Forensic Entomology:

The influence of the burning of a body on insect succession and calculation of the postmortem interval.

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The thesis is submitted in accordance with the requirements for the degree

Philosophae Doctor

in the Faculty of Natural and Agricultural Sciences,
Department of Zoology and Entomology,
at the University of the Free State

November 2009

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PREFACE

I declare that the thesis hereby submitted by me for the *Philosophae Doctor* degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further more cede copyright of the thesis in favour of the University of the Free State

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J.H. Kolver

ACKNOWLEDGEMENTS

ABSTRACT

Forensic entomology is the application of the study of insects and other arthropods which are associated with legal issues and certain suspected criminal events. Successional studies have been successfully applied in criminal cases to determine the postmortem interval (PMI). This research was done to establish the influence of burning on a body's decomposition, insect succession and calculation of the PMI.

Field trials were conducted during different seasons of successive years on the campus of the University of the Free State, Bloemfontein, South Africa. The experimental site where the field experiments were conducted, consists of 24 hectares of open grassveld with a few scattered trees. Four pig (*Sus scrofa*) carcasses were used during each trial, one carcass as control and three carcasses burnt with different volumes of LRP petrol to a CGS level 2 or 3 burn injury with varying degrees of charring. The carcasses were sampled daily for arthropod activity, carcass mass, decompositional stage and microclimate.

The control and SB (slightly burnt) carcasses decomposed at a similar rate during the warmer seasons. During the colder seasons, the SB carcass decomposed faster than the control carcass. The slowest decomposition occurred at the MB (medium burnt) and HB (heavily burnt) carcasses.

Burning had an effect on the colonisation of *Chrysomya chloropyga*, *Chrysomya marginalis* and *Chrysomya albiceps*. Oviposition occurred simultaneously at all carcasses (autumn, spring & during heavy rainfall in summer), at the burnt carcasses one day prior to the control carcass (spring & summer) and at the burnt carcasses three to five days prior to the control carcass (autumn & winter). An exception occurred during a single trial when oviposition occurred at the burnt carcasses five days after oviposition at the control carcass (winter).

During the warmer seasons oviposition time was shorter, resulting in maggots of similar age at all of the carcasses. During the colder seasons oviposition time was

extended, resulting in maggots of different ages and instars on the same carcass and between carcasses.

During all trials, except for the summer trail with heavy prolonged rainfall, only the control carcasses reached the Dry/Remains Stage. The burnt carcasses only reached the Advanced Decomposition Stage during the same timeframe.

Calliphoridae were the dominant Diptera during all trials. Dominant Diptera species, in numerical order, were *Chrysomya marginalis, Chrysomya albiceps* and *Chrysomya chloropyga.* Muscidae adults were recorded during all trials, but no maggots were observed or collected. Coleoptera were dominated by *Dermestes maculatus* (adults and larvae) and *Necrobia rufipes* (adults). Coleoptera dominance increased with the level of burning.

Differences in arthropod succession between the carcasses occurred due to the effect of burning on the time of oviposition. The PMI calculated for a burnt body would be one to five days shorter than the PMI for an unburnt body, depending on the extent of bloating of the burnt body, the season and ambient temperature. During warmer months the PMI of a burnt body and an unburnt body would essentially be the same due to simultaneous oviposition.

Laboratory trials revealed that feeding on burnt media caused *C. chloropyga* maggots to reach pupation one day faster than the control. No significant difference was found between the treatments for the development time from pupation until adult eclosion. No significant difference was found between the treatments for the mean total development time for *C. chloropyga*. A 10.6% higher survival until adulthood was found on the burnt media than the control. Morphometrics revealed a higher pupal mass for the control than the burnt media. No signifant difference was found for the adult dry mass and wing length for the control and the burnt media.

Key words: Forensic entomology; arthropod succession; oviposition; burning; postmortem interval; PMI; Diptera; Calliphoridae; Coleoptera

UITTREKSEL

Forensiese entomologie is die toepassing van die studie van insekte en ander arthropode geassosieerd met regsaspekte en sekere kriminele oortredings. Suksessiestudies word suksesvol toegepas in kriminele sake om die postmortem interval (PMI) te bereken. Hierdie navorsing is gedoen om die invloed van verbranding op die ontbinding van 'n liggaam, die inseksuksessie daarop en die invloed op die PMI berekening te bepaal.

Veldeksperimente is uitgevoer op die kampus van die Universiteit van die Vrystaat, Bloemfontein, Suid Afrika. Die proefterrein waar die veldeksperimente uitgevoer is, beslaan 24 hektaar grasveld met 'n paar bome. Vier varkkarkasse (*Sus scrofa*) is tydens elke eksperiment gebruik. Een karkas was die kontrole en die res is gebrand met verskillende volumes petrol (LRP) tot 'n CGS graad 2 of 3 brandwond met verskillende grade van verkoling. Arthropoodaktiwiteit, karkasmassa, ontbindingstadium en mikroklimaat is daagliks gemonitor op alle karkasse.

Tydens die warmer seisoene het die kontrole en SB (effens gebrande) karkasse teen 'n soortgelyke tempo ontbind. Die SB karkas het vinniger ontbind as die kontrole karkas tydens die kouer seisoene. Die stadigste ontbinding het by die MB (medium gebrande) en HB (swaar gebrande) karkasse voorgekom.

Verbranding het 'n effek gehad op die kolonisering van *Chrysomya chloropyga*, *Chrysomya marginalis* en *Chrysomya albiceps*. Eierlegging het gelyktydig by al die karkasse plaasgevind (herfs, lente & tydens swaar reenval gedurende somer), by die gebrande karkasse een dag voor die kontrole (lente & somer) en by die gebrande karkasse drie tot vyf dae voor die kontrole (herfs & winter). Een uitsondering het voorgekom tydens 'n winter eksperiment waar eierlegging by die gebrande karkasse plaasgevind het vyf dae na eierlegging by die kontrole karkas.

Die eierleggingstydperk was korter tydens die warmer as die kouer maande. Die gevolg hiervan was dat maaiers tydens die warmer maande almal ongeveer ewe oud was by al die karkasse, terwyl maaiers van verskillende ouderdomme en instars op dieselfde karkas en op verskillende karkasse voorgekom het.

Tydens alle eksperimente, behalwe tydens die somer eksperiment waar swaar aanhoudende reenval voorgekom het, het slegs die kontrole karkasse die Droë/Oorblyfsel Fase van ontbinding bereik, terwyl die gebrande karkasse slegs die Gevorderde Ontbindinstadium bereik het.

Die dominante Diptera familie was Calliphoridae. Die dominante spesies in numeriese volgorde, was *Chrysomya marginalis, Chrysomya albiceps* en *Chrysomya chloropyga.* Muscidae volwassenes is ook opgemerk, maar geen maaiers is opgemerk of versamel nie. Die dominante Coleoptera spesies was *Dermestes maculatus* (volwassenes en larwes) en *Necrobia rufipes* (volwassenes). Die dominansie van Coleoptera het verhoog met die verhoogde vlak van verbranding.

Verskille in arthropoodsuksessie tussen die karkasse het ontstaan as gevolg van die effek wat verbranding gehad het op die tyd van eierlegging. Die berekende PMI vir 'n gebrande liggaam sal een tot vyf dae korter wees as die PMI van 'n nie-gebrande liggaam, afhangend van die swelling van die gebrande liggaam, die seisoen en die omgewingstemperatuur. Tydens warmer maande sal die PMI van 'n gebrande liggaam en 'n nie-gebrande liggaam dieselfde wees as gevolg van gelyktydige eierlegging.

Laboratoriumeksperimente het getoon dat *C. chloropyga* maaiers wat op gebrande weefsel gevoed het, die papiestadium een dag voor die kontrole bereik het. Geen betekenisvolle verskille is gevind tussen die totale ontwikkelingstyd en die ontwikkelingstyd vanaf pupering tot volwassewording vir die verskillende behandelings nie. Die oorlewingsyfer van maaiers wat op gebrande weefsel gevoed het, was 10.6% hoër as by die kontrole.

Papies van maaiers wat op die kontrole gevoed het, het 'n groter massa gehad as die wat op die gebrande weefsel gevoed het. Geen betekenisvolle verskille is gevind tussen die volwasse droë massa en vlerklengtes van die verskillende behandelings nie.

Sleutelwoorde: Forensiese entomologie; arthropoodsuksessie; eierlegging; verbranding; postmortem interval; PMI; Diptera; Calliphoridae; Coleoptera

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Chapter 1: Introduction

"At the surface of the soil, exposed in the air, the hideous invasion is

possible; ay, it is the invariable rule.

For the melting down and remoulding of matter, man is no better, corpse for

corpse, than the lowest of the brutes.

Then the Fly exercises her rights and deals with us as she does with any

ordinary animal refuse.

Nature treats us with magnificent indifference in her great regenerating

factory: placed in her crucibles, animals and men, beggars and kings are

one and all alike.

There you have true equality, the only equality in this world of ours: equality

in the presence of the maggot."

- Jean Henri Fabre (18th century French entomologist)

Generally speaking, the term "forensic entomology" is the application of the knowledge of insect and other arthropod biology to legal investigations and certain suspected criminal events. These insects and arthropods are useful for uncovering information to an investigation (Keh, 1985; Erzinçlioğlu, 1989; Catts & Goff, 1992).

Lord & Stevenson (1986) identified three categories of forensic entomology, namely urban, stored-product, and medicolegal forensic entomology.

Urban forensic entomology involves civil law suits and litigations where arthropods are considered to be pests or a nuisance in the human environment, such as in homes and gardens. The misuse of pesticides and the rise of pests from livestock or similar facilities are also included in this category (Catts & Goff, 1992; Hall, 2001).

Stored-product forensic entomology generally deals with arthropod infestation or contamination of a wide range of commercial products. Some examples include beetles or their remains in candy bars, spiders in toilet tissue and fly eggs and maggots in fast food. Consumers have also planted insect evidence inside products in an attempt to defraud a restaurant or store. In such cases a forensic entomologist is required to resolve the issue. As with urban forensic entomology, stored-product forensic entomology usually involves litigation (Catts & Goff, 1992; Hall, 2001).

Medicolegal forensic entomology is the most popularized aspect of the science and also the focus of this study. It involves the use of arthropods in cases or events regarding felonies. These felonies are usually violent crimes such as murder, suicide and rape, but also include other violations such as physical and drug abuse. A more accurate name for this category is medicocriminal forensic entomology (Catts & Goff 1992). According to Hall (2001), it may also involve cases of unexplained sudden death (e.g. bee stings) or the cause of traffic accidents (e.g. bees or wasps inside a vehicle causing the driver to lose control).

One of the main functions of a forensic entomologist is to provide information on the time of death (Anderson & Van Laerhoven, 1996; Introna, Campobasso, & Di Fazio, 1998). This is achieved by studying the arrival sequence of arthropods on a decomposing body, combined with the developmental rates of the offspring of these arthropods (Lord & Burger, 1983).

The time that has elapsed since death to discovery of the body, is known as the postmortem interval or PMI. The PMI that is calculated by a forensic entomologist is the time that has elapsed since the body was exposed to insects. In some cases this PMI could differ from the actual time elapsed since death due to concealment of the body prior to exposure (Rodriguez & Bass, 1985; McKeown, 1991; Goff, 1992).

Smith (1986) made the important observation that it was always the insects that were being aged. This may or may not relate to the postmortem period, especially in cases where the insects were prevented from colonising the body immediately after death, due to some kind of physical barrier. Considerable skill is therefore required for the technique to be successfully applied.

The fundamental building blocks of the correct application of forensic entomology are four-fold. Firstly, insects species involved need to be accurately identified. Secondly, detailed knowledge of the life histories of these insects is required. Thirdly, the forensic entomologist must understand the association and interaction of these insect with the decomposing body. Lastly, the forensic entomologist must expect the unexpected, since no two cases are ever 100% identical due to differences in insects involved, geographic location and microclimate.

In cases where death has occurred shortly before discovery, medical parameters can be used to determine time of death. This becomes increasingly difficult as time passes. After an extended time (72 hours or more), forensic entomology is usually the most accurate and often the only method for determining the time of death (Anderson & Van Laerhoven, 1996). PMI calculation is most often used in cases of homicide, suicide, accidental death or unattended death due to natural causes (Smith, 1986; Catts & Haskell, 1990; Introna, *et al.*, 1998).

The knowledge base of the research scientist / forensic entomologist forms the basis of accurate PMI determination. This knowledge base includes a number of known facts, as well as assumptions concerning the biology, ecology and behaviour of specific insects (Dadour, 2000). This proves to be difficult, since the police and justice service demand an accurate PMI (Dadour, 2000; Dadour, Cook, Fissioli, & Bailey, 2001). Correct collection, preservation and rearing of entomological specimens are of paramount importance in the accurate determination of a PMI (Lord & Burger, 1983).

PMI estimation involves the setting of the maximum and minimum probable time interval between death and discovery of the body. The maximum limit is determined by the species of insects present and the weather conditions that allow these species to be active. The biology and composition of species can be used to provide an approximate estimate of the earliest time of exposure. The minimum limit is determined by estimating the age of developing immature insects at the time of discovery (Figure 1.1). The developmental rate of these immature insects is influenced by environmental variables such as temperature, humidity, rainfall and soil pH (Mann, Bass, & Meadows, 1990).

The relationship between the age of immatures and the PMI is determined from baseline studies with rates adjusted by interpolation to include the influence of climate, season, weather and location (Catts, 1992).

Body decomposition, faunal evidence analysis and environmental influences need to be considered when determining the PMI (Figure 1.1) (Hall, 1990).

Resources bound up within plant and animal remains return to the ecological system through decomposition. All around us, animals continuously die, yet the evidence is rarely seen by the untrained eye. This phenomenon is the result of a large variety of organisms that facilitate the decomposition of animals and their efficacy (Turner, 1991).
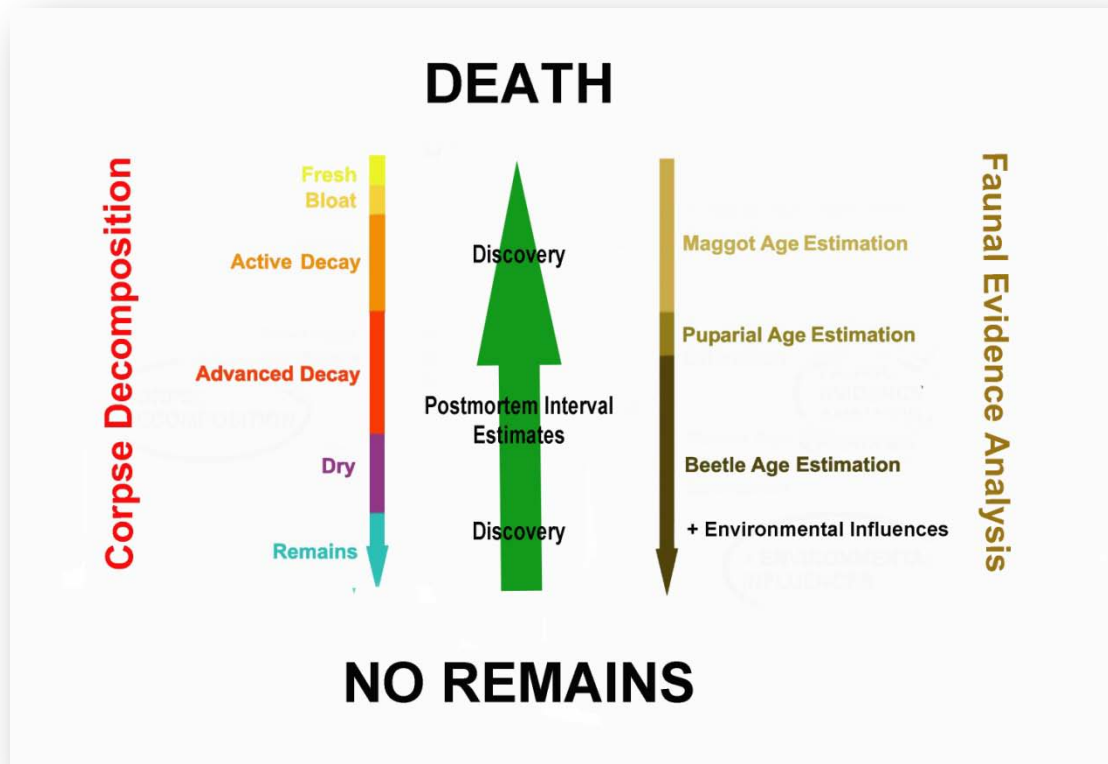
Figure 1.1. A generalized death and decomposition scenario relating to the calculation of postmortem interval (PMI) using the analysis of faunal evidence (modified from Catts & Haskell, 1990).

The decomposers are one of the most significant of all trophic assemblages, due to their numbers and the amount of material involved. The efficient and release and recycling of material during decomposition is of considerable importance (Putman, 1983). The law of entropy finally prevails at the end of life (Micozzi, 1986).

A varied and distinct community of organisms utilise dead animal matter, or carrion as a temporary and changing food source. The main component of this community are arthropods, especially insects which are the primary elements involved in the decomposition process (Richards & Goff, 1997).

Decomposing bodies go through a series of identifiable stages of decomposition. These stages are overlapping and are definitely not discrete. According to Turner (1991), the basic pattern of decomposition is influenced by three interrelated factors, namely climate, situation and access by insects.

Essentially, immediate postmortem change may be viewed as a competition between decomposition and desiccation. Decomposition consists of decay and putrefaction. External, as well as internal factors largely determine the outcome of this competition. External factors consist of temperature, humidity, and sunlight. Internal factors consist of surface area-to-volume ratio and body temperature (Micozzi, 1986).

The decomposition process is influenced by several variables. These variables include temperature, the effect of maggot-generated heat, humidity or aridity, rainfall, soil pH, and trauma to the body. Other variables include access to the body by insects, burial and depth, carnivore and rodent activity, size and weight of the body, the surface the body is placed on, clothing and embalming (Mann, *et al.*, 1990; Catts, 1992; Turner & Howard, 1992).

Insects are often the first arrivals at a death scene and they arrive in a predictable sequence called succession (Catts & Goff, 1992; Anderson & VanLaerhoven 1996). The concept of using insect succession for forensic purposes was first used by the French entomologist Pierre Mégnin in 1894 (Goff, 1991).

Blow flies will often oviposit on the body within the first few hours following death (Catts 1992), although the occurrence of strong winds, cold weather and concealment of the body in an enclosed space can delay the flies in reaching the body to oviposit. Therefore the first appearance of blow fly eggs on a body may not occur immediately after death. The result is that the first oviposition on a body indicates the minimum PMI estimation (Charabidze, Bourel, Hedouin, & Gosset, 2009).

A decomposing body, regardless of it being human or animal, undergoes a series of biological, chemical and physical changes as it decomposes from the fresh to the skeletal state. Different stages of this decomposition process are attractive to different species of insects (Catts & Goff 1992).

The decomposing body can therefore be seen as a temporary, rapidly changing resource that supports a large, dynamic arthropod community. When the colonising sequence of arthropods is known, an analysis of the arthropod fauna on a decomposing body can be used to determine the PMI (Anderson & VanLaerhoven 1996).

The primary decomposers are Diptera (flies). The adults utilise decomposing tissues as a protein source and oviposition substrate. The maggots utilise decomposing tissues as a food source. From a forensic viewpoint, the most important Diptera are in the Calliphoridae (blow flies) and Sarcophagidae (flesh flies) families. These flies are able to locate decomposing bodies over great distances. They can travel up to 20km per day, but their movement is usually within a neighbourhood, especially in urban environments. These flies could probably even cover greater distances in rural environments (Greenberg, 1991). The blow flies and flesh flies are closely related and exhibit diverse feeding strategies. Within the necrophagous fly guild, food resource partitioning occurs with different species utilising different parts or aspects of the food source. This partitioning of the food resource is driven and maintained by interspecific competition (Denno & Cothran, 1976). Severe competition for food resources occur between blow fly maggots (Fuller, 1934). Almost every aspect of their biology suggests intense competition for the rapid location and consumption of decomposing bodies (Fuller, 1934; Putman, 1983).

Some authors have suggested that the coexistence of competing species occurs to facilitate a stable equilibrium by means of specialisation on different parts of the resource. Others have suggested that competing species coexist due to mechanisms like disturbance, which reduce rates of competitive displacement (Kneidel, 1984).

Blow flies complete every stage of their life cycle on the decomposing body (Putman 1983). Therefore they are invaluable as forensic indicators and detailed knowledge of their biology is essential in PMI estimations. Diptera undergo holometabolous metamorphosis and the adults are morphologically different from the maggots. The maggots are vermiform larvae which have three larval instars before pupation. The pupae are of the coarctate type (Figure 1.2).
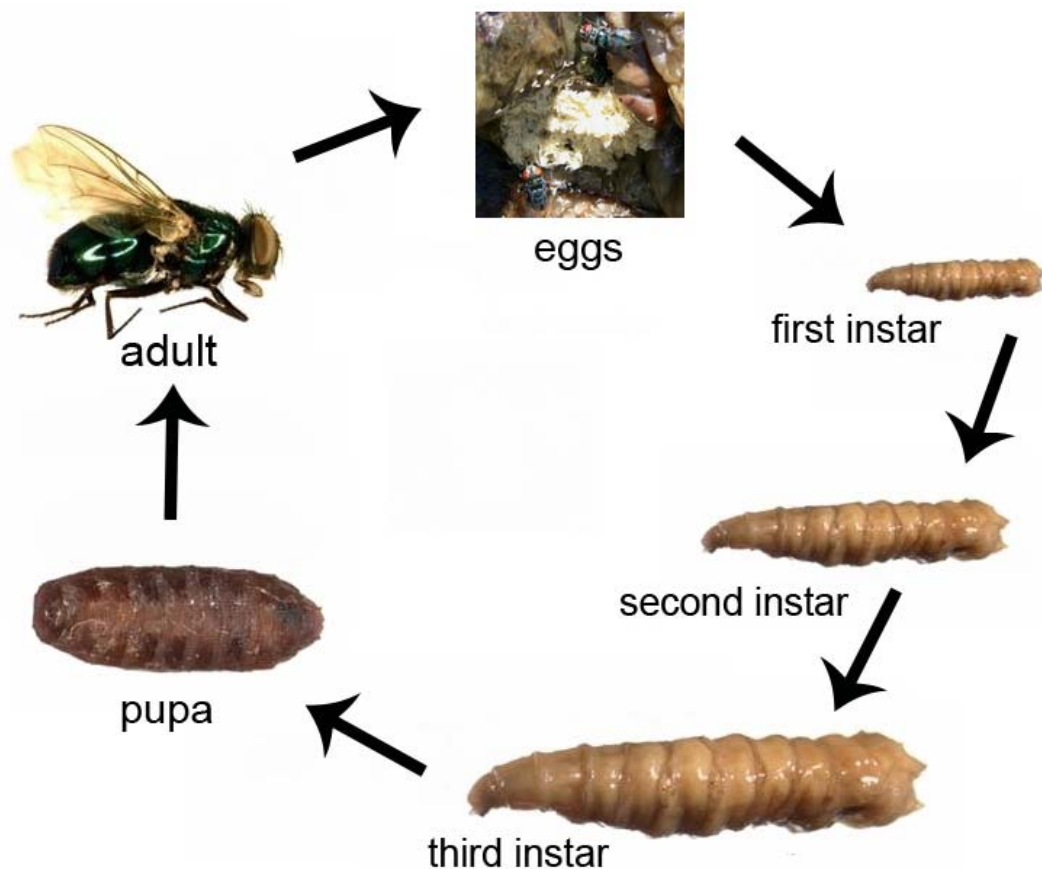
Figure 1.2 *The blow fly life cycle.*
(Composed from Cleveland Museum of Natural History, 2006 & own photograph).

The primary blow flies are the first arrivals at a decomposing body. In South Africa, these primary blow flies are *Lucilia* species, *Chrysomya marginalis* (Wiedemann) and *Chrysomya chloropyga* (Wiedemann).

Secondary blow flies arrive at the decomposing body after the primary blow flies had already oviposited and their maggots had begun feeding. This usually occurs after approximately two days in summer during Active Decomposition in the so-called "wet creamy-soupy phase". The secondary blow flies are represented by a single species in South Africa, *viz. Chrysomya albiceps* (Wiedemann).

The primary and secondary blow flies are found on the decomposing body during the wetter stages of decomposition. The tertiary "blow flies" are also called flesh flies and they occur later on the carcass alongside the beetles during the drier stages of decomposition.

In South Africa, the tertiary "blow flies" or flesh flies are represented by a single species, *viz. Sarcophaga cruentata* Meigen.

Adult blow flies visit a decomposing body to (i) find a mate of the opposite sex to mate with (males and females), (ii) take a protein meal for egg production (females) and (iii) oviposit (females).

The secondary decomposers are Coleoptera (beetles). Coleoptera feed upon the skin, hair and tendons and is the most important forensic indicators during later stages of decomposition (Boucher, 1997). The most important families are Dermestidae and Cleridae (Figure 1.3).

Coleoptera undergo holometabolous metamorphosis, with the adults morphologically different from the larvae (Figure 1.3). The larvae are campodeiform with well-developed heads and thoracic legs. The life cycles of the forensically important Coleoptera varies. Dermestidae have four to eight larval instars while Silphidae larvae moult three times. The last Silphidae instar (the prepupa) is dormant for a short period before pupation occurs (Boucher 1997).
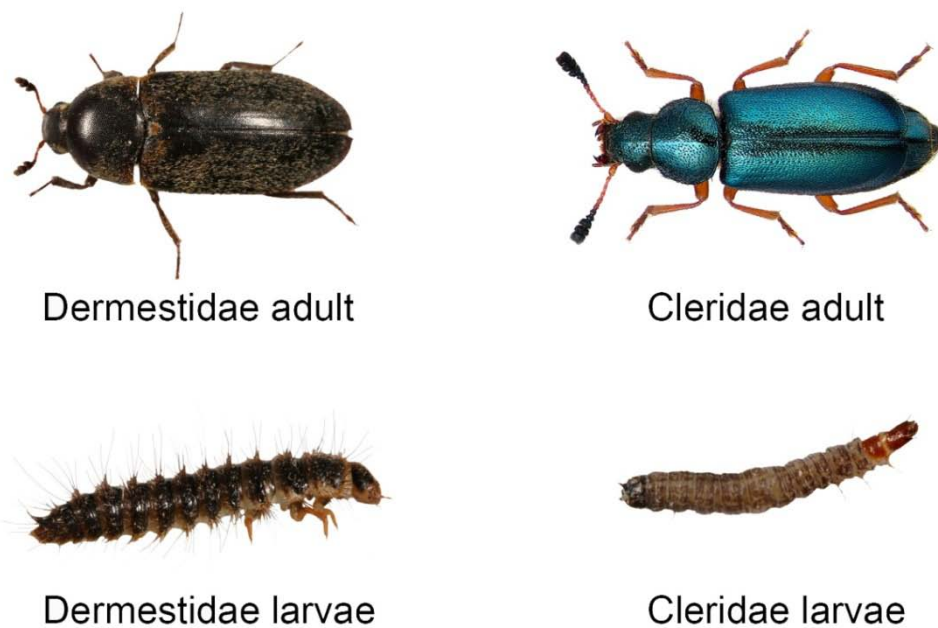
Figure 1.3 *Adult and larval Coleoptera.*
(Composed from Gross, BugGuide, 2005; Makarov, 2006 & Matz, 2009).

Adult and larval Dermestidae both occur in a variety of situations, with *Dermestes maculatus* De Geer feeding primarily on high-protein material such as stored plant products, animal carcasses, leather, feathers, hair, horns, skin, bones, cheese, dry dog food, cured meats and dry fish (Reed, 1958; Samish, Argaman, & Perelman, 1992). They may also occasionally be predators on larvae of silkworms and houseflies (Samish *et al.* 1992). They are known to feed directly on decomposing carrion, with a preference for dried carrion (Schroeder, Klotzbach, Oesterhelweg, & Püschel, 2002).

Under optimal environmental conditions (dry and warm), they can appear in large numbers. Schroeder *et al.* (2002) found that Dermestidae almost completely skeletonised the mummified corpse of a human male in less than five months at a room temperature of 25°C.

In addition to providing a PMI, a forensic entomologist can also provide added contributions to a case. These added contributions include the indication of transportation of the body and with the analysis of the pattern of insect infestation, the way in which the death was caused could possibly be determined, due to the insects colonising areas of trauma first (Erzinçlioğlu, 1989; Catts, 1992).

It is the opinion of the author that sufficient baseline studies combined with considerable experience in the field, common sense and the ability for analytical thought without any preconceived expectations should be the basis for unbiased PMI estimation.

Historical records of Forensic Entomology is somewhat sparse, but even in ancient times the importance and role of insects in decomposition was recognised. Flies prospered with animal domestication and the rise of villages, towns and cities. From a fly's perspective, civilisation is the increasing proliferation of organic waste and garbage (Greenberg, 1991). The oldest alleged records of blow flies are fossils from the Late Cretaceous period (about 70 million years ago) from Alberta, Canada. The oldest definite fossil blow fly puparia are remains found in association with Australopithecus bones in the Makapan Valley, South Africa and date from the Tertiary/Quaternary boundary, about 1-2 million years ago (Erzinçlioglu, 1996).

According to Erzinçlioglu (1996), flies appeared in ancient civilisations as amulets (Babylonia, Egypt), on cylinder seals (Mesopotamia), in legends (Epic of Gilgamesh), as a god (Baalzebub, Lord of the Flies), and as one of the plagues in the Biblical story of Exodus. Ancient Egyptians knew the effect that feeding maggots can have on a body, as well as their metamorphosis. A slip of papyrus found in the mouth of a mummy contains the following inscription: "The maggots will not turn into flies within you" (Papyrus Gizeh no. 18026:4:14). The insects that the embalmers sought to exclude are the same ones we now use to help solve murders.

In the Old Testament of the Bible (1987), Job laments on death in The Book of Job, Chapter 21, verses 23-26: "[23]One dieth in his full strength, being wholly at ease and quiet. [24]His breasts are full of milk, and his bones are moistened with marrow. [25]And another dieth in the bitterness of his soul, and never eateth with pleasure. [26]They shall lie down alike in the dust, and the worms shall cover them."

According to Davis (2001), even though humans have been capable of learning and observation since creation, "vast amounts of traditional oral knowledge" have been lost forever. This loss of knowledge continued after the invention of the written word through illiteracy, the loss of written historical records and the failure to record early events which were passed orally from generation to generation. The first published reference to blow flies appeared in a collection of cuneiform writings on clay, the Har-ra-Hubulla. This is considered to be the oldest known book on zoology and is the first to mention green and blue flies (McKnight, 1981).

The earliest record or documented case of the application of entomological evidence in a medicolegal investigation comes from thirteenth century China. Lawyer and death investigator Sung Tzu described the case of a stabbing near a rice field in 1235 AD in the medicolegal textbook "Hsi yuan chi lu" (The Washing Away of Wrongs). The investigator suspected that the lethal weapon used was a sickle. The day after the murder, he requested all the workers to lay their sickles down in front of them. Invisible traces of blood drew blow flies to a single sickle. When confronted with this evidence, the owner of the sickle confessed to the crime and "knocked his head on the floor" (Keh, 1985; Greenberg, 1991; Catts & Goff, 1992; Benecke, 2001; Davis, 2001; Hall, 2001).

In 1626, Francesco Redi disproved the idea of spontaneous generation based on the appearance of maggots on rotten meat (Hall, 1974). More than 200 years later in 1848, a pathologist named Orfila listed 30 insects and other arthropods that visited a body to feed and oviposit. This may have been the first instance of the systematisation of knowledge of arthropod succession in human bodies.

The credit of the first application of the knowledge of arthropod succession to an actual case belongs to Bergeret. In 1855 he was investigating the body of an infant found in an enclosed fireplace. He identified a flesh fly and a moth on which he based the PMI estimation. His findings exonerated the recent tenants of the premise where the infant was found. However, his PMI estimation was based on an incorrect assumption regarding the life cycles of both insects. The PMI could in fact have been much shorter, thereby possibly incriminating the recent tenants (Greenberg, 1991).

Observations on insects and other arthropods as forensic indicators were documented in Germany and France during mass exhumations in the late 1880's by Reinhard and Hofmann, who are considered to be co-founders of the discipline (Benecke 2001). With the publication of the classic work *La faune des cadavres Application de l'Entomologie a'la Medicine Legale* in Paris (Mégnin, 1894), the application of entomology to forensic medicine was firmly established. Prior to this publication, Mégnin and Yovanovitch had published two shorter works on the subject in 1888, but neither of these earlier works dealt with the subject as comprehensively as Mégnin's second book (Erzinçlioğlu, 1983).

Observations made by Niezabitowski in the Russian Empire in 1902 differed from Mégnin's and cast doubt on the application of forensic entomology in Russia (Greenberg & Kunich, 2002). After the French publication of Pierre Mégnin's popular book on the applied aspects of forensic entomology, the concept quickly spread to Canada and the U.S.A. Researchers realised that the lack of systematic observations of forensically important insects was the one thing that prevented the routine use of these insects as indicators of postmortem interval. Advances in insect taxonomy and ecology proved to be beneficial to forensic entomology during the following decades. Many early case reports involved alleged child homicides, including the suspected use of sulphuric acid. In this context, it was shown that ants, cockroaches, and freshwater arthropods could produce postmortem artifacts suggestive of child abuse (Turner, 1991; Benecke, 2001).

One of the earliest and best documented cases of the application of Forensic Entomology was the Ruxton case. Two dismembered bodies were found in Scotland on 29 September 1935. In all, 70 different pieces of the bodies were collected and the trunk of one body was never found (Figure 1.4) (Anonymous, 2005). Dr. A. G. Mearns calculated the PMI at 12-14 days and established the date on which the remains were deposited. His PMI estimation was based on the presence of third instar maggots. This evidence corroborated other evidence and led to the conviction of Dr. Ruxton for murdering and dismembering his wife and their children's nurse (Lane & Brian, 1992).
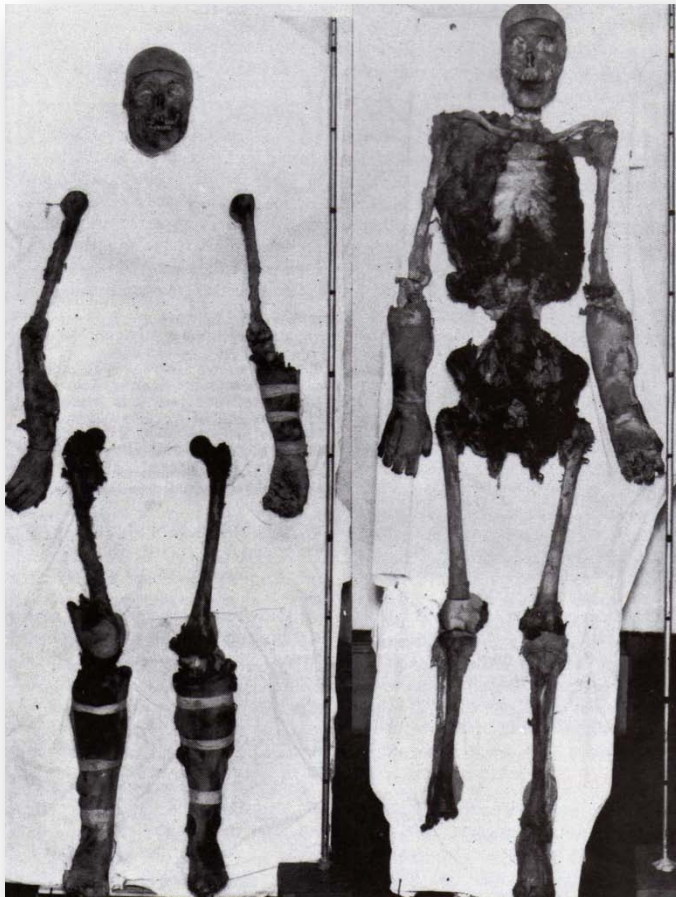
Figure 1.4 *The remains of the victims in the Ruxton-case.*
        (Anonymous, 2005)*.*

The Ruxton case was also the first to use the technique of photo-superimposition where a photograph of the victim was superimposed on to an X-ray image of one of the skulls (Figure 1.5). It was a perfect match (Anonymous, 2003).
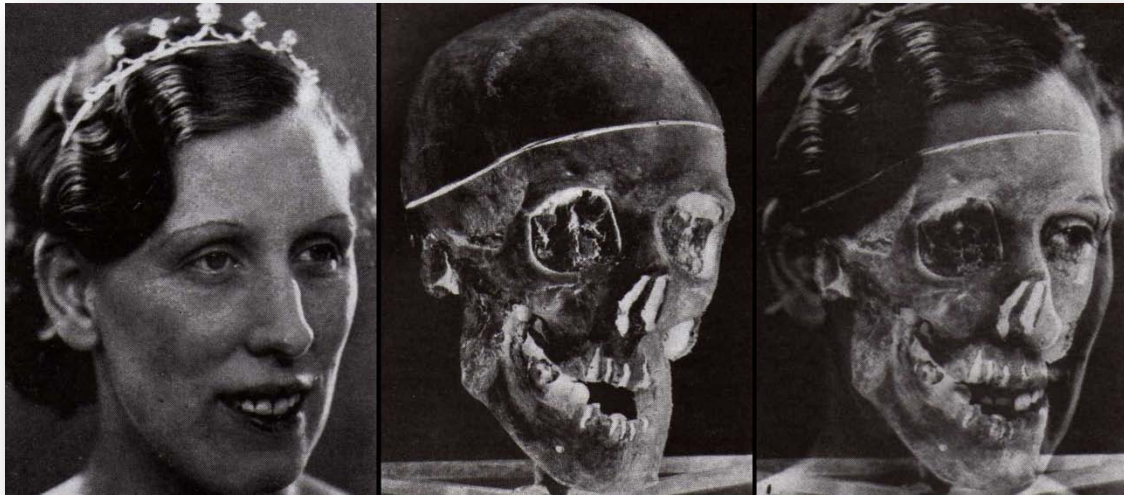
Figure 1.5 *Photo-superimposition of a portrait photograph onto an X-ray of Mrs. Ruxton. (Composed from Anonymous, 2005).*

After the World Wars, only a small number of forensic entomology cases entered the scientific literature. From the 1950's to the 1960's, Leclercq and Nuorteva were primarily responsible for maintaining the method in Central Europe, with a focus on case studies (Turner, 1991; Benecke, 2001). Since then, basic research has opened the way for the routine use of entomology in forensic investigations (Benecke, 2001).

Forensic Entomology research is vital, since one of the most important parts of the forensic sciences is the understanding of the processes of postmortem change in biological systems (Micozzi, 1986). The most commonly used animal models in forensic studies are pigs, rats and other mammals (Fuller, 1934; Micozzi, 1986; Tullis & Goff, 1987; Hewadikaram & Goff, 1991; Shean, Messinger, & Papworth, 1993; Richards & Goff, 1997; De Souza & Linhares, 1997; Avila & Goff, 1998). Lizards and toads (Cornaby, 1974), as well as human cadavers (Rodriguez & Bass, 1983). Elephants have also been used in forensic studies (Coe, 1978).

These studies were conducted during different seasons and entailed the influence of mechanical damage, exposed carcasses versus shaded carcasses, the effects of freezing and thawing and the effect of burning the carcass on decomposition.

Other global studies included the effect of excluding insects from a carcass during decomposition (Payne, 1965). The effect of water-related deaths, burial, concealment and wrapping of a body on arthropod succession patterns has also been extensively studied (Payne & King, 1972; Erzinçlioğlu, 1985; Rodriguez & Bass, 1985; Haskell, McShaffrey, Hawley, Williams, & Pless, 1989; Turner B. D., 1991; Goff, 1992; Louw & Van der Linde, 1993; Tomberlin & Adler, 1998; Hobischak & Anderson, 1999; VanLaerhoven & Anderson, 1999).

Case studies from all over the globe have revealed a wealth of unexpected information, unexpected twists and important observations and techniques into consideration when using Forensic Entomology. Although flies and beetles are the insects mainly used for PMI estimation, the PMI has also been successfully determined by using the development time required for a fly, in combination with the time required to establish a colony of ants (Goff & Win, 1997).

One case which highlights how essential knowledge of the biology of the insects found during an investigation is, came from Hawaii. Second instar maggots were recovered from the diapers of a 16-month-old child abandoned by her mother on Oahu, Hawaii. The development of these larvae indicated a minimum period of 23,5 hours of exposure to discovery of the child. Larvae of the species of fly were not normally associated with living tissues in Hawaii, but rather with faeces and remains during the early stages of decomposition. If the child had died and data not been provided detailing the site of infestation, the PMI estimate would have been significantly longer than was actually the case. This was due to the development of the larvae inside the diapers of the living child (Goff, Charbonneau, & Sullivan, 1991).

The first report where an examination of the maggots on a person showed that neglect had occurred prior to death was made by Benecke & Lessig (2001). It described a child found dead in an apartment in Central Germany. The child's body showed signs of greenish discoloration, and skin slippage. From the development times of the flies it was estimated that the anal-genital area of the child had not been cleaned for about 14 days (17-21 day range), and that death occurred only 6-8 days prior to discovery of the body. Caution should be taken in cases involving deaths of infants, the elderly, and individuals not capable of caring for themselves (Goff, *et al.*, 1991).

Forensic entomology has also been successfully applied in a case that occurred more than four centuries ago. The victim was a horse that had been killed. The insects recovered from the site arrived after the horse had died. These insects revealed that the horse was accompanied by Europeans and it died in late spring or early summer in a dry habitat while being protected from the elements. It also revealed that the horse may have been partially buried. Observations suggested that the horse had not been eviscerated or butchered for meat and that it was not moved from the place in which it was killed (Lockwood, Kumar, & Eckles, 1994).

Forensic Entomology has even been applied to wildlife in poaching cases. The calculated PMI in the illegal killing of two black bear cubs was consistent with the time that the defendants were seen at the scene. This calculated PMI was used in their conviction. This case highlights the fact that conservation officers need to be educated in this field (Anderson, 1999).

The application of insect and other arthropod evidence has become routine in forensic and medicolegal investigations and research in Europe, Australia, Canada and the USA.

South African research applicable to Forensic Entomology has been in existence since 1921, focusing on agriculture, medicine, ecology and forensics (Williams & Villet, 2006). These studies included research on the effects of scavenger mutilation on insect succession at impala carcasses (Ellison, 1990) and the community dynamics and visitation patterns of carrion-attendant arthropods (Braack, 1981, 1986, 1987). During the 1970's, the first research explicitly regarding forensic entomology was done by André Prins, culminating in a Ph.D thesis (Williams & Villet, 2006). Ongoing Forensic Entomology research started in 1992 at the University of the Free State with the founding of FEITUOFS (Forensic Entomology Investigation Team of the University of The Orange Free State) by Professors Theuns van der Linde and Schalk Louw. FEITOUFS research have included the development rate of maggots, the use of maggots as toxicological indicators, effects of freezing, clothing, wrapping, stabbing, mutilation, hanging and burning of bodies, bodies in shade *versus* sun and electron microscopy of maggots. Current research includes the use of maggots as indicators of gunshot wounds and the extraction of DNA from the gut content of maggots. More than 300 case studies have been investigated by FEITUOVS under the guidance of Van der Linde.

Subsequently, the South African Forensic Entomology Research (SAFER) laboratory came into existence at Rhodes University under the leadership of Professor Martin Villet. SAFER research have included systematics and thermo-ecology of carrion insects, an electronic key (*Identifly*) to identify South African blow flies and flesh flies, DNA sequencing to identify flies, the distribution of blow flies and developmental rates of maggots (Williams & Villet, 2006).

Although Forensic Entomology is not yet routinely used in criminal investigations in South Africa, insect evidence has been allowed in the South African courts. The first time insect evidence aided in a conviction was during 2000. Dr Mervyn Mansell testified as expert witness for the state. Mansell has been involved with South African case studies since 1995. Mansell has been working with Van der Linde and has now been involved in more than 150 case studies (Williams & Villet, 2006).

Mansell has also been co-supervisor of the author's M.Sc study regarding the influence of hanging of a body on the decomposition and insect succession (Kolver, 2003).

Very recently, the author testified in a serial killer case concerning a partially buried body (alleged to be the body of Thabang Bihi, a 13 year old child). The victim was murdered in 2004. The accused was arraigned on nine counts, namely attempted rape, three counts of murder, one count of kidnapping, two counts of theft and two counts of assault with the intent to inflict grievous bodily harm. This was a difficult case for all concerned, albeit for differing reasons. The complexity of the case was found in the circumstantial and similar fact evidence. The State, the two prosecutors and the SAPS investigating team have excelled in investigating and prosecuting this case to its ultimate successful conclusion. The first murder occurred 22 years ago (1987), the inquest docket had been destroyed and material witnesses have passed away or have retired. The same obstacles presented themselves to a lesser extent in the matter of the 2004 murder. The entomological evidence placed the defendant at the scene of the 2004 murder. Dogged persistence and diligent investigation coupled with meticulous prosecution have borne fruit for the State (The State versus Tommy Williams, 2009).

The identity of the deceased was established beyond reasonable doubt as that of Thabang Bihi. This conclusion was based on the evidence of the forensic pathologist, Dr. Liebenberg, regarding the estimated age, sex and race of the deceased; the entomological evidence presented by Mr. Kolver (the author) regarding the date of death; the identification of the clothing by the father, Mr. Bihi and the admission that Thabang Bihi had disappeared on 17 April 2004 and that no other children or males had been reported missing in the area at that time. In addition there is the version of the accused conveyed to Superintendent Verster and Inspector Luis that Mr. Motingwe had strangled Thabang Bihi in that house (The State versus Tommy Williams, 2009).

Thabang Bihi disappeared on the very same day that the accused (on his own admission) entered the Bihi residence and stole various goods from the residence (The State *versus* Tommy Williams, 2009). The third victim was partially burnt, but unfortunately the author was not involved with the case regarding the third victim. The defendant was found guilty on seven of the nine counts, including all three murder charges and was sentenced to two consecutive life sentences, plus ten years - a new record for the South African justice system. (Luis, pers. comm.)[1]. Unfortunately, this was the only case to go to trial from more than 100 case studies conducted by the author, highlighting the fact that forensic entomology is still not being routinely used in South Africa.

In some instances, cooperation from pathologists and detectives in South Africa is extremely lacking. Forensic investigations of PMI should be conducted by a multidisciplinary group comprised of forensic entomologists, pathologists and anthropologists (Schoenly, Griest, & Rhine, 1991). In reality, this has yet to happen in South Africa.

This study could make a meaningful contribution in the fight against crime in South Africa and possibly the rest of the world. A total of 18 487 persons were murdered in South Africa from January 2009 until the end of March 2009 (Keppler & Fourie, 2009). The potential application value and training possibilities in South Africa is immense. It is such a pity that there are so few forensic entomologists in South Africa.

Another problem is that forensic experts often disagree. One of the causes of disagreement is interpretive error. Interpretive error is in turn caused by inappropriate, expectation-laden observations. Observation is not interpretation. There is a marked difference. Observation is a mental experience – it is instant and requires implicit reasoning. Interpretation involves explicit reasoning and deliberate thinking (Nordby, 1992).

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[1] F. Luis, Detective Inspector, Organised Crime Unit, South African Police Service, Kimberley, South Africa.

The result of making observations that are expectation-laden is that two observers may not see the same thing, although their eyesight is normal and they are aware of the same artifact. Investigative expectations affect what count as observations. When two observers' expectations conflict, they do not see the same thing, so are not presented with the same evidence. If investigators see what they believe they should see, they may not believe what they see (Nordby, 1992).

An experienced entomologist can make a huge difference in a death investigation, but unfortunately not all entomologists are willing to become involved. In the author's opinion this has mostly been due to either disgust or fear, or both.

Criminals often try to destroy evidence in an attempt to evade prosecution. They even make the mistake of burning a body in the hope that it will be reduced to ashes. Criminal burning is mainly a means of covering up homicide or an attempt to destroy evidence (Dirkmaat, 1998; Introna, *et al.*, 1998; Suárez-Peñaranda, *et al.*, 1999; Fanton, Jdeed, Tilhet-Coartet, & Malicier, 2006). Fortunately, the destruction of evidence by burning is not as complete as seen in countless movies. In reality, the tissues of a burning body pass through various degrees of burning (Fairgrieve, 2008).

These degrees of burning can be described by using the Crow-Glassman Scale (CGS). This scale is divided into five levels, with each succeeding level depicting increasing destruction to the body from burning. (Glassman & Crow, 1996). Bodies do not burn evenly and it may be possible to see remains with different degrees of burning present (Fairgrieve, 2008).

Case reports have shown the need for reliable data in cases of burning. A case report from southern Taiwan highlights the importance of decompositional studies and the application of baseline data on actual cases. A PMI experiment was conducted with a pig carcass decomposing in the woods. The pig carcass was burned to a CGS level 2. A homicide case, very similar to the study in the woods, was investigated a month later (Pai, Jien, Li, Cheng, & Yang, 2007).

The PMI was calculated to be 50 hours from the data collected during the experiment. The murderer confessed to the crime after he was eventually arrested. According to his statement, the actual PMI was 46 hours (Pai, *et al.,* 2007).

Research on the influence of burning on the decomposition of such a body and the insect succession onto such a body has been sparse. In a study in Malaysia, two pigs were used. One pig served as control, while the other was partially burnt with 1 l of petrol. No significant difference was found between the rate of decomposition and the sequence of faunal succession on both pig carcasses. Both carcasses reached the Dry/Remains stage after nine days (Chin, Marwi, Salleh, & Jeffery, 2008). More than a decade ago, arthropod succession patterns onto burnt carrion was investigated in the Hawaiian Islands. Two pig carcasses were used during each experiment, one the control and the other burnt to a CGS level #2 burn victim. Two experiments were conducted in total, each in a contrasting habitat. No significant differences were found in the arthropods present or the length of the stages of decomposition between the carcasses at either site. Oviposition occurred one day earlier on the burnt carcass than the control carcass at one site and four days earlier on the burnt carcass than the control carcass at the other site (Avila & Goff, 1998).

As a result, the successional waves occurred one day and four days earlier on the burnt carcasses of the respective sites than at the control carcasses. Based on arthropod succession patterns, the resultant PMI estimate would differ by up to 24 hours and four days, respectively (Avila & Goff, 1998).

A number of factors influenced the decision to conduct a comprehensive study regarding the influence of burning. Firstly, published results of experiments conducted provided no statistical analysis since only one or two experiments were conducted at a time, consisting of only two pigs each. Secondly, no data existed for the influence of burning under South African conditions. Lastly, casework demanded knowledge of the influence of burning. Making educated guesses in these cases were unacceptable.

It became clear during previous decomposition studies conducted and many case studies attended, that *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae) was one of the most important forensic indicator species in the central Free State Province, especially during spring and summer. Sometimes this was the only species recovered from a body during a death investigation.

Analysis of case studies from the whole South Africa showed that *C. chloropyga* (Figure 1.6) was one of the most important forensic indicator species (Mansell, pers. comm.)[2]. However, little was known about the developmental rate of this species before 2003 (Kolver, 2003). Due to the importance of *C. chloropyga* during previous investigations, combined with research conducted on the developmental rate of this species, it was decided to use *C. chloropyga* during the laboratory experiments to determine the effect of burning on the development rate of maggots feeding on the burnt medium.

Figure 1.6 *Chrysomya chloropyga (photograph by the author).*

The key questions of this study was: What is the influence of burning of a body during different seasons on (i) the decomposition of the body?, (ii) the composition of insects associated with the body?, (iii) the insect succession on the body? and (iv) the calculation of the PMI?

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[2] M.W. Mansell, Specialist Scientist, United States Department of Agriculture, c/o US Embassy, Pretoria, South Africa.

Chapter 2: Materials and Methods

Section 2.1. Field Trials

2.1.1 Study Site

All trials were conducted in South Africa (Figure 2.1) in the Free State Province (Figure 2.2), in the city of Bloemfontein (Figure 2.3), where the University of the Free State (Figure 2.4) is situated. On the western side of the campus of the University of the Free State (29°08' S; 26°10' E), is the experimental site where all the field experiments were conducted (Figure 2.4). This site consists of approximately 24 hectares of the open grassveld, and lies approximately 1560 m above sea level. The experimental site is accessible with a vehicle and the furthest point used for placing carcasses was 3.5 kilometers from the laboratory.

The vegetation of the experimental site can be described as the central variation of the dry *Cymbopogon-Themeda* veld (Acocks, 1988). The main grass species (Family: Poaceae) were *Eragrostis lehmanniana* (Nees) (Lehmann's Love Grass), *E. capensis* (Thunb.)(Trin.) (Heart-seed Love Grass), *Aristida congesta* (Roem. & Schult.) (Tassel Three-awn), *Themeda triandra* (Forssk.) (Red Grass), *Digitaria sp.* (Haller) (Finger Grasses) and *Chloris virgata* (Feathered Chloris Grass). A few scattered *Acacia karroo* (Hayne) (Sweet Thorn, Family: Fabaceae, Subfamily: Mimosoideae) and *Rhus lancea* (L.f.) (Karee, Family: Euphorbiaceae) specimens were the only trees in the area. Since 2002, a golf driving range has been added to the north eastern corner of the experimental site (Figure 2.4). Whilst this development encroached onto the experimental site, the golfers did not interfere with the trials. However, it has lead to a breach of security as the driving range was not enclosed with a fence. Only as recently as 2008, a fence was erected around the circumference of the driving range.
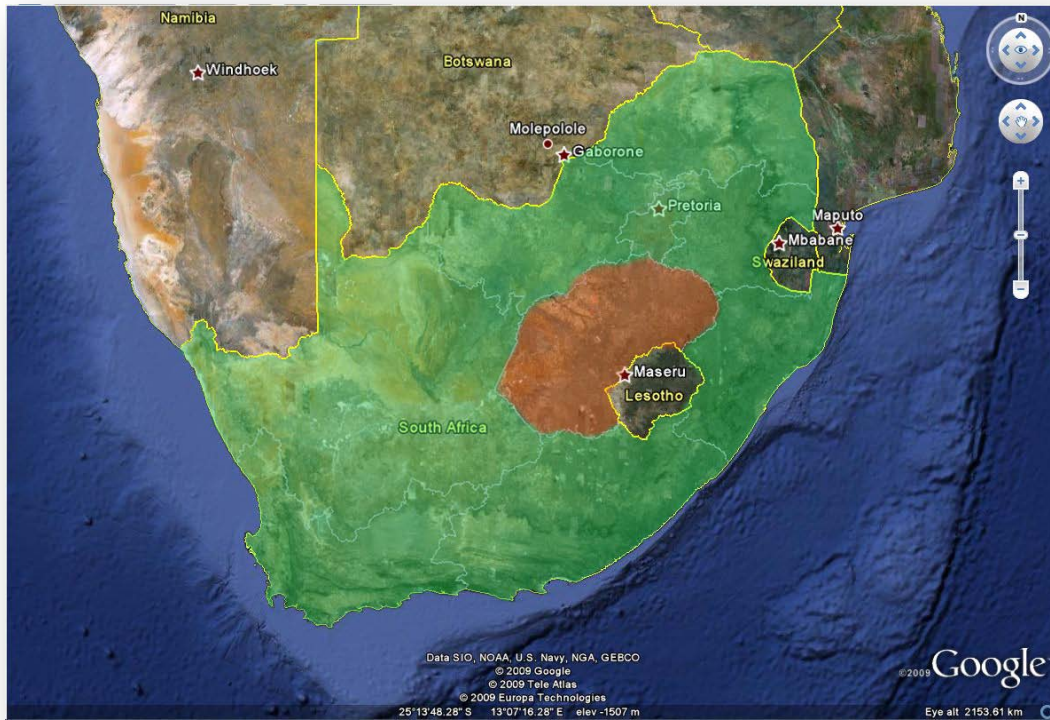
Figure 2.1 Satellite Image of South Africa (green) and the Free State (brown).
(Image altered from Google Earth, accessed 26 August 2009).
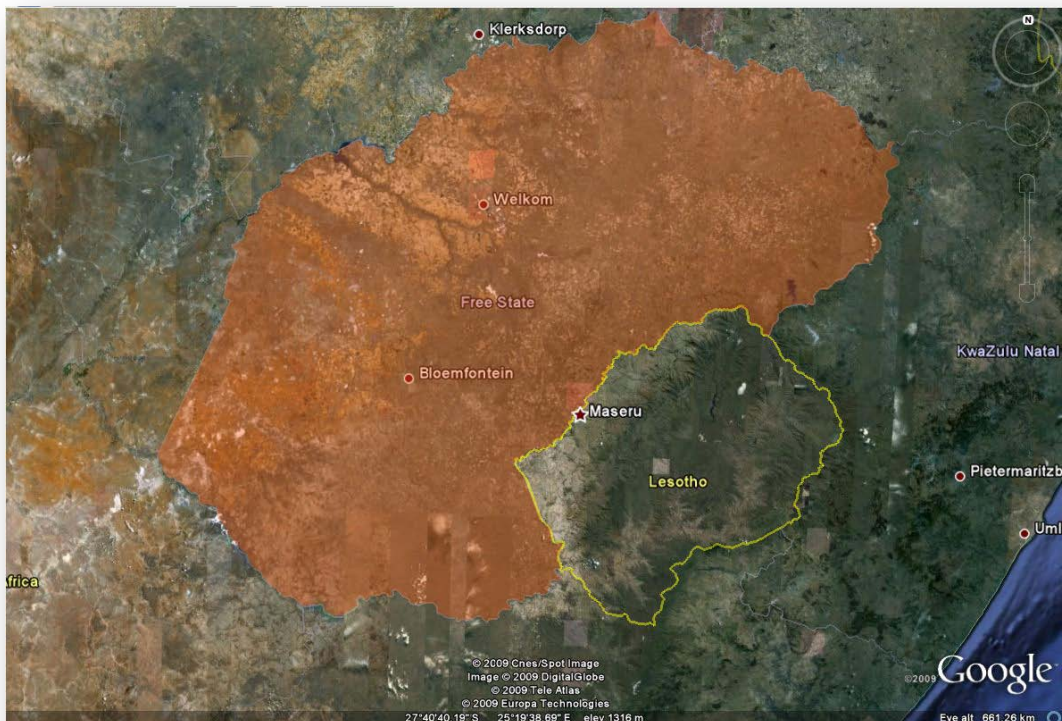
Figure 2.2 Satellite Image of the Free State (brown).
(Image altered from Google Earth, accessed 26 August 2009).
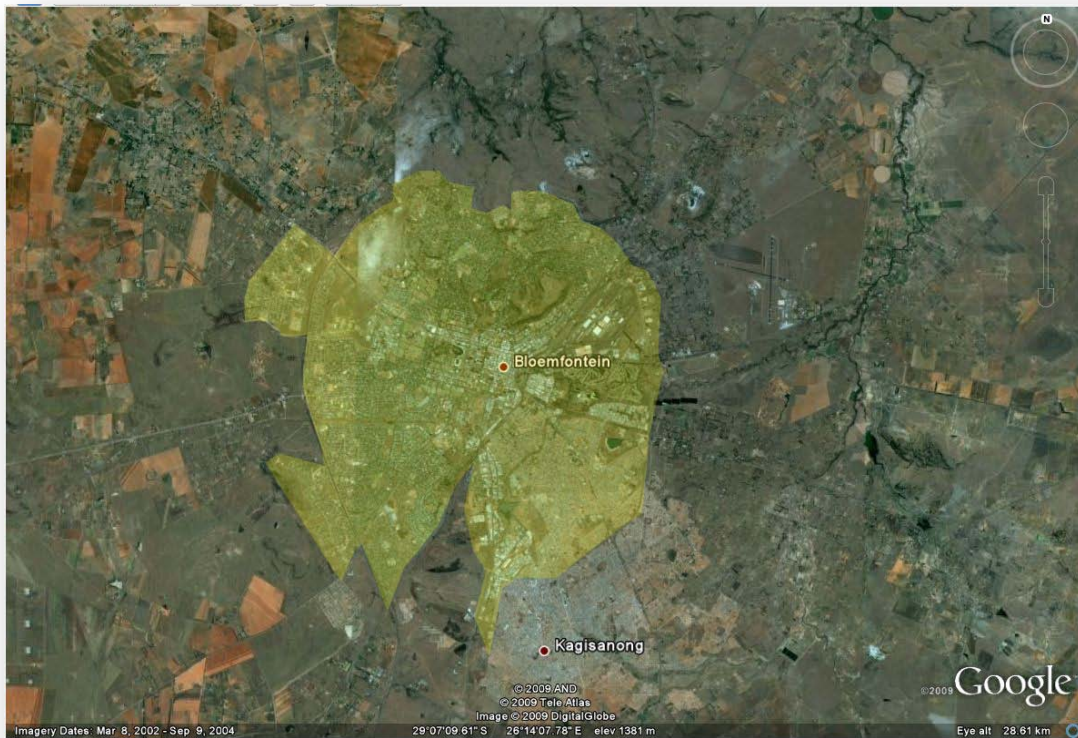
Figure 2.3 *Aerial photograph of Bloemfontein (yellow).*
(Image altered from Google Earth, accessed 26 August 2009).

Figure 2.4 *Aerial photograph of the University of the Free State (blue),*
Study Site 01 (green), Study Site 02 (yellow)
and the Golf Driving Range (red).
(Image altered from Google Earth, accessed 26 August 2009).

Quite ironically, the grassveld in this study site was periodically cut short by the groundskeeper to reduce the risk of fire. The biodiversity of tourist insect species visiting the carcasses by chance was consequently reduced due to many feeding niches being destroyed by lack of grass cover.

The Free State region has very hot summers, while the winters may be extremely cold with frost occurring regularly. The average rainfall is 450 - 500 mm per annum in this summer rainfall region.

2.1.2 Carcass Layout and Sampling Frequency

A variety of animal models has been used in decomposition studies (Payne, 1965). To study the decomposition of organic tissue, pig carcasses were used as this animal most closely approximates humans with regard to decomposition (Richards & Goff, 1997). Internationally, pig carcasses are used in forensic entomology decomposition studies as an alternative to human bodies (Catts & Goff, 1992). The pigs used in this study were killed at the experimental site, by the late Dr. Freek Potgieter, with a Pentobarbitone sodium solution (Figure 2.5), *viz.* Euthapent (200mg/ml – *Kyron Laboratories Pty Ltd*).

Figure 2.5 *Pig (Sus scrofa Linnaeus) prior to killing and Euthapent used to kill pigs.*

Trials were conducted during all four seasons. Fuller (1934) also conducted experiments during all seasons to include insects that were present at different times of the year. Anderson (2001) also stressed the importance of conducting trials during all seasons to develop a valid database for a specific area. Insects may be valuable in determining the season of death, since some species only occur during certain seasons.

During each trial, four pigs were used. Three of these pigs were burnt and one was used as a control.

The control carcass was immediately placed in the field in a prone position inside a metal-frame cage covered with 15 mm poultry mesh which allowed insects to visit the carcass without interference from large scavengers. Access to the cage was through a side door that opened from top to bottom. The carcass was placed with front and rear in a northeast-southwest orientation.

The remaining three pigs were placed in plastic bags on the back of the vehicle and driven a short distance away (±150m) from the grassveld to an area that was bare of any grass. This area was used for the burning of the carcasses to prevent veld fires. The plastic bags prevented blow flies from ovipositing on the carcasses.

The carcasses were burnt inside a metal drum (Figure 2.6), which was cut open lengthwise, laid end to end, pieces of the lid were removed and the two parts of the drum were welded together to form a long, shallow trough. Different volumes of LRP petrol were poured over the carcasses and set alight with a match. The carcasses were left to burn until the flames were extinguished.

The different volumes of petrol used resulted in carcasses burned to a CGS level 2 or CGS level 3 burn injury, with different degrees of charring. According to the Crow-Glassman Scale (CGS) of burn injury, CGS level 2 defines a body that may be recognisable but most often exhibits varying degrees of charring. Apart from charring, destruction to the body is limited to the possible absence of parts of the hands, feet, genitalia and ears.

Figure 2.6 *Pig inside trough prior to burning and during burning.*

CGS level 3 shows further destruction of the body with major portions of the arms and legs missing (Glassman & Crow, 1996).

The Slightly Burnt carcass (further denoted as "SB") was burned with 46.875ml/kg of petrol and resulted in a CGS level 2 with slight charring. The legs of this carcass were curled up due to the heat shrinking the muscles and tendons. The Medium Burnt carcass (further denoted as "MB") was burned with 156.25ml/kg of petrol and resulted in a CGS level 2 with medium charring. This carcass too exhibited curled up legs due to the effects of the heat. The Heavily Burnt carcass (further denoted as "HB") was burned with 312.5ml/kg of petrol and resulted in a CGS level 3 with heavy charring. Heat induced fractures of the legs was commonplace with this carcass to the extent that portions of the legs broke off from the rest of the carcass.

The temperature of the fire was measured with the infrared thermometer (Figure 2.13, Table 2.1) as being in excess of 470°C. The carcasses were placed at least 50m apart in the same position and orientation in the field within similar metal-frame cages covered with 15 mm poultry mesh (Figures 2.7 & 2.8).

Figure 2.7 *Burnt pig inside metal frame cage covered with poultry mesh.*

Figure 2.8 *Placement of carcasses 50m apart. Second cage encircled.*

The placement of the carcasses at least 50m apart was to limit the degree of cross contamination of insects from the other carcasses. In other decomposition studies, carcasses were placed 6m apart (Tullis & Goff, 1987), which was regarded by the current author as being too close together for any differences in the insects visiting the carcasses to manifest.

In the author's opinion, human bodies are placed too close together during decompositional studies at the famous Body Farm at the University of Tennessee. Up to 180 bodies are placed in an area of only 3 acres (World's Worst Jobs, Aired 7 May 2009). This equates to a mean distance of just over 8m between the bodies. In some instances the bodies are even closer together (Figure 2.9)

Figure 2.9   Screengrab of placement of human bodies (underneath black plastic) at the Body Farm, University of Tennessee (World's Worst Jobs, Aired 7 May 2009).

2.1.3 Sampling Methods

The control carcasses were placed in the field immediately after killing and this date and time was designated as Day 0 and marked the start of each trial. Between the time that the control carcass was placed in the field and the last burnt carcass was placed in the field, a maximum of two hours had passed. Whenever possible, the first observations commenced directly after the last burnt carcass was placed in the field. Alternatively, the first observations were made the following day (Day 1). Observations were first made at the control carcass, followed by the burnt carcasses in the order in which they were placed in the field (Figure 2.10). Between the time that the observations commenced at the control carcass and the final observations were made at the last burnt carcass, a maximum of 2 hours had passed. The observations were therefore made at each carcass with the same elapsed time since placement in the field, since the order and time of observations made were the same each day.

Observations were made twice daily for the first 14 days and thereafter once daily for the remainder of the study until blow fly activity dramatically decreased. Thereafter the carcasses were observed every 48 hours. This was done in accordance with previous decomposition studies (Shalaby, de Carvalho, & Goff, 2000; Kolver, 2003).

2.1.3.1 Carcass decomposition and arthropod composition

The first step during each visit to the carcasses was to take photographs and describe the condition of each carcass. The stage of decomposition was also noted. Each adult individual of each species at the carcasses was counted and recorded. It was almost impossible to count or make an estimate of the number of maggots at any given time on each carcass, without causing a disturbance to said maggots. Such a disturbance could severely alter the decompositional rate, which in turn could lead to misleading results. Misleading results could cause a severe error in the postmortem interval calculated, if the calculation was based on these results.

Figure 2.10 *The author making observations in the field.*

To undertake a quantitative analysis of the maggots, additional carcasses would have to be used which effectively would have to be destroyed to make a count of the maggots. Since pig carcasses are very expensive in South Africa, this was not an option. Therefore it was decided not to undertake a quantitative analysis of the maggots associated with the decomposing carcasses. However, whenever the numbers of larvae were minimal enough (less than 100) to warrant a count or estimation, this number was recorded.

In another South African study (Braack, 1981), where the aims of the study were to determine the abundance and diversity of insect species visiting decomposing wildlife carcasses, absolute counts were made. Braack (1981) decided to make absolute counts of the insects at the carcasses since it had the advantage that species present only in very low numbers would not be overlooked and the number of individual species would be reflected more accurately.

Small representative samples of larvae were collected as to limit the disturbance to the insect fauna, which could possibly have had an influence on the decomposition process and the insect succession (De Souza & Linhares, 1997). These larvae collected at each carcass were reared to adulthood in rearing containers in the laboratory for identification (Figure 2.11). The adult blow flies obtained were assimilated in the laboratory colonies of *Chrysomya marginalis* (Wiedemann)*, Chrysomya chloropyga* (Wiedemann), *Lucilia* species and *Chrysomya albiceps* (Wiedemann).

Figure 2.11 *Rearing containers used to rear maggots collected from the carcasses.*

2.1.3.2 Carcass biomass removal

The masses of the carcasses ranged between 28kg and 39kg. Carcass size has a direct relationship to the numbers of individuals and species composition of the fly population (Denno & Cothran, 1975). Carcass size also has a direct influence on the rate of decomposition (Hewadikaram & Goff, 1991). During the first trial of each season, the daily mass of each carcass was recorded with the use of a scale fixed to a metal gantry which could be placed over each cage (Figure 2.12). The whole cage was lifted with a pulley and the mass of the cage was subtracted from the total mass, revealing the carcass mass. This was only done during the first trial of each season.

Figure 2.12 *Metal gantry, pulley system and scale used to determine carcass mass.*

During successive trials, only the initial mass and the mass remaining at the termination of the trail were determined. Carcass mass was not recorded daily during successive trials, only during the first trail of each particular season. This was done to minimize the disturbance to the carcasses, which could possibly have affected the decomposition and insect succession.

2.1.3.3 Temperature and environmental factors

Ambient, internal carcass temperatures, maggot mass temperatures and the temperature underneath the carcasses were recorded with a handheld digital thermometer equipped with a probe (Figure 2.13). Ambient temperatures, maggot mass temperatures and the soil temperature underneath the carcasses were recorded with an infrared thermometer (Figure 2.13, Table 2.1). The probe of the handheld digital thermometer was inserted into five regions of the carcass, *viz.* the head (mouth), neck, thorax, upper abdomen and lower abdomen.
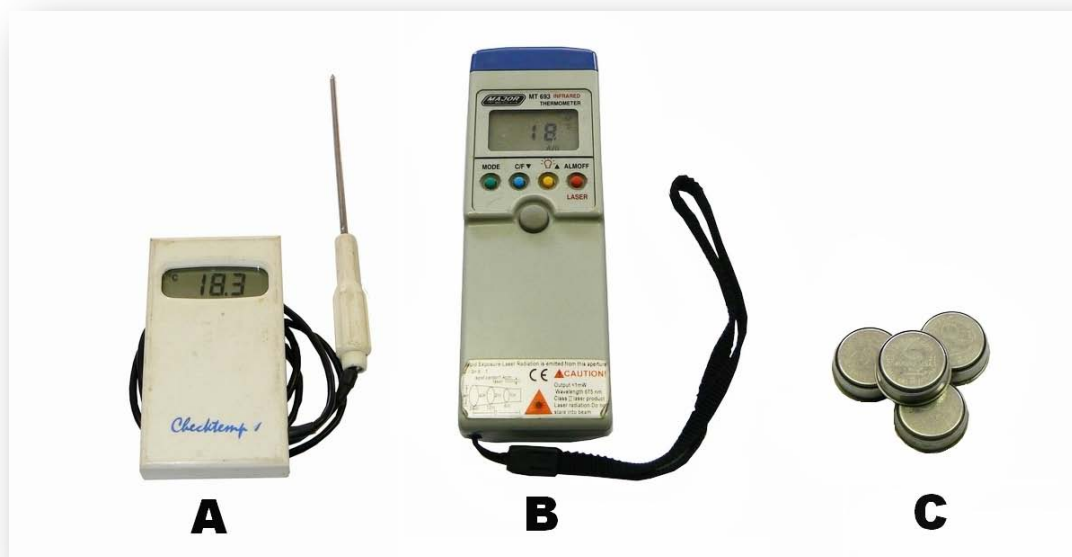
Figure 2.13 *Thermometers and iButtons used.*
(A - Digital Thermometer with probe; B - Infrared Thermometer; C - Thermochron iButtons)

Table 2.1 Specifications of Thermometers and iButtons used

	Digital Thermometer with Probe	**Infrared Thermometer**	**iButton**
Manufacturer	CheckTemp	Major Tech	Dallas Semiconductor
Product number	unknown	MT693-8886	DS1921G
Temperature Range	unknown	-40° to +500°C	-40° to +85°C
Accuracy	unknown	±2°C	-30° to +70°C (±1°C)

The probe was only inserted when and where the skin had already ruptured to prevent the creation of new oviposition sites and to prevent the escape of the decompositional gases. Escaping decompositional gases and new oviposition sites could possibly have influenced the attraction of insects to the carcasses. Internal carcass temperatures were only recorded during the active decay stage to determine the temperature of the actively feeding maggots or maggot masses. The maggot mass temperature is crucial when determining the PMI, since temperature has the greatest influence on maggot development.

Ambient temperature records and rainfall for the two nearest meteorological stations were also obtained from the South African National Weather Service in Pretoria for comparison (Figures 2.14 & 2.15). These two meteorological stations were Bloemfontein City Centre (± 2km away from experimental site, $29^0$07'01"S; $26^0$10'59"E, ± 1406 m above sea level) and Bloemfontein Airport (± 12.6km away from experimental site, $29^0$06'00"S; $26^0$18'00"E, ±1353 m above sea level).

The Bloemfontein City Centre and Bloemfontein Airport meteorological station are further denoted as "City" and "WO", respectively. Most weather stations are located at airports where the environment may differ from the locality where a body is found or an experiment is carried out (McKeown, 1991). Although not ideal, it provides an indication of weather conditions prevailing near the experimental or death site.

Only the data from the nearest meteorological station (City) were applied to this study, since ambient temperatures measured at the study site during times of observations more closely approximated the temperatures measured at the City meteorological station than at the WO meteorological station during all trials.

Thermochron iButtons (Figure 2.13, Table 2.1) were also placed inside each carcass during the initial study to record internal carcass temperatures hourly. Most of the iButtons failed due to the corrosive properties of the decompositional fluids and unfortunately they had to be discarded. During later trials, the iButtons were sealed inside plastic, but still some the iButtons failed.

It was therefore decided to discontinue their use. During a previous study conducted by the author a couple of years earlier (Kolver, 2003), dataloggers were used which provided a complete set of continuous records since one of the dataloggers was also equipped as a weather station (Figures 2.16 & 2.17). During that study, internal carcass temperatures and ambient temperatures were also measured with the same handheld digital thermometer to compare the accuracy of the two methods. The difference in temperatures measured with the datalogger and the handheld thermometer rarely exceeded 1°C (Kolver, 2003).

