Aspects of the thermal ecology of six species of carcass beetles in South Africa

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ABSTRACT

The forensic application of entomology is well known, but it is generally a field which concentrates on Diptera. Many Coleoptera also have forensic application, but are generally neglected by forensic entomology researchers. Necrophilic Coleoptera are diverse and therefore have application in estimating Post-Mortem Interval (PMI) by community composition, but they are also valuable in estimating PMI by development. In addition, Coleoptera are more common in stored product cases.

Six species of forensically important Coleoptera were studied, three from the family Dermestidae (*Dermestes haemorrhoidalis, D. maculatus* and *D. peruvianus*) and three from the family Silphidae (*Silpha punctulata, Thanatophilus micans* and *T. mutilatus*).

The effect of killing method and storage time on larval length was investigated in *T. micans*. Coleopteran larvae were shown not to behave in the same way as dipteran larvae. In contrast to dipteran larvae, it is recommended that coleopteran larvae be killed using ethanol.

A development model is presented for *T. micans*. This represents the first statistically robust development model for forensically important Coleoptera, and the first development model for forensically important Silphidae. The model offers a method of estimating PMI which can be used once Diptera are no longer present on a corpse.

Upper lethal temperature limits for four species of carcass beetle were determined. A comparison between species shows distinct differentiation between

families and species. This differentiation accounts for microhabitat differences which these species show on carcasses.

Bioclimatic models for the six species showed contrasting distributions, with both widespread and localised species. These models allow forensic investigators to assess whether the absence of a species from a corpse is forensically significant, or a result of the species distributions. Moisture-related variables were shown to be more important in predicting species distributions than temperature at a regional scale.

Forensic entomology standards can be adjusted based on the findings of this study. Length was again shown to be an inferior measurement of larval age. Coleopteran development has been shown to be useful, and should be given greater consideration in future work. *T. micans* has been shown to be capable of locating and ovipositing on carcasses promptly after death, making it a good forensic indicator. Further work is needed for the full potential of necrophilic Coleoptera to be realised.

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PREFACE

The work presented in this thesis is the result of many years of biological interest, not just the few that constituted my studies.

I will always be indebted to my supervisor, Prof. Martin Villet, for what he has taught me, throughout my studies. His enthusiasm and attitude towards entomology played a huge role in me choosing my study path at undergraduate level and his expertise and knowledge have taught me more at postgraduate level than I could ever have expected.

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DISCLAIMER

Any opinion, findings and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Research Foundation.

DECLARATION

The following thesis has not been submitted to any university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

_____ Date: _____

Chapter 1 - General Introduction

1.1 FORENSIC ENTOMOLOGY

Entomology has been used since the 19th century for forensic applications (Benecke, 2001), but despite this, research on this topic has been neglected in some parts of the world. While most northern hemisphere countries have accepted entomological evidence, many southern hemisphere countries appear to be lagging in this regard. This is partly because entomological research in these countries has not been of an explicitly forensic application until recently (Williams & Villet, 2006). Research into forensic entomology has now increased in these countries, with data now being produced for Argentina (Centeno *et al.*, 2002), Australia (Dadour *et al.*, 2001), Brazil (Carvalho *et al.*, 2000), Columbia (Barreto *et al.*, 2002) and South Africa (Williams & Villet, 2006) amoungst others.

Reviews of forensic entomology are available (Keh, 1985; Smith, 1986; Catts and Haskell, 1990; Benecke, 2001; Byrd & Castner, 2001; Catts and Goff, 1992), but tend to focus on northern species or species with a cosmopolitan distribution. These reviews focus on Diptera, neglecting necrophagous Coleoptera, despite the high diversity and prevalence of these Coleoptera (Abbot, 1937; Byrd & Castner, 2001). Forensic entomology can be divided into different categories, the most important of which are Medico-Legal and Stored Product Forensic Entomology (Williams & Villet, 2006). Medico-Legal Forensic Entomology focuses predominantly on the prediction of Post Mortem Intervals (PMIs), but insects are also used to assess the likelihood that a body has been moved post mortem (Becker *et al.*, 2007). PMI can be predicted using two general methods. The first is to assess the age of the insects on a carcass, or the post-oviposition interval, and from this the age of the carcass (Wells & LaMotte, 2001). The carcass will always be older than the post-oviposition interval, as it will normally take a few hours or even days for the arthropods to find the carcass, depending mainly on the species of insect. Detailed records of development at various temperatures are required as well as detailed succession records and local weather records to predict PMI in this way (Wells & LaMotte, 2001; Higley and Haskell, 2001). The second method is to predict PMI based on the succession of arthropods present on a carcass (LaMotte and Wells, 2000; Anderson, 2001). This requires

detailed records of the time at which each species arrives on carcasses as they decay, and not development records. Ecological successions are however dynamic and less predictable than insect development (Braack, 1981; 1987; Wells and LaMotte, 2001).

Williams and Villet (2006) review the southern African research into necrophagous insects, and also show a bias towards Dipteran necrophages in the literature. Coleoptera can be useful in forensic entomology, despite the fact that they develop on carrion in a more advanced state of decay (Catts & Haskell, 1990; Catts 1992; Byrd & Castner, 2001). The fast development times in Diptera result in them being good indicators of Post Mortem Interval (PMI) over short periods of time, but when they are not longer developing on carrion, Coleoptera can also be used in PMI estimates (Catts, 1992). At present developmental data for Coleoptera are in short supply, and the available data are not statistically robust (Coombs, 1979; Richardson and Goff, 2001). Development data for Coleoptera should therefore be created as a priority and with statistical requirements in mind. In addition to being useful in PMI estimates, Coleoptera are much more common than other insects in urban and commercial cases where stored products are damaged by insects.

1.2 THE USE OF COLEPTERA IN FORENSIC ENTOMOLOGY

Previous research in forensic entomology is heavily biased towards Diptera, and reviews have largely neglected the importance of Coleoptera. It has been assumed that because Diptera arrive soon after death they are more valuable than Coleoptera in the estimation of PMI. This depends on the state of decay in which a carcass is found. Carcasses in mid- to late decay contain few Diptera, resulting in Coleoptera being more important in predicting PMI (Braack, 1987; Anderson 2001). Coleoptera are also more diverse, with different groups feeding on carcasses in several waves, while Diptera dominate only the first wave of arthropods on a carcass. Braack (1986, 1987) found that the five most abundant families of insect to visit carcasses in South Africa were all coleopteran, which shows the value of Coleoptera as decomposers.

Adult Coleoptera are often found on carcasses before the carcasses are fit for their larvae to eat (pers. obs.). Members of the families Silphidae (*Thanatophilus micans* (Fabricius 1794)), Dermestidae (*Dermestes maculatus* (DeGeer, 1774)), Scarabaeidae (*Epirinus flagellatus* (Fabricius, 1775)) and Trogidae (*Trox sulcatus* (Thunberg, 1787)) (Braack, 1986, pers. obs.) have been observed on animal (white

rhinoceros, warthogs, bushbuck, giraffe and impala) carcasses within 48 hours of death. While none of these carcasses are human, the early colonisation observed shows that it is possible, and so prompt colonisation should not be excluded as a scenario when predicting PMI. These species represent three distinct waves of colonisation, as each feeds on carcasses of different degrees of decomposition, and suggests that the colonisation by larvae can occur as the carcass enters the correct decomposition stage, because the adults are already on the carcass when it enters the transition.

Coleoptera in South Africa colonise carrion in a distinct succession (Braack, 1981, 1987). The Silphidae are the first Coleoptera to utilise a carcass, feeding on the soft tissue and facultatively on the larvae of Diptera. After this *Dermestes* larvae feed on dried remains, and finally *Trox* larvae feed on the hair and dry skin remaining. Several other Coleoptera feed on the carcass, both directly and on other arthropods, but their presence is less predictable (Braack, 1986, 1987).

Reviews of the Coleoptera found on carrion in South Africa show great diversity at several levels. Prins (1984a, b) found ten families of Coleoptera and Braack (1986) nine. Braack (1986) also recorded 99 species of Coleoptera visiting carcasses. Larvae and pupae are used for development studies, as the adults are mobile and do not necessarily represent development completed on the carcass (Higley and Haskell, 2001). Adults can be used in cases where community analysis is used to predict PMI (Anderson, 2001), but this should be done with care, as adults can arrive before the carcass is at a stage to be utilised by their larvae (pers. obs.). The immature stages of Coleoptera are therefore of forensic use in most cases, but adults can also be informative.

Coleopteran development models offer an extended period where PMI is predictable, as they develop slower than Diptera, and often begin development later than Diptera. At present very little development data is available for Coleoptera, and the data which is available is often not statistically robust (Ikemoto and Takai, 2001), as very few temperatures are used (Richardson and Goff, 2001; Coombs 1979). If statistically robust data is generated it will be of value, provided pre-oviposition times are properly accounted for when predictions are made.

1.3 SPECIES STUDIED

Six species, from the families Silphidae and Dermestidae, were studied in this thesis. Three species of Silphidae occur in the wild in South Africa, all of which are indigenous. Of these, *Thanatophilus micans* is common throughout Africa, while *Thanatophilus mutilatus* (Castelnau, 1840) is endemic to South Africa and possibly Lesotho (Schawaller, 1987). The distribution of the third species, *Silpha punctulata* (Olivier, 1790), is not clear. Schawaller (1987) reports that Portevin (1926) claims the distribution to extend to East Africa, but did not examine any East African specimens for his review. South African museum collections contain no specimens from outside the fynbos region of South Africa (pers. obs.) and Prins (1984a) reported that he collected this species only where fynbos grew. Both *Thanatophilus* species are commonly collected on carcasses, but *Silpha punctulata* is collected much less often, as it is apparently a rarer species.

There are about 30 species of Dermestidae known from South Africa in four genera (Scholtz and Holm, 1985). This thesis focuses on species of *Dermestes*, as they are most common in forensic cases (e.g. Schroeder *et al.*, 2002). Three species of *Dermestes* occur in the wild in South Africa, all of which are introduced. *Dermestes maculatus* is the most common dermestid in the region, and is the only species regularly collected from carcasses. *Dermestes haemorrhoidalis* (Kuster, 1852) and *D. peruvianus* (Castelnau, 1840) are most often found in stored products, but *D. haemorrhoidalis* has been collected from carcasses in the Grahamstown region (pers. obs.), and *D. peruvianus* from White-browed Sparrow-weaver (*Plocepasser mahali*) nests near Cradock (S.M. Beck, pers. comm.).

South Africa has a rich necrophilic coleopteran community (Braack, 1987), but many species are not necrophagous (Braack, 1984). Non-necrophagous coleopterans, such as Scarabaeidae, Histeridae and Staphylinidae, were not considered for this study, as their larvae are not limited to a carcass to complete development. Species which take extremely long periods to develop, such as Trogidae, and species which only arrive on very old carcasses, such as the dermestid genera *Anthrenus* and *Attagenus* were also not considered, due to their limited forensic application. The groups chosen for this study contain closely related species and widespread species.

1.4 THESIS OUTLINE

To increase the forensic application of Coleopteran biology in South Africa, I undertook several experiments, which are presented in this thesis. I focussed on *T. micans*, for two reasons. The Silphidae develop faster than the Dermestidae, and thus predictions of their development are likely to be used more often than data from Dermestidae. As *T. micans* is the most common silphid in South Africa, it will be relevant in more situations.

Change in length over time was measured in *T. micans* larvae stored in ethanol, as larval length is often used as a surrogate for age. Several authors (Tantawi and Greenberg 1993; Adams and Hall, 2003; Amendt *et al.*, 2007) have noted that dipteran larvae change length when stored in ethanol, and so for coleopteran length data to be regarded in the same way as dipteran larvae, it is necessary to assess if the change is similar or different in the former.

A development model was created for *T. micans*. By better understanding the development of *T. micans*, this species can be used as a PMI predictor, either in addition to Diptera found on a carcass, or alone if Diptera are no longer present.

The two most common species of Silphidae and the two species of Dermestidae that are known to occur on carcasses in southern Africa were used in experiments to compare upper lethal temperature limits. These physiological limits may be used to assess distributions at a national level, or at a microhabitat level on carcasses.

Predictive geographic distribution models were developed for the six species. Built into the models are methods of assessing the affect of climatic variables, and these were used to test the importance of temperature in determining species distributions. These models provide a guide for assessing if the absence of a species on a carcass in a particular investigation is due to the decomposition stage of a carcass, or because it does not occur in a given region. In addition to this, whether a carcass has been moved can be determined if a species is found which should not occur in a given region. This is also true for stored product infestations, where the source of infestation can be assessed.

1.5 AIMS

Forensic entomology is still an underdeveloped science in South Africa (Williams and Villet, 2006), and Coleoptera as forensic indicators are neglected

throughout the world. This thesis presents a collection of data on several important aspects of the biology of forensically important Coleoptera in South Africa. By performing not only the base research required by forensic entomologists, but also following more recent research trends, South African forensic entomology can develop to the same level as other countries. With this in mind, particular studies were undertaken to meet these aims.

Adams and Hall (2003) performed studies on the change in length of samples stored in 70% ethanol, using Holarctic species. By performing similar experiments on Afrotropical species, South African forensic entomology can develop as a science, keeping up to date with research in other countries.

Braack (1981; 1986; 1987) presented data on coleopteran succession in South Africa, but to date no studies have presented data on development in forensically important Coleoptera in South Africa. The developmental model in this thesis presents African forensic entomologists with a new tool for predicting PMIs.

Braack (1981; 1986; 1987) also only presented data for a limited part of South Africa. The predictive geographical models generated for this study allow forensic entomology to be useful across a larger part of South Africa. By linking this information to thermal tolerances, the importance of temperature in determining species distribution can be assessed.

Chapter 2 - The effect of killing method on post-mortem change in length of larvae of *Thanatophilus micans* stored in 70% ethanol

2.1 SUMMARY

It has recently been recommended that insect larvae collected for forensic purposes should be killed using the same method as was used to create existing models of rate of development. Certain killing methods have been shown to be preferable because they cause less distortion of the specimens, but these are not always practicable in a particular case, and so a method of correcting for killing method is required. Larvae of all instars of *Thanatophilus micans* (Fabricius, 1794) (Coleoptera: Silphidae) were measured and then killed by emersion in ethanol, emersion in hot water or freezing. Samples were re-measured immediately after death, then stored in excess 70% ethanol and re-measured after one week and again after four weeks. The change in length was significantly different from zero in all samples ($t = -9.07022$, $p < 0.001$). An ANCOVA showed that instar, killing method and storage time all had a significant effect on the change in length. The results showed that *T. micans* larvae have a great potential for change in length during storage, but that the change is not predictable, as the magnitude and sign of the change are variable.

2.2 INTRODUCTION

Forensic entomology is a sufficiently new field that its techniques are still under development. Many techniques also require refinements to make them more accurate and precise. It has recently been recommended that insect samples should be killed using the same method as was used in the construction of the model that will be used to estimate the age of the insect larvae (Amendt *et al.*, 2007). In the construction of the model in Chapter Three of this thesis, live animals were measured, necessitating a method of correcting measurements of larval length depending on the killing method used in order to make the model as practically useful as possible. If a correction method can be developed, then the model would be applicable regardless of the killing method used in the field. While it is preferable to use developmental landmarks to estimate age, this is not always possible because larvae are often collected by non-experts or during autopsy. This results in dead samples being delivered to forensic entomologists, preventing them from rearing the larvae to the next landmark. Length is a widely used surrogate for age, despite the limitations of this method, particularly in young specimens (Dadour *et al.*, 2001; Gaudry *et al.*, 2001). Small specimens could be young or stunted, but length measurements do not take this into account (Gaudry *et al.*, 2001). Dadour *et al.* (2001) state that there is natural variation in insect length, resulting in sampling error in length measurement, and that larvae of the same species moult at different lengths, meaning that length does not trigger moulting, and therefore is not a direct substitute for age.

