mirrored in the parasitoid. In

general, the adaptations that this species possesses and displays were largely uninvestigated. Chapter 4 assessed the presence and function of internal and external adaptations to water inundation using scanning electron microscopy, sectioning of specimens, critical thermal limit and respirometry methodologies, allowing the assessment of adaptations at multiple levels. Firstly, the species showed a large critical thermal range, from sub-zero to 48ºC. While the upper limit is understandable when taking the climatic conditions outlined by data assimilation in Chapter 2, the lower limit is particularly low. This is thought to indicate ancestors or relatives in areas where conditions reached much colder levels than in South Africa. The observation that a thin silvery film covered submerged specimens, which quickly stopped motion and displayed reduced, yet observable removal of oxygen from the surrounding water, supported the original hypotheses of Masner (1968) and van Noort *et al.* (2014) that *E. lamorali* is capable of physical gill formation. Furthermore, the species appears to enter a quiescent state when immersed, a possible adaptation to reduce oxygen consumption, such that the physical gill is capable of meeting the oxygen needs (Foster & Treherne, 1976). In the event of disruption or depletion of this structure, other adaptations are generally lacking, suggesting that the wasp is only capable of very short periods of submersion. Internally, taenidia in the tracheal tubes would provide support in the event of pressure changes associated with submersion (Lamoral, 1968), although these would not be assisted by any external adaptations.

5.2.4 Systematic affinities

Understanding the systematic affinities of a species is important for all aspects of its life history (Caterino *et al.,* 2000). They could explain their current distribution, patterns in their basic behaviour or unusual adaptations and physiological limits (Caterino *et al.,* 2000).

_____

Although generally little investigated, the Scelioninae have a turbulent history, with frequent, yet often vague changes to the hypothetical higher taxonomic relationships within the sub-family and between it and the remainder of the Platygastridae (Austin *et al.,* 2005; Murphy *et al.*, 2007). Much of the confusion can be attributed to the use of morphological characters to determine the relationships, which, if the data set contains homology as a result of convergent morphological adaptation, can be misleading in a group full of small, cryptic species such as the Platygastridae (Austin *et al.,* 2005; van Noort *et al.,* 2014).

As such, and much like the van Noort *et al.* (2014) paper that focused on 37 foreign specimens from 17 genera, this study originally aimed at determining the systematic affinities of *Echthrodesis* within the African Scelioninae using genetic techniques. These were, unfortunately, ultimately unsuccessful, but the attempts made to elucidate the systematic affinities are worth mentioning here.

The cytochrome C oxidase subunit 1 (CO1) and elongation factor 1 alpha (EF1α) genetic regions were chosen to amplify in order to determine the species (Savolainen *et al.,* 2005) and deeper-level affinities (Keeling & Doolittle, 1996) respectively. Furthermore, CO1 sequences can be fed into a larger barcoding project through the use of GenBank (Savolainen *et al.,* 2005). Van Noort *et al.* (2014) found *Echthrodesis* to be a sister clade of *Embidobia* Ashmead 1896*, Mirobaeus* Dodd 1914 and *Mirobaeoides* Dodd 1914 (Hymenoptera: Platygastridae, Scelioninae), all of which attack web-spinning hosts, and this study was hypothesized to exhibit the same patterns. Extractions were attempted on 116 dried specimens (representing 34 genera of 3-19 year old specimens collected using five different collection methods [Malaise trapping, sweeping, yellow pan trapping, Winkler bag extraction

from leaf litter and pitfall trapping] from six different African countries) and 96 wet specimens that had been stored in 96% ethanol since their collection in 96% ethanol or propylene glycol (18 South African genera ranging from one to four years old and collected using only two methods: malaise traps and Winkler bag extraction from leaf litter) that were withdrawn from the Iziko South African Museum's entomological collection. All dry specimens were originally killed in either 96% ethanol or propylene glycol and subsequently preserved in 96% ethanol. These specimens were then stored in a dark air-conditioned wet collection room, where the temperature was maintained at 16ºC for a varying number of years prior to rapid heat accelerated air drying and mounting on card points. Much effort was exerted while attempting to generate usable, accurate sequences. This included using four different extraction techniques and kits (sonication, prepGEM, DNEasy and CTAB); nine primers for the CO_1 region, four of which were designed for the study; six EF1α primers, of which two were designed; 15 CO_1 polymerase chain reaction (PCR) cocktails using three different Taq brands and one cocktail for the EF1α region; 14 different PCR cycling conditions in an attempt to amplify the CO_1 region (including cold starts, lowered annealing temperatures, increased cycles and altered temperatures for each primer set) and three cycling condition attempts for EF1α that each had different annealing temperatures depending on the primer pair, as well as a lowered temperature to encourage annealing; and finally gels were developed using both SYBRSafe DNA gel stain and ethidium bromide. All sequencing was performed using Sange Dideoxy sequencing at the University of Stellenbosch sequencing unit, whereafter sequences were cleaned in BioEdit Version 7.2.5 (Hall, 1999), aligned using ClustalW 2 (Larkin *et al.,* 2007) and maximum likelihood, neighbour joining and maximum parsimony tress constructed in MEGA6 (Tamura *et al.,* 2013).

The only extraction technique that produced sequences was the CTAB method, but only for 81 of the wet specimens. No gene regions from dried specimens, nor any EF1α regions could be amplified. Furthermore, only 37 of these wet specimen sequences (comprising 10 genera) were of a length and quality that could be used for tree development. Analysis of the trees revealed severe problems within the sequences. For the 81 specimens, 106 parsimonious characters were identified. This is particularly high for the sample size and reveals errors within the sequences themselves. One large clade was formed with all identical sequences, despite the inclusion of a variety of genera. The other groupings all contained a mixture of taxa, with no clear patterns and low support for many branches. These issues may be the result of identification errors, a common problem amongst the Scelioninae, which are generally miniscule and often cryptic (Austin *et al.,* 2005; van Noort *et al.,* 2014), but the overall evidence pointed towards a clear overwhelming problem within the sequences themselves.

The failure to produce any sequences of sufficient quality for analysis can be explained by the relative size and treatment of the specimen during- and post-euthanization (Gilbert *et al.,* 2007; Wandeler *et al.,* 2004; Wang *et al.,* 2004; Wandeler *et al.,* 2007; Hunter *et al.,* 2008). Other studies have found that smaller specimens generally host fewer cells and therefore less DNA (Hunter *et al.,* 2008). Where less DNA is available from the start, the chance of getting enough strands of sufficient length for amplification during PCR is greatly reduced (Wang *et al.,* 2004). Despite the high success of certain extraction kits on small specimens in other studies (Dittrich-Schröder *et al.,* 2012), the same kit used here with no success suggests another issue other than just specimen size. The method and substance used to euthanize an insect; time in storage; and the amount of water, oxygen, heat and contaminants it was exposed to during storage will all impact the DNA quality and quantity, contributing to the

success or failure of any attempts at amplification (Gilbert *et al.,* 2007; Wandeler *et al.,* 2004; Wandeler *et al.,* 2007).

If such a study were to be attempted again, recommendations are made to use fresher specimens, focus on a different gene region or attempt different extraction and amplification methods (Wang *et al.,* 2004; Wandeler *et al.,* 2007). The need for use of fresher specimens is unfortunate, as museums host substantial pools of specimens that stand as a great resource for such investigations (Wandeler *et al.,* 2004; Wandeler *et al.,* 2007). These can be, and are, usefull sources for a variety of genetic investigations on larger specimens, but it would appear that the smaller ones require assessment shortly after euthanization. Wandeler *et al.* (2007) also recommend the use of single nucleotide polymorphisms over the CO_1 and other gene regions in old and degraded specimens. This method was designed with a known mutational model and generally demonstrates a higher genotyping efficiency, possibly as a result of the shorter sequence lengths that are generated (Wandeler *et al.,* 2007). Finally, the use of a balanced PCR may improve the chance of successfull amplification (Wang *et al.,* 2004). During a balanced PCR, the DNA is extracted during step 1, after which all steps take place in the same tube (Wang *et al.,* 2004). This decreases the chance of contamination and fragment loss that can be common during tube-to-tube movements involved with other extraction and PCR methodologies (Wang *et al.,* 2004).

5.3 General trends

Mainly as a result of their small size, the Scelioninae are generally a neglected group in the literature (Austin *et al.,* 2005). Furthermore, as a result of their scarcity, intertidal insects are

also largely uninvestigated (Cheng, 1976). The data collected for this thesis contribute a significant portion of information on very broad aspects of the life history of *E. lamorali* at a multi-scale level from multi-population down to that of the individual. At the scale of the individual, *E. lamorali* provided a model example of the interwoven components of the life history of a species, with trends at one scale of investigation greatly impacting those observed at another. At the scale of a single ecosystem, *E. lamorali* did not exert top-down control of *D. formidabilis* populations as theorised. Rather, the species was governed by both biotic and abiotic components and therefore was proven to not be a keystone species in natural habitat dynamics. Finally, at the scale of multiple populations and ecosystems, the full distributional range of this species was found to be under the umbrella conservation of the Table Mountain National Park, ensuring a continuation of populations for the foreseeable future. As Austin *et al.* (2005) pointed out, the structures, patterns and observations collected here can be broadened to other parasitoids in a variety of areas of research.