Figure 2.14 *Calculation of distance between study sites and City Centre Weather Station.*

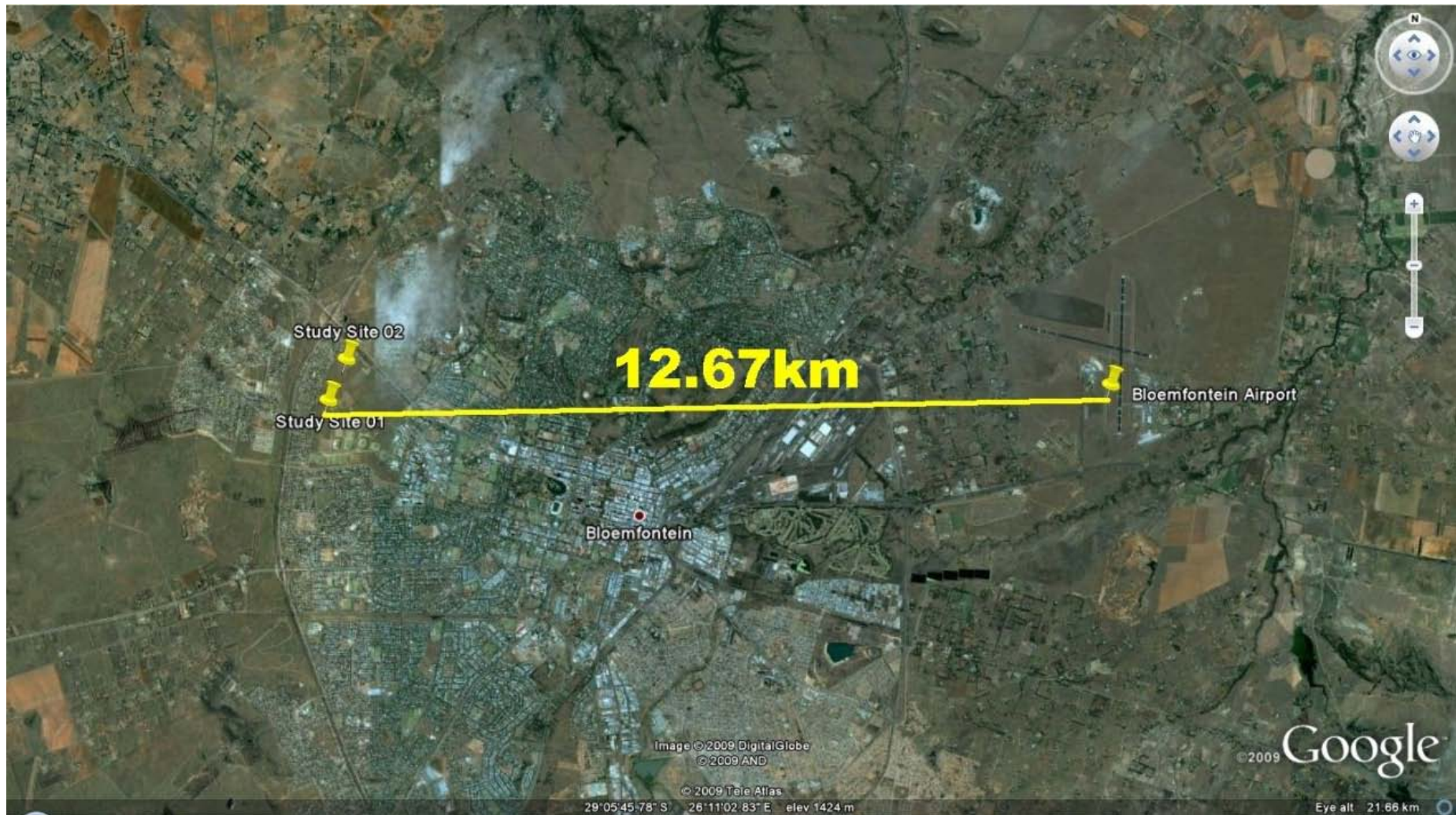*(Image altered from Google Earth, accessed 26 August 2009).*

Figure 2.15 *Calculation of distance between study sites and Bloemfontein Airport.*

(Image altered from Google Earth, accessed 26 August 2009).

Figure 2.16 *Datalogger and weather station used in a previous study (Kolver, 2003).*

Figure 2.17 *Datalogger used in a previous study (Kolver, 2003).*

Unfortunately, these dataloggers were not available as they were being used by another student during this study. Financial constraints prevented the acquisition of additional dataloggers. One datalogger became available during the winter 2004 trial, but unfortunately it had multiple failures and could not be repaired in time. It was therefore decided to only use the temperatures recorded during the actual times of observation.

2.1.4 Data and Statistical Analysis

The application of statistical methods that could apply to forensic entomological data was first published by Schoenly (1992). The chosen similarity coefficient was the Jaccard Metric method. This method is not quantitative. It only requires the absence and presence of arthropods. It allows the data to be quantified as the daily species changes of the arthropods associated with carcasses. Schoenly's methods were chosen due to the fact that the results obtained from this method may be compared to other work (e.g. Schoenly, Goff, Wells, & Lord, 1996; Boucher, 1997; Tabor, Brewster, & Fell, 2004).

The analysis was taken one step further by Tabor *et al* (2004). They used a correlation coefficient to test the data from the Jaccard Metric method to determine the degree of similarity in the species occurrence for the different experimental carcasses. Both methods were applied to the data obtained from the field trials. The data were tested for all species found on the carcasses and forensic indicator species. These analyses were applied to data within the same trial (control carcasses *versus* burnt carcasses) and data between trials (each season).

2.1.4.1 Analysis 1: Jaccard Metric

A Jaccard similarity matrix was derived. The matrix was square (n x n) and symmetric because the similarity of taxa between sampling intervals *i* and *j* is the same as between *j* and *i*, and the similarity of taxa within an interval with itself (i.e. *i* = *j*) is 1.0.

Jaccard similarity coefficients were calculated as follows:

Jaccard metric: $s_{ij} = a/(a + b + c)$

Where s_{ij} is the degree of similarity between any pair of time-specific samples *i* and *j*.

a is the number of taxa common to both samples

b is the number of taxa found in sample *i* but not in *j*

c is the number of taxa found in sample *j* but not in *i*

The Jaccard Metric ranges in value between zero, when two samples fail to match on any taxa, to unity when they match perfectly (Ludwig & Reynolds, 1988; Schoenly, 1992). The analysis was done with a computer program written by Mr. Sean van der Merwe, Department of Mathematical Statistics, UFS (See Appendix).

2.1.4.2 Analysis 2: Correlation coefficient

The matrices were tested using a correlation coefficient. The coefficient was calculated with Graphpad Instat. A correlation coefficient of 1 indicated 100% similarity with a coefficient of 0 indicating 100% difference. The two-tailed P value indicated whether these calculated correlation coefficients were statistically significant.

Section 2.2 Laboratory Trials

2.2.1 Experimental setup

The laboratory trials were conducted to determine the influence of burning the feeding medium with petrol on the developmental rate of maggots feeding on this medium. Six *4.5kg Marvello Medium Fat Spread* tubs were used as rearing containers (Figure 2.18) for *C. chloropyga* larvae. These containers contained sawdust as suitable substrate for pupation of the larvae. Absorbent paper was folded and placed on top of the sawdust to absorb excess moisture from the feeding medium.

The feeding mediums used were Chacma or Cape baboon, *Papio ursinus* (Kerr) hind legs (Figure 2.19). The baboons were acquired from the Experimental Animal Unit of the University of the Free State. Pig carcasses were very scarce and extremely expensive due to the pig flu epidemic during 2007 and 2008. Baboon carcasses were procured at no cost and it was decided to use the available baboon carcasses. Six baboon hind legs were used in the experiment. One leg was used as a control and the remaining legs were doused with petrol, ignited and left to burn (Figure 2.20).

Figure 2.18 *Rearing containers used for laboratory trials.*

Figure 2.19 *Chacma baboons grooming* (Stanton-Reid, 2007)*.*

Figure 2.20 *Burning of the baboon hind legs.*

The petrol to mass ratio used was 156.25ml/kg to simulate the same level of burning as with the medium burnt carcasses during the field trials (CGS Level 2).

The mass of each of the baboon legs were recorded prior to burning, after burning, as well as after the experiment (Figure 2.21). The burnt legs were allowed to cool for 24 hours before the start of each experiment. In the insectarium, chicken liver was placed inside gauze-covered metal frame cages containing *C. chloropyga* adults. The chicken liver served as oviposition substrate.

Figure 2.21 *Mass determination of hind legs.*

The resultant eggs were closely monitored and 100 newly hatched (less than one hour old) larvae were placed on each leg inside the rearing containers by using a small brush. The legs were loosely covered with aluminium foil to minimise desiccation and still allow heat generated by the maggot masses to escape (Figure 2.22).

Figure 2.22 *Rearing container with aluminium foil loosely covering media.*

These containers were placed in incubators set to a constant temperature of 35°C (Figure 2.23). The decision to rear at 35°C was made due to rearing *C. chloropyga* on chicken liver in a previous study (Kolver, 2003), where it was found that rearing *C. chloropyga* larvae at 35°C proved to be the optimum temperature with the fastest development rate and the highest percentage survival to adulthood.

2.2.2 Sampling

Sampling was done every 24 hours (Figure 2.24). The rearing containers were checked for pupae. If any pupae were found, their number was recorded, as well as their individual mass (Figure 2.25). These pupae were transferred to emergence containers, which were placed back into the incubators and were also checked every 24 hours for emerging adults.

Figure 2.23 Rearing and emergence containers inside incubator.

Figure 2.24 Sampling - checking for pupae.

Figure 2.25 *Ohaus microbalance used for determination of individual pupae and adult dry mass.*
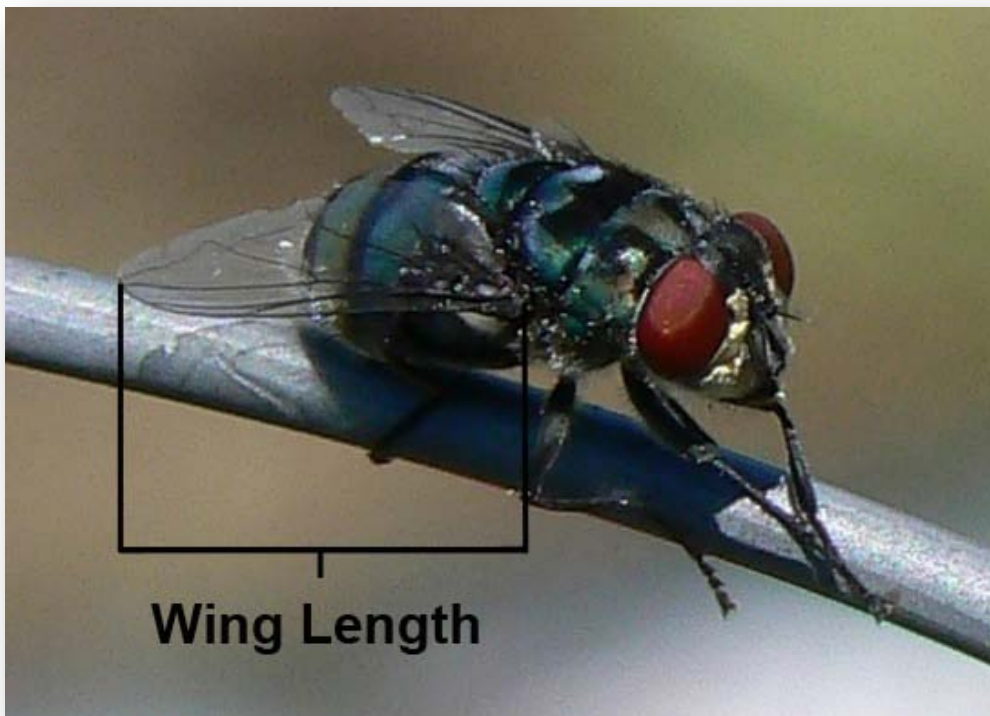
Figure 2.26 *Front wing length measurement of C. chloropyga.*

The emerged adults were killed by freezing and were placed in a 70°C oven for 24 hours to desiccate. These adults were transferred to a desiccation chamber and their dry masses were recorded individually with a microbalance (Figure 2.25). Front wing length was also measured (Figure 2.26) with a stereo dissection microscope and a digital caliper.

2.2.3 Statistical analysis

Numbers of pupae and adults, pupal mass, adult mass and wing length were statistically analysed with two tests by using Graphpad InStat. Since the results were not paired and were not sampled from a Gaussian distribution, ordinary ANOVA and nonparametric tests were used. The Kruskal-Wallace test showed whether significant differences were found and the Dunn's Multiple Comparisons test showed where the significant differences were, if any.

Chapter 3: Results and Discussion

Section 3.1 Stages of Decomposition

As the decomposition of a body progresses, it goes through a series of different stages. There are as many different stages as there are authors describing these stages.

Rodriguez & Bass (1983) recognised four different stages that included: fresh, bloated, decay and the final dry stage. Five stages of decomposition were recognised by Tullis & Goff (1987). These were: fresh, bloated, decay, post-decay, and remains. Payne (1965) identified six different stages of decay, namely fresh, bloated, active decay, advanced decay and dry and skeletal remains. Anderson & VanLaerhoven (1996) identified the same decay stages as those recognised by Tullis & Goff (1987), the only differences were that they called the decay stage the active decay stage and the post-decay stage the advanced decay stage. Schoenly & Reid (1987) used 11 published studies of carrion communities to form the basis of their statistical analysis. They identified 29 decay stage boundaries. Only 14 of these were associated with major faunal changes. They found that named decay stages have descriptive utility in carrion studies. They felt compelled to alert ecologists and forensic entomologists to the inadequacies of decay stages in summarising patterns of faunal succession in carrion arthropod investigations.

In reality, the stages of decomposition are a series of continuum changes that occur as the body decomposes. Discreet stages of decomposition do not occur in nature, but only exist in theory. Decomposition is continuous and there are no distinct dividing lines between stages (Goddard & Lago, 1985).

The stages identified by Anderson & VanLaerhoven (1996), *i.*e. fresh, bloated, active decay, advanced decay and dry/remains were applied during field trials: However, the following alterations were made to the defining parameters of these stages of decomposition:

- **Fresh:** From the time the pigs were killed until the first signs of bloating were observed. During this stage the limbs of the carcass were flexible and the body was soft to the touch. This stage had a short duration and no odour of decomposition could be discerned. During the fresh stage of decay, blow flies and muscid flies were the primary insects observed. Their activities comprised feeding and reproduction. Oviposition by adult blow flies first occurred in the area of the face, in the nasal openings, ears, mouth and eyes. Towards the end of the fresh stage, eggs were deposited in the genital area. Rodriguez & Bass (1983) also observed this phenomenon. Calliphoridae maggots are important to the onset of decay in the carcasses. Johnson (1975) emphasized this in a study where carcasses were placed in an area during the time of year when the blow flies were not breeding. The carcasses went through a different decomposition process that skipped the decay stage.

- **Bloated:** From the time that the first signs of bloating were observed until the first instar maggots hatched. These first signs included blood forming bubbles at the nose of the carcass and the inflation of the abdomen due to the buildup of decompositional gases. The limbs were distended and appeared to be suspended in the air. The body was hardened to the touch and livor mortis was apparent. Oviposition by blow flies occurred during this stage. Anderson & VanLaerhoven (1996) defined this stage as the time since bloating commenced due to the accumulation of gases until the carcass started to deflate due to loss of decompositional gases. During some trials, carrion beetles were observed on the carcasses during the bloated stage. This supports findings by Rodriguez & Bass (1983).

- **Active decay:** From the time that the first instar larvae hatched until the maggot masses moved away from the carcass to pupate. The majority of authors have suggested that this stage commences when penetration of the skin by larvae occur (Payne 1965; Anderson & VanLaerhoven 1996). This idea was abandoned during study due to larvae hatching inside the mouth and feeding on the inside of the mouth as soon as they have hatched. In effect this stage of decomposition had already started by the time the larvae had hatched. During this stage, the limbs were not as stiff as during the bloated stage and returned to the resting position. The body was also softer to the touch. Skin slippage allowed maggots to feed on decompositional fluids that collected inside blisters that formed on the skin. Decompositional fluids collected underneath the carcasses and a strong odour of decomposition was prominent. The carcass also deflated due to the loss of decompositional gases through holes caused by the feeding activity of the maggot masses. The greatest percentage of carcass biomass was removed during this stage due to the feeding activity of the maggot masses, supporting findings by Richards & Goff (1997). According to Putman (1977), the feeding activities of the maggot masses totally dominate the pattern of decomposition during the active decay stage.

- **Advanced decay:** From the time that the maggots started to migrate from the carcass to pupate until only skin and bone remained. The carcass still appeared moist, but in most cases the head was completely dried out with the skull visible. The torso and limbs were skeletonised with the ribs, leg bones and spinal column visible. The intestines were still recognisable. The odours of decomposition were less discernable as during the active decay stage. Carrion beetles (*Dermestes maculatus* & *Necrobia rufipes* De Geer) were dominant during this stage of decomposition. Tullis & Goff (1987) defined this stage as beginning when most large Diptera larvae have departed from the carcass, leaving behind bones, cartilage, hair, small portions of tissue and a mass of viscous material that constitutes by-products of decay (BOD).

- **Dry/Remains:** Only hair, skin and bone remained. The carcass was completely dried out. The remaining skin was found in small patches across the body. Carrion beetles were observed feeding on the remaining dry tissue and hair, supporting findings by Rodriguez & Bass (1983). Tullis & Goff (1987) characterised this stage by bones with little cartilage remaining, while Shalaby *et al.* (2000) characterised this stage by the presence of only dried skeletal material.

These stages of decomposition are summarized in Table 3.1.

In the succession diagrammes (See Sections 3.2.3, 3.3.3, 3.4.3 & 3.5.3), transitional stages are included for the duration of times when the differences between the physical characteristics of the stages were not always as clear. During these transitional stages, physical characteristics from the adjoining decomposition stages were present. This application of transitional stages is supported by Megyesi, Nawrocki, & Haskell (2005), who suggested that decomposition should be treated as a variable, albeit semi-continuous process.

The different stages of decomposition for each of the carcasses were similar with the only differences observed being the duration of each stage and the level of overlap between the stages (Figures 3.1 – 3.4).

Table 3.1. A generalised summary of decomposition stages with the characteristics of each stage and the dominant arthropods present during each stage

| **Fresh** Commenced with killing of the pig Body soft & flexible No odour Oviposition occurred	**Diptera adults** Sarcophagidae: *Sarcophaga cruentata* Muscidae: *Musca domestica*
Bloated Commenced with the observation of the first signs of bloating Blood flowing & bubbling from nose & mouth Body inflated due to gas build-up Limbs distended Livor mortis apparent Oviposition occurred	**Diptera adults** Calliphoridae: *Lucilia* spp. Calliphoridae: *Chrysomya chloropyga* Calliphoridae: *Chrysomya marginalis* Calliphoridae: *Chrysomya albiceps* Sarcophagidae: *Sarcophaga cruentata* Muscidae: *Musca domestica* Muscidae: *Hydrotea capensis*
Active Decay Commenced with the hatching of the first instar maggots Carcass started deflating Limbs returned to resting position Strong odour of decay Skin slippage with maggots feeding underneath loose skin Carcass moist Area underneath carcass saturated with body fluid (BOD)	**Diptera adults & maggots** Calliphoridae: *Chrysomya chloropyga* Calliphoridae: *Chrysomya marginalis* Calliphoridae: *Chrysomya albiceps* Sarcophagidae: *Sarcophaga cruentata* **Diptera maggots** Calliphoridae: *Lucilia* spp. **Coleoptera adults** Dermestidae: *Dermestes maculatus* Cleridae: *Necrobia rufipes* Silphidae: *Thanatophilus micans* (predators)
Advanced Decay Commenced with the maggots leaving the carcass to pupate Little carcass tissue remaining Carcass dry, some moisture remaining Odours of decomposition less Skeleton visible along skull & torso Maggots left the carcass to pupate	**Diptera adults & maggots** Piophilidae: *Piophila casei* **Coleoptera adults** Cleridae: *Necrobia rufipes* **Coleoptera adults & larvae** *Dermestidae: Dermestes maculatus* Silphidae: *Thanatophilus micans* (predators)
Dry/Remains Hair & skeleton remaining Skin remaining in a few patchy areas Carcass completely dried out	**Coleoptera adults** Cleridae: *Necrobia rufipes* **Coleoptera adults & larvae** Dermestidae: *Dermestes maculatus*
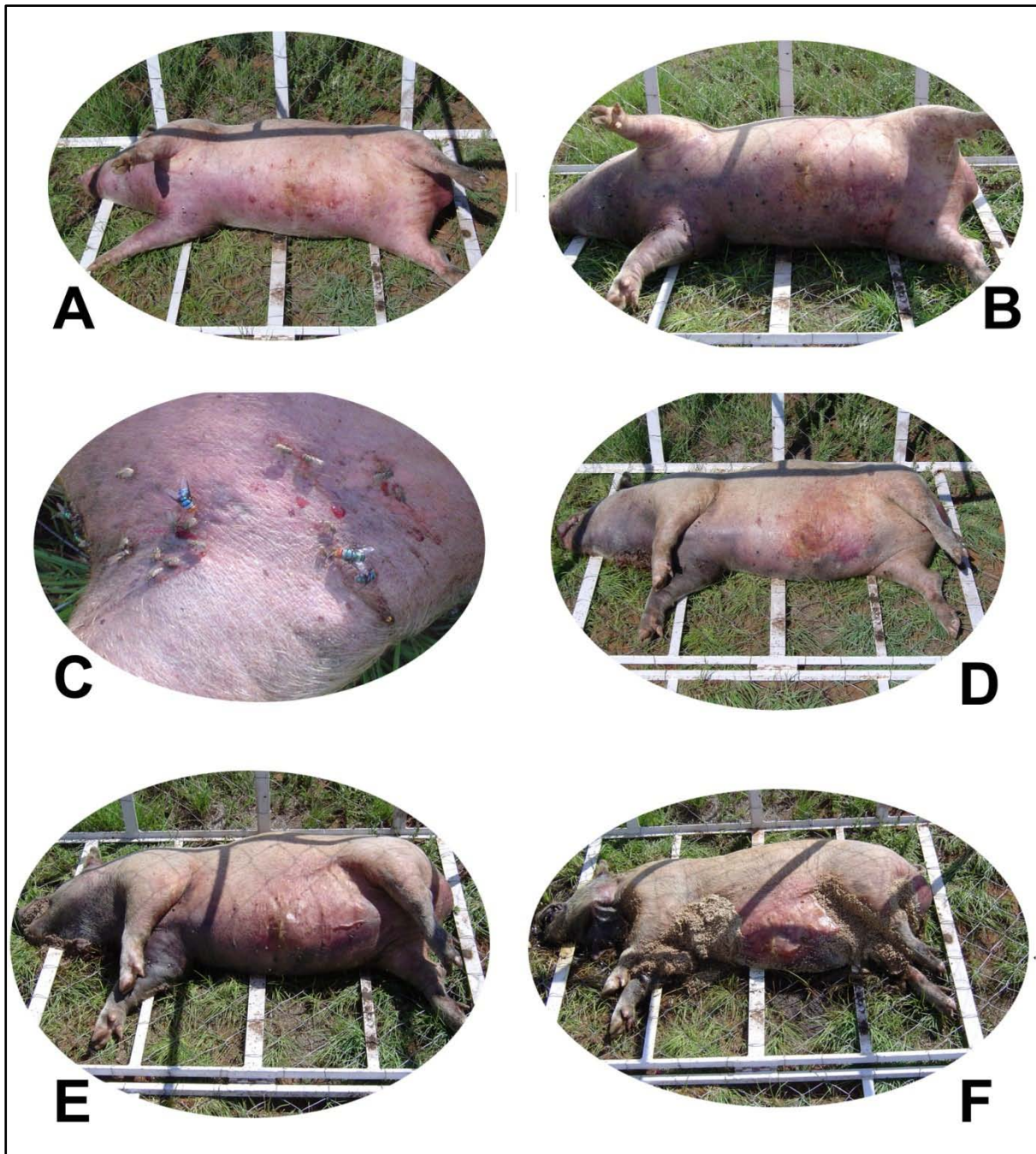
Figure 3.1 *Generalised decomposition of a Control Carcass.*

A - Start of the Bloated Stage
B - Bloated
C - Adult Diptera visiting the carcass
D - Active Decomposition started – small maggot mass in mouth
E - Active Decomposition – larger maggot mass in mouth & head
F - Active Decomposition – larger maggot mass in mouth, head, torso & abdomen

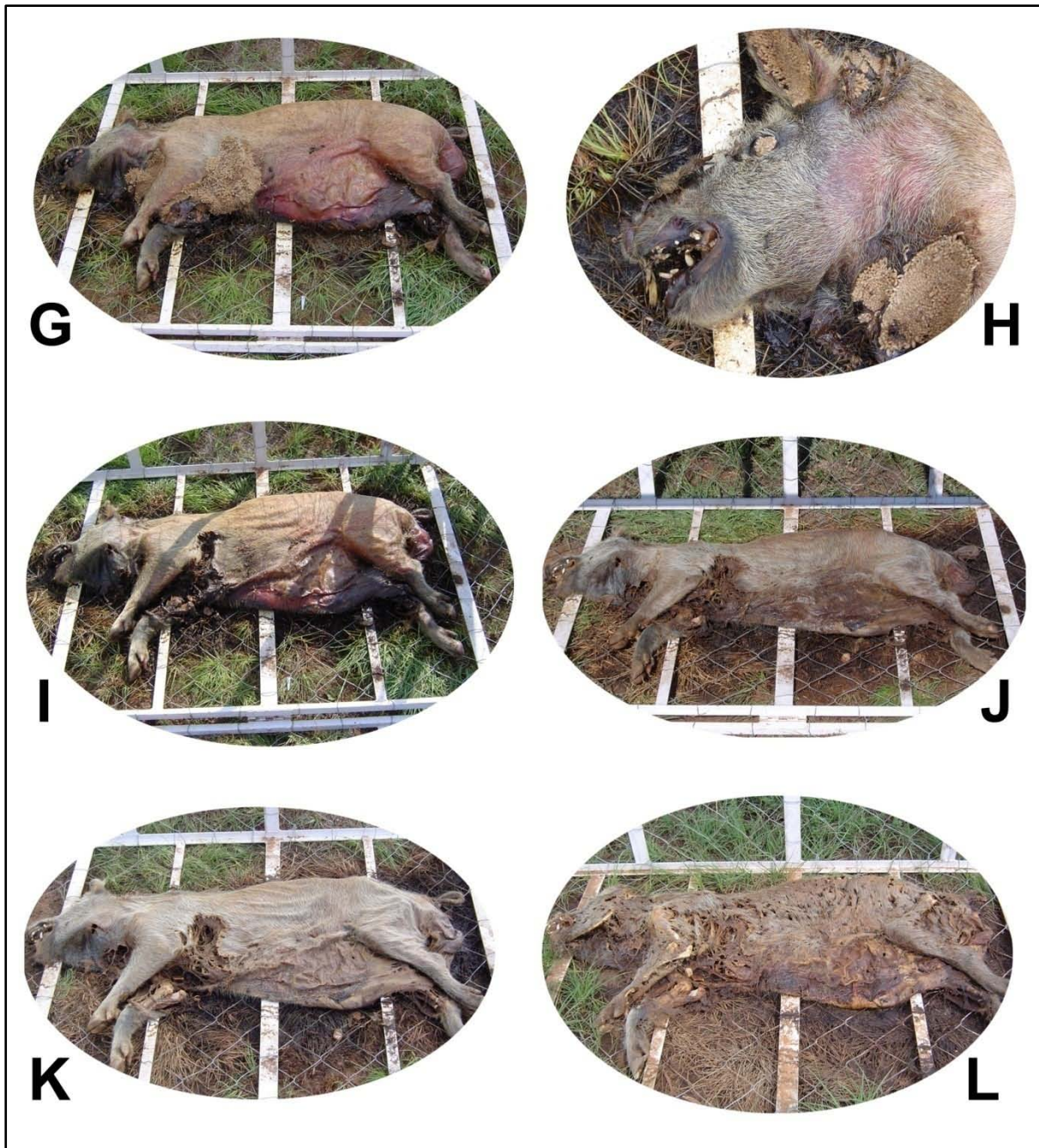*Figure 3.1 (continued)* Generalised decomposition of a Control Carcass.

G - Active Decomposition – larger maggot mass in mouth, head, torso
H - Active Decomposition – maggot mass in mouth & head
I - Advanced Decomposition – maggots started leaving to pupate
J - Advanced Decomposition – maggots left to pupate
K - Advanced Decomposition
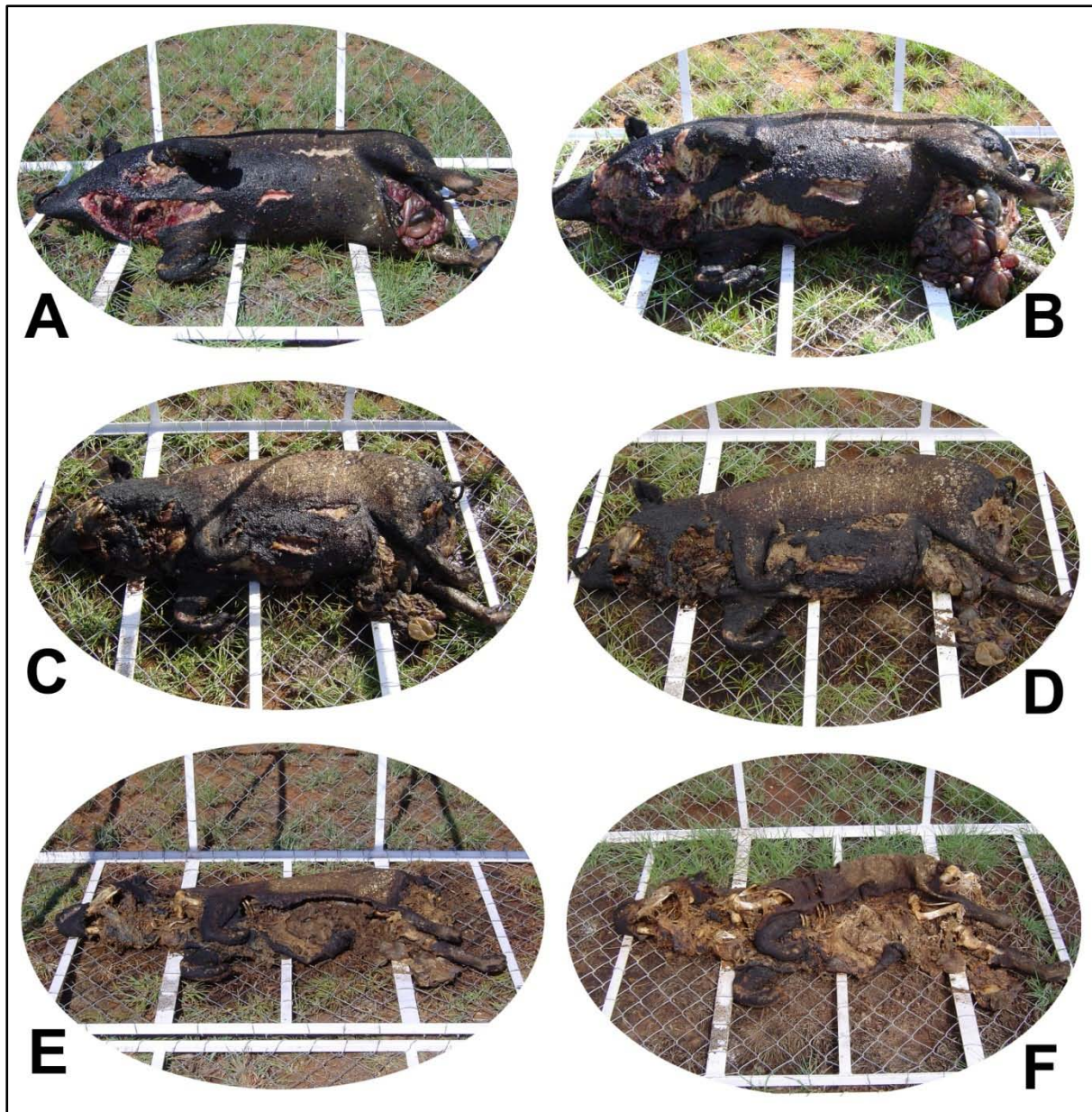L - Dry/Remains showing beetle feeding damage

Figure 3.2 *Generalised decomposition of a Slightly Burnt Carcass.*

A - Bloated - skin ruptured
B - Bloated - skin ruptured, intestines protruding
C - Active Decomposition – small maggot masses
D - Active Decomposition – larger maggot masses
E - Advanced Decomposition – maggots left to pupate
F - Advanced Decomposition
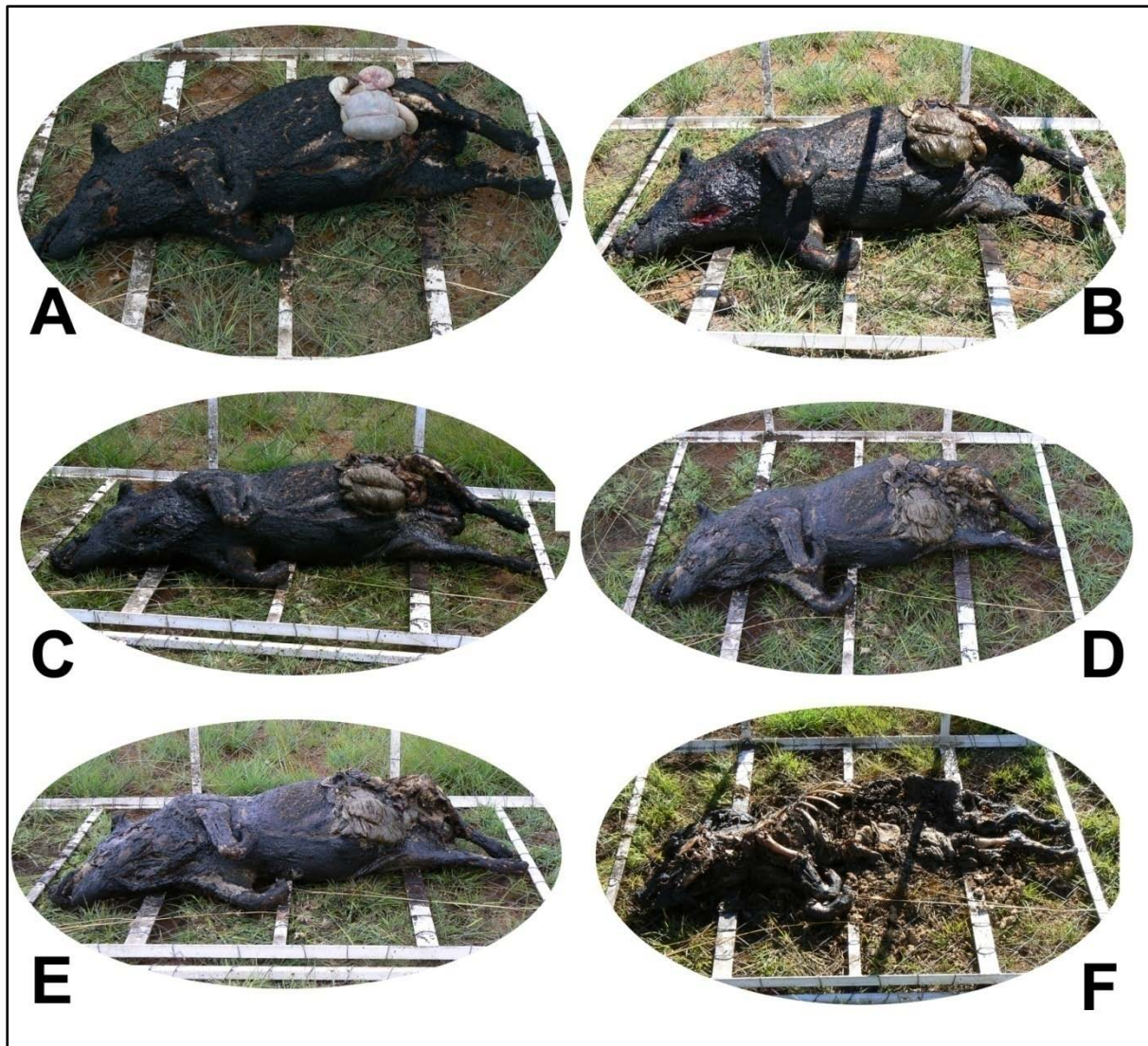
Figure 3.3 *Generalised decomposition of a Medium Burnt Carcass.*

A - Bloated - skin ruptured, intestines protruding
B - Active Decomposition – small maggot masses
C - Active Decomposition – larger maggot masses
D - Advanced Decomposition – maggots left to pupate
E - Advanced Decomposition
F - Advanced Decomposition

Figure 3.4 *Generalised decomposition of a Heavily Burnt Carcass.*

A - Bloated - skin ruptured
B - Bloated – skin ruptured, intestines protruding
C - Bloated & Active Decomposition
D - Active Decomposition
E - Advanced Decomposition - maggots left to pupate
F - Advanced Decomposition

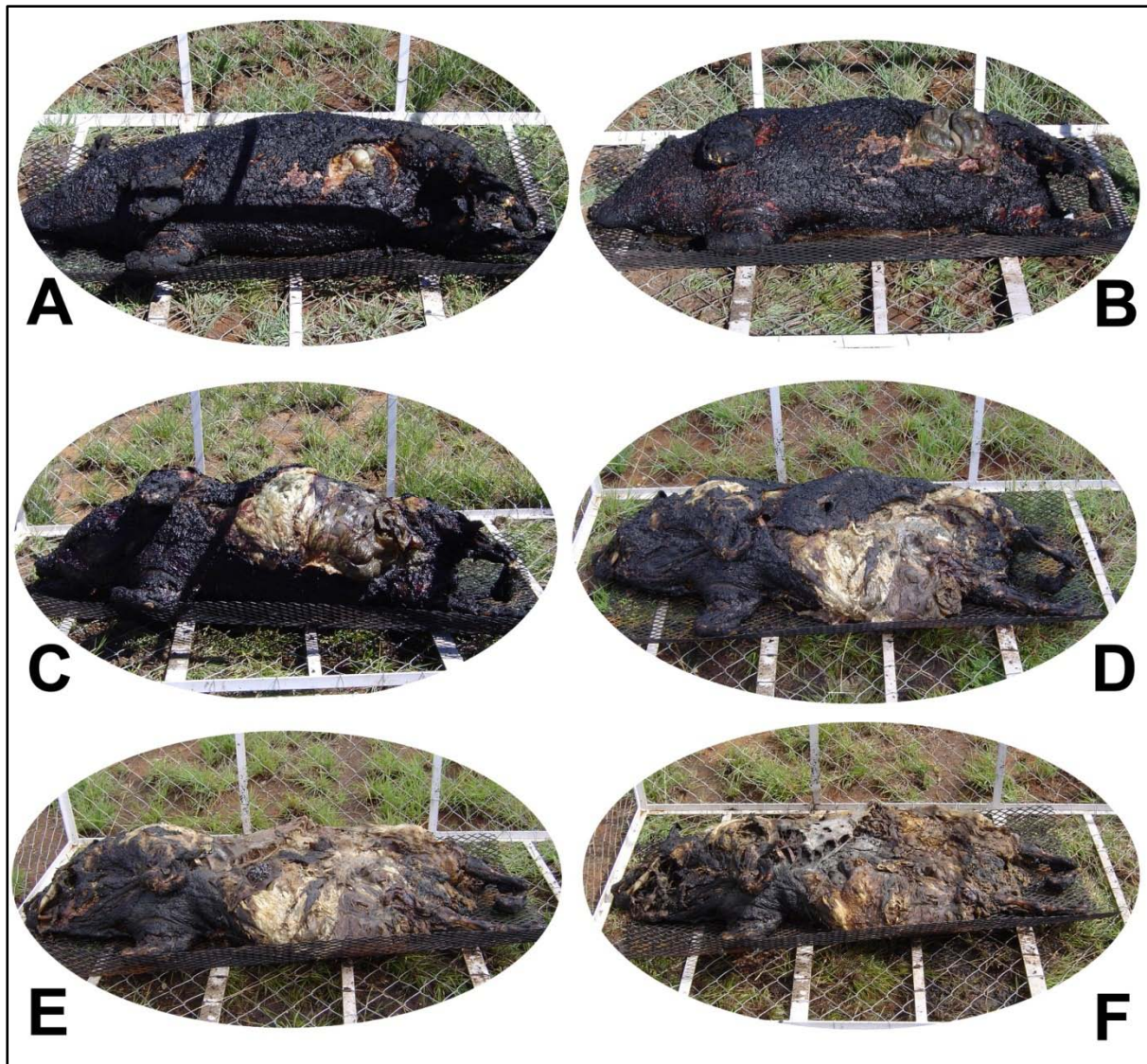# Section 3.2 The influence of burning on carcass decomposition and arthropod succession: Three Summer Studies.

3.2.1 Decomposition of the carcasses

The control carcasses were placed in the field immediately after killing and this date and time was designated as Day 0 and indicated the start of each trial (Table 3.2).

Table 3.2. Commencement dates and times of the Summer Trials

Year	Date	Time
2004	26 January	14:30
2005	18 January	14:00
2006	01 February	14:45

During the first observations, the control carcasses showed the first signs of rigor mortis, with the limbs starting to become stiff. The burnt carcasses showed signs of bloating during the first observations, with the abdominal wall ruptured in most cases. The intestines protruded from the ruptured abdomens of the burnt carcasses. The burnt carcasses had cooled down completely by the time the first observations were made.

The control carcasses remained fresh for less than one day, similar to Payne (1965). This was shorter than the period a control carcass stayed fresh in the northern hemisphere (Anderson & Van Laerhoven, 1996; Richards & Goff, 1997). The difference can be attributed to the climatic and geographic differences between the study sites. Databases should be developed in which insects are used to determine the PMI for every biogeoclimatic zone (Anderson & VanLaerhoven 1996; Anderson, 2001).

Oviposition occurred at the burnt carcasses one day prior to oviposition at the control carcass during 2004 and 2005 (Figure 3.5), supporting the findings of Avila and Goff (1998). However, due to cloudy conditions and heavy rainfall during 2006, oviposition occurred simultaneously at all the carcasses (Figure 3.5).

This could be due to lesser environmental temperature extremes during cloudy conditions causing less bloating to occur due to a smaller build up of decompositional gases. With less bloating at the burnt carcasses, decompositional gases would not have the same effect as olfactory stimulant to attract adult blow flies.

Only the days when major changes in the appearance of the carcasses and/or major changes in the insect fauna associated with the carcasses occurred, are discussed.

3.2.1.1 2004 Summer Trial

Day 1:

- Bloating was evident for the control carcass (Figures 3.5 & 3.6). Blood filled blisters were observed on the right front leg. Blood trickled from the left ear and livor mortis was also evident. Blow fly eggs were found inside the mouth. According to Avila & Goff (1998), the start of arthropod succession on a carcass is marked by oviposition. A delay in initial oviposition may consequently slow down the appearance of each successive taxon, although this effect will diminish shortly after invasion of the initial waves of arthropods.
- At the SB carcass, skin ruptures occurred on the neck, thorax and between the hind legs (Figure 3.7). Another skin rupture stretched from the neck to the abdomen. The intestines protruded from the rupture on the abdomen. Blow fly eggs and newly hatched first instar maggots were found inside the mouth. At the burnt carcasses, the active decay stage lasted from Day 1 until Day 6 (Figure 3.5).
- The MB carcass was bloated, the thoracic and abdominal skin ruptured and the intestines protruding from the abdominal skin rupture (Figure 3.8). A wet, foamy substance dripped from the neck. The burnt skin was flaking off the carcass at the genital area and the left hind leg. Blow fly eggs and first instar maggots were found inside the mouth.
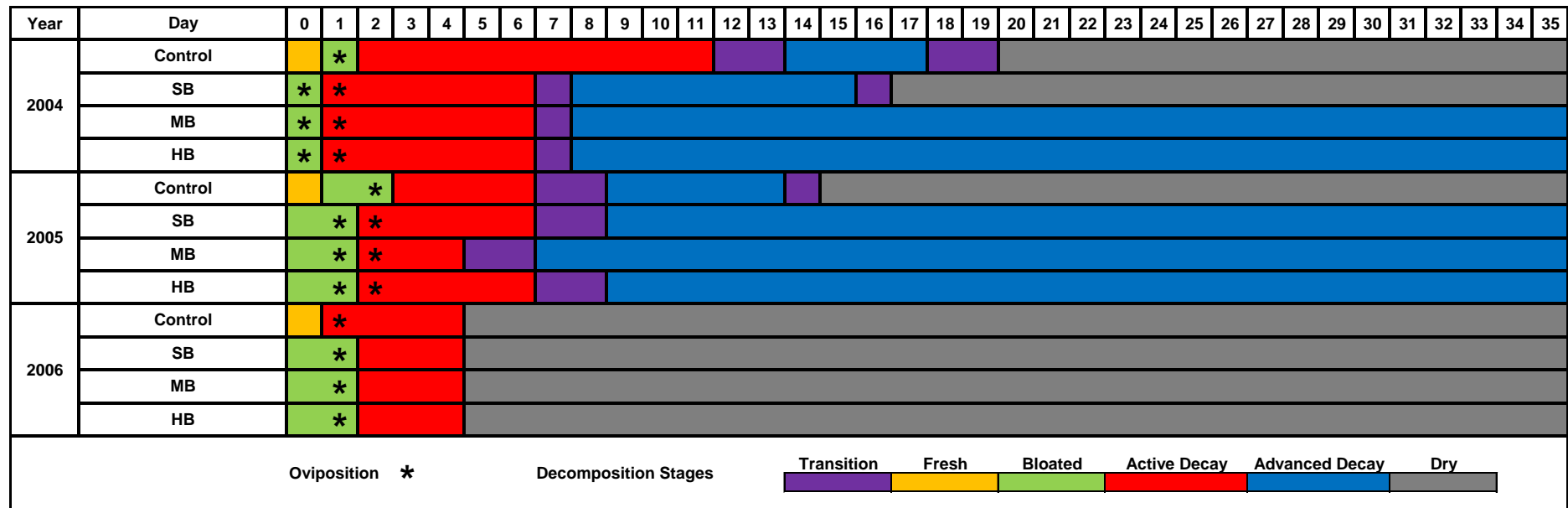
Figure 3.5 *Decomposition stages of carcasses and days of oviposition during the summer trials.*

Figure 3.6 *Bloating of the control carcass on Day 1 during the Summer 2004 Trial*

Figure 3.7 *Bloating of the SB carcass on Day 1 during the Summer 2004 Trial*

Figure 3.8 *Bloating of the MB carcass on Day 1 during the Summer 2004 Trial*

Figure 3.9 *Bloating of the HB carcass on Day 1 during the Summer 2004 Trial*

Day 1 (continued):

- The HB carcass was bloated. Skin ruptures were found on the neck, thorax and abdomen with the intestines protruding from the abdominal skin rupture (Figure 3.9). The carcass surface appeared to be moister than the MB carcass due to body fat and tissue fluid dripping from the carcass. Blow fly eggs and first instar maggots were observed inside the mouth. Formicidae carried pieces of burnt skin to their nest.

Day 2:

- The intestines of the control carcass protruded from the anus, similar to a rectal prolapse. Bloating had started to recede. Second instar maggots were feeding inside the mouth, underneath the head, on the neck and at the carcass-ground interface at the thorax. The active decay stage lasted from Day 2 until Day 11 at the control carcass. By the afternoon of Day 2, skin slippage had occurred on the right front leg and a few third instar maggots were feeding inside the mouth.
- At the SB carcass, the bloating had started to recede. First and second instar maggots were feeding underneath the head and thorax. Second instar maggots were feeding inside the mouth.
- The bloating of the MB carcass had receded. First and second instar maggots were found at the carcass-ground interface underneath the protruding intestines. According to Anderson & VanLaerhoven (1996), this is caused by the possible attraction of gravid female blow flies which are ready to oviposit to the areas of body fluid accumulation and seepage. Second instar maggots and a few third instar maggots remained inside the mouth.
- Bloating had started to recede at the HB carcass. Blow fly eggs, first and second instar maggots were found inside the mouth. Blow fly eggs were also observed in the skin rupture of the thorax.

Day 3:

- Bloating had receded even further at the control carcass. The teeth were falling out of the mouth. Third instar maggots were actively feeding on the thorax, between the hind legs and at the anus.
- Third instar maggots were feeding underneath the SB carcass.
- Third instar maggots were found in the abdominal cavity and underneath the MB carcass.
- A single third instar maggot was found on the thorax of the HB carcass. Dead third instar maggots were also found inside the mouth.

Day 4:

- The whole of the control carcass was deflated in appearance. Third instar maggots were feeding deep inside the head. They could not be seen, but their movement/feeding activity could be heard with the naked ear placed at the mouth of the carcass. Third instar maggots were also feeding in the thorax and between the hind legs.
- Third instar maggots were feeding underneath the SB carcass.
- A large mass of third instar maggots was found inside the head and thoracic cavity of the MB carcass.
- Third instar maggots were found underneath the head at the HB carcass.

Day 5:

- The maggot masses on the control carcass were smaller.
- Third instar maggots were found in the skin rupture on the head and underneath the head at the HB carcass. Dead third instar maggots were also found on the carcass surface.

Day 7:

- Dead maggots were found on the control carcass surface on Day 7. This phenomenon could be due to high carcass surface temperatures exceeding the upper thermal tolerance limit of the maggots.
- Third instar maggots and postfeeding maggots were found underneath the SB carcass.
- Postfeeding *C. marginalis* and *C. albiceps* maggots were found underneath the MB carcass.

The maggots started to migrate away from the control carcass to pupate on Day 12 and Day 13, starting the Advanced Decay stage. Advanced Decay had started at the SB carcass on Day 8 and lasted until Day 15. The Dry/Remains stage commenced at the control and SB carcasses on Days 20 and 17, respectively (Figures 3.5, 3.10 & 3.11). This stage was not reached by the MB and HB carcasses (Figures 3.12 & 3.13), where more than 50% of the biomass remained (See 3.2.1.4).

3.2.1.2 2005 Summer Trial

Bloating lasted for two days at the carcasses. At the control carcass this was Day 1 and Day 2 and at the burnt carcasses Day 0 and Day 1 (Figure 3.5).

Day 1:

- Livor mortis was evident at the control carcass. The blood in the blood vessels turned green due to the invasion of the blood vessels by anaerobic bacteria from the abdomen. This occurred in a mosaic pattern and gave the carcass surface a marble-like appearance, also known as marbling (Goff, 2009).
- As in 2004, the burnt carcasses show greater bloating, with skin ruptures on the thorax and abdomen, with the intestines protruding from the ruptured abdominal wall. Bodily fluid seeped out of the skin ruptures, leaving a fatty residue on the skin surface.

Figure 3.10 *Control carcass in Dry/Remains stage at the end of the Summer 2004 trial*

Figure 3.11 *SB carcass in Dry/Remains stage at the end of the Summer 2004 trial*

Figure 3.12 *MB carcass in Advanced Decomposition stage at the end of the Summer 2004 trial*

Figure 3.13 *HB carcass in Advanced Decomposition stage at the end of the Summer 2004 trial*

Day 2:

- The abdominal skin of the control carcass was ruptured. The intestines and liver protruded from this rupture. Blow fly eggs were found in the ears, and at the carcass-ground interface at the thorax, just behind the front legs.
- At the SB carcass, the skin was ruptured on the neck, between the front legs, on the thorax and left hind leg. First and second instar maggots were feeding in the mouth and skin rupture on the hind leg. First and second instar maggots were also feeding at the carcass-ground interface at the abdominal rupture.
- Skin ruptures was also found at the MB carcass on the neck, thorax, back and abdomen. Blow fly eggs were observed on the ground at the carcass-ground interface all along the carcass. First instar maggots were feeding in the skin ruptures on the thorax. First and second instar maggots were feeding inside the mouth and the skin rupture on the neck.
- At the HB carcass, skin ruptures had occurred on the abdomen and from the neck to the lower abdomen. Blow fly eggs were present in the skin ruptures and first instar maggots were feeding inside the mouth.
- The Active Decay stage lasted from Day 2 until Day 6 at the burnt carcasses, except at the MB carcass where it lasted until Day 4 (Figure 3.5).

Day 3:

- The eggs in the ears of the control carcass had hatched with first instar maggots feeding in the ears, mouth and on the head. This effectively started the Active Decay stage which lasted until Day 6.
- At the burnt carcasses, the bloating had started to recede.
- Second instar maggots were found at the SB and MB carcasses, with third instar maggots at the HB carcass.

The Advanced Decay stage started on Day 9 for all the carcasses, except at the MB carcass where it started on Day 7. This stage lasted until Day 13 for the control carcass. The burnt carcasses remained in the Advanced Decay stage until the end of the trial (Figure 3.5).

Three, two and four postfeeding *C. albiceps* maggots were respectively found underneath the control, MB and HB carcasses on Day 10.

On Day 15 the Dry/Remains stage commenced at the control carcass (Figure 3.5). This stage was not reached at the burnt carcasses due to the muscle, body fat and viscous tissue remaining at each of these carcasses. The burnt carcasses were in the Advanced Decomposition stage (Figure 3.5). Formicidae predated on the newly emerged adult blow flies at the SB carcass on Day 15.

3.2.1.3 2006 Summer Trial

On Day 0 the control carcass was still fresh, whilst bloating was evident for the burnt carcasses.

Day 1:

- At the control carcass, first instar maggots hatched from a large egg mass underneath the head and right shoulder at the carcass-ground interface. A small egg mass was also present inside the mouth. The active decay stage lasted from Day 1 until Day 4 at the control carcass (Figure 3.5).
- Larger bloating was evident with skin ruptures on the neck of the SB carcass. Blood and clear fluid dripped from the skin rupture. Blood was also dripping from of the mouth and head. A small egg mass was visible inside the mouth.
- Bloating was evident at the MB carcass with a skin rupture on the neck. A piece of burnt skin was flaking off the carcass. Oviposition had occurred in the mouth and on the thorax.
- Bloating was evident with skin ruptures on the neck, thorax and abdomen of the HB carcass. The intestines protruded from the skin rupture and were burnt (happened during the burning of the carcass. A small egg mass was found between the hind legs.

Bloating lasted until Day 4 at the burnt carcasses (Figure 3.5).

Day 2:

- The control carcass still showed signs of bloating. The blood collected on the underside of the body (livor mortis) and the blood in the blood vessels had turned green, creating a marble-like appearance on the carcass surface. First instar maggots were actively feeding inside the mouth and in the area of the neck at the carcass-ground interface.

- The SB carcass was bloated with the abdomen ruptured in two places. The intestines protruded from both these ruptures. First instar maggots were feeding inside the mouth, on the thorax surface, the right front leg and the area of the neck and thorax at the carcass-ground interface, where there still was a mass of unhatched eggs.

- The MB carcass was still bloated with ruptured skin on the thorax and abdomen. First and second instar maggots were actively feeding on the surface of the head, neck and thorax.

- The HB carcass showed less bloating, with first and second instar maggots feeding on and inside the thoracic skin rupture. Second instar maggots were feeding inside the mouth, head and on the surface of the back.

No observations were made on Days 3 and 4 due to prolonged rain which made the study site inaccessible. During previous fieldwork when less rainfall was recorded, the study site became so slippery with thick layers of mud that the vehicle became stuck. Rather than risk a repeat of such a scenario, it was decided to try and investigate on foot. No viable route could be found leading to the carcasses. Due to the severity of the mud the author had to throw away his shoes and abandon his efforts of trying to reach the carcasses.

Observations resumed on Day 5 when the ground was drier with less mud. The carcasses were reached on foot and observations revealed the carcasses to be stripped of all utilisable tissue by feeding maggot masses. Skin and bone was all that remained of the carcasses. The maggots had already migrated from the carcasses to pupate which had signaled the onset of the advanced decay stage.

This stage could not have lasted for more than a day due to the fact that only skin and bone remained at all the carcasses on Day 5. This effectively placed all of the carcasses in the Dry/Remains stage (Figure 3.5), even the MB and HB carcasses which previously had not reached this stage (Figures 3.14 & 3.15). This could be due to the very heavy rainfall that occurred which had kept the carcass tissue moist enough to be consumed by the maggot masses.

The Dry/Remains stage was a bit of a misnomer, since none of the carcasses were technically dry due to being saturated with water after the rain. Observations continued to Day 15 with little to no beetle activity observed at any of the carcasses.

This rapid decomposition was attributed to the preceding heavy rainfall (See Section 3.2.5 & Figure 3.38) and extremely large maggot masses that were feeding on the carcasses. Together with the rainfall, a constant blanket of stratocumulus clouds helped keep the ambient temperature relatively constant at an average of 21.6°C, perfect conditions for large maggot masses to thrive. Blow fly maggots are the initial and major consumers of carrion and the most important entomological indicator in evaluating human decomposition (Shean *et al.* 1993). The heavy rainfall did not seem to have an effect on the maggot activity, but adult blow fly activity dramatically decreased. The maggots remained hidden inside the body cavity and continued feeding. This observation supports findings by Mann *et al.* (1990).

3.2.1.4 Mass Loss

As mentioned in Section 2.1.3.2, the mass of each carcass was recorded daily during the first trial of each season. This was only done during the first trial of each season to minimize the disturbance to the carcasses and the possible effect of this disturbance on decomposition and insect succession.

Figure 3.14 MB carcass in Dry/Remains stage at the end of the 2006 Summer trial.

Figure 3.15 HB carcass in Dry/Remains stage at the end of the 2006 Summer trial.

During the 2004 trial, the fastest decomposition occurred at the control carcass (Figure 3.16). This carcass also had the most tissue removed. The SB and MB carcass decomposed at a similar rate, with slightly more tissue removed from the SB carcass than the MB carcass. The slowest decomposer with the most tissue remaining was the HB carcass(Figure 3.16). Payne's findings (1965) showed similar decomposition to the control carcass for all his exposed carcasses.
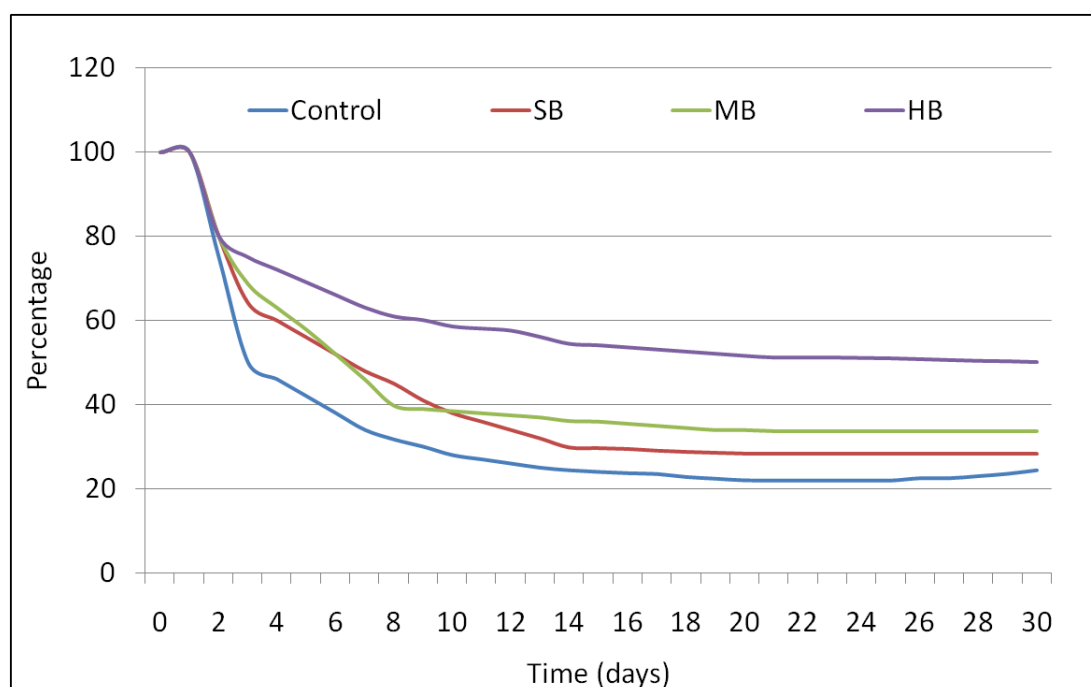

Figure 3.16 *Percentage body mass remaining of carcasses during the Summer 2004 trial.*

During 2005 and 2006, the carcasses did not always follow the abovementioned decompositional trend. No data are available for the actual rate of decomposition, since the mass of each carcass was not recorded daily to limit disturbance to the carcass fauna. Visual observations confirmed that the SB carcass decomposed quicker than the control carcass during 2005. The MB and HB carcasses decomposed the slowest.

According to Tullis & Goff (1987), the rapid mass loss of the carcasses is the result of conversion of carcass biomass to dipteran larval biomass and the subsequent departure of these larvae from the remains to pupate. They made no mention of the contribution of desiccation/fluid loss to mass loss.

The gain of mass during decomposition may be attributed to arthropod arrival on the carcass combined with rainfall (Tullis & Goff 1987).

Richards & Goff (1997) found that the greatest percentage of biomass was removed during the decay stage because of the maggot feeding masses.

3.2.2 Arthropod Composition

According to Catts & Goff (1992), the ecological roles of the arthropods visiting the carcasses may be placed into four categories:

(i)   necrophagous (species feeding on corpse tissue)

(ii)   predators and parasites (*e.g.* mantids, robberflies, beetles, ants and wasps)

(iii) omnivores (*e.g.* ants, wasps, and some beetles that feed on both the corpse and associated fauna)

(iv)   incidentals/tourists (arthropods that used the corpse as an extension of their normal habitat)

These categories were also used during this study, the only difference is that the term "tourists" is preferred to incidentals.

3.2.2.1 Orders

3.2.2.1.1 2004 Summer Trial

The dominant order during 2004 at the control carcass were Diptera. The dominant order at the burnt carcasses were Coleoptera. The decrease in dominance of Diptera increased with the degree of burning of the carcasses (Figure 3.17).
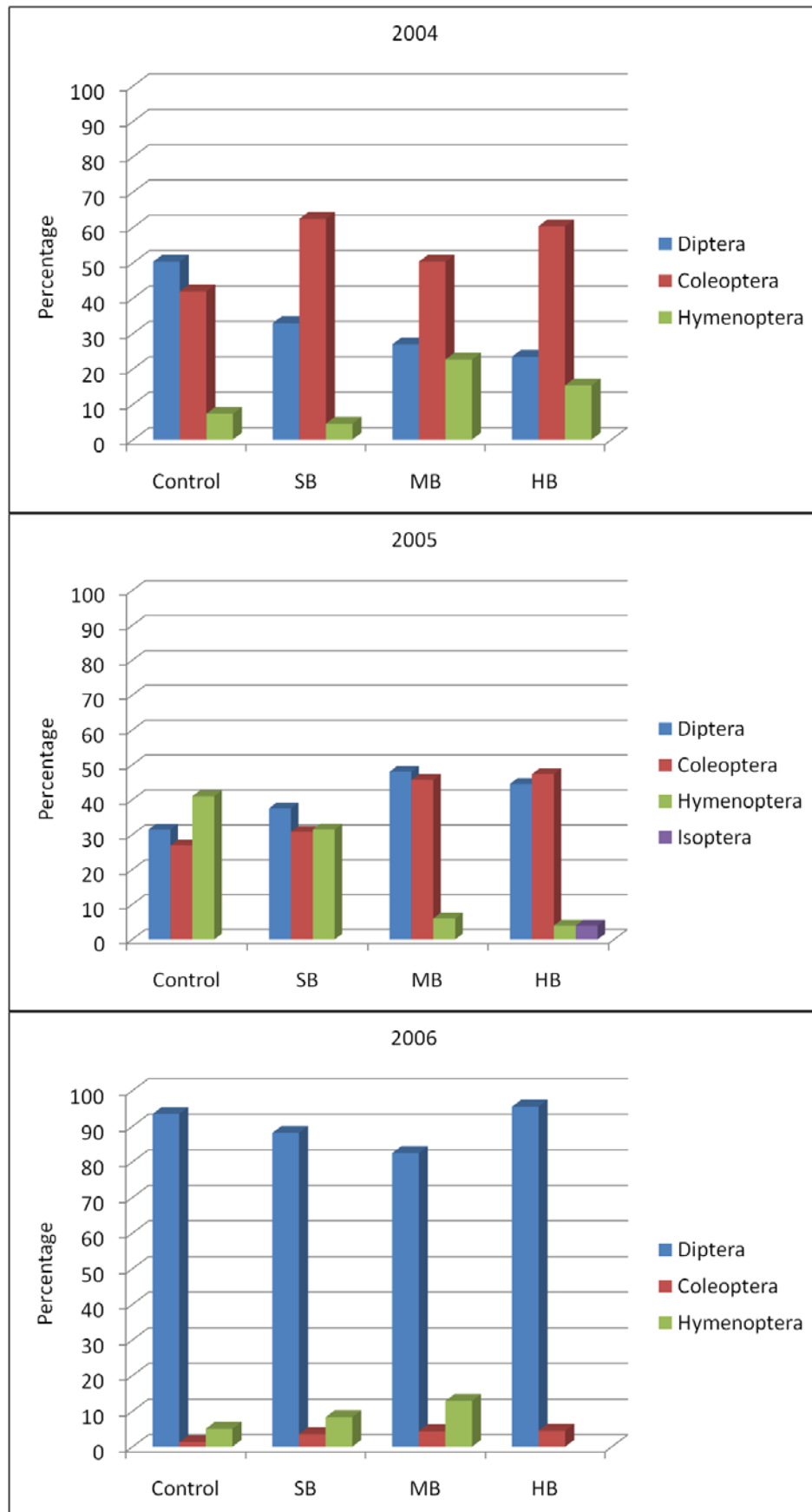
Figure 3.17 *Order composition at each carcass during the different summer trials*

3.2.2.1.2 2005 Summer Trial

During 2005 the dominant order at the control carcass were Hymenoptera. Since the Hymenoptera did not directly influence decomposition, the dominant decomposer at the control carcass were Diptera. Diptera were also dominant at the SB and MB carcasses, whilst Coleoptera were dominant at the HB carcass. The dominance of Coleoptera increased with the degree of burning of the carcasses due to the carcasses being increasingly dry with the increase in the degree of burning (Figure 3.17).

3.2.2.1.3 2006 Summer Trial

Diptera were dominant at all the carcasses during 2006. Hymenoptera were recorded at all the carcasses, except for the HB carcass. The dominance of Hymenoptera increased with the degree of burning of the carcasses. Low numbers of Coleoptera were recorded at all the carcasses due to the rapid decomposition (Figure 3.17).

3.2.2.2 Diptera

Muscidae were not observed to breed in any of the carcasses. Therefore no Muscidae maggots were observed to be necrophagous and thus had no effect on the decomposition process. Adult Muscidae were observed to feed on bodily fluids dripping from the carcasses (BOD).

3.2.2.2.1 2004 Summer Trial

Calliphoridae were the dominant necrophagous family at the control and the MB carcasses, with Muscidae dominant at the SB and HB carcasses (Figure 3.18). The dominant necrophagous family at the SB and HB carcasses were Calliphoridae (Figure 3.18).
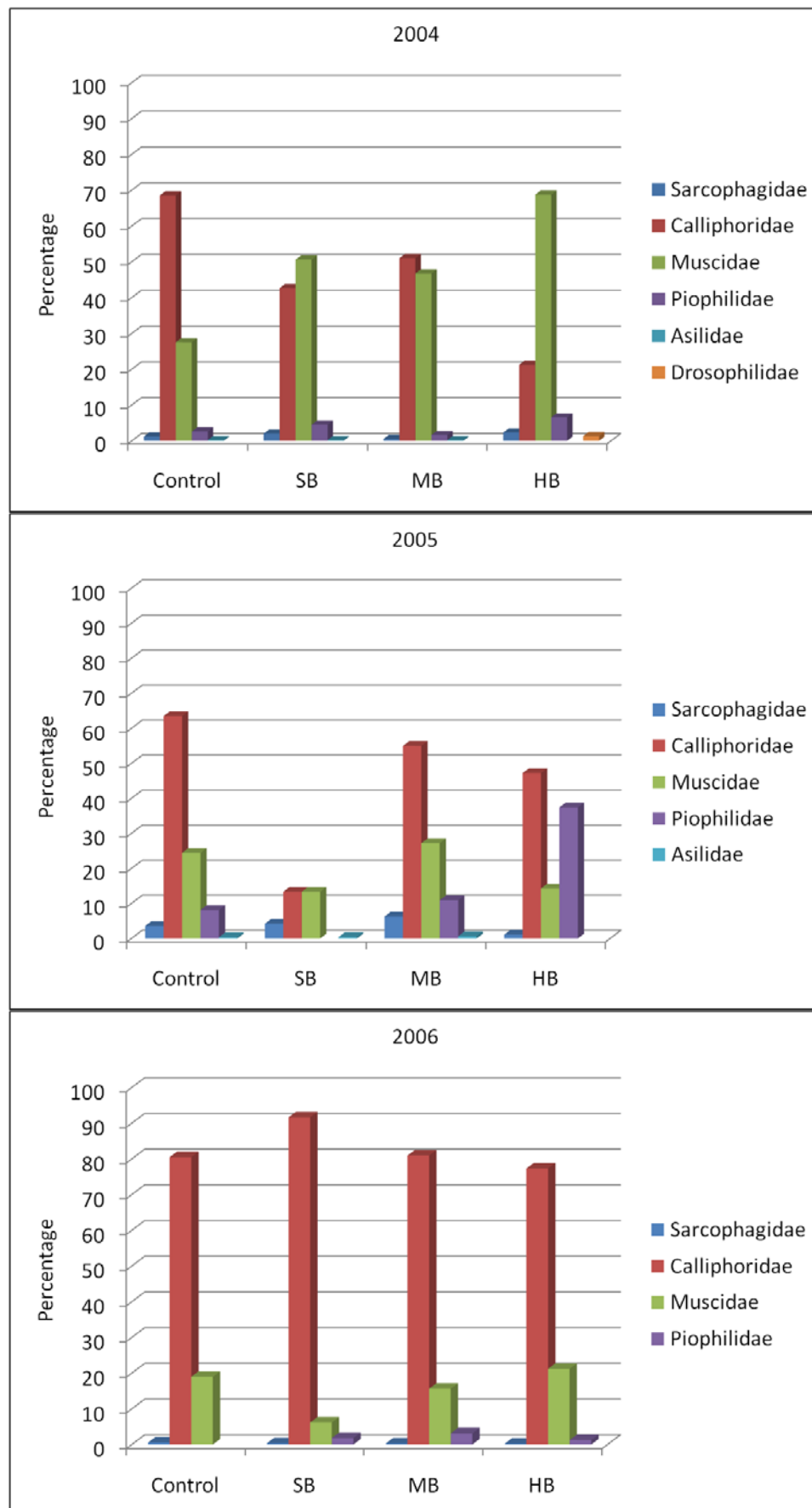
Figure 3.18 *Diptera composition at each carcass during the different summer trials*

3.2.2.2.2 2005 Summer Trial

The dominant necrophagous Diptera species at the control carcass were *C. marginalis* and *C. albiceps* (Figure 3.19). The dominant Diptera species at the SB and HB carcasses were *Musca domestica* Linnaeus (Figure 3.19). The dominant necrophagous insects at the SB and MB carcasses were *C. marginalis* and *C. albiceps.* At the HB carcass the dominant necrophagous insects were *Lucilia sp, C. marginalis* and *Piophila casei* (L.) (Figure 3.19).

3.2.2.2.3 2006 Summer Trial

Calliphoridae were the dominant family at all carcasses (Figure 3.18), with *C. marginalis* the dominant species at all carcasses (Figure 3.19).

3.2.2.3 Coleoptera

Dermestidae were represented by a single species, *viz. Dermestes maculatus*, as were Cleridae by *Necrobia rufipes*. Braack (1987) also recorded the representation of Dermestidae and Cleridae by one species each.

During this study, *N. rufipes* occurred on the carcasses with the dermestids and was observed feeding only on carrion, supporting observations by Payne & King (1970). *Necrobia rufipes* has also been recorded as a predator of the cheese skipper (Piophilidae) and Dermestidae larvae (Reed, 1958).

Silphidae (necrophagous and predaceous) were represented by *Thanatophilus micans* L. Histeridae (predaceous) species were unidentified.
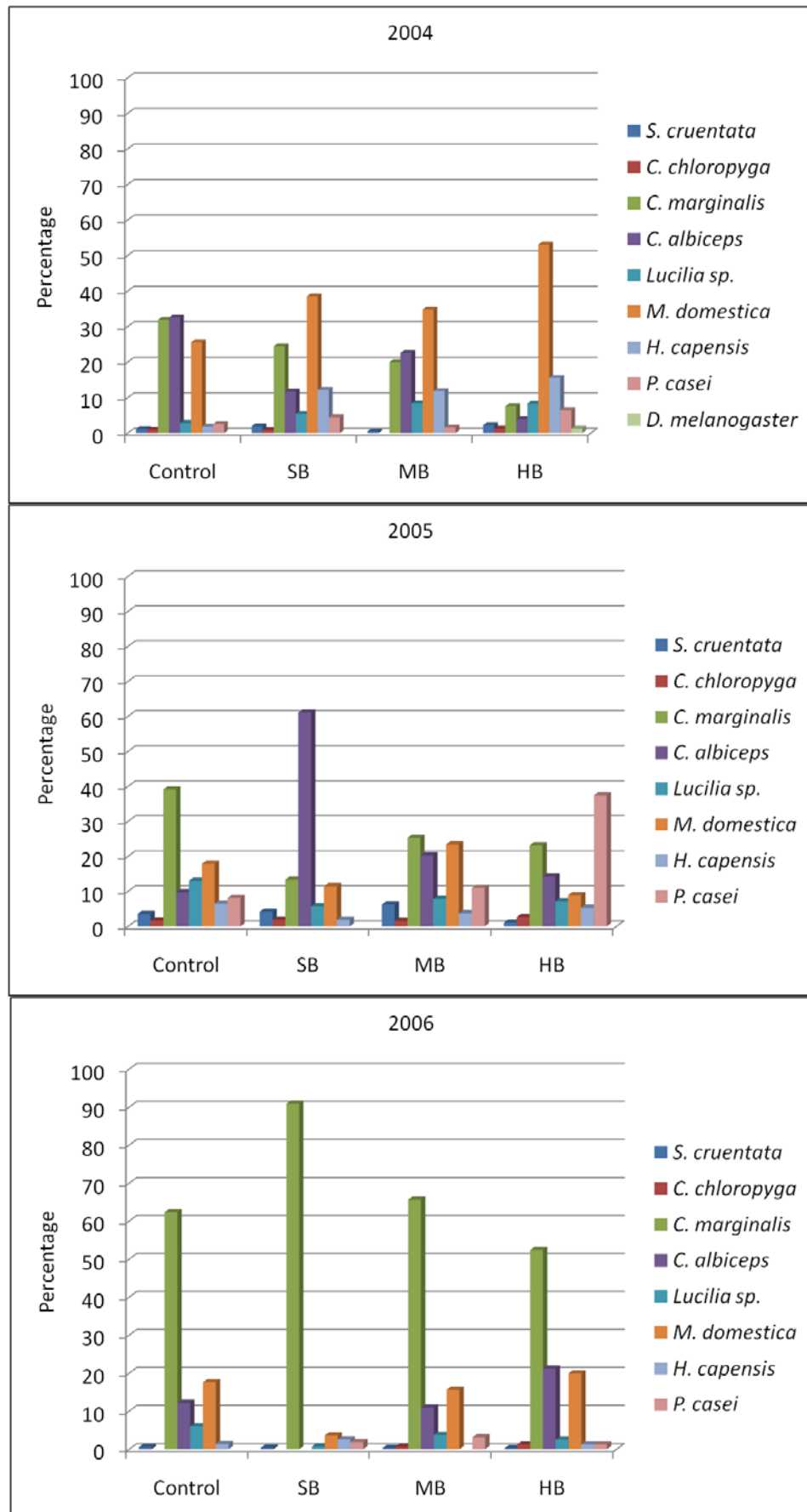
Figure 3.19 *Diptera species composition at each carcass during the different summer trials*

3.2.2.3.1 2004 Summer Trial

Coleoptera were dominated by Dermestidae at all the carcasses. With the exception of the control carcass, the dominance of Dermestidae increased and the dominance of Cleridae diminished with an increase in the degree of burning of the carcasses. Low numbers of Histeridae were also recorded at all carcasses (Figure 3.20).

3.2.2.3.2 2005 Summer Trial

Dermestidae dominated at all the carcasses, with the highest dominance at the HB carcass and the lowest at the MB carcass. A higher incidence of Cleridae and Dermestidae were recorded at all the carcasses than was the case during 2004. Low numbers of Silphidae were recorded at the MB carcass (Figure 3.20).

3.2.2.3.3 2006 Summer Trial

The only Coleoptera recorded at the control carcass were Histeridae. The only occurrence of Histeridae at the burnt carcasses was at the HB carcass in low numbers. Cleridae were dominant at the burnt carcasses. Low numbers of Dermestidae occurred at the SB and MB carcasses. Low numbers of Silphidae and Scarabaeidae were observed at the SB and HB carcasses (Figure 3.20). These numbers could be misleading due to the extremely short duration of decomposition, together with two days during which no observations were made due to rain.