Certain killing methods are preferable because they result in less change in length than others (Amendt *et al.*, 2007). Several authors (Tantawi and Greenberg 1993; Adams and Hall, 2003; Amendt *et al.*, 2007) have noted that larvae killed in ethanol tend to change length more than those killed by other methods, but these studies refer to maggots, and no data are available on the change of length of beetle larvae. Beetle larvae are generally more sclerotised than maggots, which should mean that any change in length would be different from published dipteran patterns.

To validate some of the methods used in subsequent chapters of this thesis, this chapter therefore examines which killing method results in the least change in beetle larvae length when stored in 70% ethanol.

2.3 MATERIALS AND METHODS

Thirty larvae of each instar (1st, 2nd and 3rd) of *Thanatophilus micans* (Fabricius, 1974) were collected from a culture maintained at the Department of Zoology and Entomology at Rhodes University. All of the larvae were measured to the nearest 0.1mm using a measurement triangle (Villet 2006). Ten larvae of each instar were killed by immersing them in water at 90˚C for one minute (Amendt *et al.*, 2007), freezing at -20˚C for one hour (Amendt *et al.*, 2007), or immersing them in 70% ethanol for one minute. The larvae were then immediately re-measured and placed in excess 70% ethanol for storage. The stored larvae were re-measured after one and four weeks. These times were chosen to mimic actual cases, with one week representing a first laboratory analysis, and four weeks a reanalysis at the time of a trial going to court.

The difference between the live length and the three other measurements was then calculated, and converted to a percentage of live length. The arcsine-transformed data were analysed using a one-sample t-test and ANCOVA (with live length as the covariate) in Statistica 7.

2.4 RESULTS

Hot-water-killed individuals tended to curl tightly, making them difficult to measure, as straightening was required. The bodies in these animals were stiff, in strong contrast to the freeze-killed individuals, whose bodies remained loose and supple. The bodies of the ethanol-killed individuals were of intermediate stiffness. In addition to this, neither the ethanol-killed nor freeze-killed individuals curled.

Plots of the data show more variable change in larger animals, both in terms of total change and proportional change (Figure 2.1). There was also a general tendency for smaller and larger individuals to decrease in size more than individuals of intermediate length. While the data for storage time showed a high level of overlap, particularly between one and four weeks, it is apparent that there was an increase in length as storage time increased.

The ANCOVA results showed significant differences in the change in length for instar, killing method and storage time (Table 2.1, Figure 2.2). First instar larvae showed the most change, relative to initial length, while third instar showed the least. Larvae killed in hot water showed the least change, relative to initial length, and those

killed by freezing the most, while ethanol-killed larvae showed highly variable changes. The samples shrank initially, but generally increased in length as storage time increased, with the exception of the ethanol-killed larvae after four weeks.

In addition to this, significant interactions were present between Instar and storage time, and between killing method and storage time (Table 2.1). The interaction between instar and storage time is due to extension of second and third instar larvae over time, while first instar larvae remained the same length. (Newman-Keuls Test, Table 2.2). The interaction between killing method and storage time is due to the strong initial shrink in freeze-killed individuals, where other killing methods did not show this pattern as strongly. In addition to this, the extension of ethanol-killed and freeze-killed individuals over time resulted both being different from ethanol-killed individuals after zero weeks and the ethanol-killed individuals after four weeks being different from all hot-water-killed individuals, which did not change over time (Newman-Keuls Test, Table 2.3).

2.5 DISCUSSION

The results obtained show that larval length has a great potential for change after killing. This change cannot be accurately predicted because the magnitude and even the sign of the changes observed differed greatly in this experiment (Fig 1, 2). The wide range of observed changes shows that length of preserved larvae can not be considered to be an accurate indicator of their live length, and therefore of larval age. That larvae of intermediate length change less than those of more extreme lengths is also concerning because this shows a non-linear relationship between initial length and change in length. Corrections based on a linear relationship would result in underestimations of age for small and large individuals.

It has been reported that the killing method that results in the lowest change in length of maggots is emersion in hot water (above 80˚C) (Adams and Hall, 2003; Amendt *et al.*, 2007; Tantawi and Greenberg 1993). This study showed that this is not necessarily the case for beetle larvae. While the larvae changed very little over time after being killed in hot water, there was still an initial change from live length to the length immediately after death. Only the third instar larvae showed a mean length close to the initial mean after being killed, but there was a high degree of variation

around this mean. This stability in length is probably due to the muscles being fixed by the heat, in effect cooked.

Freezing has been proposed as an alternative to emersion in hot water (Amendt *et al* 2007). This study shows that freezing has a strong initial effect on larval length, which lessens over time. Animals that are frozen should therefore be measured after at least one week of storage. The initial shrinkage in frozen animals is probably a behavioural response. While hot water and ethanol are relatively quick killing methods, requiring at most one minute in *T. micans*, freezing requires up to an hour. In this time the larvae appear to contract, decreasing their surface area to volume ratio, which improves heat retention. Behavioural thermoregulation has previously been reported in many insects (Ono *et al.* 1995; Richards *et al.*, 2005).

Ethanol-killed individuals show the most erratic change, but the mean change of these individuals was less than that under the other two methods. If large samples of individuals are taken, then a mean length calculated after ethanol-killing is the best measure of live length in silphid larvae, in contrast to what has been found regarding maggots (Tantawi and Greenberg 1993; Adams and Hall, 2003). Amendt *et al.*, (2007) made a general recommendation about all insect larvae, but this study shows that beetle larvae should be treated differently to fly larvae.

The relative change at each instar shows a strong pattern, with first instar larvae shrinking the most, then second instar larvae shrinking less and third instar larvae showing a slight extension. Tantawi and Greenberg (1993) reported that maggots of the same length but different ages underwent different amounts of shrinkage, but these comparisons were made relative to lengths after death, not live length. It is likely that the pattern found in this study is also due to age difference, accounting for some of the variation within each group, as well as the difference between instars.

Due to the changes observed in all killing methods, it is recommended that beetle larvae be measured while still alive, whenever possible. The range in magnitude of length change makes it impossible to correct for length change, as confidence in corrected data would be too low for the data to be useful, even for large samples. In addition to this, the non-linear relationship between live length and length change would result in corrected lengths under-estimating the age of small and large larvae.

Table 2.1: ANCOVA results for the change over time in length of *T. micans* larvae of different instars following killing by various methods. Bold values are statistically significant ($\alpha = 0.05$).

	SS	d.f.	MS	F	p
Intercept	0.106	1	0.106	**19.469**	**0.000**
Live Length	0.185	1	0.185	**33.900**	**0.000**
Instar	0.333	2	0.166	**30.543**	**0.000**
Killing Method	0.078	2	0.039	**7.195**	**0.001**
Time	0.246	2	0.123	**22.584**	**0.000**
Instar * Killing Method	0.050	4	0.013	2.299	0.060
Instar * Time	0.080	4	0.020	**3.688**	**0.006**
Killing Method * Time	0.242	4	0.061	**11.107**	**0.000**
Instar * Killing Method * Time	0.044	8	0.005	1.003	0.435
Error	1.319	242	0.005		

Table 2.2: Newman-Keuls tests for the interaction between storage time and instar, after an ANCOVA of change in length over time in *Thanatophilus micans* larvae showed significant differences occurred. Bold values indicate significant differences.

Treatment	1st *0 Weeks	1st *1 Week	1st *4 Weeks	2nd *0 Weeks	2nd *1 Week	2nd *4 Weeks	3rd *0 Weeks	3rd *1 Week
1st *1 Week	0.882							
1st *4 Weeks	0.351	0.328						
2nd *0 Weeks	0.949	0.872	0.277					
2nd *1 Week	**0.000**	**0.000**	**0.004**	**0.000**				
2nd *4 Weeks	**0.000**	**0.000**	**0.011**	**0.000**	0.627			
3rd *0 Weeks	0.591	0.509	0.560	0.343	0.001	0.003		
3rd *1 Week	**0.001**	**0.002**	**0.038**	**0.002**	0.403	0.424	0.021	
3rd *4 Weeks	**0.000**	**0.000**	**0.000**	**0.000**	0.221	0.201	0.000	0.058

Table 2.3: Newman-Keuls results for the interaction between killing method and storage time, after an ANCOVA of length change over time in *Thanatophilus micans* larvae showed significant differences occurred. Bold values indicate significant differences.

Treatment	Cold*0Week	Cold*1Week	Cold*4Week	Etoh*0Week	Etoh*1Week	Etoh*4Week	HWK*0Week	HWK*1Week
Cold*1Week	**0.000**							
Cold*4Week	**0.000**	0.166						
Etoh*0Week	**0.000**	0.435	0.047					
Etoh*1Week	**0.000**	0.707	0.151	0.479				
Etoh*4Week	**0.000**	0.066	0.513	0.010	0.092			
HWK*0Week	**0.000**	0.705	0.069	0.988	0.645	**0.014**		
HWK*1Week	**0.000**	0.819	0.079	0.995	0.723	**0.015**	0.939	
HWK*4Week	**0.000**	0.829	0.063	0.993	0.708	**0.010**	0.964	0.854

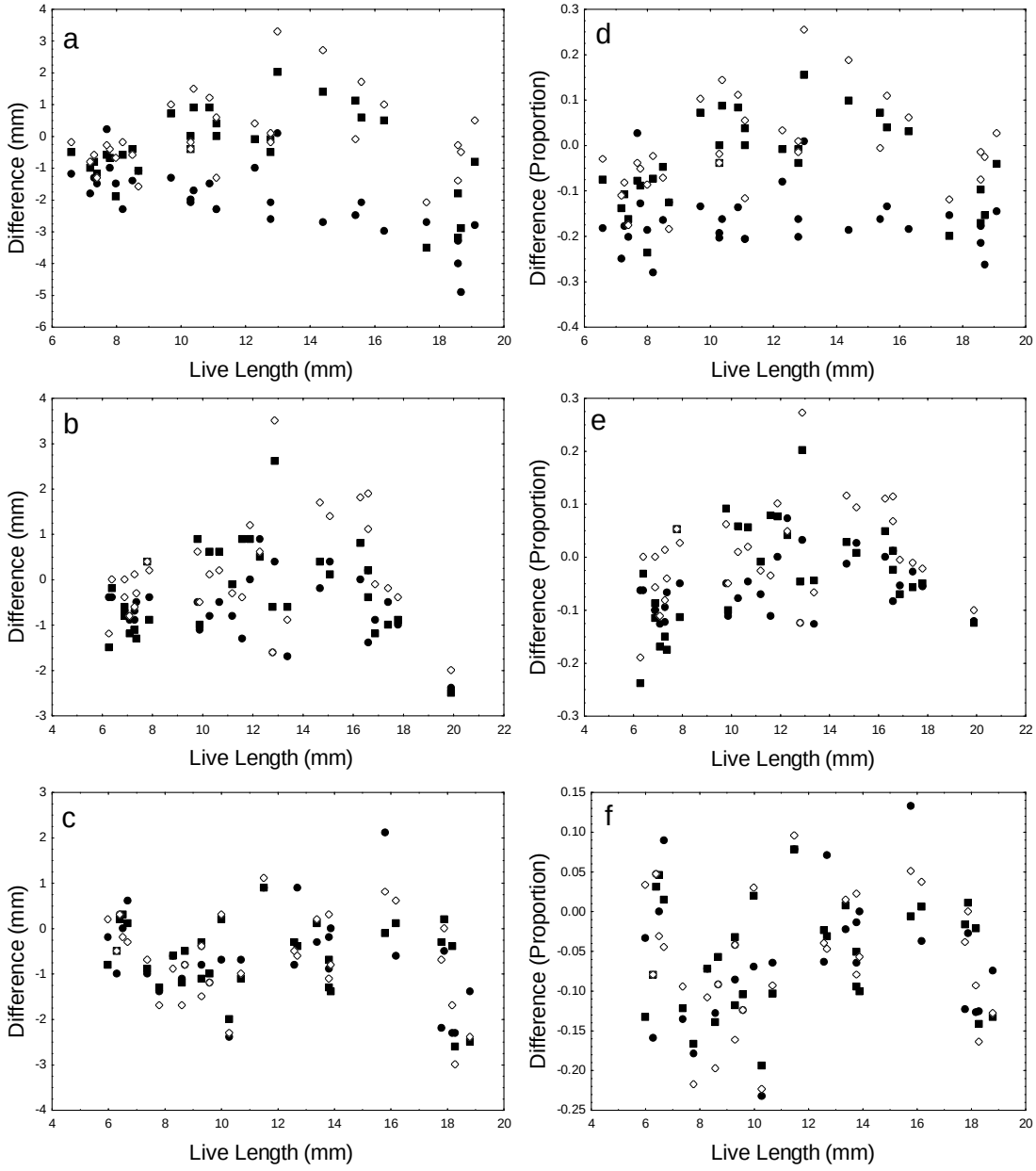

Figure 2.1: A diagram of the length change of *Thanatophilus micans* larvae, (a), (b) and (c) show the change in millimetres, while (d), (e) and (f) show the change as a proportion of live length. Freeze-killed larvae are shown in figures (a) and (d), ethanol -killed larvae in figures (b) and (e) and hot-water -killed larvae in figures (c) and (f). Measurements after 0 weeks are indicated by (●), 1 week by (■) and 4 weeks by (◊).

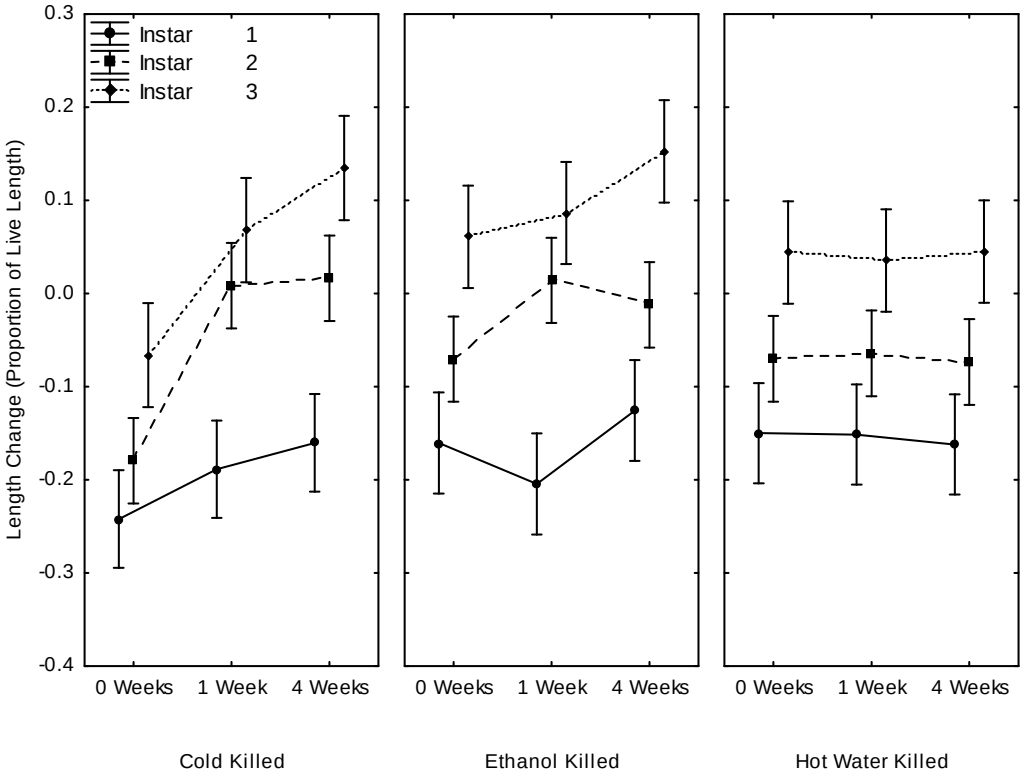

Figure 2.2: The mean change (± 95% confidences interval) in *Thanatophilus micans* larval length (proportion of live length), showing significant changes for (a) killing method, (b) storage time and (c) larval instar.