5.3.1 All aspects of the life history of *Echthrodesis lamorali* are linked

Carducci & Jakob (2000) discuss how all aspects of an organism's life history are linked. *Echthrodesis lamorali* illustrated this point perfectly. In Chapter 2, climatic conditions, macro-scale maximum temperatures in particular, were seen to control the distribution of the species to some extent. This can be directly linked to its physiological capabilities, with maximum temperatures on a shore coming close to the wasp's upper critical thermal limit, but lower temperatures not extending anywhere near as low as the lower thermal limit (as investigated in Chapter 4). Its morphological adaptations (Chapter 4) also greatly impact dispersal and behaviour by limiting how far an individual can travel and how many egg-sacs it can enter before depleting its energy stores (Chapter 3). This thus influences the biology (as

detailed in Chapter 3) and distribution (outlined in Chapter 2) of the species. As stated in Chapter 1 of this thesis, "it is important that all aspects be fully investigated before we can truly understand the species as a whole". The data acquired in this thesis on the large variety of aspects of the life history of *E. lamorali* greatly contributes to "a more holistic understanding of the life history of a species as a whole" (Chapter 1).

Links between different components of the life history of an organism arise as the result of feedback processes (Crespi, 2004). Feedbacks can be positive, where the development or presence of one factor promotes another (Crespi, 2004), or negative, where one process is inhibited by another (Huang *et al.,* 2007). Each trait that drives or inhibits the selection for changes in another is associated with underlying genetic characters (Crespi, 2004). In the case of *E. lamorali*, the limitation to a small distribution as a result of the organism's physiological capabilities (critical thermal limitation) or behaviour provides an example of a negative feedback, while the development of quiescent behaviour when submerged allowed for better physical gill functioning and therefore the persistence of the species within the intertidal region (a positive feedback). Negative feedbacks do appear to be the more common system for the aspects of the wasp's life history as assessed here, however, with behaviour, physiological capabilities and morphological adaptations reducing the distribution of the organism. From the perspective of varying scales, a recurring theme in this thesis, positive feedback is hypothesised to act at finer scales producing heterogeneity that is then stabilised by broad-scale negative feedback dynamics (Levin, 2000). Thus, the observation that negative feedbacks dominate the processes investigated in this thesis point towards broad-scale observations. The feedbacks identified here are not as short-lived as 'organism-learning' where an individual gathers information from its environment and adapts its behaviour based on this (Carducci & Jakob, 2000), but are long-term, effectively influencing

ingrained factors of a species, and thus its evolution. Short-term changes are important though, as they aggregate to start forming patterns at intermediate scales, as was found for *Phidippus audax* Hentz 1845 (Araneae: Salticidae) individuals, which exhibited different behaviour when exposed to video footage of prey items depending on the environment in which they lived prior to the commencement of the study (Carducci & Jakob, 2000). While the mechanisms leading to these differences were unclear, Carducci & Jakob (2000) hypothesise them to be linked to morphological changes in the central nervous system, eye structures and muscle characteristics during development in the original environment, as well as that of learning or experience. Small scale aspects of the biology of *E. lamorali,* such as intra-specific interactions or dispersal behaviour, still need to be determined.

5.3.2 The effect of *Echthrodesis lamorali* on the intertidal ecosystem

In Chapter 1 it was stated that "[t]he arthropods described in this thesis form a small feeding chain within a larger food web. This chain is poorly known and even less observable to the untrained or incurious eye". The dynamics between the parasitoid and host have now been better outlined, indicating a smaller impact on the spider than previously thought. Firstly, the wasp does not occur throughout the entire distribution of the host, allowing for a source of possible spider immigration to the Peninsula from other regions in the event of any population reductions. This is unlikely, however, as various models used in the study revealed *D. formidabilis* to be controlled by bottom-up, rather than top-down impacts. This trend holds for the wasp, with habitat characteristics, temperatures and host dynamics influencing their population patterns.

Bottom-up effects encompass a system where changes in productivity at the scale of the host or abiotic components of an ecosystem affect the predators or parasitoids 'higher up' in the food web (Moon & Stiling, 2002; Hoekman, 2010). In contrast, systems where predators and parasitoids strongly regulate the population sizes of prey and hosts respectively are said to experience mostly top-down effects (Hoekman, 2010). An example of clear top-down population size control of the host by a parasitoid is provided by an unnamed generalist parasitoid on *Aphis glycines* Matsumura 1917 (Hemiptera: Aphididae) (Costamagna & Landis, 2006), while bottom-up control is illustrated by *Aphidius rhopalosiphi* DeStefani-Peres 1902 (Hymenoptera: Braconidae, Aphidiinae), a parasitoid on *Rhopalosiphum padi* L. (Hemiptera: Aphididae) (Hempel *et al.* 2009). In this study, the parasitoid demonstrated reduced developmental times and larger body sizes at eclosion as a result of the foundress attacking aphids feeding on plants that had been inoculated with a mycorrhizal fungus (Hempel *et al.,* 2009). The prevalence of either of these controlling forces in natural systems is a cause of some debate, however.

Firstly, spatial and temporal variation in the dynamics of top-down or bottom-up effects is not commonly assessed, although the differences can be striking (Gratton & Denno, 2003; Costamagna & Landis, 2006; Gripenberg & Roslin, 2007; Abdala-Roberts *et al.,* 2010; Hoekman, 2010). The direction and strength of these population-structuring effects can be greatly affected by ecosystem complexity and productivity; host efficiency and life history (Moon & Stiling, 2002; Gratton & Denno, 2003; Costamagna & Landis, 2006). Whether a system exhibits bottom-up or top-down structuring is usually taken as a result of biotic components of the system, but abiotic factors could play a large influence too (Hoekman, 2010; Moon & Stiling, 2002). For example, higher temperatures increase metabolic rates in parasitoids, thereby increasing their need for resource acquisition and thus their foraging,

consumption, digestion and growth rates (Hoekman, 2010). These will all result in a greater effect on the host and may switch a system from a bottom-up one to top-down (Hoekman, 2010). Taking spatial components into consideration in this study supported the findings of others that the direction and strength of community-processes can differ vastly (Gratton & Denno, 2003; Costamagna & Landis, 2006; Gripenberg & Roslin, 2007; Abdala-Roberts *et al.,* 2010; Hoekman, 2010), but also proved that they can be complex and unclear.

Furthermore, the general patterns in top-down versus bottom-up dynamics do not translate well between theoretical and natural, realised systems. In 1960, Hairston *et al.* proposed the "green world hypothesis". This hypothesis theorised that natural enemies of herbivores maintain the host populations at sufficiently low levels to prevent the devastation of global flora (Hairston *et al.,* 1960), suggesting that top-down control of herbivores by predators and parasitoids is cosmopolitan. This concept forms the basis of all biological control efforts using these organisms (Mills, 2001), but Hawkins *et al.* (1999) argues that top-down control by parasitoids is not as commonly observed in natural systems, and even less so where the interaction involves a specialist species. Top-down control of species in natural systems may actually be more prevalent in predator-prey systems than parasitoid-host ones (Costamagna *et al.*, 2007). To further impact these conflicting concepts, in the context of the intertidal region on which this thesis focussed, Mills (2001), Karimzadeh *et al.* (2004) and Karimzadeh & Wright (2008) state that top-down control is more prevalent in aquatic systems, whereas terrestrial ecosystems are generally more controlled by bottom-up effects. With the dual-nature of the intertidal region being both aquatic and terrestrial at different temporal and spatial scales, general trends become difficult to determine. Ultimately, the findings here support the theory postulated by Polis & Strong (1996) and Sinclair *et al.* (2000) that both

top-down and bottom-up dynamics can be present at the same time and are in constant flux depending on temporal and spatial scales.

5.3.3 Conservation of *Echthrodesis lamorali*

Traut (2005) states that an "[u]nderstanding [of] the transition between the components of a landscape can help increase effective management of these systems". Based on the lack of controlling force exerted by *E. lamorali* over the spider populations, as well as the inclusion of the full distributional range of this species in the Table Mountain National Park, where a large proportion of activities that may negatively impact intertidal, marine and terrestrial fauna and flora are strongly prohibited (South African National Parks, 2015), it can be concluded that this specific interaction requires no further management.

Both *E. lamorali* and *D. formidabilis* are being effectively conserved through an umbrella effect provided by the Table Mountain National Park. This conservation area was established as a floral refuge, as is exemplified on the park's website (South African National Parks, 2015), which highlights that "[m]any of the plants here are endemic... [with] 1406 threatened plant species, 300 of which are endangered or critically endangered". These plants can be considered to fit within the umbrella-species concept, which theorises that actions taken for the conservation of a single or complex of target species may maintain populations of other non-target organisms (New, 1997; Branton & Richardson, 2010). Good examples of umbrella-conservation of one or a complex of species by another are provided for the Lepidoptera, which are usually attractive to the general public, thereby fostering sympathy and a call for action (New, 1997). The active conservation focussed on *Acrodipsas*

_____

myrmecophila Waterhouse & Lyell 1913 (Lepidoptera: Lycaenidae) and *A. brisbanensis* Miskin 1884 (Lepidoptera: Lycaenidae) on Mount Piper (Victoria, Australia) led to an observable increase in populations of other taxa in the region, for example (New, 1997). While the effectiveness of the variety of processes employed to select umbrella species are greatly contended (Roberge & Anglestam, 2004; Branton & Richardson, 2010), cases such as that of *E. lamorali* in the Table Mountain National Park prove that the empirical concept is sound.

5.4 Future research considerations

There is still much to learn about *E. lamorali*, which has repeatedly demonstrated unique adaptations and life history characteristics throughout the course of the investigations in this thesis. The true causal factors behind their restricted distributional pattern still need to be tested through experimentation. Whether differences exist in *D. formidabilis* populations between the Peninsula and surrounding areas from a morphological, behavioural and genetic view point would either support or refute the theory that host biology is limiting the parasitoid. This will tie in well with investigations into host-finding behaviour of the parasitoid, a still largely-unknown phenomenon for this species that may prove to be of interest from an evolutionary adaptation perspective. These will test the hypothesis that their orthokineses are limiting them to the current distributional range. Although one study conducted during the course of this thesis demonstrated that *E. lamorali* formed a physical gill when submerged, this structure stills needs to be thoroughly investigated, particularly in terms of duration that it remains functional for. Finally, appropriate methodology for amplification and sequencing of the genetic components of the African Scelioninae still needs to be found, such that the internal relationships within the group can be assessed.