3.2.3 Arthropod succession on the carcasses

A carcass of a dead animal or human corpse is a uniform ecological unit. Although the insects inhabiting the body are different, the ecological principles for exploiting this resource are the same throughout the world. Insects occupy the niche of scavengers. The carcass supports an association of insects that are brought together by common feeding or reproductive functions, along with their predators and parasites. The carcass represents a microcosm that can be conveniently studied and analysed (Fuller, 1934).
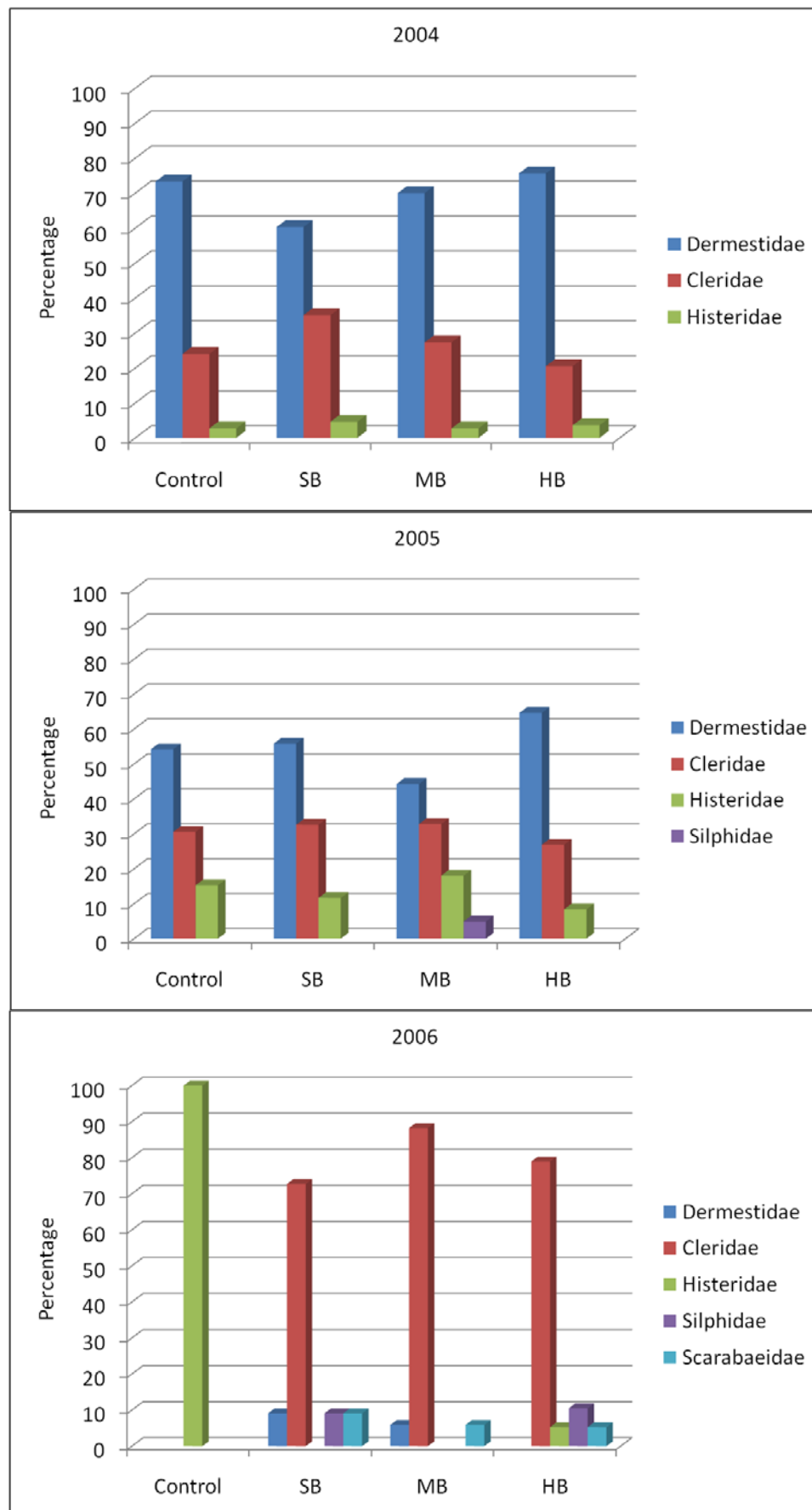
Figure 3.20 *Coleoptera composition at each carcass during the different summer trials*

The most abundant species observed at the carcasses during summer were C. *marginalis, C. albiceps, Lucilia sp., M. domestica, D. maculatus* and *N. rufipes* (Figure 3.21).

Adult Calliphoridae and Sarcophagidae were the first insects to arrive, within five minutes of exposure. These observations were in agreement with many previous studies (Hall & Doisy, 1993; Tullis & Goff, 1987; Hewadikaram & Goff, 1991). Shean *et al*. (1993) observed that blow flies were attracted to carcasses within 20 minutes of death and oviposition was observed 2 to 3 hours later. Sarcophagidae were represented by only one species – *Sarcophaga cruentata* Meigen. Sarcophagidae are strong fliers and will often arrive first at a carcass during conditions that will hamper the flight capability of other Diptera (Byrd & Castner, 2001). These conditions include strong winds and rain.

Calliphoridae were initially present at the carcasses in large numbers for two consecutive days. In a study by Schoenly & Reid (1983), adult Diptera were collected in large numbers shortly after carcass deposition (days 2-4) when tissues were fresh and at a time when the carcass presumably reached peak levels of attractiveness for feeding, oviposition, and larviposition.

Chrysomya marginalis generally fed on and inside the carcasses, while *C. albiceps* fed underneath the carcasses. This partitioning of a single resource, according to succession within the carcass, will permit the co-existence of several species of carrion flies (Schoenly & Reid 1987). *Chrysomya albiceps* maggots were also observed to predate on *C. marginalis* larvae on two occasions. Richards & Goff (1997) noted that larvae of *C. rufifacies* were facultative predators of *C. megacephala* and remained under the carcass even after the carcass resource was depleted. Because of this potential threat, other species of maggots may leave the carcass before *C. rufifacies* to avoid predation. Braack (1987) also noted partitioning of the carcass resource. In Braack's study, conducted in Africa, two species in the family Calliphoridae, C*. marginalis* and *C. albiceps* mimic the pattern observed in Hawaii in *Chrysomya megacephala* and *Chrysomya rufifacies*, respectively (Richards & Goff, 1997).
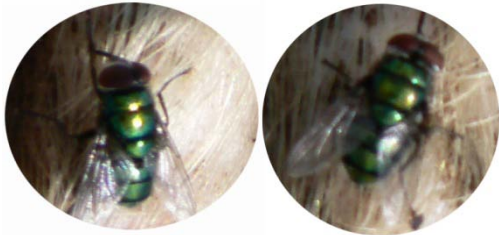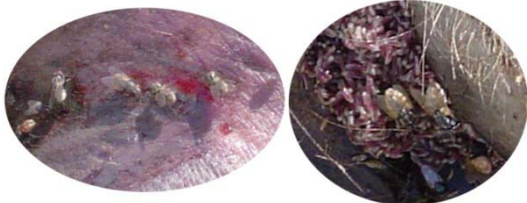
Figure 3.21 *Most abundant species observed during the Summer Trials.*

Photographs not taken by the author:
    i (Gross, 2005)
    ii (Gross, 2005)
    iii (London Natural Science Museum, 2004)
    iv (Makarov, 2006)

These patterns were not only found in the departure from the carcass but also in the sequence of initial oviposition. In Hawaii, *C. megacephala* is generally the first species to deposit eggs, followed by *C. rufifacies* (Richards & Goff, 1997). Braack (1987) and Kelly (2006) noted that *C. marginalis* would oviposit first, followed by the predatory *C. albiceps*. This also occurred during the current study.

Decomposing carcasses are quickly found by insects and colonised in a predictable sequence called insect succession (Payne, 1965; Smith, 1986; Tullis & Goff, 1987; Catts & Haskell, 1990). As the carcass ages, it progresses through a series of different decomposition stages, each attractive to a different group of insects (guilds). This phenomenon has been known for over 100 years since Mégnin identified the fauna on cadavers. The study by Mégnin (1894) is generally regarded as the classic work in the field of carcass-inhabiting insects (Payne 1965; Tullis & Goff 1987).

Each insect species colonising or using the decomposing carcasses at any particular stage did so because the particular conditions at that time suited its specific requirements. In its exploitation of the carcass, that species altered the carrion medium and changed its characteristics. This occurred to such an extent that conditions became less suitable for the insects own continued occupation. At the same time, the carrion changed to such an extent that it became more suitable for another species that were unable to use the carrion in its previous state. Insect succession can therefore be ascribed to a combination of factors, such as the alteration of the medium by earlier inhabitants, rendering the medium more favourable to their successors.

It could also be attributed to the stimulation of different species to oviposit at different stages, combined with the effect of competition, or both (Boucher, 1997).

Succession at carcasses is viewed as being inherently different from the traditional concept of succession because the habitat is non-replenishing and does not lead to a climax community. Carrion therefore represents an ephemeral resource with no steady progression to a stable climax community with a reasonable prospect of long-term existence (Braack, 1987).

Only the numbers of Coleopteran larvae are indicated, since it was easier to count these larvae than was the case with the maggots. All fauna referred to are adults, unless otherwise indicated Figures 3.22 – 3.24).

3.2.3.1 Control

Sarcophagidae, Calliphoridae, Muscidae and Formicidae were the first arrivals at the carcass during 2004 and 2006 (Figures 3.22 & 3.24). During 2005, the first arrivals were Sarcophagidae, Muscidae and Formicidae on Day 1, with Calliphoridae present at the carcass on Day 2 (Figure 2.23).

A second record of large numbers of adult Calliphoridae (*C. marginalis* & *C. albiceps*) were found at the carcass during 2004 from Day 14 (Figure 3.22). These adults were in the vicinity of the carcass when they emerged from the puparia and visited the carcasses to take a protein meal. It was evident that these adults had recently emerged from the puparia since not all of them were fully sclerotised. Large numbers of adults were also found on the surrounding vegetation. *Chrysomya albiceps* and *C. marginalis* are crucial species because of the ability of the immature stages (maggots) to rapidly consume all carcass soft tissue. By their presence and action on the carcass they can drastically influence other members of the carrion community (Braack, 1986).
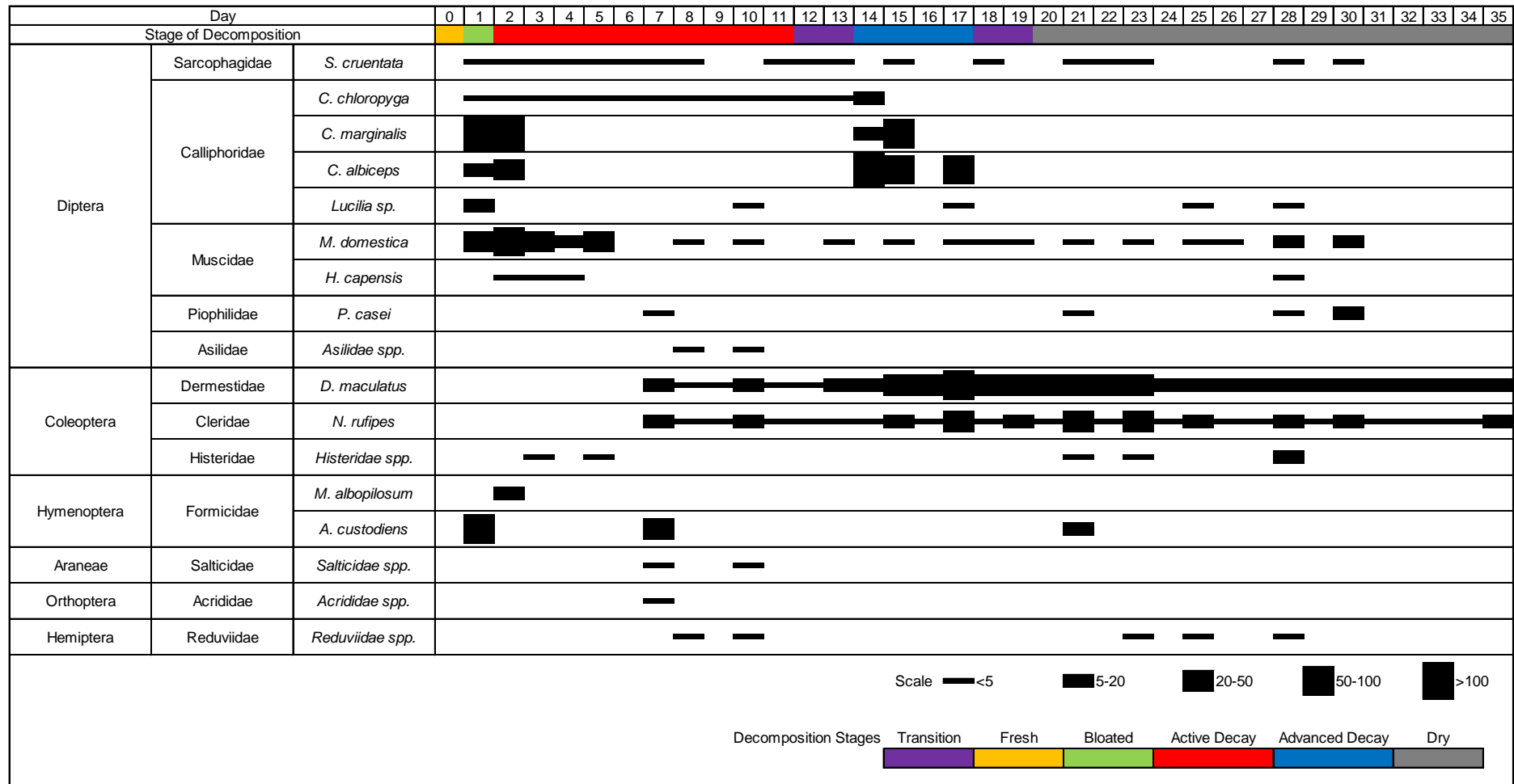
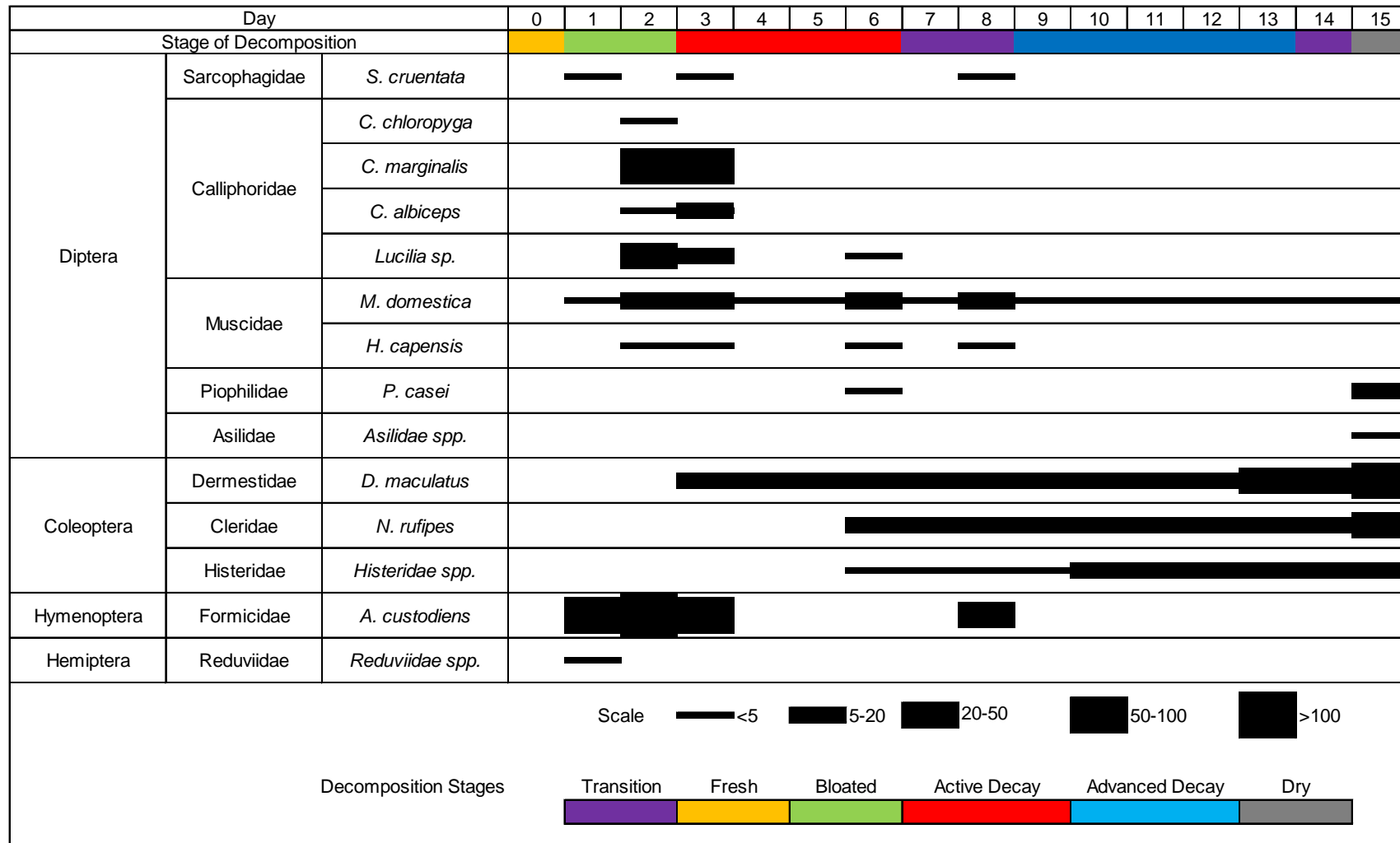**Figure 3.22** *Arthropod Succession on the control carcass during the Summer 2004 Trial.*

Figure 3.23 *Arthropod Succession on the control carcass during the Summer 2005 Trial.*

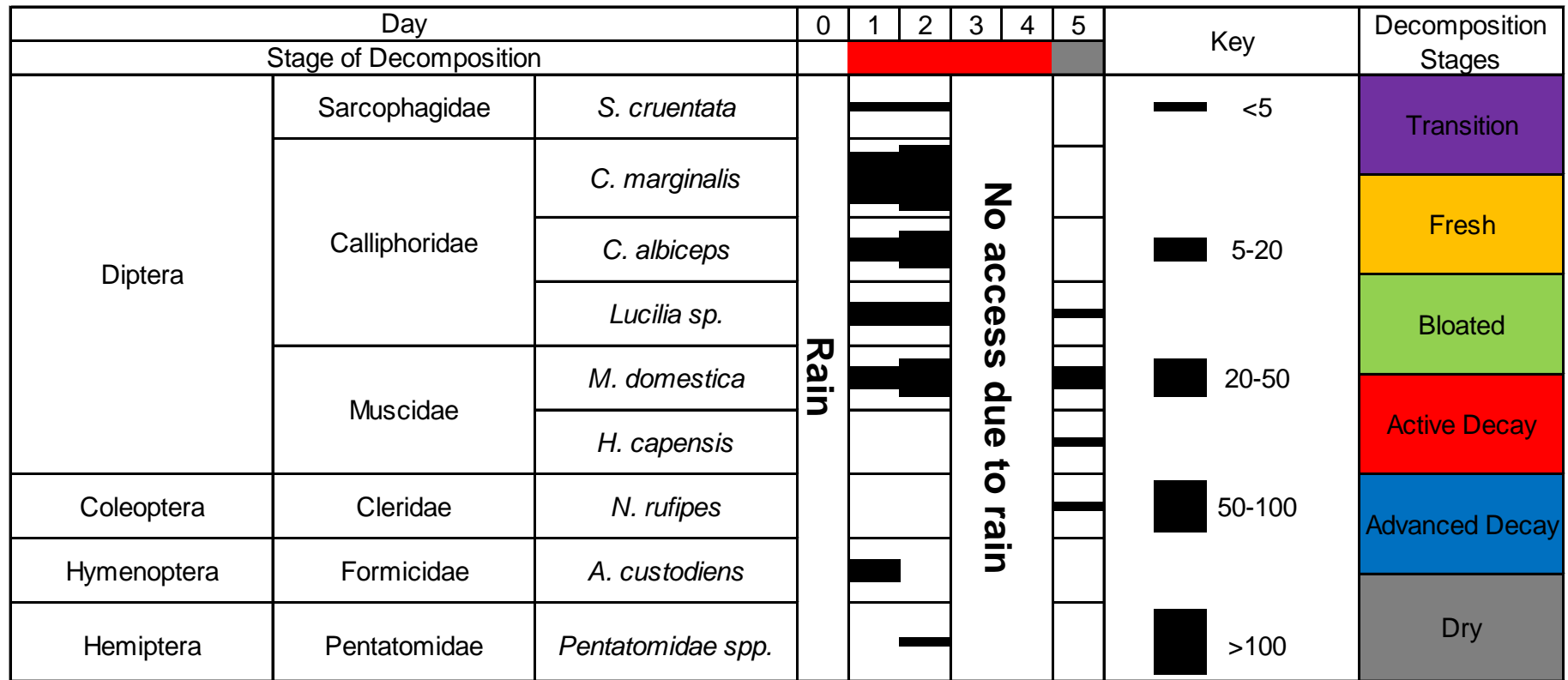| Day			0	1	2	3	4	5	Key	Decomposition Stages
Stage of Decomposition										
Diptera	Sarcophagidae	*S. cruentata*	Rain			No access due to rain			▬ <5	Transition
	Calliphoridae	*C. marginalis*								Fresh
		C. albiceps							▬ 5-20	
		Lucilia sp.								Bloated
	Muscidae	*M. domestica*							■ 20-50	
		H. capensis								Active Decay
Coleoptera	Cleridae	*N. rufipes*							■ 50-100	Advanced Decay
Hymenoptera	Formicidae	*A. custodiens*								Dry
Hemiptera	Pentatomidae	*Pentatomidae spp.*							■ >100	

Figure 3.24 Arthropod Succession on the control carcass during the Summer 2006 Trial.

Muscidae were continually observed in the vicinity of the carcass, but did not breed on the carcasses (Figures 3.22 & 3.23).

Dermestidae were observed on the carcass from Day 7 during 2004 and from Day 3 during 2005 and remained on the carcass for the remainder of each trial (Figures 3.22 & 3.23). Dermestidae were not observed to breed on the carcass and were not observed at all during 2006 due to the carcasses being constantly wet during the excessive rain (Figure 3.24). Hewadikaram & Goff (1991) first observed beetles of the families Dermestidae, Histeridae, Staphylinidae and Tenebrionidae associated with carcasses between Days 3 and 5 after death.

Cleridae were not observed to breed on the carcass, but were observed from Day 7, Day 6 and Day 5 during 2004, 2005 and 2006, respectively (Figures 3.22 – 3.24).

Small numbers of Histeridae were also found on the carcass during 2004 and 2005 (Figures 3.22 & 3.23)

Two observations during 2004 revealed predatory Salticidae on the carcass. Salticidae are diurnal hunting spiders (Dippenaar-Schoeman & Joqué, 1997). Bornemissza (1957) also found spiders of the family Salticidae associated with decomposing carcasses.

Formicidae were present on the carcasses and predated on the eggs, maggots and beetles (see Section 3.2.7). Reduviidae were not observed to predate on any of the carcass fauna during 2004 and 2005 (Figures 3.22 & 3.23), whereas Salticidae were observed to predate on the carcass fauna during 2004 (Figures 3.22).

3.2.3.2 SB

Sarcophagidae, Calliphoridae and Formicidae were the first to arrive at the carcass on Day 1 (Figures 3.25 – 3.27). Mostly single Sarcophagidae were observed on one or two instances where two or three individuals were observed at the carcass.
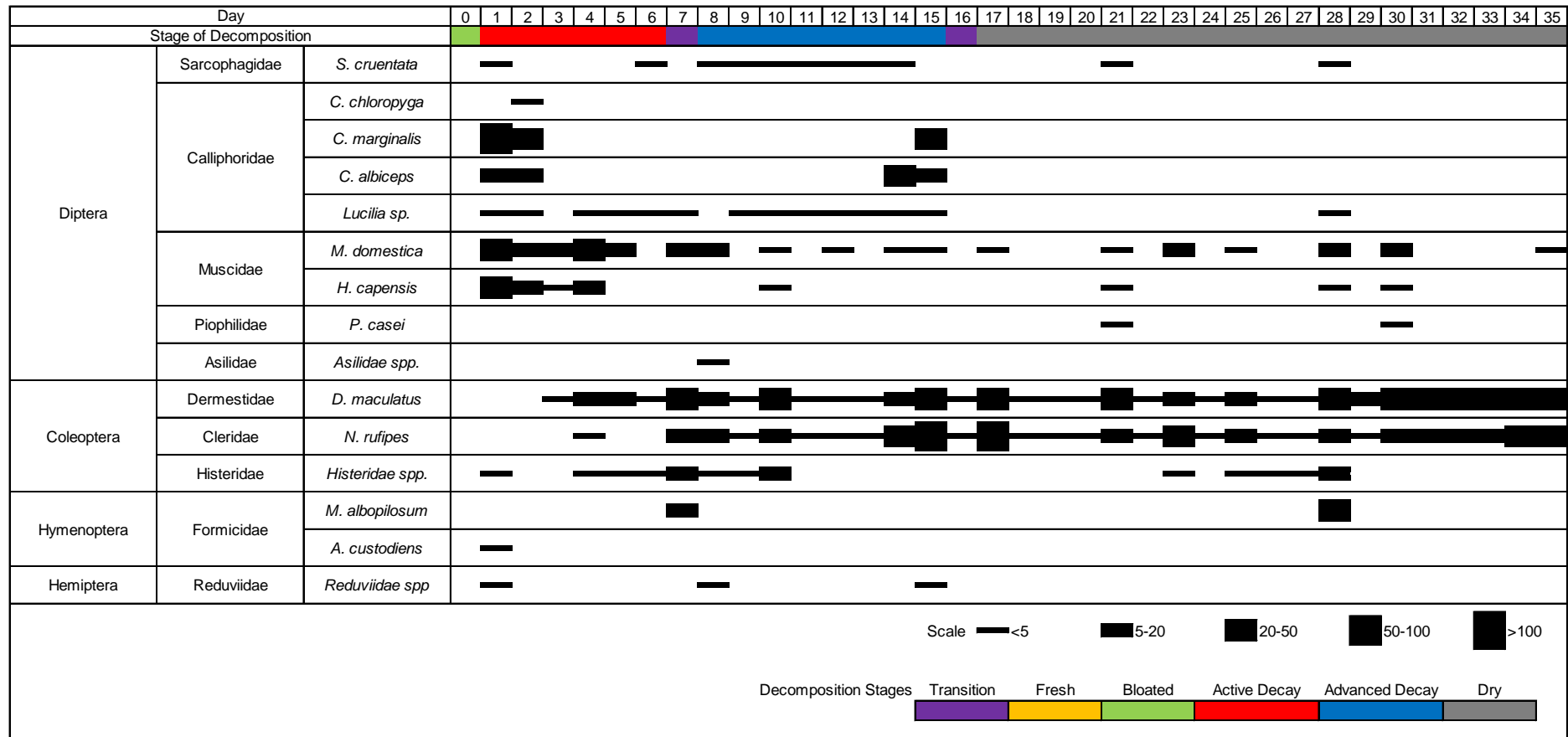
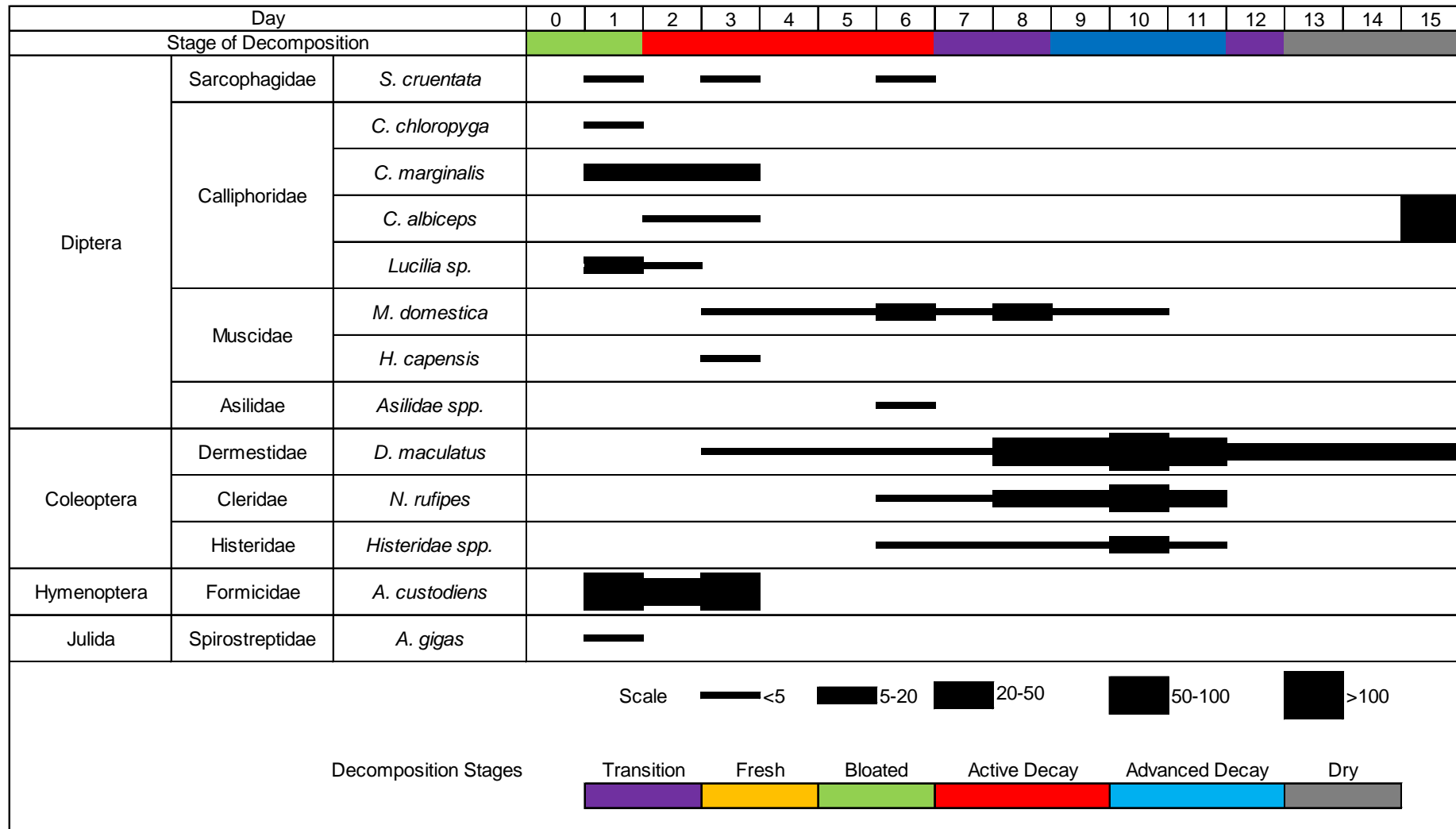**Figure 3.25** *Arthropod Succession on the SB carcass during the Summer 2004 Trial.*

Day			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Stage of Decomposition																		
Diptera	Sarcophagidae	*S. cruentata*																
	Calliphoridae	*C. chloropyga*																
		C. marginalis																
		C. albiceps																
		Lucilia sp.																
	Muscidae	*M. domestica*																
		H. capensis																
	Asilidae	*Asilidae spp.*																
Coleoptera	Dermestidae	*D. maculatus*																
	Cleridae	*N. rufipes*																
	Histeridae	*Histeridae spp.*																
Hymenoptera	Formicidae	*A. custodiens*																
Julida	Spirostreptidae	*A. gigas*																

Scale —— <5 ■ 5-20 ■ 20-50 ■ 50-100 ■ >100

Decomposition Stages: Transition Fresh Bloated Active Decay Advanced Decay Dry

Figure 3.26 *Arthropod Succession on the SB carcass during the Summer 2005 Trial.*

Day			0	1	2	3	4	5	Key	Decomposition Stages
Stage of Decomposition				🟩	🟥			⬜		
Diptera	Sarcophagidae	*S. cruentata*	Rain	—	No access due to rain				— <5	**Transition** (purple)
	Calliphoridae	*C. marginalis*		■ 200					■ 5-20	**Fresh** (orange)
		Lucilia sp.		—						
	Muscidae	*M. domestica*						■	■ 20-50	**Bloated** (green)
		H. capensis		—						
	Piophilidae	*P. casei*							■ 50-100	**Active Decay** (red)
Coleoptera	Dermestidae	*D. maculatus*								
	Cleridae	*N. rufipes*		—				■	■ 50-100	**Advanced Decay** (blue)
		N. rufipes larvae								
	Scarabaeidae	*Scarabaeidae spp.*							■ >100	
Hymenoptera	Formicidae	*M. albopilosum*		■						**Dry** (grey)
		A. custodiens		—						

Figure 3.27 *Arthropod Succession on the SB carcass during the Summer 2006 Trial.*

Similar to the control carcass, the same pattern of large numbers of adult Calliphoridae occurring on the carcass for two consecutive days was observed.

Formicidae were observed to take pieces of burnt skin from the carcass and predate on the eggs, maggots and beetles (see Section 3.2.7).

Muscidae were also attracted to the carcass on Day 1 during 2004 (Figure 3.25), and Day 3 during 2005 (Figure 3.26). It was unclear when exactly Muscidae arrived during 2006, due to the inaccessibility of the carcass during heavy rain (Figure 3.27).

Similar to findings at the control carcass, a second record of large numbers of adult Calliphoridae, namely *C. albiceps*, a secondary blow fly which had recently emerged from the puparia, was found during 2005 from Day 15 (Figure 3.26). This phenomenon was not observed for *C. marginalis* or *C. chloropyga*, the primary blow flies. The reason for this could be two-fold. Firstly, an extraordinarily large number of Formicidae predated extensively on the eggs and maggots of the primary blow flies from Days 1 – 3 (see Section 3.2.7) and secondly, secondary *C. albiceps* maggots predated on the maggots of the primary blow flies, since *C. albiceps* adults only oviposited after the primary blow fly maggots had already been feeding for two or more days.

As was the case with the control carcass, Muscidae were more or less continually observed in the vicinity of the carcass, but did not breed on the carcasses (Figures 3.25 & 3.26).

Dermestidae were recorded as early as Day 2 and Day 3, being attracted to the dry burnt skin and remained on the carcass for the remainder of each trial (Figures 3.25 & 3.26). Adult Dermestidae did not breed on the carcass and were only observed on Day 5 during 2006 (Figure 3.27).

Cleridae were present from Day 4, Day 6 and Day 2 during 2004, 2005 and 2006, respectively (Figures 3.25 – 3.27). Cleridae larvae were only found once during 2006 on Day 5 (Figure 3.27).

Small numbers of Histeridae were also found on the carcass during 2004 and 2005 (Figures 3.25 & 3.26)

3.2.3.3 MB

The first arrivals at the carcass were Calliphoridae, Muscidae and Formicidae (Figures 3.28 – 3.30). Adult Sarcophagidae were observed on Day 1 during 2005 and 2006 (Figures 3.29 & 3.30).

A large second record of adult *C. marginalis* and *C. albiceps* was observed during 2004 from Days 11 – 15 (Figure 3.28). Similar to the SB carcass, a large second record of only adult *C. albiceps* was observed during 2005 (Figure 3.29), possibly due to the same circumstances, predation by *C. albiceps* and Formicidae (see Section 3.2.7).

Dermestidae were observed at the carcass for the first time on Day 4, Day 2 and Day 5 during 2004, 2005 and 2006, respectively and remained on the carcass for the remainder of each trial (Figures 3.28 – 3.30). Dermestidae larvae were only found on the carcass during 2005 on Day 15 (Figure 3.29).

Cleridae were found on the carcass for the first time on Day 4, Day 6 and Day 5 during 2004, 2005 and 2006, respectively and remained on the carcass for the remainder of each trial (Figures 3.28 – 3.30).

Small numbers of Histeridae were found intermittently on the carcass from Day 4 during 2004 (Figure 3.28). During 2005, Histeridae first occurred on the carcass from Day 3 and remained on the carcass for the remainder of the trial (Figure 3.29).
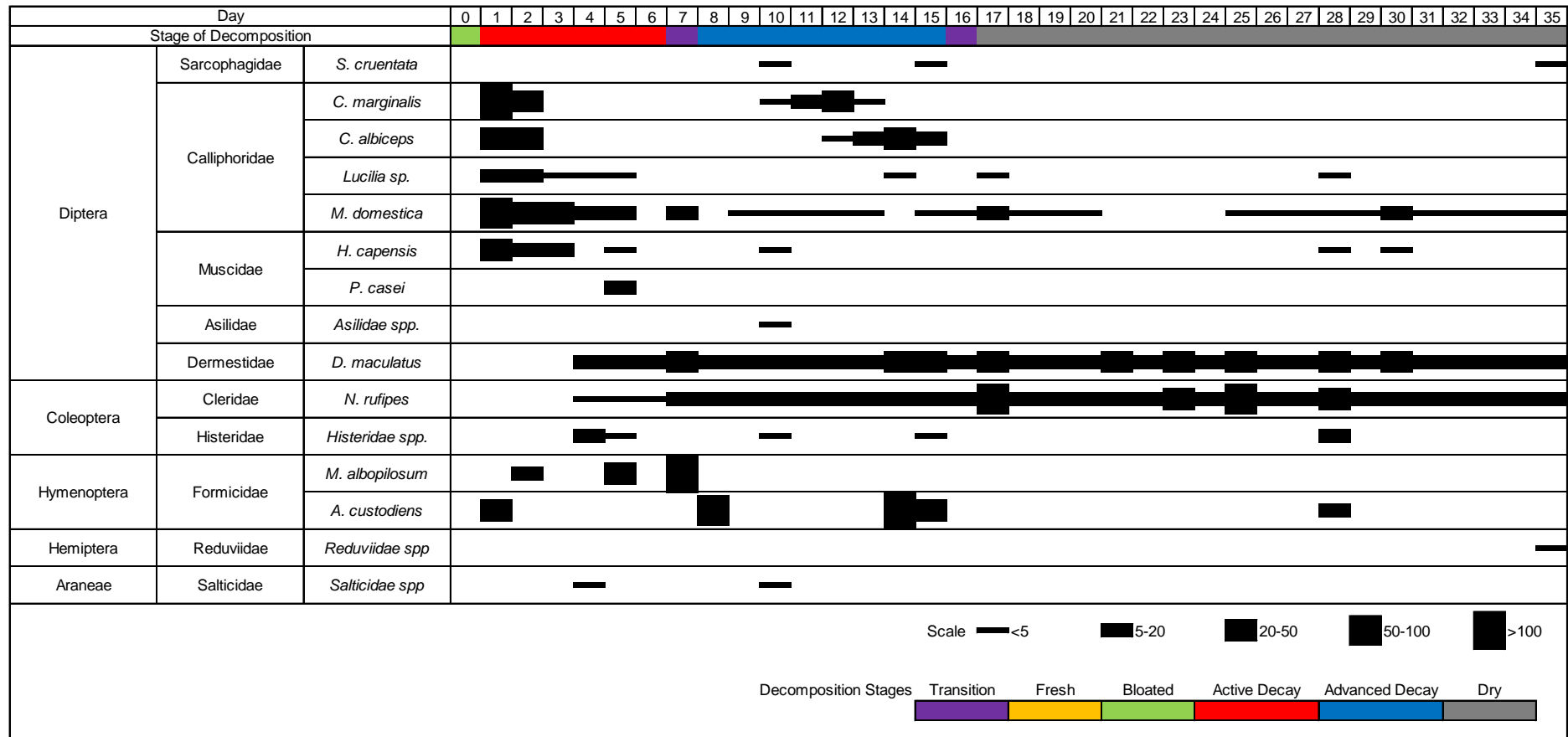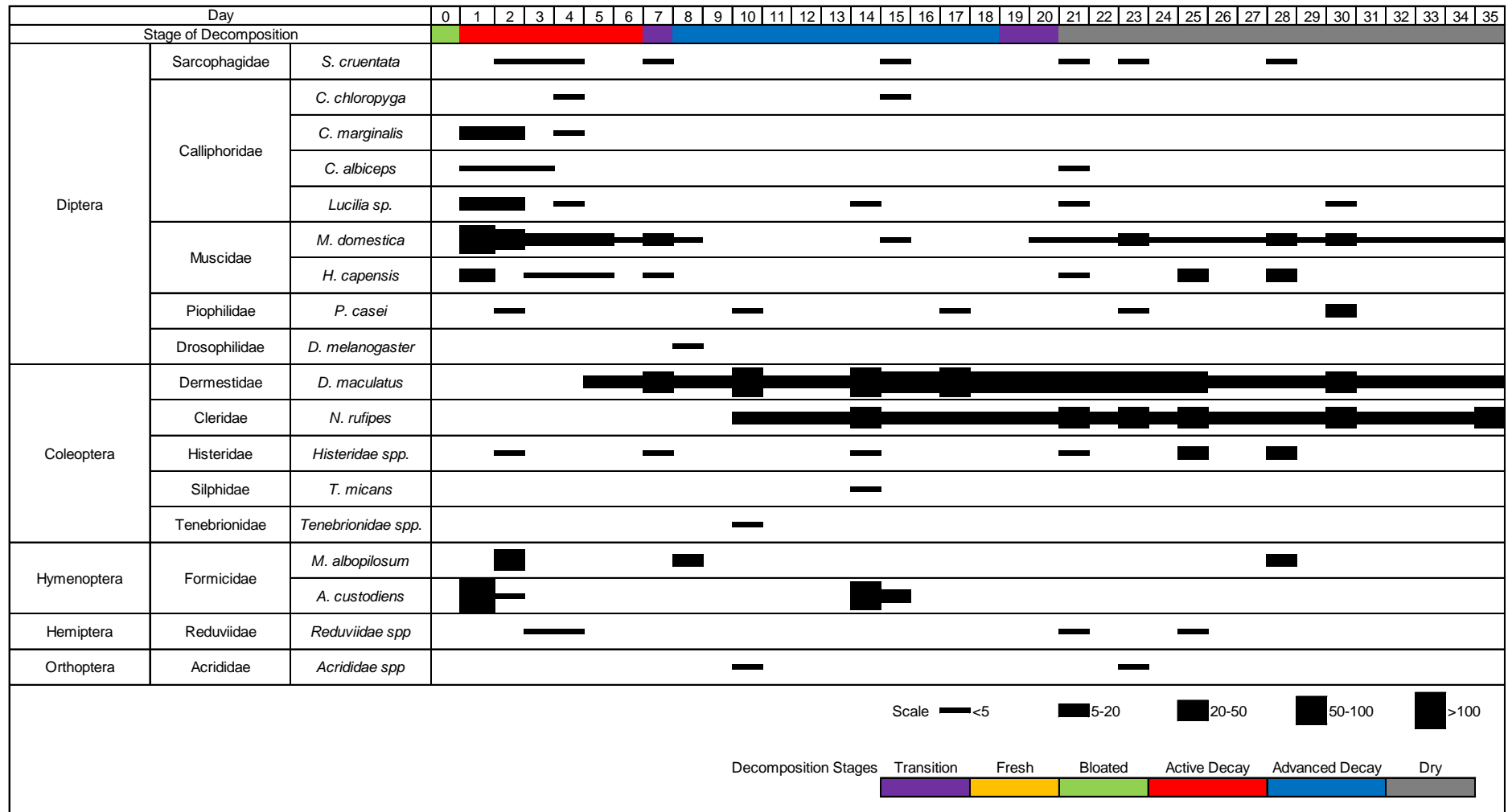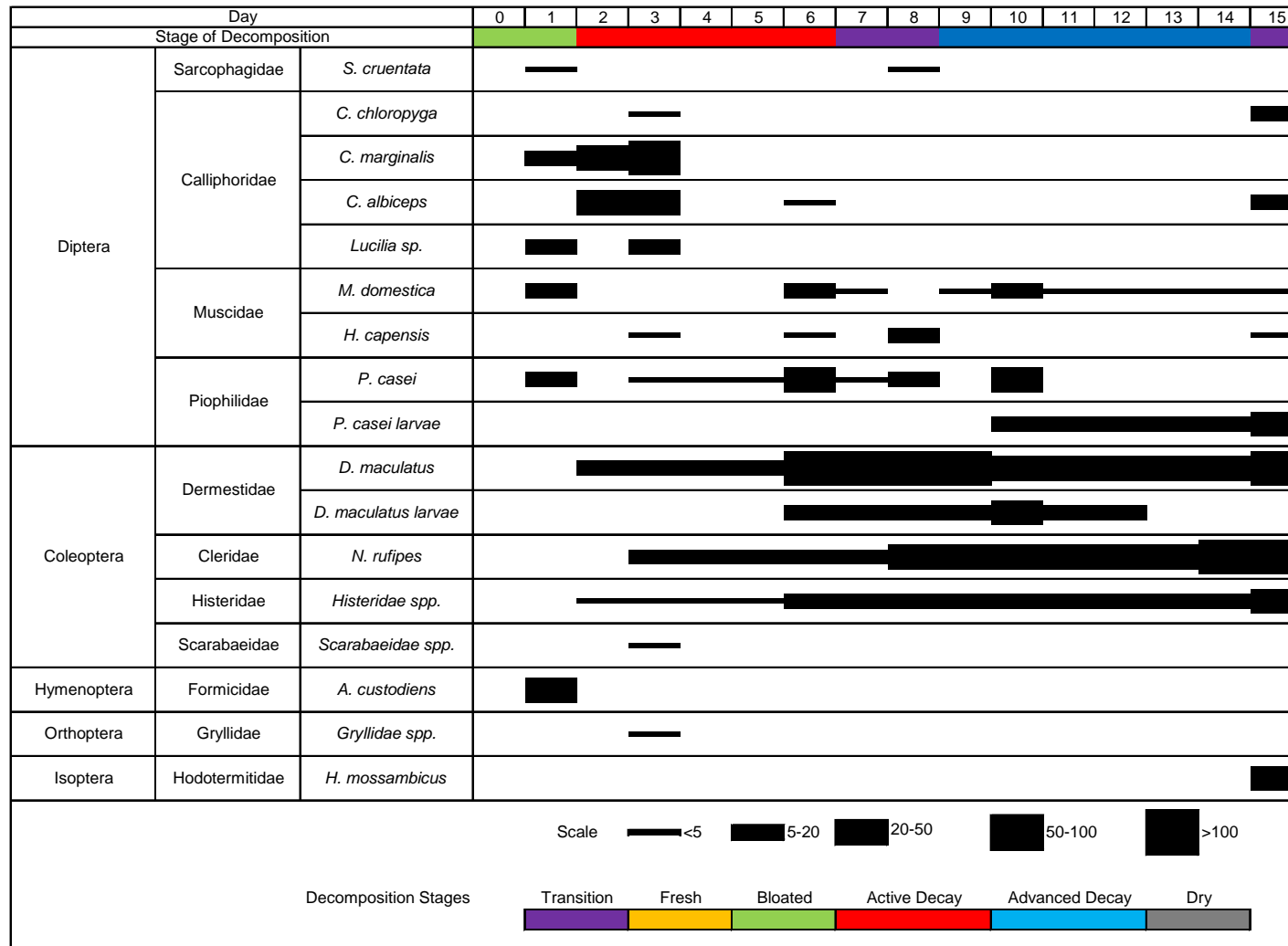
Figure 3.28 *Arthropod Succession on the MB carcass during the Summer 2004 Trial.*
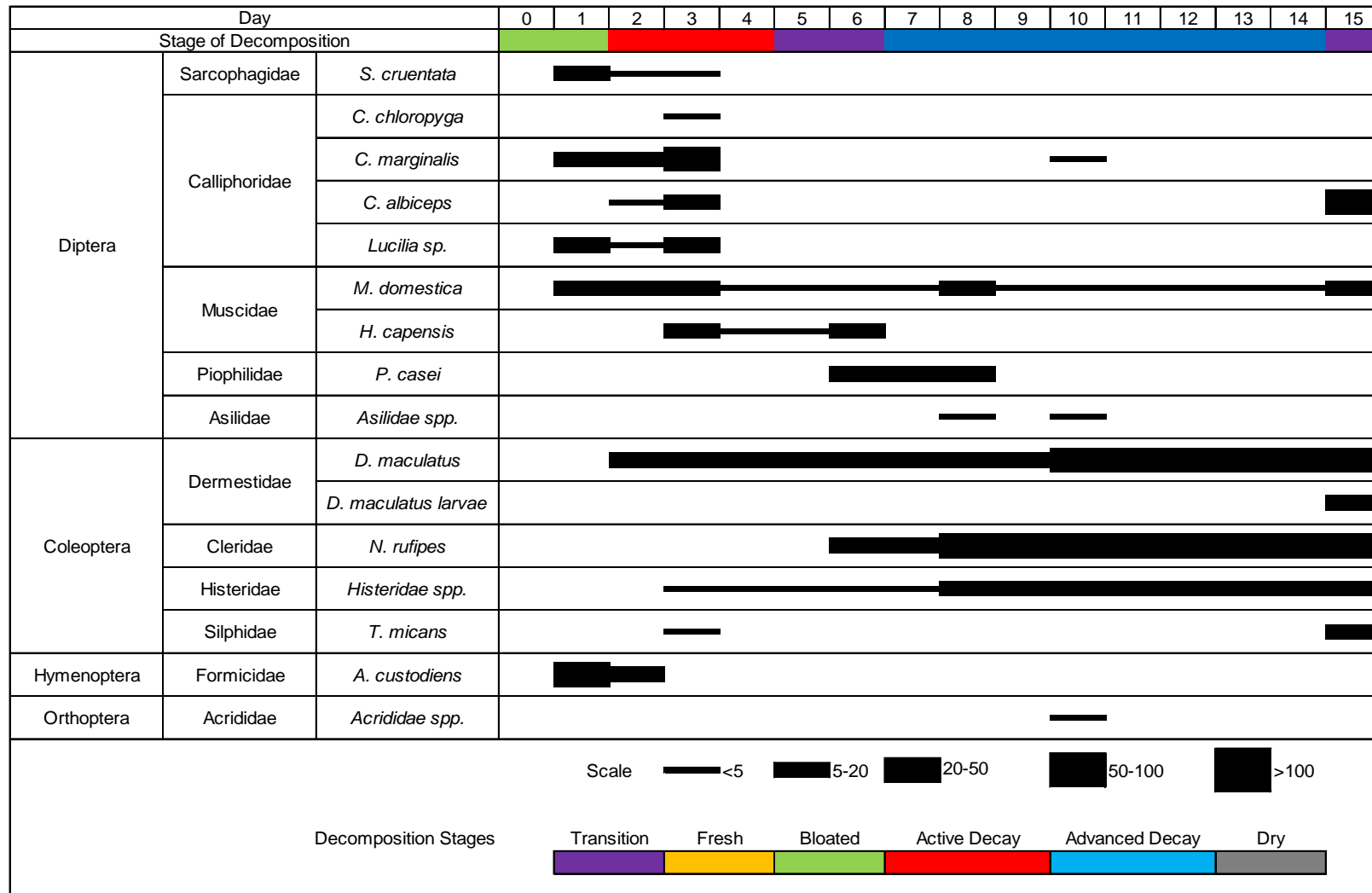
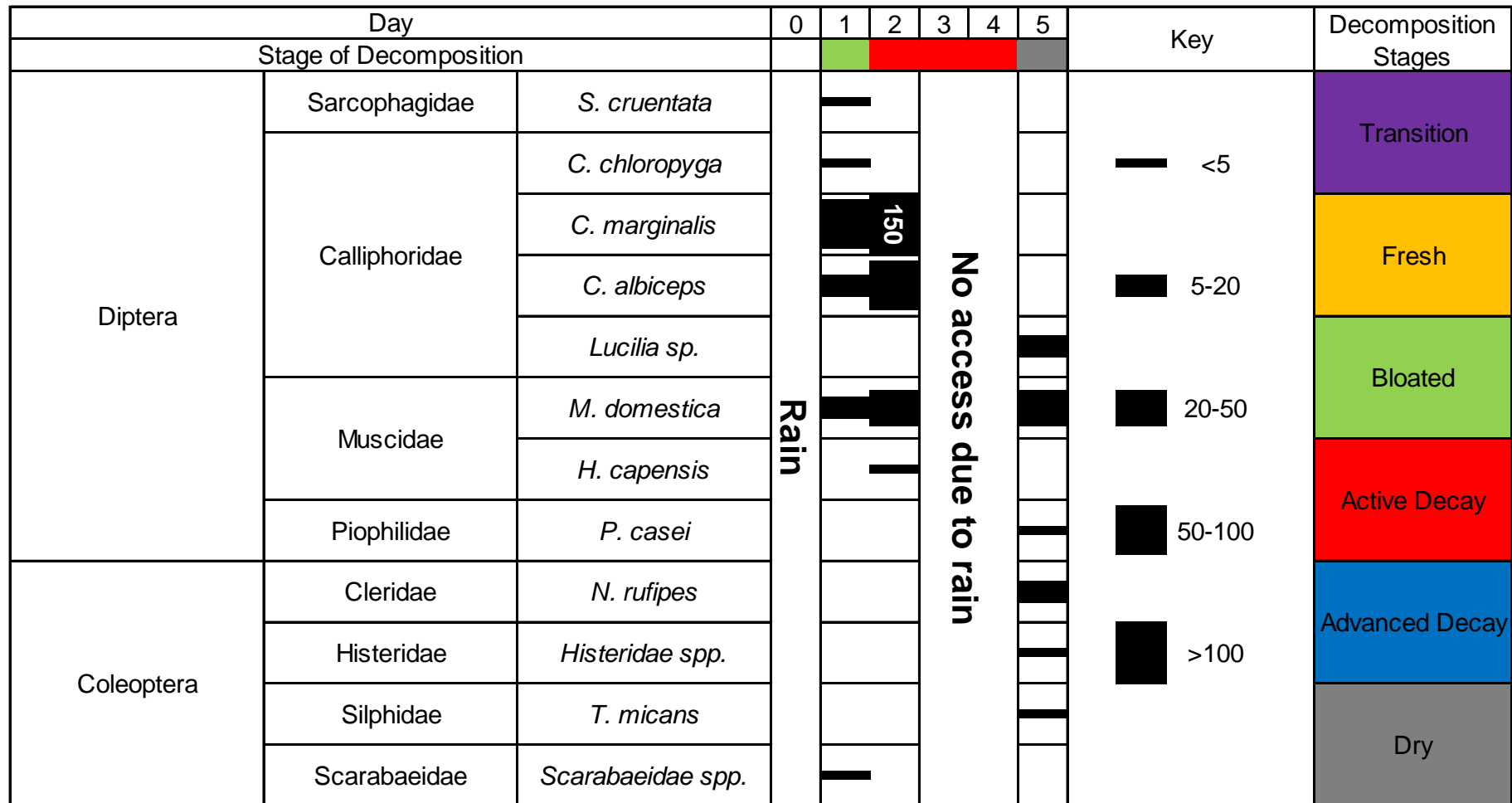**Figure 3.29** *Arthropod Succession on the MB carcass during the Summer 2005 Trial.*

Day			0	1	2	3	4	5	Key	Decomposition Stages
Stage of Decomposition				🟩	🟥			⬜		
Diptera	Sarcophagidae	*S. cruentata*	Rain	—		No access due to rain			— <5	Transition
	Calliphoridae	*C. chloropyga*		—						
		C. marginalis		▬ 200					▬ 5-20	Fresh
		C. albiceps			▬					
		Lucilia sp.		—				▬		Bloated
	Muscidae	*M. domestica*		—▬				▬	▬ 20-50	
	Piophilidae	*P. casei*						▬		Active Decay
Coleoptera	Dermestidae	*D. maculatus*						▬	▬ 50-100	
	Cleridae	*N. rufipes*						▬		Advanced Decay
	Scarabaeidae	*Scarabaeidae spp.*						▬	▬ >100	
Hymenoptera	Formicidae	*M. albopilosum*		▬						Dry
Orthoptera	Acrididae	*Acrididae spp.*		—						

Figure 3.30 *Arthropod Succession on the MB carcass during the Summer 2006 Trial.*

As with the control carcass, two observations during 2004 revealed the presence of Salticidae on the carcass during 2004 (Figure 3.28).

3.2.3.4 HB

Calliphoridae, Muscidae and Formicidae were among the first insects to arrive at the carcass on Day 1 (Figures 3.31 – 3.33).

A large second occurrence of *C. chloropyga* and *C. albiceps* was found at the carcass on Day 15 during 2005 (Figure 3.32).

Piophilidae only occurred infrequently and in small numbers at the other carcasses (Figures 3.22, 3.23, 3.25, 3.27 - 3.30). However at this carcass, Piophilidae occurred more frequently during 2004 and 2005 (Figures 3.31 & 3.32). Piophilidae larvae were only recorded during 2005 from Day 10 until the end of the trial (Figure 3.32).

Sarcophagidae arrived at the carcass on Day 1 during 2005 and 2006 (Figures 3.32 & 3.33) and on Day 2 during 2004 (Figure 3.31).

Anoplolepis custodiens Smith and *Monomorium albopilosum* Emery (Formicidae) were present in large numbers on Days 1 and 2 during 2004 and 2005 (Figures 3.31 & 3.32).

Dermestidae were only observed during 2004 and 2005 and for the first time on Day 5 and Day 2, respectively (Figures 3.31 & 3.32). Dermestidae larvae were only observed during 2005 from Day 6 to Day 12 (Figure 3.32).

Cleridae were present in relatively large numbers on the carcass from Day 10, Day 3 and Day 5 during 2004, 2005 and 2006, respectively (Figures 3.31 – 3.33).

A more frequent incidence of Histeridae was found from Day 2 onwards during 2005 than found during 2004 (Figures 3.31 & 3.32).

Figure 3.31 Arthropod Succession on the HB carcass during the Summer 2004 Trial.

Figure 3.32 *Arthropod Succession on the HB carcass during the Summer 2005 Trial.*

Day			0	1	2	3	4	5	Key	Decomposition Stages
Stage of Decomposition				🟩	🟥			⬜		
Diptera	Sarcophagidae	*S. cruentata*		▬					▬ <5	Transition
	Calliphoridae	*C. chloropyga*		▬					■ 5-20	Fresh
		C. marginalis		■ 150		No access due to rain				
		C. albiceps		■					■ 20-50	Bloated
		Lucilia sp.						■		
	Muscidae	*M. domestica*		■				■	■ 50-100	Active Decay
		H. capensis		■						
	Piophilidae	*P. casei*						■	■ >100	Advanced Decay
Coleoptera	Cleridae	*N. rufipes*						■		
	Histeridae	*Histeridae spp.*						■		
	Silphidae	*T. micans*						■		Dry
	Scarabaeidae	*Scarabaeidae spp.*		■						

Figure 3.33 Arthropod Succession on the HB carcass during the Summer 2006 Trial.

3.2.4 Statistical analysis of arthropod succession

3.2.4.1 Analysis 1: Jaccard Metric

The Jaccard occurrence matrices were created by using data from the succession diagrams. Mean faunal similarity was calculated for each day (See 2.1.4.1.). These faunal similarities were plotted (Figures 3.34 & 3.35).

Except for 2006, the graphs did not show the characteristic horseshoe-shape as described by Schoenly (1992). These carcasses were consumed faster than during any other trial and species diversity was low during 2006.

According to Schoenly (1992), similar shapes should be manifested by plots of mean similarities and a general property of the dynamic daily changes that occur during carrion-arthropod succession should be reflected by the ranges. This was evident during each trial (Figure 3.35), but not over successive trials (Figure 3.34).

The faunal similarity values calculated for the summer trials were between 0.125 - 0.833, 0.222 – 1 and 0.2 – 1 during 2004, 2005 and 2006, respectively. According to Schoenly (1992), the comparison of a variety of studies revealed faunal similarity values ranging from 0.218 to 0.808, where the variation could be ascribed to different types of carcasses that were essentially nonhuman. In the current study, the variation might be due to the different degrees of charring due to burning.

3.2.4.2 Analysis 2: Correlation coefficient

The matrices were tested using a correlation coefficient. The coefficient was calculated with Graphpad Instat. The Pearson's coefficient statistical analysis (Table 3.3) conducted on the carcass similarity matrices for all taxa showed that there were no significant differences in the overall arthropod succession between the same treated carcasses (e.g. control 2004 *versus* control 2005 *versus* control 2006) over successive trials, suggesting that the arthropod succession on each of the differently treated carcasses was the same during successive trials for each treatment.
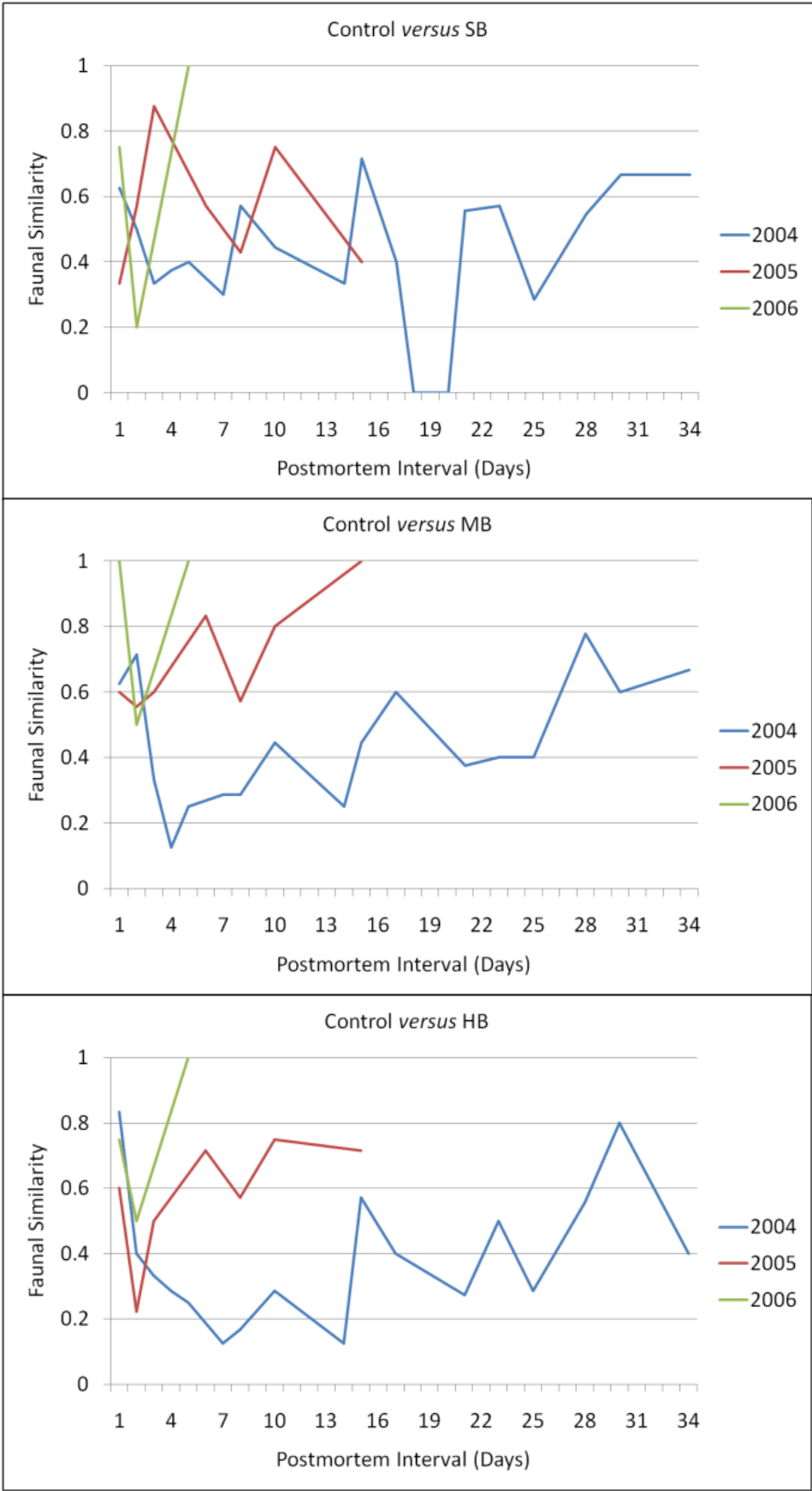
Figure 3.34 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession over the three summer trials.*
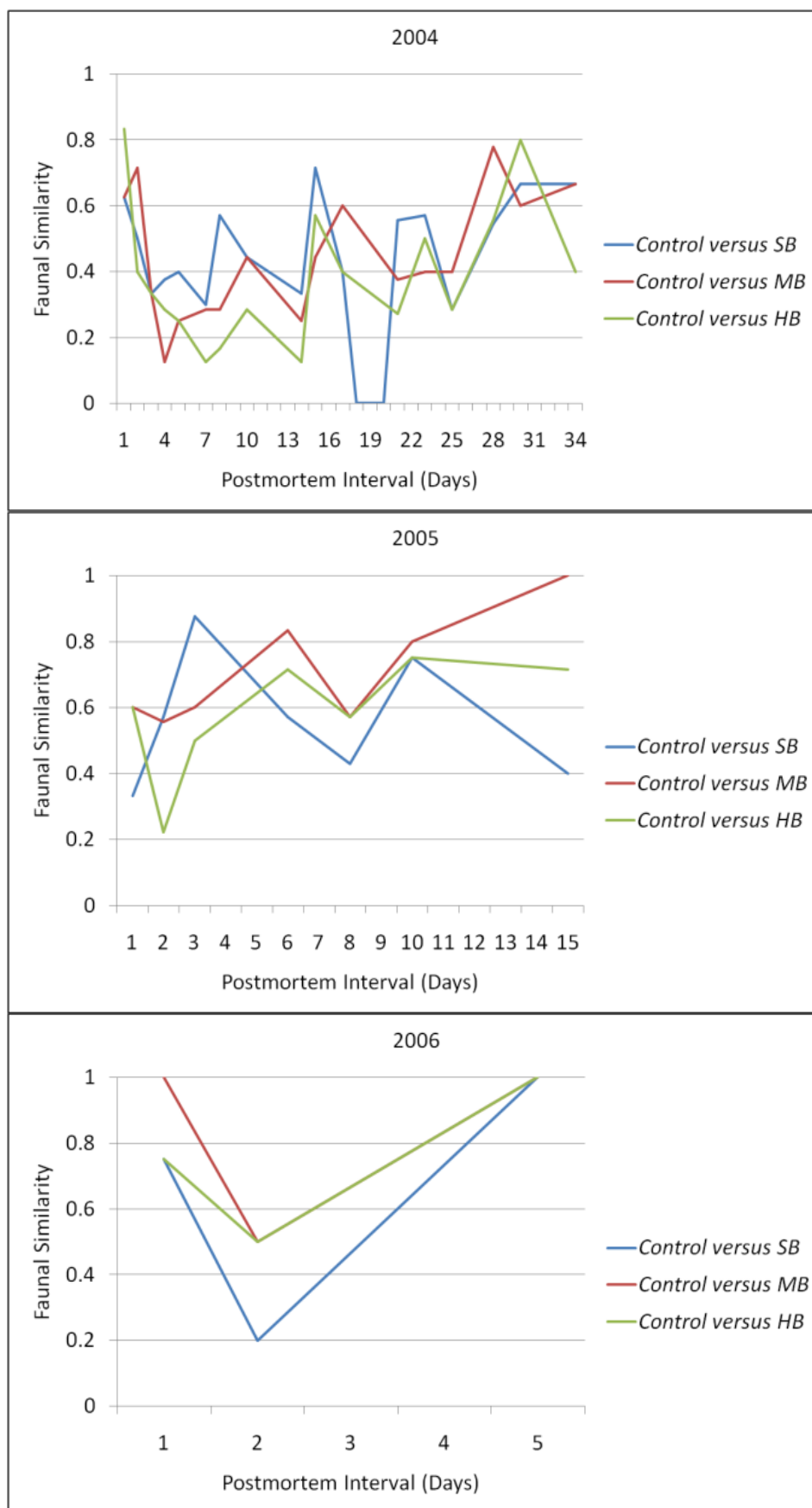
Figure 3.35 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession during each summer trial.*

Table 3.3 Similarity matrix analysis of each treatment over successive summer trials

2004/2005/2006	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
Control	0.06941	-0.009152 to 0.1471	0.0832	not significant
SB	0.01139	-0.06717 to 0.08982	0.7763	not significant
MB	0.04751	-0.03274 to 0.1272	0.2456	not significant
HB	-0.01157	-0.08999 to 0.06700	0.7731	not significant

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for forensic indicator species showed differences for *C. chloropyga* between carcasses during each trial (Table 3.4). The differences found during 2004 and 2005 were not statistically significant. This suggested that the level of burning had an influence on the colonisation of *C. chloropyga* on the carcasses during 2006.

Table 3.4 Similarity matrix analysis of *C. chloropyga* at all carcasses during successive summer trials

C. chloropyga	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	-0.05764	-0.3454 to 0.2400	0.7069	not significant
2005	-0.08348	-0.3681 to 0.2154	0.5856	not significant
2006	0.5345	0.03051 to 0.8218	0.0401	significant

The Pearson's coefficient showed significant differences for *C. marginalis* between carcasses during 2004 and 2005. (Table 3.5). This suggested that the level of burning had an influence on the colonisation of *C. marginalis* on the carcasses.

Table 3.5 Similarity matrix analysis of *C. marginalis* at all carcasses during successive summer trials

C. marginalis	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.6374	0.5055 to 0.7401	< 0.0001	significant
2005	0.7338	0.5611 to 0.8453	< 0.0001	significant
2006	*	*	*	*
* Divide by zero. A computation has divided a number by zero which is illegal. This may be caused by unusual data or an unanticipated circumstance.				

The Pearson's coefficient showed significant differences for *C. albiceps* between carcasses during successive trials. (Table 3.6). This suggested that the level of burning had an influence on the colonisation of *C. albiceps* on the carcasses.

Table 3.6 Similarity matrix analysis of *C. albiceps* at all carcasses during successive summer trials

C. albiceps	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.5696	0.4218 to 0.6879	< 0.0001	significant
2005	0.9354	0.8848 to 0.9642	< 0.0001	significant
2006	0.6124	0.1457 to 0.8561	0.0152	significant

3.2.5 Ambient temperatures and rainfall

As pointed out in Section 2.1.3.3, ambient temperature records and rainfall for the two nearest meteorological stations (Figures 2.14 & 2.15) were obtained from the South African National Weather Service in Pretoria.

The Bloemfontein City Centre (City) and Bloemfontein Airport (WO) meteorological station are situated ± 2km and 12.6km from the study site (Figures 2.14 & 2.15). Temperature records from local meteorological stations are likely to differ considerably from the temperatures actually experienced by fly larvae on or in a decomposing body (Turner & Howard, 1992).

It is therefore extremely important to determine and study the temperatures at which maggots develop inside the decomposing body. Since there may be a large difference in the temperatures experienced by the larvae in a decomposing body *versus* the ambient temperature, it may have a significant influence of the calculation of the PMI.

As was the case in this study, Shean *et al.* (1993) found that the decomposition rates of pig carcasses were affected primarily by feeding of the Calliphoridae larvae and their relative rate of development, which in turn was related to ambient air temperature.

Major differences in meteorological data from these two stations are highlighted below. Only the data from the nearest meteorological station (City) were applied to this study, since ambient temperatures measured at the study site during times of observations more closely approximated the temperatures measured at the City meteorological station than at the WO meteorological station.

3.2.5.1 2004 Summer Trial

The average maximum and minimum City temperature was 32.5°C and 19.2°C, respectively. The average City temperature was 25.9°C. Average maximum and minimum WO temperature was 30.3°C and 15.2°C, respectively. The average WO temperature was 22.7°C.

City rainfall occurred on Days 7, 15, 18, 20, 23, 27, 31 and 35 (Figure 3.36), with a total of 16.2mm.

WO rainfall occurred on Days 4, 11, 15, 16, 17, 24, 25, 26, 27, 31, 32 and 35 (Figure 3.36), with a total of 44mm.

3.2.5.2 2005 Summer Trial

The average maximum City temperature was 31.5°C and the minimum 17.3°C, while the average City temperature was 24.3°C. Average maximum and minimum WO temperature was 29.9°C and 14.8°C, respectively. The average WO temperature was 22.3°C.

City rainfall occurred on Days 2, 3, 10, 12 and 13 (Figure 3.37), with a total of 14.6mm.

WO rainfall occurred on Days 1, 2, 3, 10, 12, 13 and 14 (Figure 3.37), with a total of 26.5mm.
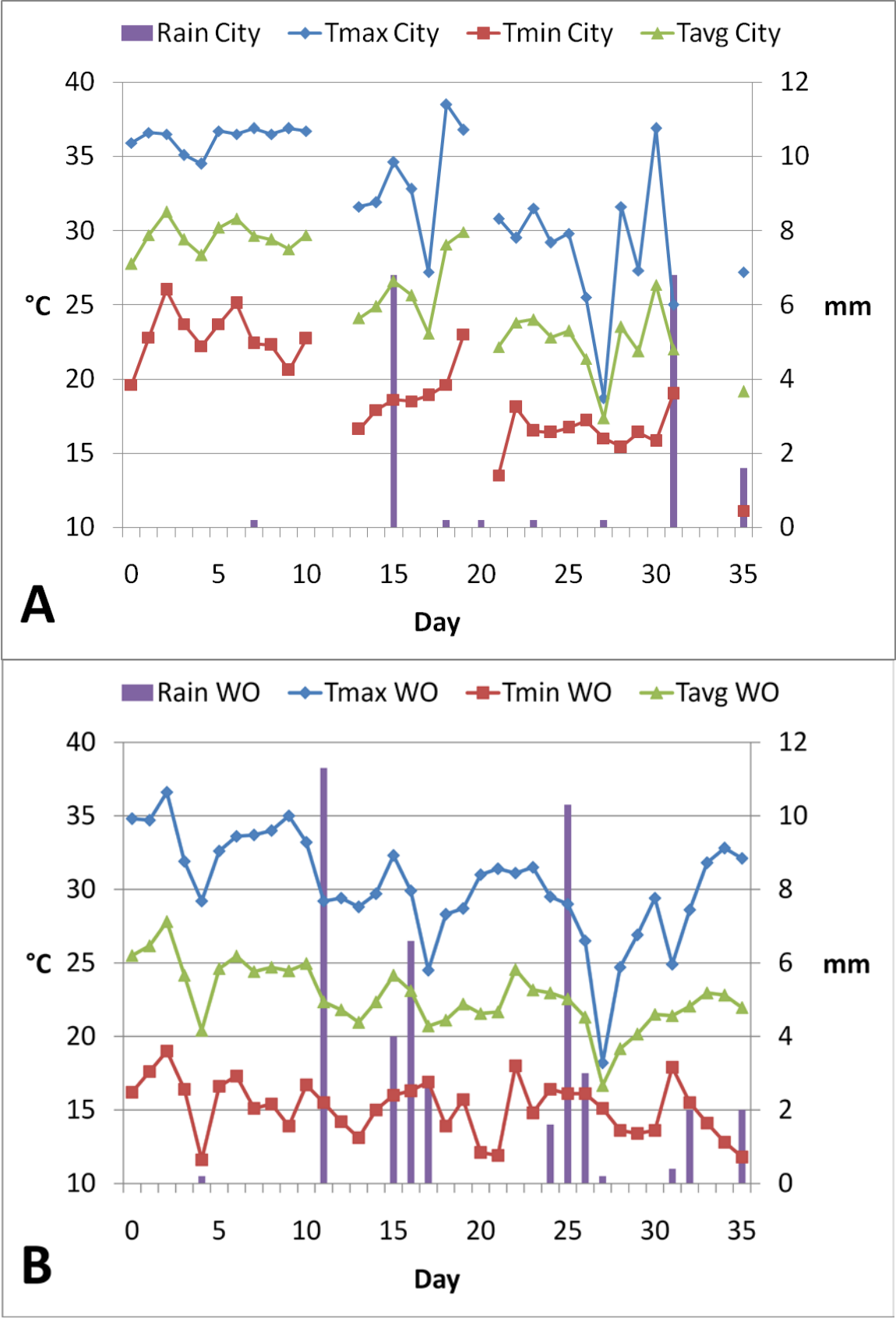
Figure 3.36 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Summer 2004 Trial.*
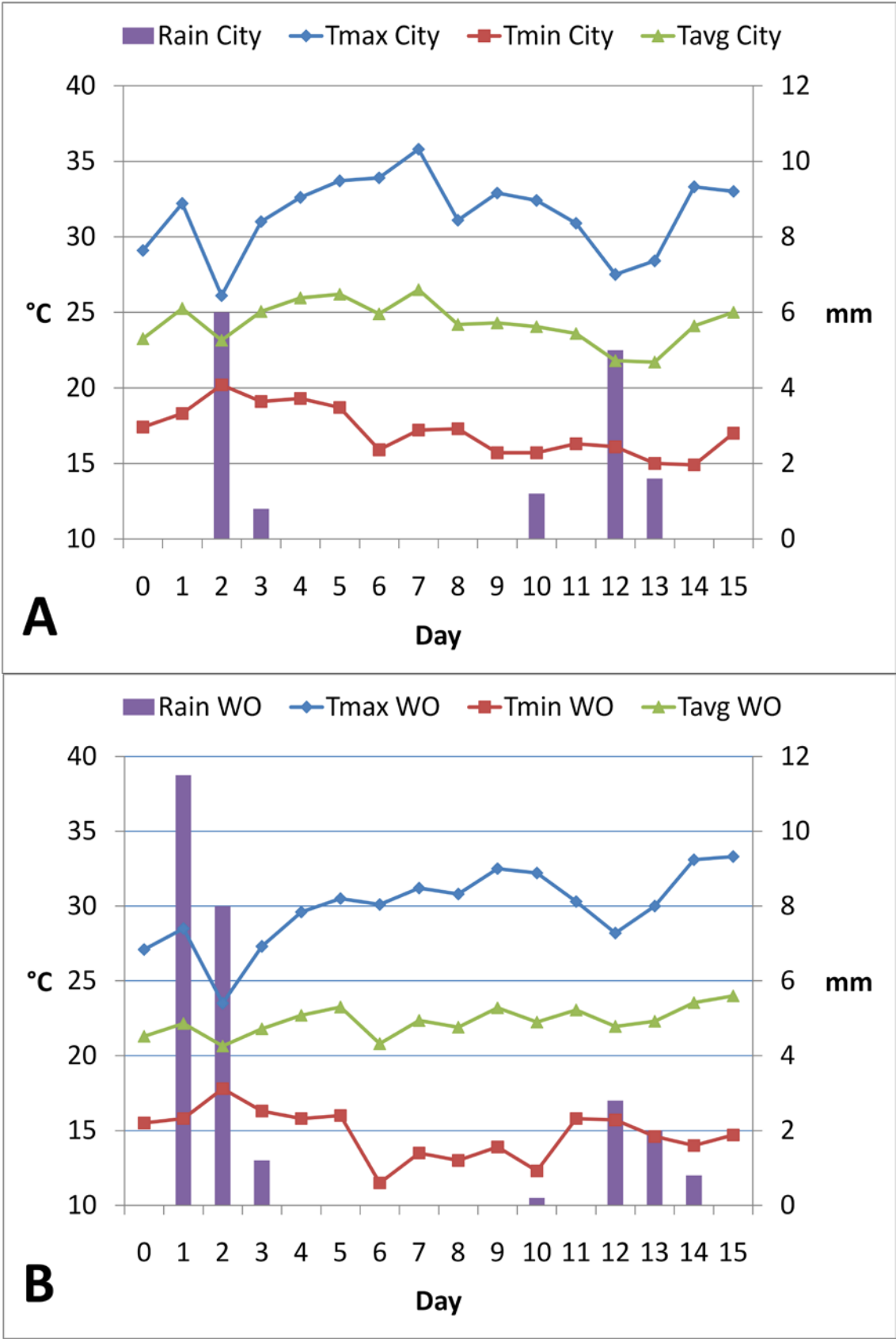
Figure 3.37 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Summer 2005 Trial*

3.2.5.3 2006 Summer Trial

The average maximum and minimum City temperature was 27.4°C and 16°C, respectively. The average City temperature was 21.7°C. Average maximum and minimum WO temperature was 26.7°C and 15°C, respectively. The average WO temperature was 20.9°C.