Chapter 3 - Development of *Thanatophilus micans* at constant temperatures

3.1 SUMMARY

Development of carrion feeding insects is commonly used as an indicator of post mortem interval. *Thanatophilus micans* is capable of finding corpses at least as quickly as most fly species and, as the most widespread of the Silphidae in Africa, offers a useful model for PMI once flies have matured to the point of leaving a corpse. Larvae were reared at 10 constant temperatures from 15˚C to 35˚C and their length measured every four, eight or twelve hours depending on the instar. Note was made of when the individuals progressed past developmental milestones. Length was compared between temperatures using ANCOVA and developmental constants were generated for each milestone using major axis regression. Length increased with increased rearing temperature, but decreased at extremely high temperatures. Development took longer at lower temperatures. Development times are presented. Only nine of the 59 data points generated for the calculation of developmental constants could not be used because they did not form a linear relationship or were from a malfunctioning incubator. Developmental threshold values differed between milestones, but not significantly. Development took longer than published development data on flies, but took less time than published data on Dermestidae. This model therefore covers an important time frame in estimating PMI which was not previously available to forensic entomologists.

3.2 INTRODUCTION

The development of carrion-breeding insects is widely accepted as a useful indicator of post mortem interval (PMI) (Smith, 1986; Catts & Goff, 1992; Byrd & Castner 2001). Most estimates of PMI are made using the development of maggots, as adult flies are capable of finding corpses within hours of death (Smith, 1986). For this reason most of the research into carrion-related insect development has focussed on maggots, and beetles have been neglected in this regard.

Beetles are also capable of locating corpses soon after death, and adult *Thanatophilus micans* (Silphidae) and *Dermestes maculatus* (Dermestidae) have been observed on animal carcasses within 24 hours of death (personal observation). While *T. micans* larvae have been observed on these carcasses within a few days of death, *D. maculatus* larvae do not normally appear until the carcass has dried substantially. Beetles typically develop more slowly than flies, and therefore offer an opportunity to predict PMI from developmental data after maggots have left the carcass. PMI estimates based on beetle development are reputedly less precise (days or weeks) than those based on maggot development (hours or days) (Catts, 1992), but once maggots are no longer present on a carcass they are still suitable.

Given that *T. micans* is the most common and widespread silphid species in Africa (Schawaller, 1987) and that it can locate carcasses soon after death, a development model for this species is of forensic value. This chapter presents such a model, developed from the growth rate of *T. micans* at ten constant temperatures, to illustrate the worth of coleopteran developmental data to forensic entomology in general.

3.3 MATERIALS AND METHODS

A culture of *T. micans* was established by collecting adults from various dead animals in the Grahamstown district, South Africa. Pairs of adults were placed at a range of temperatures (15˚C, 17˚C, 18˚C, 19˚C, 20˚C, 25˚C, 28.4˚C, 35˚C) with a small amount of food (Shallow water hake, *Merluccius capensis*) and oviposition substrate. Freshly hatched first instar larvae were separated into smaller containers and reared at ten constant temperatures (oviposition temperatures and 22.5˚C, 32.5˚C), ten individuals per temperature, until adults emerged from the pupation

substrate. The containers used were plastic Petri dishes, turned on their sides. These were narrow enough to allow monitoring of the pupal chamber, allowing pupation and eclosion times to be noted, but also providing enough space for the animals to move and feed in.

Developmental "milestones" were identified and the period between milestones noted by checking all individuals at regular intervals based on development stage (eggs 8-hourly; 1st instar 4-hourly; 2nd instar 8-hourly; 3rd instar, pupae and adults 12-hourly). Beetle larvae were measured using a measurement triangle (Villet, 2006) at the same intervals, giving a total of 3398 measurements. The milestones identified were: *Oviposition*; *Dig 1*, when 1st instar larvae dug out of the oviposition substrate; *Ecdysis 1*; *Ecdysis 2*; *Dig 2*, when 3rd instar larvae dug into the pupation substrate; *Pupation*; *Eclosion* and *Dig 3*, when the adults dug out of the pupation substrate.

Lengths were compared between temperatures using ANCOVA with age as a covariate. Only temperatures where development was completed were used in the analysis. Developmental constants for each milestone were estimated using major axis regression as described by Ikemoto and Takai (2001). Data from the 28.4˚C experiment were not used due to incubator malfunctions during the experiment, but are still presented.

3.4 RESULTS

Growth curves at all temperatures were sigmoidal (Fig 3.1a, b, c). Mortality ranged from 20% to 100%, with the highest mortality at extreme temperatures and lower mortality at intermediate temperatures (Figure 3.2). Larval length differed significantly between temperatures ($F = 355.34$, $p < 0.001$), showing five distinct groups (Figure 3.3). Larvae reared at low temperatures (15˚C and 17˚C) were significantly shorter than other temperatures and each other, while the higher temperatures formed three overlapping groups (Table 3.1), showing an increase in body length with increased temperature (Figure 3.3), even after taking age into account. This is seen in the isomorphen diagram, where developmental contours intersect several body length contours on the isomegalen diagram (Figure 3.4). Ecdysis 1 and Ecdysis 2 have the fewest intersections (three contours) and Dig 2 the most (four contours).

Egg development took 5.33 days (128 hours) at 17˚C to 1.66 days (40 hours) at 35˚C. Development from hatching to adult took between 63.16 days (1516 hours) at 15˚C and 19.33 days (464 hours) at 28.4˚C to be completed. Development was not completed above 28.4˚C, and at 28.4˚C mortality was high (Table 3.1). At 17˚C and 15˚C mortality was also high (Table 3.1).

Thermal summation models were constructed for each previously identified landmark, using as many co-linear temperatures as possible (Table 3.2, Figure 3.5). Egg development (Dig 1) was modelled separately from other landmarks, because oviposition did not occur at all temperatures. Development for all other landmarks therefore represents the time between digging to the surface (Dig 1) and the specific landmark, and not time from oviposition to the landmark. In order to obtain an accurate model, only data points which fall in the optimal temperature range should be used (Ikemoto and Takai 2001). The optimal temperature range has a near-linear relationship with the development rate, and points that deviate from this linear relationship should not be used in the generation of development models (Higley and Haskell 2001, Ikemoto and Takai 2001). Of the 59 data points presented, 50 were used in the seven models. Three points were rejected because they did not form a linear relationship and six points were rejected because they were from the 28.4˚C data set.

3.5 DISCUSSION

Larval development (oviposition to pupation) in *Thanatophilus micans* took 15.7 days at 25˚C. This is longer than several forensically important Diptera, including *Sarcophaga tibialis*, which takes 4.5 days at 25˚C (Villet *et al*. 2006), *Lucilia sericata*, which takes 6.6 days at 25˚C (Grassberger and Rieter 2001) and *Protophormia terraenovae*, which takes 9.6 days at 25˚C (Grassberger and Rieter 2002). Development also took longer than *Chrysomya albiceps*, which takes 22.5 days at 17.5˚C (Richards *et al*., In Press), while T. micans takes 29.5 days at 17˚C. Other forensically important Coleoptera take longer to develop than *T. micans*, including *Dermestes haemorrhoidalis*, which takes 40.6 days at 25˚C (Coombs 1979) and *Dermestes peruvianus*, which takes 52.5 days at 25˚C (Coombs 1979). *Dermestes maculatus* takes 59.2 days at 25˚C to complete development from egg to adult (Richardson and Goff 2001) while *T. micans* takes 22.2 days at 25˚C. The

development time of Silphidae therefore fills an important gap in our ability to predict PMI. In field observations around Grahamstown, *T. micans* larvae were found on carcasses within 4 days of death. This corresponds to the egg development times in this study; given that egg development took 3 days at 25˚C, which implies that like blowflies, *T. micans* can lay eggs on freshly dead carcasses and corpses.

Intermediate temperatures produced lower mortality, with the exception of 22.5˚C, where mortality was 50% (Table 3.1). This suggests a wide range of temperatures where *T. micans* survives well. The optimal temperature for survivorship was 20˚C, where mortality was lowest. Larval length was significantly shorter at 15˚C and 17˚C than all other temperatures, but the remaining temperatures formed three overlapping groups, showing a steady increase in larval length through the temperature range at which *T. micans* survives well. Larger female beetles tend to produce more offspring than smaller beetles (Honek 1993; Czypionka and Hill 2007), which means that while the optimal temperature for individual survivorship is 20˚C; population growth might well be higher at increased temperatures. Further work is needed to determine the optimal temperature for population growth (as opposed to individual growth) in *T. micans.*

The intersected contours on the overlaid isomorphen and isomegalen diagrams (Fig. 3.4) give good evidence that length is an ambiguous indicator of physiological age. This is especially true at low temperatures, where the ranges of body sizes at each developmental event were very large (Fig. 3.4). This has been noted in Diptera by previous authors (Dadour *et al.*, 2001; Gaudry *et al.*, 2001; Richards *et al.*, In Press), and the same is apparently true for Coleoptera.

Developmental threshold (D_0) values ranged by 1.6˚C between developmental landmarks, but the D_0 values for the life stages that occur above ground differed by only 0.4˚C, while the stages below ground differed by 1.0˚C (Table 3.2). These two groups also showed no overlap, with the epigeal stages having D_0 values 0.2˚C higher than the hypogeal groups. The observed differences are not significant, as the 95% confidence intervals overlap in all cases. It is likely that the observed differences are due to the subsurface environment buffering the organisms from temperature fluctuations that occur above ground.

The D_0 values are not unexpected, given the geographical distributions predicted in Chapter 5, but cannot be compared to other silphid species, as such data are not published. Richardson and Goff (2001) present some developmental data for

D. maculatus, from which a D_0 value for *D. maculatus* can be calculated. This value is however not statistically robust, as only three data points fall on the linear section of the graph. The D_0 value calculated from these data is 12.48˚C, almost 2.5˚C higher that the value for *T. micans*. Coombs (1979) presents data for both *D. haemorrhoidalis* and *D. peruvianus*, from which D_0 values can also be calculated, but these are also not statistically robust, with only four and three points on the linear section respectively. The D_0 value for *D. haemorrhoidalis* is 12.99˚C, which is almost 3˚C higher than *T. micans* and 12.32˚C for *D. peruvianus*, which is almost 2.4˚C higher. Given that the three D_0 values calculated for the *Dermestes* species are all not statistically robust, it is not wise to compare them too rigorously because they cover a relatively small range. The difference between the *Dermestes* species and *T. micans* is large enough to compare, and this pattern is consistent with that of the LT_{50}s calculated in previous chapters. Definitive data should be generated before definitive comparisons can be made.

While comparisons between many parameters of this model are possible, the most valuable aspect of this study is the fact that it provides a model for a time period where forensic entomologists were previously unequipped to make PMI estimates.

Table 3.1: Tukey HSD test showing significant differences after ANCOVA was performed on larval mature length data. Age (hours) was used as a covariate.

Temperature Treatment	15˚C	17˚C	18˚C	19˚C	20˚C	22.5˚C	25˚C
17.0˚C	**0.0001**						
18.0˚C	**0.0001**	**0.0152**					
19.0˚C	**0.0001**	**0.0003**	0.9378				
20.0˚C	**0.0001**	**0.0002**	0.7997	0.9999			
22.5˚C	**0.0001**	**0.0001**	0.1845	0.8201	0.9606		
25.0˚C	**0.0001**	**0.0001**	**0.0001**	**0.0038**	**0.0228**	0.4783	
28.4˚C	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0004**	0.2207

Table 3.2: Summary of development constants for the seven developmental milestones defined in the text.

Event	Temperature Range	R^2	Df	K		D_0 (SE)
				Degree Days (SE)	Degree Hours (SE)	
Dig 1	17.0˚C - 35.0˚C	0.9323	5	1.59 (0.25)	38.1 (5.9)	10.76 (1.43)
Ecdysis 1	15.0˚C - 35.0˚C	0.9828	7	0.82 (0.06)	19.7 (1.4)	11.31 (0.53)
Ecdysis 2	15.0˚C - 32.5˚C	0.9902	7	2.26 (0.14)	54.2 (3.3)	11.05 (0.45)
Dig 2	15.0˚C - 32.5˚C	0.9868	6	4.61 (0.49)	110.6 (11.7)	10.90 (0.78)
Pupation	15.0˚C - 25.0˚C	0.9806	6	7.62 (0.63)	182.9 (15.1)	10.30 (0.64)
Eclosion	15.0˚C - 25.0˚C	0.9880	6	12.08 (0.69)	289.8 (16.5)	9.73 (0.48)
Dig 3	15.0˚C - 25.0˚C	0.9921	6	13.31 (0.65)	319.5 (15.7)	10.00 (0.39)

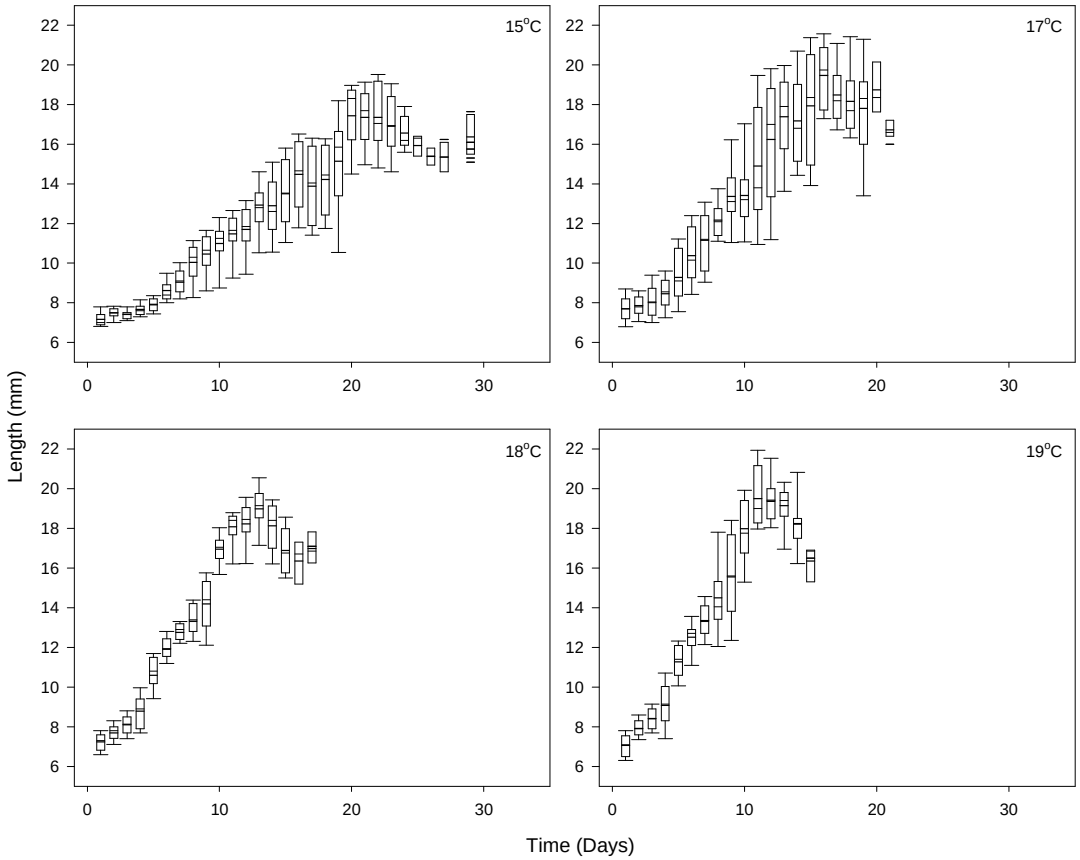

Figure 3.1a: Growth curves of *Thanatophilus micans* larvae at four constant temperatures, 15˚C, 17˚C, 18˚C and 19˚C. All curves are sigmoidal, showing slow initial growth, followed by an accelerated growth phase and tailing off as larval development is completed.