References

ABDALA-ROBERTS, L., PARRA-TABLA, V., SALINAS-PEBA, L. and DÍAZ-CASTELAZO, C. 2010. Spatial variation in the strength of a trophic cascade involving *Ruellia nudiflora* (Acanthaceae), an insect seed predator and associated parasitoid fauna in Mexico. *Biotropica* **42**: 180-187.

ABUSHAALA, N. M., SHAIBI, T. and HOWAEGE, H. M. 2014. The abundance and diversity of benthic crustaceans along the coastal zone of Western Libya. *Journal of Black Sea/Mediterranean Environment* **20**: 33-45.

AGUIAR, A. P., DEANS, A. R., ENGEL, M. S., FORSHAGE, M., HUBER, J. T., JENNINGS, J. T., JOHNSON, N. F., LELEJ, A. S., LONGINO, J. T., LOHRMANN, V., MIKÓ, I., OHL, M., RASMUSSEN, C., TAEGER, A. and YU, D. S. K. 2013. Order Hymenoptera. *Zootaxa* **3703**: 51-62.

ALLEN, J. R. L. and DUFFY, M. J. 1998. Medium-term sedimentation on high intertidal mudflats and salt marshes in the Severn Estuary, SW Britain: the role of wind and tide. *Marine Geology* **150**: 1-27.

ALTON, L. A., WHITE, C. R., WILSON, R. S. and FRANKLIN, C. E. 2012. The energetic cost of exposure to UV radiation for tadpoles is greater when they live with predators. *Functional Ecology* **26**: 94-103.

ANDERSEN, N. M. and WEIR, T. A. 1994. *Austrobates rivularis* gen. et sp. nov., a freshwater relative of *Halobates* Eschscholtz (Hemiptera: Gerridae), with a new perspective on the evolution of sea skaters. *Invertebrate Taxonomy* **8**: 1-15.

AOYAGI, M. and ISHII, M. 1991. Host acceptance behavior of the Japanese aquatic wasp, *Agriotypus gracilis* (Hymenoptera: Ichneumonidae) toward the caddisfly host, *Goerajaponica* (Trichoptera: Limnephilidae). *Journal of Ethology* **9**: 113-119.

ARAÚJO, M. B., FERRI-YÁÑEZ., F., BOZINOVIC, F., MARQUET, P. A., VALLADARES, F. and CHOWN, S. L. 2013. Heat freezes niche evolution. *Ecology Letters* **16**: 1206-1209.

ATKINS, M. D. 1980a. Chapter 2: Basic responses and patterns of behaviour. In: ATKINS, M. D. 1980. *Introduction to insect behaviour*. Macmillan Publishing Co., Inc.: New York. pp 26-44.

ATKINS, M. D. 1980b. Chapter 11: Population behavior. In: ATKINS, M. D. 1980. *Introduction to Insect Behaviour*. Macmillan Publishing Co., Inc.: New York. pp 167-175.

ATKINS, M. D. 1980c. Chapter 12: Behavior, genetics, evolution, and speciation. In: ATKINS, M. D. 1980. *Introduction to Insect Behaviour*. Macmillan Publishing Co., Inc.: New York. pp 176-192.

AUSTIN, A. D. 1983. Morphology and mechanics of the ovipositor system of *Ceratobaeus* Ashmead (Hymenoptera: Scelionidae) and related genera. *International Journal of Insect Morphology and Embryology* **12**: 139-155.

AUSTIN, A. D. 1984. The fecundity, development and host relationships of *Ceratobaeus* spp. (Hymenoptera: Scelionidae), parasites of spider eggs. *Ecological Entomology* **9**: 125-138.

AUSTIN, A. D. 1985. The function of spider egg sacs in relation to parasitoids and predators, with special reference to the Australian fauna. *Journal of Natural History* **19**: 359-376.

AUSTIN, A. D. 1988. A new genus of baeine wasp (Hymenoptera: Scelionidae) from New Zealand associated with moss. *New Zealand Journal of Zoology* **15**: 173–183.

AUSTIN, A. D. 1995. New species of scelionid wasps (Hymenoptera: Scelionidae: Baeini) from Western Australia, parasitic on spider eggs. *Records of the Western Australian Museum* **52**: 253–263.

AUSTIN, A. D. and DOWTON, M. 2010. Part 1: The Hymenoptera: an introduction. In: AUSTIN, A. D. and DOWTON, M. 2010. *Hymenoptera: evolution, biodiversity and biological Control*. CSIRO: Canberra. pp 3-10.

AUSTIN, A. D., JOHNSON, N. F. and DOWTON, M. 2005. Systematics, evolution, and biology of scelionid and platygastrid wasps. *Annual Review of Entomology* **50**: 553-582.

BAIRD, D. 2006. *Estuaries of South Africa*. [Online] Available at: http://nest.su.se/mnode/africa/S_Africa/safrica.html. Accessed: 13/07/2012.

BALIRAINE, F. N., BONIZZONI, M., GUGLIELMINO, C. R., OSIR, E. O., LUX, S. A., MULAA, F. J., GOMULSKI, L. M., ZHENG, L., QUILICI, S., GASPERI, G. and MALACRIDA, A. R. 2004. Population genetics of the potentially invasive African fruit fly species, *Ceratitis rosa* and *Ceratitis fasciventris* (Diptera: Tephritidae). *Molecular Ecology* **13**: 683-695.

BARBER, A. D. 2009. Littoral myriapods: a review. *Soil Organisms* **81**: 735-760.

BARTON, K. 2015. MuMIn: Multi-Model Inference. *R package version 1.13.4*. [Online] Available at: http://CRAN.R-project.org/package=MuMIn. Accessed: 17/06/2015.

BEREC, L., BOUKAL, D. S. and BEREC, M. 2001. Linking the allee effect, sexual reproduction, and temperature-dependent sex determination via spatial dynamics. *The American Naturalist* **157**: 217-230.

BERGERON, Y. and FLANNIGAN, M. D. 1995. Predicting the effects of climate change on fire frequency in the Southeastern Canadian boreal forest. *Air, Water and Soil Pollution* **82**: 437-444.

BEZEMER, T. M., HARVEY, J. A. and MILLS, N. J. 2005. Influence of adult nutrition on the relationship and reproductive parameters in a parasitoid wasp. *Ecological Entomology* **30**: 571-580.

BLACKLEDGE, T. A. and WENZEL, J. W. 2000. Silk mediated defense by an orb web spider against predatory mud-dauber wasps. *Behaviour* **138**: 155-171.

BLANCHARD, D. and BOURGET, E. 1999. Scales of coastal heterogeneity: influence on intertidal community structure. *Marine Ecology Progress Series* **179**: 163-173.

BLANCHETTE, C. A., MINER, C. M., RAIMONDI, P. T., LOHSE, D., HEADY, K. E. K. and BROITMAN, B. R. 2008. Biogeographical patterns of rocky intertidal communities along the Pacific coast of North America. *Journal of Biogeography* **35**: 1593-1607.

BODASING, M., CROUCH, T. and SLOTOW, R. 2002. The influence of starvation on dispersal in the social spider, *Stegodyphus mimosarum* (Araneae, Eresidae). *Journal of Arachnology* **30**: 373-382.

BRAGG, D. E. 1974. Ecological and behavioral studies of *Phaeogenes cynarae*: ecology; host specificity; searching and oviposition; and avoidance of superparasitism. *Annals of the Entomological Society of America* **67**: 931-936.

BRANCH, M. and BRANCH, G. 1981. *The living shores of southern Africa*. C. Struik, Cape Town. pp 200-201.

BRANCH, G. M. and STEFFANI, C. N. 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). *Journal of Experimental Marine Biology and Ecology* **300**: 189-215.

BRANTON, M. and RICHARDSON, J. S. 2010. Assessing the value of the umbrella-species concept for conservation planning with meta-analysis. *Conservation Biology* **25**: 9-20.

BRIGGS, C. J. and HOOPES, M. F. 2004. Stabilizing effects in spatial parasitoid-host and predator-prey models: a review. *Theoretical Population Biology* **65**: 299-315.

BROITMAN, B. R., BLANCHETTE, C. A., MENGE, B. A., LUBCHENCO, J., KRENZ, C., FOLEY, M., RAIMONDI, P. T., LOHSE, D. and GAINES, S. D. 2008. Spatial and temporal patterns of invertebrate recruitment along the west coast of the United States. *Ecological Monographs* **78**: 403-421.

BUCHWALTER, D. B., JENKINS, J. J. and CURTIS, L. R. 2003. Temperature influences on water permeability and chlorpyrifos uptake in aquatic insects with differing respiratory strategies. *Environmental Toxicology and Chemistry* **22**: 2806-2812.

BUSTAMANTE, R. H., BRANCH, G. M. and EEKHOUT, S. 1997. The influences of physical factors on the distribution and zonation patterns of South African rocky-shore communities. *South African Journal of Marine Science* **18**: 119-136.

BUSTMANTE, R. H., BRANCH, G. M., EEKHOUT, S., ROBERTSON, B., ZOUTENDYK, P., SCHLEYER, M., DYE, A., HANEKOM, N., KEATS, D., JURD, M. and MCQUAID, C.

1995. Gradients of intertidal primary productivity around the coast of South Africa and their relationships with consumer biomass. *Oecologia* **102**: 189-201.