City rainfall occurred on Days 0, 1, 2, 3 and 5 (Figure 3.38), with a total of 31mm.

WO rainfall occurred on Days 0, 1, 2, 3 and 5 (Figure 3.38), with a total of 23.1mm

3.2.6 Ambient, external and internal carcass temperatures

During the summer 2006 trial, the carcasses were inaccessible due to constant rainfall and no temperatures were recorded at the carcasses on Days 2 - 4.

Overall, the ambient temperature measured during observations follow the trend of the daily average ambient temperature for the nearest meteorological station, i.e. City (Figures 3.39 & 3.40).

Great fluctuations occurred for the temperature of the head, thorax, and abdomen with the internal temperatures elevated above the ambient temperature. This suggests that the maggot masses feeding in these sites produced metabolic heat generation and regulated the temperature of these masses with their feeding behaviour by constantly feeding at the bottom of the mass and then moving to the surface to cool down (Figures 3.39 & 3.40). Maggots in a mass constantly move down to the feeding site and back out to the exterior of the mass (Anderson & VanLaerhoven 1996). Temperature plays a significant role in the development of carrion flies, with maggot-mass temperatures often becoming elevated above ambient temperatures (Joy, Herrel, & Rogers, 2002).
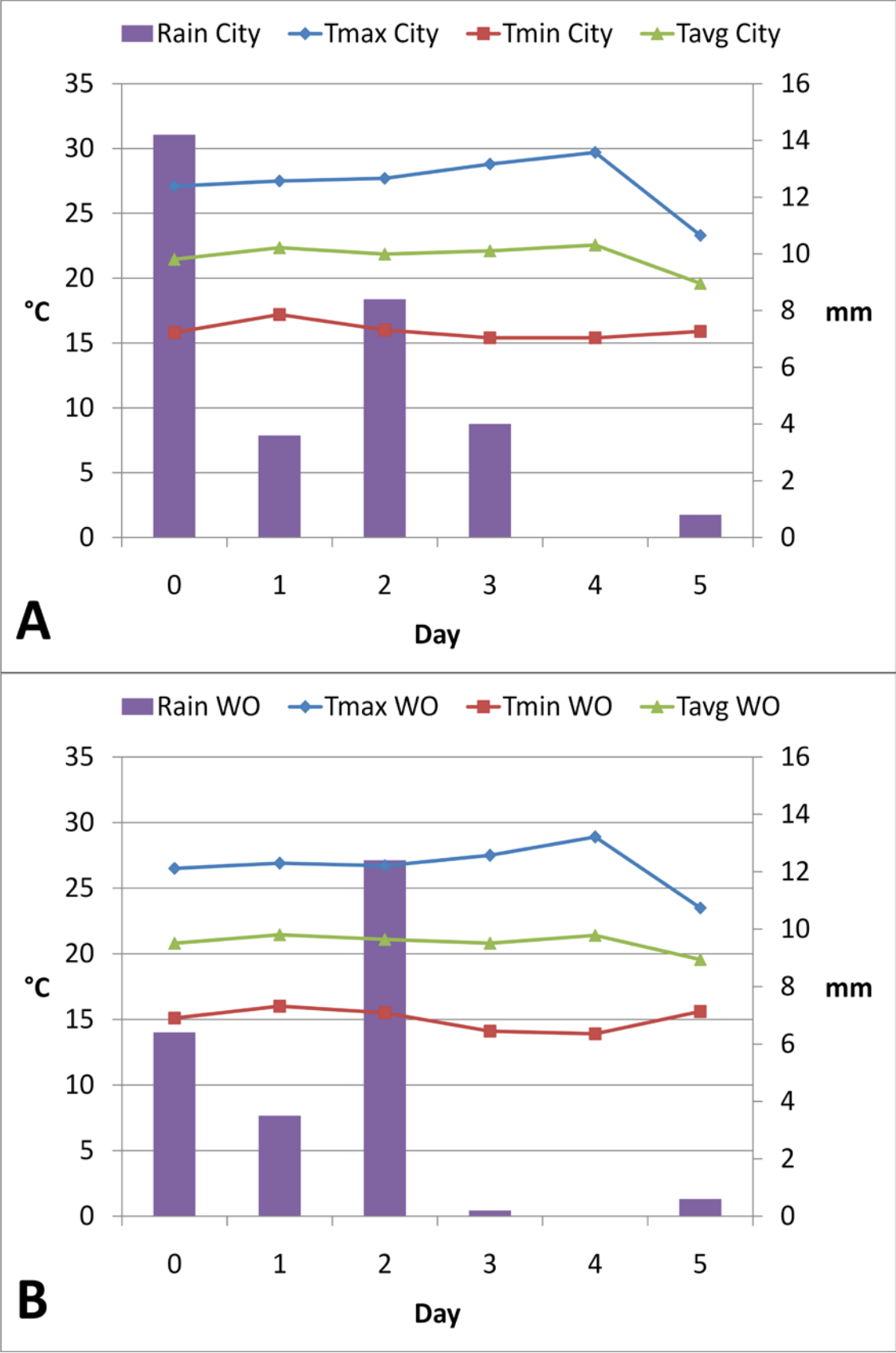
Figure 3.38 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Summer 2006 Trial*
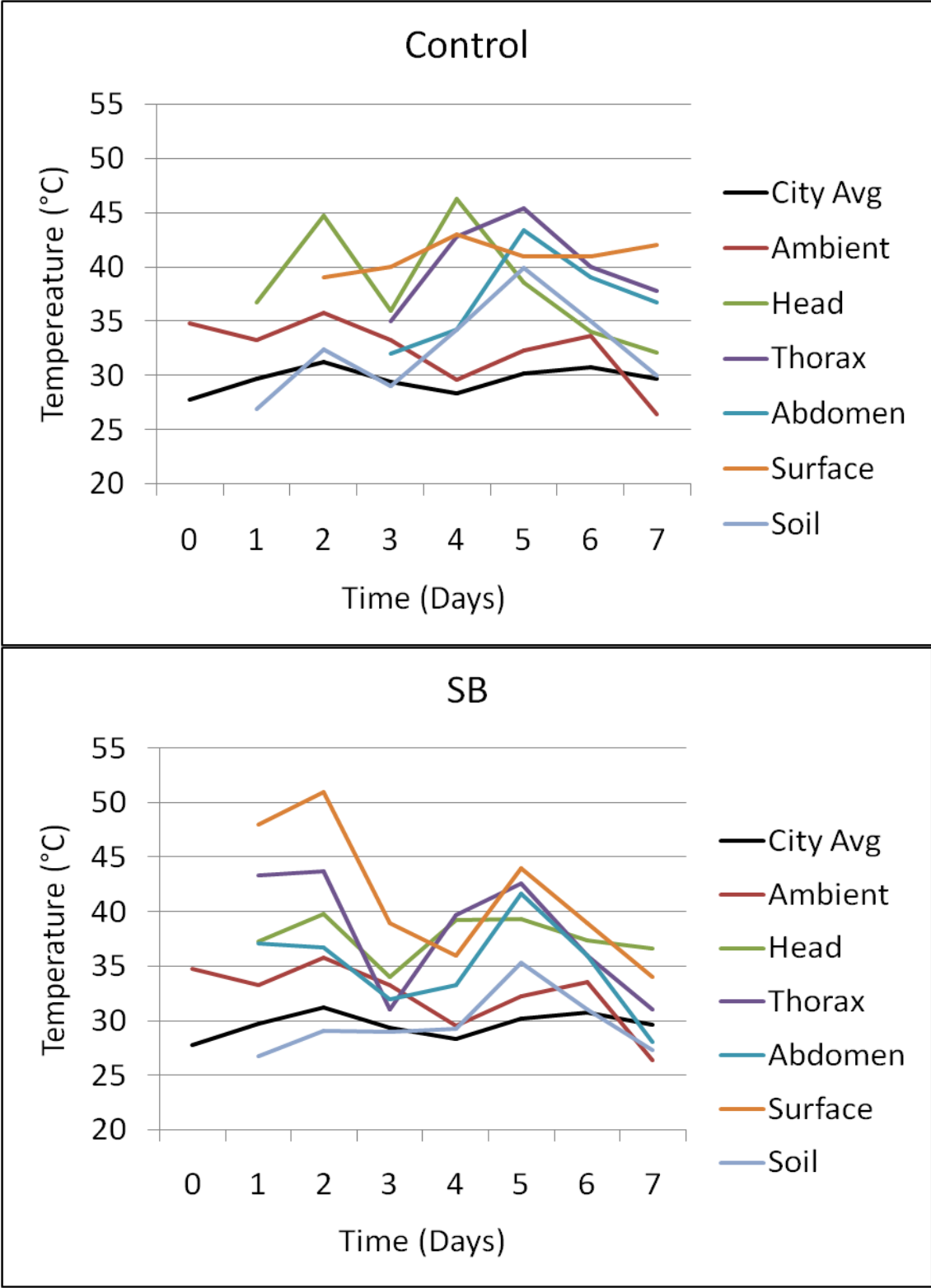
Figure 3.39 *Ambient & internal carcass temperatures during the Summer 2004 Trial.*
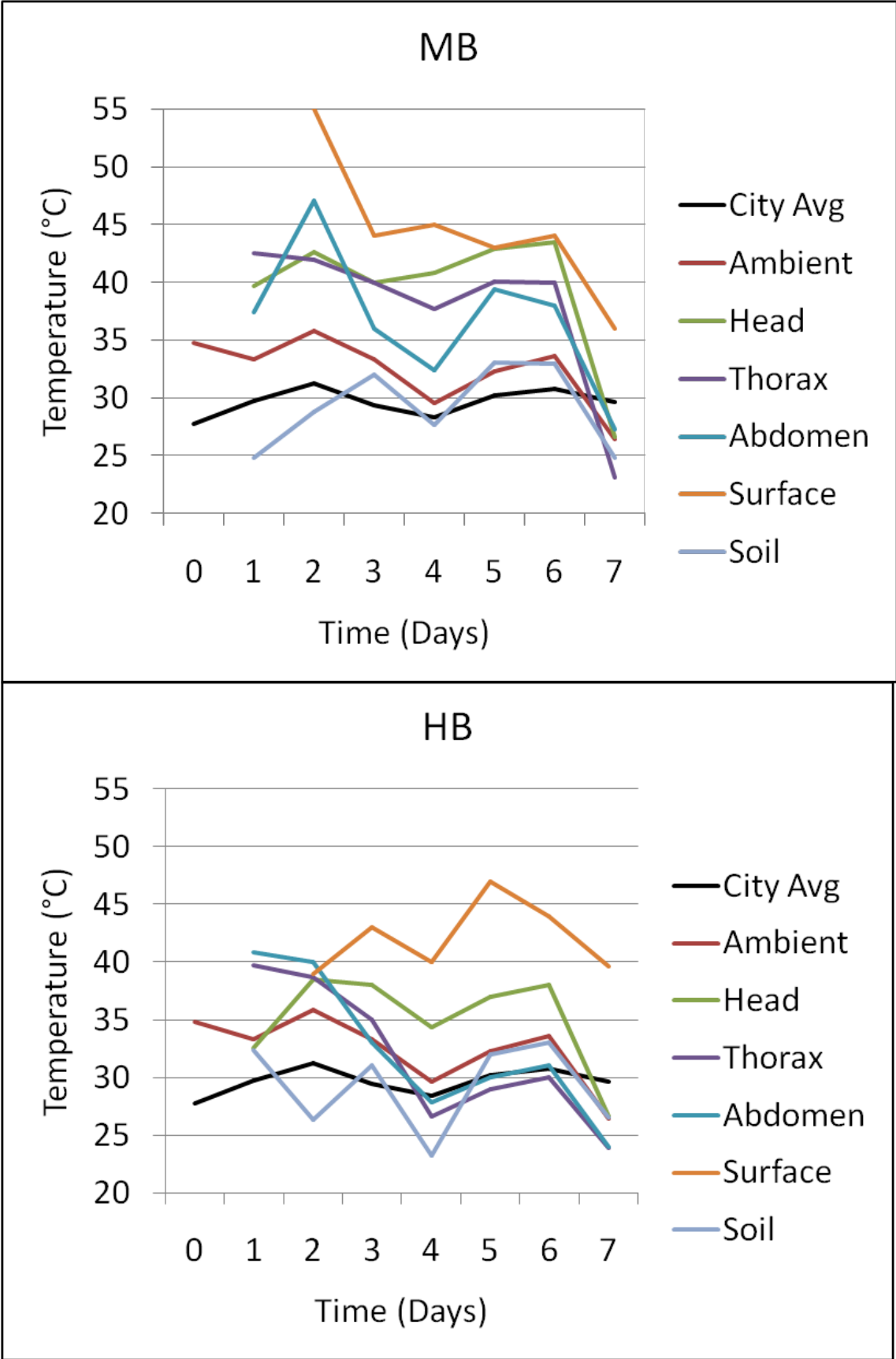
Figure 3.39 (continued) *Ambient & internal carcass temperatures during the Summer 2004 Trial.*
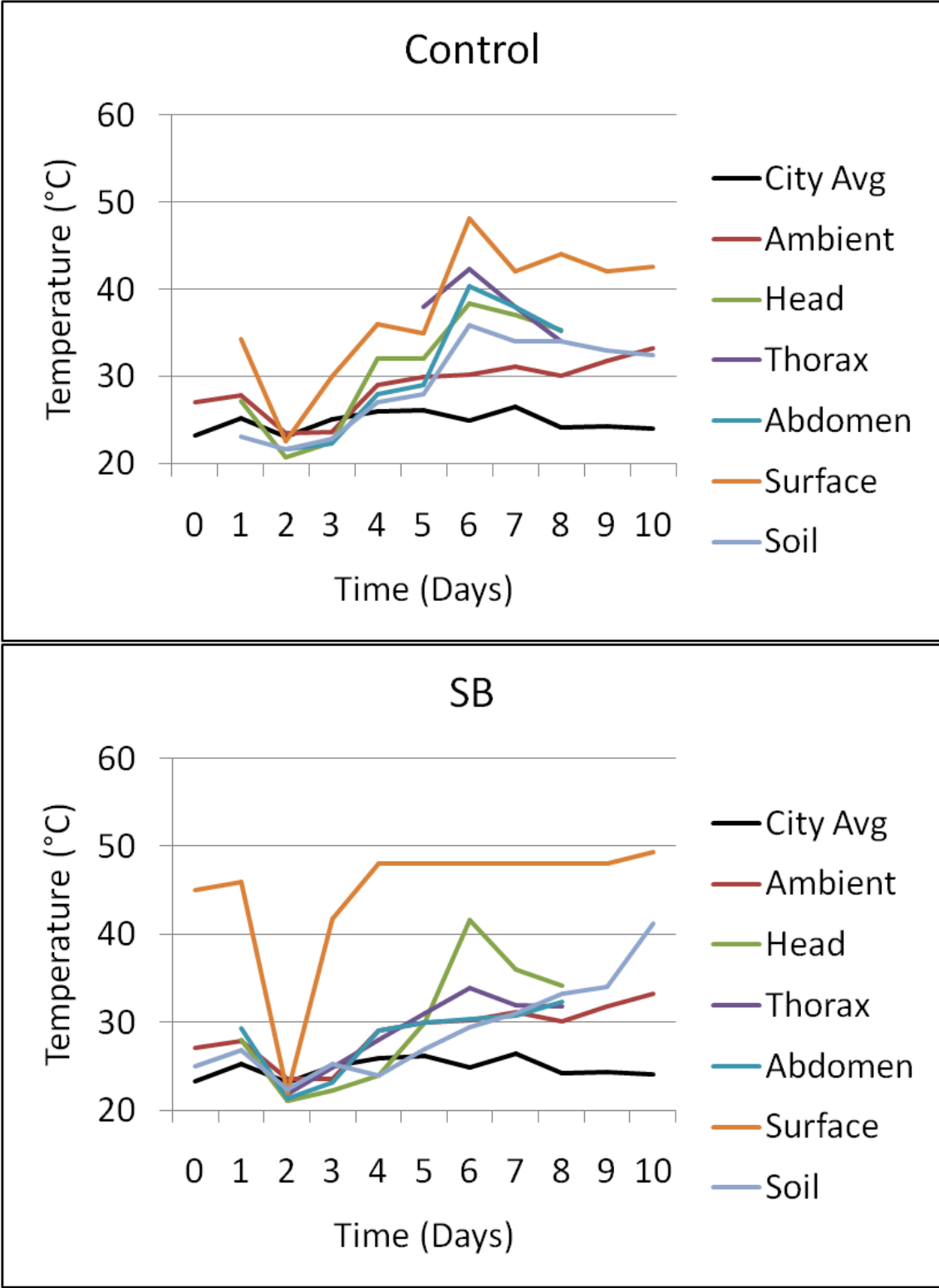
Figure 3.40 *Ambient & internal carcass temperatures during the Summer 2005 Trial.*
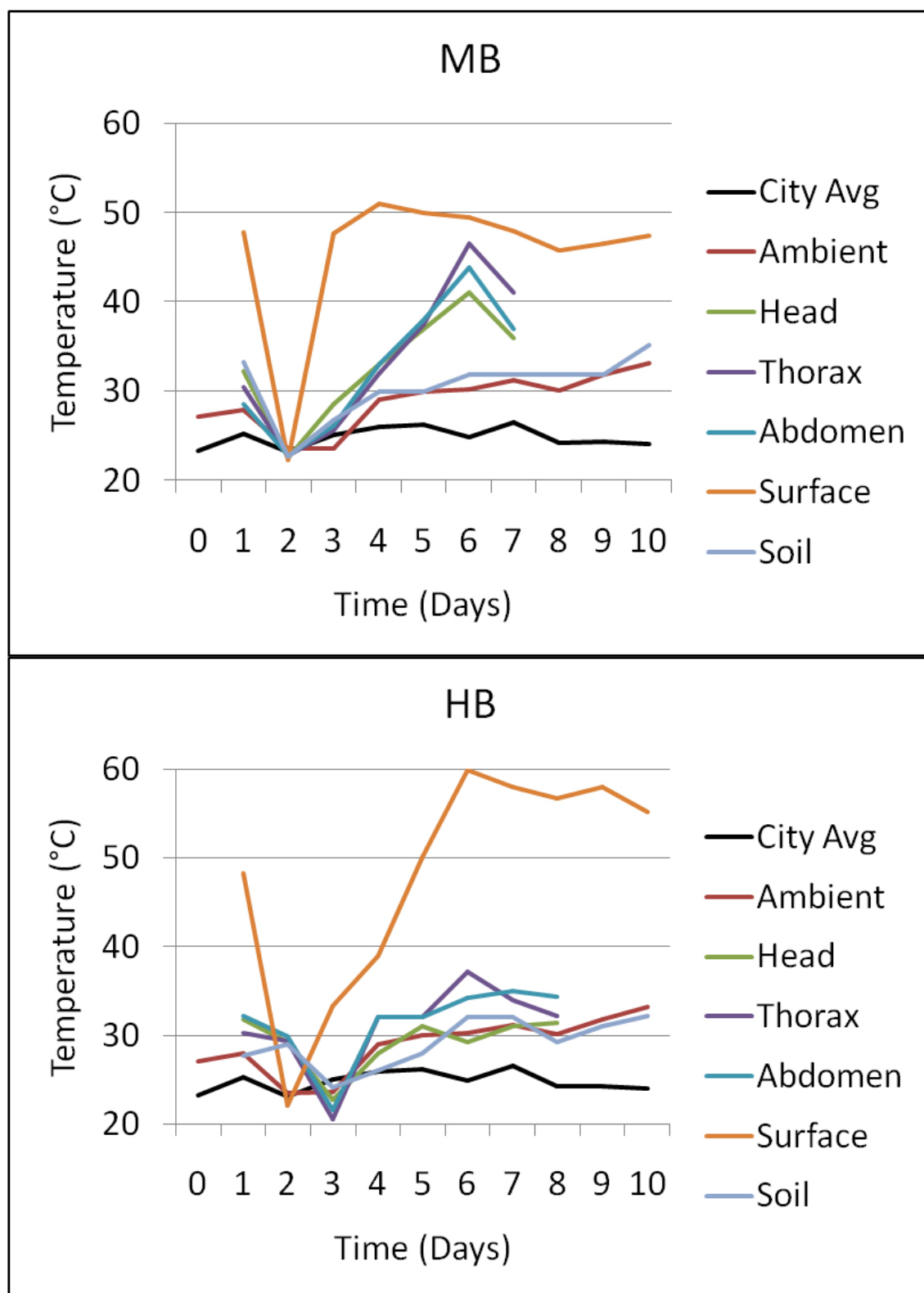
Figure 3.40 (continued) *Ambient & internal carcass temperatures during the Summer 2005 Trial.*

The temperature at which maggots develop in a decomposing body is often much higher than the ambient temperature, and varies according to the stage of decomposition and location within the decomposing body (Deonier, 1940; O'Flynn, 1983). When investigating the insects associated with a decomposing body, ambient temperature and the internal temperature (of the body and maggot mass) are the temperature measurements required (West, 1951). Ambient temperature appears to have the greatest effect on the rate of decay of a body or carcass. Maggots within body cavities such as the head, chest, abdomen and vagina will continue to feed and develop even in freezing weather because they generate their own metabolic heat through their large numbers (Mann, *et al.*, 1990; Turner & Howard, 1992). The rate of development of the insect is faster at higher temperatures (Dadour *et al.*, 2001), and the thermal history of maggots is the key to estimating postmortem interval (Greenberg, 1991). During the current study, the internal carcass temperatures were as much as 9.2°C higher than the ambient during the summer trials, due to maggot masses producing metabolic heat. This observation supported findings by Deonier (1940), who reported raised temperatures in horse, sheep and goat carcasses infested by maggots. Greenberg (1991) reported the temperature of a maggot mass feeding on ground beef, packed inside a human skull, to be 18°C above ambient.

Unfortunately, as mentioned in Section 2.1.3.3, no dataloggers were available for use during this study as during the previous study regarding the effect of orientation of the body or carcass (Kolver, 2003). The Thermochron iButtons also proved to be unreliable. Thus there is no record of carcass temperatures during times other than that of the actual observations. This may yield an incomplete picture of larval development and fluctuations in the ambient and the maggot mass temperatures, since nocturnal internal temperatures might have been considerably lower at the experimental site. This could have retarded decomposition rates. This was also noted by De Jong & Chadwick (1999). However, this was not a major concern during the summer trials, but of considerable importance during the cooler months of the year.

During decomposition, internal carcass temperatures are the combined results of the high metabolic rates of the bacteria (putrefaction) (Payne 1965) and arthropod activity (Tullis & Goff 1987; Hewadikaram & Goff 1991). During the fresh and bloated stages, autolysis and putrefaction are the primary heat generating sources. During this period, the carcass is subject to influence by external temperatures.

With the onset of arthropod activity, primarily the feeding activities of maggots, carcass temperatures were elevated above ambient and remained high until the carcasses deflated and the advanced decay stage began, at which point maggot masses left the carcasses to pupate and the internal temperatures dropped to approximate ambient temperatures. This supports findings by Tullis & Goff (1987), Greenberg (1991) and Hewadikaram & Goff (1991).

Soil temperature underneath the carcasses also varied greatly due to the effects of earth radiation and the carcass insulating the soil with a blanket effect, combined with the warming of the soil by feeding maggot masses (Figures 3.39 & 3.40).

The most extreme temperature by far was the carcass surface temperature, being constantly much higher than the ambient temperature. The only exception was on Day 2 during 2005 when it was completely overcast with a blanket of low level stratocumulus cloud. (Figure 3.41).

During 2005, the difference in carcass surface temperature and ambient temperature during times of observations increased with the level of burning of the carcasses (Tables 3.7 - 3.9). This phenomenon can be attributed to the carcass skin. With an increase in the level of burning, at least from control to SB and from SB to MB and HB, the skin was darker due to prolonged burning. This darkened or blackened skin absorbed more of the radiation from the sun and thus became hotter.

Table 3.7 Maximum difference (°C) in ambient and carcass surface temperature during the Summer Trials

	Control	SB	MB	HB
2004	15.6 (Day 7)	15.2 (Day 2)	19.2 (Day 2)	14.7 (Day 5)
2005	17.9 (Day 6)	19 (Day 4)	24.1 (Day 3)	29.7 (Day 6)

Table 3.8 Mean difference (°C) in ambient and measured temperature during the 2004 Summer Trial

	Head	Thorax	Abdomen	Surface	Soil
Control	6.3	9.2	6	9.2	0.4
SB	5.6	6.1	2.9	9.5	-2.4
MB	7.4	5.9	4.8	12.7	-2.9
HB	2.9	-0.2	0.3	10.3	-2.8

Table 3.9 Mean difference (°C) in ambient and measured temperature during the 2005 Summer Trial

	Head	Thorax	Abdomen	Surface	Soil
Control	2.5	7.7	2.4	8.6	0.1
SB	1.5	0.8	0.1	15.9	0.2
MB	5	6.3	4.8	16.6	1.5
HB	1.1	2.8	3.2	19	0.1

Figure 3.41 *Completely overcast conditions on Day 2 during the Summer 2005 Trial.*

The most difficult data to obtain is surely the most important too, namely the microclimatic conditions within the decomposing body. These are also the conditions in which the insects develop. A further complication results from the presence of dipteran larvae that change the microclimate. Because the internal carcass temperature fluctuates greatly during a single day, it is important to recognise that a single reading at a death scene during an investigation may yield an erroneously high reading for overall analysis (Anderson & VanLaerhoven 1996).

3.2.7 Formicidae Predation

As mentioned in Sections 3.2.3.2 and 3.2.3.3, Formicidae predated on the eggs, maggots and beetles, but also fed on the burnt skin and soft tissue of the burnt carcasses, causing feeding damage in the form of a characteristic pitted appearance formed by the removal of minute portions of tissue. This supports findings at unburnt carcarcasses by other authors (Schoenly & Reid, 1983; Braack, 1987; Anderson & Van Laerhoven, 1996). According to Braack (1987), ants only rarely have the opportunity to gain access to the carcass during summer because the carcass is then covered in a dense, highly active layer of maggots (Braack 1987).

The current study has shown that Formicidae were present at the carcasses during early decomposition. They usually arrive long before maggots have developed (Mansell, pers. comm.)[3].

Formicidae were represented by *A. custodiens* and *M. albopilosum* and predated on the carcass fauna as individuals and also in groups.

Eggs were taken from the carcasses soon after oviposition, supporting findings by Schoenly & Reid (1983) and Anderson & Van Laerhoven (1996). Predation on the maggots mostly occurred on postfeeding maggots which started to migrate from the carcasses to find a suitable place to pupate (Figure 3.42).

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Figure 3.42 *Single A. custodiens (Formicidae) predating on a postfeeding maggot.*

Figure 3.43 *Group of A. custodiens (Formicidae) predating on a Dermestidae adult.*

Migration of the maggots occurred over distances of up to 20 meters, similar to previous studies (Van der Linde, pers. comm.)[4]

In some instances, Formicidae were observed to predate on Dermestidae and Histeridae adults, which were carried to the nest by groups of formicids of between 10 and 20 individuals (Figures 3.43 & 3.44). Formicidae even predated on the newly emerged adult blow flies during 2005 at the SB carcass on Day 15.

Figure 3.44 *Group of A. custodiens (Formicidae) predating on a Histeridae adult.*

In a previous study in Hawaii, it was found that the presence of ants at a carcass lengthened the duration of the bloated and decay stages (Early & Goff, 1986). This finding could neither be refuted nor confirmed during the summer trials of the current study.

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Early and Goff (1986) has demonstrated that ants can dramatically retard dipteran larval activities and extend the time necessary for decomposition, or cause carcasses to remain untouched by maggots and available to vertebrate scavengers. This phenomenon was not observed during the current study, possibly due to the large maggot masses found on the carcasses. Predation of the blow fly eggs was not sufficient to have such an effect on the size of the maggot masses. However, predation on the postfeeding maggots, together with possible *C. albi*ceps predation on the other maggots had an influence on the number of adult blow flies emerging from the pupae.

Section 3.3. The influence of burning on carcass decomposition and arthropod succession: Two Autumn Studies.

3.3.1. Decomposition of the carcasses

Immediately after killing, the control carcasses were placed in the field and this date and time was designated as Day 0 and indicated the start of each trial (Table 3.10).

Table 3.10 Commencement dates and times of the Autumn Trials

Year	Date	Time
2004	25 March	14:30
2005	11 April	14:30

The control carcasses showed the first signs of rigor mortis, with the limbs starting to become stiff during the first observations, whereas the burnt carcasses showed signs of bloating. The burnt carcasses had cooled down completely by the time the first observations were made.

The Bloated stage was shorter at the control carcass than at the burnt carcasses (Figure 3.45).

During 2004, oviposition occurred simultaneously at all of the carcasses, except for the HB carcass where it occurred one day later (Figure 3.45). This was contradictory to findings by Avila and Goff (1998), but during 2005 oviposition occurred at the burnt carcasses three to four days earlier than at the control carcass (Figure 3.45), supporting the findings of Avila and Goff (1998).

The Active Decay stage was shorter at the burnt carcasses than the control carcass (Figure 3.45) due to maggots gaining access to utilisable tissue far quicker than at the control carcass. This rapid access was possible due to the numerous oviposition sites and access routes established by the rupture of the burnt skin.
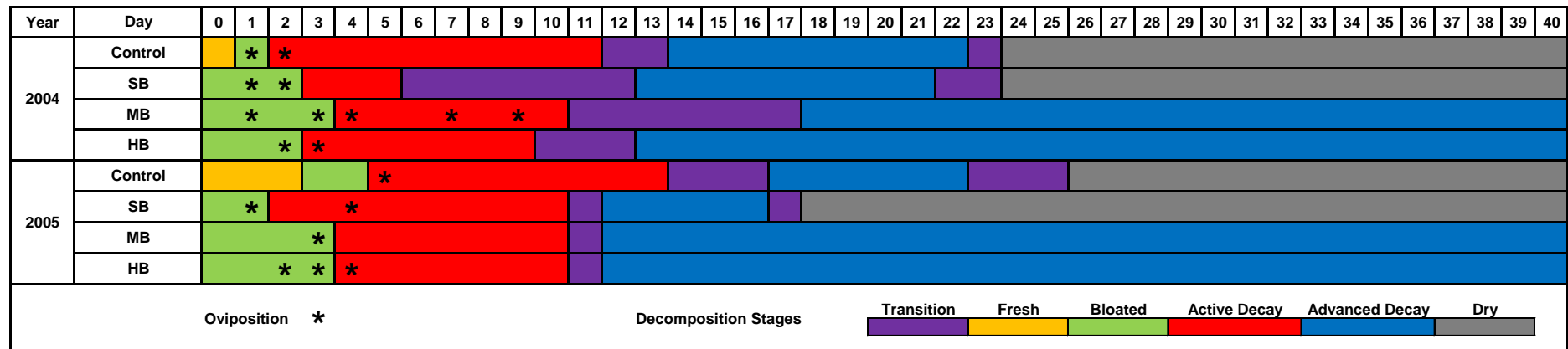
Figure 3.45 *Decomposition stages of carcasses and days of oviposition during the autumn trials.*

The Advanced Decay stage occurred earlier at the burnt carcasses than at the control carcass, with the exception of the MB carcass during 2004 (Figure 3.45). The Dry/Remains stage was not reached by the MB and HB carcasses (Figure 3.45) due to muscle tissue remaining.

Only the days when major changes in the appearance of the carcasses and/or major changes in the insect fauna associated with the carcasses occurred, are discussed.

3.3.1.1 2004 Autumn Trial

Day 0:

- The control carcass still appeared fresh with blood dripping from the nose.
- All of the burnt carcasses showed signs of bloating (Figure. 3.45).
- The SB and MB carcasses showed slight bloating, while the HB carcass showed extensive bloating with the abdomen ruptured.
- The HB carcass also had heat fractures to the distal parts of the legs.

Day 1:

- No blow fly eggs were visible at the control carcass during the morning observations (±09:49).
- The control carcass had started to bloat (Figure 3.45) and showed signs of livor mortis. Rigor mortis was evident for the hind legs whilst the front legs had started to lose some of the stiffness of rigor mortis.
- During the afternoon observations (±16:17), newly hatched first instar maggots was observed in the mouth of the control carcass and indicated the start of the Active Decay stage. The Active Decay stage lasted until Day 11 at the control carcass (Figure 3.45).
- The SB and MB carcasses showed slight bloating (Figure 3.45), with oviposition having occurred in the mouth of the SB carcass.

Day 2:

- The carcasses still appeared bloated (Figure 3.45).

- At the control carcass, the intestines protruded from the rectum due to the pressure built up from putrefaction (Figure 3.46). The blood in the blood vessels on the lower side had turned green to create a marbling effect on the skin surface. Oviposition had occurred at the head-soil interface at the control carcass. First instar maggots were feeding inside the mouth.

- The SB carcass had a skin rupture on the back due to bloating. Blood was foaming from the nose. Blow fly eggs were found inside the mouth and underneath the head of the SB carcass

- The skin on the thorax and abdomen of the MB carcass had ruptured due to bloating and the intestines protruded from the abdominal skin rupture.

- The intestines of the HB carcass protruded from the abdominal rupture. Other skin ruptures were found at the HB carcass on the neck, the thorax between the front legs and on the back (Figure 3.47). Oviposition had occurred in the mouth and between the hind legs
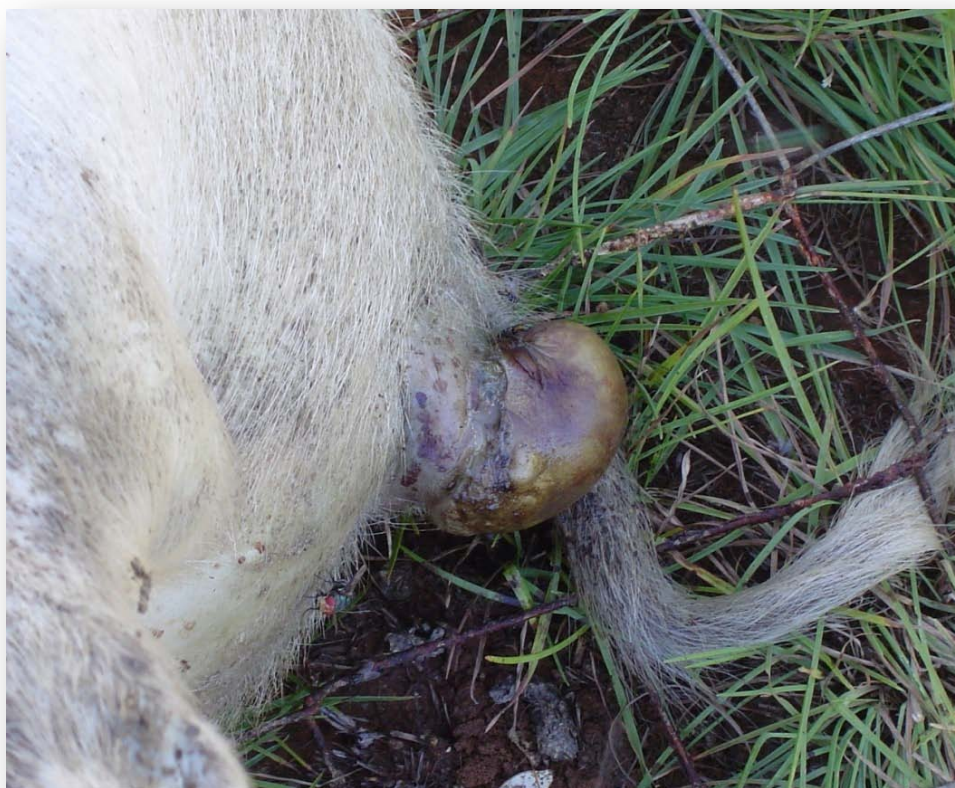
Figure 3.46 *Intestines of the control carcass protruding from the anus due to bloating during the Autumn 2004 Trial.*

Figure 3.47 *Skin ruptures on the HB carcass due to bloating (front and back) during the Autumn 2004 Trial.*

Day 3:

- First and second instar maggots were feeding inside the mouth and underneath the head of the control carcass.
- The SB carcass had new skin ruptures on the neck, thorax and abdomen. The carcass was still bloated, but blow fly eggs and first instar maggots were found feeding in the mouth and the ruptures of the neck and abdomen, thus starting the Active Decay Stage which lasted until Day 5 (Figure 3.45).
- The skin rupture on the thorax of the MB carcass had enlarged. Oviposition occurred inside the mouth of this carcass.
- Oviposition had occurred at the HB carcass in the thoracic skin rupture. First instar maggots were actively feeding on carcass tissue in the mouth and between the hind legs. The Active Decay stage lasted from Day 3 until Day 7 at the HB carcass (Figure 3.45).

Day 4:

- Blow fly eggs and first instar maggots were found inside the mouth of the MB carcass, one day after the first beetle larvae (Dermestidae) were observed on the carcass.
- Despite this finding, the MB carcass was still considered to be in the Bloated Stage until Day 3, with the Active Decay Stage commencing on Day 4. (Figure 3.45).

Day 5:

- Second and third instar maggots were feeding underneath the areas of skin slippage on the right front leg and abdomen at the control carcass.
- At the SB carcass, third instar maggots were actively feeding all over the carcass. The carcass appeared more deflated due to the feeding activity of the maggots (Figure 3.48).

Figure 3.48 *SB carcass flatter in appearance due to large mass of feeding third instar larvae during the Autumn 2004 Trial.*

Day 5 (continued):

- Second instar maggots were feeding in the mouth of the MB carcass.
- Dead second instar maggots were found on the surface of the HB carcass. Second instar maggots were active in the mouth, head, abdomen and skin fold of the abdomen of the HB carcass.

Day 6:

- Large third instar maggot masses were feeding on the tissue of the head, right front leg and abdomen of the control carcass. The carcass appeared deflated with the hind legs in a resting position. The thorax was slightly turned with the carcass almost on its back. The left front leg was still in the air.

Day 6 (continued):

- The large third instar maggot mass feeding all over the SB carcass was still present. The SB carcass appeared almost completely flat with most of the tissue removed by the feeding activity of the large maggot mass (Figure 3.45). Postfeeding maggots had started to migrate from the carcass to pupate, 6 days earlier than at the control carcass.

- Normally this would signal the start of the Advanced Decay Stage, but this migrating process continued for 6 more days until Day 12 with the Advanced Decay stage commencing at the SB carcass on Day 13, one day earlier than at the control carcass (Figure 3.45). The Advanced Decay Stage lasted until Day 21 at the SB carcass.

- A small number of second instar maggots were feeding inside the mouth and on the head of the MB carcass. Third instar maggots were found feeding in a skin fold on the right side of the neck of the MB carcass.

- At the HB carcass, third instar maggots were active in the head and thoracic cavity (Figure 3.49).

Day 7:

- Freshly oviposited blow fly eggs were found at the thorax-soil interface at the MB carcass.

- Large third instar maggot masses were feeding in the head, thoracic cavity and abdominal cavity of the HB carcass. Freshly oviposited blow fly eggs were found on the left hind leg of the HB carcass.

Day 9:

- Newly oviposited blow fly eggs were found at the thorax-soil interface at the MB carcass.
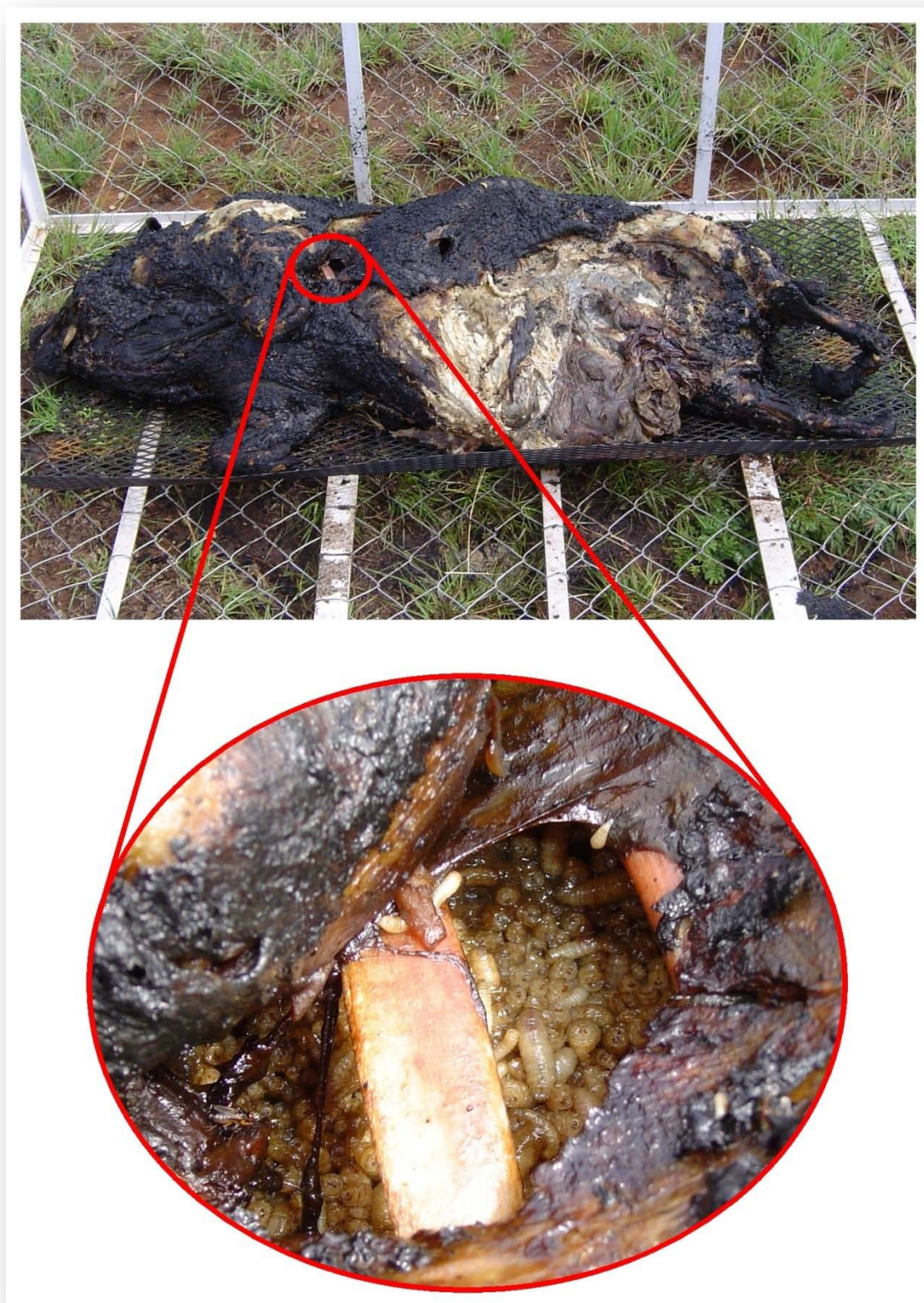
Figure 3.49 *Active third instar maggots in the thoracic cavity of the HB carcass on Day 6 during the Autumn 2004 Trial.*

Day 12:

- Postfeeding maggots started to move away from the control carcass to pupate, starting the Advanced Decay stage which lasted until Day 22 (Fig 3.45).
- Second instar maggots were feeding in a skin fold between the front legs and at the thorax-soil interface at the MB carcass.

Piophilidae maggots (cheese skippers) were observed underneath the control carcass during the Active Decay stage from Day 16 for the remainder of the study.

On Day 17, postfeeding maggots started to migrate from underneath the MB carcass. The Advanced Decay stage followed at the MB carcass (Figure 3.45). Cheese skipper larvae were observed from Day 18 on the MB carcass.

The Dry/Remains Stage commenced on Day 24 at the control and SB carcasses Figure 3.45).

3.3.1.2 2005 Autumn Trial

Day 1:

- The control carcass showed no signs of bloating and still appeared fresh.
- The burnt carcasses were bloated (Figure 3.45). Oviposition had occurred in the mouth of the SB carcass

Day 2:

- The control carcass still appeared fresh and was in rigor mortis on Day 2, but showed no bloating (Figure 3.45).
- The SB carcass was bloated with a skin rupture on the neck. First instar maggots were feeding inside the mouth, signifying the onset of the Active Decay stage.

Day 2 (continued):

- The MB and HB carcasses were bloated with skin ruptures on the left side of the thorax.
- Oviposition had occurred in the mouth of the HB carcass.

Day 3:

- Bloating commenced at the control carcass (Figure 3.45).
- Blow fly eggs were found inside the mouths of the MB and HB carcasses.

Day 4:

- At the control carcass, rigor mortis had ended, but the legs were in the air due to bloating. A prolapse of the rectum and vagina had also developed.
- The SB carcass had skin ruptures on the neck, thorax, abdomen, between the hind legs and directly below the rectum. The intestines protruded from the skin rupture below the rectum. Oviposition had occurred in the skin rupture between the hind legs and underneath the right ear. First and second instar maggots filled the cavity of the mouth.
- The MB carcass had skin ruptures on the neck, thorax and abdomen. The intestines protruded from the abdominal skin rupture. Blow fly eggs and first instar maggots were found inside the mouth.
- At the HB carcass, skin ruptures were observed on the neck, thorax and abdomen. The intestines protruded from the abdominal skin rupture. A large mass of blow fly eggs were found underneath the head. First instar maggots were active inside the mouth.
- The Active Decay Stage commenced on Day 4 at the MB and HB carcasses (Figure 3.45)

Day 5:

- Oviposition had occurred at the carcass-soil interface in the area of the thorax and abdomen of the control carcass.
- These blow fly eggs later hatched and maggots were feeding on the carcass by the time the afternoon observations were made.
- This indicated the start of the Active Decay stage (Figure 3.45).

Day 8:

- At the control carcass, a small mass of second and third instar maggots were active on the right front leg, thorax and at the abdominal carcass-soil interface.
- Active second and third instar maggots were observed at the SB carcass inside or on the head, neck, thorax, and the abdomen at the carcass-soil interface.
- Third instar maggots were found on the thorax of the MB carcass.
- The head of the HB carcass was skeletonised and a large active mass of third instar maggots was found on the abdomen.

Day 9:

- Second and third instar maggots were active on the right front leg and the rectum of the control carcass.
- The SB carcass was flattened and the head completely skeletonised. Third instar maggots covered the entire carcass.
- The MB carcass had third instar maggots feeding inside the abdominal cavity and on the thorax between the front legs.

Day 11:

- The control carcass was still more or less intact, with the exception of the right front leg being skeletonised by the feeding of the maggots. Third instar maggots were observed inside the head and on the thorax between the front legs.
- Postfeeding maggots started to migrate away from the burnt carcasses to pupate.

The Advanced Decay stage commenced at the burnt carcasses on Day 12 (Figure 3.45). Postfeeding maggots started to migrate from the control carcass on Day 14, with the Advanced Decay stage commencing on Day 17 (Figure 3.45). A large mass of third instar and postfeeding maggots was observed underneath the control carcass on Day 17. The Dry/Remains stage commenced on Days 26 and 18 at the control and SB carcasses, respectively (Figure 3.45).

3.3.1.3 Mass Loss

The mass of each carcass was recorded daily only during the first trial of each season (see Section 2.1.3.2). This was done to minimize the disturbance to the carcasses and the possible effect of this disturbance on decomposition and insect succession.

The fastest decomposition occurred at the SB carcass during the 2004 trial (Figure 3.50). This carcass also had the most tissue removed. The MB carcass decomposed faster than the Control and HB carcasses during the first 4 days. Thereafter, the control carcass decomposed faster than the MB and HB carcasses. The MB and HB carcass decomposed at a similar rate, with slightly more tissue removed from the MB carcass than the HB carcass. The slowest decomposer with the most tissue remaining was the HB carcass (Figure 3.50), due to less tissue remaining after burning which could be utilised/consumed by the feeding maggots.
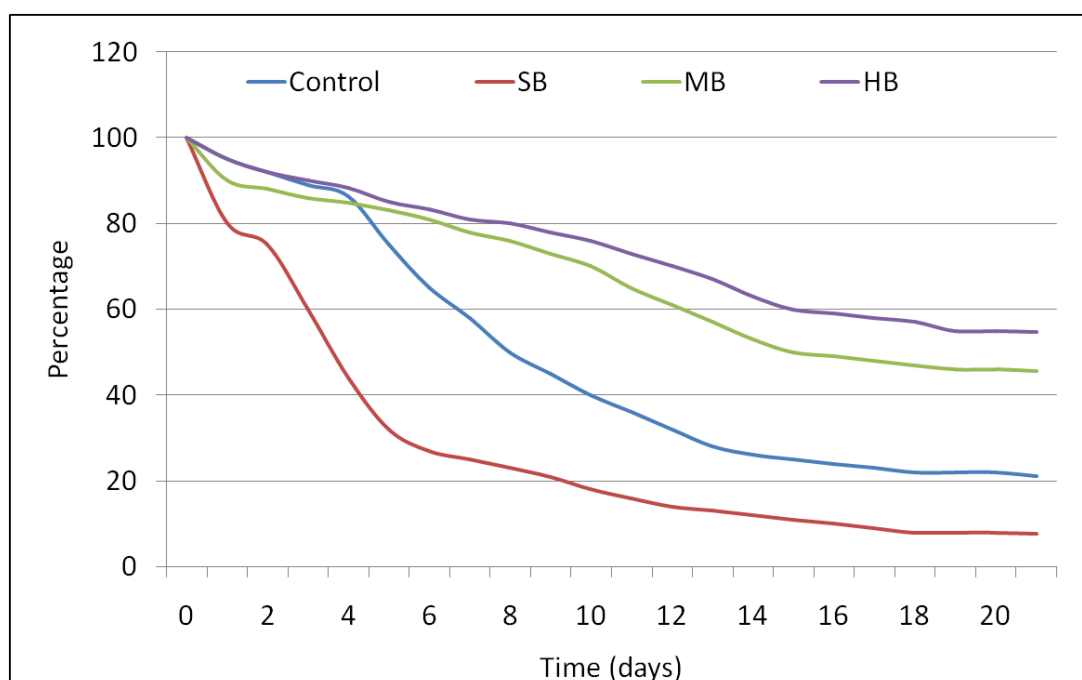
Figure 3.50 *Percentage body mass remaining of carcasses during the Autumn 2004 Trial.*

During 2005, the carcasses followed the same decompositional trend as during 2004. No data were available for the actual rate of decomposition during the Autumn 2005 trial, since the mass of each carcass was not recorded daily to limit disturbance to the carcass fauna. Visual observations confirmed that the SB carcass decomposed quicker than the control carcass during 2005, since decomposition had progressed further at the SB carcass than at the control carcass during same time frame. The MB and HB carcasses decomposed the slowest, with a lesser degree of decomposition reached within the same time as at the control and SB carcasses.

3.3.2. Arthropod Composition

3.3.2.1 Orders

3.3.2.1.1 2004 Autumn Trial

The dominant necrophagous order at all the carcasses was Diptera, followed by Coleoptera. No discernable pattern was found in the dominance at the carcasses.

Predators were present in low numbers viz. Hymenoptera (ants), Isoptera and Araneae. Tourist insects comprised Orthoptera, Hemiptera, and Lepidoptera. (Figure 3.51).
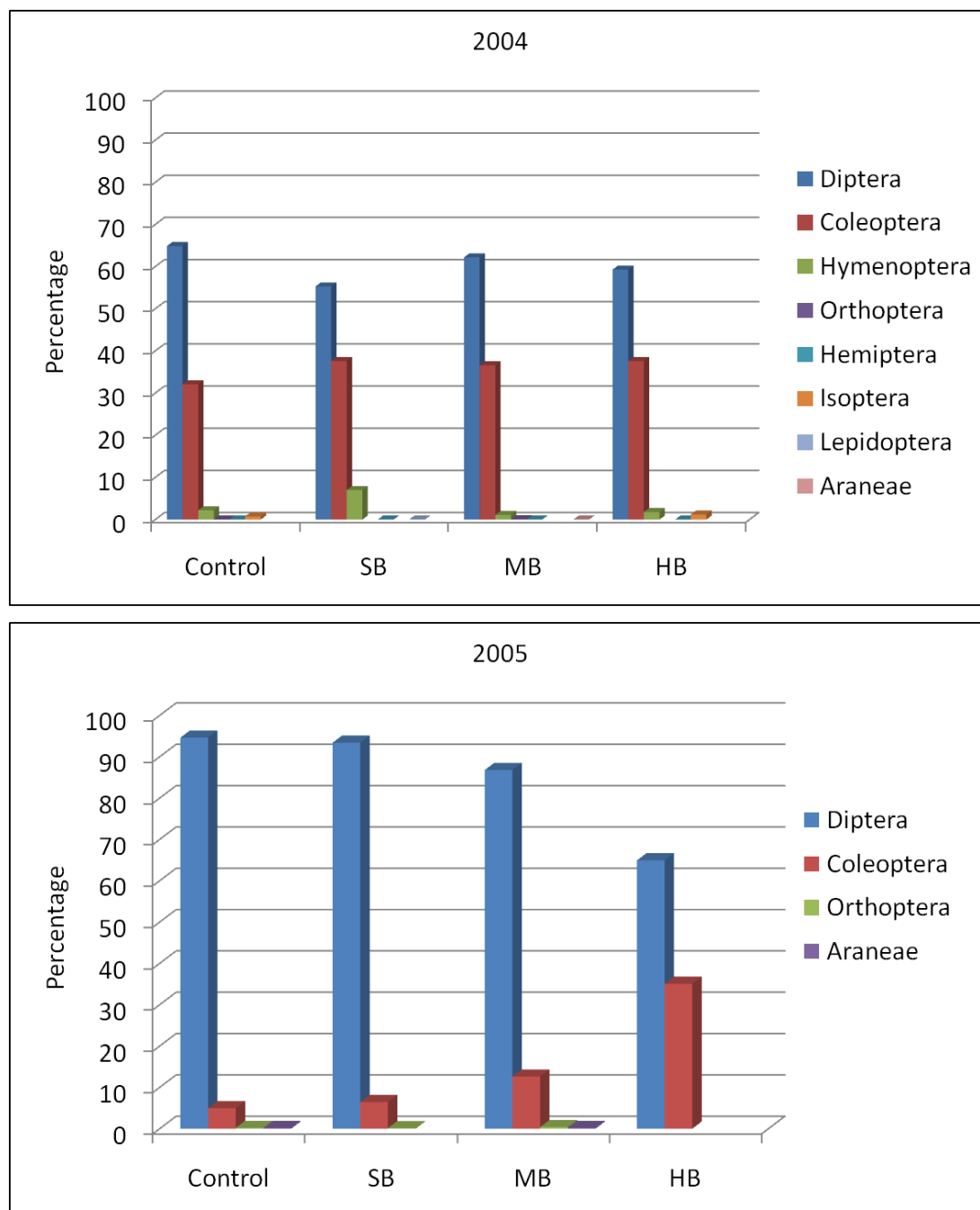
Figure 3.51 *Order Composition during the autumn trials.*

3.3.2.1.2 2005 Autumn Trial

As found during 2004, Diptera were the dominant necrophagous order, followed by Coleoptera. The dominance of Diptera decreased as the dominance of Coleoptera increased with an increase in the level of burning of the carcass. Low numbers of tourist Orthoptera and predatory Araneae were also observed (Figure 3.51).

3.3.2.2 Diptera

Muscidae were not observed to breed in any of the carcasses. Therefore no Muscidae maggots were observed to be necrophagous and thus had no effect on the decomposition process. Adult Muscidae were observed to feed on decompositional fluids dripping from the carcasses (BOD).

3.3.2.2.1 2004 Autumn Trial

Calliphoridae were the dominant necrophagous family at all the carcasses (Figure 3.52). Lower numbers of Muscidae, Piophilidae, Sarcophagidae and Asilidae were also observed. A slightly higher dominance of Piophilidae than Muscidae was found at the MB carcass (Figure 3.52).

The dominant necrophagous Diptera species at all the carcasses were *C. marginalis* and *C. albiceps*, with *C. marginalis* being most dominant (Figure 3.53).

3.3.2.2.2 2005 Autumn Trial

Diptera dominance was almost the same as during 2004, with larger numbers of Calliphoridae observed (Figure 3.52). Dominance of Calliphoridae decreased as the dominance of Muscidae increased with the increase in burn level at the burnt carcasses(Figure 3.52). The dominance of Piophilidae increased with the increase of burn level at all carcasses (Figure 3.52).
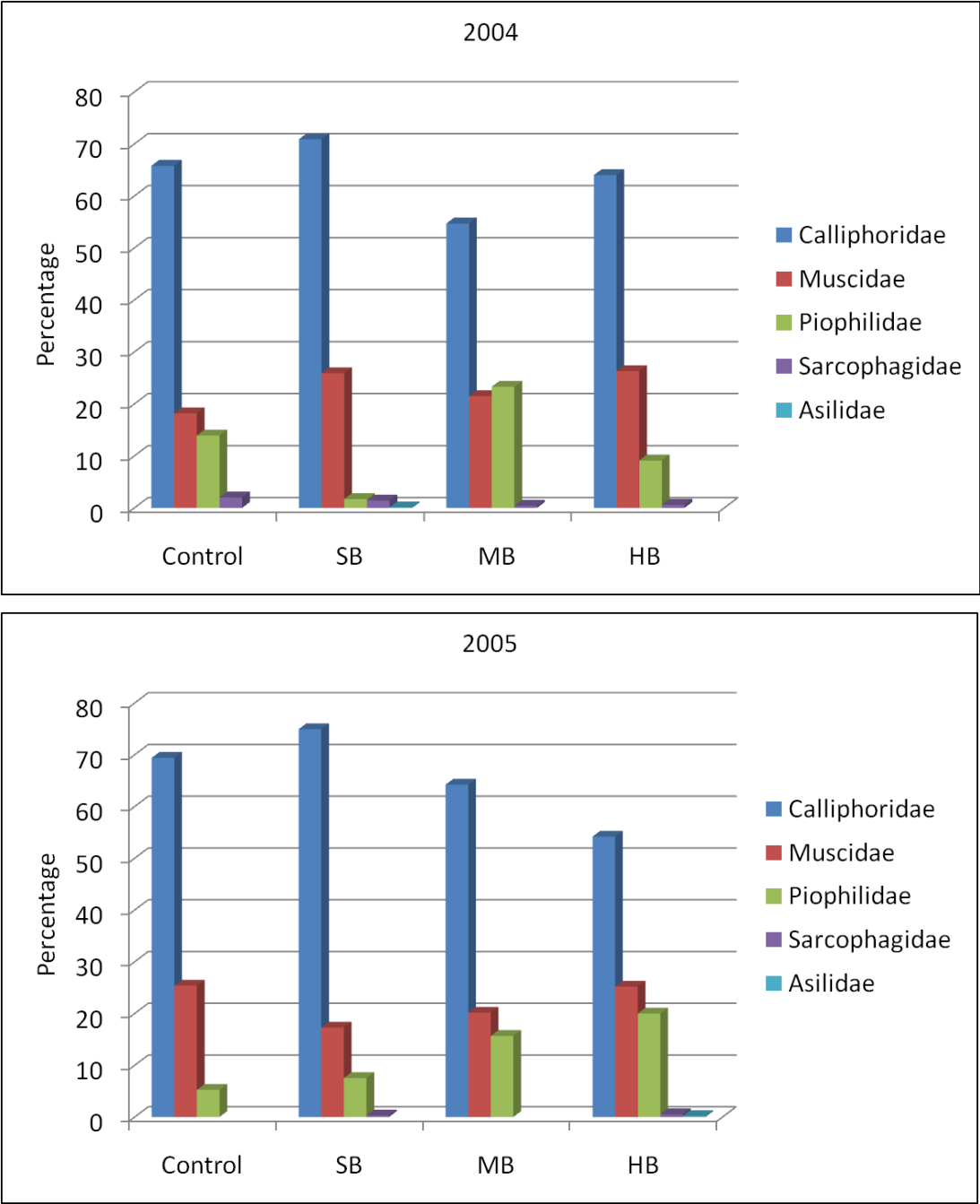
Figure 3.52 *Diptera Composition during the autumn trials.*
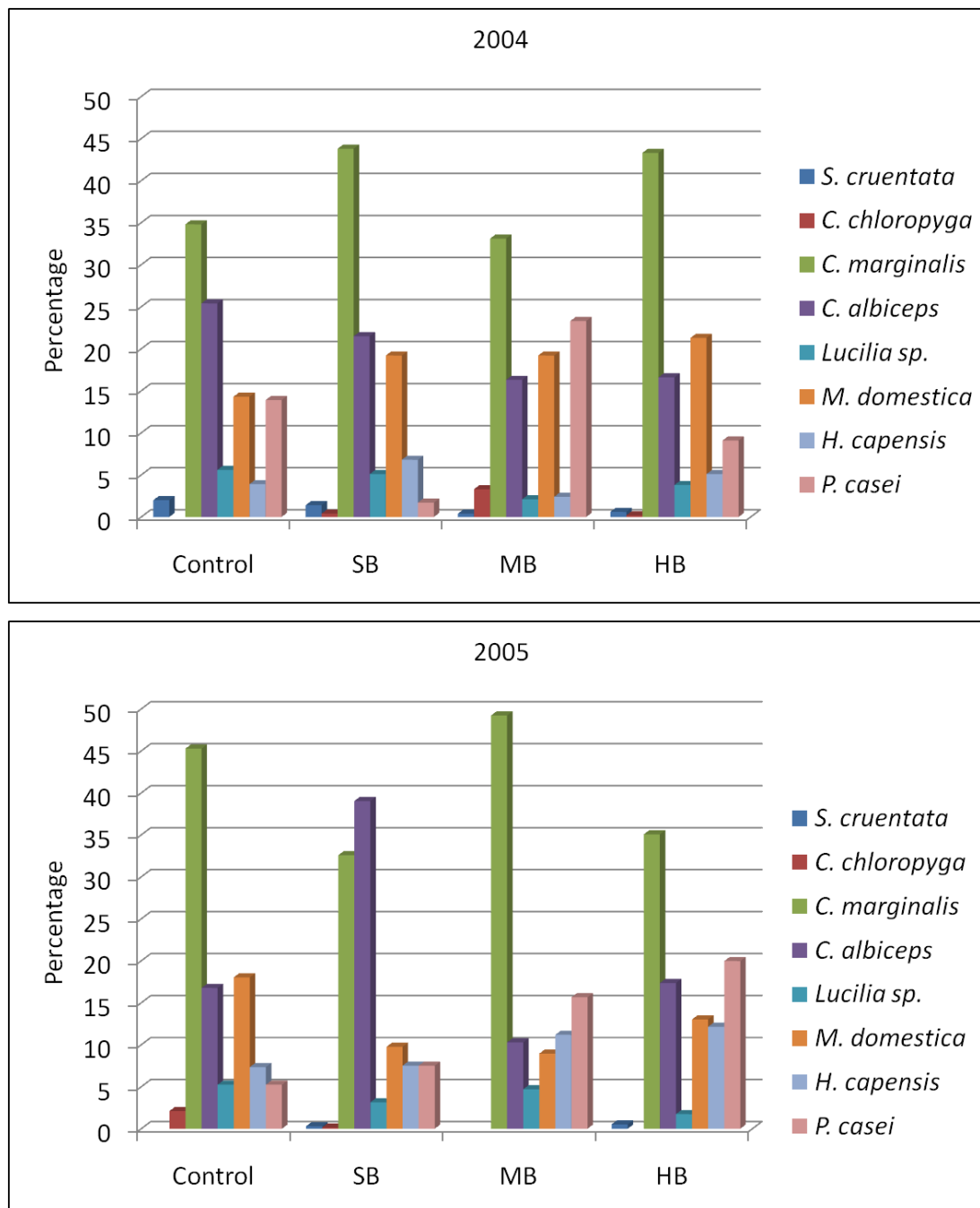
Figure 3.53 *Diptera Species Composition during the autumn trials.*

Chrysomya marginalis was the dominant Diptera species at the control, MB and HB carcasses. *Chrysomya albiceps* was the dominant Diptera species at the SB carcass (Figure 3.53).

3.3.2.3 Coleoptera

3.3.2.3.1 2004 Autumn Trial

Histeridae were dominant at the control carcass and Dermestidae at the SB carcass. Cleridae were the dominant Coleoptera family at the MB and HB carcasses. Silphidae and Histeridae were present at all the carcasses in varying numbers (Figure 3.54).

The dominance of Dermestidae decreased and the dominance of Cleridae and Histeridae increased with an increase in the level of burning at the burnt carcasses. The dominance of Silphidae at the burnt carcasses was significantly greater at the burnt carcasses than at the control carcass (Figure 3.54).

3.3.2.3.2 2005 Autumn Trial

Dermestidae were dominant at the control carcass while Cleridae were dominant at the MB and HB carcasses. Dermestidae and Cleridae were equally dominant at the SB carcass. Histeridae were not present at the MB carcass, while Silphidae were only present at the SB and HB carcasses (Figure 3.54).

The dominance of Dermestidae decreased with the level of burning of the carcasses, with the highest dominance at the control carcass and the lowest at the HB carcass. With the exception of the HB carcass, the dominance of Cleridae increased with the increased level of burning of the carcasses. The lowest dominance of Cleridae occurred at the control carcass and the highest at the MB carcass (Figure 3.54).
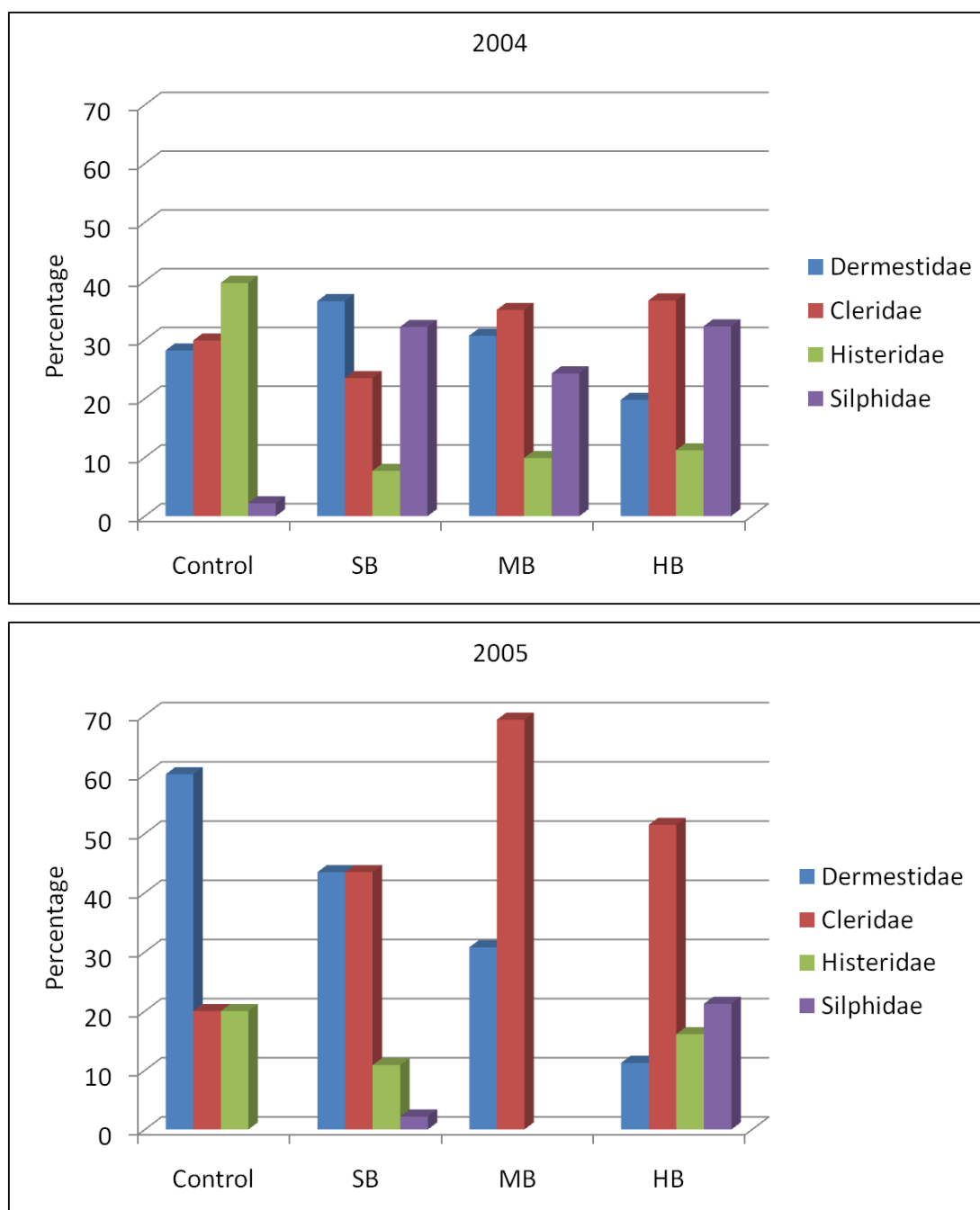
Figure 3.54 *Coleoptera Composition during the autumn trials.*

Adult Calliphoridae, Sarcophagidae and in some instances, Formicidae were the first insects to arrive, within five minutes of exposure.

3.3.3. Arthropod succession on the carcasses

The most abundant species observed at the carcasses during autumn were C*. marginalis, C. albiceps, M. domestica, P.casei, D. maculatus, N. rufipes, T. micans* (Figure 3.55) and unidentified Histeridae beetles.

Calliphoridae were initially present at the carcasses in large numbers for two to three consecutive days. As was found during the summer trials, *C. marginalis* generally fed on and inside the carcasses, while *C. albiceps* fed underneath the carcasses. *Musca domestica* never bred on the carcasses and subsequently no *M. domestica* larvae were observed or collected.

Only the numbers of Coleopteran larvae are indicated, since it was easier to count these larvae than was the case with the maggots. All fauna referred to are adults, unless otherwise indicated.

3.3.3.1 Control

Calliphoridae and Muscidae were the first arrivals at the carcass during 2004 and 2005 (Figures 3.56 & 3.57). During 2004, the first arrivals were less than five adults of *S. cruentata, C. marginalis, C. albiceps* and *Lucilia sp. Musca domestica* adults were initially represented by less than 20 individuals at the control carcass (Figure 3.56). Their numbers increased over the next 3 days. Less than 50 Formicidae individuals occurred at the carcass on Days 1 and 2, respectively.

During 2005, the first arrivals were less than five adults of *Lucilia sp.*, *M. domestica* and *Salticidae sp.*(Figure 3.57). *Chrysomya marginalis,* and *C. chloropyga* were first observed at the carcass on Day 2 and *C. albiceps* on Day 4.