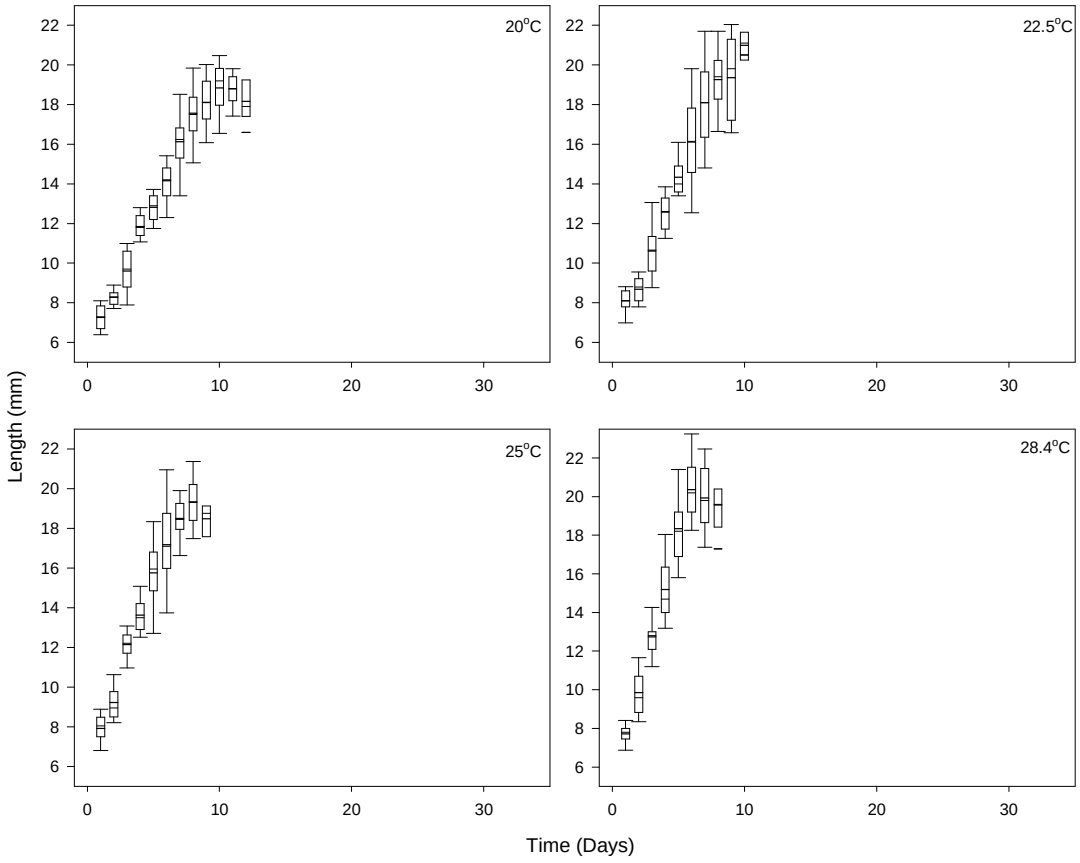

Figure 3.1b: Growth curves of *Thanatophilus micans* larvae at four constant temperatures, 20.0˚C, 22.5˚C, 25.0˚C and 28.4˚C. All curves are sigmoidal, showing slow initial growth, followed by an accelerated growth phase and tailing off as larval development is completed.

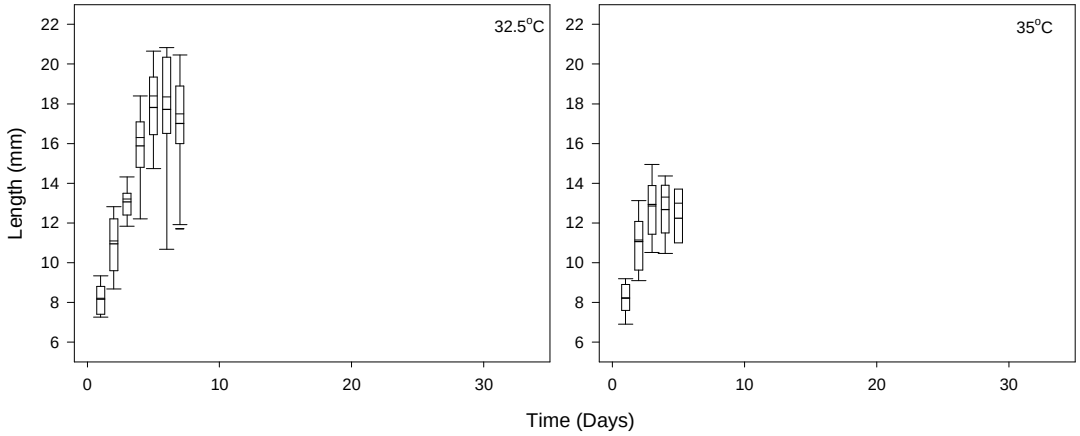

Figure 3.1c: Growth curves of *Thanatophilus micans* larvae at two constant temperatures, 32.5˚C and 35.0˚C. All curves are sigmoidal, showing slow initial growth, followed by an accelerated growth phase and tailing off as development nears completion. Data should be interpreted with care as larval development was not completed.

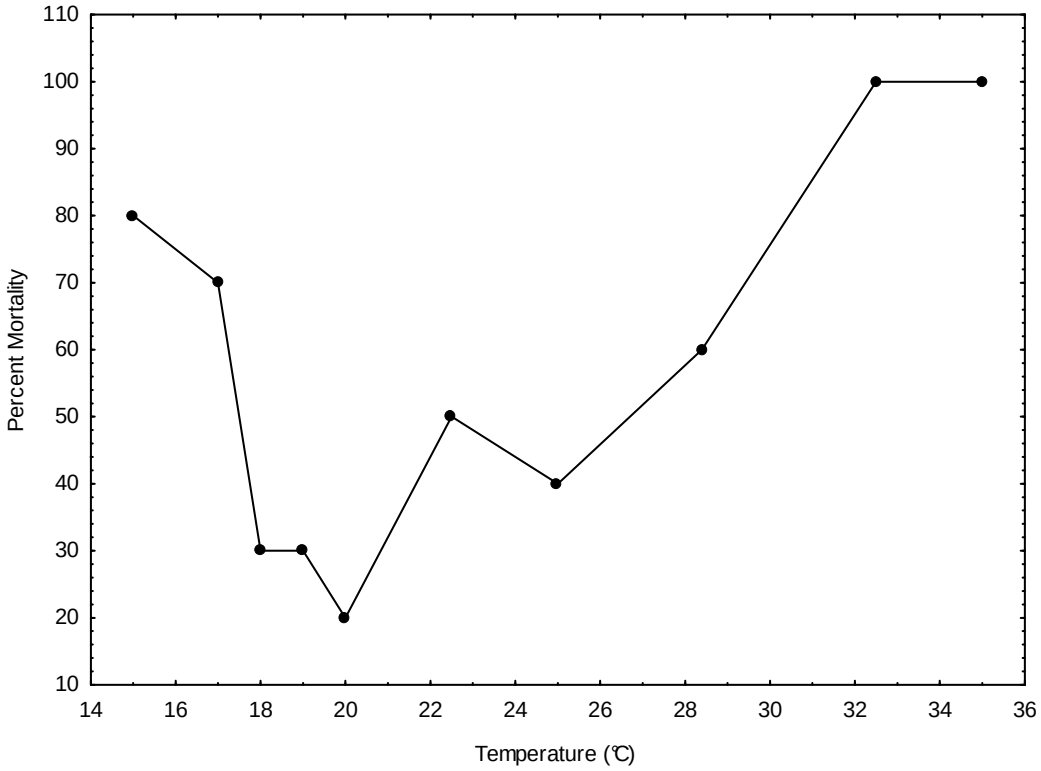

Figure 3.2: Mortality rate in *Thanatophilus micans* at ten constant temperatures.

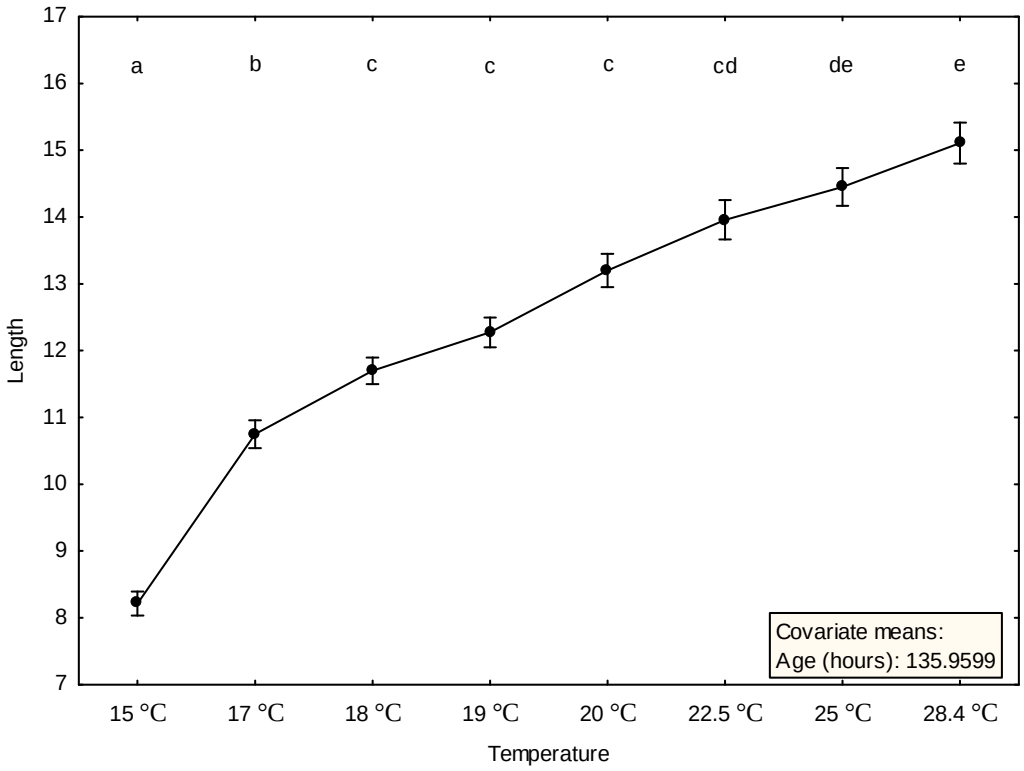

Figure 3.3: Means (± 95% CI) of body length of mature larvae of *T. micans* at different constant temperatures. Letters above the points indicate statistically significant differences (Table 1). Length at low temperatures (15˚C, 17˚C) was significantly shorter than all other temperatures, while higher temperatures formed overlapping groups, showing a more gradual increase in length.

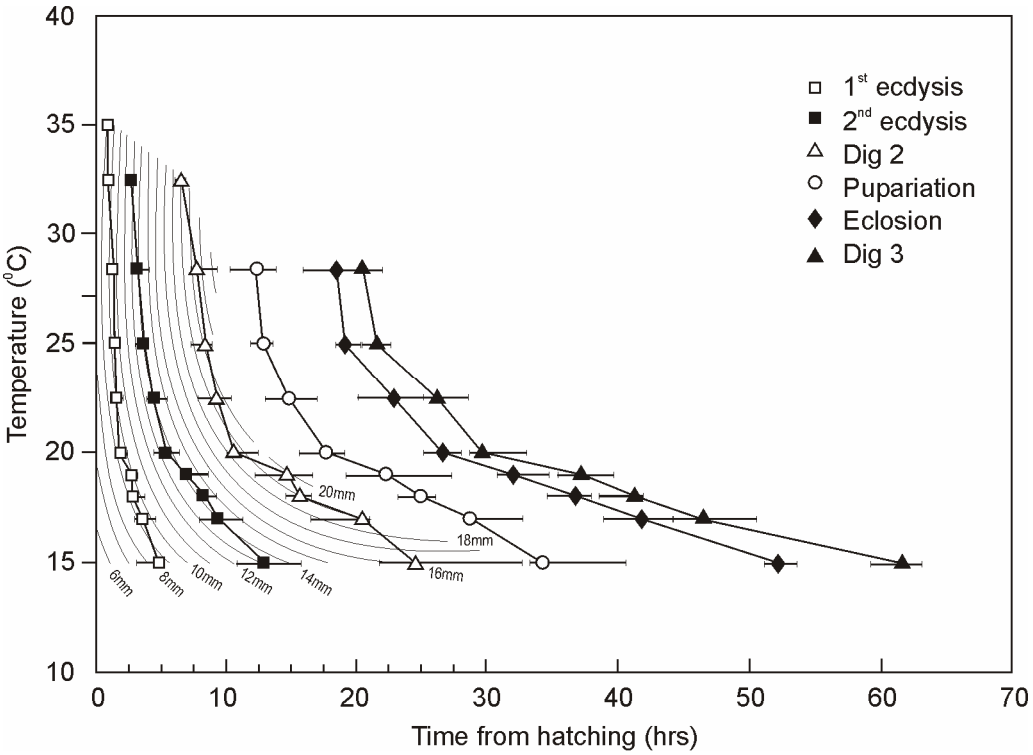

Figure 3.4: Overlaid isomorphen and isomegalen diagrams showing intersections of developmental and body length contours. The isomorphen diagram was constructed using the median times to each developmental event at each temperature; error bars denote range of time at given event and temperature. The isomegalen contours were constructed using spine interpolation.