CARDUCCI, J. P. and JAKOB, E. M. 2000. Rearing environment affects behaviour of jumping spiders. *Animal Behaviour* **59**: 39-46.

CAREY, D., MURPHY, N. P. and AUSTIN, A. D. 2006. Molecular phylogenetics and the evolution of wing reduction in the Baeini (Hymenoptera: Scelionidae): parasitoids of spider eggs. *Invertebrate Systematics* **20**: 489-501.

CASTELLA, J. C., KAM, S. P., QUANG, D. D., VERBURG, P. H. and HOANH, C. T. 2007. Combining top-down and bottom-up modelling approaches of land use/cover change to support public policies: Application to sustainable management of natural resources in northern Vietnam. *Land Use Policy* **24**: 531-545.

CATERINO, M. S., CHO, S. & SPERLING, F. A. H. 2000. The current state of insect molecular systematics: a thriving tower of Babel. *Annual Review of Entomology* **45**: 1-54.

CHAPMAN, A. D. 2009. Numbers of living species in Australia and the world. *Report for the Australian Biological Resources Study.* Commonwealth of Australia, Canberra.

CHEMINEE, A., FRANCOUR, P. and HARMELIN-VIVIEN, M. 2011. Assessment of *Diplodus* spp. (Sparidae) nursery grounds along the rocky shore of Marseilles (France, NW Mediterranean). *Scientia Marina* **75**: 181-188.

CHEN, B. and WISE, D. H. 1999. Bottom-up limitation of predaceous arthropods in a detritus-based terrestrial food web. *Ecology* **80**: 761-772.

CHENG, L. 1973. Marine and freshwater skaters: Differences in surface fine structures. *Nature* **242**: 132-133.

CHENG, L. 1976. Insects in marine environments. In: CHENG, L. 1976. *Marine Insects.* North-Holland Publishing Company: Amsterdam. pp 1-4.

CHOWN, S. L., DUFFY, G. A. and SØRENSEN, J. G. 2015. Upper thermal tolerance in aquatic insects. *Current Opinion in Insect Science* **11**: 1-6.

CHOWN, S. L., JUMBAM, K. R., SØRENSEN, J. G. and TERBLANCHE, J. S. 2009. Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology* **23**: 133-140.

CHOWN, S. L., LEE, J. E. and SHAW, J. D. 2008. Conservation of Southern Ocean Islands: invertebrates as exemplars. *Journal of Insect Conservation* **12**: 277-291.

CLIFFORD, C. W. and WOODRING, J. P. 1986. The effects of virginity and ovariectomy on growth, food consumption, fat body mass and oxygen consumption in the house cricket, *Acheta domesticus. Journal of Insect Physiology* **32**: 425-431.

COLLINS, D. P. 2013. Termite behavior: Measuring the postanoxic consumption rates of landscape mulches by Eastern subterranean termites. *The American Biology Teacher* **75**: 41-45.

COMBES, C. 1996. Parasites, biodiversity and ecosystem stability. *Biodiversity and Conservation* **5**: 953-962.

COOMBES, M. A., NAYLOR, L. A., VILES, H. A. and THOMPSON, R. C. 2013. Bioprotection and disturbance: seaweed, microclimatic stability and conditions for mechanical weathering in the intertidal zone. *Geomorphology* **202**: 4-14.

COSTAMAGNA, A. C. and LANDIS, D. A. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. *Ecological Applications* **16**: 1619-1628.

COSTAMAGNA, A. C., LANDIS, D. A. and DIFONZO, C. D. 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications* **17**: 441-451.

COUDRAIN, V., HERZOG, F. and ENTLING, M. H. 2013. Effects of habitat fragmentation on abundance, larval food and parasitism of a spider-hunting wasp. *Plos One* **8**: 1-7.

COX, T. E., PHILIPPOFF, J., BAUMGARTNER, E., ZABIN, C. J. and SMITH, C. M. 2013. Spatial and temporal variation in rocky intertidal communities along the main Hawaiian islands. *Pacific Science* **67**: 23-45.

CRAFFORD, J. E., SCHOLTZ, C. H. and CHOWN, S. L. 1986. The insects of sub-Antarctic Marion and Prince Edward Islands with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84.

CRESPI, B. J. 2004. Vicious circles: positive feedback in major evolutionary and ecological transitions. *TRENDS in Ecology and Evolution* **19**: 627-633.

CROSS, R. H. M. and PINCHUCK, S. 1987. *A handbook on the preparation of biological material for electron microscopy.* Rhodes University, Grahamstown.

DALZIEL, I. W. D. 1991. Pacific margins of Laurentia and East Antarctic-Australia as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology* **19**: 598-601.

DAY, J. H. 1974. *A guide to marine life on South African shores.* 2nd Edition. A. A. Balkema: Cape Town. pp 122.

DEBACH, P. and ROSEN, D. 1991. *Biological Control by Natural Enemies, 2nd Edition.* Cambridge University Press, Cambridge. pp 456.

DEEVEY, JR., E. S. 1947. Life Tables for Natural Populations of Animals. *The Quarterly Review of Biology* **22**: 283-314.

DENNO, R. F., GRATTON, C., PETERSON, M. A., LANGELLOTTO, G. A., FINKE, D. L. and HUBERTY, A. F. 2002. Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology* **83**: 1443-1458.

DIPPENAAR-SCHOEMAN, A. S. and JOCQUÉ, R. 1997. *African spiders: an identification manual.* Plant Protection Research Institute Handbook No. 9, Agricultural Research Council, Pretoria. pp 101-103; 145-147.

DITTRICH-SCHRÖDER, G., WINGFIELD, M. J., KLEIN, H. & SLIPPERS, B. 2012. NDA extraction techniques for DNA barcoding of minute gall-inhabiting wasps. *Molecular Ecology Resources* **12**: 109-115.

DOBEL, H. G., DENNO, R. F. and CODDINGTON, J. A. 1990. Spider (Araneae) community structure in an intertidal salt marsh: effects of vegetation structure and tidal flooding. *Environmental Entomology* **19**: 1356-1370.

DOBSON, M. 2013. Family-level keys to freshwater fly (Diptera) larvae: a brief review and a key to European families avoiding use of mouthpart characters. *Freshwater Reviews* **6**: 1-32.

DONADI, S., VAN DER HEIDE, T., VAN DER ZEE, E. M., EKLÖF, J. S., VAN DE KOPPEL, J., WEERMAN, E. J., PIERSMA, T., OLFF, H. and ERIKSSON, B. K. 2015. Cross-habitat interactions among bivalve species control community structure on intertidal flats. *Ecology* **94**: 489-498.

DORCHIN, N., MIFSUD, D. and ASKEW, R. 2014. Saltbush-associated *Asphondylia* species (Diptera: Cecidomyiidae) in the Mediterranean Basin and their chalcidoid parasitoids (Hymenoptera: Chalcidoidea). *Zootaxa* **3869**: 383-396.

EASON, R. R., PECK, W. B. and WHITCOMB, W. H. 1967. Notes on spider parasites, including a reference list. *Journal of Kansas Entomological Society* **40**: 422-434.

EDGERLY, J. S., SZUMIK, C. A. and MCCREEDY, C. N. 2007. On new characters of the eggs of Embioptera with the description of a new species of *Saussurembia* (Anisembiidae). *Systematic Ecology* **32**: 387-395.

EMANUEL, B. P., BUSTAMANTE, R. H., BRANCH, G. M., EEKHOUT, S. and ODENDAAL, F. J. 1992. A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. *South African Journal of Marine Science* **12**: 341-354.

ESLIN, P. and PRÉVOST, G. 2000. Racing against host's immunity defenses: a likely strategy for passive evasion of encapsulation in *Asobara tabida* parasitoids. *Journal of Insect Physiology* **46**: 1161-1167.

ESRI. 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

EVANS, P. D., RUSCOE, C. N. E. and TREHERNE, J. E. 1971. Observations on the biology and submergence behaviour of some littoral beetles. *Journal of the Marine Biological Association of the United Kingdom* **51**: 375-386.

FERNÁNDEZ, J. E., MORENO, F., GIRÓN, I. F. and BLÁZQUEZ, O. M. 1997. Stomatal control of water use in olive tree leaves. *Plant and Soil* **190**: 179-192.

FERREIRA, C. E. L., GONÇALVES, J. E. A. and COUTINHO, R. 2001. Community structure of fishes and habitat complexity on a tropical rocky shore. *Environmental Biology of Fishes* **61**: 357-369.

FIELD, S. A. and AUSTIN, A. D. 1994. Anatomy and mechanics of the telescopic ovipositor system of *Scelio* Latreille (Hymenoptera: Scelionidae) and related genera. *International Journal of Insect Morphology and Embryology.* **23**: 135-158.

FILMER, M. R. 1995. *Southern African spiders: an identification guide.* Struik Publishers, Cape Town. pp 74-75; 79-80.

FILMER, M. R. and LARSEN, N. In press. *Filmer's spiders: an identification guide for Southern Africa.* Struik Nature, Cape Town. pp 72-74; 82-83.

FOSTER, W. A. and TREHERNE, J. E. 1976. Insects of marine saltmarshes: problems and adaptations. In: CHENG, L. 1976. *Marine insects.* North-Holland Publishing Company, Amsterdam. pp 5-42.

FOX, J. and WEISBERG, S. 2011. An {R} *Companion to Applied Regression, Second Edition.* Thousand Oaks CA: Sage. [Online] Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. Accessed: 17/06/2015.

FOX, L. R., LETOURNEAU, D. K., EISENBACH, J. and VAN NOUHUYS, S. 1990. Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia* **83**: 414-419.