During 2004, *P. casei* was observed at the carcass from Day 6, with larvae from Day 16 (Figure 3.56).
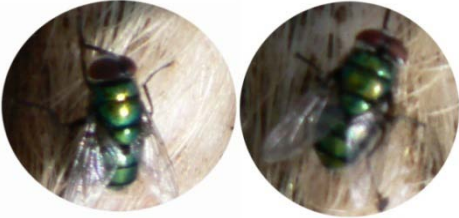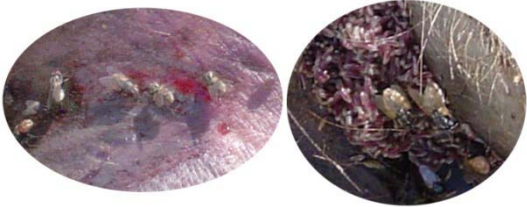
Figure 3.55 *Most abundant species observed during the autumn trials.*

Photographs not taken by the author: *i (Gross, 2005)*

                                                     ii (Gross, 2005)

                                                iii (London Natural Science Museum, 2004)

                                                iv (Makarov, 2006)

                                                v (Falatico)
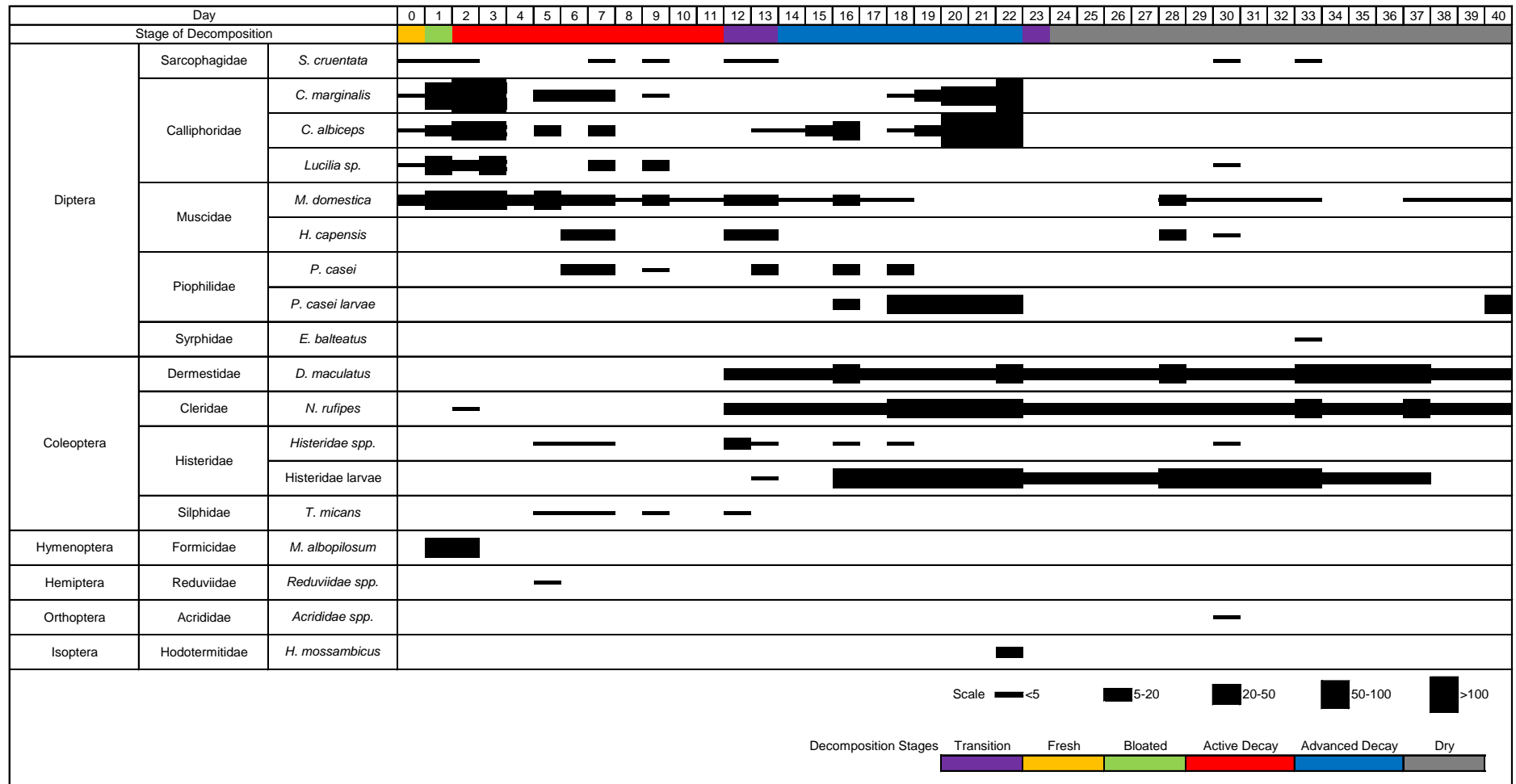
Figure 3.56 *Arthropod Succession on the control carcass during the Autumn 2004 Trial.*
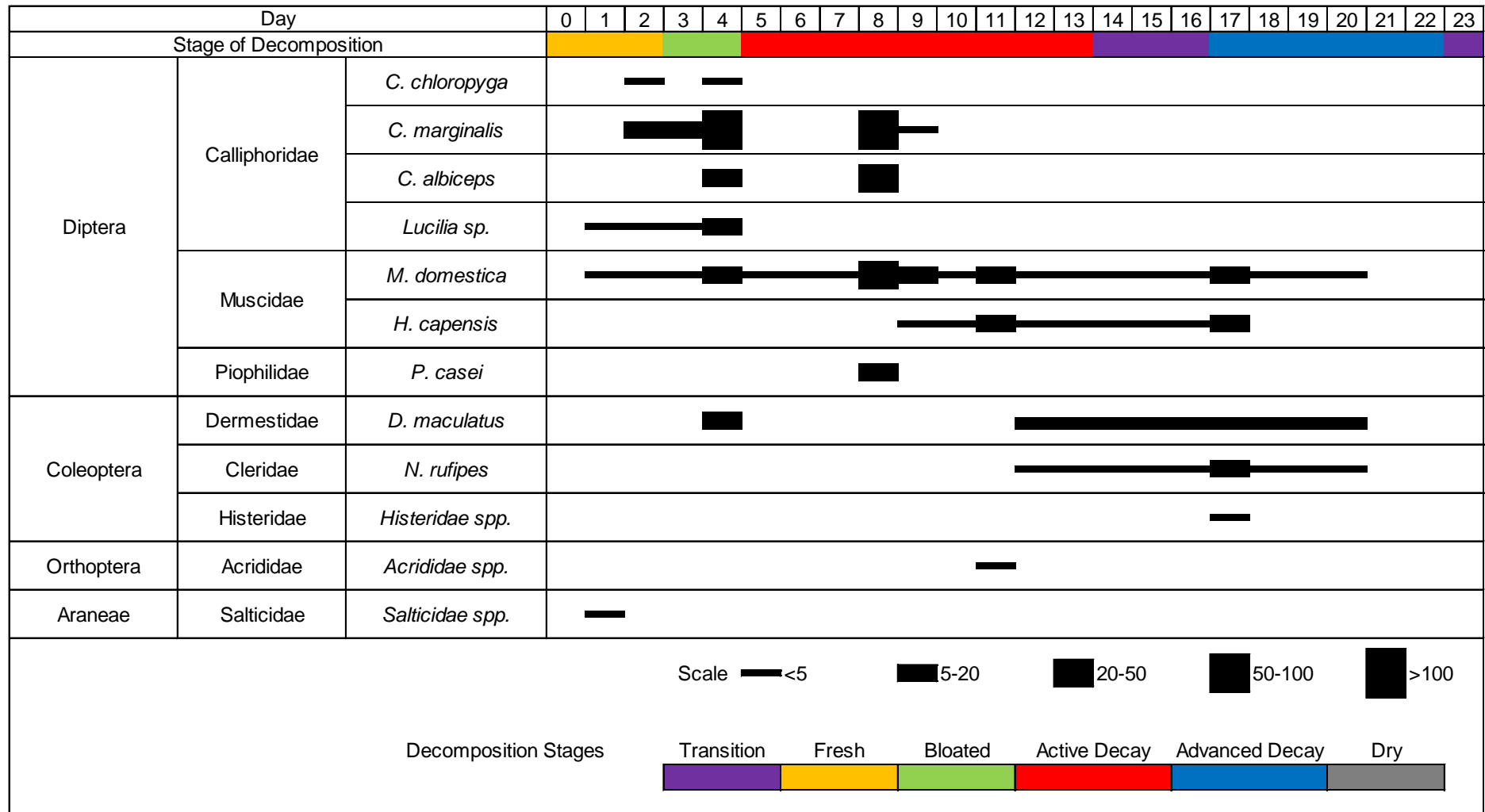
Figure 3.57 *Arthropod Succession on the control carcass during the Autumn 2005 Trial.*

A second occurrence (second generation) of large numbers of adult Calliphoridae (*C. marginalis* & *C. albiceps*) was found at the carcass during 2004 and 2005 from Day 20 and Day 8, respectively (Figures 3.56 & 3.57). These were the adult blow flies that had emerged from the pupae.

As during the summer trials, Muscidae were continually observed in the vicinity of the carcass (Figures 3.56 & 3.57).

Dermestidae occurred on the carcass from Day 12 to Day 40 during 2004 and from Day 12 to Day 20 during 2005 (Figures 3.56 & 3.57). Dermestidae larvae were not found at the carcass.

Isoptera, represented by *Hodotermes mossambicus*, were observed only once during the 2004 trial and was predating on blow flies emerging from the pupae. Cleridae were observed at the carcass at the same time as Dermestidae. Cleridae also were not observed to breed on the carcass (Figures 3.56 & 3.57).

Small numbers of Histeridae were also periodically found on the carcass from Day 5 during 2004 and only once on Day 17 during 2005. However, large numbers of Histeridae larvae were found at the carcass from Day 16 until Day 37 during 2004 (Figures 3.56 & 3.57).

3.3.3.2 SB

Calliphoridae (*C. marginalis, C. albiceps* and *Lucilia sp.*) and Muscidae were the first arrivals at the carcass during both trials (Figures 3.58 & 3.59). *Chrysomya chloropyga* was found only once and in small numbers (less than 5) during each trial. During 2004, a second occurrence of large numbers of adult Calliphoridae which had recently emerged from the puparia, was found for *C. marginalis* from Days 15 - 17 and for *C. albiceps* from Days 20 – 22 (Figure 3.58).

Formicidae were only observed during 2004 (Figure 3.58) and predated on the eggs and maggots.
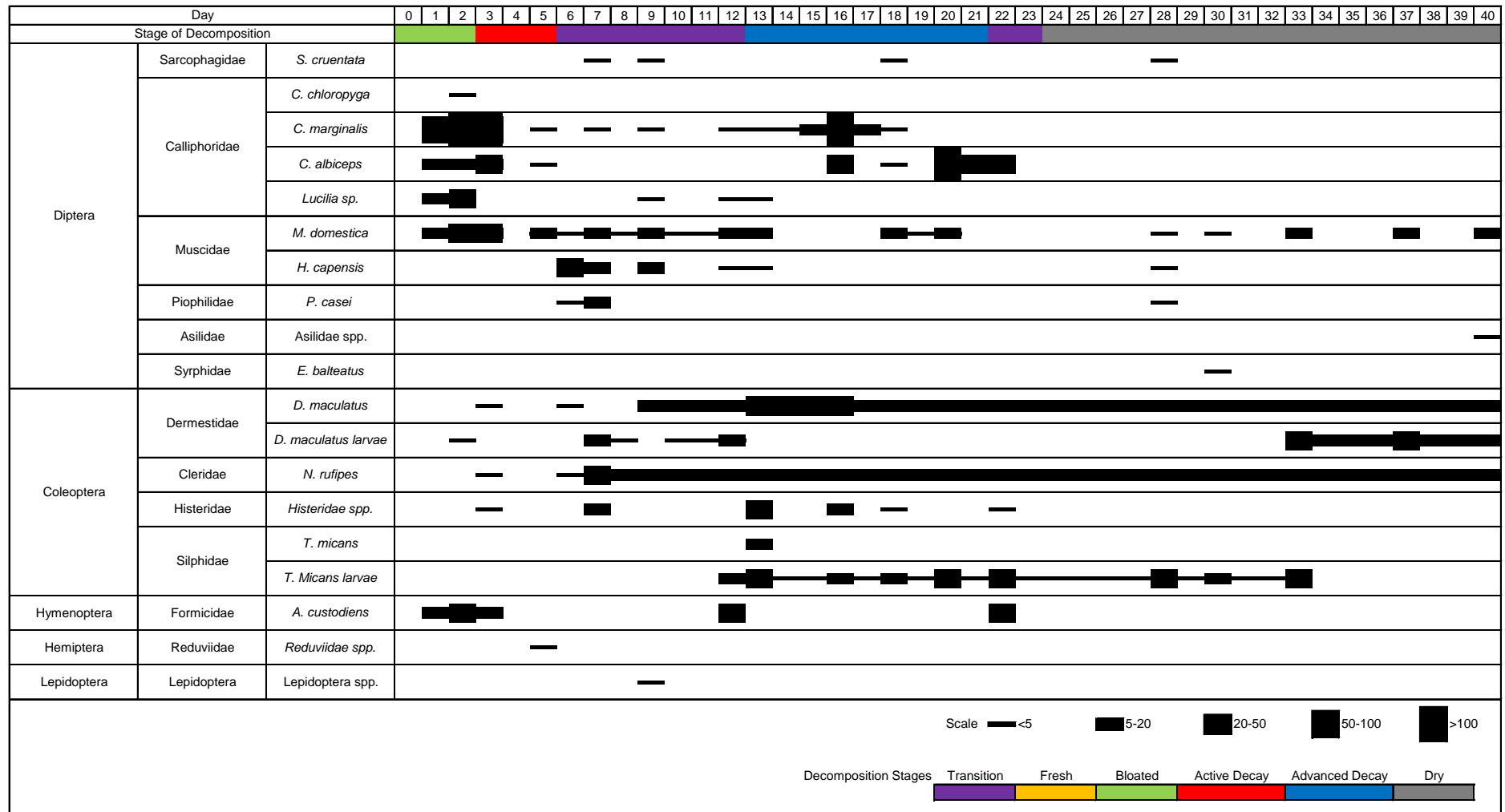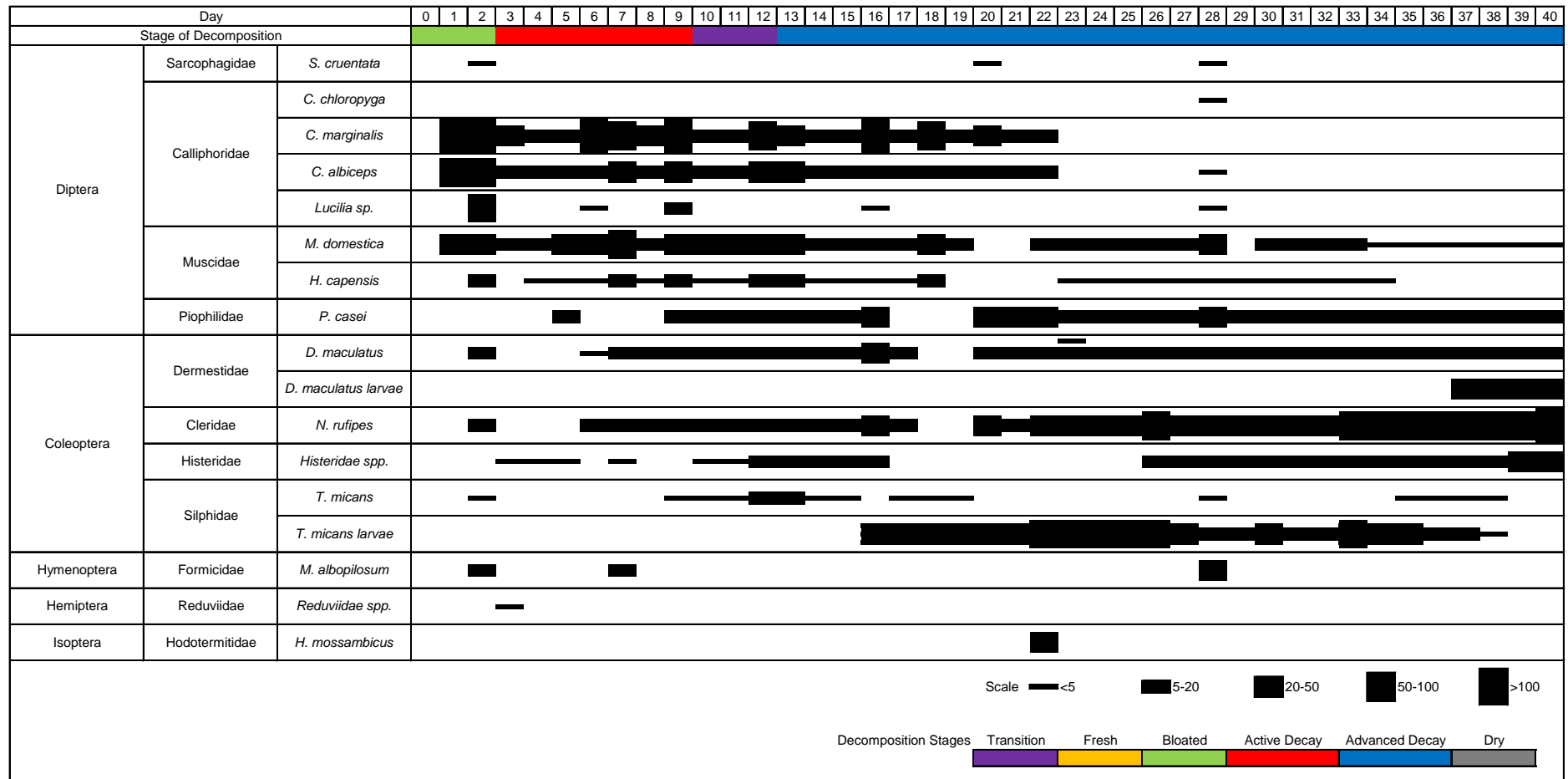
Figure 3.58 Arthropod Succession on the SB carcass during the Autumn 2004 Trial.

151
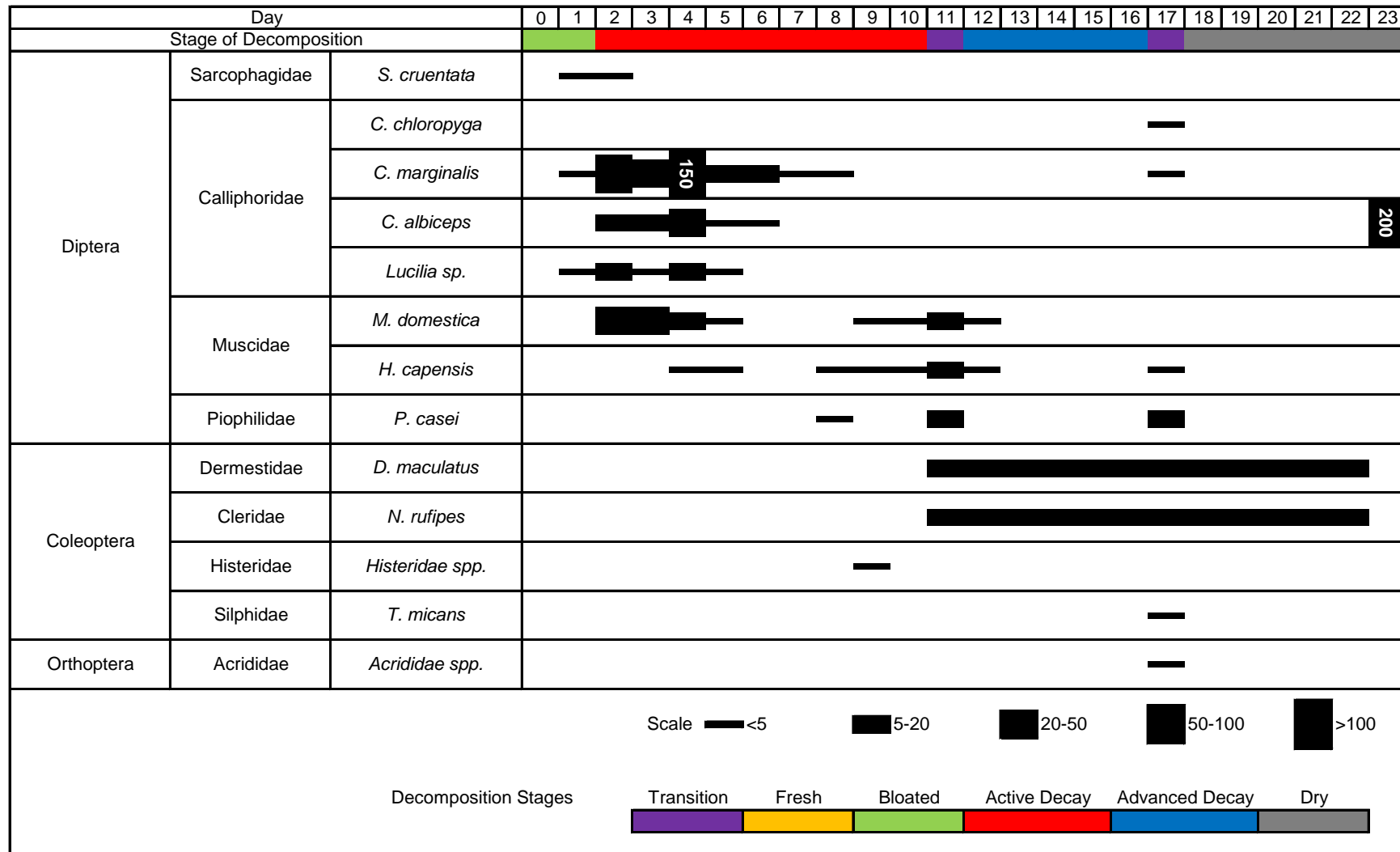
**Figure 3.59** *Arthropod Succession on the SB carcass during the Autumn 2005 Trial.*

The same pattern manifested during 2005, but only for *C. albiceps* on Day 23 (Figure 3.59). This phenomenon of only large numbers of *C. albiceps* adults emerging from the pupae, could be due to same reasons as during the summer trials, *i.e.* predation by Formicidae (see Section 3.2.7) and/or *C.albiceps* maggots predating on *C. marginalis* maggots.

Dermestidae larvae were found before adult Dermestidae were recorded at the carcass during 2004. The larvae were found on Days 2, 7, 8, 10 – 12, and 33 – 40 (Figure 3.58). No larvae were observed during 2005 (Figure 3.59).

Cleridae were present at the same time as Dermestidae during both trials (Figures 3.58 & 3.59).

Silphidae were recorded only once during each trial (Figures 3.58 & 3.59), but larvae were only found during 2004 from Day 12 for 22 consecutive days (Figures 3.58).

3.3.3.3 MB

The first insects at the carcass during both trials were *Lucilia sp.* on Day 1 (Figures 3.60 & 3.61). Piophilidae, Dermestidae and Cleridae were also present on Day 1 during 2004, although only in very small numbers (Figure 3.60).

Chrysomya marginalis, C. albiceps and *M. domestica* were only observed from Day 2 (Figures 3.60 & 3.61). The second generation of *C. marginalis* and *C. albiceps* emerged from the pupae during 2004 on Day 16 and Day 23, respectively (Figure 3.60).

During 2004, the carcass fauna was dominated by *P. casei, D. maculatus, N. rufipes* and *T. micans* larvae from Day 13. Dermestidae larvae were only observed on Day 3 and Day 40 (Figure 3.60).

Dermestidae were present on the carcass from Day 4 during 2005, whilst Cleridae were only observed from Day 17 (Figure 3.61).
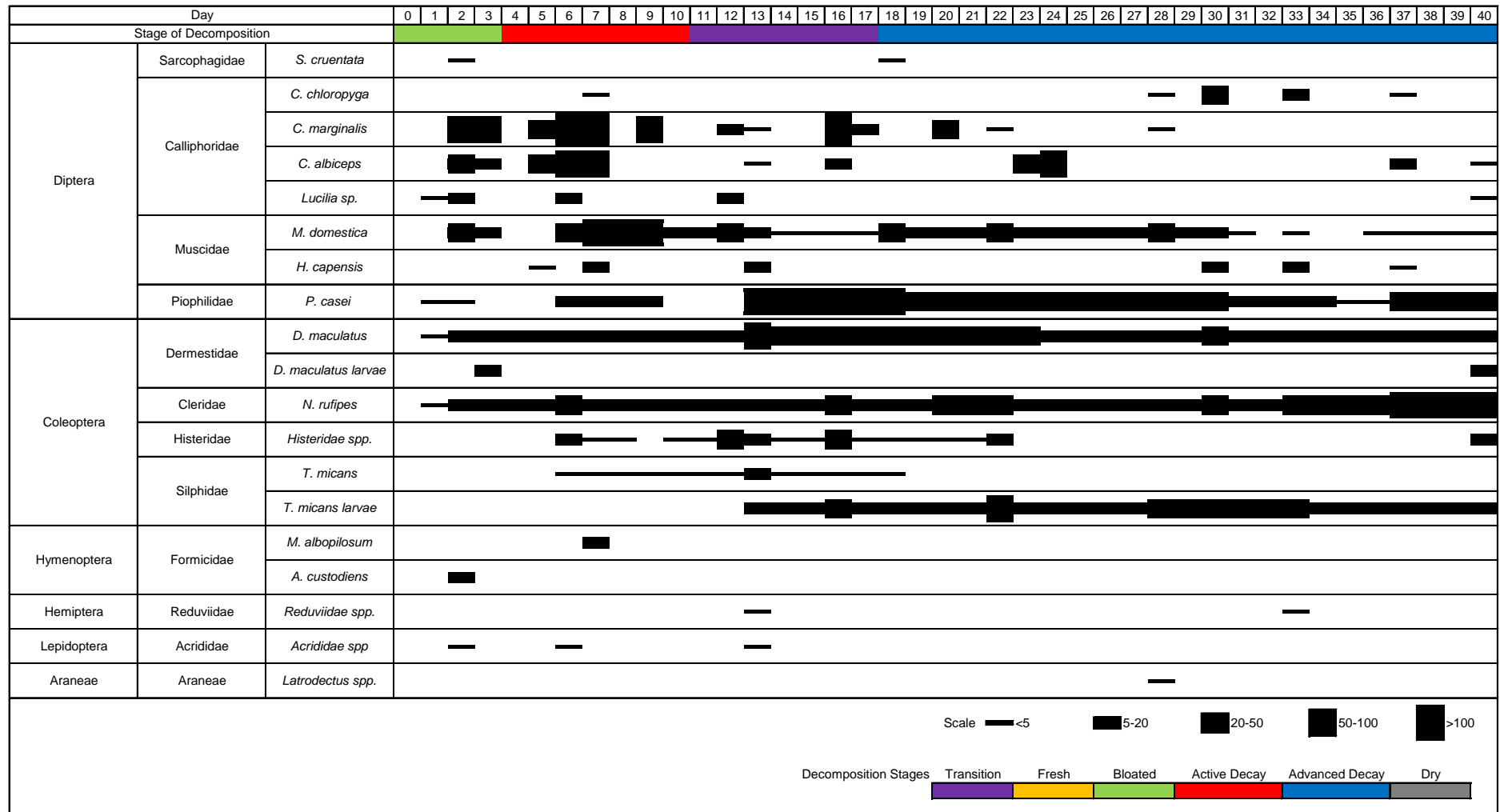
Figure 3.60 *Arthropod Succession on the MB carcass during the Autumn 2004 Trial.*
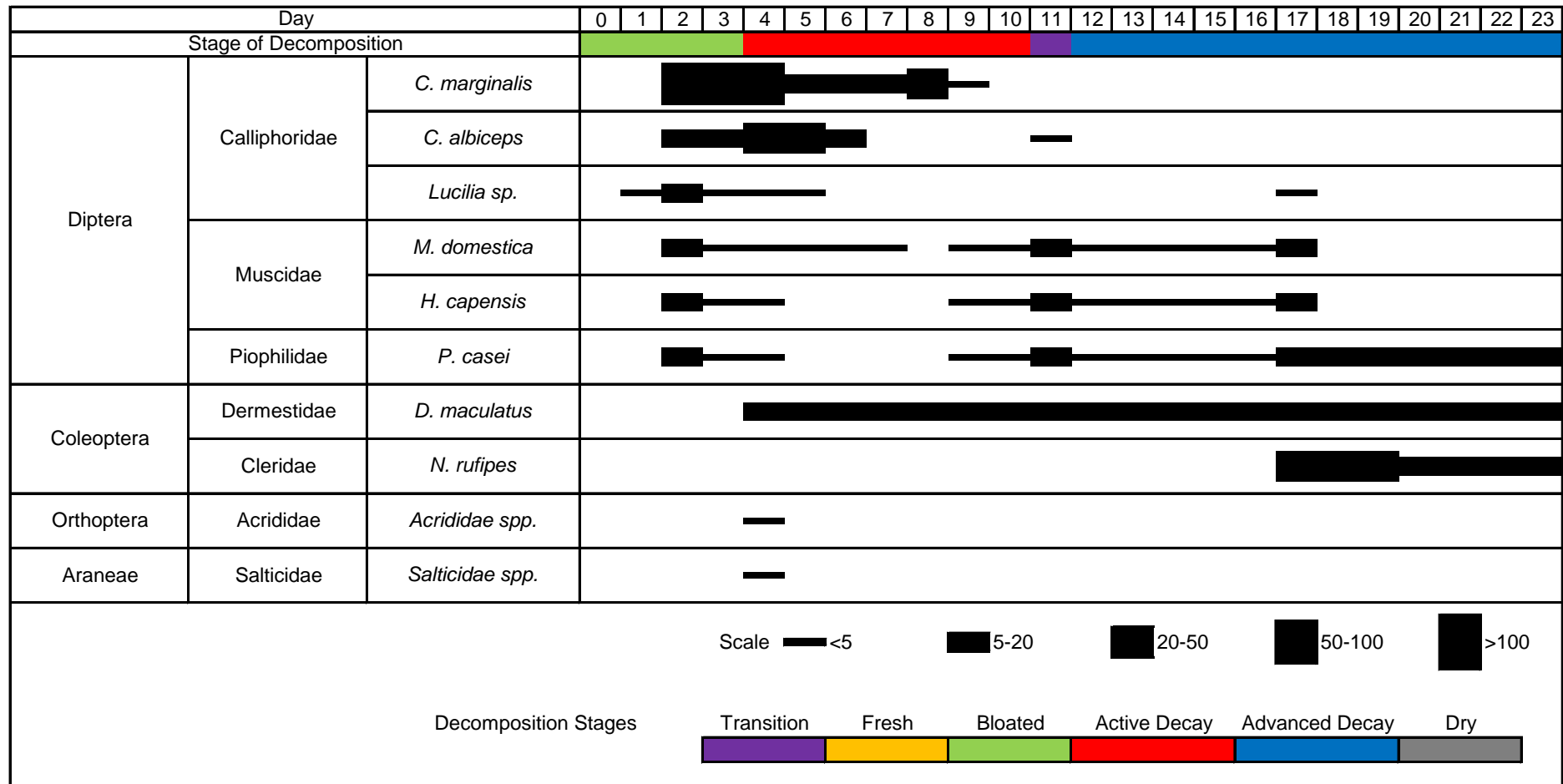
Figure 3.61 *Arthropod Succession on the MB carcass during the Autumn 2005 Trial.*

3.3.3.4 HB

Calliphoridae and Muscidae were the first insects to arrive at the carcass on Day 1 (Figures 3.62 & 3.63).

During 2004, *C. marginalis* and *C. albiceps* were continually observed at the carcass from Day 1 until Day 22 (Figure 3.62). This was unlike any of the other findings at the other carcasses during the autumn trials (Figures 3.56 – 3.61 & 3.63).

Piophilidae occurred during both trials, but no Piophilidae maggots (cheese skippers) were found (Figures 3.62 & 3.63).

Dermestidae and Cleridae were observed simultaneously, but Cleridae outnumbered Dermestidae during both trials. Small numbers of Silphidae were observed, but during both trials a much larger number of larvae were observed (Figures 3.62 & 3.63).

Only during 2004 were less than 50 individuals of Formicidae periodically observed (Figure 3.62).

As with the control carcass during 2004 (Figure 3.56), Isoptera were found at the HB carcass during 2004 (Figure 3.62). They came from a nearby nest opening to be opportunistic predators.

3.3.4. Statistical analysis of arthropod succession

3.3.4.1 Analysis 1: Jaccard Metric

The Jaccard occurrence matrices were created by using data from the succession diagrams. Mean faunal similarity was calculated for each day (See 2.1.4.1.). These faunal similarities were plotted (Figures 3.64 & 3.65).

Figure 3.62 *Arthropod Succession on the HB carcass during the Autumn 2004 Trial.*

157
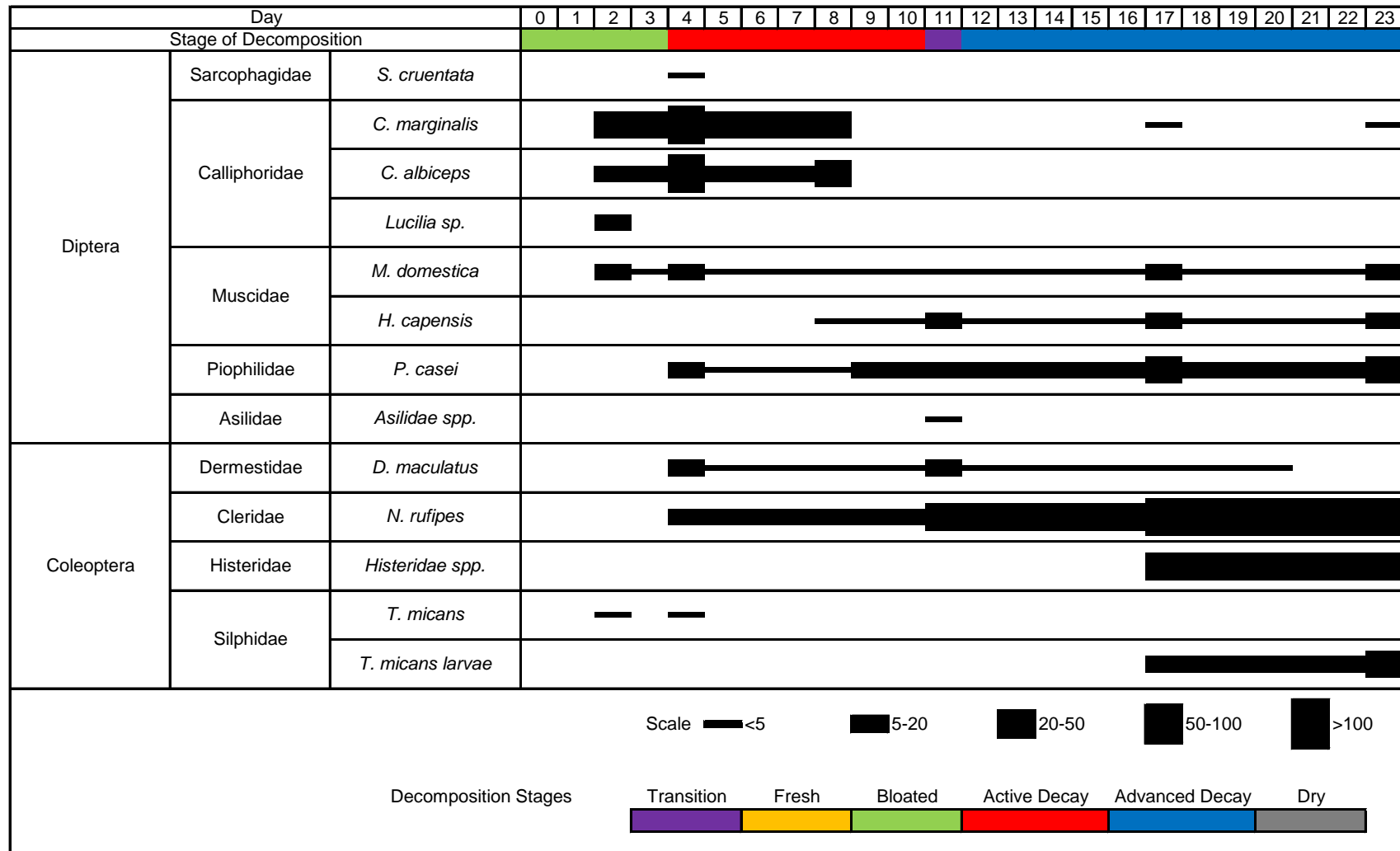
Figure 3.63 *Arthropod Succession on the HB carcass during the Autumn 2005 Trial.*
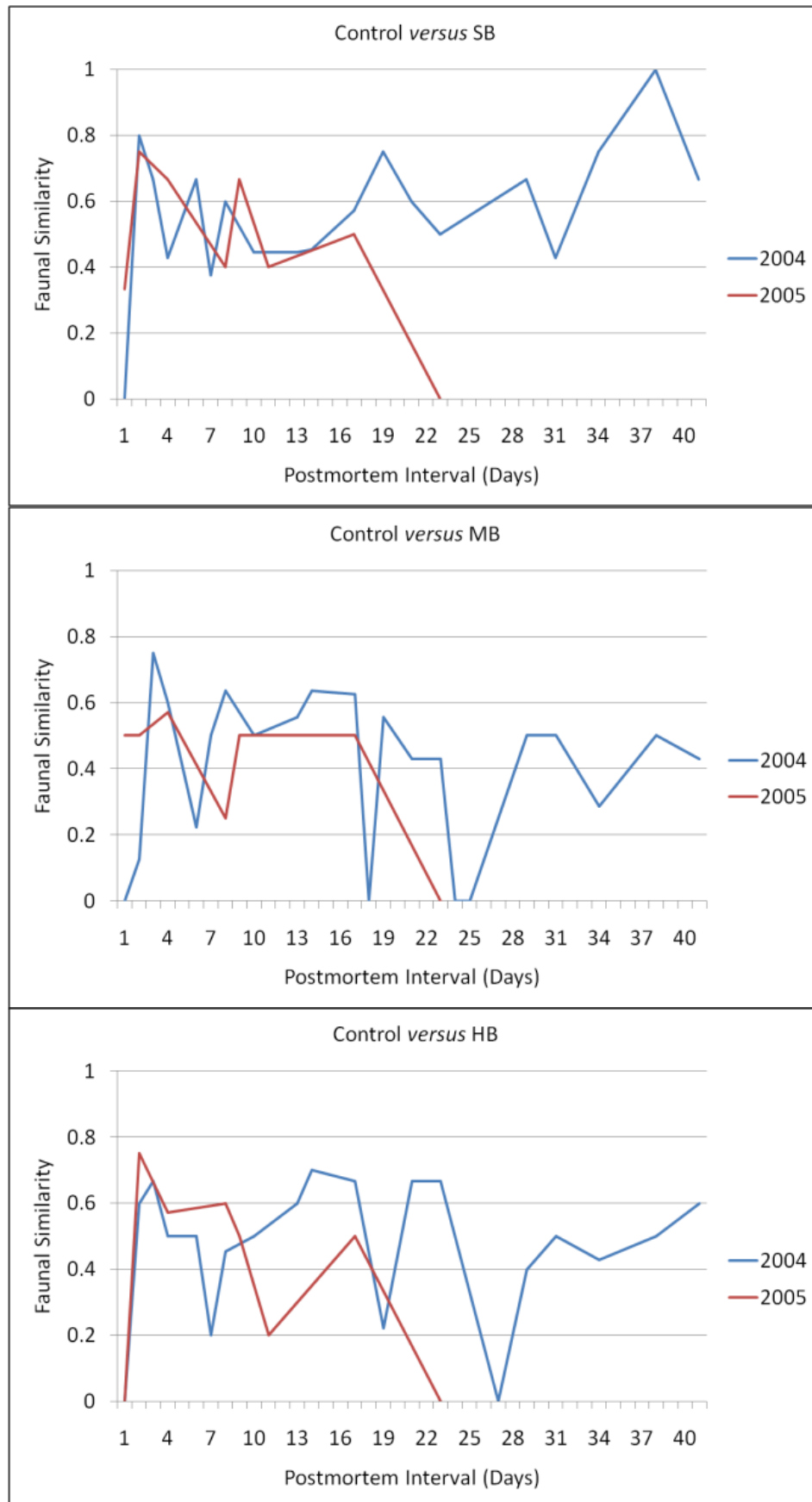
Figure 3.64 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession over the two trials.*
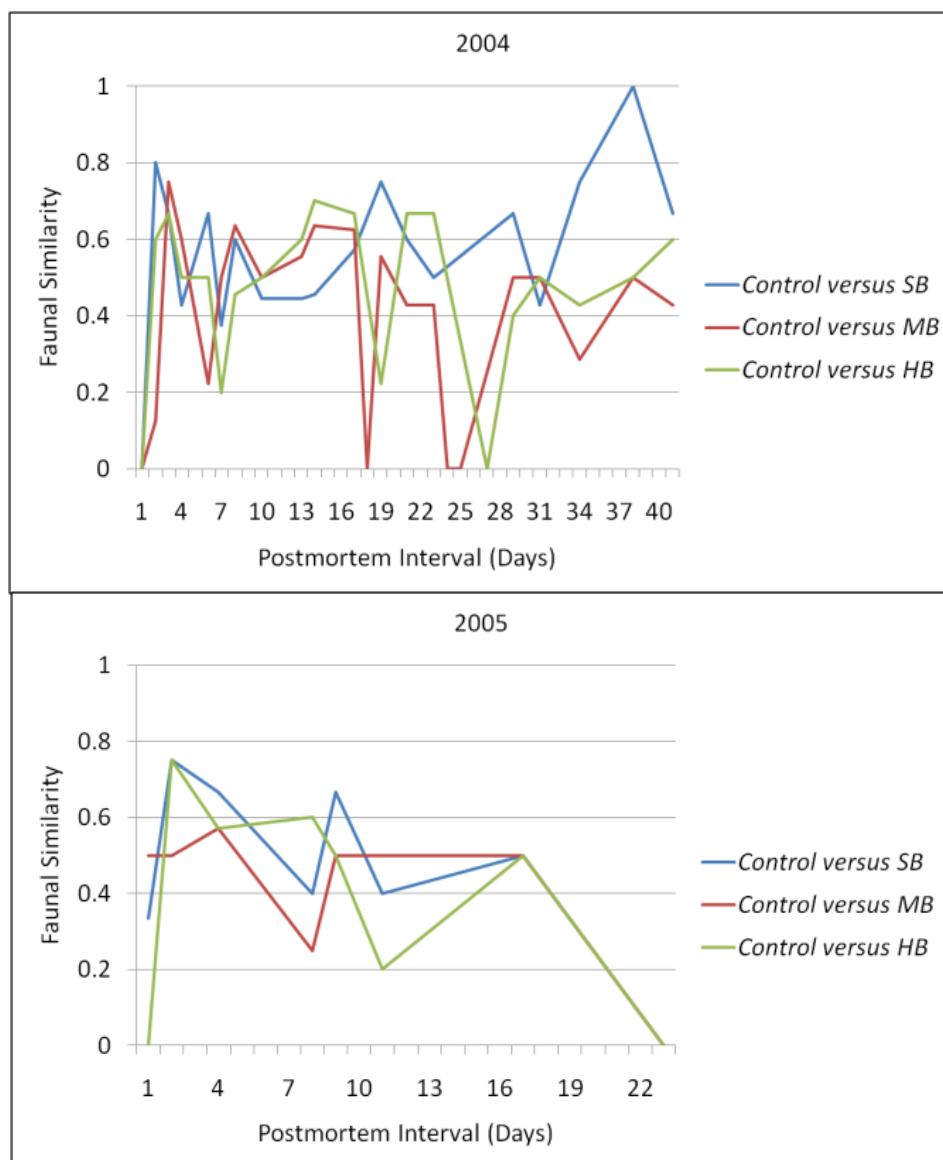
Figure 3.65 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession during each trial.*

The graphs did not show the characteristic horseshoe-shape as described by Schoenly (1992). Similar shapes should be manifested by plots of mean similarities and a general property of the dynamic daily changes that occur during carrion-arthropod succession should be reflected by the ranges (Schoenly, 1992). As found during the summer trials, this was evident during each autumn trial (Figure 3.65), but not over successive trials (Figure 3.64).

The comparison of a variety of studies revealed faunal similarity values ranging from 0.218 to 0.808 (Schoenly, 1992). The faunal similarity values during the autumn trials were between 0.125 - 1, and 0.2 – 0.75 during 2004 and 2005, respectively. According to Schoenly (1992), the variation could be due to different types of carcasses that are essentially nonhuman. In this study, the variation may be due to the different degrees of charring due to burning.

3.3.4.2 Analysis 2: Correlation coefficient

The matrices were tested using a correlation coefficient. The coefficient was calculated with Graphpad Instat.

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for all taxa showed that there were differences in the overall arthropod succession between the same treated carcasses (e.g. control 2004 *versus* control 2005) over successive trials (Table 3.11). Only the results for the control carcass were statistically significant.

Table 3.11 Similarity matrix analysis of each treatment over successive trials

2004/2005	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
Control	0.1606	0.07178 to 0.2470	0.0004	significant
SB	-0.05341	-0.1426 to 0.03666	0.2448	not significant
MB	-0.06323	-0.1523 to 0.02682	0.1684	not significant
HB	0.05101	-0.03983 to 0.1410	0.2707	not significant

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for forensic indicator species showed no significant differences for *C. chloropyga* between carcasses during each trial (Table 3.12). This suggests that burning had no effect on *C. chloropyga* colonisation.

Table 3.12 Similarity matrix analysis of *C. chloropyga* at all carcasses during successive
trials

C. chloropyga	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	-0.03581	-0.2115 to 0.1422	0.6942	not significant
2005	-0.03742	-0.2718 to 0.2011	0.7601	not significant

The Pearson's coefficient showed significant differences for *C. marginalis* between
carcasses during 2004 and 2005. (Table 3.13). This suggested that the level of
burning had an influence on the colonisation of *C. marginalis* on the carcasses.

Table 3.13 Similarity matrix analysis of *C. marginalis* at all carcasses during successive
trials

C. marginalis	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.6187	0.4960 to 0.7172	< 0.0001	significant
2005	0.659	0.5003 to 0.7748	< 0.0001	significant

Statistically significant differences were found in the Pearson's coefficient for *C.
albiceps* between carcasses during successive trials. (Table 3.14). This suggested
that the level of burning had an influence on the colonisation of *C. albiceps* on the
carcasses.

Table 3.14 Similarity matrix analysis of *C. albiceps* at all carcasses during successive trials

C. albiceps	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.6029	0.4767 to 0.7047	< 0.0001	significant
2005	0.4914	0.2882 to 0.6522	< 0.0001	significant

3.3.5 Ambient temperatures and rainfall

As pointed out in Section 2.1.3.3, ambient temperature records and rainfall for the
two nearest meteorological stations in Bloemfontein (Figures 2.14 & 2.15) were
obtained from the South African National Weather Service in Pretoria.

Major differences in meteorological data from these two stations are highlighted below. Only the data from the nearest meteorological station (City) were applied to this study, since ambient temperatures measured at the study site during times of observations more closely approximated the temperatures measured at the City meteorological station than at the WO meteorological station.

3.3.5.1 2004 Autumn Trial

The average maximum and minimum City temperature was 24.3°C and 9.6°C, respectively. The average City temperature was 16.9°C. Average maximum and minimum WO temperature was 24.2°C and 8.3°C, respectively. The average WO temperature was 16.2°C.

City rainfall occurred on Days 4, 5, 6, 11, 12, 23, 26 and 27 (Figure 3.66), with a total of 70mm

WO rainfall occurred on Days 4, 5, 6, 10, 11, 16, 23 and 26 (Figure 3. 66), with a total of 71mm.

3.3.5.2 2005 Autumn Trial

The average maximum City temperature was 20.7°C and the minimum 6.6°C, while the average City temperature was 13.69°C. Average maximum and minimum WO temperature was 21.2°C and 5.7°C, respectively. The average WO temperature was 13.4°C.

City rainfall occurred on Days 1, 8, 11, 22 and 23 (Figure 3.67), with a total of 28.2mm.

WO rainfall occurred on Days 4, 5, 7, 8, 11, 22 and 23 (Figure 3.67),with a total of 28.4mm.
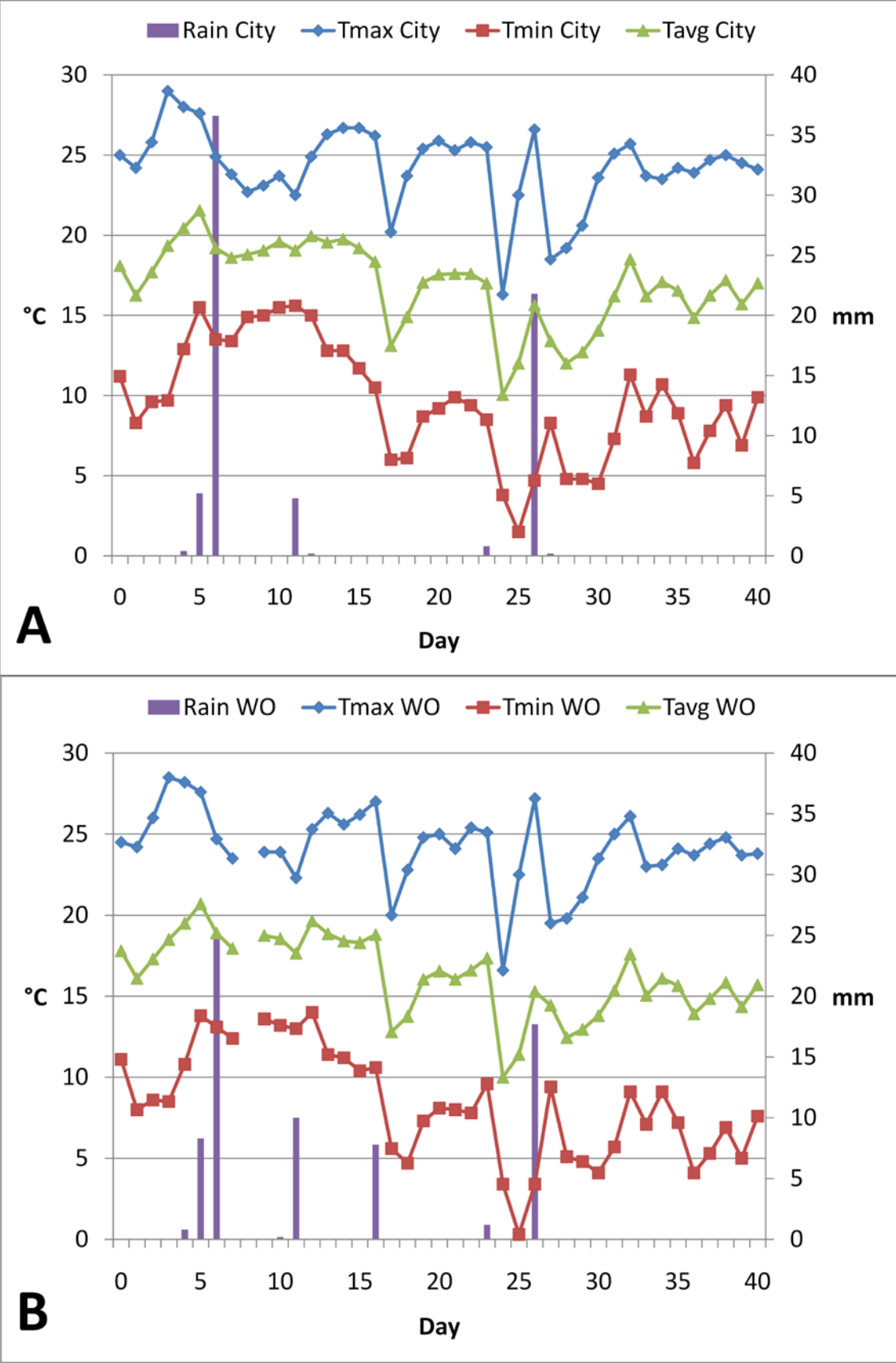
Figure 3.66 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Autumn 2004 Trial*
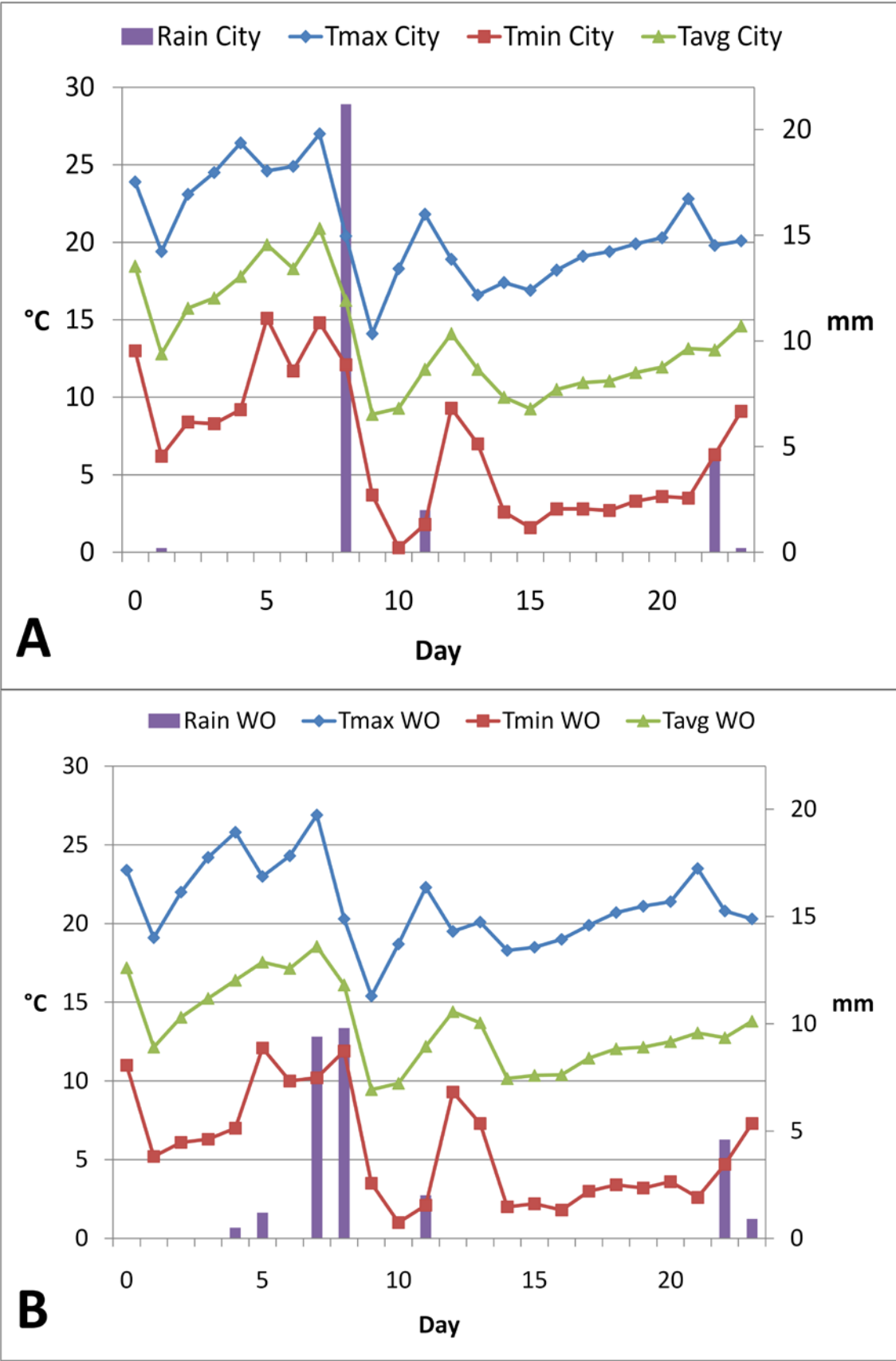
Figure 3.67 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Autumn 2005 Trial*

3.3.6. Ambient, external and internal carcass temperatures

The ambient temperature measured during observations follow the trend of the daily average ambient temperature for the nearest meteorological station, i.e. City (Figures 3.68 & 3.69).

As during the summer trials, fluctuations occurred for the temperature of the head, thorax and abdomen. Temperatures were only measured in these areas once the skin had ruptured and the bubbles of fluid could be seen and the escaping gas could be heard. This was done to prevent the fabrication of holes through which gas could escape, which in turn could influence the insect succession. Escaping gas is one of the chemical cues blow flies use to locate a body or carcass.

Soil temperature underneath the carcasses also varied greatly. The largest difference in temperature was between the ambient and carcass surface temperature (Figures 3.68 & 3.69; Tables 3.15 – 3.17).

Table 3.15 Maximum difference (°C) in ambient and carcass surface temperature

	Control	SB	MB	HB
2004	19.9 (Day 13)	33.5 (Day 2)	25.7 (Day 3)	28.2 (Day 2)
2005	17.9 (Day 1)	27.0 (Day 8)	16.0 (Day 9)	11.0 (Day 4)

Table 3.16 Mean difference (°C) in ambient and measured temperature during 2004

	Head	Thorax	Abdomen	Surface	Soil
Control	12.1	12.7	10	12.7	4.4
SB	3.5	7.8	3.4	17.7	4.2
MB	3.1	5.3	4.6	11	-1.5
HB	0.7	3	1.2	11.4	-2.1
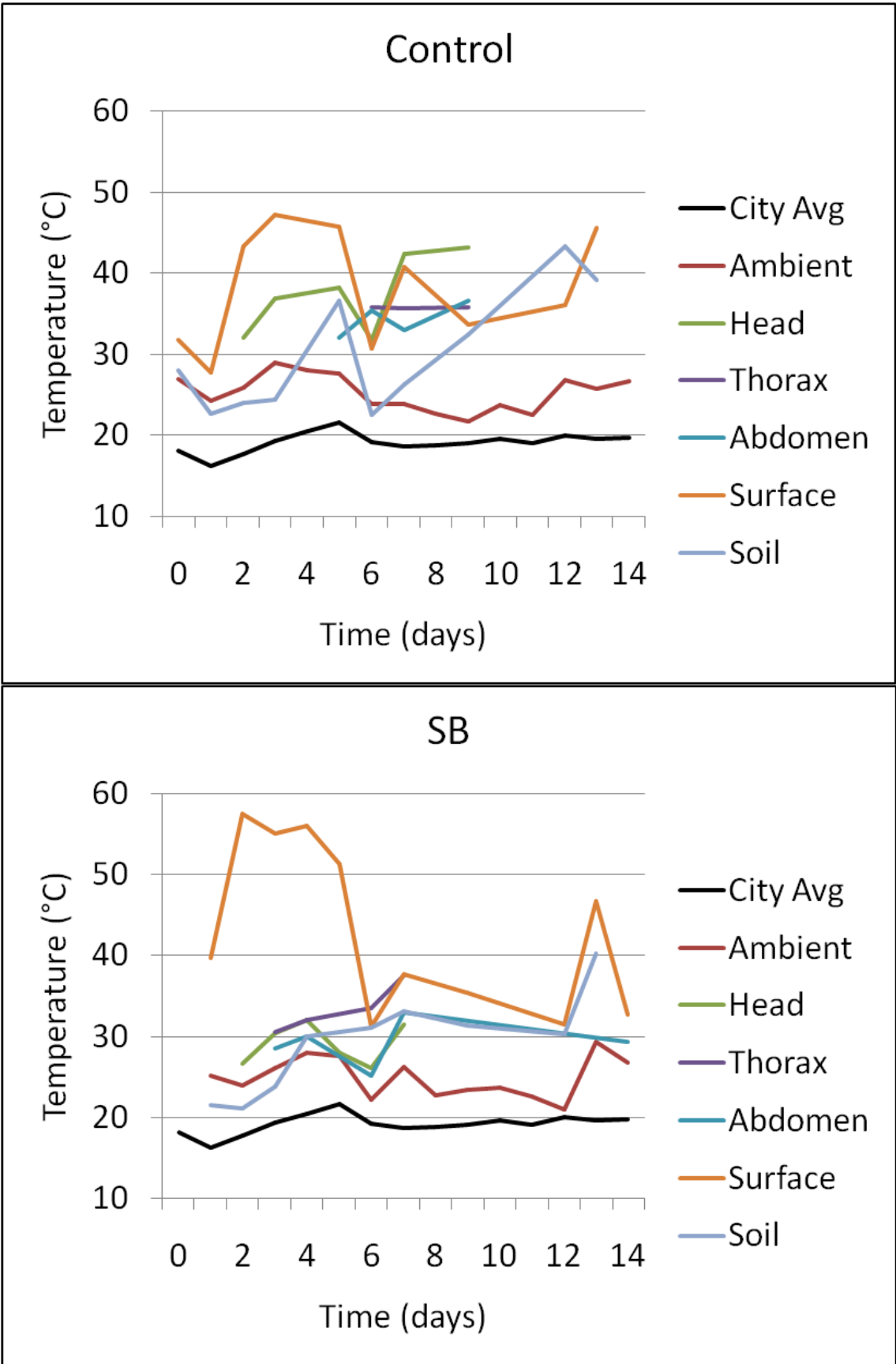
Figure 3.68 *Ambient & internal carcass temperatures during the Autumn 2004 Trial.*
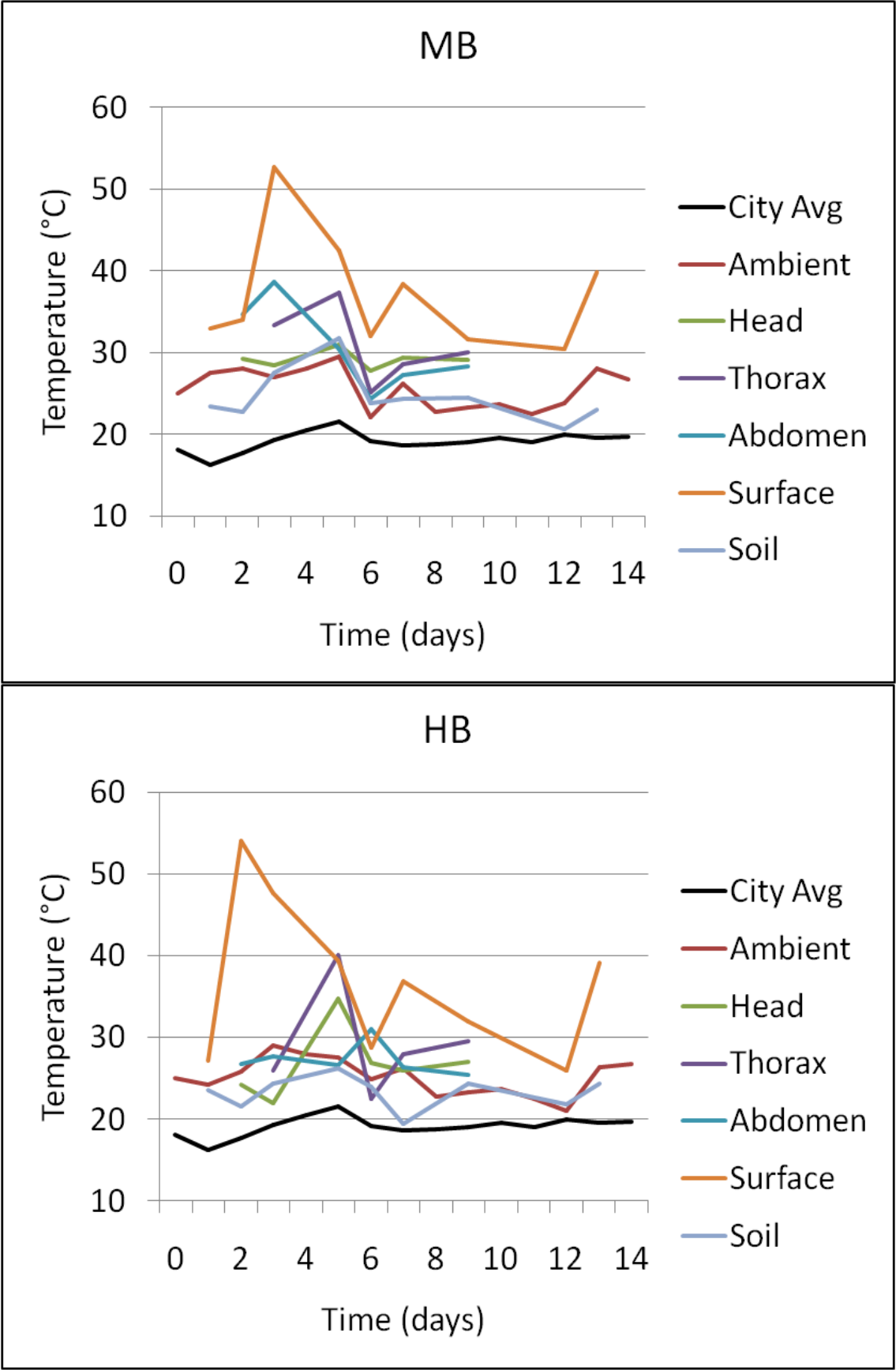
Figure 3.68 (continued) *Ambient & internal carcass temperatures during the Autumn 2004 Trial.*
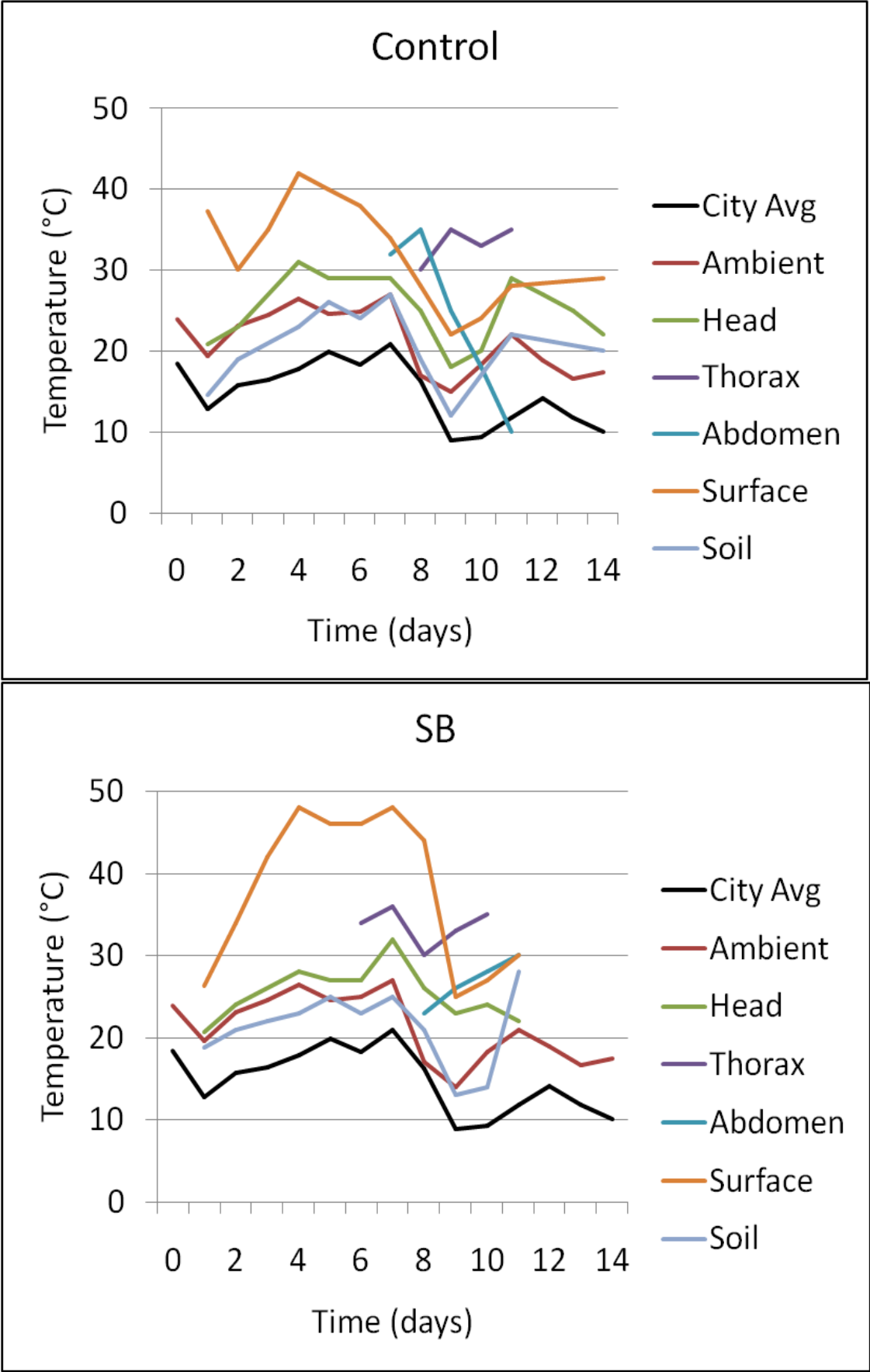
Figure 3.69 *Ambient & internal carcass temperatures during the Autumn 2005 Trial.*
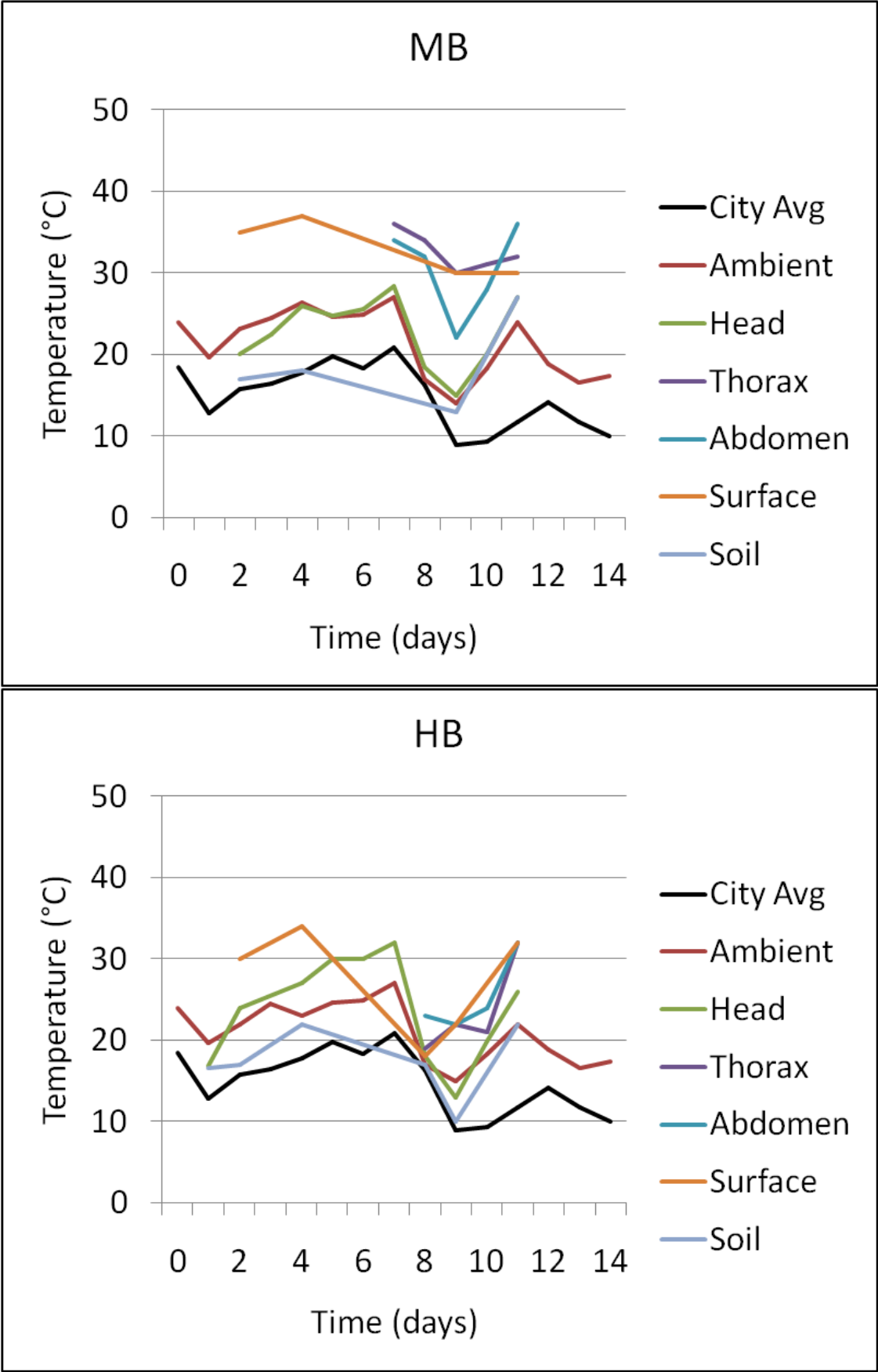
Figure 3.69 (continued) *Ambient & internal carcass temperatures during the Autumn 2005 Trial.*

Table 3.17 Mean difference (°C) in ambient and measured temperature during 2005

	Head	Thorax	Abdomen	Surface	Soil
Control	4.4	15.2	4.1	10.6	-1.4
SB	3.8	13.4	9.2	16	-0.6
MB	0.4	12.6	10.3	11.1	-3.1
HB	3.8	13.4	9.2	16	-0.6

Carcass temperatures were lower than any other time on Day 6 and Day 9 during 2004 and 2005, respectively (Figures 3.68 & 3.69). This was due to the high rainfall recorded on Day 6 and Day 8 during 2004 and 2005, respectively (Figures 3.66 & 3.67).

Section 3.4. The influence of burning on carcass decomposition and arthropod succession: Two Winter Studies.

3.4.1. Decomposition of the carcasses

Immediately after killing, the control carcasses were placed in the field and this date and time was designated as Day 0 and marked the start of each trial (Table 3.18).

Table 3.18 Commencement dates and times of the Autumn Trials

Year	Date	Time
2004	6 July	14:30
2005	4 August	14:00

As found during the summer and autumn trials, the control carcasses showed the first signs of rigor mortis, with the limbs starting to become stiff during the first observations, whereas the burnt carcasses showed signs of bloating. The burnt carcasses had cooled down completely by the time the first observations were made.

During 2004, the Bloated stage was shorter at the control carcass than at the burnt carcasses. The duration of the bloated stage increased with the level of burning of the carcass. During 2005, the duration of the bloated stage was similar for all the carcasses (Figure 3.70). The carcasses never became inflated to the extent they were during the summer or autumn trials.

Oviposition occurred 5 days later at the burnt carcasses than at the control carcass during 2004 (Figure 3.70). This was contradictory to findings by Avila and Goff (1998), probably due to lesser bloating caused by lower ambient temperatures, resulting in fewer skin ruptures (fewer new oviposition sites & less olfactory stimulant for attracting blow flies). During 2005 oviposition occurred at the burnt carcasses three to five days earlier than at the control carcass (Figure 3.70), supporting the findings of Avila and Goff (1998) and observations made during the autumn trials. Maggots were only found to feed in small areas on the carcasses.
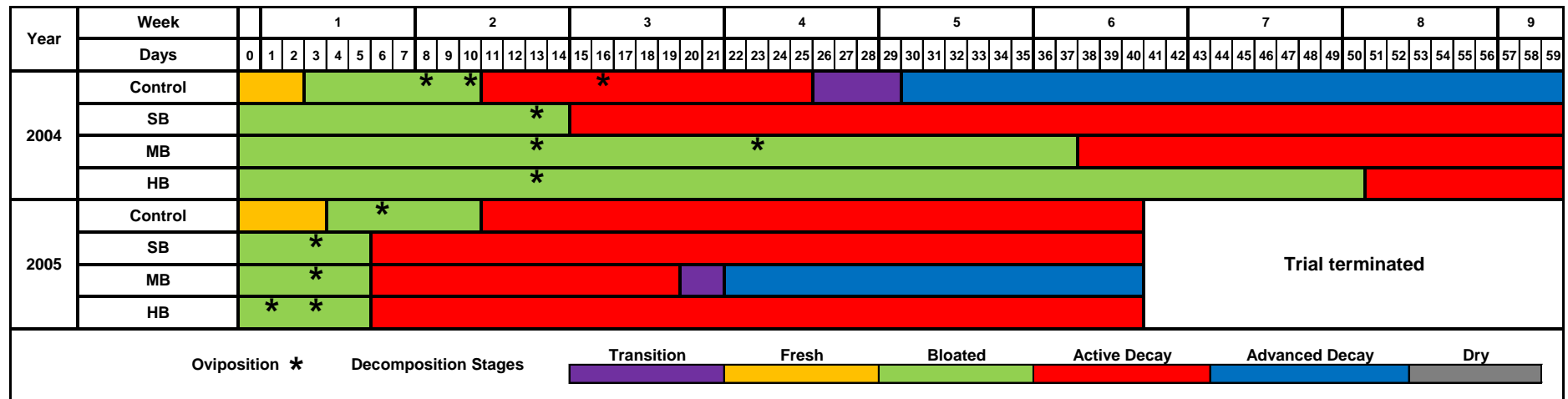
Figure 3.70 *Decomposition stages of carcasses and days of oviposition during the winter trials.*

During 2004, only the control carcass reached the Advanced Decay stage. During 2005 only the MB carcass reached this stage (Figure 3.70).

The Dry/Remains stage was not reached by any of the carcasses. This could be due to the 2004 and 2005 trials being terminated on Days 59 and 40, respectively (Figure 3.70). This was done since it was already early to mid-September (spring in South Africa). Alternatively, due to smaller maggot masses feeding on the carcasses, the carcass tissue became desiccated before the maggot masses could consume the carcass.

Only the days when major changes in the appearance of the carcasses and/or major changes in the insect fauna associated with the carcasses occurred, are discussed.

3.4.1.1 2004 Winter Trial

Day 1:

- The control carcass still appeared fresh (Figure 3.70).
- Rigor mortis was evident at the control carcass and mucus formed bubbles at the nose.
- The SB and MB carcasses had started to bloat (Figure 3.70).
- The skin on the thorax of the MB carcass had ruptured. Blood and mucus dripped from the nose. The burnt skin on the rear of the carcass had separated from the body and totally exposed the rear (buttocks).
- The skin of the HB carcass was totally incinerated with the underlying muscle visible. Heat fractures of the legs were observed and were totally skeletonised.