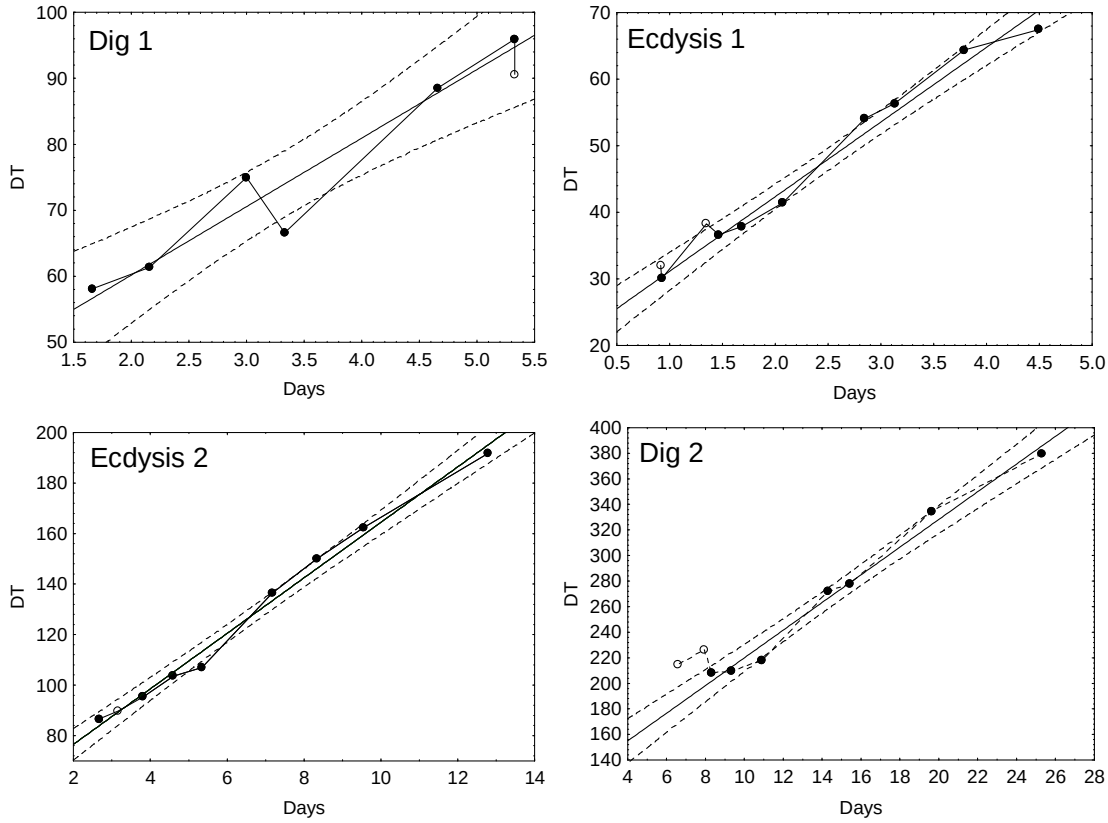

Figure 3.5a: Regression lines and 95% confidence intervals for four of the seven development milestones identified for *Thanatophilus micans*. Fifty of the 59 points generated were used in the analyses: closed symbols were used in the calculations and open symbols were excluded.

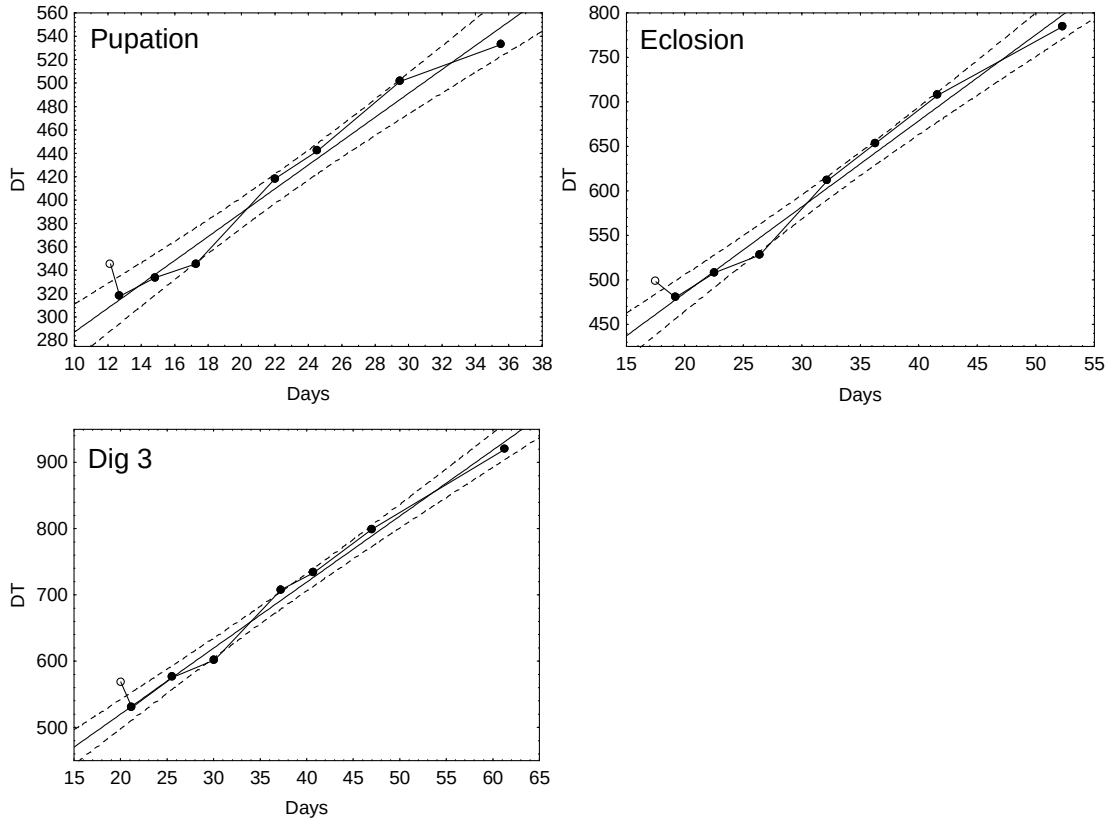

Figure 3.5b: Regression lines and 95% confidence intervals for three of the seven development milestones identified for *Thanatophilus micans*. Fifty of the 59 points generated were used in the analyses: closed symbols were used in the calculations and open symbols were excluded.

Chapter 4 - Temperature-induced mortality in four species of carcass beetle

4.1 SUMMARY

Environmental temperature affects ectothemic animals in many ways, including their biology and distribution. The community of arthropods present on a corpse can be influenced by both of these, and therefore is dependant on ambient temperature. The effect of temperature on four species of carcass beetle was tested by exposing individuals to a range of temperatures and measuring their mortality. Median upper lethal temperature thresholds were calculated and compared between species and life stages. *Dermestes* species were more tolerant than *Thanatophilus* species, and female *Thanatophilus* more than conspecific males. Older larvae were more tolerant than younger larvae, and *Thanatophilus* larvae were more tolerant that *Thanatophilus* adults. The higher thermal tolerance and D_0 values in the *Dermestes* species correspond to their known distributions, both global and local, as do the differences in biology between *Thanatophilus* and *Dermestes*. Temperature therefore directly influences carcass beetle distribution and biology.

4.2 INTRODUCTION

Ambient temperature can have strong effects on ectothermic animals in many ways. One of the more obvious effects is on the geographical distribution of animals, but their behaviour and physiology will also be influenced. This is demonstrated by the recent changes in insect distribution and phenology due to climate change (Menéndez, 2007). It is widely accepted that tropical animals cannot live in polar regions, and that polar species cannot live in tropical regions (Schmidt-Nielsen, 1997). On a continental scale, certain species are restricted to tropical areas and others to sub-tropical or temperate areas, depending on the continent. This pattern should follow through to a more localised spatial scale, be it at sub-continental or country level. The thermal tolerance of a species is therefore useful in the prediction of the distribution of the species.

Forensic scientists use community composition on corpses to predict PMI, based on the animals that are present on the corpse (Smith, 1986; Anderson, 2001). Corpses exposed to extreme conditions decompose differently from those in more normal conditions (Schroeder *et al.*, 2002). For this reason it is important to know which species are tolerant to high temperatures, to correct PMI estimates. These should be the animals which occur once the corpse has dried, as there will be less evaporative cooling on a dry corpse.

The community composition on a corpse also depends on what species are present in a particular area (Anderson, 2001). Geographic predictions in the following chapter of this thesis assist forensic scientists to assess whether the absence of a species is due to the decomposition stage of a corpse, or if it is due to the animal not occurring in the area where the corpse is found. Geographic models can be either correlative or mechanistic. Correlative models are easier to construct, as they link distribution to climatic variables based on known locality data. While mechanistic models are more difficult to construct, they are considered to be more reliable, as they link physiological data to climatic variables (Guisan and Zimmermann, 2000). The predictions in Chapter Five are made from correlative models, but the results in this chapter give mechanistic insights to these predictions. Maximum thermal tolerances for the species tested will show which species are more capable of withstanding high temperatures. These species should be more common in areas with high maximum temperatures. The models produced in Chapter Five should predict higher suitabilities

for the more tolerant species in warmer parts of the country, for example, the north-central and north-western interior. If the models produce the same pattern as the maximum thermal tolerances, then temperature can be considered to be an important variable. In addition to this, different tolerances to temperature can account for interspecific interactions at carrion. If certain species are more tolerant to high temperatures, they could out-compete less tolerant species and this should be considered when PMI is estimated from community analyses. Thermal tolerance will probably be higher in more widespread species, as they naturally occur at a greater range of temperatures.

This chapter investigates maximum thermal tolerance of four species of carcass beetle, with the aim of explaining geographical distributions generated in the following chapter and giving insight into their comparative biology.

4.3 MATERIALS AND METHODS

Adult male and female *T. micans*, *T. mutilatus*, *D. maculatus* and *D. haemorrhoidalis*; first, second and third instar larvae of *T. micans* and *T. mutilatus* and mature larvae of *D. maculatus* and *D. haemorrhoidalis* were obtained from cultures maintained at the Rhodes University Department of Zoology and Entomology. Because *Dermestes* species show flexibility in the number of instars that they will undergo before pupation (Coombs, 1979), large, mature larvae were chosen, rather than selecting a specific instar.

Ten animals were placed in Labcon 3104U incubators at temperatures of 35 ˚C, 37.5 ˚C, 40 ˚C, 42.5 ˚C, 45 ˚C, 47.5 ˚C, 50 ˚C and 52.5 ˚C (a total of 80 animals) and their mortality was measured after 30 minutes. Animals were considered dead if they did not move after 30 minutes at room temperature after the exposure period.

The data were then plotted in Sigmaplot 8.0 and fitted with a three-parameter logistic curve. Median lethal temperatures (LT_{50}) and their 95% confidence intervals were calculated to compare species and life stages.

4.4 RESULTS

All four species showed unique patterns, although a general pattern is evident for each genus tested (Figure 4.1). Adults of the *Dermestes* species were significantly more tolerant of high temperature than adults of the *Thanatophilus* species, with *D. maculatus* being more tolerant than *D. haemorrhoidalis*. The adult *Thanatophilus* species did not show a clear difference, but *T. micans* larvae were more tolerant than *T. mutilatus* larvae. While males and females of neither *Dermestes* species showed a significantly different LT_{50}, male *Thanatophilus* have a significantly lower tolerance than females in both species.

First instar larvae of both *Thanatophilus* species were significantly less heat-tolerant than second instar larvae. Mature larvae of *T. micans* were more tolerant than second instar, but this pattern was not present in *T. mutilatus*. The mature and second instar larvae of *T. mutilatus* were the only larvae that were not significantly different from each other. No patterns were observed in larvae of the *Dermestes* species.

4.5 DISCUSSION

It is not surprising that *Dermestes* species are more tolerant to high temperature than *Thanatophilus* species, as they feed on drier carcasses (Prins, 1984a, b). Wet carcasses will be cooler than dry carcasses due to evaporative cooling. *Dermestes* species have hairs on their bodies (larvae dorsally, adults dorsally and ventrally) which would also insulate the animals, making them more resistant to temporary heating, as would be experienced around midday in hot regions. The D_0 values calculated in Chapter Three follow this pattern, with the *T. micans* D_0 value lower than both *Dermestes* species. No development data are available for *T. mutilatus*.

The fact that *D. maculatus* is more tolerant than *D. haemorrhoidalis* in all life stages and both sexes is also expected, as it is a more widespread species because it is able to survive a wider range of temperatures. The D_0 values calculated in Chapter Three confirm this pattern. This explains the observed difference in global distribution, and the frequency of collections in South Africa (pers. obs., museum collections). *Dermestes maculatus* now has a cosmopolitan distribution (Azab *et al.*, 1972), but while its region of origin is unknown, it is generally accepted that it is an

introduced species in South Africa. *Dermestes haemorrhoidalis* is also an introduced species in South Africa, but has a more limited global distribution (Coombs, 1979).

A more interesting pattern is found in the *Thanatophilus* species, where the males and females show a strong difference in tolerance, but not between species. Schawaller (1981) stated that females of both *T. micans* and *T mutilatus* are larger than males. While size measurements were unfortunately not taken in this study, measurements of a few pinned *T. micans* individuals (male n = 4, female n = 5) showed that females were larger. The size difference is probably responsible for the difference in tolerance, as large individuals heat up more slowly and are therefore more tolerant to relatively brief exposure to extreme temperatures. Larger individuals are also more resistant to desiccation, as they contain more water. In a natural situation, heating to extreme temperatures is likely to be relatively brief, as extreme heat is likely to be present at midday.

Thanatophilus micans larvae showed much higher tolerance than *T. mutilatus*, which appears to be the reason for *T. micans* being more widespread, as the adults show very similar thermal tolerances in these species. *Thanatophilus micans* larvae are larger than *T. mutilatus* larvae, particularly in their third instar (Schawaller, 1981; pers. obs.), which explains the higher tolerance. The difference in tolerance is greatest in the third instar, which is also the instar where the greatest difference in size is observed (Schawaller, 1987; pers. obs.). The fact that *T. micans* is more widespread than *T. mutilatus* makes *T. micans* more forensically valuable in development studies, but *T. mutilatus* of more valuable in community composition studies (Anderson, 2001).

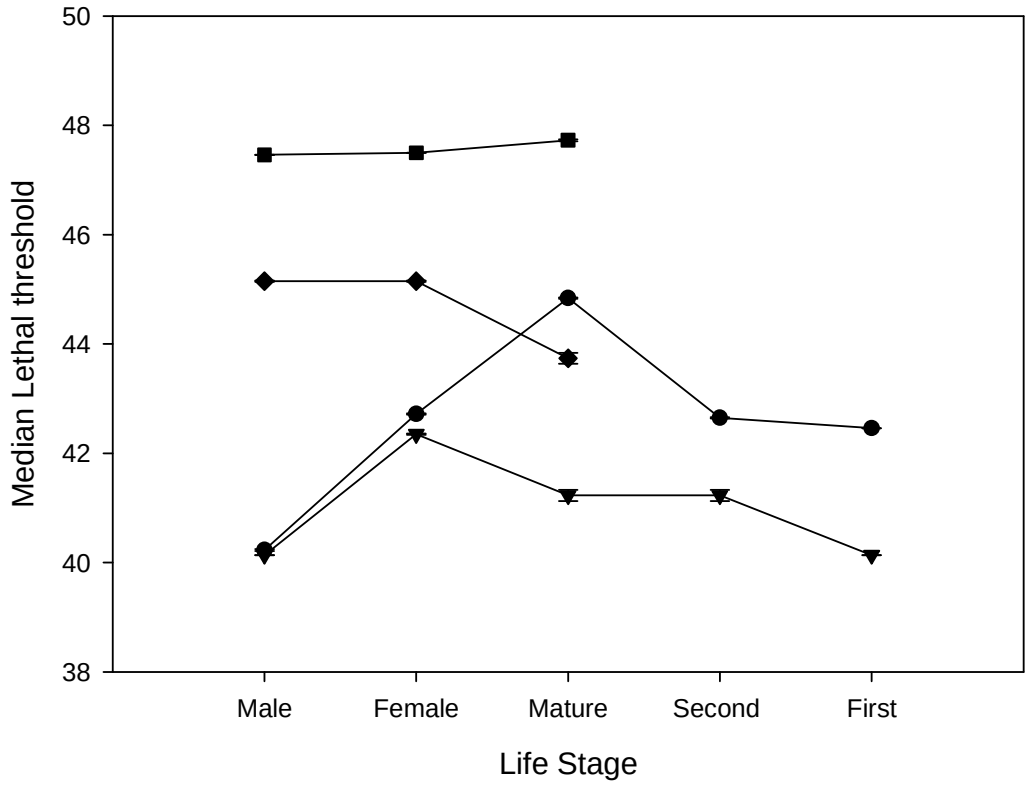

Figure 4.1: Median maximum thermal tolerance (with 95%CIs) of four species of carcass beetle: *Thanatophilus micans* (●), *Thanatophilus mutilatus* (▼), *Dermestes maculatus* (■) and *Dermestes haemorrhoidalis* (♦). Larvae were considered to be mature when they had reached their final instar (third for *Thanatophilus* spp, fifth to seventh for *Dermestes* spp.). *Thanatophilus micans* male and *T. mutilatus* male; *D. maculatus* male and female; *D. haemorrhoidalis* male and female and *T. mutilatus* second and third instar larvae are not significantly different form each other. All other points are significantly different from each other ($\alpha = 0.05$).