GADGIL, M. and BOSSERT, W. H. 1970. Life historical consequences of natural selection. *The American Naturalist* **104**: 1-24.

GAINEY, JR., L. F. 1984. Osmoregulation in the larvae of *Odontomyia cincta* (Diptera: Stratiomyidae). *Physiological Zoology* **57**: 663-672.

GANTZ, J. D. and LEE, JR, R. E. 2015. The limits of drought-induced rapid cold-hardening: Extremely brief, mild desiccation triggers enhanced freeze-tolerance in *Eurosta solidaginis* larvae. *Journal of Insect Physiology* **73**: 30-36.

GASPERI, G., BONIZZONI, M., GOMULSKI, L. M., MURELLI, V., TORTI, C., MALACRIDA, A. R. and GUGLIELMINO, C. R. 2002. Genetic differentiation, gene flow and the origin of infestations of the medfly, *Ceratitis capitata*. *Genetica* **116**: 125-135.

GERMANO, M. D. and PICOLLO, M. I. 2015. Reproductive and developmental costs of deltamethrin resistance in the Chagas disease vector *Triatoma infestans*. *Journal of Vector Ecology* **40**: 59-65.

GILBERT, M. T. P., MOORE, W., MELCHIOR, L. & WOROBEY, M. 2007. DNA extraction from dry museum beetles without conferring external morphological damage. *Plos One* **2**: e272. doi:10.1371/journal.pone.0000272.

GRATTON, C. and DENNO, R. F. 2003. Seasonal shift from bottom-up to top-down impact in phytophagous insect populations. *Oecologia* **134**: 487-495.

GREEN, R. F., GORDH, G. and HAWKINS, B. A. 1982. Precise sex ratios in highly inbred parasitic wasps. *The American Naturalist* **120**: 653-665.

GRIPENBERG, S. and ROSLIN, T. 2007. Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos* **116**: 181-188.

GROSELL, M., BLANCHARD, J., BRIX, K. V. and GERDES, R. 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. *Aquatic Toxicology* **84**: 162-172.

HADDAD, C. R. 2003. Aspects of the biology of *Heliophanus pistaciae*, an agrobiont jumping spider in pistachio orchards in South Africa (Araneae: Salticidae) (Chapter 5). In: HADDAD, C. R. 2003. *Spider ecology in pistachio orchards in South Africa*. MSc Thesis, University of the Free State, Bloemfontein. pp 174.

HADDAD, C. R. and DIPPENAAR-SCHOEMAN, A. S. 2009. A checklist of the non-Acarine Arachnids (Chelicerata: Arachnida) of the De Hoop Nature Reserve, Western Cape Province, South Africa. *Koedoe* **51**, Art. #149.

HADDAD, C. R. and LOUW, S. V. D. M. 2006. Phenology, ethology and fecundity of *Heliophanus pistaciae* Wesolowska (Araneae: Salticidae), an agrobiont jumping spider in South African pistachio orchards. *African Plant Protection* **12**: 1-11.

HAIRSTON, N. G., SMITH, F. E. and SLOBODKIN, L. B. 1960. Community structure, population control, and competition. *The American Naturalist* **94**: 421-425.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

HAMILTON, W. D. 1987. Kinship, recognition, disease, and intelligence: constraints of social evolution. In: ITO, Y., BROWN, J. L. and KIKKAWA, J. 1987. *Animal societies: theories and facts*. Japan Scientific Societies Press: Japan. pp 81-102.

HANSEN, J., RUEDY, R., SATO, M. and LO, K. 2010. Global surface temperature change. *Reviews of Geophysics* **48**: 1-29.

HAWKINS, B. A., MILLS, N. J., JERVIS, M. A. and PRICE, P. W. 1999. Is the biological control of insects a natural phenomenon? *Oikos* **86**: 493-506.

HAZELL, S. P., PEDERSEN, B. P., WORLAND, M. R., BLACKBURN, T. M. and BALE, J. S. 2008. A method for the rapid measurement of thermal tolerance traits in studies of small insects. *Physiological Entomology* **33**: 389-394.

HEIMPEL, G. E. and CASAS, J. 2008. Chapter 3: Parasitoid foraging and oviposition behaviour in the field. In: WAJNBERG, É., BERNSTEIN, C. and VAN ALPHEN, J. 2008. *Behavioural Ecology of Insect Parasitoids*. Blackwell Publishing Ltd.: Malden. pp 51-70.

HELMUTH, B., MIESZKOWSKA, N., MOORE, P. and HAWKINS, S. J. 2006. Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**: 373-404.

HEMPEL, S., STEIN, C., UNSICKER, S. B., RENKER, C., AUGE, H., WELSSER, W. W. and BUSCOT, F. 2009. Specific bottom-up effects of arbuscular mycorrhizal fungi across a plant-herbivore-parasitoid system. *Oecologia* **160**: 267-277.

HERBERSTEIN, M. E., CRAIG, C. L., CODDINGTON, J. A. and ELGAR, M. A. 2000. The functional significance of silk decorations of orb-web spiders: a critical review of the empirical evidence. *Biological Reviews* **75**: 649-669.

HICKMAN, V. V. 1967. New Scelionidae (Hymenoptera) which lay their eggs in those of spiders. *Journal of the Entomological Society of Australia* **4**: 15-39.

HINTON, H. E. 1976. Respiratory adaptations of marine insects. In: CHENG, L. 1976. *Marine Insects*. North-Holland Publishing Company, Amsterdam.

HOEKMAN, D. 2010. Turning up the heat: temperature influences the relative importance of top-down and bottom-up effects. *Ecology* **91**: 2819-2825.

HOFFMANN, A. A., CHOWN, S. L. and CLUSELLA-TRULLAS, S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology* **27**: 934-949.

HOLDEN, H. A. and STOREY, K. B. 2011. Reversible phosphorylation regulation of NADPH-linked polyol dehydrogenase in the freeze-avoiding gall moth, *Epiblema scudderiana*: role in glycerol metabolism. *Archives of Insect Biochemistry and Physiology* **77**: 32-44.

HUANG, G., CHEN, S., LI, S., CHA, J., LONG, C., LI, L., HE, Q. and LIU, Y. 2007. Protein kinase A and casein kinases mediate sequential phosphorylation events in the circadian negative feedback loop. *Genes and Development* **21**: 3283-3295.

HUBER, J. T. 2009. Chapter 12: Biodiversity of Hymenoptera. In: FOOTTIT, R. and ADLER, P. 2009. *Insect Biodiversity: Science and Society.* Blackwell Publishing, Oxford. pp 303-323.

HUEY, R. B., CRILL, W. D., KINGSOLVER, J. G. and WEBER, K. E. 1992. A method for rapid measurement of heat or cold resistance of small insects. *Functional Ecology* **6**: 489-494.

HUNTER, S. J., GOODALL, T. I., WALSH, K. A., OWEN, R. & DAY, J. C. 2008. Non-destructive DNA extraction from blackflies (Diptera: Simuliidae): retaining voucher specimens for DNA barcoding projects. *Molecular Ecology Resources* **8**: 56-61.

IKAWA, T., OKABE, H. and CHENG, L. 2012. Skaters of the seas – comparative ecology of nearshore and pelagic *Halobates* species (Hemiptera: Gerridae), with special reference to Japanese species. *Marine Biology Research* **8**: 915-936.

IQBAL, M. and AUSTIN, A. D. 2000. A preliminary phylogeny for the *Baeini* (Hymenoptera: Scelionidae): endoparasitoids of spider eggs. In: AUSTIN, A. D. & DOWTON, M., (eds.) *The Hymenoptera: evolution, biodiversity and biological control.* CSIRO Publishing, Melbourne. pp 178-191.

IYER, P. V. and ANANTHANARAYAN, L. 2008. Enzyme stability and stabilization - aqueous and non-aqueous environment. *Process Biochemistry* **43**: 1019-1032.

JACKSON, D. J. 1958. Observations on the biology of *Caraphractus cinctus* walker (Hymenoptera: Mymaridae), a parasitoid of the eggs of Dytiscidae. *Transactions of the Royal Entomological Society of London* **110**: 533-554.

JANSSEN, A. 1989. Optimal host selection by *Drosophila* parasitoids in the field. *Functional Ecology* **3**: 469-479.

JANZEN, F. J. and MORJAN, C. L. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour* **62**: 73-82.

JAPOSHVILI, G. and RUSSELL, E. 2012. A new parasitization record of *Haliaspis spartinae* (Diaspididae) and *Encarsia ellisvillensis* sp. nov. (Chalcidoidea: Aphelinidae) from the United States. *Annals of the Entomological Society of America* **105**: 493-497.

JOHNSON, N. F. 2015. *Hymenoptera Online Database.* [Online] Available at: http://hol.osu.edu/agent-full.html?id=2. Accessed 01/12/2015.

JOHNSON, N. F. and MASNER, L. 2004. The genus *Thoron* Haliday (Hymenoptera: Scelionidae), egg-parasitoids of waterscorpions (Hemiptera: Nepidae), with key to world species. *American Museum Novitates* **3452**: 1-16.

_____

JOHNSON, N. F., MUSETTI, L., ZEINNER, V., HEMLY, S. E. and CORA, J. 2014. *Phylogenetic concentration and provenance of host data for parasitic wasps of the superfamily Platygastroidea (Hymenoptera).* Entomological Society of America Annual Meeting at the Portland State University, Oregon, USA. 15-19 November.

KARIMZADEH, J. and WRIGHT, D. J. 2008. Bottom-up cascading effects in a tritrophic system: interactions between plant quality and host-parasitoid immune responses. *Ecological Entomology* **33**: 45-52.