The control carcass still appeared fresh on Day 2 (Figure 3.70). Blood dripped from the nose of the carcass. Blood formed bubbles at the nose of the control carcass on Day 3, indicating that bloating had started. However, the carcass still appeared fresh (Figure 3.70).

Day 6:

- A slight smell of decomposition was noted at the control carcass.
- Skin ruptures were found on the thorax and neck of the MB carcass.

Day 8:

- Newly oviposited blow fly eggs were found inside the mouth of the control carcass.
- The rectum of the control carcass protruded due to bloating.
- The SB carcass was bloated (up to Day 14, Figure 3.70) with a rupture of the skin of the rear (buttocks) of the carcass.

On Day 10, freshly oviposited eggs were found on the left hind leg of the control carcass. These eggs hatched on Day 11, starting the Active Decay stage (Figure 3.70).

Second and third instar maggots were found in the rectum of the control carcass on Day 13. Blow fly eggs were also found in the mouths of the burnt carcasses. The eggs inside the mouths of the MB and HB carcasses did not hatch for the remainder of the study, probably due to desiccation.

The skin on the right hind leg of the SB carcass ruptured on Day 15. Fat dripped from the rupture. First instar maggots were found in the mouth of the carcass, signaling the onset of the Active Decay stage at the SB carcass (Figure 3.70). These maggots were not observed again for the rest of the study, probably due to the maggots moving further down the oral cavity and chest to feed. The SB carcass effectively remained in the Active Decay stage for the remainder of the study (Figure 3.70).

Freshly oviposited blow fly eggs were found in the mouth and ears of the control carcass on Day 16.

Day 17:

- First and second instar maggots were found in the mouth of the control carcass on Day 17. Second and third instar maggots were feeding on the hind leg.
- A skin rupture occurred underneath the left hind leg of the HB carcass and the intestines protruded from this rupture.

Third instar maggots were feeding on the hind leg of the control carcass on Day 21.

Day 23:

- Second instar maggots were feeding inside the mouth of the control carcass. Some of the third instar maggots that were found on the hind leg the previous day, gathered inside the skin fold between the hind legs. Maggots were also feeding inside the abdomen. These maggots could not be seen, since the carcass skin had not ruptured at all. It was deduced from the movement of the abdomen and the sound that there were in fact maggots feeding inside the abdomen.
- Freshly oviposited eggs were found on the neck of the MB carcass.

By Day 27, the maggots feeding on the left hind leg of the control carcass had left the carcasses to pupate, signaling the onset of the Advanced Decay stage (Figure 3.70).

On Day 31, second instar maggots were found on the thorax underneath the left front leg of the control carcass. These were Sarcophagidae maggots, explaining the absence of eggs. Sarcophagidae does not oviposit, but deposit newly hatched first instar maggots.

The Active Decay Stage commenced at the MB carcass on Day 38 with first instar maggots found in an abdominal skin rupture (Figure 3.70). At the HB carcass, the skin of the neck had ruptured.

Day 41:

- Third instar maggots were found inside the mouth and underneath the left front leg and abdomen of the control carcass.
- Second instar maggots were feeding in a skin rupture on the abdomen of the MB carcass.

A very small group of blow fly eggs were found underneath the abdomen at the carcass-ground interface at the HB carcass on Day 49. These eggs hatched on Day 50, starting the Active Decay Stage (Figure 3.70). A sample of these maggots was identified as *C. chloropyga.*

3.4.1.2 2005 Winter Trial

Day 1:

- The control carcass appeared fresh (Figure 3.70) and rigor mortis had commenced.
- The abdominal skin of the HB carcass had ruptured during burning and the intestines protruded from this rupture. Blow fly eggs were deposited on the protruding intestines.

Day 3:

- Rigor mortis was no longer observed at the control carcass. Skin slippage and livor mortis had occurred.
- At the SB and MB carcasses, the skin of the thorax had ruptured between the front legs. Blow fly eggs were found inside the mouth of this carcass.
- The skin on the abdomen of the MB carcass had also ruptured. Blow fly eggs were also found inside the mouth of this carcass.
- Oviposition had occurred inside the mouth of the HB carcass.

Day 6:

- The control carcass was severely bloated (Figure 3.70). Skin slippage occurred on the abdomen, the rectum protruded and the ears were swollen shut. Blow fly eggs were oviposited in the mouth of this carcass.

- The SB carcass showed signs of bloating with skin ruptures occurring on the abdomen and neck. Blow fly eggs, as well as first and second instar maggots were found inside the mouth of this carcass. The presence of maggots signified the start of the Active Decay stage at this carcass (Figure 3.70).

- Skin ruptures occurred at the MB carcass in the same place as at the SB carcass. The MB carcass also appeared bloated and first and second instar maggots were found inside the mouth of the carcass.

- Bloating had started to recede at the HB carcass. Skin ruptures were found on the back and abdomen of the carcass. Tissue fluid and liquid fat had seeped out of the back of the carcass. First and second instar maggots were also found inside the mouth of this carcass, effectively starting the Active Decay stage (Figure 3.70).

Day 11:

- The control carcass was still bloated. Skin ruptures were found on the abdomen between the hind legs and the rectum was protruding. Skin slippage occurred on the thorax and abdomen, with blood filled blisters on the front legs. First instar maggots were found on the thorax just underneath the left front leg. This was the start of the Active Decay stage at this carcass (Figure 3.70).

- The SB carcass was severely bloated and new skin ruptures were found on the thorax and between the front legs.

- A rich foamy mucus was observed inside the mouth of the MB carcass due to the feeding activity of third instar maggots.

- Less than five dead third instar maggots were found between the hind legs of the HB carcass. Third instar maggots were found inside the mouth of this carcass.

Day 15:

- Third instar maggots were found inside the mouth of the control carcass.
- Third instar maggots were found underneath the skin of the neck and the thorax between the front legs of the MB carcass

Day 20:

- Gas continued to escape due to bloating at the SB carcass. Second instar maggots were found on the protruding intestines and third instar maggots underneath the protruding intestines.
- The Advanced Decay stage had commenced at the MB carcass since most of the maggots had migrated from the carcass to pupate (Figure 3.70). A single third instar maggot was found on the hind leg of the MB carcass and approximately 10 third instar maggots were found underneath the carcass.

Day 40:

- Small numbers of actively feeding maggots (less than 10 individuals) were found on the thorax of the control carcass just underneath the left front leg, as well as underneath the carcass
- Similar numbers of third and second instar maggots, as well as termites, were found underneath the SB carcass.
- A few empty pupal cases were found underneath the carcasses.
- The trial was terminated in mid-September (spring in South Africa).

3.4.1.3 Mass Loss

As mentioned in Section 2.1.3.2, the mass of each carcass was recorded daily during the first trial of each season. This was only done during the first trial of each season minimize the disturbance to the carcasses and the possible effect of this disturbance on decomposition and insect succession.

Decomposition was similar for the 2004 and 2005 trials. The fastest decomposition with the largest mass loss occurred at the SB carcass (Figure 3.71).
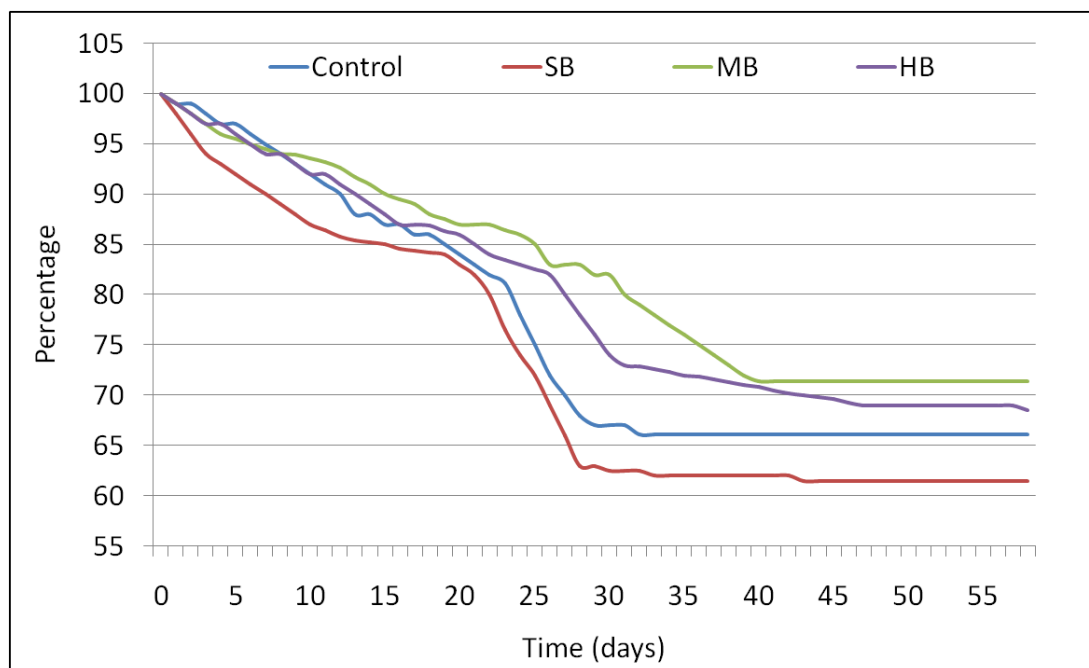
Figure 3.71 *Percentage body mass remaining of carcasses during the Winter 2004 Trial.*

For the first 20 days, the control, MB and HB carcasses showed a similar rate of decomposition. Thereafter a larger difference was seen in the rate of decomposition. The control carcass decomposed faster than the MB and HB carcasses and more tissue was removed by the necrophagous insects. This was due to a larger number of maggots on the control carcass which consumed more carcass tissue than the small numbers of maggots on the MB and HB carcasses. The slowest decomposition with the least tissue removed occurred at the HB carcass, where the smallest number of maggots was observed and the largest portion of the tissue was unfit for consumption by maggots due to burning. The difference in tissue remaining between the fastest and the slowest decomposition and the end of the trial was 10% (Figure 3.71). The condition of the carcasses at the end of the study (Day 59) is shown in Figures 3.72 – 3.75.

Figure 3.72 *The control carcass at the end of the Winter 2004 trial (Day 59).*

Figure 3.73 *The SB carcass at the end of the Winter 2004 trial (Day 59).*

Figure 3.74 *The MB carcass at the end of the Winter 2004 trial (Day 59).*

Figure 3.75 *The HB carcass at the end of the Winter 2004 trial (Day 59).*

3.4.2. Arthropod Composition

3.4.2.1 Orders

3.4.2.1.1 2004 Winter Trial

Coleoptera were dominant over Diptera at all of the carcasses, except at the SB carcass. Except for the control carcass, the dominance of Coleoptera increased with the increasing level of burning of the carcasses. The dominance of Diptera decreased with an increase in level of burning of the carcasses, except for the control carcass (Figure 3.76). This was due to increasingly less tissue being fit for consumption by maggots with an increase in the level of burning.

3.4.2.1.2 2005 Winter Trial

Diptera were dominant over Coleoptera at all of the carcasses, possibly due to low environmental temperatures. No discernible pattern of dominance was observed. Small numbers of Hymenoptera (at the control and SB carcass) and Hemiptera (at the SB carcass) were also observed (Figure 3.76).

3.4.2.2 Diptera

Calliphora vicina Robineau-Devoidy was observed for the first time during the winter trials, but unfortunately no maggots were found at, on or underneath any of the carcasses. This does not exclude the possibility that they actually did oviposit on the carcasses. This species has been observed to oviposit at night (Singh & Bharti, 2001), but the extremely low temperatures during the Free State winter may have proved to be too cold for this species to actually oviposit during the night.
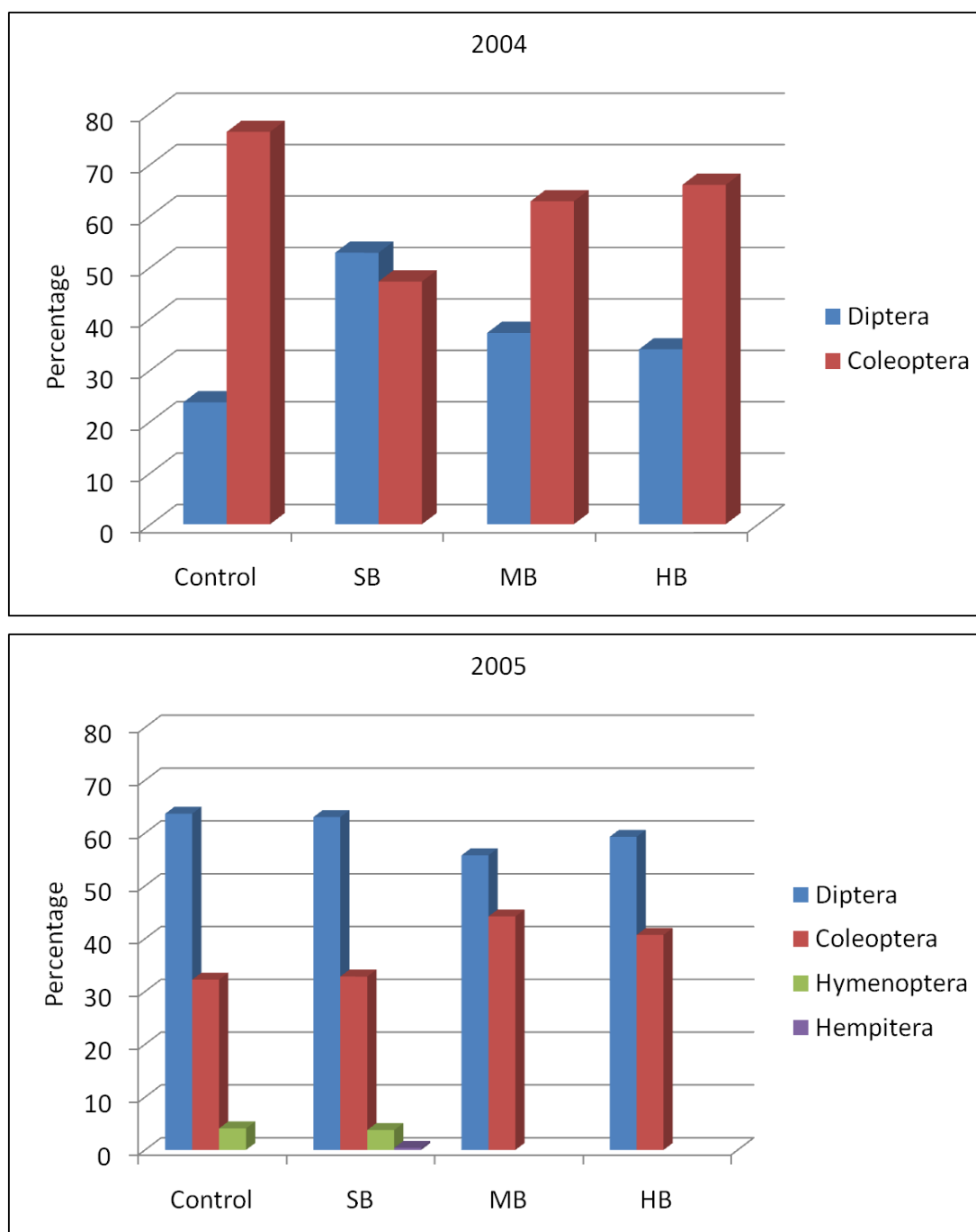
Figure 3.76 *Order Composition during the winter trials.*

3.4.2.2.1 2004 Winter Trial

Calliphoridae were dominant at the control carcass, with Piophilidae dominant at the burnt carcasses. The dominance of Calliphoridae seemed to diminish with an increase in the level of burning, except for the HB carcass (Figure 3.77).
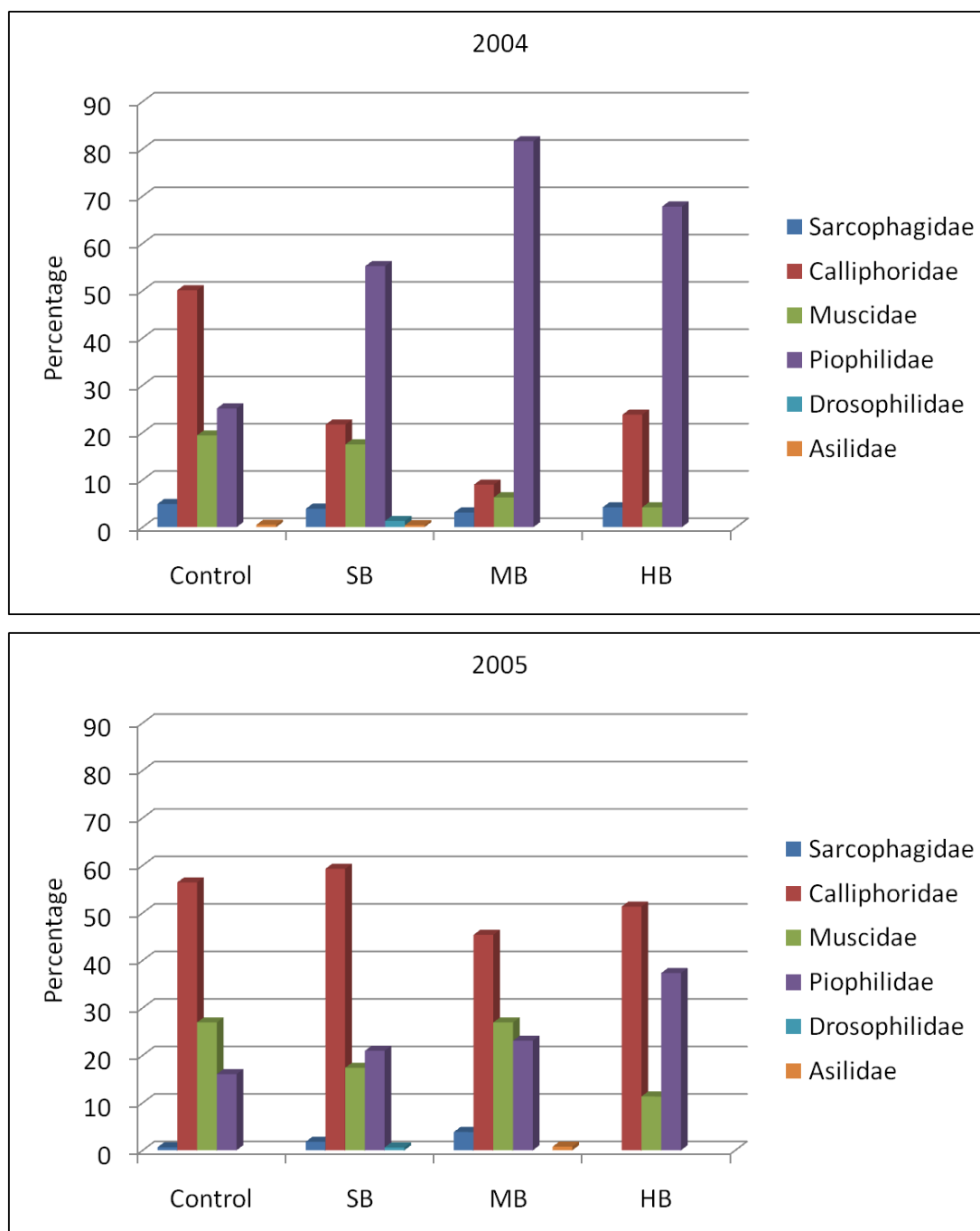
Figure 3.77 *Diptera Composition during the winter trials.*

The level of dominance of Piophilidae increased with the increasing level of burning of the carcasses, except for the HB carcass. Sarcophagidae were found to breed on the control carcass, but in extremely low numbers (Figure 3.77). The dominant Diptera species at all the carcasses was *C. marginalis* (Figure 3.78).
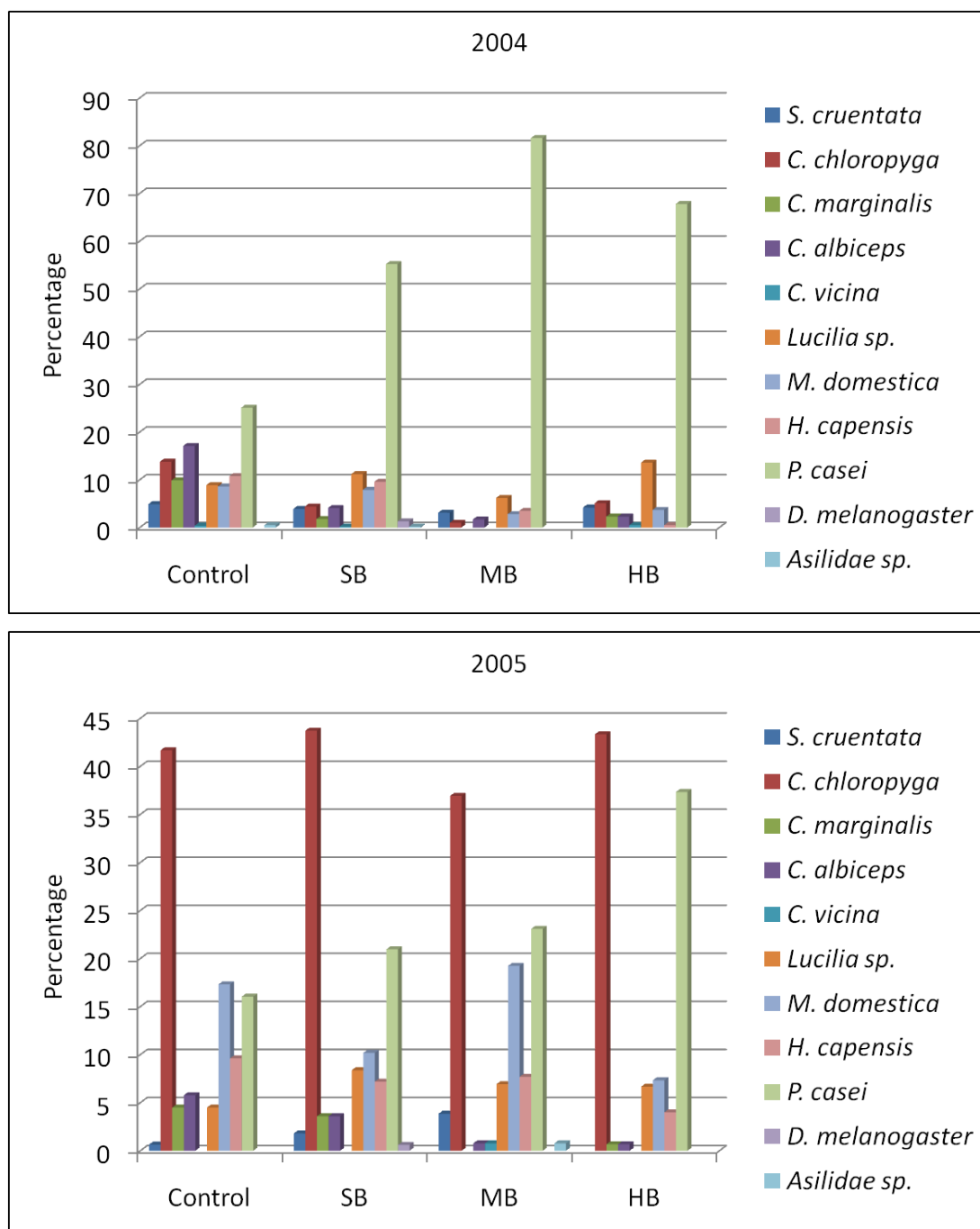
Figure 3.78 *Diptera Species Composition during the winter trials*

3.4.2.2.2 2005 Trial

Calliphoridae dominated the numbers of Diptera, with lesser numbers of Muscidae and Piophilidae observed. The level of dominance of Piophilidae increased with an increased level of burning (Figure 3.77), possibly due to more fat and bodily fluids seeping from the burnt carcasses which had more skin ruptures due to the increased

level of burning. The dominant Diptera species at all the carcasses was *C. chloropyga*, with lesser numbers of *C. marginalis* (Figure 3.78).

3.4.2.3 Coleoptera

3.4.2.3.1 2004 Trial

Dermestidae were dominant at all of the carcasses, with lesser numbers of Cleridae and Histeridae being observed. At the burnt carcasses, the level of dominance of Dermestidae decreased and the level of dominance of Histeridae increased with an increase in the level of burning (Figure 3.79).

3.4.2.3.2 2005 Trial

Similar to findings during the 2004 trial, Dermestidae were dominant at all of the carcasses, with lesser numbers of Cleridae, Histeridae and Silphidae being observed. However, during 2005 the level of dominance of Dermestidae at the burnt carcasses increased with an increase in the level of burning (Figure 3.79). This could be attributed to three factors. Firstly, the larger numbers of Diptera at the carcasses during 2005, compared to 2004. Secondly, larger and more maggot masses were found on the carcasses during 2005 than during 2004. Thirdly, the feeding activity of these maggots on the carcass had the effect of the carcasses being moister and the carcasses did not dry out as rapidly as during 2004. Therefore the carcasses may not have been as attractive to Coleoptera during the 2005 trial as during the 2004 trial

3.4.3. Arthropod succession on the carcasses

The most abundant Diptera species observed at the carcasses during winter were *C. chloropyga,* C*. marginalis, C. vicina, M. domestica* and *P.casei* (Figure 3.80).

Dermestes maculatus and N. rufipes were the most abundant Coleoptera observed during the winter trials (Figure 3.81).
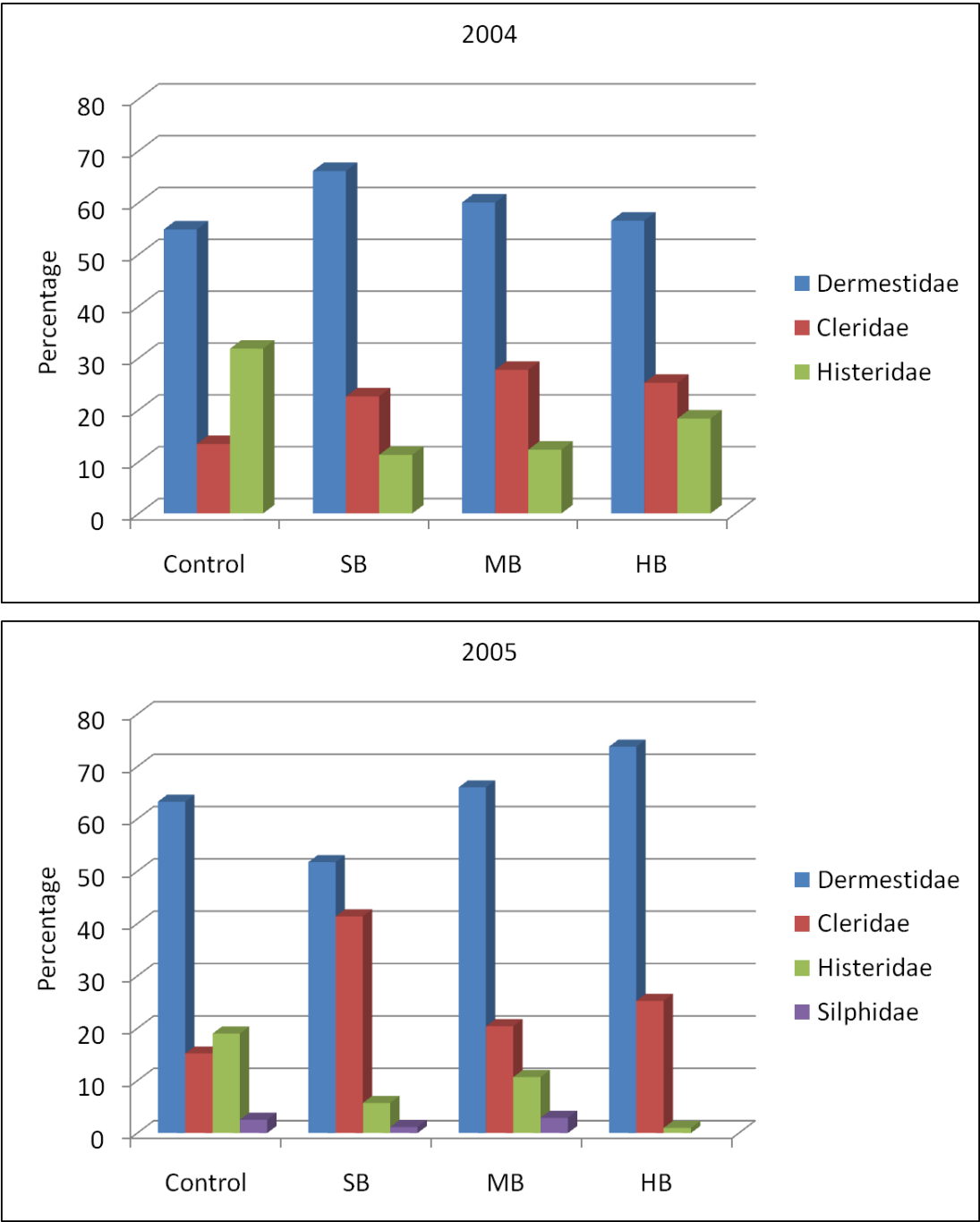
Figure 3.79 *Coleoptera Composition during the winter trials.*
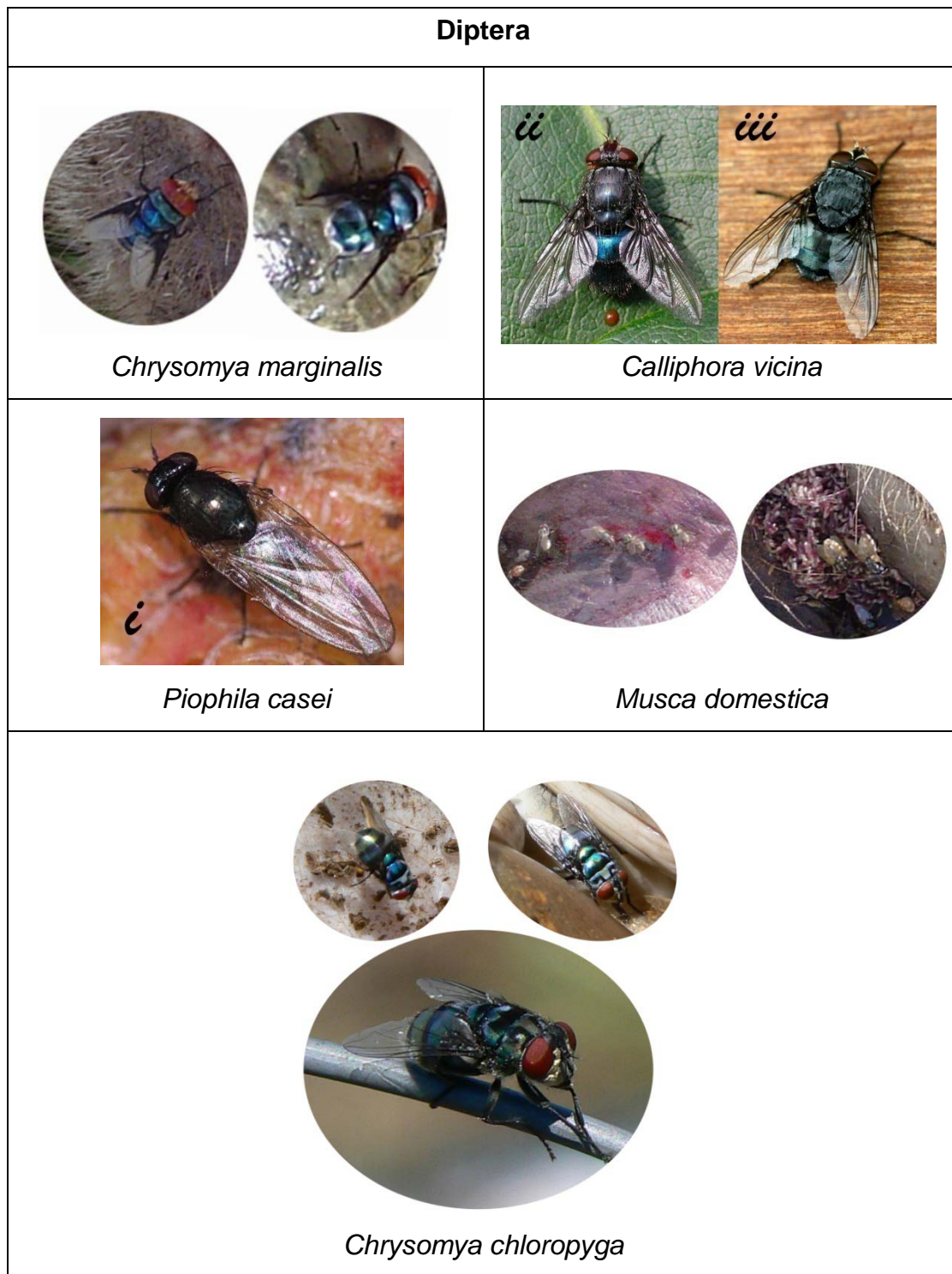
Figure 3.80 *Most abundant Diptera species observed during the winter trials.*

Photographs not taken by the author: *i* (Falatico)

ii (Berdys, 2007)

iii (bio Natura, 2008)

Coleoptera	
Dermestes maculatus	*Necrobia rufipes*
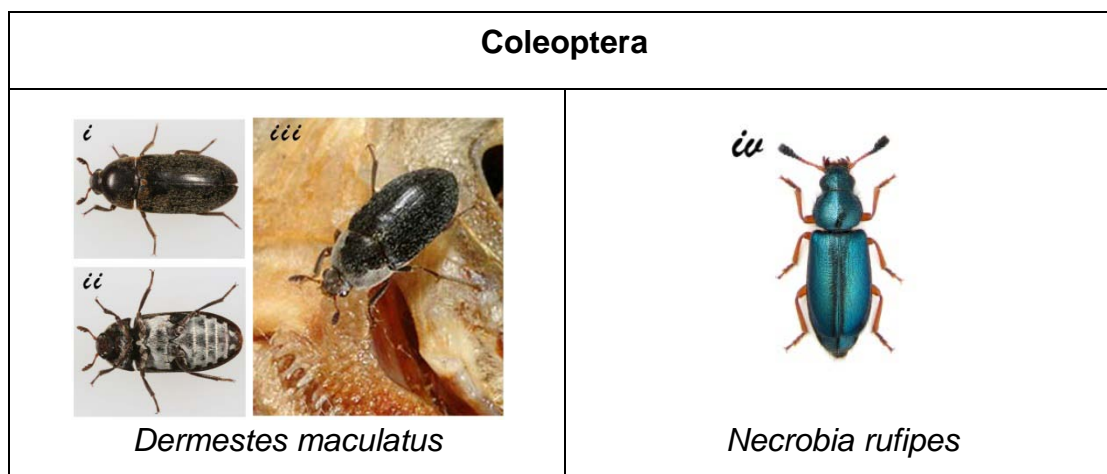
Figure 3.81 *Most abundant Coleoptera species observed during the winter trials.*

Photographs not taken by the author: *i (Gross, 2005)*

ii (Gross, 2005)

iii (London Natural Science Museum, 2004)

iv (Makarov, 2006)

Adult Calliphoridae, Sarcophagidae and Muscidae, were the first insects to arrive at the carcasses. In some instances this occurred within 5 minutes of the start of the trial and in other instances it only occurred after three days.

The pattern of Calliphoridae being initially present at the carcasses in large numbers for two to three consecutive days that was found during the summer and autumn trials, also manifested during the winter trials. During 2004, this only happened at the end of week two or the start of week three. Oviposition occurred to a far lesser extent than during the summer and autumn trials, probably due to lower ambient temperatures and less active females.

Calliphoridae maggots were feeding inside skin ruptures. Sarcophagidae maggots were feeding in the area of the thorax immediately underneath the left front leg. *Musca domestica* maggots were not found on the carcasses.

For the duration of the trials, the insects on the carcasses seemed to occur at the same time. One possible explanation of this phenomenon could be the fact that the maggots only fed on very small areas of the carcasses, which left the bulk of the carcasses to be colonised and utilised by other arthropods, such as *Dermestes maculatus* and *Necrobia rufipes*. Another explanation could be the difference in micro niches utilised between the Diptera and Coleoptera. Coleoptera utilised the dried tissues of the carcass, while Diptera utilised the slightly wetter tissues.

A higher diversity of species was found during winter than during the summer and autumn trials, but far lower numbers of these species occurred at the carcasses at any given time.

All fauna referred to are adults, unless otherwise indicated.

3.4.3.1 Control

Muscidae, Asilidae and Sarcophagidae were the first arrivals in very low numbers at the carcass. Low numbers (less than five individuals) of Calliphoridae were observed throughout the trials. Higher numbers (20 to 50 individuals) of Calliphoridae were observed at the start of week three (Day 15) during 2004 (Figure 3.82), but high numbers of *C. chloropyga* was observed in the middle of week one (Days 3 and 4) during 2005 (Figure 3.83).

Piophilidae were observed in large numbers (less than 50 individuals) from the end of week three (Day 21) to the end of week six (Day 42) during 2004 (Figure 3.82). During 2005, Piophilidae were observed in low numbers (less than 20 individuals) from the middle of week two (Day 11) until the end of week six (Day 40), which was at the end of the trial (Figure 3.83).
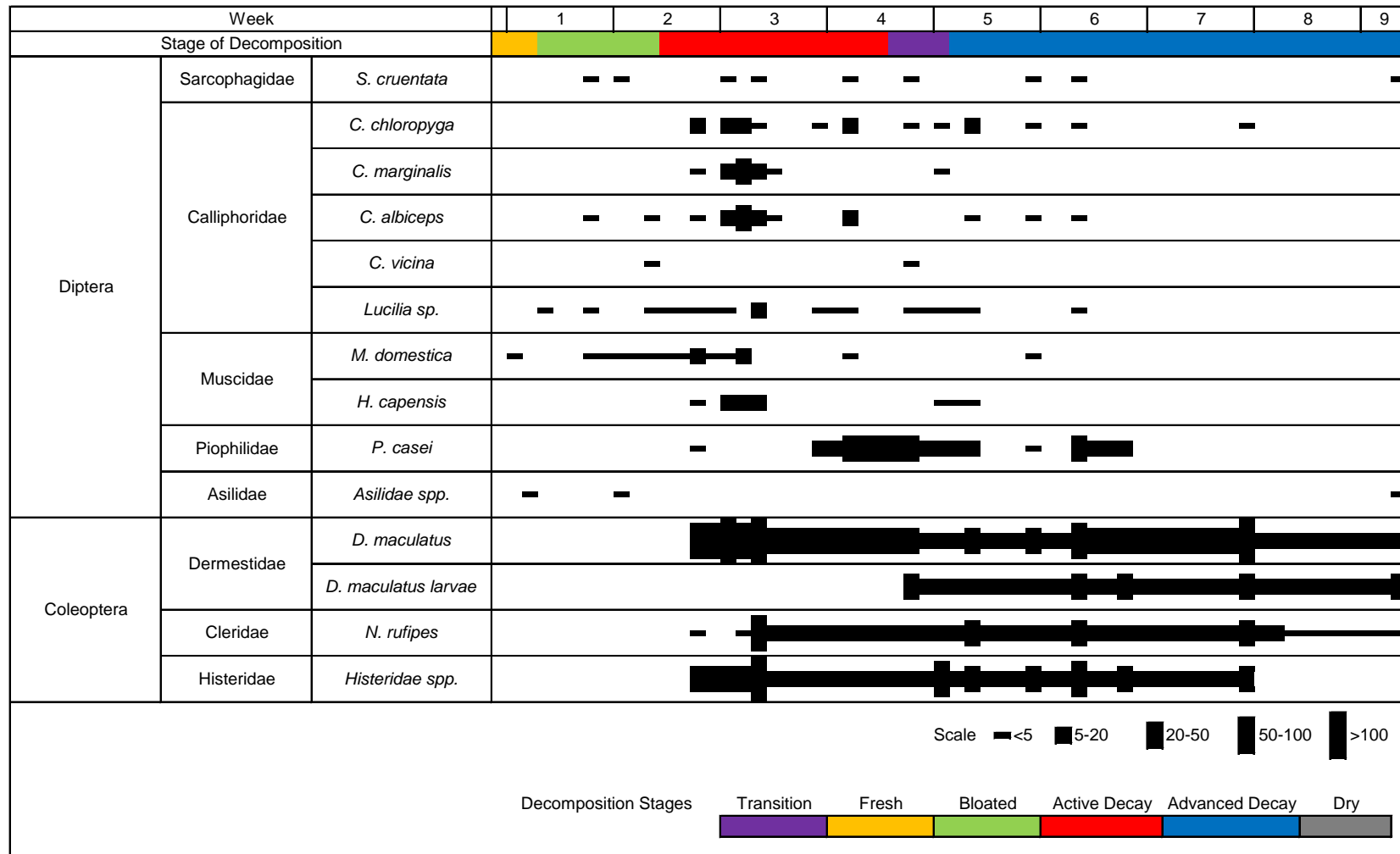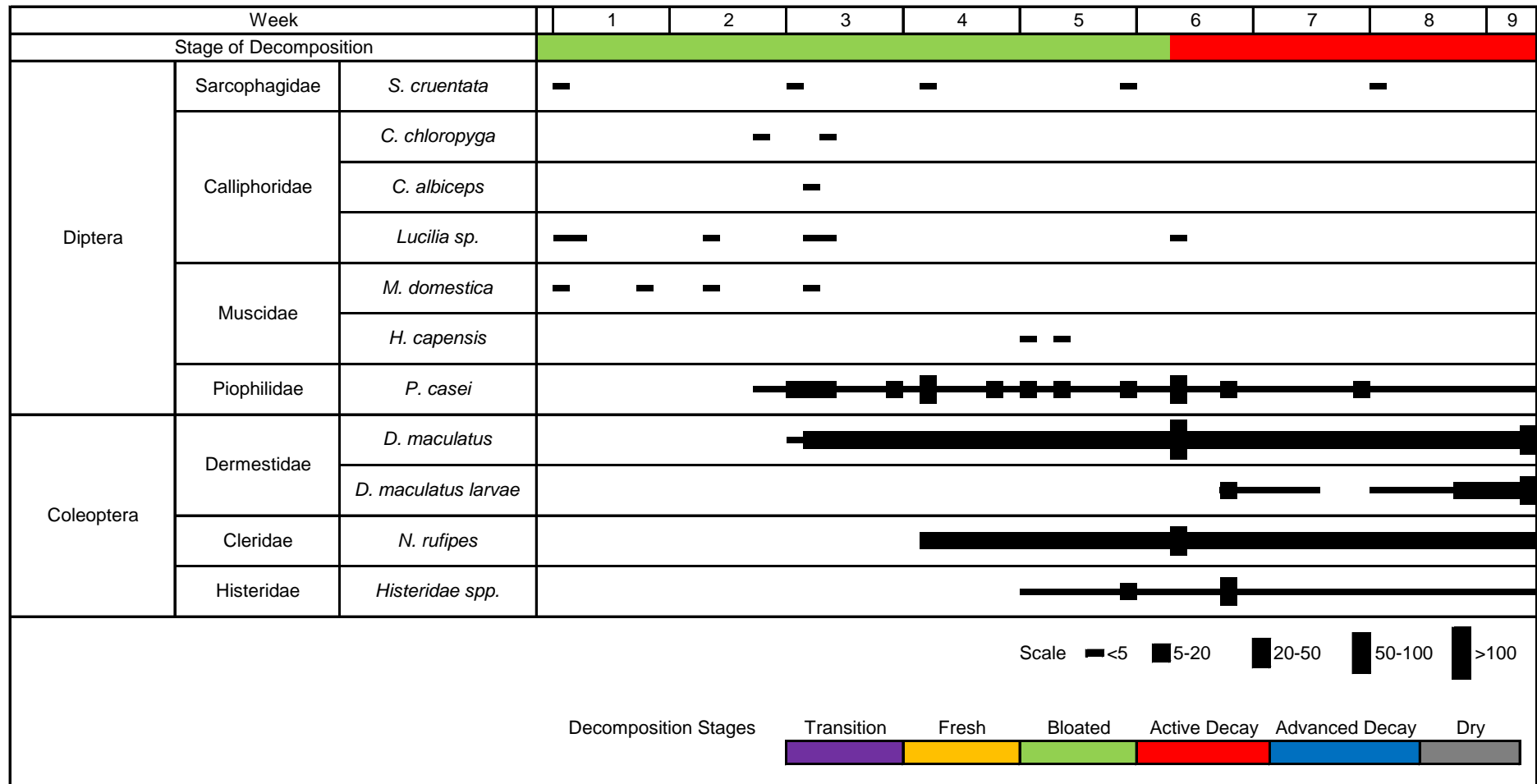
Figure 3.82 *Arthropod Succession on the control carcass during the Winter 2004 Trial.*
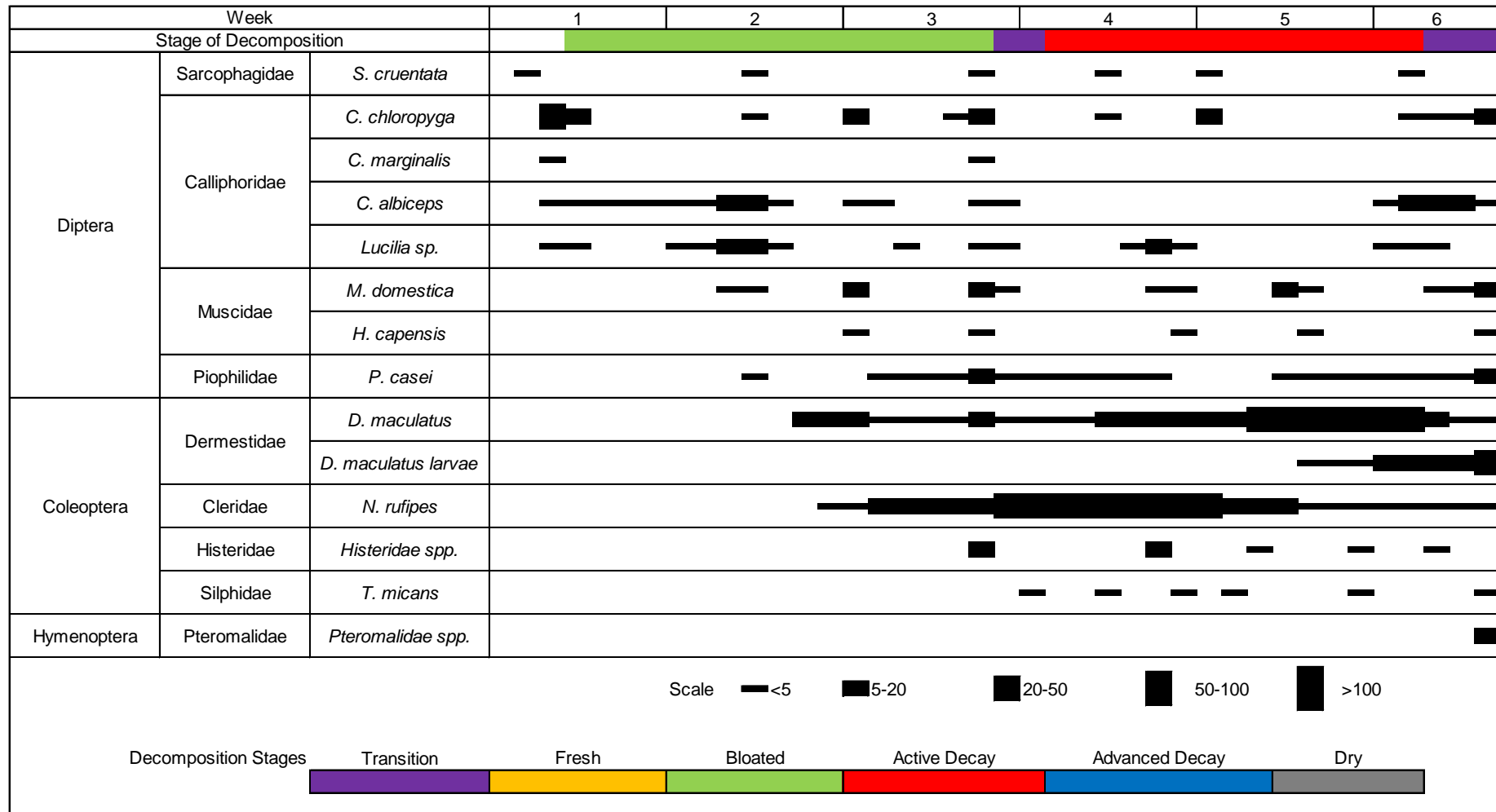
Figure 3.83 *Arthropod Succession on the control carcass during the Winter 2005 Trial.*

High numbers of Dermestidae were observed from the end of week two (Day 13) until the end of the trial (Day 59) during 2004 (Figure 3.82). At the same time, large numbers of Cleridae and Histeridae were observed. Dermestidae and Cleridae were also observed from the end of week two (Day 13) until the end of the trial (Day 40) during 2005 (Figure 3.83). Dermestidae larvae were observed from the end of week four (Day 27) and the middle of week five (Day 33) during 2004 (Figure 3.82) and 2005 (Figure 3.83), respectively. Histeridae were only periodically observed during 2005 (Figure 3.83).

3.4.3.2 SB

Even less insect activity was observed at the carcass than at the control carcass during both trials. The first arrivals during 2004 were Sarcophagidae, Calliphoridae and Asilidae during week one (Figure 3.84). Calliphoridae were only periodically observed during 2004 (Figure 3.84) from the end of week two (Day 13). During 2005, larger numbers of Calliphoridae were initially observed during weeks one and two (Figure 3.85).

Small numbers of Muscidae were observed from the beginning of week two (Day 8) during both trials (Figures 3.84 & 3.85). Piophilidae occurred at the carcass from week two (Day 13 during 2004 and Day 11 during 2005) onwards during both trials (Figures 3.84 & 3.85). However, Piophilidae maggots were observed only once at the beginning of week three (Day 15) during 2005 (Figure 3.85). It is possible that they moved inside the carcass thereafter and as such could not be observed.

Dermestidae occurred at the carcass from the middle of week two (Day 10) until the end of each trial (Figures 3.84 & 3.85). Dermestidae larvae were observed from the end of week five during 2004 (Day 35) (Figure 3.84), but were only observed at the end of week six (Day 40) at the end of the trial during 2005 (Figure 3.85).

Cleridae were found on the carcasses 2 to 3 days after Dermestidae were observed for the first time (Figures 3.84 & 3.85).
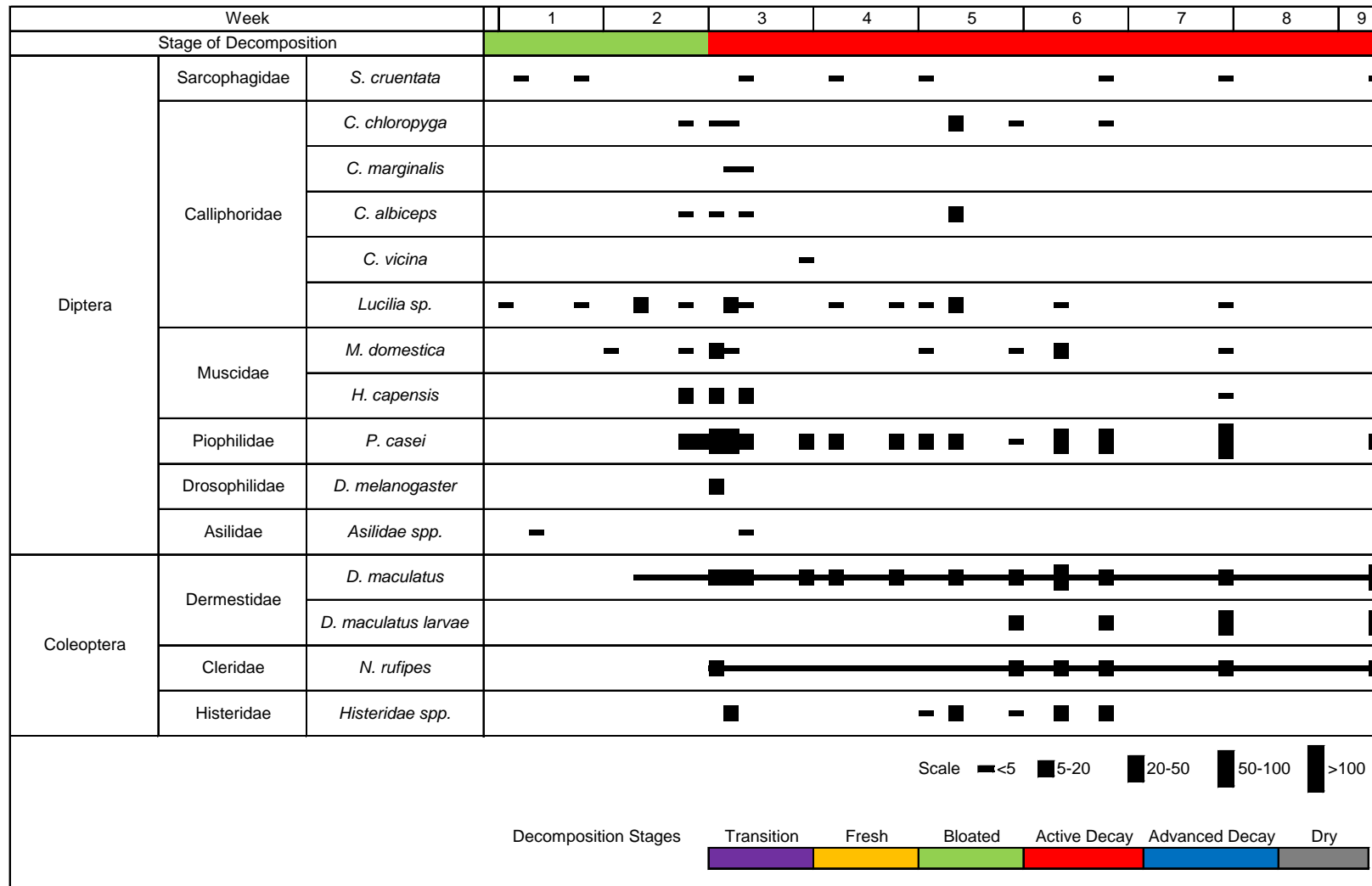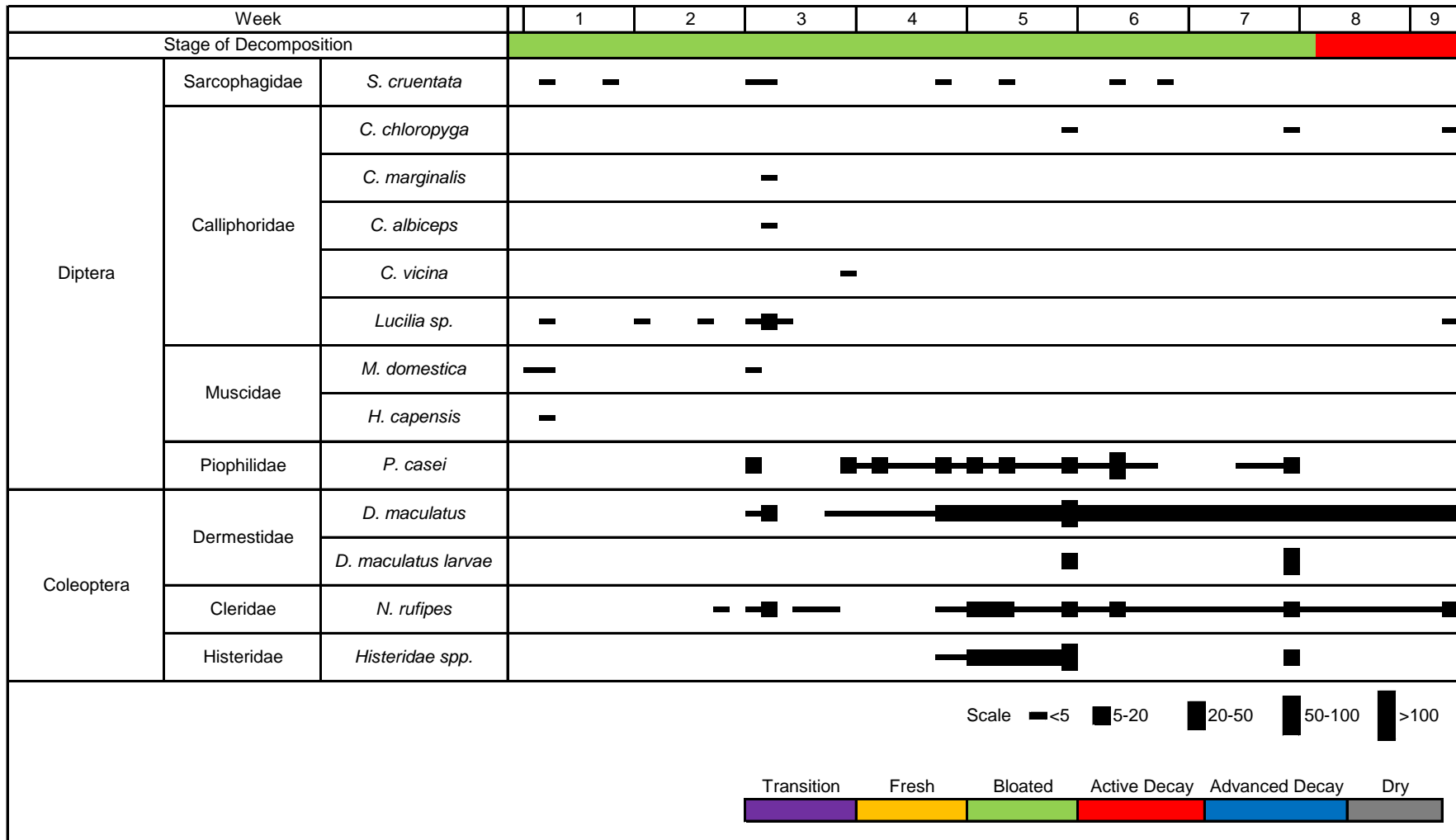
Figure 3.84 *Arthropod Succession on the SB carcass during the Winter 2004 Trial.*
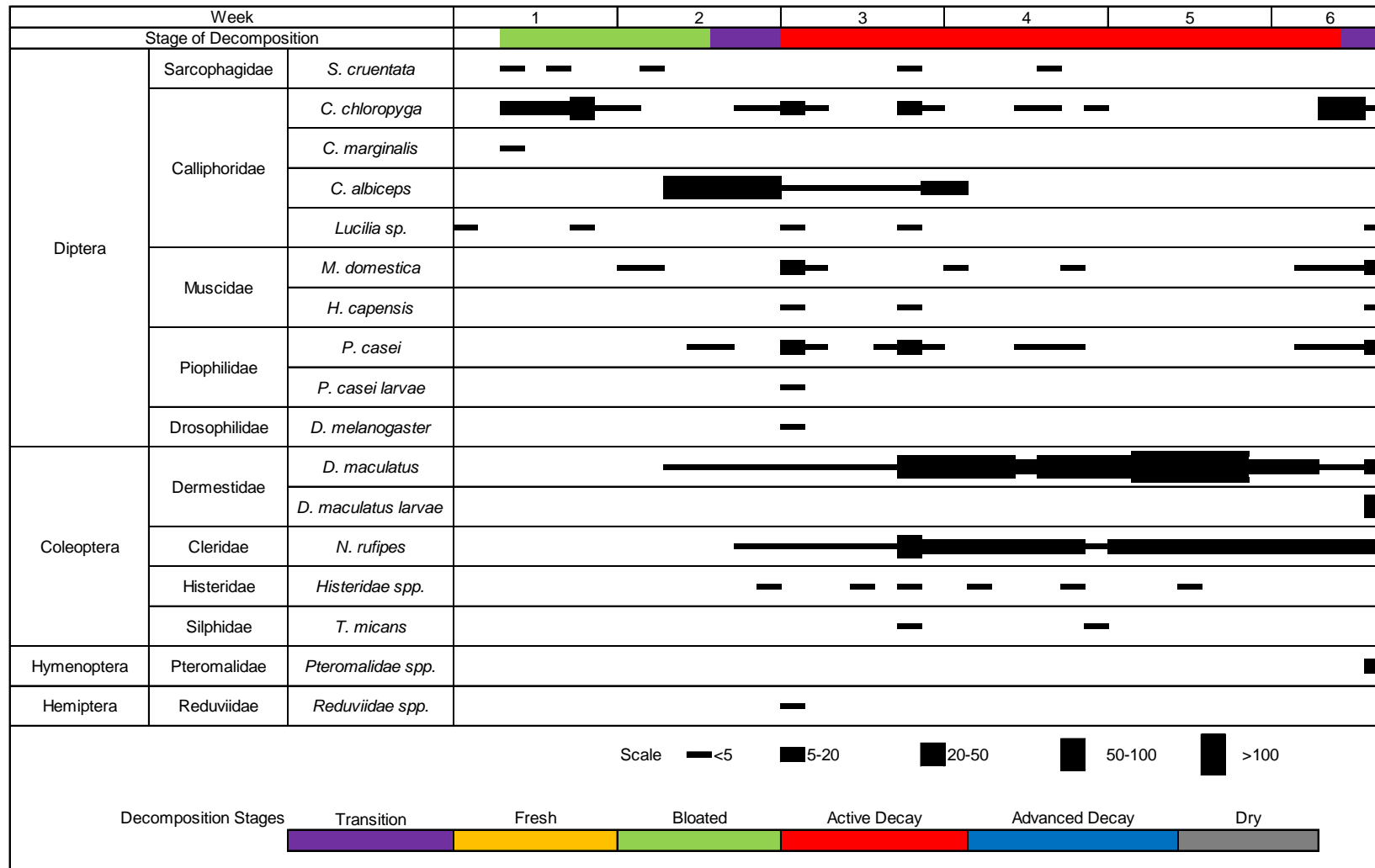
Figure 3.85 *Arthropod Succession on the SB carcass during the Winter 2005 Trial.*

Histeridae were only periodically observed during both trials from the end of week two (Day 14) during 2004 (Figure 3.84) and the beginning of week three during 2005 (Day 16) (Figure 3.85).

3.4.3.3 MB

Sarcophagidae, Calliphoridae and Muscidae were the first to arrive at the carcass on Day 1 during 2004 (Figure 3.86). Compared to the Coleoptera, Diptera only occurred periodically and in small numbers, except for Piophilidae during 2004 (Figure 3.86). During 2005, Calliphoridae (represented by *C. chloropyga*) were the first arrivals at the carcass on Day 3 (Figure 3.87). This could be due to the low ambient temperatures causing the bloated stage to commence on Day 3 (Figure 3.70). When bloating started, the first decompositional gases started to build up and were released in small amounts, serving as olfactory cues to attract blow flies in the area.

Dermestidae were found at the carcass from the beginning of week three (Day 15) in 2004 (Figure 3.86) and the middle of week two (Day 12) during 2005 (Figure 3.87). Dermestidae larvae were found from the end of week six (Day 41) and the middle of week five (Day 33) during 2004 (Figure 3.86) and 2005 (Figure 3.87), respectively. These larvae were observed until the end of each trial, with their numbers steadily increasing.

Cleridae were only found from week four (Day 23) during 2004 (Figure 3.86) and from the end of week two (Day 14) during 2005 (Figure 3.87) and were observed until the end of each trial.

Interestingly, Histeridae were only observed at the carcass from the beginning of week five (Day 29) until the end of the trial during 2004 (Figure 3.86). They were however observed since the end of week one (Day 6) during 2005 (Figure 3.87).

Silphidae were only periodically observed from the end of week two (Day 13) during 2005 (Figure 3.87).

Figure 3.86 *Arthropod Succession on the MB carcass during the Winter 2004 Trial.*
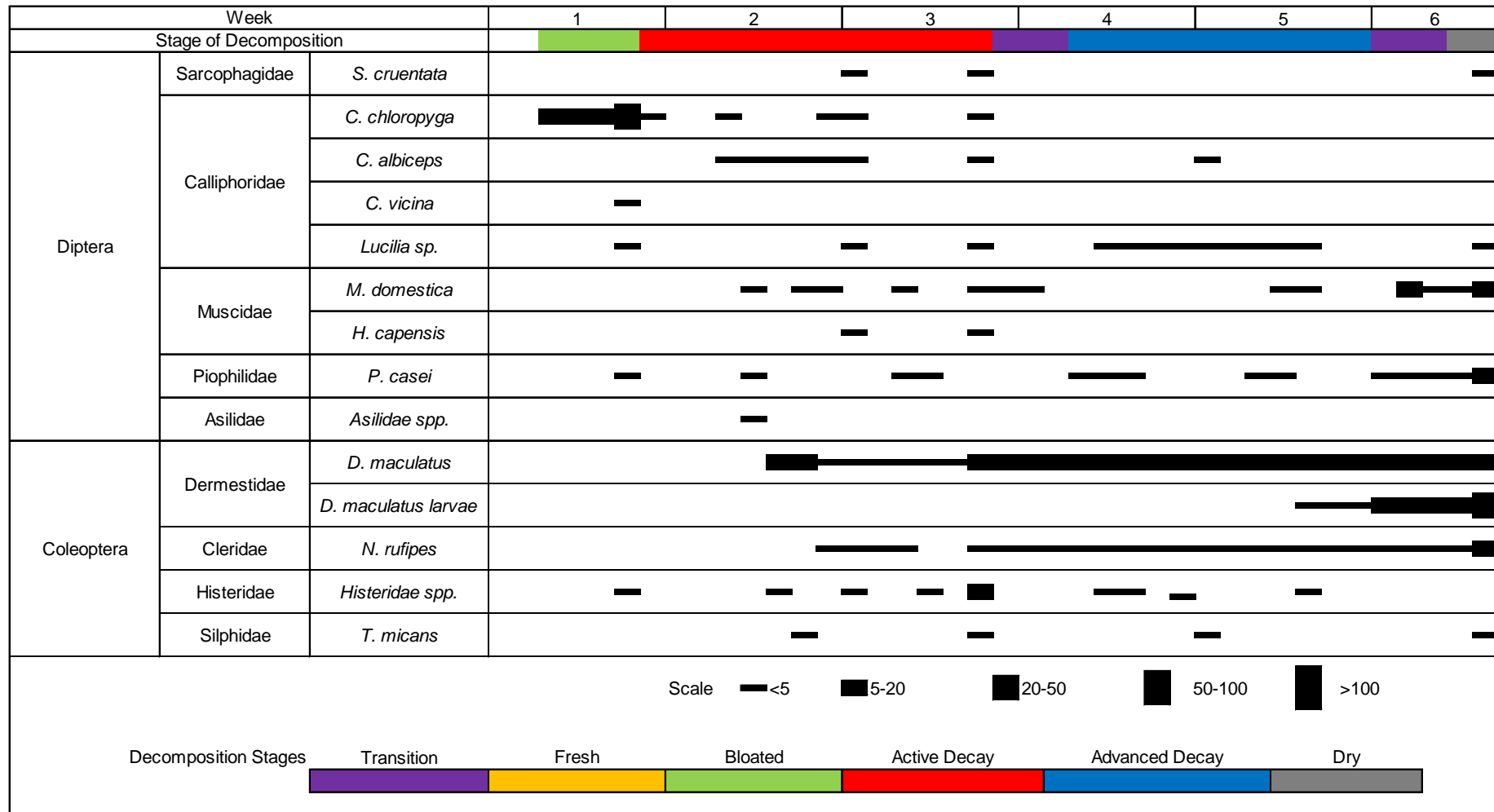
Figure 3.87 *Arthropod Succession on the MB carcass during the Winter 2005 Trial.*

3.4.3.4 HB

Sarcophagidae, Calliphoridae and Muscidae were the first insects arriving in extremely low numbers (less than five individuals) at the carcass during 2004 (Figure 3.88). During 2005, Calliphoridae were the first arrivals and between five and 50 individuals of *C. chloropyga* were observed during the first two weeks (Figure 3.89).

Piophilidae were found at the carcass from the beginning of week three (Day 15) during 2004 (Figure 3.88), and from the end of the first week (Day 6) during 2005 (Figure 3.89). This difference could be due to the winter 2004 trial commencing during the middle of winter and the 2005 trial commencing during the second half of winter when ambient temperatures were starting to rise.

Dermestidae were observed from the start of week three (Day 15) during 2004 (Figure 3.88) and from the middle of week two (Day 12) during 2005 (Figure 3.89). Dermestidae larvae were only found twice during 2004 (Figure 3.88), at the end of week five (Day 35) and the end of week seven (Day 49). During 2005 (Figure 3.89), Dermestidae larvae were found from the end of week five (Day 35) until the trial was terminated in week six (Day 40).

Cleridae occurred earlier than Dermestidae on the carcass during 2004 (Figure 3.88), but later than Dermestidae during 2005 (Figure 3.89).

Larger numbers of Histeridae were observed on the carcass during 2004 (Figure 3.88) from the end of week four (Day 27) to the end of week five (Day 35). During 2005, Histeridae were periodically observed since the middle of week two (Day 12) until the end of the trial (Figure 3.89).

Figure 3.88 *Arthropod Succession on the HB carcass during the Winter 2004 Trial.*
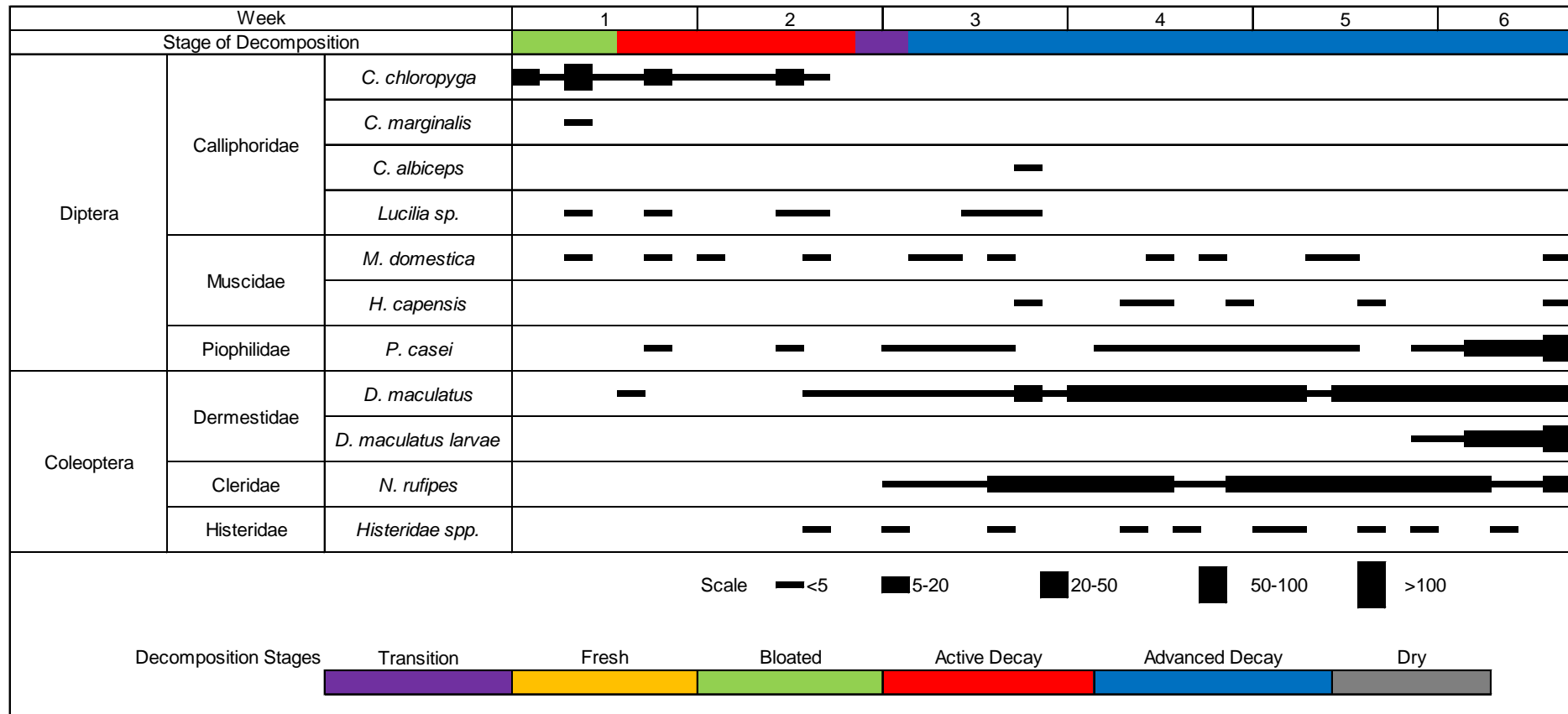
Figure 3.89 *Arthropod Succession on the HB carcass during the Winter 2005 Trial.*

3.4.4. Statistical analysis of arthropod succession

3.4.4.1 Analysis 1: Jaccard Metric

The Jaccard occurrence matrices were created by using data from the succession diagrams. Mean faunal similarity was calculated for each day (See 2.1.4.1.). These faunal similarities were plotted (Figures 3.90 & 3.91).

The characteristic horseshoe-shape did not manifest in the graphs as described by Schoenly (1992). Due to the low numbers of individuals present at the carcasses, the species was not recorded in successive days, which could account for the difference.

Similar shapes should be manifested by plots of mean similarities and a general property of the dynamic daily changes that occur during carrion-arthropod succession should be reflected by the ranges (Schoenly 1992),. This was evident during each trial (Figure 3. 91), but not over successive trials (Figure 3.90).

The comparison of a variety of studies revealed faunal similarity values ranging from 0.218 to 0.808 (Schoenly, 1992). The faunal similarity values during the winter trials were between 0.125 – 1 and 0.16 – 1 during 2004 and 2005, respectively. According to Schoenly (1992), the variation could be due to different types of carcasses that are essentially nonhuman. In this study, the variation may be due to the different degrees of charring due to burning.