Chapter 5 - Bioclimatic niche modelling of carcass beetle distributions

5.1 SUMMARY

Estimates of Post Mortem Interval (PMI) can be based on the structure of the insect community present on a carcass. This can only be done accurately if species' distributions are known. Predictive geographic modelling of a species' bioclimatic niches offers an easy method of determining its distribution for such PMI estimates. Various models are available, and so the maximum entropy, principal component analysis and fuzzy logic models were chosen to assess distributions of six species using eleven climatic predictor variables. Predictor variables were generated by PCA from data presented by Shulze *et al.* (1997). Suitability plots for the three models and six species show differences in distribution for the species. Area Under Curve statistics showed good fit for the models. These distributions are valuable for forensic entomologists, as they give insight to PMI predictions, moved corpses and stored product cases. Jackknife analysis showed that temperature is not as important a predictor variable as previously thought. Moisture-related variables are more important in predicting carcass beetle distributions than temperature in most species.

5.2 INTRODUCTION

In many investigations of Post Mortem Interval (PMI) it is possible to use the arthropod community to estimate the PMI qualitatively (Smith, 1986; Catts & Haskell, 1990) or even probabilistically (Schoenley, 1992; Schoenley *et al.*, 1996). This can however be complicated by a lack of knowledge of the probability that particular arthropods are present in a given area. South African carcass beetles are poorly sampled, making it difficult to assess whether a species is absent from a corpse because of some characteristic of the corpse or because the species does not occur in a given geographical area.

A solution to this problem is predictive geographic modelling. Predictive modelling allows geographical distributions to be predicted from museum records and limited field sampling (Franklin, 1995; Guisan and Zimmermann, 2000), rather than exhaustive field sampling, which uses time, labour and money.

Predictive models can be grouped into two categories, namely correlative and mechanistic models (Robertson *et al.*, 2003). Correlative models use locality data to predict distributions by correlating known presence or absence data to maps of variables, often climatic variables, and predicting distributions based on the values of these variables (Beerling *et al*., 1995). Mechanistic models use physiological data like the thresholds described in Chapter 4 to predict distributions. Correlative models are more used more regularly, as they are easier to construct and require less complicated input than mechanistic models (Robertson *et al.*, 2003).

Another way of grouping predictive models is into presence-only and presence-absence models. Presence-only data are easier to collect than presence-absence data. The reason for this is that reliable absence data is relatively difficult to collect (Guisan and Thuiller, 2005). Absence data can only be used for the area examined when data were collected, while presence data can be scaled up (Guisan and Thuiller, 2005). If an organism is found anywhere in an area of one hectare, it is said to be present in that hectare, but for the same organism to be said to be absent from an area of one hectare, the entire area must be searched to confirm this. The presence data generated from this can be used for a larger area which incorporates the initial hectare, but to do the same for absence data, the entire larger area must be surveyed. A species can be present or absent in a given area for several reasons. Presence can be due to migration, introduction or natural occurrence, while absence data can be due to

season, migration, local extinctions, rarity, inaccessible terrain, or genuine non-occurrence. These factors show that presence data are more reliable than absence data, simply because fewer factors can affect their quality (Fielding and Bell, 1997).

Many different correlative models are available, including genetic algorithms (implemented in the GARP software package: Anderson *et al.*, 2003), maximum entropy analysis (implemented in the MAXENT software package: Phillips *et al.*, 2006), principal component analysis (PCA) (Robertson *et al.*, 2001), fuzzy logic (Robertson *et al.*, 2004) and various others, each with advantages and disadvantages (Guisan and Zimmermann, 2000). It is therefore desirable to use more than one model, particularly when there are few localities available to build a model, as is the case with many carcass beetles. The MAXENT package offers the advantage of providing information for each variable used in the analysis. Each variable is assessed to determine its contribution to the overall prediction and how much unique information it contains. When using physiologically relevant variables, this can give insight into the mechanisms that control species distribution, despite the fact that the model is correlative. For this reason MAXENT was chosen, as well as two other models, a PCA-based niche model (Robertson *et al.*, 2001) and a Fuzzy Envelope model based on fuzzy set theory (Robertson *et al.*, 2004).

Two types of errors are common when few data points are available, with certain models being more prone to each error type (Anderson *et al.*, 2003). Commission, or over-prediction, errors occur when the predicted distribution includes areas where the target organism is not present, while omission, or over-fitting, errors occur when the predicted distribution does not include all of the areas in which the target organism is present. By using more than one model for prediction, commission and omission can be assessed between models to give a better prediction of the distribution.

5.3 MATERIALS AND METHODS

5.3.1 Locality Data

The distributions of six species of carcass beetle from two families (Silphidae, Dermestidae) were modelled. The Silphidae used for the study were *S. punctulata* (25 localities), *T. micans* (164 localities) and *T. mutilatus* (105 localities). The

Dermestidae used were *D. maculatus* (151 localities), *D. peruvianus* (7 localities) and *D. haemorrhoidalis* (31 localities). Locality data were collected from the Albany Museum (Grahamstown), the National Collection of Insects (Pretoria), the Rhodes University Insect Collection (Grahamstown), the South African Museum (Cape Town) and the Transvaal Museum (Pretoria). From these collections, areas with low sampling effort were identified and field trips were undertaken to these areas to provide better sample coverage for the entire area being modelled and to ameliorate the inevitable bias in museum records. These samples were taken by examining carcasses found on the roadside while driving through the field trip area. Data collected in this way have a distinct temporal limit, and so can be used only for their value as presence data and not absence data. The absence data also assess only a single point or carcass, not a large area, and therefore cannot be scaled up.

5.3.2 Climatic Variables

Climate maps for South Africa, Lesotho and Swaziland were prepared by Schulze *et al.* (1997), at one-minute resolution. Shulze *et al.* (1997) present the maps in sets of twelve, representing monthly data for each climate variables. Each set of twelve maps was processed in a principal component analysis to achieve data compression, and the first two PCA axes were used in subsequent modelling (Robertson *et al.*, 2001; 2003; 2004). The first of these axes generally gives a measure of the magnitude of the climatic variable, while the second gives a measure of the variability, which is usually seasonal. The PCA therefore compressed twelve coverages into two for each climatic variable. This approach reduces computing time without discarding the seasonal information contained in monthly maps. The total number of frost days per year was not processed in a PCA, because it is not monthly data only one map was presented by Shulze *et al.* (1997). The predictor variables are listed in Table 4.1.

5.3.3 Statistical Assessment

The PCA and FE Models were implemented in MATLAB (Moller, 1980), and the results viewed in IDRISI32 (Eastman, 1999), while the Maximum Entropy Model was implemented and viewed using MAXENT version 2.2. Jackknife analysis was

performed in MAXENT 2.2, as it is built into the program. The jackknife results allow one to rank each variable in terms of importance. These rankings were averaged for all the species to give an average importance for each variable.

To assess the fit of the models, the Area Under Curve (AUC) for the Receiver Operator Characteristic (ROC) was calculated (Fielding and Bell, 1997). This was performed for the maximum entropy models in MAXENT version 2.2 and for the FE and PCA models using R version 2.6.0.

5.4 RESULTS

5.4.1 Distribution predictions

Dermestes haemorrhoidalis was collected at scattered locations around South Africa, along the southern and eastern coasts and in the "highveld" region in the interior (Figure 5.1). The areas with high predicted suitability in the FE model were the southern and eastern coast, and the highveld, but represented a larger region in these areas than the collected data covered (Figure 5.1a). It is unclear if this represents a lack of data collection or a commission error. *Dermestes haemorrhoidalis* is not a common species in South Africa and shows seasonal population peaks in winter, which are most likely the reasons for the lack of locality data. The PCA model predicted a wider distribution, with low suitability predicted only on the southern mountain ranges of South Africa and Lesotho, and the interior to the immediate north of the mountains (Figure 5.1b). The MAXENT model predicted low suitability over Lesotho and the south-western mountains, and the western Kalahari region (Figure 5.1c).

Collection records for *D. maculatus* covered a wider area, with records collected from everywhere except the south-western interior, Lesotho and areas in the north-western interior (Figure 5.2). The predicted suitability for the FE model closely matches the collection records, with low suitability only in Lesotho and the south-western interior (Figure 5.2a). The PCA model predicts high suitability throughout South Africa, with the exception of the mountain ranges from the south-west to north-east, including Lesotho (Figure 5.2b). The MAXENT model predicts similar suitabilities to the FE model, with only Lesotho and the south-western interior having low predicted suitability (Figure 5.2c).

The area covered by collection records for *D. pervianus* is very limited. Records from the coast are sparse, and records from the interior are found on the highveld and the north-eastern interior and north-central interior (Figure 5.3). The FE model predicts high suitability in a thin band along the south-eastern and eastern coasts, as well as the north-eastern interior and highveld (Figure 5.3a). This pattern is contrasted by the PCA model, which shows high suitability along all coastal areas and the immediate interior and the northern interior (Figure 5.3b). The only areas with low suitability are the south-western interior, the southern mountains and Lesotho. The MAXENT model predicts low suitability only in the south-western interior and the Lesotho highlands (Figure 5.3c).

Silpha punctulata records are limited to the "fynbos" region along the southern and western coasts (Figure 5.4). This pattern was also evident in the suitabilities predicted by the FE model, where only the fynbos region had high predicted suitability (Figure 5.4a). The PCA model predicts a wider suitable range, extending further east and north along the coasts and further into the interior (Figure 5.4b). The MAXENT model predicts high suitabilities over most of South Africa, with the exception of the north-central interior and the Lesotho highlands (Figure 5.4c).

Collection records for *T. micans* cover the entire coast and the north-eastern interior, with a few records from the central interior (Figure 5.5). The FE model predicts high suitability over the eastern coast and the north-eastern interior, but low suitability over the central and western interior, the south-western coast and the Lesotho highlands (Figure 5.5a). The PCA and MAXENT models predict a more limited area of low suitability, covering only the north-western and south-western interior and the Lesotho highlands (Figure 5.5b & 5.5c).

Thanatophilus mutilatus records are concentrated in the south-western fynbos region, but scattered records occur in the south-eastern interior and mountains, the eastern interior and eastern escarpment (Figure 5.6). The predicted suitability for the FE model is high in the fynbos region, and intermediate in the south-eastern interior and along the eastern escarpment, but low in the northern interior (Figure 5.6a). The PCA model predicts low suitability over the Lesotho highlands, the eastern coastal belt and the extreme north-central and north-eastern interior (Figure 5.6b). The MAXENT model predicts low suitability over the north-central interior and areas of the north-eastern interior, particularly the western section (Figure 5.6c).

5.4.2 Receiver Operator Characteristic (ROC)

An AUC value of 1.0 indicates a perfect fit, while an AUC value of 0.5 indicates a statistically random fit (Fielding and Bell, 1997). The Area Under Curve (AUC) for the Receiver Operator Characteristic (ROC) for all models was significantly (one-sample t-test, $t = 19.6$, $p = 0.000$) greater than 0.5 (Table 5.2), which indicates that all models are statistically not random. The average AUC value for all of the models implemented and species used was 0.855 (SD = 0.076). This means that 85.5% of positive predictions on the maps are true positives (Fielding and Bell, 1997). The average for each of the models implemented was similar (within one standard deviation) to this value (Table 5.2), indicating that none of the models performed better than the others. This was confirmed by a one-way ANOVA ($F = 0.327$; $p = 0.726$).

The AUC averages for the species used did show significant differences ($F = 7.722$; $p = 0.002$). These differences were caused by the higher AUC values for *S. punctulata* and *T. mutilatus*. The AUC values for *S. punctulata* (average: 0.978) were significantly higher than all species except *T. mutilatus* (average: 0.845), while all other species were not significantly different (Table 5.3).

Of the individual models and species, only the PCA model of *D. peruvianus* and the three models of *D. maculatus* had AUC values of less than 0.800, or 80% true positive prediction (Table 5.2).

5.4.3 Jackknife testing of MAXENT model

Each climatic variable was given a ranking for every species model from the jackknife analyses based on the amount of unique data it contained about the model prediction and how well the variable matched the total prediction (Table 5.4). In addition to this, the overall value of each variable to carcass beetle distribution prediction was assessed, by creating a median ranking for each variable for the amount of unique data and how well the variable prediction matched the total model prediction (Table 5.5). This ranking was generated by taking the median rank of each variable for each of the six species. These were combined to give a median jackknife ranking. The median ranking ranged from 3.0 to 9.5 (out of 11) for the model match and from 2 to 8.75 for the unique data. The importance of each variable was assessed from these rankings to give an importance ranking and these were combined to form

an average importance ranking. Based on the importance ranking the most important variables were Annual frost days (2.75), Rainfall factor one (3.25) and Rainfall factor two (3.75). The three least important variables were Maximum temperature factor one (10), Humidity factor two (9) and Evaporation factor one (8).

5.5 DISCUSSION

The models used appear to give good fits for the species used (Table 5.1), but certain issues must be taken into account. The FE model appears to over-fit predictions, with several areas where locality data are present having low predicted suitability (Figure 5.1a, 5.2a, 5.3a, 5.4a, 5.5a & 5.6a). By contrast both the PCA (Figure 5.1b, 5.2b, 5.3b, 5.4b, 5.5b & 5.6b) and MAXENT models (Figure 5.1c, 5.2c, 5.3c, 5.4c, 5.5c & 5.6c) over-predict the distributions. This emphasizes the fact that different models are needed to give a true reflection of the distributions of species (Guisan and Zimmermann, 2000). By looking at all three models simultaneously a more accurate predicted distribution can be obtained.