KARIMZADEH, J., BONSALL, M. B. and WRIGHT, D. J. 2004. Bottom-up and top-down effects in a tritrophic system: the population dynamics of *Plutella xylostella* (L.)-*Cotesia plutellae* (Kurdjumov) on different host plants. *Ecological Entomology* **29**: 285-293.

KEELING, P. J. & DOOLITTLE, W. F. 1996. A non-canonical genetic code in an early diverging eukaryotic lineage. *The EMBO Journal* **5**: 2285-2290.

KELLERMANN, V., OVERGAARD, J., HOFFMANN, A. A., FLØJGAARD, C., SVENNING, J. C. and LOESCHCKE, V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proceedings of the National Academy of Sciences* **109**: 16228-16233.

KING, B. H. 1987. Offspring sex ratios in parasitoid wasps. *The Quarterly Review of Biology* **62**: 367-396.

KÖSTER, M. and PAFFENHÖFER, G. A. 2012. Oxygen consumption of fecal pellets of doliolids (Tunicata, Thaliacea) and planktonic copepods (Crustacea, Copepoda). *Journal of Plankton Research* **0**: 1-14.

KÖSTER, M., KRAUSE, C. and PAFFENHÖFER, G. A. 2008. Time-series measurements of oxygen consumption of copepod nauplii. *Marine Ecology Progress Series* **353**: 157-164.

KREMEN, C. 2005. Managing ecosystem services: what do we need to know about their ecology? *Ecology Letters* **8**: 468-479.

LACY, R. C. 1993. VORTEX: a computer simulation model for population viability analysis. *Wildlife Research* **20**: 45-65.

LAEGDSGAARD, P., MONAMY, V. and SAINTILAN, N. 2006. Investigating the presence of threatened insectivorous bats on coastal NSW saltmarsh habitat. *Wetlands (Australia)* **22**: 29-41.

LAING, J. 1937. Host-finding by insect parasitoids. 1. Observations on the finding of hosts by *Alysia manducator, Mormoniella vitripennis* and *Trichogramma evanescens. Journal of Animal Ecology* **6**: 298-317.

LAMORAL, B. H. 1968. On the ecology and habitat adaptations of two intertidal spiders, *Desis formidabilis* (O.P. Cambridge) and *Amaurobioides africanus* Hewitt, at "The Island" (Kommetjie, Cape Peninsula), with notes on the occurrence of two other spiders. *Annals of the Natal Museum* **20**: 151-193.

LANDIS, D. A., WRATTEN, S. D. and GURR, G. M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* **45**: 175-201.

LARKIN, M. A., BLACKSHIELDS, G., BROWN, N. P., CHENNA, R., MCGETTIGAN, P. A., MCWILLIAM, H., VALENTIN, F., WALLACE, I. M., WILM, A., LOPEZ, R., THOMPSON, J. D., GIBSON, T. J. & HIGGINS, D. G. 2007. ClustalW Version 2. *Bioinformatics* **23**: 2947-2948.

LARSEN, N. 2012. Predatory behaviour of Afrotropical Palpimanidae. *Newsletter of the African Arachnological Society* **25**: 9.

LEVIN, S. A. 2000. Multiple scales and the maintenance of biodiversity. *Ecosystems* **3**: 498-506.

LEVIS, N. A., YI, S. X. and LEE, JR, R. E. 2012. Mild desiccation rapidly increases freeze tolerance of the goldenrod gall fly, *Eurosta solidaginis*: evidence for drought-induced rapid cold-hardening. *The Journal of Experimental Biology* **215**: 3768-3773.

LI, D., KOK, L. M., SEAH, W. K. and LIM, M. L. M. 2003. Age-dependent stabilimentum-associated predator avoidance behaviours in orb-weaving spiders. *Behaviour* **140**: 1135-1152.

LIGHTON, J. R. B., SCHILMAN, P. E. and HOLWAY, D. A. 2004. The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *The Journal of Experimental Biology* **207**: 4463-4471.

LILLYWHITE, H. B., SHEEHY III, C. M. and ZAIDAN III, F. 2008. Pitviper scavenging at the intertidal zone: an evolutionary scenario for invasion of the sea. *BioScience* **58**: 947-955.

LOBDELL, C. E., YONG, T. H. and HOFFMANN, M. P. 2005. Host color preferences and short-range searching behavior of the egg parasitoid *Trichogramma ostriniae. Entomologia Experimentalis et Applicata* **116**: 127-134.

LUBKE, R. A. 1998. Chapter 11: Marine ecology. In: LUBKE, R. A. and DE MOOR, I. (Eds.) 1998. *Field guide to the Eastern and Southern Cape coasts*. University of Cape Town Press, Cape Town. pp 170-179.

LYONS, P. J., CRAPOULET, N., STOREY, K. B. and MORIN, P. J. 2015. Identification and profiling of miRNAs in the freeze-avoiding gall moth *Epiblema scudderiana* via next-generation sequencing. *Molecular and Cellular Biochemistry* **410**: 155-163.

MADDRELL, S. H. P. 1998. Why are there no insects in the open sea? *The Journal of Experimental Biology* **201**: 2461-2464.

MARCOTT, S. A., SHAKUN, J. D., CLARK, P. U. and MIX, A. C. 2013. A reconstruction of regional and global temperature for the past 11,300 years. *Science* **339**: 1198-1201.

MARSHALL, S. D. and GITTLEMAN, J. L. 1994. Clutch size in spiders: is more better? *Functional Ecology* **8**: 118-124.

MASNER, L. 1968. A new Scelionid wasp from the intertidal zone of South Africa (Hymenoptera: Scelionidae). *Annals of the Natal Museum* **20**: 195-198.

MASNER, L. 1976. Revisionary notes and keys to world genera of Scelionidae (Hymenoptera: Proctotrupoidea). *Memoirs of the Entomological Society of Canada* **108**: 1-87.

MASNER, L. 1993. Chapter 14: Superfamily Platygastroidea. In: GOULET, H. and HUBER, J. T. 1993. *Hymenoptera of the world: an identification guide to families*. Canada Communication Group: Ottawa. pp 558-565.

MASON, W. R. M. and HUBER, J. T. 1993. Chapter 2: Order Hymenoptera. In: GOULET, H. and HUBER, J. T. 1993. *Hymenoptera of the world: an identification guide to families*. Canada Communication Group: Ottawa. pp 4-12.

MATSUO, K., HIROSE, Y. and JOHNSON, N. F. 2014. A taxonomic issue of two species of Trissolcus (Hymenoptera: Platygastridae) parasitic on eggs of the brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae): resurrection of *T. plautiae*, a cryptic species of *T. japonicus* revealed by morphology, reproductive isolation and molecular evidence. *Applied Entomology and Zoology* **49**: 385-394.

MATTHEWS, P. G. D. and SEYMOUR, R. S. 2006. Diving insects boost their buoyancy bubbles. *Nature* **441**: 171.

MATTIACCI, L., VINSON, S. B., WILLIAMS, H. J., ALDRICH, J. R. and BIN, F. 1993. A long-range attractant kairomone for egg parasitoid *Trissolcus basalis*, isolated from defensive secretion of its host, *Nezara viridula*. *Journal of Chemical Ecology* **19**: 1167-1181.

MAXIME, V., PEYRAUD-WAITZENEGGER, M., CLAIREAUX, G. and PEYRAUD, C. 1990. Effects of rapid transfer from sea water to fresh water on respiratory variables, blood acid-base status and O_2 affinity of haemoglobin in Atlantic salmon (*Salmo salar* L.). *Journal of Comparative Physiology B* **160**: 31-39.

MENGE, B. A., GOUHIER, T. C., HACKER, S. D., CHAN, F. and NIELSEN, K. J. 2015. Are meta-ecosystems organized hierarchically? A model and test in rocky intertidal habitats. *Ecological Monographs* **85**: 213-233.

MERCER, R. D., CHOWN, S. L. and MARSHALL, D. J. 2000. Mite and insect zonation on a Marion Island rocky shore: a quantitative approach. *Polar Biology* **23**: 775-784.

MILLS, N. J. 2001. Factors influencing top-down control of insect pest populations in biological control systems. *Basic and Applied Ecology* **2**: 323-332.

MOON, D. C. and STILING, P. 2002. The influence of species identity and herbivore feeding mode on top-down and bottom-up effects in a salt marsh system. *Oecologia* **133**: 243-253.

MORAND, S. and GONZALEZ, E. A. 1997. Is parasitism a missing ingredient in model ecosystems? *Ecological Modelling* **95**: 61-74.

MUNKITTRICK, K. R. and MCCARTY, L. S. 1995. An integrated approach to aquatic ecosystem health: top-down, bottom-up or middle-out? *Journal of Aquatic Ecosystem Health* **4**: 77-90.

MURDOCH, W. W. 1966. Population stability and life history phenomena. *The American Naturalist* **100**: 5-11.

MURPHY, N. P., CAREY, D., CASTRO, L. R., DOWTON, M. and AUSTIN, A. D. 2007. Phylogeny of the platygastroid wasps (Hymenoptera) based on sequences from the 18S rRNA, 28S rRNA and cytochrome oxidase I genes: implications for the evolution of the ovipositor system and host relationships. *Biological Journal of the Linnean Society* **91**: 653-669.

NADEAU, N. J., MARTIN, S. H., KOZAK, K. M., SALAZAR, C., DASMAHAPATRA, K. K., DAVEY, J. W., BAXTER, S. W., BLAXTER, M. L., MALLET, J. and JIGGINS, C. D. 2013. Genome-wide patterns of divergence and gene flow across a butterfly radiation. *Molecular Ecology* **22**: 814-826.