3.4.4.2 Analysis 2: Correlation coefficient

The matrices were tested using a correlation coefficient. The coefficient was calculated with Graphpad Instat.
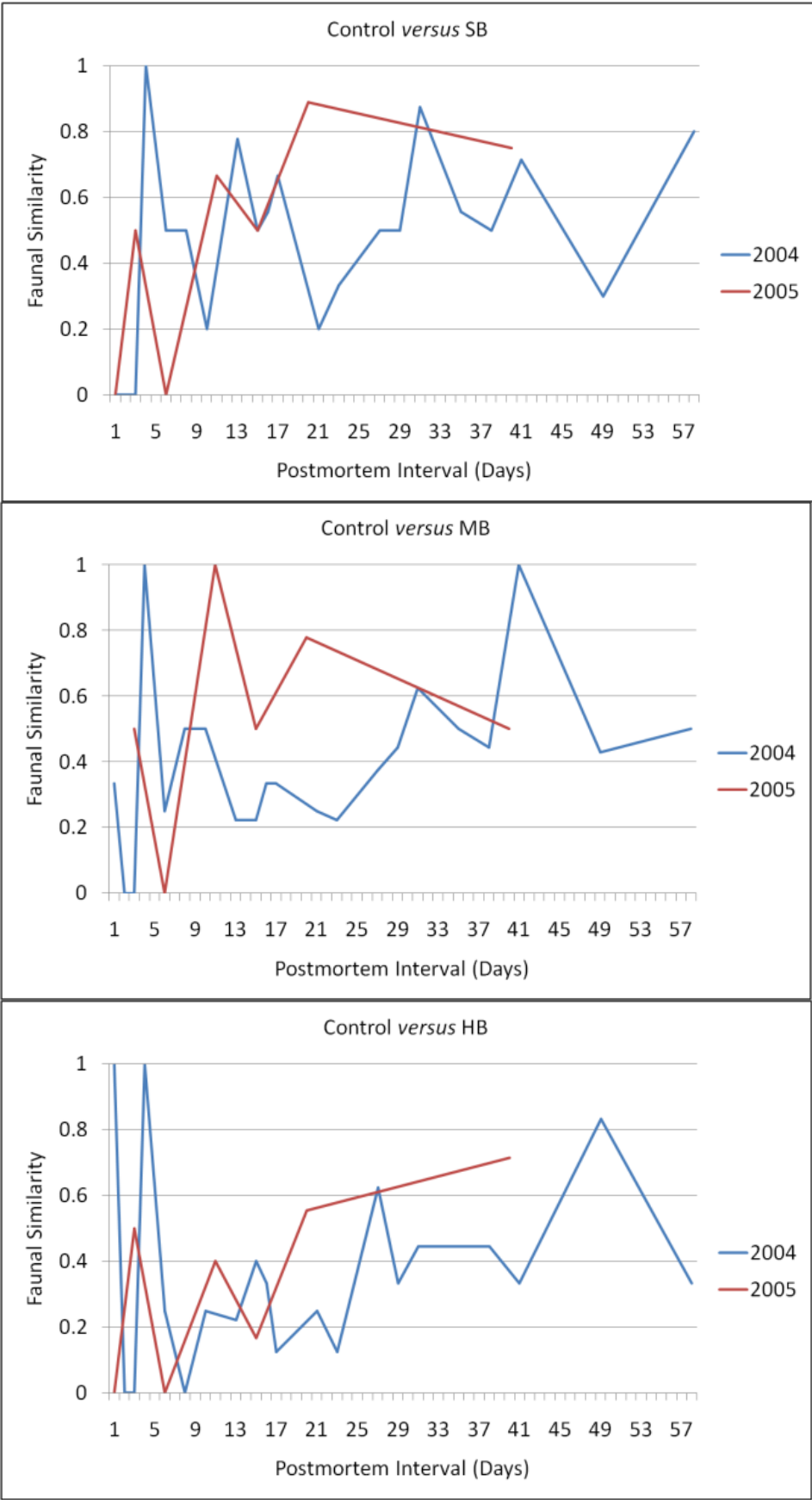
Figure 3.90 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession over the two winter trials.*
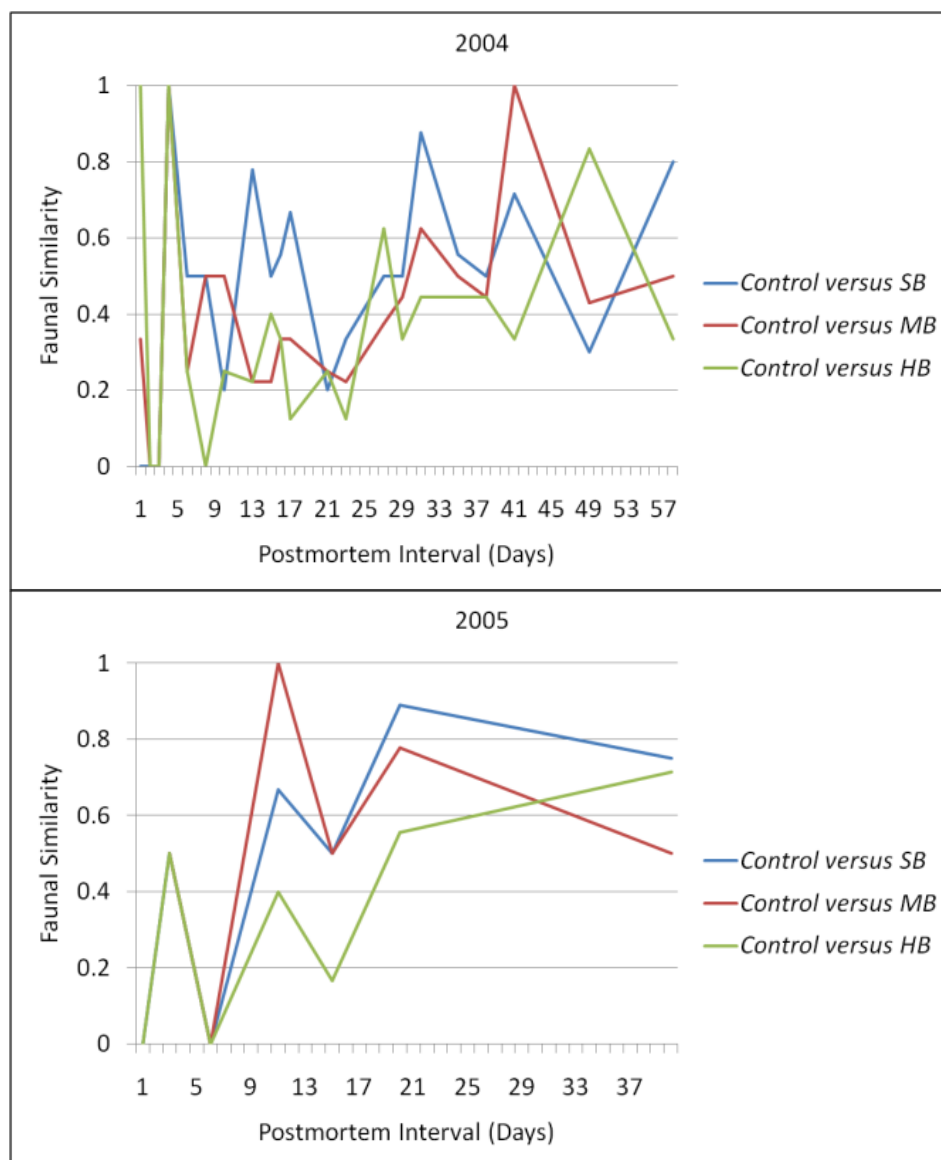
Figure 3.91 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession during each winter trial.*

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for all taxa showed that there were significant differences in the overall arthropod succession between the same treated carcasses of the control and MB carcasses (Table 3.19). No significant difference could be found in the overall arthropod succession between the same treated carcasses of the SB and HB carcasses. This could be due to different daily mean ambient temperatures during successive trials, resulting in a difference in duration and onset of bloating. During 2004 the daily mean temperature was 10.1°C and during 2005 it was 13.6°C.

Table 3.19 Similarity matrix analysis of each treatment over successive winter trials

2004/2005	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
Control	0.08887	0.001165 to 0.1752	0.047	significant
SB	0.05424	-0.03363 to 0.1413	0.226	not significant
MB	0.09883	0.01122 to 0.1849	0.0271	significant
HB	0.0297	-0.05817 to 0.1171	0.5076	not significant

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for forensic indicator species showed significant differences for *C. chloropyga*, *C. marginalis* and *C.albiceps* for each trial (Tables 3.20 - 3.22). This suggested that the colonisation of *C. chloropyga*, *C. marginalis* and *C.albiceps* were influenced by the level of burning of the carcasses.

Table 3.20 Similarity matrix analysis of *C. chloropyga* at all carcasses during successive winter trials

C. chloropyga	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.392	0.2583 to 0.5110	< 0.0001	significant
2005	0.7412	0.6481 to 0.8126	< 0.0001	significant

Table 3.21 Similarity matrix analysis of *C. marginalis* at all carcasses during successive winter trials

C. marginalis	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.4312	0.3018 to 0.5451	< 0.0001	significant
2005	0.4531	0.2980 to 0.5849	< 0.0001	significant

Table 3.22 Similarity matrix analysis of *C. albiceps* at all carcasses during successive winter trials

C. albiceps	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2004	0.414	0.2826 to 0.5302	< 0.0001	significant
2005	0.6521	0.5355 to 0.7444	< 0.0001	significant

3.4.5 Ambient temperatures and rainfall

As pointed out in Section 2.1.3.3, ambient temperature records and rainfall for the two nearest meteorological stations (Figures 2.14 & 2.15) were obtained from the South African National Weather Service in Pretoria.

The Bloemfontein City Centre (City) and Bloemfontein Airport (WO) meteorological station are situated ± 2km and 12.6km from the study site (Figures 2.14 & 2.15). Major differences in meteorological data from these two stations are highlighted below. Only the data from the nearest meteorological station (City) was applied to this study, since ambient temperatures measured at the study site during times of observations more closely approximated the temperatures measured at the City meteorological station than at the WO meteorological station.

3.4.5.1 2004 Winter Trial

The average maximum City temperature was 18.9°C and the minimum 1.3°C, while the average City temperature was 10.1°C. Average maximum and minimum WO temperature was 19.2°C and -0.5°C, respectively. The average WO temperature was 9.3°C.

City rainfall occurred on Days 45, 46, 49 and 50 (Figure 3.92), with a total of 12.22mm.

No rainfall was recorded at WO (Figure 3.92).
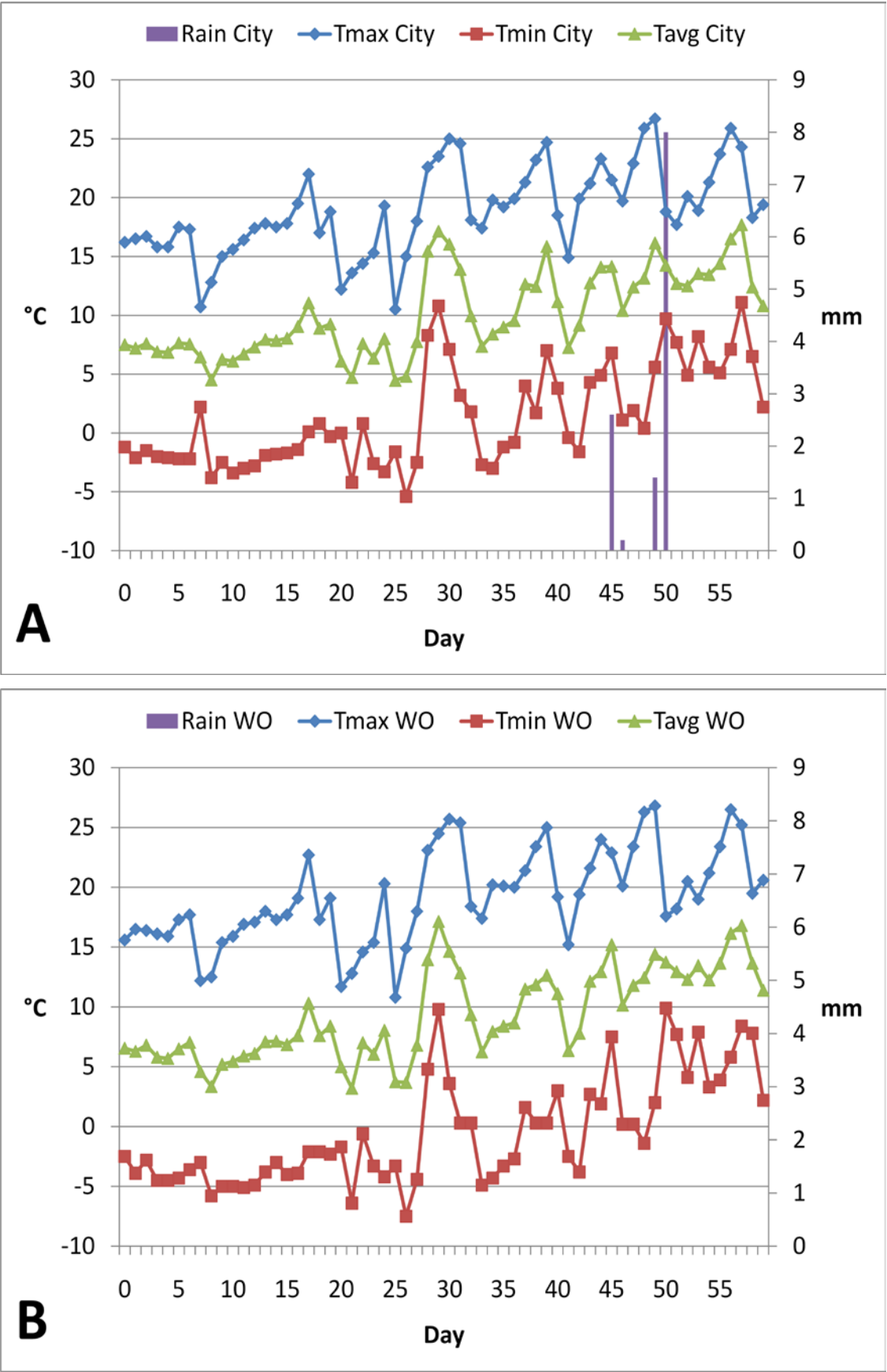
Figure 3.92 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Winter 2004 Trial.*

3.4.5.2 2005 Winter Trial

The average maximum and minimum City temperature was 22.7°C and 4.5°C, respectively. The average City temperature was 13.6°C. Average maximum and minimum WO temperature was 22.8°C and 1.9°C, respectively. The average WO temperature was 12.4°C.

City rainfall occurred on Days 5, 6, 12 and 33 (Figure 3.93), with a total of 3mm.

WO rainfall occurred on Days 12, 33 and 34 (Figure 3. 93), with a total of 10.6mm.

3.4.6. Ambient, external and internal carcass temperatures

The ambient temperature measured during observations follow the trend of the daily average ambient temperature for the nearest meteorological station, i.e. City (Figures 3.94 & 3.95). The exception to this was during times of observations when cloud cover, rain and windy conditions caused the ambient temperature to differ from the temperature measured at the nearest meteorological station.

As during the summer trials, fluctuations occurred for the temperature of the head. The temperature of the head was almost consistently lower than the ambient temperature during 2004 (Figure 3.94). This was due to the volume to surface ratio of the head (large surface compared to the small volume), causing the head to rapidly cool down. The difference between the head and the ambient temperature was greater during 2004 (Figure 3.94) than during 2005 (Figure 3.95). As during the summer and autumn trials, temperatures were only measured in these areas once the skin had ruptured and the bubbles of fluid could be seen and the escaping gas could be heard. This was done to prevent the fabrication of holes through which gas could escape, which in turn could influence the insect succession. Escaping gas is one of the chemical cues blow flies use to locate a body or carcass. Unfortunately, no escaping gas or bubbles of fluid was observed at any of the carcasses during the winter trials. Therefore measurements of the thoracic and abdominal temperatures were not made (Figures 3.94 & 3.95).
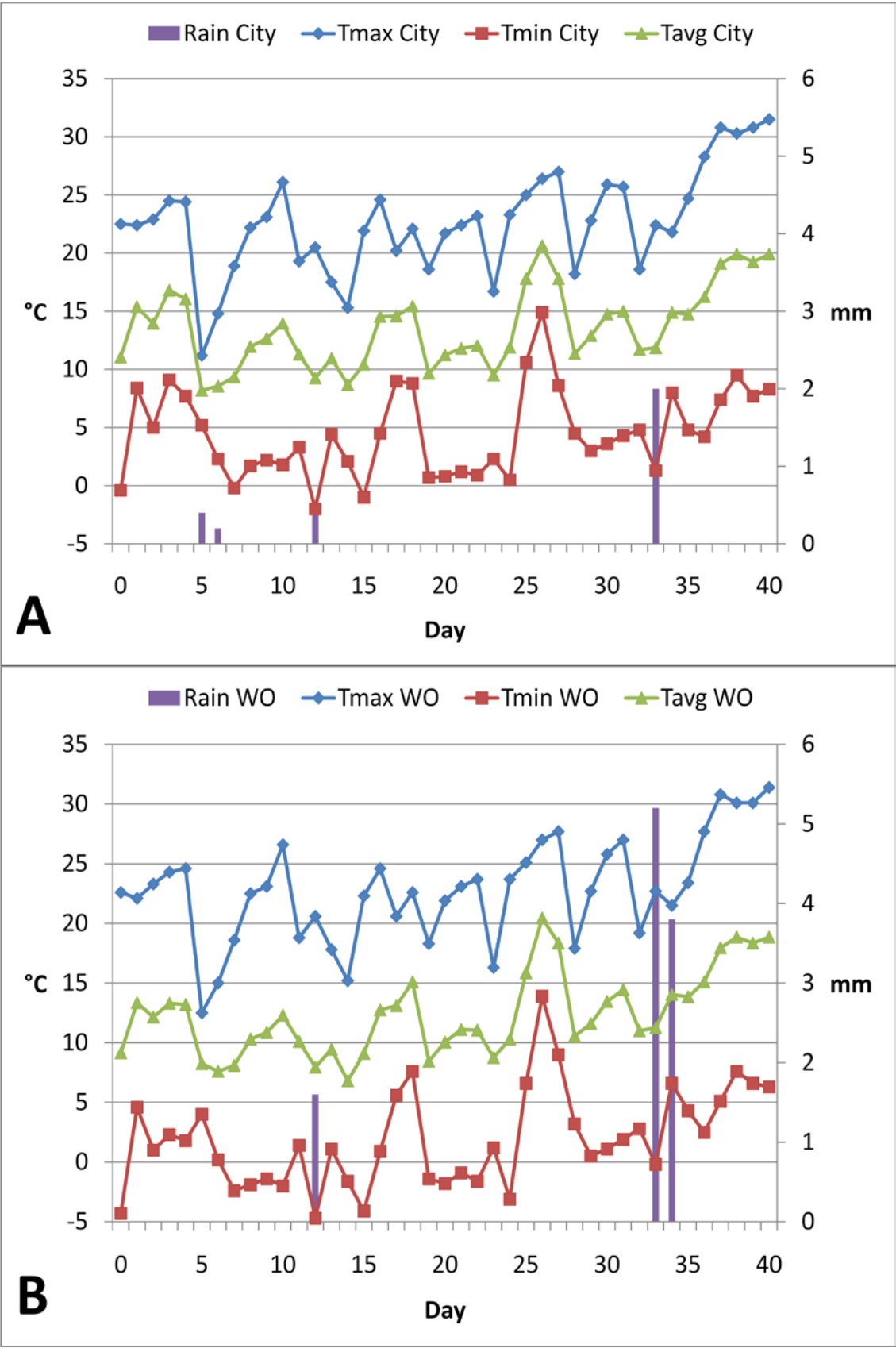
Figure 3.93 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Winter 2005 Trial.*
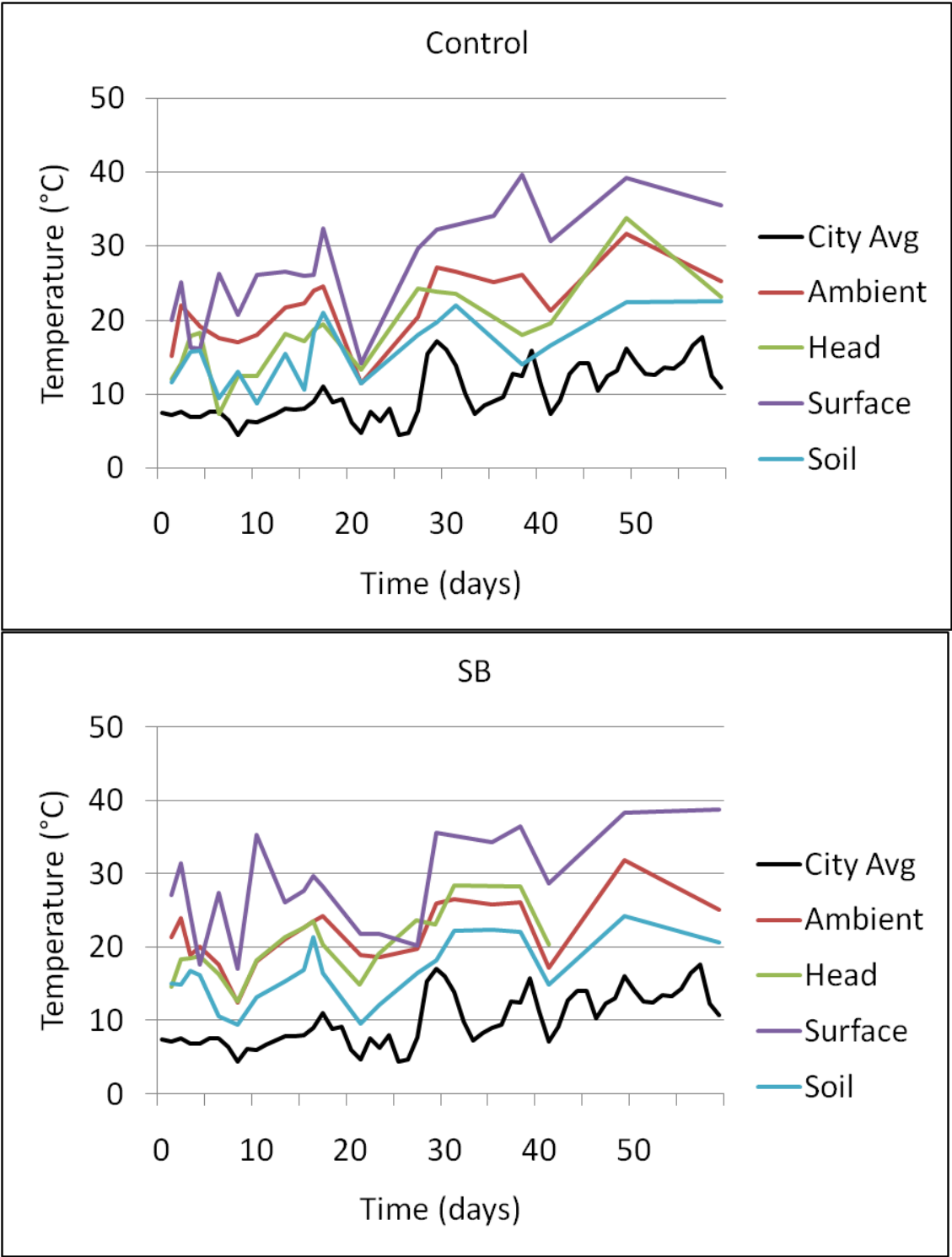
Figure 3.94 *Ambient & internal carcass temperatures during the Winter 2004 Trial.*
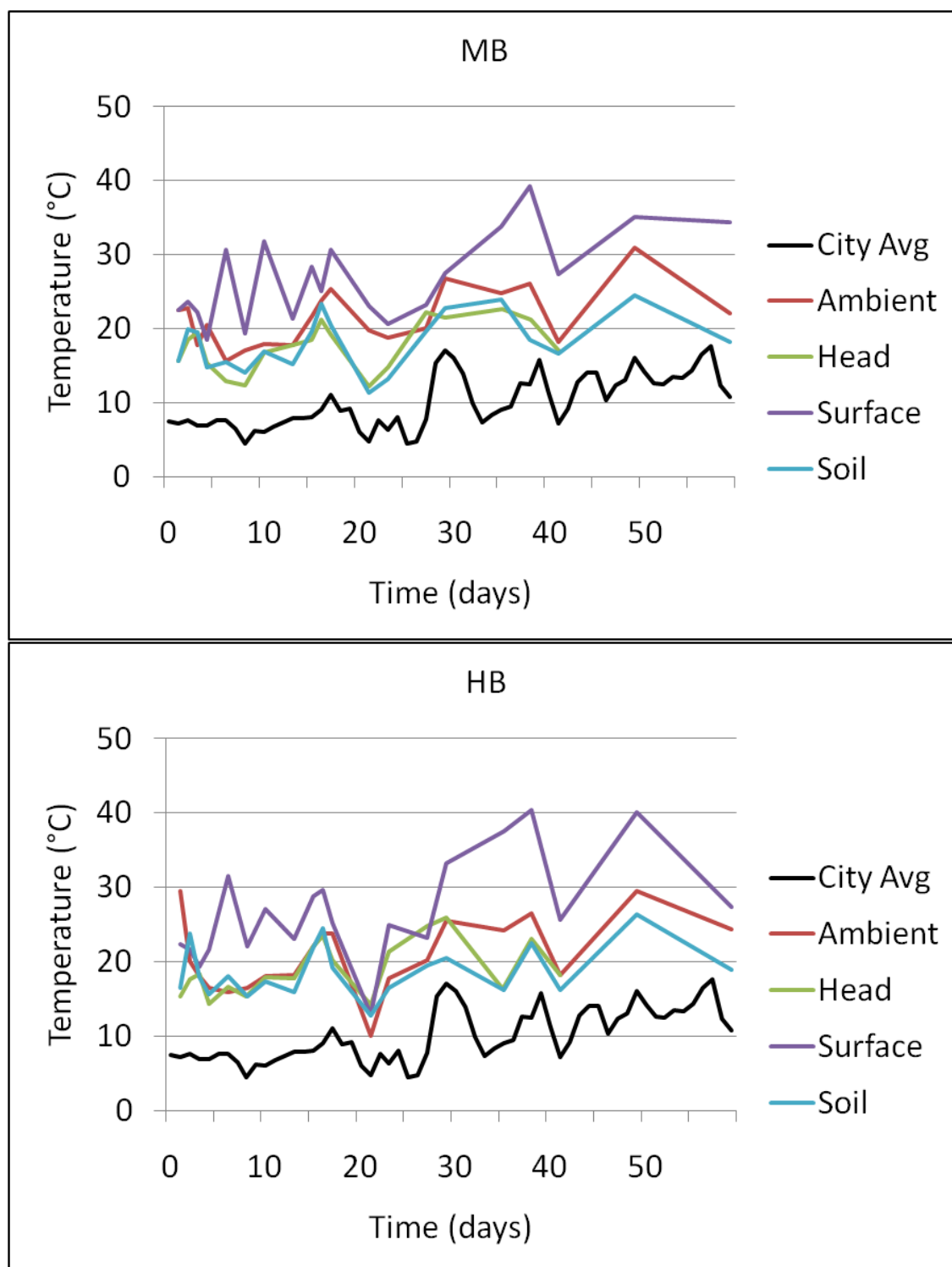
Figure 3.94 (continued) *Ambient & internal carcass temperatures during the Winter 2004 Trial.*
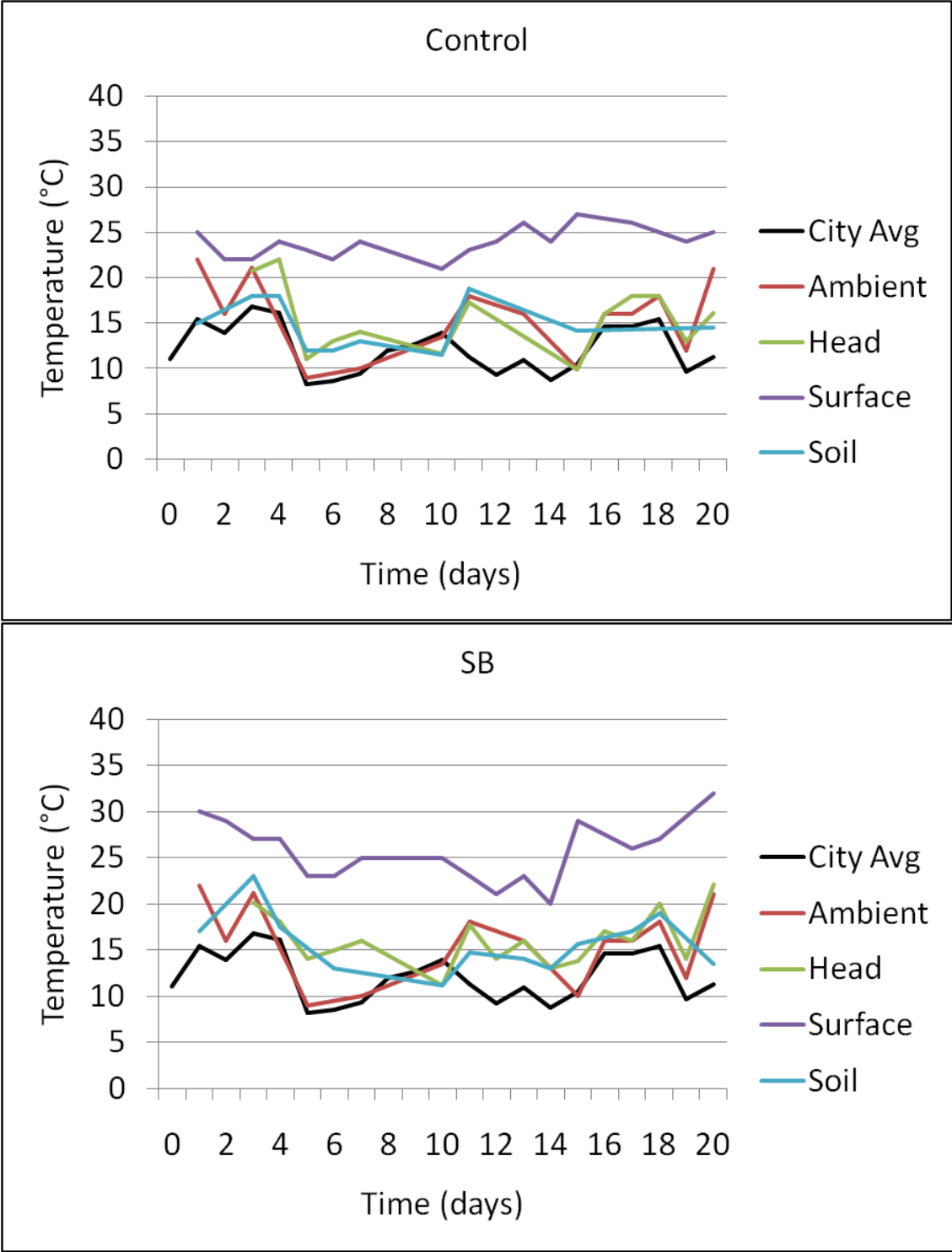
Figure 3.95 *Ambient & internal carcass temperatures during the Winter 2005 Trial.*
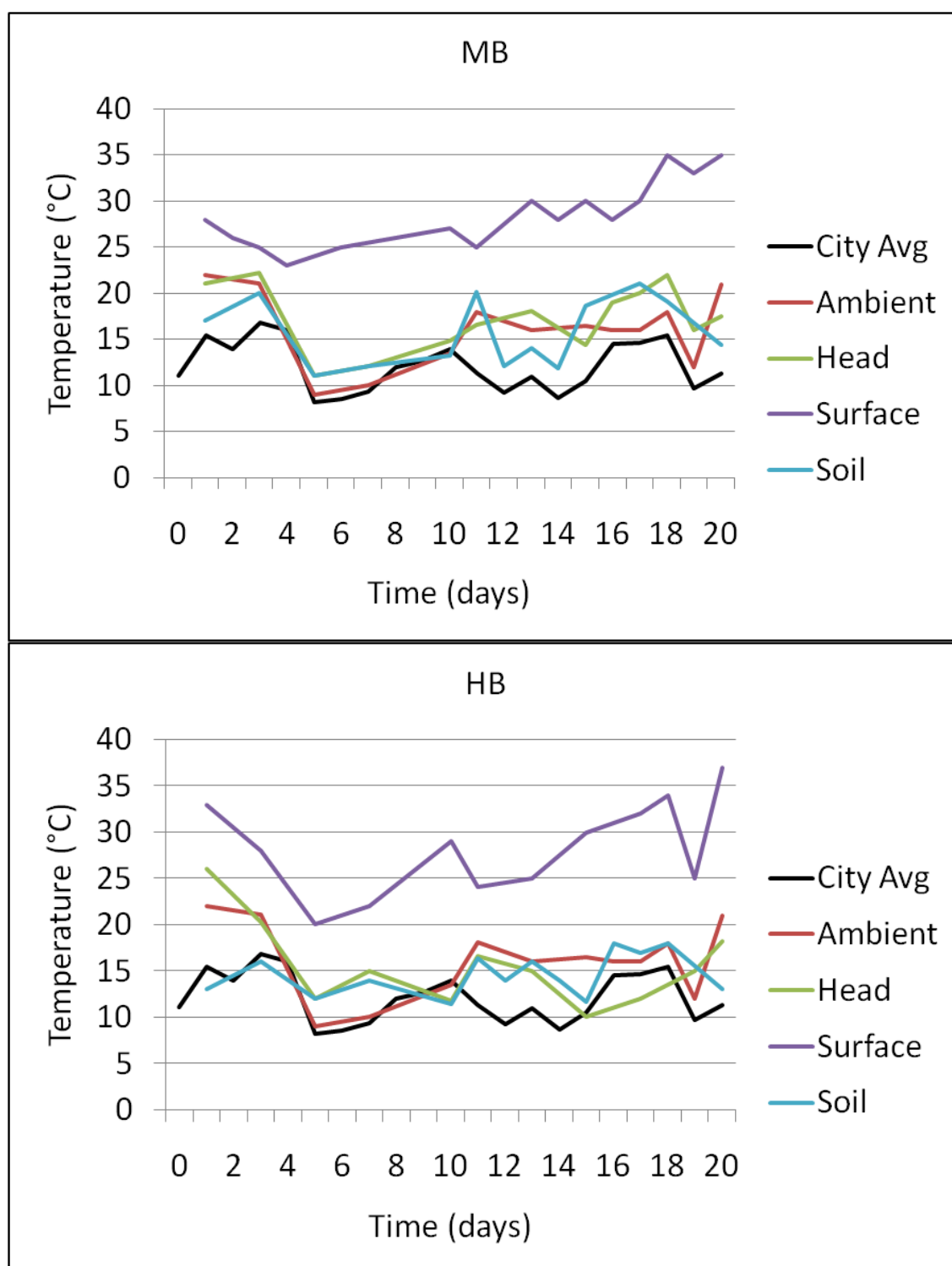
Figure 3.95 (continued) *Ambient & internal carcass temperatures during the Winter 2005 Trial.*

Soil temperature underneath the carcasses also varied greatly, with the greatest difference occurring during 2004 (Figure 3.94). During 2005, the soil temperature more closely approximated the ambient temperature (Figure 3.95).

The largest difference in temperature was between the ambient and carcass surface temperature (Figures 3.94 & 3.95; Tables 3.23 – 3.25).

Table 3.23 Maximum difference (°C) in ambient and carcass surface temperature

	Control	SB	MB	HB
2004	13.5 (Day 38)	17.3 (Day 10)	13.8 (Day 10)	15.6 (Day 6)
2005	17.0 (Day 15)	19.0 (Day15)	17.0 (Day 18)	16.0 (Days 17 & 20)

Table 3.24 Mean difference (°C) in ambient and measured temperature during 2004

	Head	Surface	Soil
Control	-3.5	5.4	-5.7
SB	-0.8	6.7	-5.1
MB	-3.2	5.4	-3.4
HB	-1.1	6	-2.1

Table 3.25 Mean difference (°C) in ambient and measured temperature during 2005

	Head	Surface	Soil
Control	2.8	10.7	0.6
SB	2.5	11.6	0.2
MB	1.2	13.7	0.9
HB	-0.3	12.2	-1.7

The prolonged presence of maggots on the carcasses had the effect that the active decay stage lasted longer than during the summer and autumn trials. This was due to the effect of the low temperatures during the winter trial, since maggot development slows down significantly at lower temperatures (Deonier, 1940; Davies & Ratcliffe, 1994; Ames & Turner, 2003). However, an increase in temperature can be found in even small maggot masses, *i.e.* more than four maggots per gram of substrate (Goodbrod & Goff, 1990).

Temperatures are typically higher at an exposed site during the day but often fall below those of shaded sites during the night and just before dawn. Extreme maximum and minimum temperatures at exposed locations occur more readily than at protected locations (Shean, Messinger, & Papworth, 1993).

These differences in temperature patterns may have a profound effect upon the decomposition rate of a body, which will directly influence the estimation of the postmortem interval.

Section 3.5. The influence of burning on carcass decomposition and arthropod succession: Two Spring Studies.

The Spring 2003 trial was the very first trial of this study. Only three carcasses were used, *i.e.* control, SB and MB carcasses. At the end of the spring 2003 trial, it was decided to introduce a fourth carcass, namely the HB carcass to see what the effect of considerable more charring would be on the insect succession and decomposition of this carcass, if any. Since the MB carcass was burnt with 156.25ml/kg of petrol, which resulted in a CGS level 2 with medium charring, it was decided that the HB carcass would be burnt with 312.5ml/kg of petrol. This resulted in a CGS level 3 with heavy charring. The very first time that the HB carcass was used in the study was during the Summer 2004 trial (See Section 3.2).

3.5.1. Decomposition of the carcasses

Immediately after killing, the control carcasses were placed in the field and this date and time was designated as Day 0 and marked the start of each trial (Table 3.26).

Table 3.26 Commencement dates and times of the Spring Trials

Year	Date	Time
2003	22 September	14:00
2004	2 November	14:00

The control carcasses showed the first signs of rigor mortis during the first observations. The burnt carcasses showed signs of bloating. The bloated stage lasted one day for the control carcass during 2003 and all of the carcasses during 2004. At the SB and MB carcasses, the bloated stage lasted for three days during 2003 (Figure 3.96).

During 2003, oviposition occurred simultaneously at each of the carcasses, except at the MB carcass where it occurred one day earlier. During 2004, oviposition occurred at the burnt carcasses one day prior to oviposition at the control carcass (Figure 3.96).

Year	Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2003	Control		*	*				*															
	SB		*	*		*																	
	MB	*	*	*						*	*												
2004	Control		*																				
	SB	*	*																				
	MB	*	*																				
	HB	*	*																				

Decomposition Stages

Transition   Fresh   Bloated   Active Decay   Advanced Decay   Dry
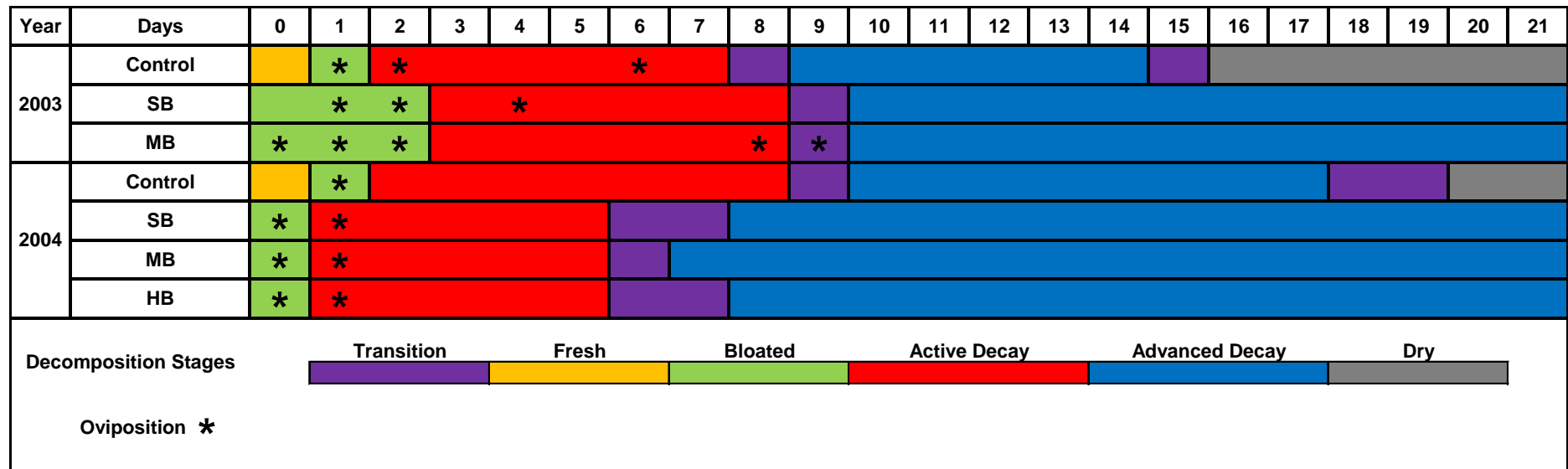
Oviposition *

Figure 3.96 *Decomposition stages of carcasses and days of oviposition during the spring trials.*

The Dry/Remains stage was not reached by any of the burnt carcasses (Figure 3.96). The 2003 trial was terminated after 30 days and the 2005 trial after 21 days. This was done due to the fact that all of the maggots had left the carcasses to pupate and the adults had already emerged from the pupae. No further oviposition had taken place at the carcasses. Even if oviposition had occurred, none of the remaining carcass tissue could be utilised by maggots due to desiccation. Therefore, the trials were terminated.

Only the days when major changes in the appearance of the carcasses and/or major changes in the insect fauna associated with the carcasses occurred, are discussed.

3.5.1.1 2003 Spring Trial

Day 1:

- The control carcass still appeared fresh during the morning observations. However, bloating had commenced with a rupture on the abdominal wall by the afternoon observations.
- Oviposition had occurred inside the mouths of the SB and MB carcasses.

Day 2:

- Oviposition occurred at the control carcass before the morning observations, most likely during the late afternoon of Day 1. Actively feeding first instar maggots were found inside the mouth during the afternoon observations. This indicated the start of the Active Decomposition stage at the control carcass (Figure 3.96).

Day 2 (continued):

- The oviposited eggs inside the mouths of the SB and MB carcasses had not hatched yet. Oviposition also occurred in fold of the left thigh of the MB carcass.

Day 3:

- First and second instar maggots were feeding inside the mouth of the control carcass. Green marbling of postmortem lividity was observed on the abdomen. Newly oviposited blow fly eggs were found between the thighs.
- The Active Decay stage commenced at the burnt carcasses on this day (Figure 3.96).
- First and second instar maggots, as well as newly oviposited blow fly eggs, were found inside the mouth of the SB carcass. Bloodstains were observed underneath the mouth of this carcass.
- First and second instar maggots were found inside the mouth of the MB carcass. The eggs oviposited in the left thigh had not hatched yet.

Day 4:

- Second instar maggots were found inside the mouth of the control carcass. Newly oviposited blow fly eggs were also observed underneath the right hand side of the carcass.
- In addition to the second and third instar maggots inside the mouth of the SB carcass, newly oviposited blow fly eggs were found in the left ear and on the thorax underneath the left front leg. The skin on the thorax started to rupture due to bloating.
- All of the maggots inside the mouth of the MB carcass had progressed to the second instar.

Day 5:

- All the carcasses were still smelly and bloated.
- The skin had ruptured along the abdomen and thorax of the SB carcass and on the back of the MB carcass.
- The intestines of the MB carcass protruded from the skin rupture.

Day 6:

- Large egg masses were oviposited at the control carcass from the chin to the hind leg along the carcass ground interface.
- The control carcass was still bloated, but the legs were starting to collapse back into the resting position.

Day 7:

- Third instar maggots were feeding inside the mouth of the control carcass. Second instar maggots were found in an area of skin slippage on the neck. Oviposition had occurred at the carcass-ground interface. Although the carcass was in the Active Decay stage (Figure 3.96), the carcass still appeared bloated.
- In the mouth of the SB carcass, third instar maggots were actively feeding. The skin over the abdomen had ruptured and a piece of skin was hanging loose with second instar maggots feeding underneath this loose piece of skin. The skin inside the left thigh had also ruptured.
- A soapy discharge was observed on the back of the MB carcass, with skin ruptures on the head and neck, right buttock and back of this carcass. Second and third instar maggots were actively feeding inside the mouth.

Day 8:

- Postfeeding maggots started to migrate from the control carcass to pupate, indicating the onset of the Advanced Decay stage (Figure 3.96).

Day 8 (continued):

- Second instar maggots were found in his skin flap on the right side of the SB carcass. Second and third instar maggots were feeding underneath an area of skin slippage of the right front leg, with third instar maggots actively feeding inside the mouth of this carcass.
- New skin ruptures appeared at the MB carcass on the thorax underneath the left front leg and the abdomen. Oviposition occurred in a small ground depression underneath the abdomen of the carcass where the intestines had protruded. Third instar maggots were observed inside the mouth and underneath the skin of the abdomen and left front leg.

Day 9:

- Maggots started to migrate away from the two burnt carcasses to pupate, starting the transition to the Advanced Decay stage (Figure 3.96).
- Oviposition had occurred on the thorax just above the right front leg of the MB carcass.
- Even though the burnt carcasses were in the advanced decay stage from this day forward, second & third instar maggots and postfeeding maggots were found at these carcasses until Day 18.

On Day 10, second and third instar maggots were observed actively feed all over the MB carcass.

Although the greatest mass of maggots had already migrated from the control carcass, second and third instar maggots were found underneath the carcass on Day 12. This was due to oviposition occurring at different times on different parts of the carcass.

On Day 17, the control carcass was already in the Dry/Remains stage, while the burnt carcasses were still in the Advanced Decay stage (Figure 3.96).

From Days 17 to 31 (Figure 3.96), the second generation of blow flies emerged from the pupae, whilst postfeeding maggots (*C. albiceps*) were found underneath the carcass.

From this observation it can be deduced that competition for living space and food source was limited to a minimum by the occurrence of oviposition at different times and on different sites at the carcass. Predation by *C. albiceps* maggots, if any, must have been minimal (none was observed). Figure 3.96 only shows the 2004 trial up to Day 21 for comparison with the 2005 trial.

3.5.1.2 2004 Spring Trial

Day 1:

- The control carcass was bloated (Figure 3.96), with the skin of the abdomen ruptured. Blood was dripping from the mouth and blood filled blisters were present on the right front leg. Oviposition occurred on the thorax just above the right front leg and at the carcass-ground interface

- The burnt carcasses were bloated, with the intestines protruding from abdominal skin ruptures.

- A skin rupture formed on the neck of SB carcass. Oviposition occurred on the protruding intestines of SB carcass. During the afternoon observations the eggs on the protruding intestines were hatching and first instar maggots were observed.

- Oviposition occurred on the intestines of the MB carcass and first instar maggots were actively feeding inside the mouth.

- Unlike the control and SB carcasses, the legs of the MB carcass were not lifted into the air due to bloating.

- Oviposition occurred inside the mouth and on the abdomen of the HB carcass. The eggs inside the mouth were hatching and a few first instar maggots were found inside the mouth during the afternoon observations.

- The presence of first instar maggots at the burnt carcasses indicated the start of the Active Decomposition stage (Figure 3.96).

Day 2:

- First instar maggots were found on the thorax just above the right front leg and at the carcass-ground interface, indicating the start of the Active Decomposition stage at the control carcass (Figure 3.96).

- The control carcass was still bloated, with the intestines protruding from a blood filled blister on the abdomen (Figure 3.97). One of the eyes was also protruding from the eye socket.

- First instar maggots were feeding at the protruding intestines (Figure 3.98) of the SB carcass.

- Second instar maggots and a few first instar maggots were found inside the mouth of the MB carcass. The eggs oviposited on the intestines (Figure 3.99) did not hatch yet.

- Inside the mouth of the HB carcass, second instar maggots were found. The skin rupture on the HB carcass was not as extensive as on the MB carcass (Figure 3.100).

Third instar maggots were found on the thorax between the front legs, inside the mouth and abdomen of the control carcass on Day 3.

Day 5:

- Second and third instar maggots were found at the intestines protruding from the SB carcass.

- A very small number of second instar maggots were feeding underneath the MB carcass. One live and one dead postfeeding maggot were also found underneath the MB carcass.

- At the HB carcass, second and third instar maggots were found on the ground underneath the abdomen and protruding intestines.

- Dead third instar maggots were also found on the surface of the abdomen and thorax directly above the right front leg, probably due to the high surface temperature of the carcass (See Section 3.5.6).

Figure 3.97 The control carcass on Day 2 during the Spring 2004 Trial
(blood filled blister indicated by arrow).

Figure 3.98 The SB carcass on Day 2 during the Spring 2004 Trial.
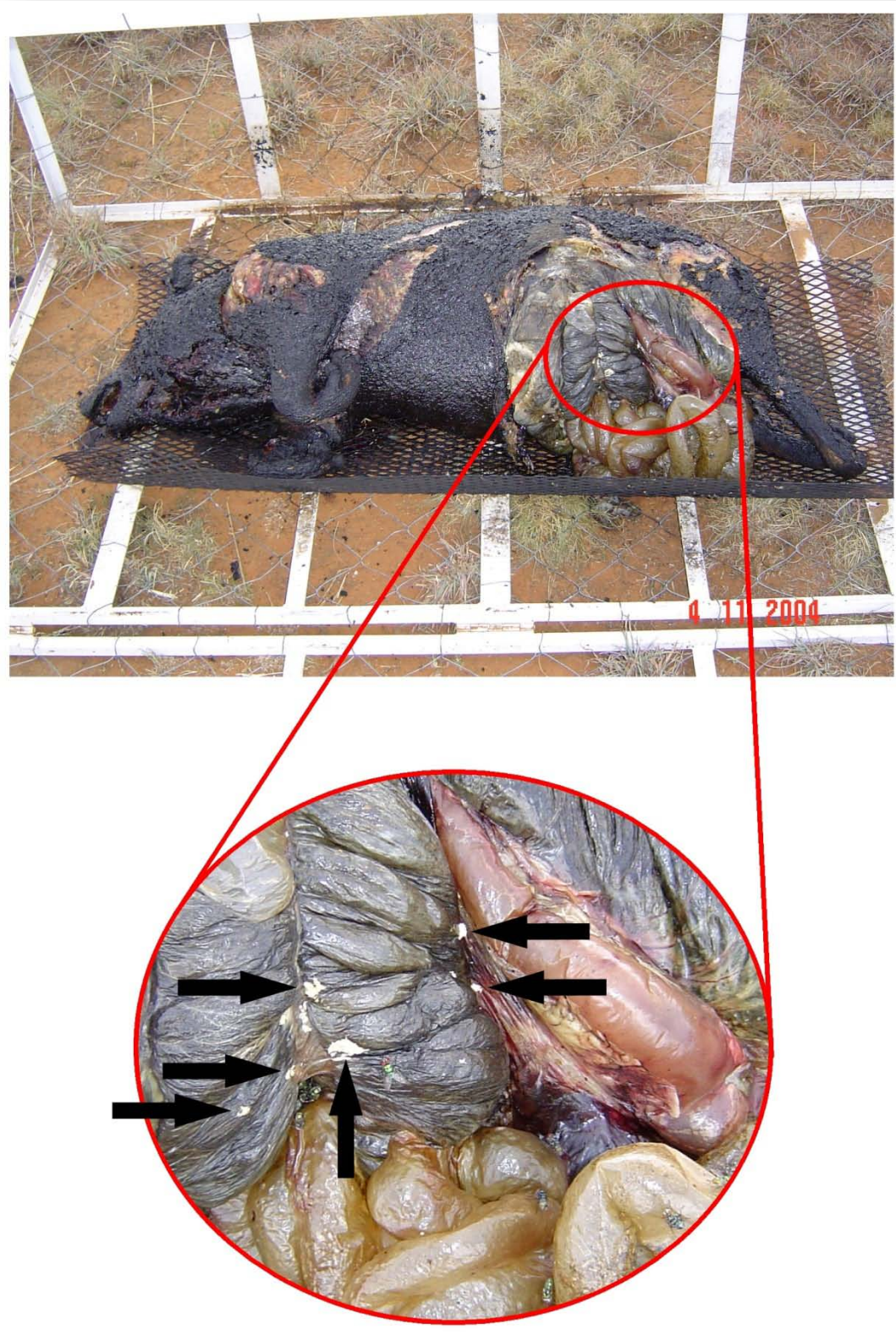
Figure 3.99 *The MB carcass on Day 2 during the Spring 2004 Trial (Enlargement shows eggs oviposited on the protruding intestines, eggs indicated by arrows).*

Figure 3.100 *The HB carcass on Day 2 during the Spring 2004 trial.*

Day 7:

- Third instar maggots were found underneath the carcass at the thorax and abdomen of the SB carcass. A number of dead third instar maggots were also observed on the surface of the abdomen, probably due to the high surface temperature of the carcass (See Section 3.5.6).
- A very small number of third instar maggots were found on the surface of the abdomen of the MB carcass.
- At the HB carcass, second and third instar maggots were found in a small area of the abdominal cavity. Third instar maggots were also found underneath the abdomen of the carcass.

The control carcass was flatter in appearance on Day 8.

Postfeeding maggots left the control carcass to pupate on Day 9. This event marked the start of the Advanced Decomposition stage (Figure 3.96).

Day 10:

- A few third instar maggots were found underneath the control and SB carcasses on Day 10.
- Blow flies had emerged from the pupae at the HB carcass.

Blow flies had emerged from the pupae at the control carcass on Day 17. Some of these blow flies were not fully sclerotised yet.

3.5.1.3 Mass Loss

Due to the fact that there was no HB carcass during 2003 (Figure 3.101), the mass of each of the four carcasses used during 2004 was also continually monitored (Figure 3.102).

During 2003, the control carcass decomposed the slowest and the least amount of tissue was removed from it. The burnt carcasses decomposed at a similar rate, except during the latter part of the study when the SB carcass decomposed the fastest. Roughly the same percentage of tissue of the burnt carcasses remained at the end of the 2003 trial (Figure 3.101).

The control carcass decomposed the fastest and the majority of tissue was removed from it during 2004. The SB, MB and HB carcasses followed in order of the fastest decomposition to slowest decomposition. Accordingly, at the burnt carcasses, the most tissue was removed from the SB carcass and the least from the HB carcass (Figure 3.102).
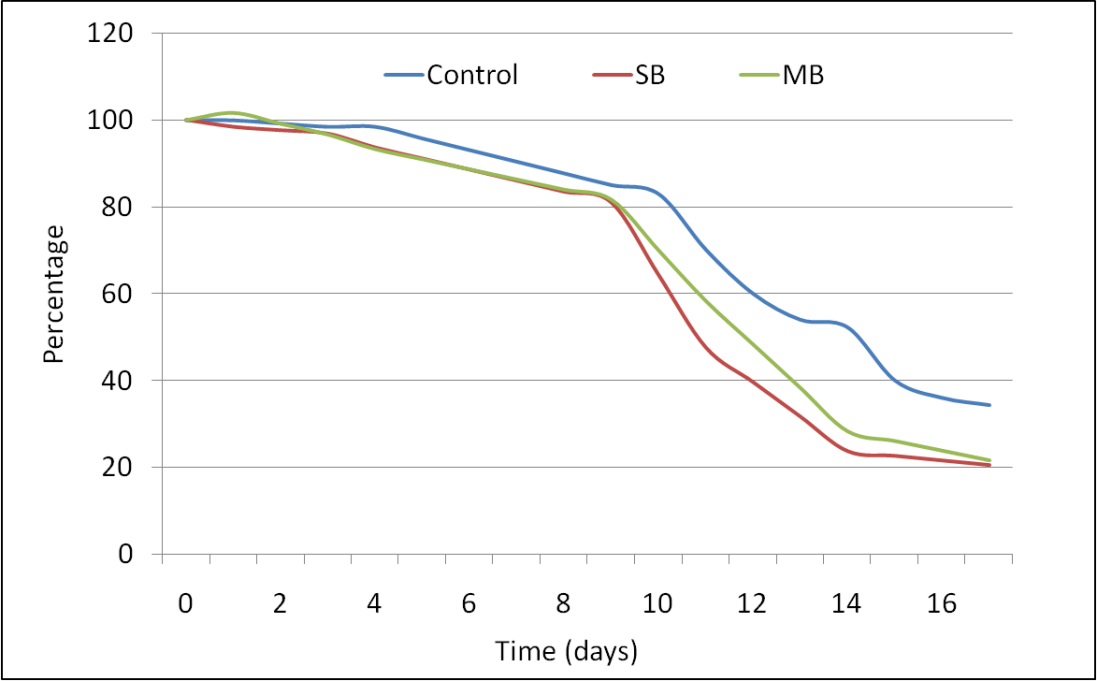
Figure 3.101 *Percentage body mass remaining of carcasses during the Spring 2003 Trial.*
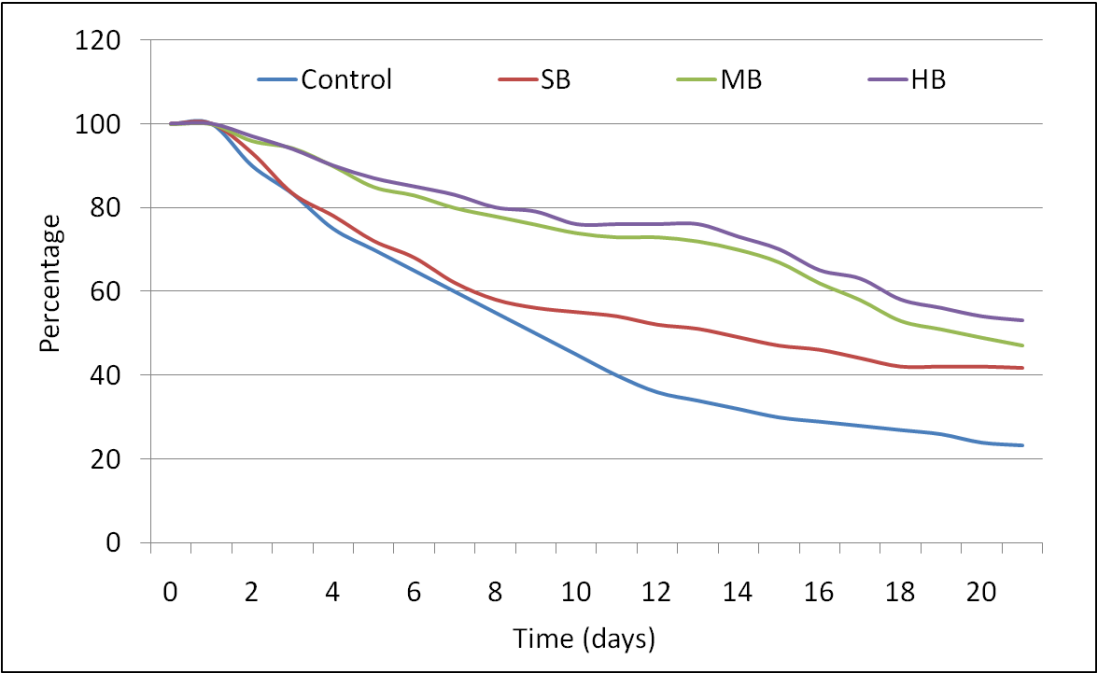
Figure 3.102 *Percentage body mass remaining of carcasses during the Spring 2004 Trial.*

3.5.2. Arthropod Composition

3.5.2.1 Orders

3.5.2.1.1 2003 Spring Trial

Diptera were the dominant order at each of the carcasses, with very low numbers of Coleoptera. At the MB carcass, Hymenoptera were dominant (Figure 3.103).

3.5.2.1.2 2004 Spring Trial

Diptera were only dominant at the control carcass, with Coleoptera dominant at the burnt carcasses (Figure 3.103).

3.5.2.2 Diptera

3.5.2.2.1 2003 Spring Trial

The dominant family was Calliphoridae at each of the carcasses, comprising between 80% and 90% of the total Diptera (Figure 3.104). *Chrysomya chloropyga* was the dominant species at each of the carcasses (Figure 3.105).

3.5.2.2.2 2004 Spring Trial

Calliphoridae was the dominant family at the control and SB carcasses, with the dominance of Calliphoridae being secondary to the dominance of Muscidae at the MB and HB carcasses. As during all the other trials, no Muscidae maggots were observed on the carcasses, therefore Muscidae were not considered to be necrophagous. Calliphoridae was the dominant necrophagous Diptera family at these carcasses. With the exception of the HB carcass, the dominance of Calliphoridae decreased and the dominance of Muscidae increased with an increase in the level of burning of the carcass. Lower numbers of Piophilidae were also observed at all the carcasses (Figure 3.104).
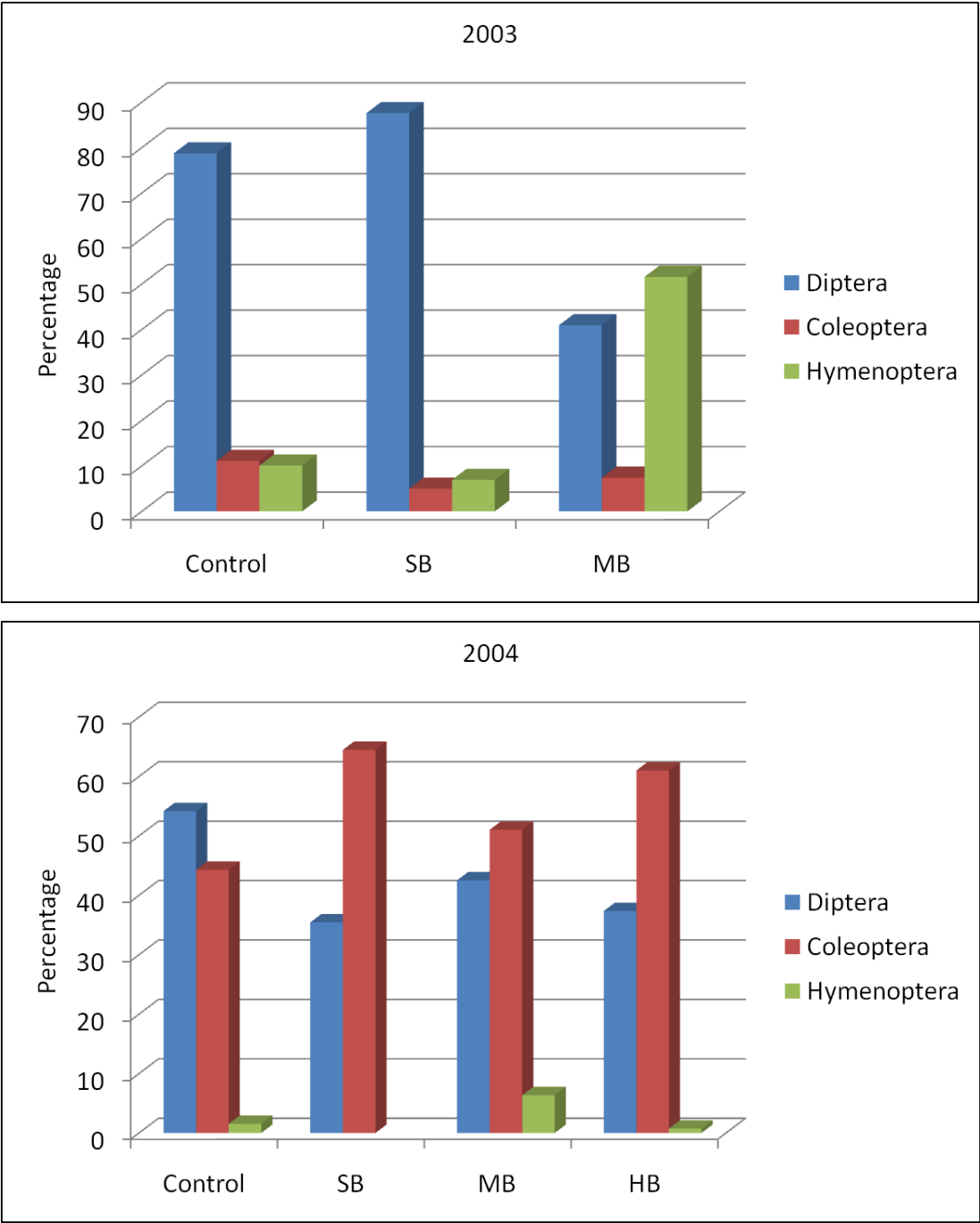
Figure 3.103   *Order Composition during the spring trials.*
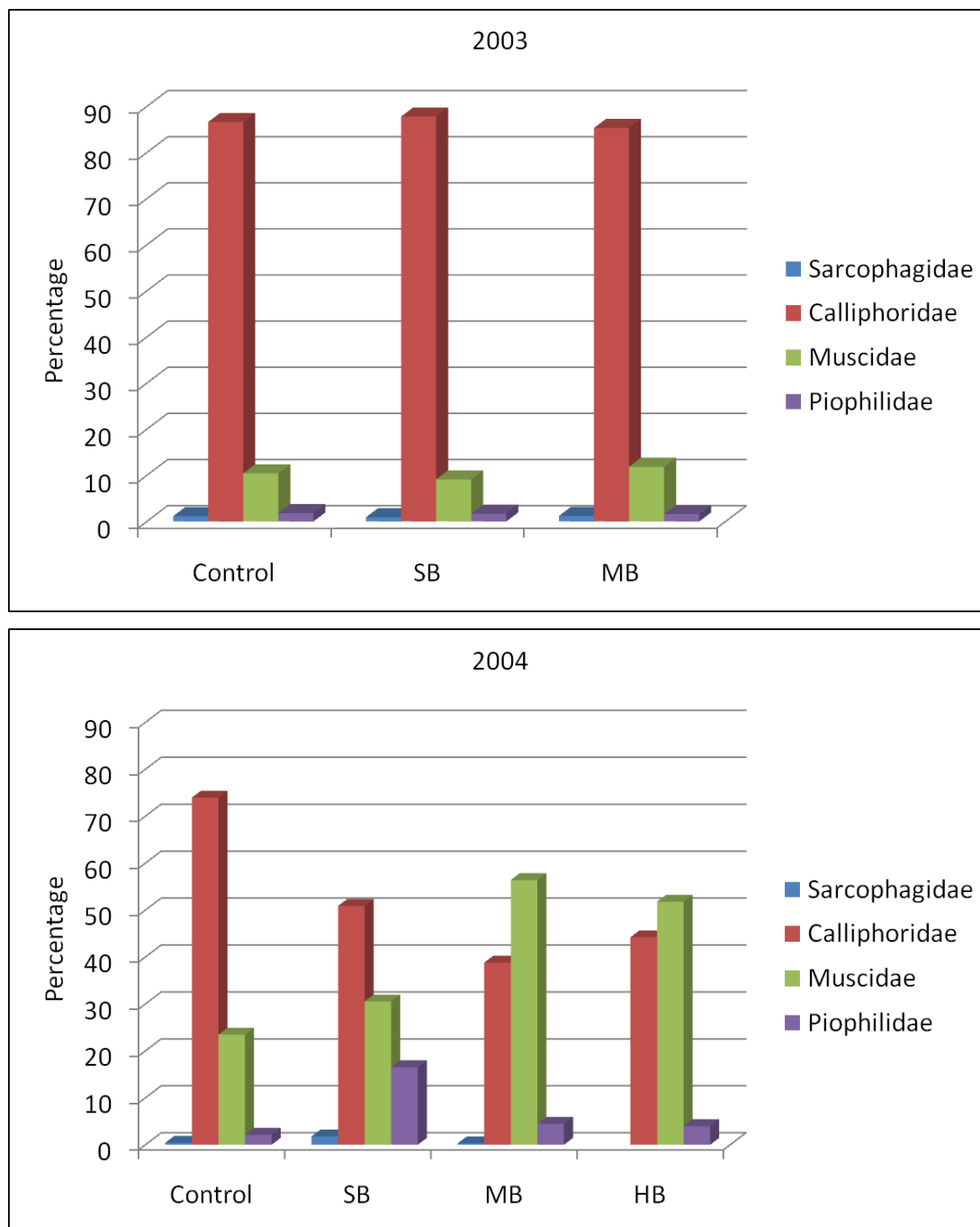
Figure 3.104 *Diptera Composition during the spring trials.*
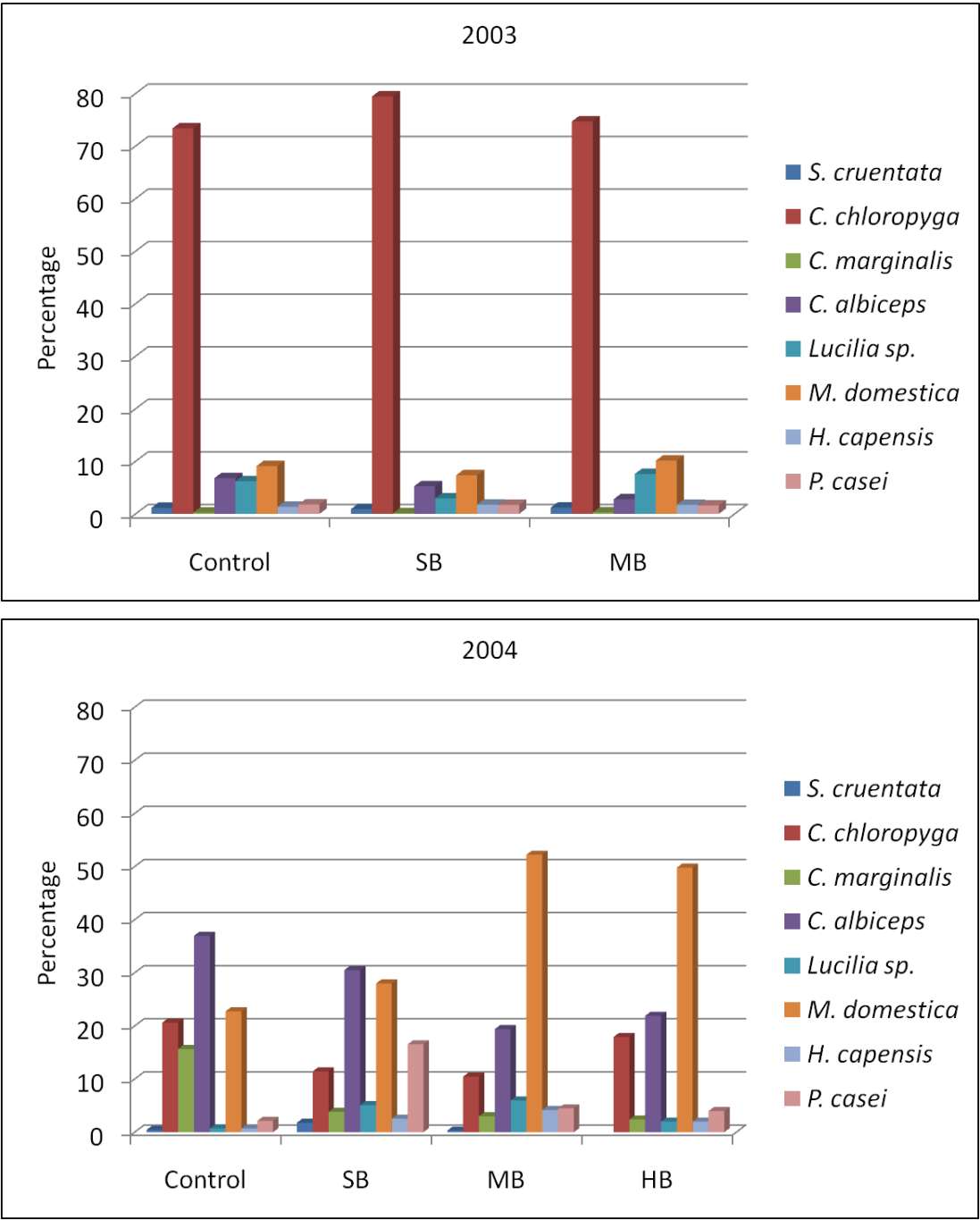
Figure 3.105 *Diptera Species Composition during the spring trials.*

The dominant necrophagous species at each of the carcasses was *C. albiceps*, with lower numbers of *C. chloropyga* observed (Figure 3.105).

3.5.2.3 Coleoptera

3.5.2.3.1 2003 Spring Trial

Dermestidae were only dominant at the control carcass, with Cleridae being dominant only at the SB carcass. Histeridae and Dermestidae were relatively equal in dominance at the MB carcass (Figure 3.106).

3.5.2.3.2 2004 Spring Trial

Except for the MB carcass, Dermestidae were dominant over Cleridae and Histeridae at all of the carcasses. At the MB carcass, Dermestidae and Cleridae were almost equally dominant (Figure 3.106).

3.5.3. Arthropod succession on the carcasses

Chrysomya chloropyga, C*. marginalis, C. albiceps* and *M. domestica* (Figure 3.107) were the most abundant Diptera species observed at the carcasses during spring.

Dermestes maculatus and Necrobia rufipes (Figure 3.107) were the most abundant Coleoptera observed during the spring trials.

As was the case during other seasons, the first insects to arrive at the carcasses were adult Calliphoridae, Sarcophagidae and Muscidae. The pattern of Calliphoridae being initially present at the carcasses in large numbers for two to three consecutive days that was found during the other seasons, also manifested during the spring trials. The only exception was during 2003 where Calliphoridae were initially present in large numbers for seven days.

All fauna referred to are adults, unless otherwise indicated.
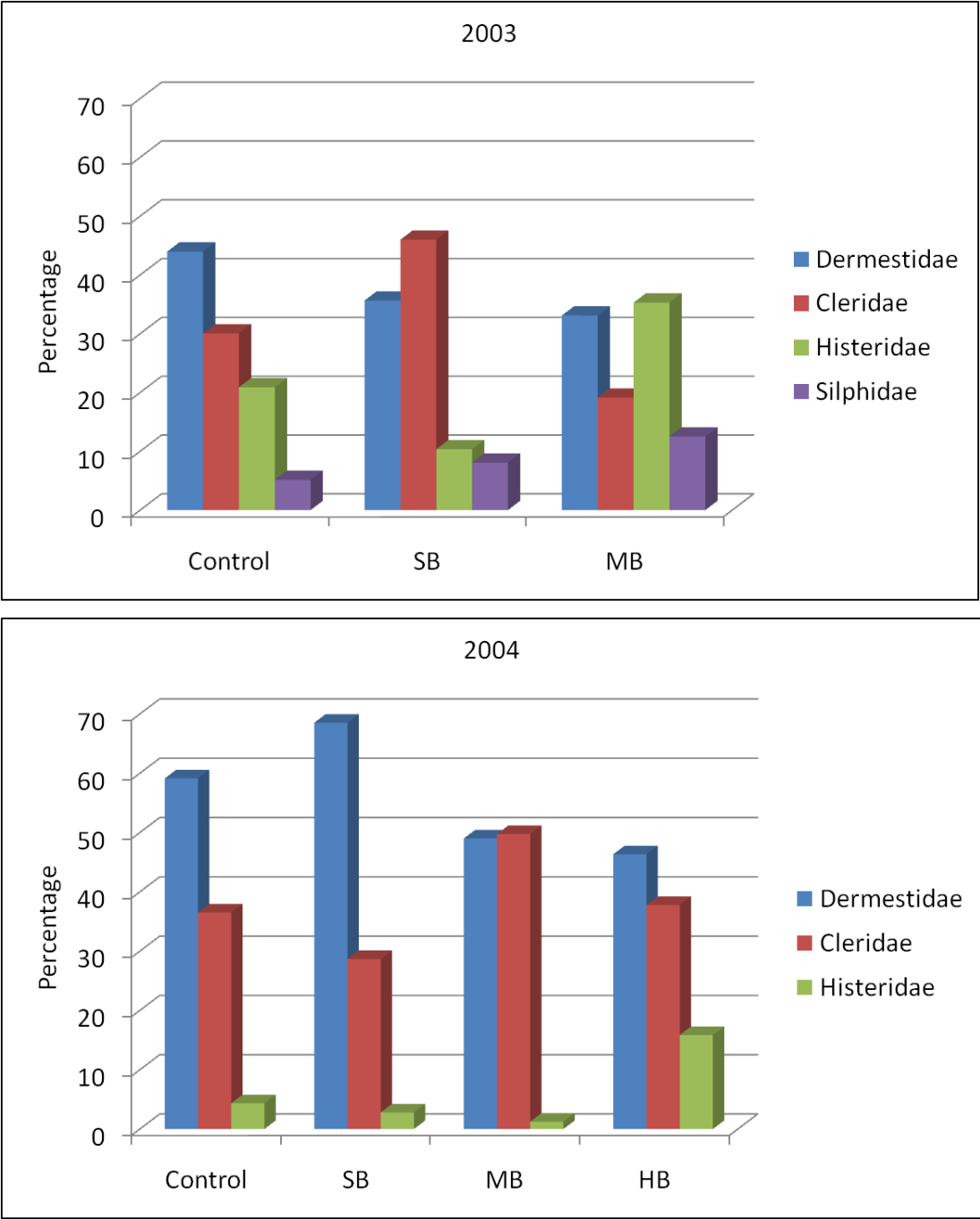
Figure 3.106 *Coleoptera Composition during the spring trials.*
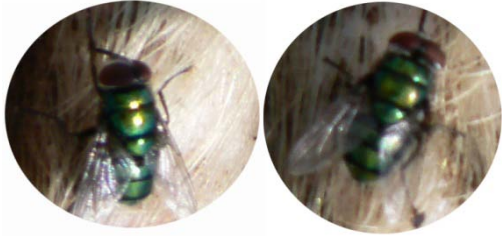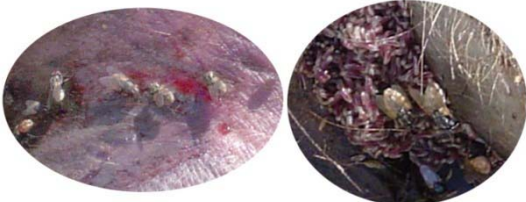
Figure 3.107 *Most abundant species observed during the spring trials.*

Photographs not taken by the author: *i (Gross, 2005)*

                                           ii (Gross, 2005)

                                         iii (London Natural Science Museum, 2004)

                                         iv (Makarov, 2006)

3.5.3.1 Control

Calliphoridae were observed on the carcass in extremely large numbers for the first nine days during 2003 (Figure 3.108). During 2004, Calliphoridae were initially observed at the carcass from Days 1 to 3 (Figure 3.109). The blow flies emerged from the pupae and were found in large numbers on the carcass from Day 16 during 2003 (Figure 3.108). During 2004, the blow flies emerged from the pupae from days 14 to 16. The number of blow flies that emerged from the pupae was less than the blow flies that initially visited the carcass (Figure 3.109).