Despite the differences observed in the predicted suitability maps, the models all give good AUC values. A possible reason that the AUC values for *D. maculatus* were so low is the wide distribution of this species. This results in a higher percentage of the randomly generated absence data falling in areas where the species would normally be present, resulting in a lower AUC value. The fact that the absence data is randomly generated should be considered when interpreting the AUC values presented here. Randomly generated absence data will give lower AUC values, as false absences will be generated in most cases. The restricted range of *S. punctulata* results in the high AUC values obtained in a similar way, as only a small percentage of the generated absence data would fall in the range where the species is present. *Silpha punctulata* is the only species with a restricted range, and was the only species with a significantly higher AUC value. The intermediate ranges of the other species result in the intermediate AUC values.

The jackknife analysis showed no particular climatic variable that dictated the distribution of all carcass beetles (Table 5.4), but frost, humidity and rainfall are consistently important (Table 5.5). The importance of humidity and water balance in beetle development has been noted (Coombs, 1979) and the amount of water available to carcass beetles appears to be an important factor governing their distribution. It has

been assumed that temperature is an important factor governing insect distributions, but this does not appear to be the case. The temperature variables tested in the jackknife analysis were ranked 4 (5), 5 (5.75), 7.5 (6.25) and 11 (10) in the importance ranking, out of eleven. This shows that temperature is not a strong predictor of distribution and is only of average importance. In some species, temperature is important (*D. peruvianus*, all Silphidae, Table 5.4), and should therefore not be discounted when assessing distributions. This analysis shows that temperature is not as important in predicting coleopteran distributions as previously thought (Higley and Haskell 2001; Ames and Turner 2003; Menéndez, 2007) and that moisture-related variables, particularly rainfall, should be given greater consideration.

During personal collecting trips it was also noted that in dry conditions fewer species of carcass beetle were collected. The species that were collected were beetles that would usually arrive only once carcasses have dried significantly, such as *Dermestes maculatus*. It is possible that the rapid drying of carcasses prevents beetles that feed on wet carcasses from completing development. Under laboratory conditions desiccation was a major cause of mortality in *Thanatophilus* cultures (pers. obs.).

All of the models generated show areas where the target species are unlikely to occur. This can help in the elimination of species from carcass communities based on their distributions, which will allow more accurate PMI estimates (Anderson, 2001). In addition to this, bodies that have been moved can sometimes be identified based on the insect species found on them (Becker *et al.*, 2007), as certain species are absent from large parts of the country. Should a body be found with a species feeding on it which should not occur in the area, the body may well have been moved (Becker *et al.*, 2007). When stored products are found with an infestation of a species which should not occur in a given area, this may point to the infestation occurring prior to the consignment arriving at the point of discovery.

Table 5.1: Codes and descriptions for the eleven predictor variables used in the models. The first axis of the PCA can be interpreted as a measure of the general magnitude of the variable, while the second axis gives a measure of its variability, which is usually its seasonal pattern.

Predictor Variables	Description
Rainz1	First axis of PCA of monthly rainfall
Rainz2	Second axis of PCA of monthly rainfall
Mintz1	First axis of PCA of monthly minimum temperature
Mintz2	Second axis of PCA of monthly minimum temperature
Maxtz1	First axis of PCA of monthly maximum temperature
Maxtz2	Second axis of PCA of monthly maximum temperature
Humdz1	First axis of PCA of monthly humidity
Humdz2	Second axis of PCA of monthly humidity
frost	Annual total frost days
Evapz1	First axis of PCA of monthly evaporation
Evapz2	Second axis of PCA of monthly evaporation

Table 5.2: Area Under Curve (AUC) values of Receiver Operator Characteristic (ROC) curves for the three models implemented. A value of 0.5 indicates a random fit, and 1.0 a perfect fit.

Species	Fuzzy Envelope	Principal Component Analysis	Maximum Entropy	Average
Dermestes haemorrhoidalis	0.849	0.818	0.876	0.848
Dermestes maculatus	0.762	0.771	0.777	0.770
Dermestes peruvianus	0.878	0.704	0.829	0.804
Silpha punctulata	0.990	0.976	0.967	0.978
Thanatophilus micans	0.826	0.854	0.875	0.852
Thanatophilus mutilatus	0.825	0.892	0.915	0.845
Average	0.855	0.836	0.873	0.855

Table 5.3: Results of Tukey's HSD test on AUC values for the six species modelled. The test was performed after a significant result was obtained in a one-way ANOVA. Bold values indicate significant differences.

Species	*Dermestes haemorrhoidalis*	*Dermestes maculatus*	*Dermestes peruvianus*	*Silpha punctulata*	*Thanatophilus micans*
Dermestes maculatus	0.331				
Dermestes peruvianus	0.824	0.932			
Silpha punctulata	**0.035**	**0.001**	**0.005**		
Thanatophilus micans	1.000	0.285	0.769	**0.042**	
Thanatophilus mutilatus	0.959	0.098	0.382	0.133	0.978

Table 5.4: Unique and best-matched variables for each species according to jackknife analysis.

Species	Most Unique Data	Best-Matched Variable
Dermestes haemorrhoidalis	Rainfall Axis 1	Rainfall Axis 1
Dermestes maculatus	Rainfall Axis 1	Humidity Axis 1
Dermestes peruvianus	Minimum Temperature Axis 1	Minimum Temperature Axis 1
Silpha punctulata	Minimum Temperature Axis 2	Rainfall Axis 2
Thanatophilus micans	Rainfall Axis 1	Maximum Temperature Axis 2
Thanatophilus mutilatus	Evaporation Axis 1	Minimum Temperature Axis 2

Table 5.5: Variable importance according to jackknife analyses of the maximum entropy models.

Predictor Variables	Median rank within Jackknife models			Importance rank across models		
	Best Matched Variable	Most Unique Data	Average	Best Matched Variable	Most Unique Data	Average
frost	5	3.5	4.25	3.5	2	2.75
Rainz1	6.5	2	4.25	5.5	1	3.25
Evapz2	3	5.75	4.375	1.5	5	3.25
Mintz2	3	6.75	4.875	1.5	6	3.75
Rainz2	6.5	5.5	6	5.5	4	4.75
Evapz1	5	8	6.5	3.5	8.5	6
Mintz1	9.5	4.5	7	11	3	7
Humdz2	7	7.75	7.375	7.5	7	7.25
Maxtz2	8.5	8	8.25	9.5	8.5	9
Maxtz1	7	8.75	7.875	7.5	10.5	9
Humdz1	8.5	8.75	8.625	9.5	10.5	10

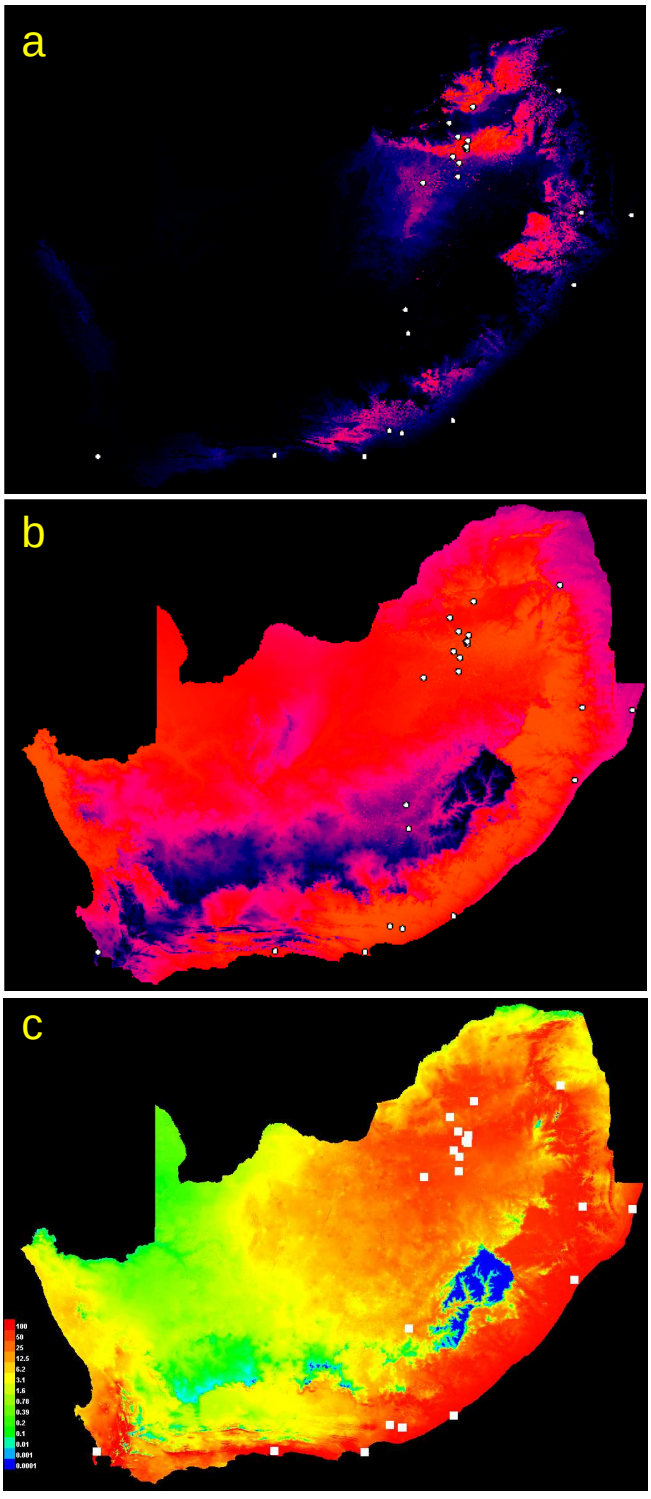

Figure 5.1: Suitability plots for *Dermestes haemorrhoidalis* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model.

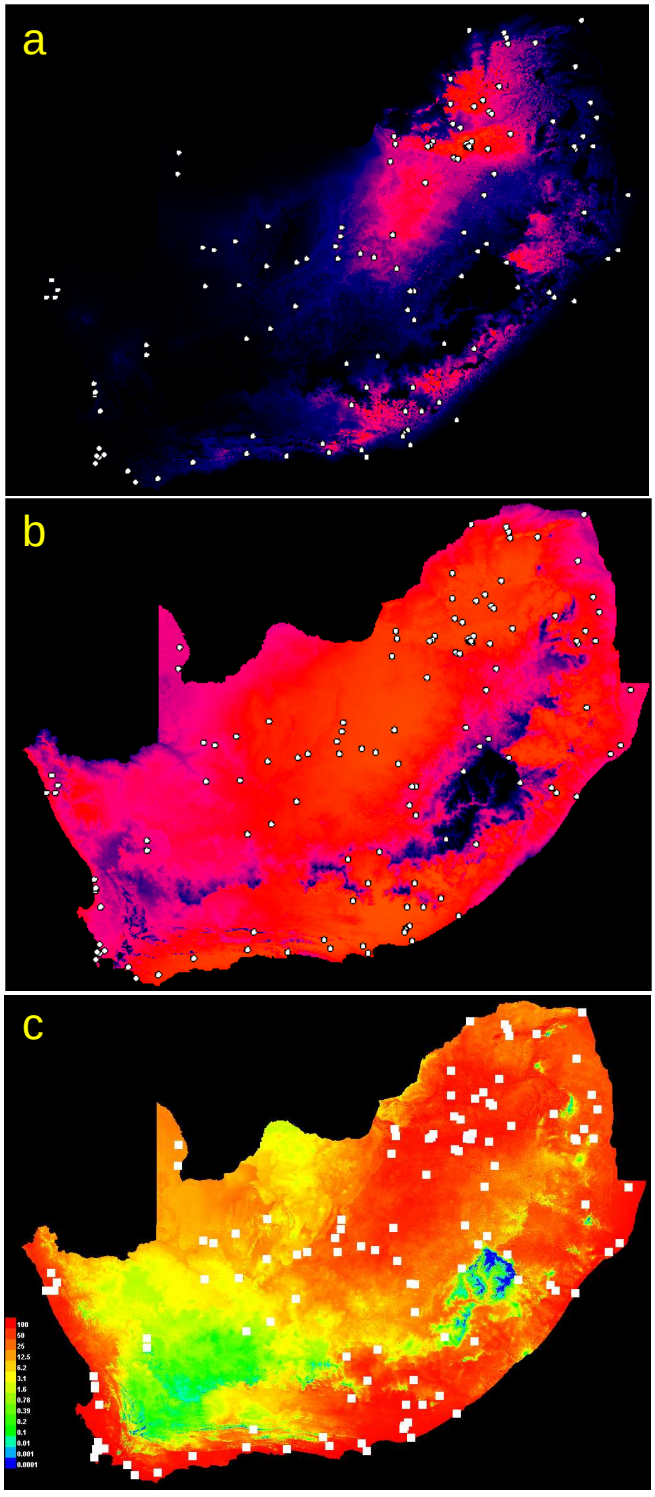

Figure 5.2: Suitability plots for *Dermestes maculatus* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model

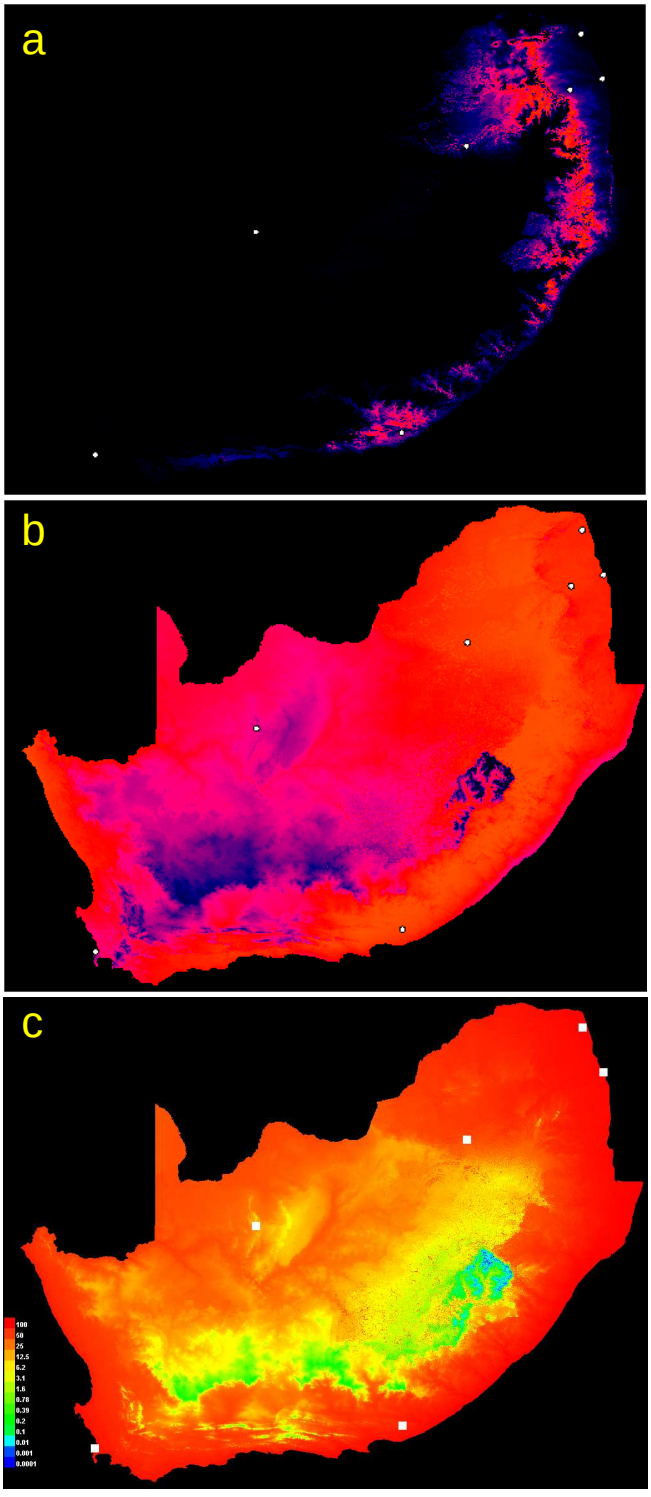

Figure 5.3: Suitability plots for *Dermestes peruvianus* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model.