NAKAOKA, M., ITO, N., YAMAMOTO, T., OKUDA, T. and NODA, T. 2006. Similarity of rocky intertidal assemblages along the Pacific coast of Japan: effects of spatial scales and geographic distance. *Ecological Research* **21**: 425-435.

NAVARRO-BARRANCO, C., GUERRA-GARCÍA, J. M., SÁNCHEZ-TOCINO, L., ROS, M., FLORIDO, M. and GARCÍA-GÓMEZ, J. C. 2015. Colonization and successional patterns of the mobile epifaunal community along an environmental gradient in a marine cave. *Marine Ecology Progress Series* **521**: 105-115.

NEUMANN, D. and WOERMANN, D. 2009. Physical conditions for trapping air by a microtrichia-covered insect cuticle during temporary submersion. *Naturwissenschaften* **96**: 933-941.

NEW, T. R. 1997. Are Lepidoptera an effective 'umbrella group' for biodiversity conservation? *Journal of Insect Conservation* **1**: 5-12.

NEWS24. 2015. Cape Town fire out. [Online] Available at: http://www.news24.com/SouthAfrica/News/Cape-Town-fire-out-20150309. Accessed: 30/06/2015.

NONDULA, N., MARSHALL, D. J., BAXTER, R., SINCLAIR, B. J. and CHOWN, S. L. 2004. Life history and osmoregulatory ability of *Telmatogeton amphibious* (Diptera, Chironomidae) at Marion Island. *Polar Biology* **27**: 629-635.

OAKES, J. M. and EYRE, B. D. 2015. Wastewater nitrogen and trace metal uptake by biota on a high-energy rocky shore detected using stable isotopes. *Marine Pollution Bulletin* **100**: 406-413.

O'CONNOR, J. P. 1996. Further records of Irish *Pteromalidae* (Hymenoptera: Chalcidoidea), including ten species new to Ireland. *The Irish Naturalists' Journal* **25**: 254-257.

OWEN, C. A., COETZEE, J. A. and VAN NOORT, S. 2014. Distributional range of the South African maritime spider-egg parasitoid wasp, *Echthrodesis lamorali* (Hymenoptera: Platygastridae: Scelioninae). *African Invertebrates* **55**: 323-332.

PAETZOLD, A., LEE, M. and POST, D. M. 2008. Marine resource flows to terrestrial arthropod predators on a temperate island: the role of subsidies between systems of similar productivity. *Oecologia* **157**: 653-659.

PEDERSEN, O. and COLMER, T. D. 2012. Physical gills prevent drowning of many wetland insects, spiders and plants. *The Journal of Experimental Biology* **215**: 705-709.

PEKÁR, S., CODDINGTON, J. A. and BLACKLEDGE, T. A. 2012. Evolution of stenophagy in spiders (Araneae): evidence based on the comparative analysis of spider diets. *Evolution* **66**: 776-803.

PENNACCHIO, F. and STRAND, M. S. 2006. Evolution of developmental strategies in parasitic Hymenoptera. *Annual Review of Entomology* **51**: 233-258.

PHAKATHI, B. 2015. Cape Town Fire and Rescue spearheads team as fire spreads. [Online] Available at: http://www.bdlive.co.za/national/2015/03/02/cape-town-fire-and-rescue-spearheads-team-as-fire-spreads. Accessed: 30/06/2015.

PIERCE, S. K. 1982. Invertebrate cell volume control mechanisms: a coordinated use of intracellular amino acids and inorganic ions as osmotic solute. *The Biological Bulletin* **163**: 405-419.

PIZZOL, J., DESNEUX, N., WAJNBERG, E. and THIÉRY, D. 2012. Parasitoid and host egg ages have independent impact on various biological traits in a Trichogramma species. *Journal of Pest Science* **85**: 489-496.

POLIS, G. A. and STRONG, D. R. 1996. Food web complexity and community dynamics. *The American Naturalist* **147**: 813-846.

POLIS, G. A., HURD, S. D., JACKSON, T. and SANCHEZ-PIÑERO, F. 1998. Multifactor population limitation: variable spatial and temporal control of spiders on Gulf of California islands. *Ecology* **79**: 490-502.

POMPANON, F., DE SCHEPPER, B., MOURER, Y., FOUILLET, P. and BOULETREAU, M. 1997. Evidence for a substrate-borne sex pheromone in the parasitoid wasp, *Trichogramma brassicae. Journal of Chemical Ecology* **23**: 1349-1360.

PÖRTNER, H. O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**: 137-146.

PÖRTNER, H. O. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology Part A* **132**: 739-761.

PRZHIBORO, A. A. 2000. Synusia of the turf inhabitants of monocotyledones: a poorly known component of the lake macrobenthos. *Annual Reports of the Zoological Institute RAS.* [Online] Available at: http://www.zin.ru/annrep/2000/19.html. Accessed 05/11/2015.

PUGH, P. J. A. 2004. Biogeography of spiders (Araneae: Arachnida) on the islands of the Southern Ocean. *Journal of Natural History* **38**: 1461-1487.

R CORE TEAM. 2015. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Austria. Available at: http://www.R-project.org/. Accessed: 17/06/2015.

RAGG, N. L. C. and TAYLOR, H. H. 2006. Oxygen uptake, diffusion limitation, and diffusing capacity of the bipectinate gills of the abalone, *Haliotis iris* (Mollusca: Prosobranchia). *Comparative Biochemistry and Physiology* **143**: 299-306.

RAINA, A. K., WERGIN, W. P., MURPHY, C. A. and ERBE, E. F. 2000. Structural organization of the sex pheromone gland in *Helicoverpa zea* in relation to pheromone production and release. *Arthropod Structure and Development* **29**: 343-353.

RAVINESH, R. and BIJUKUMAR, A. 2013. Comparison of intertidal biodiversity associated with natural rocky shore and sea wall: a case study from the Kerala coast, India. *Indian Journal of Geo-Marine Science* **42**: 223-235.

ROBERGE, J. M. and ANGELSTAM, P. 2004. Usefulness of the umbrella species concept as a conservational tool. *Conservation Biology* **18**: 76-85.

ROTH, V. D. and BROWN, W. L. 1976. Other intertidal air-breathing arthropods. In: CHENG, L. 1976. *Marine Insects*. North-Holland Publishing Company, Amsterdam. pp 119-150.

ROVNER, J. S. 1987. Nests of terrestrial spiders maintain a physical gill: flooding and the evolution of silk constructions. *Journal of Arachnology* **14**: 327-337.

SANFORD, E. 2002. Water temperature, predation, and the neglected role of physiological rate effects in rocky intertidal communities. *Integrative and Comparative Biology* **42**: 881-891.

SAPA. 2015. Cape Town fire under control. [Online] Available at: http://www.timeslive.co.za/local/2015/03/06/cape-town-fire-under-control. Accessed: 30/06/2015.

SAPEOPLE. 2015. Cape Town Photos Today as Fires Continue on Hottest Day in 100 Years! [Online] Available at: http://www.sapeople.com/2015/03/03/cape-town-photos-today-fires-continue/. Accessed: 30/06/2015.

SAVOLAINEN, V., COWAN, R. S., VOGLER, A. P., RODERICK, G. K. & LANE, R. 2005. Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B* **360**: 1805-1811.

_____

SEITZ, R. D., WENNHAGE, H., BERGSTRÖM, U., LIPCIUS, R. N. and YSEBAERT, T. 2014. Ecological value of coastal habitats for commercially and ecologically important species. *ICES Journal of Marine Science* **71**: 648-665.

SHARKEY, M. J. 2007. Phylogeny and classification of Hymenoptera. *Zootaxa* **1668**: 521-548.

SINCLAIR, A. R. E., KREBS, C. J., FRYXELL, J. M., TURKINGTON, R., BOUTIN, S., BOONSTRA, R., SECCOMBE-HETT, P., LUNDBERG, P. and OKSANEN, L. 2000. Testing hypotheses of trophic level interactions: a boreal forest ecosystem. *Oikos* **89**: 313-328.

SINCLAIR, B. J., VERNON, P., KLOK, C. J. and CHOWN, S. L. 2003. Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution* **18**: 257-262.

SINK, K. J., BRANCH, G. M. and HARRIS, J. M. 2005. Biogeographic patterns in rocky intertidal communities in KwaZulu-Natal, South Africa. *African Journal of Marine Science* **27**: 81-96.

SKINNER, S. W. 1982. Maternally inherited sex ratio in the parasitoid wasp *Nasonia vitripennis*. *Science* **215**: 1133-1134.

SMIT, A. J., ROBERTS, M., ANDERSON, R. J., DUFOIS, F., DUDLEY, S. F. J., BORNMAN, T. G., OLBERS, J. and BOLTON, J. J. 2013. A coastal seawater temperature dataset for biogeographical studies: large biases between *in situ* and remotely-sensed data sets around the coast of South Africa. *Plos One* **8**: 1-13.

SOMERO, G. N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integrative and Comparative Biology* **42**: 780-789.

SOMERO, G. N. 2005. Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* **2**: 1-9.

SOUTH AFRICAN NATIONAL PARKS. 2015. Table Mountain National Park. [Online] Available at: http://www.sanparks.co.za/parks/table_mountain/tourism/activities.php. Accessed 13/11/2015.

_____

SOUTHWOOD, T. R. E. and HENDERSON, P. A. 2000. Chapter 10: Estimation of natality, mortality, and dispersal. In SOUTHWOOD, T. R. E. and HENDERSON, P. A. 2000. *Ecological Methods (3rd Edition)*. Blackwell Science Ltd.: Oxford. pp 375-403.

STATSOFT, INC. 2011. Statistica 10. Oklahoma, United States of America.