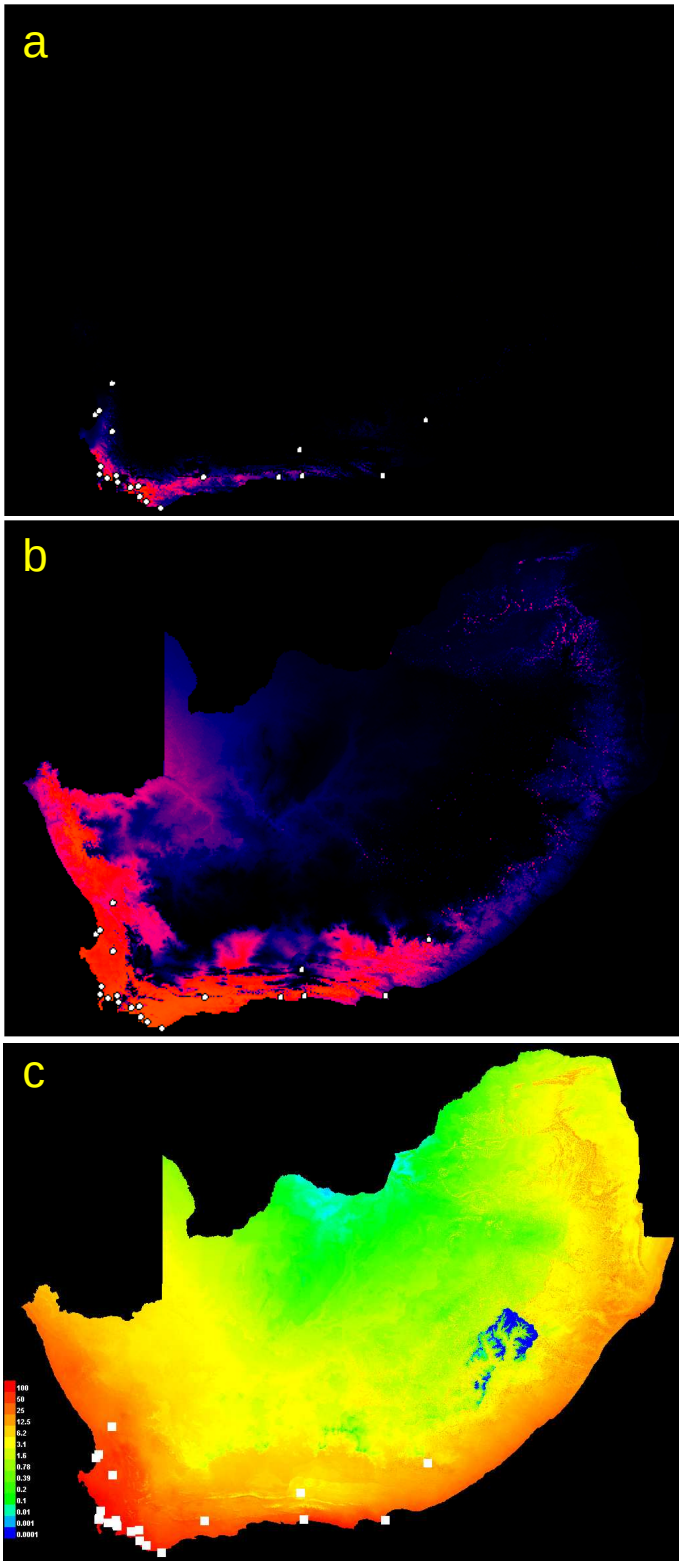

Figure 5.4: Suitability plots for *Silpha punctulata* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model.

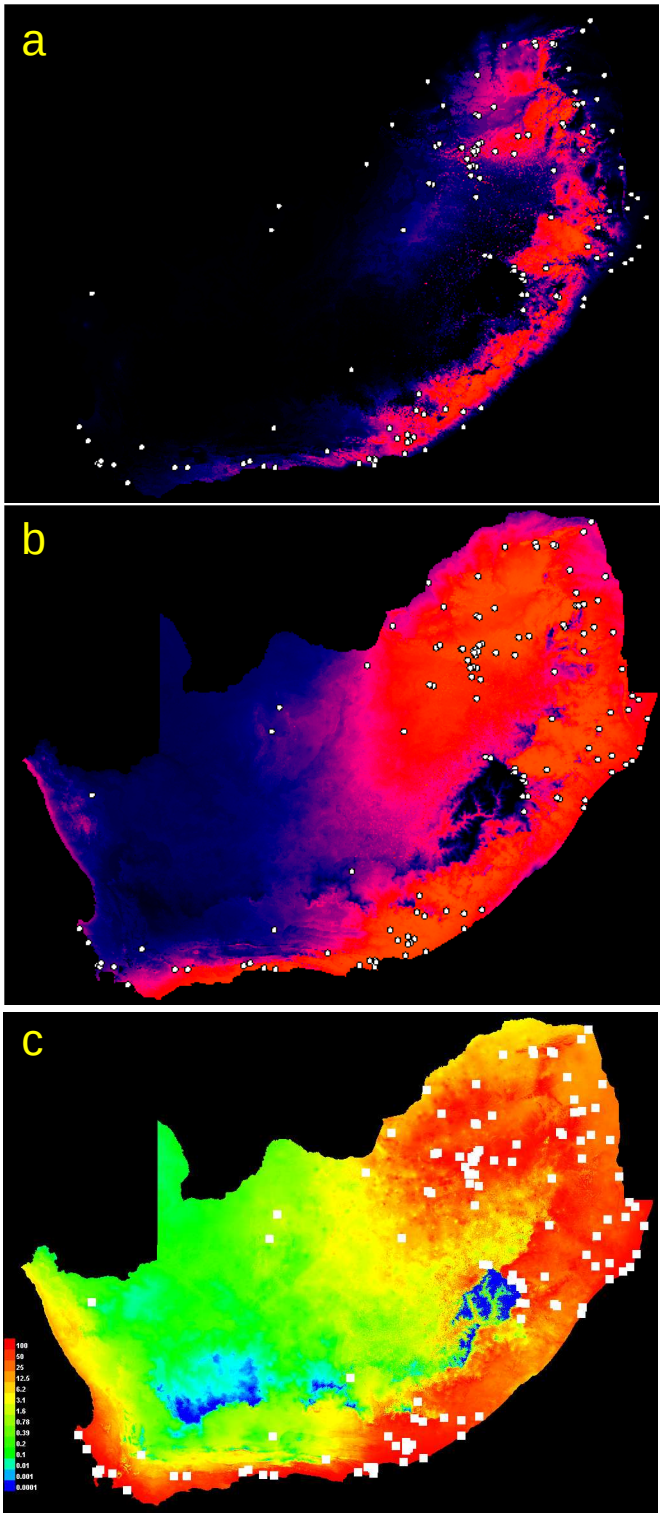

Figure 5.5: Suitability plots for *Thanatophilus micans* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model.

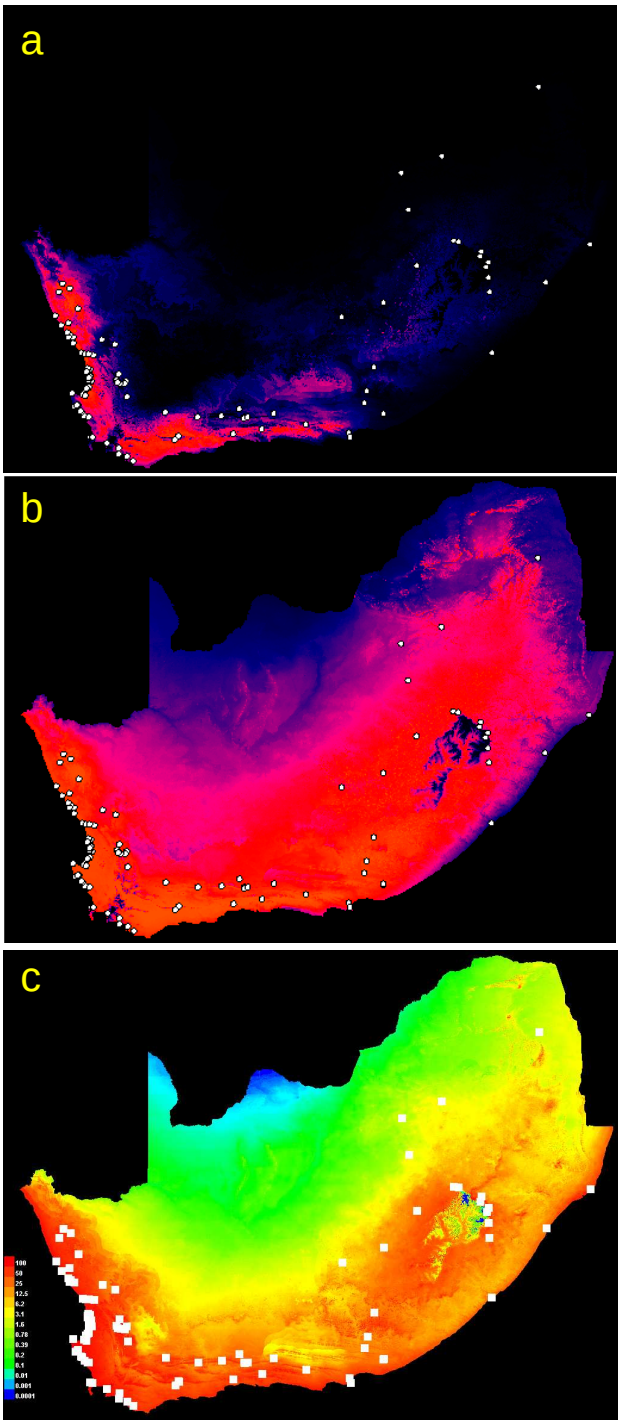

Figure 5.6: Suitability plots for *Thanatophilus mutilatus* for the three models implemented. Red indicates high suitability, blue low suitability and black no suitability. White dots indicate locality records. (a) Fuzzy Logic Model, (b) Principal Component Analysis Model and (c) Maximum Entropy Model.

Chapter 6 - Synthesis, Recommendations and Conclusions

While forensic entomology is still a developing science, it is reaching the level of maturity where standard protocols can be recommended (Amendt *et al.*, 2007). There is still room for these standards to be revised and in some cases tested. The results of this thesis represent not only new data for forensic entomologists, but also refinements on recommended standards (Amendt *et al.*, 2007).

6.1 SHRINKAGE AND THE USE OF DEVELOPMENT IN ESTIMATING PMI

Development rate of insect larvae is an accepted method of estimating PMI (Higley and Haskell, 2001). Most entomological forensic investigations have used dipteran development to estimate PMI, mainly due to the lack of information on coleopteran development. This thesis presents such information for the development of the most widespread silphid species in Africa (Chapter 3). The new data can be used to determine age either from body length, or preferably (Dadour *et al.*, 2001; Gaudry *et al.*, 2001; Richards *et al.*, In Press; Chapter 3) from developmental stage. If collections are made by police officials, it is not always possible to use development stage, as the larvae may have been preserved. When this is the case, larval length should be used, but it must be interpreted with care, based on the killing method and storage time (Chapter 2). The error in predictions made from larval length is not to be underestimated, and this should be considered when estimations are made. Length has inherent biological error or variability (Chapter 3) and the unpredictability of changes

during storage (Chapter 2) only exaggerate the imprecision due to this error. It is recommended that the measurement of length is not made with undue and misleading precision. It is also recommended that specimens should be killed by emersion in ethanol, and that the largest manageable sample of the largest larvae be taken and the mean length used for calculating PMI.

These results contrast with recommendations (Amendt *et al.*, 2007) based on Holarctic Diptera and show the danger of broad statements in a still developing science.

6.2 DEVELOPMENT OF BEETLES

Carcass beetles are clearly important in South African forensic entomology. Carcass communities are dominated by Coleoptera (Braack, 1981; 1986; 1987) and so are important in estimating PMI by community analysis (Anderson, 2001). *Thanatophilus micans* adults are capable of finding carcasses within 24 hours of death (pers. obs.) and *T. micans* larvae are observed within four days, corresponding to the egg development if the eggs were laid within 24 hours of death (Chapter 3). This shows that *T. micans* is valuable in predicting PMI by development, as their development is highly predictable and begins promptly after death (Chapter 3).

Length was again (Dadour *et al.*, 2001; Gaudry *et al.*, 2001) shown to be an inferior measure of age. Not only is length highly variable at any given age (Chapter 3) but length also changes during storage (Chapter 2). Developmental milestones are not only more predictable, but they do not change during storage.

These results show the value of *T. micans* to forensic investigations, and samples of *T. micans* should be analysed whenever they are collected from corpses as

a standard practice. Development models for other species of carcass beetle should be developed for use when *T. micans* is not collected on corpses.

6.3 THE RELEVANCE OF THRESHOLDS AND DISTRIBUTIONS

Temperature has always been thought to have a strong influence on insect distributions (Menéndez, 2007). In Chapter 5 this was shown to not regularly be the case. In some species temperature is important, but in other cases moisture-related variables, such as rainfall and humidity, are more important. For carcass beetles as a group, water-related variables are more important than temperature. The thresholds produced in Chapter 4 are still useful, as the distributions of *T. micans* and *T. mutilatus* (and other species) are still influenced by temperature. These are two closely related species, and their behaviour on carcasses is likely to be influenced by temperature. Interactions between these species are likely to be influenced by temperature, with microhabitat separation likely to be determined by temperature. The differences observed in their geographic distributions can be explained by the upper lethal temperatures determined in Chapter 4 and the jackknife analysis in Chapter 5. The jackknife analysis showed that for *T. micans* maximum temperature was most important and for *T. mutilatus* minimum temperature and the upper lethal limits showed that *T. micans* was more tolerant to high temperatures. The matching of these two results shows that temperature is important in predicting the distribution of *Thanatophilus*, but the effect of water availability should not be discounted, as it is still important in these species, and more important in the other species studied.

The implications of these findings for forensic entomology are, first, that while weather station data may be used to estimate the temperature at which insects develop on a carcass, allowances may need to be made for microhabitat specialization

and, second, that when applying community analysis to estimate a PMI, bioclimatic assessments of which species could potentially be present (or ought to be absent) need to take into account more than just temperature.

6.4 CONCLUSIONS

This thesis provides a developmental model for *T. micans* for the first time, and one of the first coleopteran developmental models ever to have direct forensic applicability. In addition, the interpretation of this data has been refined by assessing different practices in entomological evidence collection and making recommendations as to which perform best. These recommendations, as well as the recommendations for the use of distribution data at the regional and microhabitat level are internationally applicable.

Diptera will always be used more in forensic entomology than Coleoptera, but Coleoptera still show promise as forensic indicators, and more work in this field will confirm this. More developmental models are needed, for different species of carcass beetle. Lethal limits for a variety of climatic variables are needed to better understand species distributions, particularly for water-related variables.

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