STEPHENSON, T. A. and STEPHENSON, A. 1949. The universal features of zonation between tide-marks on rocky coasts. *Journal of Ecology* **37**: 289-305.

STILLMAN, J. H. and SOMERO, G. N. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (Genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. *The Journal of Experimental Biology* **199**: 1845-1855.

STIVE, M. J. F. 1986. A model for cross-shore sediment transport (Chapter 114). In: STIVE, M. J. F. 1986. *Proceedings of the 20th International Conference on Coastal Engineering*. American Society of Civil Engineers, New York. pp 1550-1564.

STRAND, M. R. 1986a. Chapter 4: The physiological interactions of parasitoids with their hosts and their influence on reproductive strategies. In: WAAGE, J. and GREATHEADS, D. 1986. *Insect parasitoids*. Academic Press: London. pp 97-136.

STRAND, M. R. 1986b. Chapter 5: Parasitoid mating behaviour. In: WAAGE, J. and GREATHEADS, D. 1986. *Insect Parasitoids*. Academic Press: London. pp 137-163.

STRAND, M. R. 1988. Variable sex ratio strategy of *Telenomus heliothidis* (Hymenoptera: Scelionidae): adaptation to host and conspecific density. *Oecologia* **77**: 219-224.

STRAND, M. R. 2010. Part 2: The effects of life history on development of the Hymenoptera. In: AUSTIN, A. D. and DOWTON, M. 2010. *Hymenoptera: Evolution, Biodiversity and Biological Control*. CSIRO: Canberra. pp 11-16.

STRAND, M. R., MEOLA, S. M. and VINSON, S. B. 1986. Correlating pathological symptoms in *Heliothis virescens* eggs with development of the parasitoid *Telenomus heliothidis*. *Journal of Insect Physiology* **32**: 389-402.

STRAND, M. R. and PECH, L. L. 1995. Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology* **40**: 31-56.

SYMONDS, M. R. E. and MOUSSALLI, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioural and Ecological Sociobiology* **65**: 13-21.

TAKADA, Y., KAWAMURA, S. and TANAKA, T. 2000. Biological characteristics: growth and development of the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) on the cabbage armyworm *Mamestra brassicae* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* **35**: 369-379.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.

TEAL, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. *Ecology* **43**: 614-624.

TERBLANCHE, J. S., DEERE, J. A., CLUSELLA-TRULLAS, S., JANION, C. and CHOWN, S. L. 2007. Critical thermal limits depend on metholodological context. *Proceedings of the Royal Society B* **274**: 2935-2942.

TESKE, P. R., MCQUAID, C. D., FRONEMAN, P. W. and BARKER, N. P. 2006. Impacts of marine biogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. *Marine Ecology Progress Series* **314**: 283-293.

TESKE, P. R., VON DER HEYDEN, S., MCQUAID, C. D. and BARKER, N. P. 2011. A review of marine phylogeography in southern Africa. *South African Journal of Science* **107**: 43-53.

THAW, R. & MALGAS, N. 2015. Fires rage in Cape Town's deep south. [Online] Available at: http://ewn.co.za/2015/03/02/Fires-rage-in-Cape-Towns-deep-south. Accessed: 30/06/2015.

TIETZ, R. M. and ROBINSON, G. A. 1974. *Tsitsikama shore*. Wallachs Printing Co., Pretoria.

_____

TRAUT, B. H. 2005. The role of coastal ecotones: a case study of the salt marsh/upland transition zone in California. *Journal of Ecology* **93**: 279-290.

TRUCHOT, J. P. 1987. *Comparative aspects of extracellular acid-base balance*. Springer-Verlag, Berlin. pp 41-48.

TURNBULL, A. L. 1973. Ecology of the true spiders (Araneomorphae). *Annual Review of Entomology* **18**: 305-348.

TURPIE, J. K., BECKLEY, L. E. and KATUA, S. M. 2000. Biogeography and the selection of priority areas for conservation of South African coastal fishes. *Biological Conservation* **92**: 59-72.

USINGER, R. L. 1957. Marine insects. *Geological Society of America Memoirs* **67V1**: 1177-1182.

VAN ACHTERBERG, HEMMINGA, M. A. and VAN SOELEN, J. 1990. New host record of *Bracon intercessor* Nees f. *megasomides* Strand (Hymenoptera: Braconidae), a parasite of *Agapanthia villosoviri-descens* DeGeer (Coleoptera: Cerambycidae) in salt marsh. *Zoologische Mededelingen* **64**: 25-29.

VAN ASCH, M. and VISSER, M. E. 2007. Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annual Reviews of Entomology* **52**: 37-55.

VAN DE KOPPEL, J., VAN DER HEIDE, T., ALTIERI, A. H., ERIKSSON, B. K., BOUMA, T. J., OLFF, H. and SILLIMAN, B. R. 2015. Long-distance interactions regulate the structure and resilience of coastal ecosystems. *Annual Review of Marine Science* **7**: 139-158.

VAN NOORT, S. 2009. The maritime platygastrid *Echthrodesis lamorali* Masner. *Skaphion* **3**: 1-3.

VAN NOORT, S. 2011. Caught in the act – *Echthrodesis* laying eggs. *Skaphion* **5**: 1-2.

VAN NOORT, S., MASNER, L., POPOVICI, O., VALERIO, A. A., TAEKUL, C., JOHNSON, N., MURPHY, N. P. and AUSTIN, A. D. 2014. Systematics and biology of the

aberrant intertidal parasitoid wasp *Echthrodesis lamorali* Masner (Hymenoptera: Platygastridae s.l.): a parasitoid of spider eggs. *Invertebrate Systematics* **28**: 1-16.

VARIOUS CONTRIBUTORS. 2015. *Echthrodesis lamorali* on: *Hymenoptera Online (HOL).* [Online] Available at: http://hol.osu.edu/index.html?id=4283. Accessed 13/11/2015.

VEENSTRA, A. A., MICHALCZYK, A. and KOLESIK, P. 2011. Taxonomy of two new species of gall midge (Diptera: Cecidomyiidae) infesting *Tecticornia arbuscula* (Salicornioideae: Chenopodiaceae) in Australian saltmarshes. *Australian Journal of Entomology* **50**: 393-404.

VERBERK, W. C. E. P., BARTOLINI, F., MARSHALL, D. J., PÖRTNER, H. O., TERBLANCHE, J. S., WHITE, C. R. and GIOMI, F. 2015. Can respiratory physiology predict thermal niches? *Annals of the New York Academy of Sciences* **Issue: Respiratory Science**: 1-16.

VERMEIJ, G. J. and DUDLEY, R. 2000. Why are there so few evolutionary transitions between aquatic and terrestrial ecosystems? *Biological Journal of Linnean Society* **70**: 541-554.

VETTER, R. S. and CARROLL, D. P. 2013. An identification key for eggs and egg sacs of spiders of potential agroeconomic importance: a feasibility study. *The Journal of Arachnology* **41**: 176-183.

VON DER MEDEN, C. E. O., PORRI, F., ERLANDSSON, J. and MCQUAID, C. D. 2008. Coastline topography affects the distribution of indigenous and invasive mussels. *Marine Ecology Progress Series* **372**: 135-145.

WAAGE, J. K. 1986. Chapter 3: Family planning in parasitoids: adaptive patterns of progeny and sex allocation. In: WAAGE, J. and GREATHEADS, D. 1986. *Insect parasitoids*. Academic Press: London. pp 63-95.

WANDELER, P., HOECK, P. E. A. & KELLER, L. F. 2007. Back to the future: museum specimens in population genetics. *TRENDS in Ecology and Evolution* **22**: 634-642.

WANDELER, P., SMITH, S., MORIN, P. A., PETTIFOR, A. & FUNK, S. M. 2004. Patterns of nuclear DNA degeneration over time – a case study in historic teeth samples. *Molecular Ecology* **12**: 1087-1093.

WANG, G., MAHER, E., BRENNAN, C., CHIN, L., LEO, C., KAUR, M., ZHU, P., ROOK, M., WOLFE, J. L. & MAKRIGIORGOS, G. M. 2004. DNA amplification method tolerant to sample degradation. *Genome Research* **14**: 2357-2366.

WARD, D. and LUBIN, Y. 1993. Habitat selection and the life history of a desert spider, *Stegodyphus lineatus* (Eresidae). *Journal of Animal Ecology* **62**: 353-363.

WERMELINGER, B. and SEIFERT, M. 1999. Temperature-dependent reproduction of the spruce bark beetle *Ips typographus*, and analysis of the potential population growth. *Ecological Entomology* **24**: 103-110.

WHITEHOUSE, M., AGNARSSON, I., MIYASHITA, T., SMITH, D., CANGIALOSI, K., MASUMOTO, T., LI, D. and HENAUT, Y. 2002. *Argyrodes*: phylogeny, sociality and interspecific interactions - a report on the *Argyrodes* symposium, Badplaas 2001. *Journal of Arachnology* **30**: 238-245.

WIGGLESWORTH, V. B. 1953. Surface forces in the tracheal systems of insects. *Quarterly Journal of Microscopical Science* **94**: 507-522.

WU, Y. T., WANG, C. H., ZHANG, X. D., ZHAO, B., JIANG, L. F., CHEN, J. K. and LI, B. 2009. Effects of saltmarsh invasion by *Spartina alterniflora* on arthropod community structure and diets. *Biological Invasions* **11**: 635-649.

YOUSSEF, N. N. 1969. Musculature, nervous system and glands of metasomal abdominal segments of the male of *Nomia melanderi* Ckll. (Hymenoptera: Apoidea). *Journal of Morphology* **129**: 59-79.

ZUUR, A. F., IENO, E. N. and ELPHICK, C. S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* **1**: 3-14.