Muscidae were observed in variable numbers for the whole duration of the study during both trials (Figures 3.108 & 3.109). However, no Muscidae maggots were observed.

Piophilidae were observed during the second half of the 2003 trial and were observed only once on Day 2 during the 2004 trial (Figures 3.108 & 3.109).

Dermestidae and Cleridae were found on the carcass from Day 2 during 2004 and remain on the carcass for the duration of the study, whereas it was only observed from Day 8 onwards during the 2003 trial (Figures 3.108 & 3.109). Dermestidae larvae were only found during 2004 (Figure 3.109).

Large numbers of Formicidae (Figure 3.108) were observed to predate on the Calliphoridae eggs, maggots and newly emerged adults during 2003.
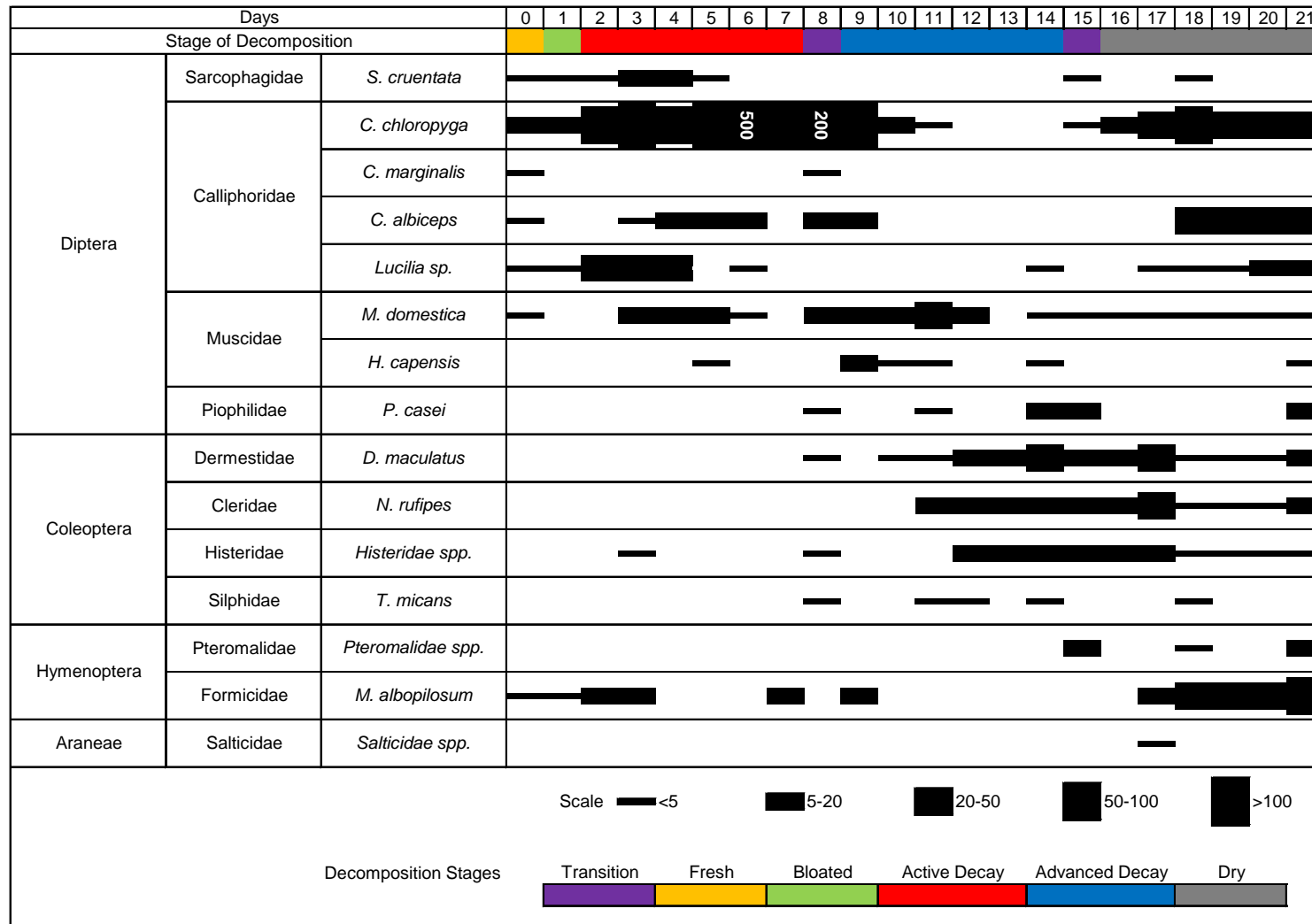
Figure 3.108 *Arthropod Succession on the control carcass during the Spring 2003 Trial.*
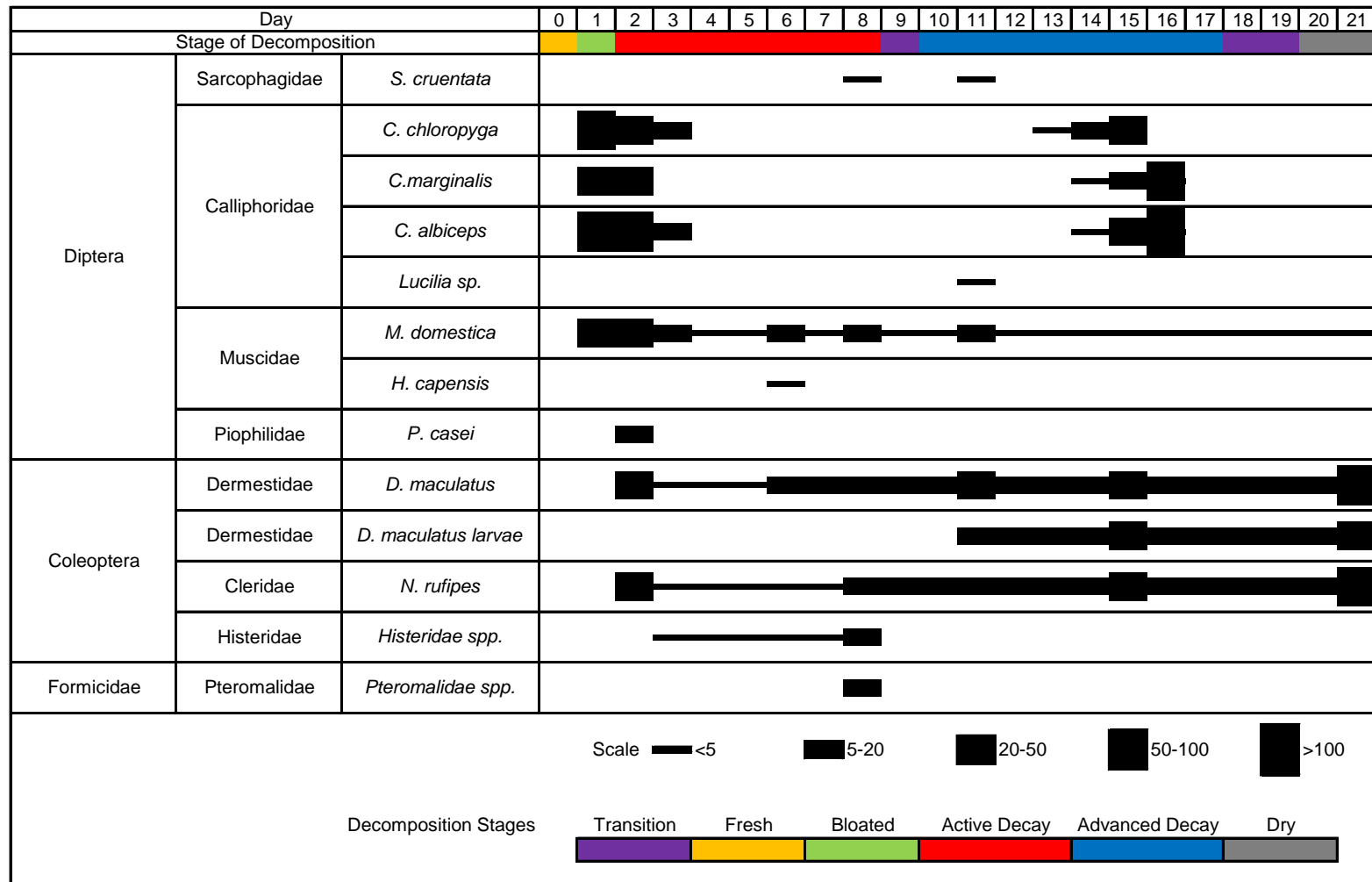
Figure 3.109 *Arthropod Succession on the control carcass during the Spring 2004 Trial.*

3.5.3.2 SB

Chrysomya chloropyga was observed in very large numbers during the first nine days and again from Day 18 during 2003 (Figure 3.110). *Chrysomya albiceps* was observed in lower numbers. Other Diptera were only observed in low numbers. During 2004, the numbers of these two species were approximately similar (Figure 3.111).

Very low numbers of Dermestidae were found on Day 3 and from Day 9 onwards during 2003. Cleridae were present in slightly higher numbers, but were only observed from Day 11 (Figure 3.110). During 2004, Dermestidae and Cleridae were present in larger numbers from Day 2 until the end of the trial. Dermestidae larvae were found on the carcass from Day 14 until the end of the trial (Figure 3.111).

Tourist insects, such as Hemiptera, Blattidae and Acrididae were observed only once during 2003 (Figure 3.110).

A higher diversity of insect species occurred during 2003 than during 2004 (Figures 3.110 & 3.111). Lower numbers of individuals for each species was observed than during 2004.

3.5.3.3 MB

Chrysomya chloropyga was present on the carcass during 2003 from day 0 until Day 11, with the second generation emerging from the pupae on Day 21 (Figure 3.112). However, during 2004 it was only observed twice in low numbers (Figure 3.113). Low numbers of other Calliphoridae were only observed during the first two days during 2004 (Figure 3.113).

Extremely large numbers of Formicidae were observed during 2003 (Figure 3.112). These ants were predatory on the Calliphoridae eggs, maggots and newly merged adults. They also took pieces of burnt skin from the carcass. During 2004, Formicidae were only observed once (Figure 3.113).
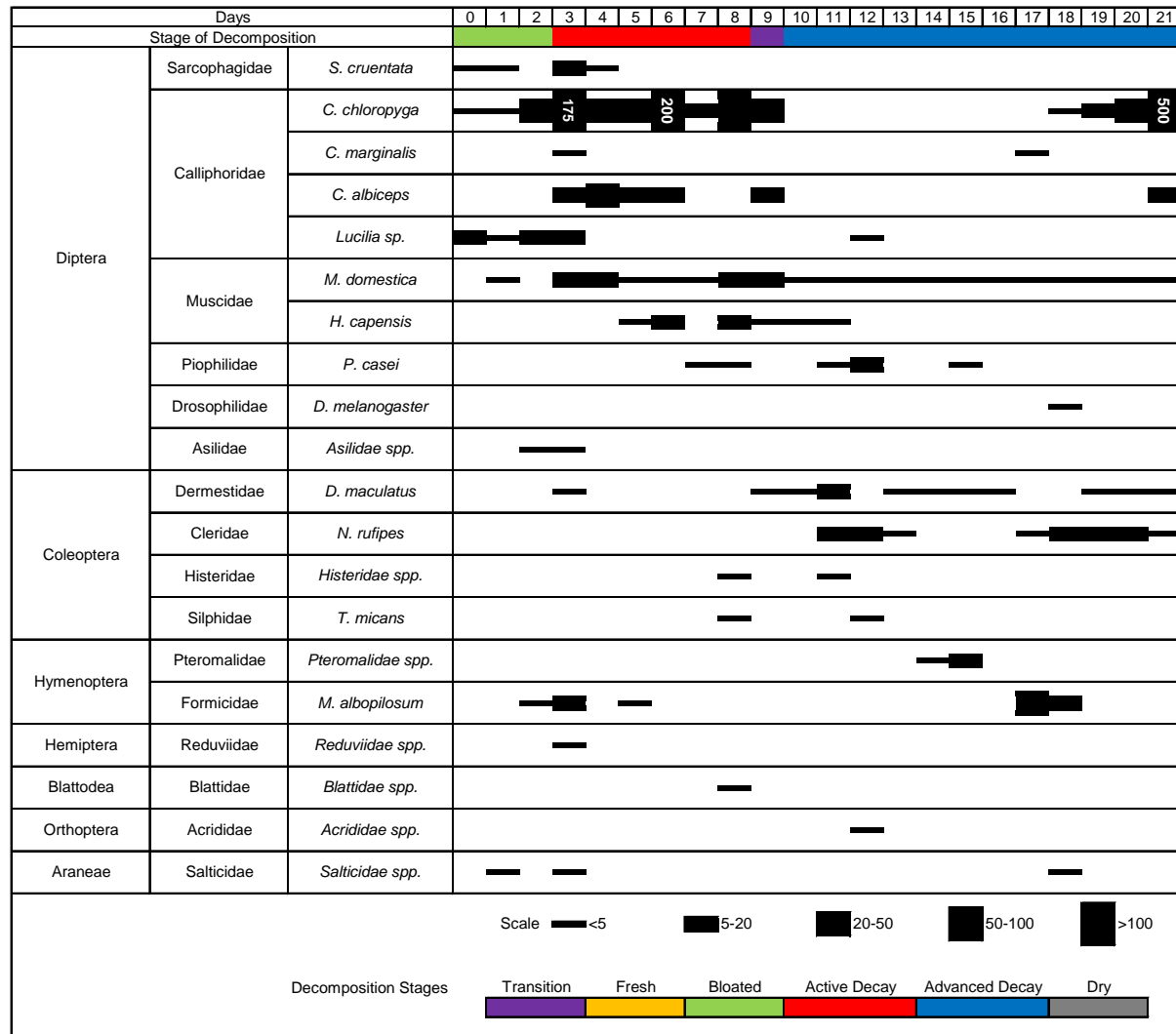
Figure 3.110 *Arthropod Succession on the SB carcass during the Spring 2003 Trial.*
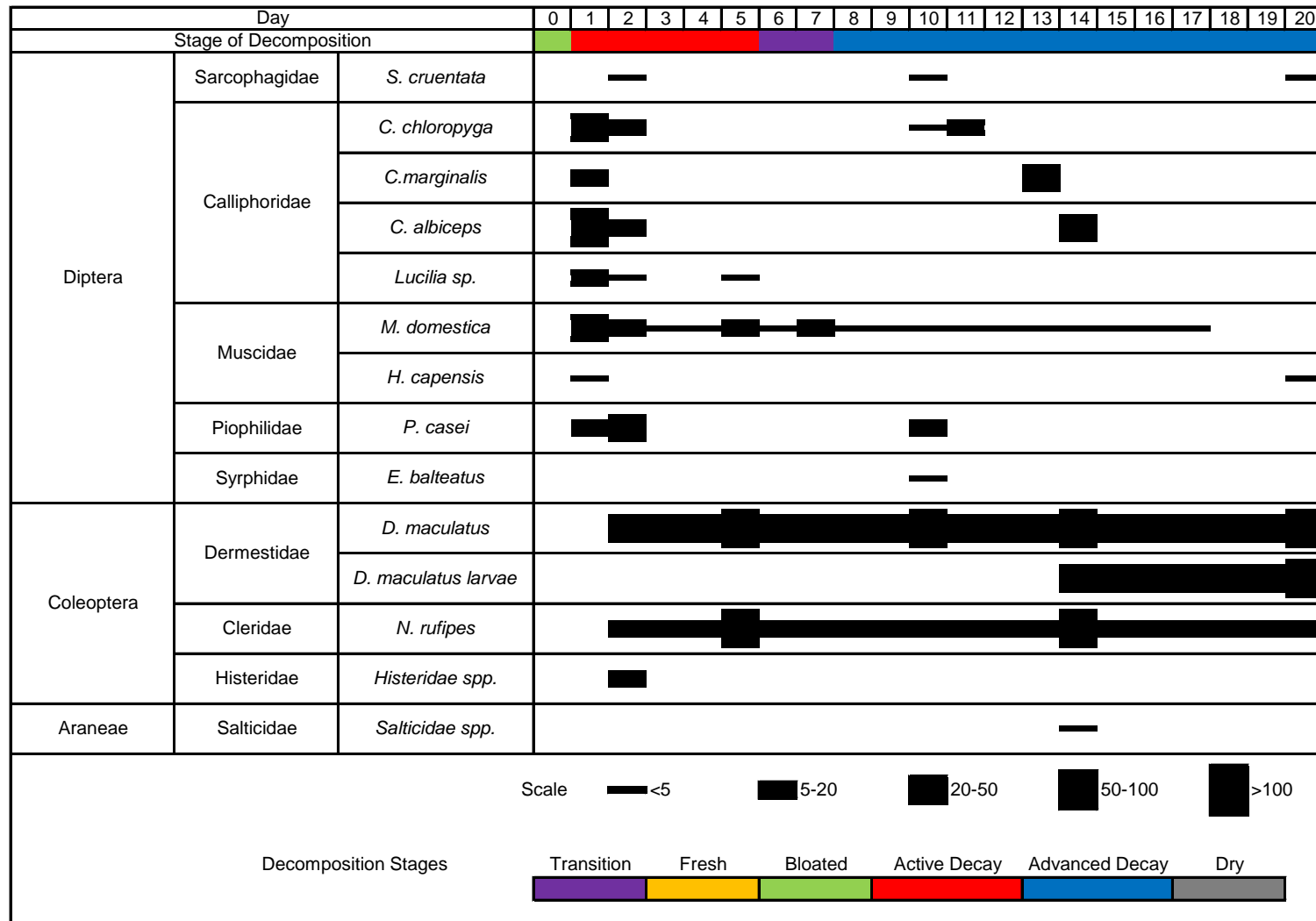
Figure 3.111 *Arthropod Succession on the SB carcass during the Spring 2004 Trial.*
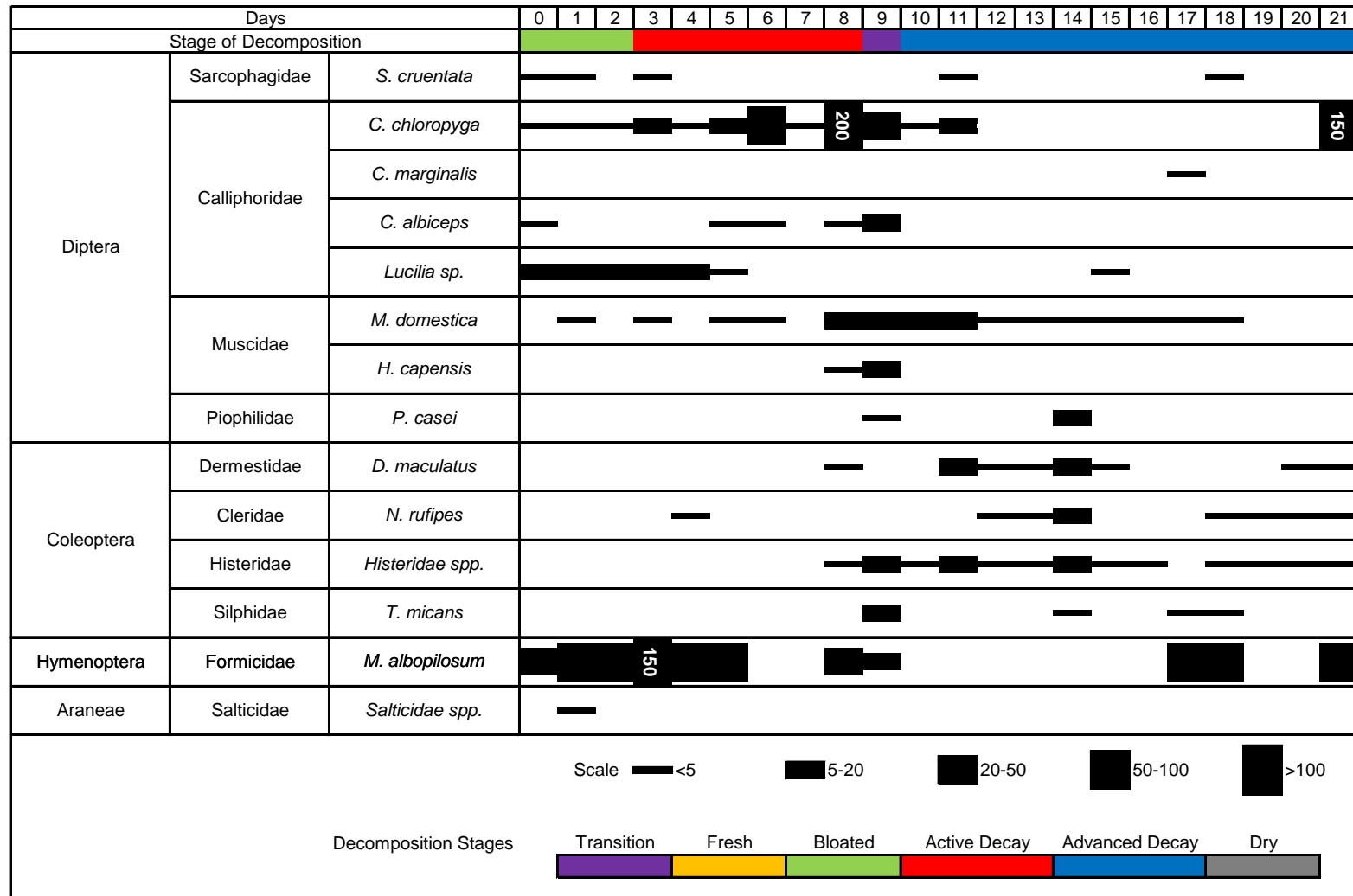
Figure 3.112 *Arthropod Succession on the MB carcass during the Spring 2003 Trial.*
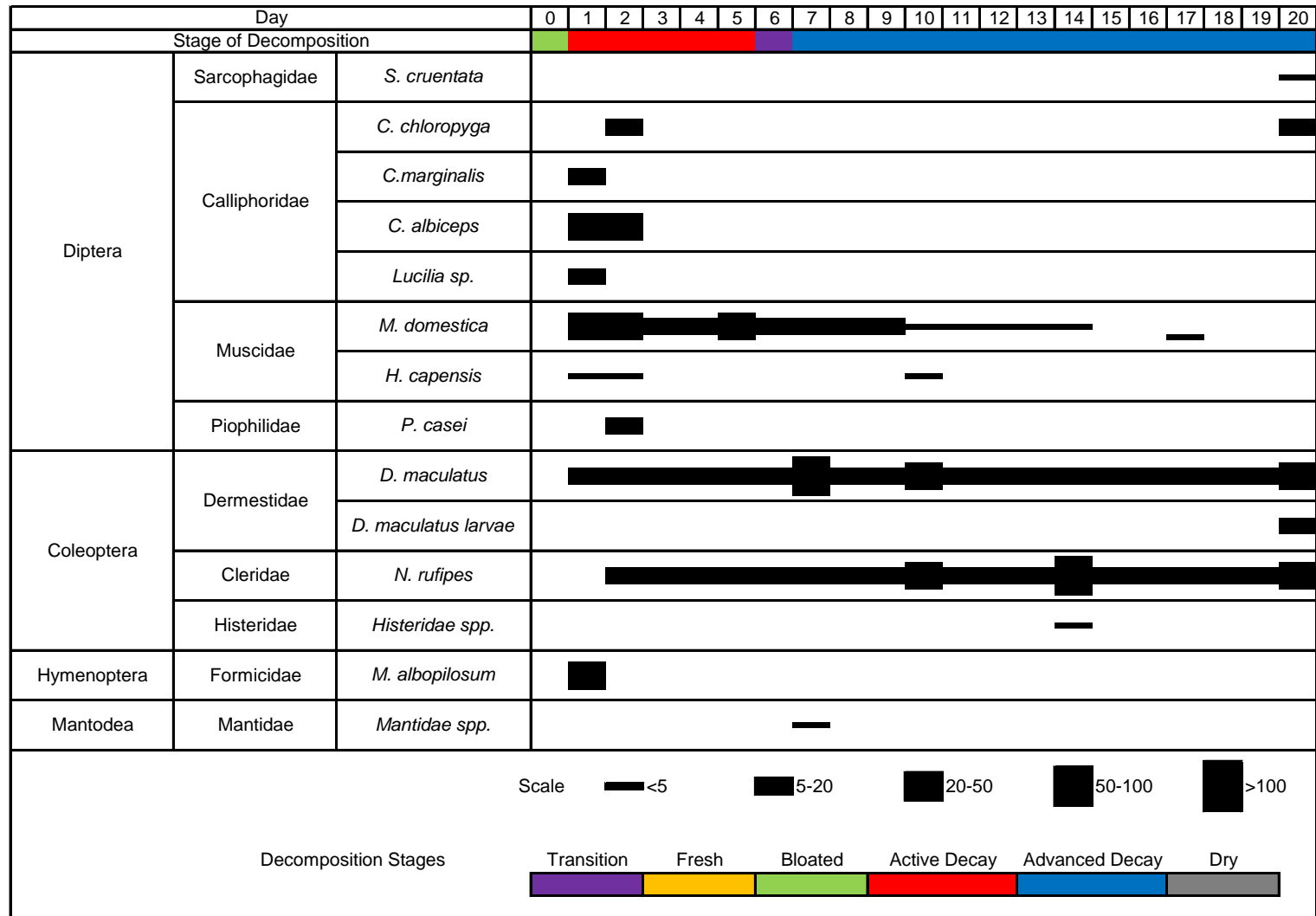
Figure 3.113 *Arthropod Succession on the MB carcass during the Spring 2004 Trial.*

During 2003, Dermestidae were observed on Days 8, 11 to 15, 20 and 21. Cleridae were observed on Days 4, 12 to 14 and 18 to 21 (Figure 3.112). Dermestidae and Cleridae were observed from Days 1 and 2, respectively until Day 20 during 2004 (Figure 3.113).

Histeridae were observed from Day 8 until Day 21 during 2003 (Figure 3.112), but were observed only once during 2004 on Day 14 (Figure 3.113).

Other insects found at the carcass during 2003 were only present in very low numbers (Figure 3.112).

3.5.3.4 HB

During 2003, only three carcasses were used. Therefore, no HB carcass data exists for 2003.

Calliphoridae were observed for two days at the start of the 2004 trial, namely Days 1 and 2. Less than five individuals of adult Calliphoridae were observed at this carcass during the latter part of the 2004 trial. *Musca domestica* was present in reasonable numbers during the entire trial (Figure 3.114).

Cleridae and Histeridae were found at this carcass two days before Dermestidae were observed for the first time on Day 7. Dermestidae larvae were only found on the carcass on the last day of the trial, namely Day 20. Adult Dermestidae and Cleridae were observed until the end of the trial, while Histeridae were only observed until Day 13 (Figure 3.114).
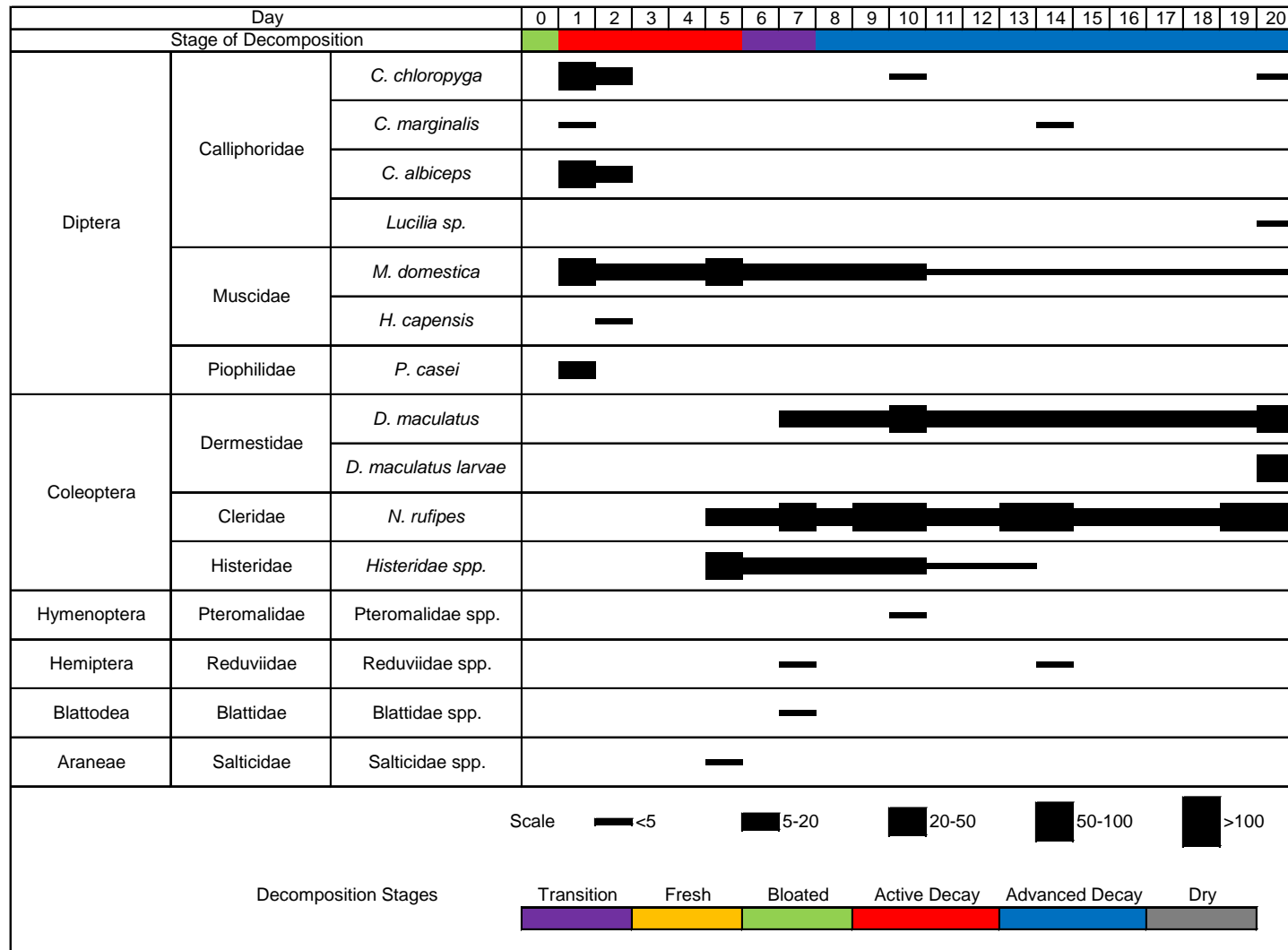
Figure 3.114 *Arthropod Succession on the HB carcass during the Spring 2004 Trial.*

3.5.4. Statistical analysis of arthropod succession

3.5.4.1 Analysis 1: Jaccard Metric

The Jaccard occurrence matrices were created by using data from the succession diagrams. Mean faunal similarity was calculated for each day (See 2.1.4.1.). These faunal similarities were plotted (Figures 3.115 & 3.116).

The characteristic horseshoe-shape as described by Schoenly (1992) is not evident in these graphs. According to Schoenly (1992), similar shapes should be manifested by plots of mean similarities. The ranges should reflect a general property of the dynamic daily changes that occur during carrion-arthropod succession (Schoenly, 1992). This was evident during the 2004 trial (Figure 3.116), but not over successive trials (Figure 3.115).

The comparison of a variety of studies revealed faunal similarity values ranging from 0.218 to 0.808 (Schoenly, 1992).

The faunal similarity values during the spring trials were between 0.1 - 0.8 and 0.29 – 0.75 during 2003 and 2004, respectively. These values were between 0.125 – 1 during the summer, autumn and winter trials. According to Schoenly (1992), the variation could be due to different types of carcasses that are essentially nonhuman. In this study, the variation may be due to the different degrees of charring due to burning.

3.5.4.2 Analysis 2: Correlation coefficient

The matrices were tested using a correlation coefficient. The coefficient was calculated with Graphpad Instat.
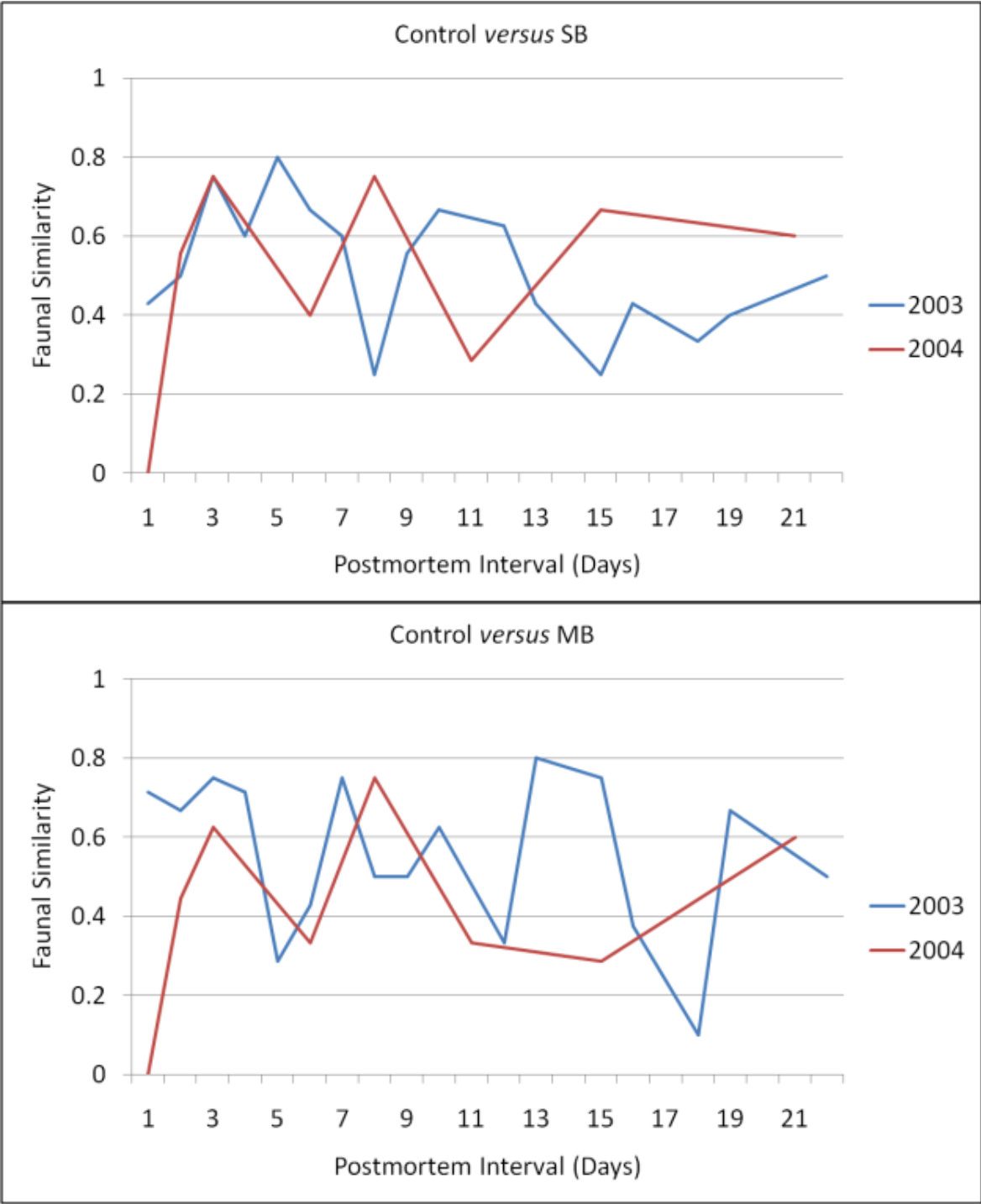
Figure 3.115 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession over the two spring trials.*
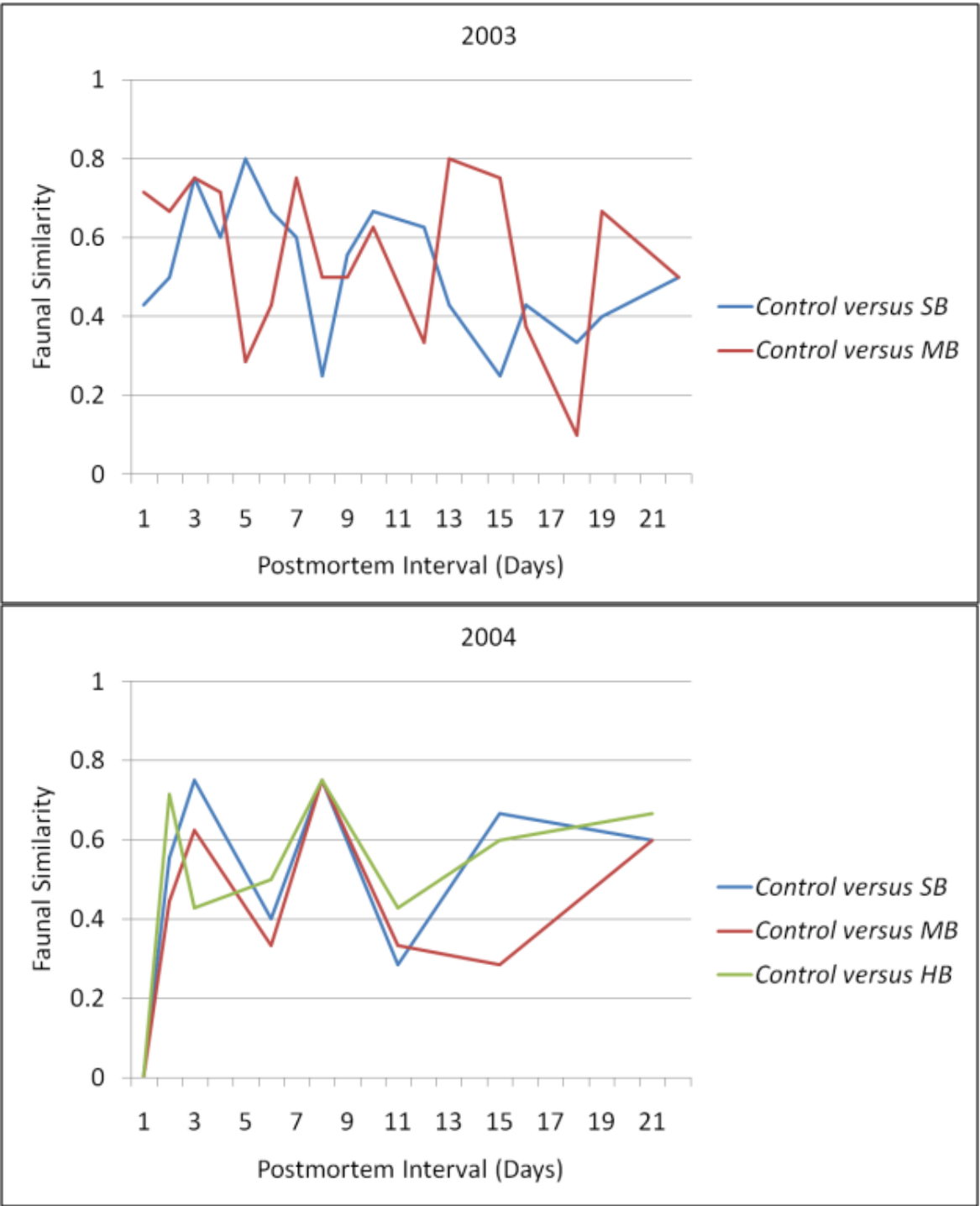
Figure 3.116 *Plots of pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession during each spring trial.*

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for all taxa showed that there were significant differences in the overall arthropod succession between the same treated carcasses (e.g. control 2003 *versus* control 2004) over successive trials (Table 3.27). These significant differences were found for the control and MB carcasses. No significant differences were found for the MB carcasses. No results could be obtained for the HB carcasses since there was no HB carcass during the 2003 trial.

Table 3.27 Similarity matrix analysis of each treatment over successive spring trials

2003/2004	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
Control	0.125	0.03766 to 0.2104	0.0051	significant
SB	0.06545	-0.02422 to 0.1541	0.1522	not significant
MB	0.1832	0.09328 to 0.2701	< 0.0001	significant
HB	n.a	n.a	n.a	n.a

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices for forensic indicator species showed significant differences for *C. chloropyga* and *C.albiceps* for each trial (Tables 3.28 & 3.30). Significant differences for *C. marginalis* were only found during 2003 (Table 3.29). This suggested that the colonisation of *C. chloropyga*, *C. marginalis* and *C.albiceps* was influenced by the level of burning of the carcasses.

Table 3.28 Similarity matrix analysis of *C. chloropyga* at all carcasses during successive spring trials

C. chloropyga	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2003	0.7483	0.5803 to 0.8552	< 0.0001	significant
2004	0.4815	0.2653 to 0.6516	< 0.0001	significant

Table 3.29 Similarity matrix analysis of *C. marginalis* at all carcasses during successive spring trials

C. marginalis	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2003	-0.1066	-0.4205 to 0.2301	0.5361	not significant
2004	0.6378	0.4632 to 0.7647	< 0.0001	significant

Table 3.30 Similarity matrix analysis of *C. albiceps* at all carcasses during successive spring
trials

C. albiceps	Pearson's correlation coefficient	95% confidence interval	Two-tailed P value	Significance
2003	0.6939	0.5000 to 0.8216	< 0.0001	significant
2004	0.7289	0.5871 to 0.8273	< 0.0001	significant

3.5.5 Ambient temperatures and rainfall

As pointed out in Section 2.1.3.3, ambient temperature records and rainfall for the
two nearest meteorological stations (Figures 2.14 & 2.15) were obtained from the
South African National Weather Service in Pretoria.

The Bloemfontein City Centre (City) and Bloemfontein Airport (WO) meteorological
station are situated ± 2km and 12.6km from the study site (Figures 2.14 & 2.15).
Major differences in meteorological data from these two stations are highlighted
below. Only the data from the nearest meteorological station (City) were applied to
this study, since ambient temperatures measured at the study site during times of
observations more closely approximated the temperatures measured at the City
meteorological station than at the WO meteorological station.

3.5.5.1 2003 Spring Trial

The average maximum and minimum City temperature was 27.2°C and 9.8°C,
respectively. The average City temperature was 18.5°C. Average maximum and
minimum WO temperature was 28.2°C and 7.9°C, respectively. The average WO
temperature was 18.1°C.

City rainfall occurred on Days 5, 7, 20 and 24 (Figure 3.117), with a total of 15.8mm.

WO rainfall occurred on Days 5, 7, 20 and 32 (Figure 3. 117), with a total of 15.2mm.
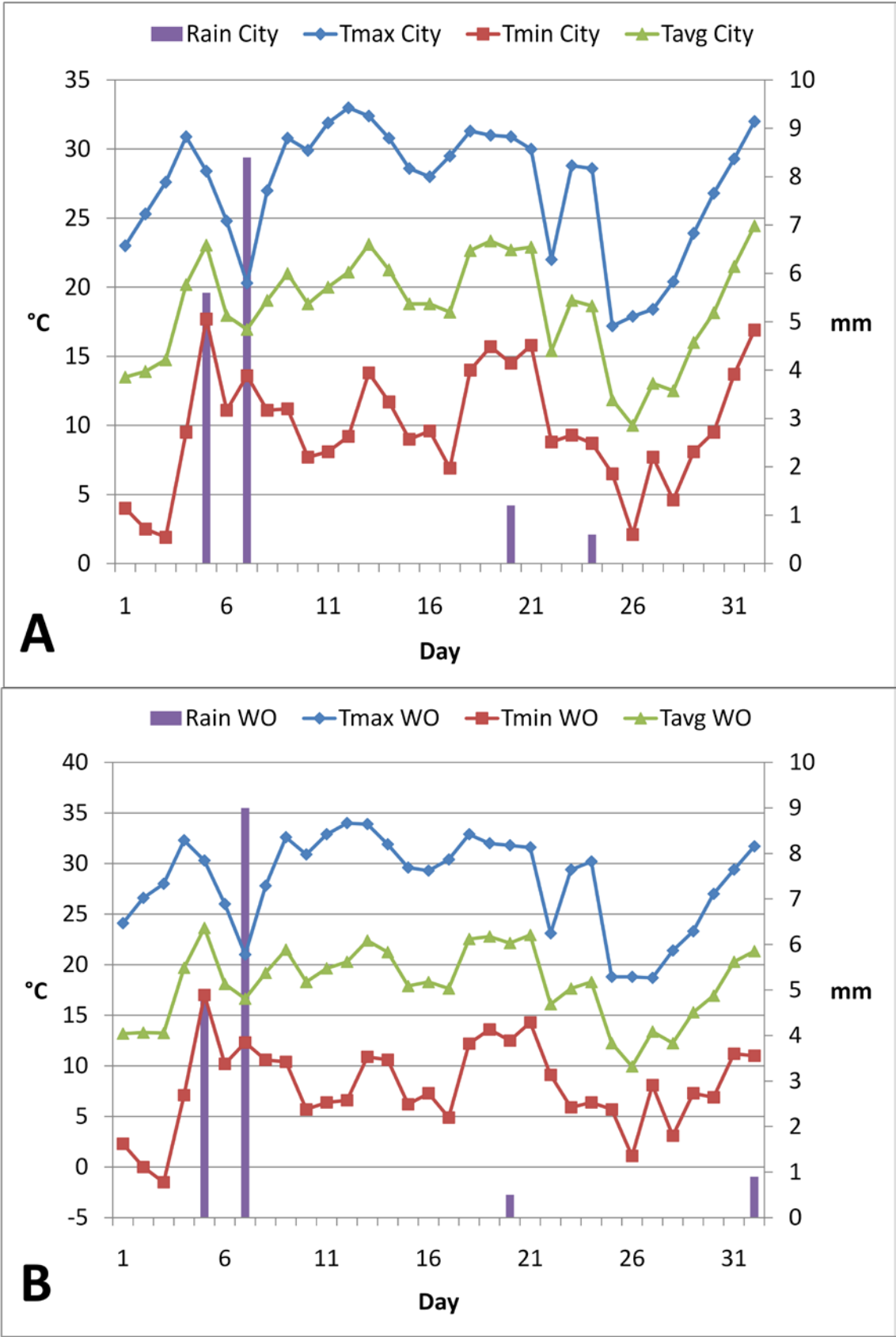
Figure 3.117 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Spring 2003 Trial.*

3.5.5.2 2004 Spring Trial

The average maximum City temperature was 33.5°C and the minimum 18.3°C, while the average City temperature was 25.9°C. Average maximum and minimum WO temperature was 32°C and 15.3°C, respectively. The average WO temperature was 23.7°C.

City rainfall occurred on Days 3, 17, 18 and 19 (Figure 3.118), with a total of 7mm.

WO rainfall occurred on Days 17, 18 and 19 (Figure 3.118), with a total of 13.5mm.

3.5.6. Ambient, external and internal carcass temperatures

The ambient temperature measured during observations followed the trend of the daily average ambient temperature for the nearest meteorological station, i.e. City for the first eight days during 2003 (Figure 3.119). Thereafter the measured temperatures varied considerably. This could be attributed to the extremely large masses of maggots that were feeding on the carcasses. Maggot masses produce their own metabolic generated heat (Mann, *et al.*, 1990). The large maggot masses produced sufficient heat to produce radically different internal carcass temperatures from the ambient. From day 18 onwards, the measured internal carcass temperatures followed the trend of the daily average ambient temperature. This was after all the maggots had left the carcasses to pupate. During 2004 (Figure 3.120), the measured internal carcass temperatures more or less followed the trend of the daily average ambient temperature. The carcass surface temperature significantly exceeded the other measured temperatures (Table 3.31). Unfortunately, during 2003 no carcass surface temperatures were measured. The variation between the internal carcass temperatures was greater during 2004 than during 2003. The largest difference in temperature during 2004 was between the ambient and carcass surface temperature (Figures 3.119 & 3.120 ; Tables 3.31 – 3.33).
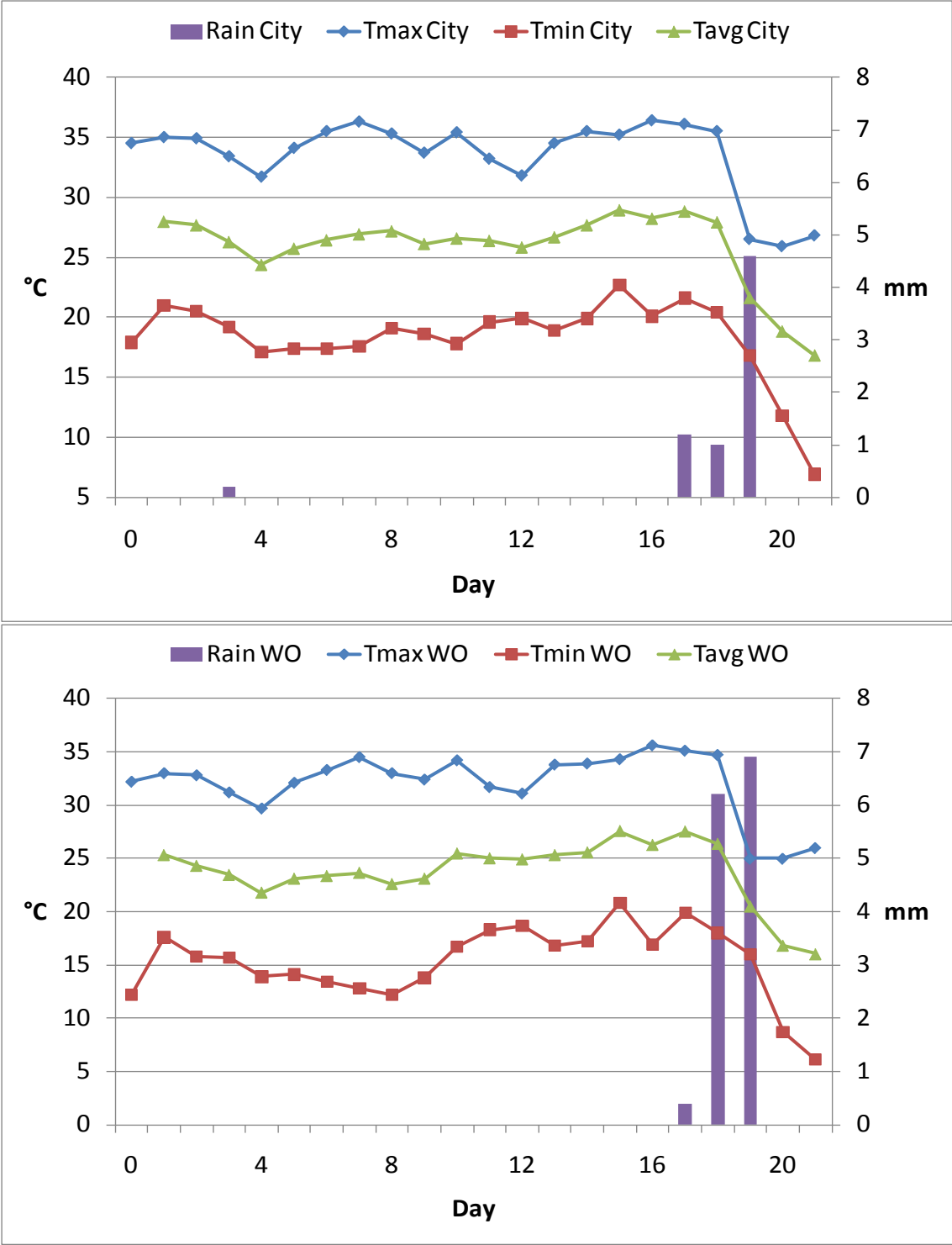
Figure 3.118 *Ambient temperatures and rainfall recorded at the City (A) and WO (B) meteorological stations during the Spring 2005 Trial.*
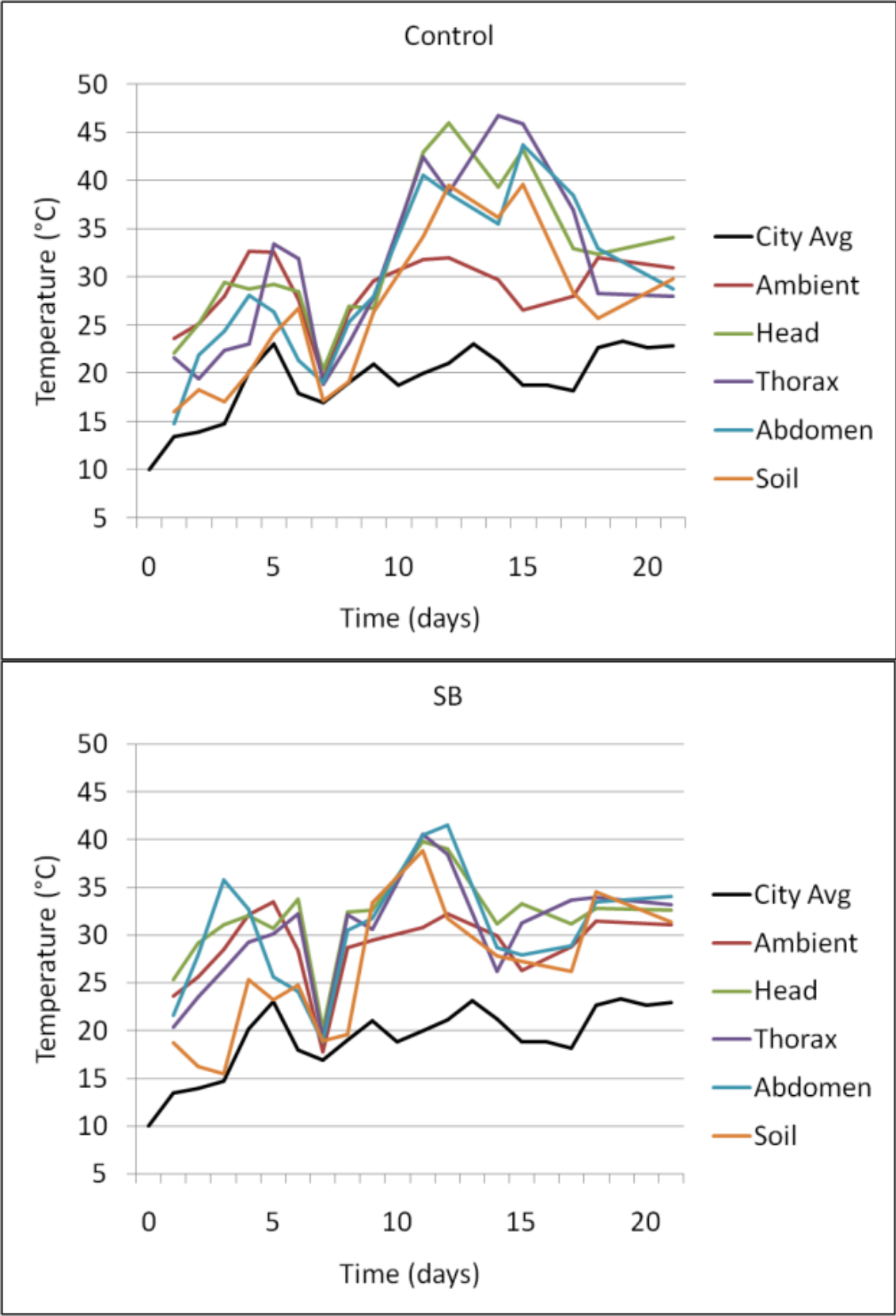
Figure 3.119 *Ambient & internal carcass temperatures during the Spring 2003 Trial.*
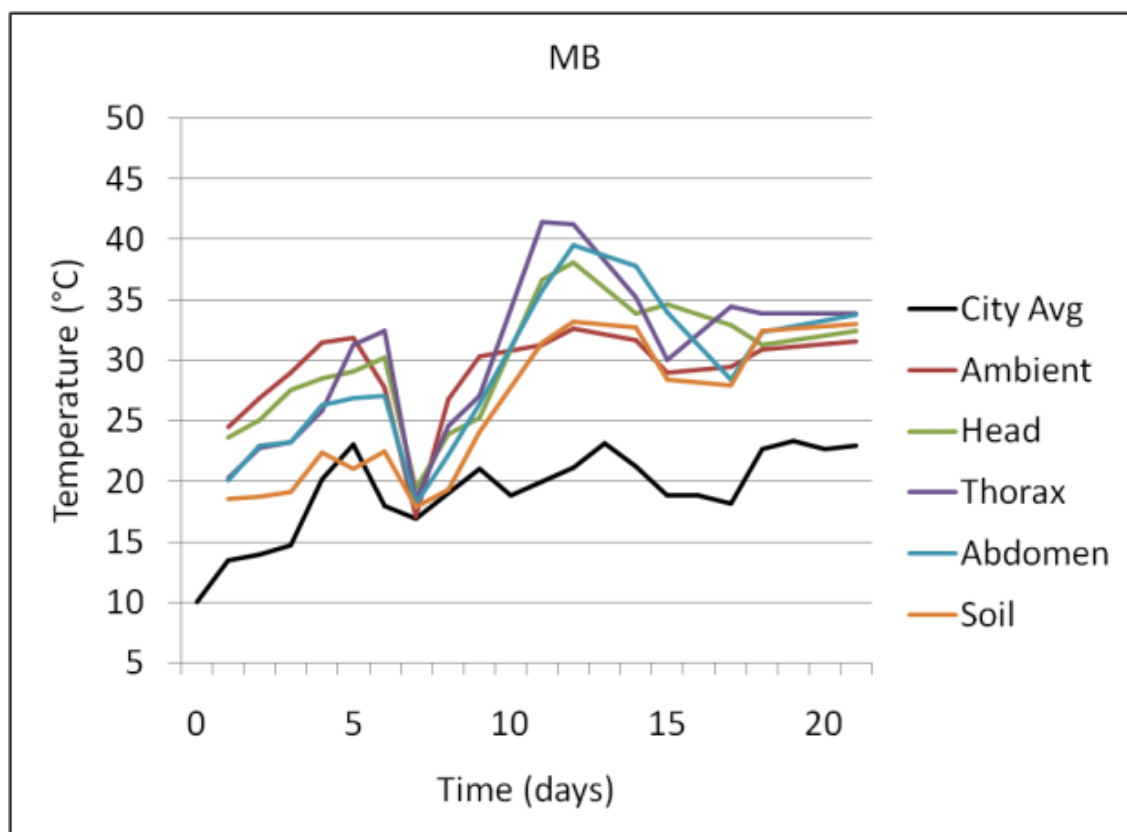
Figure 3.119 (continued) *Ambient & internal carcass temperatures during the Spring 2003 Trial.*

Table 3.31 Maximum difference (°C) in ambient and carcass surface temperature

	Control	SB	MB	HB
2003	n.a	n.a	n.a	n.a
2004	18.4 (Days 7 & 8)	23.7 (Day 7)	22.9 (Day 7)	27.4 (Day 7)

Table 3.32 Mean difference (°C) in ambient and measured temperature during 2003

	Head	Thorax	Abdomen	Soil
Control	3.3	2.1	0.8	-2.4
SB	3.1	1.4	1.6	-2.8
MB	0.6	0.9	-0.5	-3.7

Table 3.33 Mean difference (°C) in ambient and measured temperature during 2004

	Head	Thorax	Abdomen	Surface	Soil
Control	0.6	11.5	9.6	12.3	-2.2
SB	0.7	7.5	9.7	11.1	-0.9
MB	1.2	5.4	6	16.7	-3.7
HB	2.6	5.2	3.4	14.8	-0.7
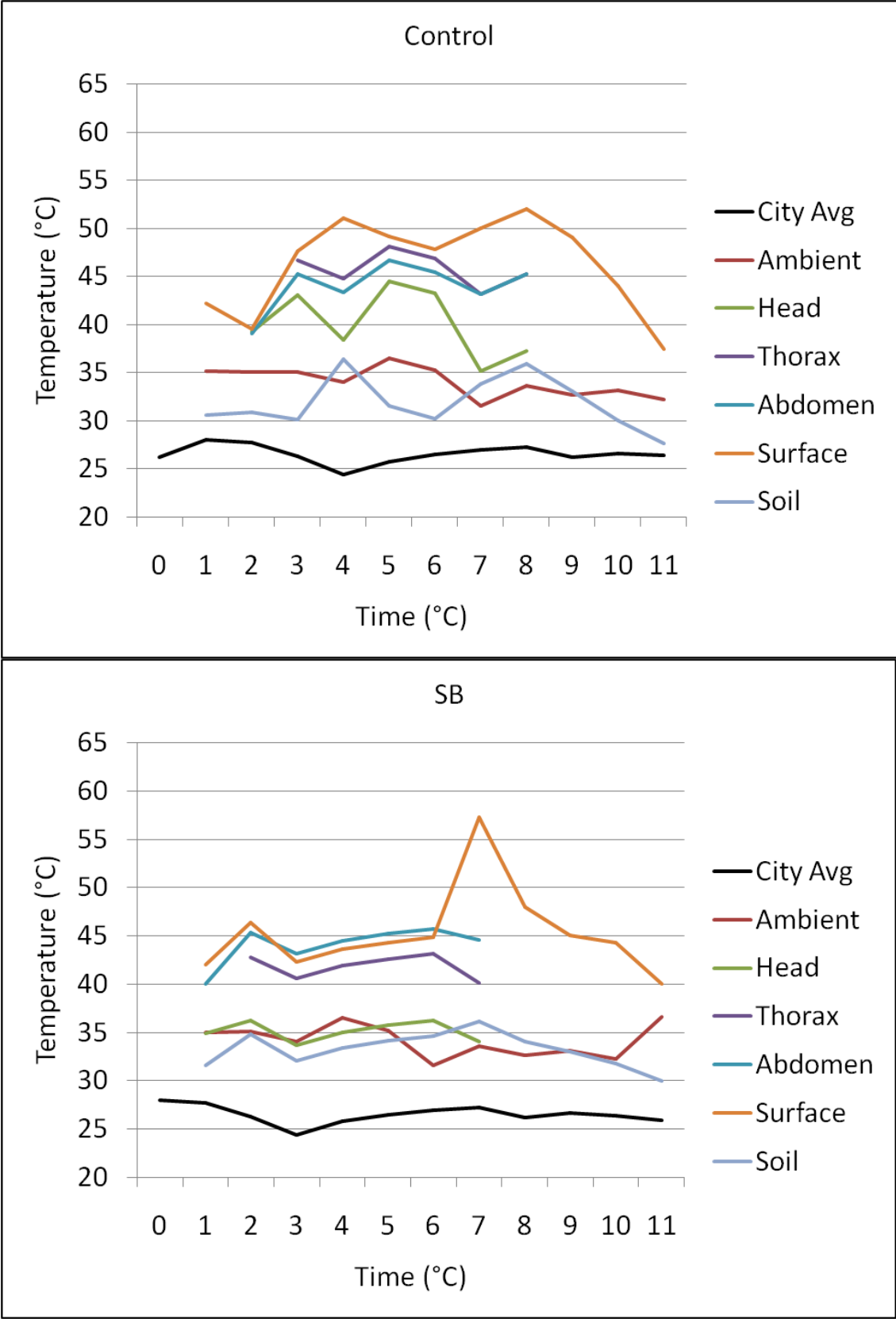
Figure 3.120 *Ambient & internal carcass temperatures during the Spring 2004 Trial.*
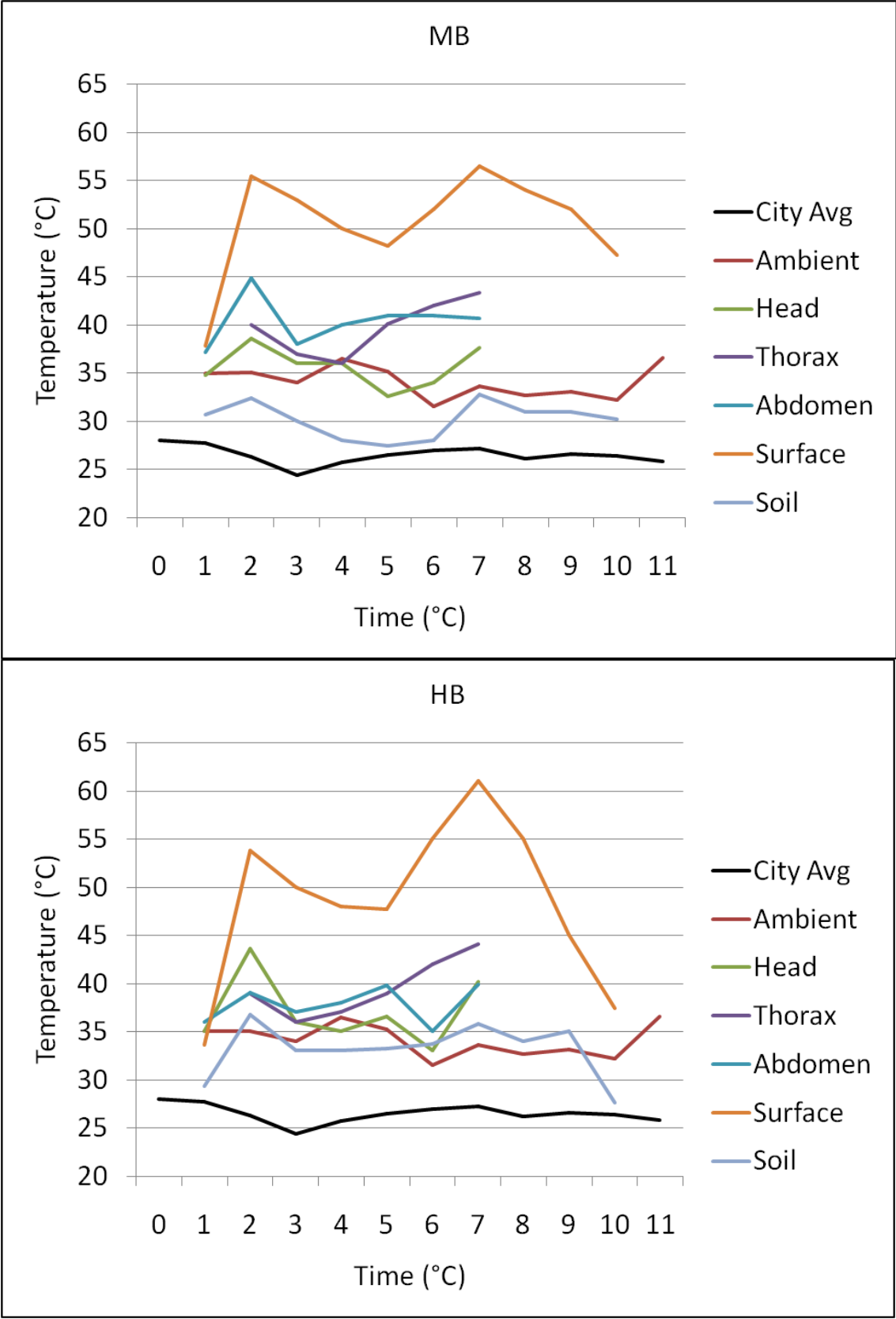
Figure 3.120 (continued) *Ambient & internal carcass temperatures during the Spring 2004 Trial*

Section 3.6. The influence of burning the feeding medium with petrol on the development and morphometrics of *Chrysomya chloropyga* reared on this medium at 35°C.

It was evident from the field trials that burning the carcasses with petrol had a profound effect on the carcass skin, causing the skin to become hardened and to split/rupture. The skin ruptures created new oviposition sites for blow flies, which in some instances cause the burnt carcasses to decompose faster than the control carcasses. However, this did not answer the question on what the influence of burning is on the developmental rate of blow flies feeding on the burnt carcasses.

Even though *Chrysomya marginalis* and *Chrysomya albiceps* were the dominant forensic indicator species during the field trials, *Chrysomya chloropyga* was used in this experiment due to it being one of the most important forensic indicator species in the Central Free State region, often being the only forensic indicator species found during case studies.

For a long time, the rates of development at known temperatures of eggs, maggots and pupae of necrophagous calliphorid flies have formed the basis for producing minimum estimates of PMI in forensic entomological studies (Davies & Ratcliffe, 1994). Fundamentally, insect growth rates are influenced by temperature. Insects' metabolic rates and rate of growth is influenced by the temperature of their immediate surroundings since they are exothermic (Turner, 1991).

The experiment was conducted at 35°C, which is the optimal temperature for the development of *C. chloropyga* (Table 3.34; Kolver, 2003).

3.6.1 Mass Loss of Rearing Media

The mass of the baboon hind legs were determined as mentioned in Section 2.2.1. The mass of each individual hind leg was determined prior to burning (initial mass), after burning and after the experiment had ended (mass after rearing).

Table 3.34 Rearing of *C. chloropyga* at different constant temperatures
(adapted from Kolver, 2003)

Temperature	Development time (days)			Survival (Percentage)	
	one hour old maggots to pupation	pupation to eclosion	one hour old maggots to eclosion	one hour old maggots to pupation	one hour old maggots to eclosion
10°C	33 ± 0.00	37.00 ± 2.65	72.67 ± 4.16	1.33 ± 2.00	1.33 ± 2.00
15°C	28.41 ± 3.71	22.25 ± 2.18	48.17 ± 1.53	16.89 ± 9.96	5.33 ± 9.59
20°C	9.58 ± 1.16	8.03 ± 1.18	17.59 ± 0.82	52.44 ± 12.07	52.00 ± 12.17
25°C	6.11 ± 1.10	5.10 ± 1.27	11.11 ± 0.95	57.77 ± 21.46	55.56 ± 20.24
30°C	5.41 ± 0.64	3.98 ± 0.84	9.43 ± 0.66	62.67 ± 10.58	56.44 ± 10.67
35°C	**5.80 ± 0.81**	**3.77 ± 0.89**	**9.55 ± 0.86**	**67.11 ± 5.93**	**58.22 ± 9.40**
40°C	6.55 ± 0.91	not applicable	not applicable	41.78 ± 10.60	not applicable

The percentage mass remaining was calculated after each time the mass was determined (Figure 3.121). The control revealed a mass loss of 53.2% due to the feeding activity of the maggots. Burning initially caused an average mass loss of 6.5 %, with the feeding activity of the maggots causing a further average mass loss of 42%. The average total mass loss due to burning and feeding was 51.7 per cent. The highest percentage of mass loss due to burning and feeding was 57.9% (Figure 3.121).
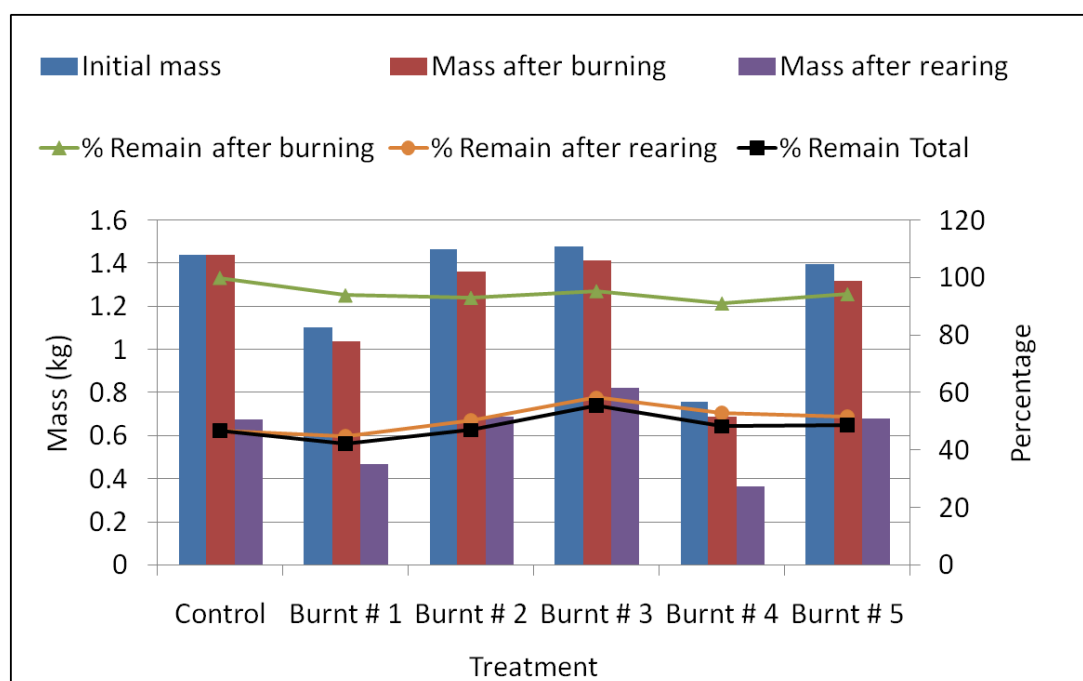
Figure 3.121 *Mass and percentage of rearing media remaining after burning and rearing*

3.6.2 Development time from one hour old maggots until pupation at 35°C

The development time at the control from one-hour-old maggots until pupation was 9.04 ± 1.01 days, with 73 % survival. The mean development time at the burnt media was 8.07 ± 0.72 days, with 59.6 % survival (Table 3.35 A). This was well within the larval development range of 6.75 – 9.58 days for *C. chloropyga* (Prins, 1982). The maggots feeding on the burnt media developed a day faster than the maggots feeding on the control, but 13% less reached the pupal stage. Due to 100 maggots used per treatment, "n" denotes percentage survival (Table 3.35A).

Significant differences occurred between the control treatment (#1) and burnt treatments # 2, #3 and #4, with $P < 0.001$. A significant difference was also found between the control treatment (#1) and burnt treatment # 6, with $P < 0.05$. No significant difference was found between the control treatment (#1) and burnt treatment # 5, with $P > 0.05$ (Table 3.35 B).

3.6.3 Development time from pupation until eclosion at 35°C

No significant difference was found between the treatments for the development time from pupation until eclosion (Table 3.36). Due to 100 maggots used per treatment, "n" denotes percentage survival (Table 3.36).

The P value was 0.0980, considered not significant. Variation among column medians was not significantly greater than expected by chance.

3.6.3 Total development time until eclosion at 35°C

The total development time from one-hour-old maggots until eclosion at the control was 12.00 ± 1.16 days, with 13 % survival until eclosion. The average development time at the burnt media was 11.91 ± 1.28 days, with 23.6 % survival until eclosion (mean survival of burnt treatments). Due to 100 maggots used per treatment, "n" denotes percentage survival (Table 3.37 A).

Table 3.35 A Mean development time in days of *Chrysomya chloropyga* from one hour old maggots until pupation at 35°C

Treatment	n	Mean ± Std. Dev
1 - Control	73	9.04 ± 1.01
2 - Burnt	64	7.34 ± 0.59
3 - Burnt	64	7.19 ± 0.71
4 - Burnt	98	7.612 ± 0.741
5- Burnt	17	10.00 ± 1.06
6 - Burnt	55	8.20 ± 0.52
ANOVA: Kruskal-Wallis test (p<0.0001)		

Table 3.35 B Comparison of mean development time in days of *Chrysomya chloropyga* from one hour old maggots until pupation at 35° C (shows were the significant differences were, if any)

Comparison of treatments	Mean Rank Difference	Significance	P value
1- Control vs. 2 - Burnt	160.44	significant	P<0.001
1- Control vs. 3 - Burnt	175.52	significant	P<0.001
1- Control vs. 4 - Burnt	132.37	significant	P<0.001
1- Control vs. 5- Burnt	-45.633	not significant	P>0.05
1- Control vs. 6 - Burnt	58.586	significant	P<0.05
2 - Burnt vs. 3 - Burnt	15.086	not significant	P>0.05
2 - Burnt vs. 4 - Burnt	-28.07	not significant	P>0.05
2 - Burnt vs. 5- Burnt	-206.07	significant	P<0.001
2 - Burnt vs. 6 - Burnt	-101.85	significant	P<0.001
3 - Burnt vs. 4 - Burnt	-43.156	not significant	P>0.05
3 - Burnt vs. 5- Burnt	-221.15	significant	P<0.001
3 - Burnt vs. 6 - Burnt	-116.94	significant	P<0.001
4 - Burnt vs. 5- Burnt	-178	significant	P<0.001
4 - Burnt vs. 6 - Burnt	-73.779	significant	P<0.001
5- Burnt vs. 6 - Burnt	104.22	significant	P<0.01
Post-Test: Dunn's Multiple Comparisons Test			

Table 3.36 Mean development time in days of *Chrysomya chloropyga* from pupation until eclosion at 35°C

Treatment	n	Mean ± Std. Dev
1 - Control	13	4.38 ± 0.65
2 - Burnt	42	4.36 ± 0.85
3 - Burnt	38	3.97 ± 0.82
4 - Burnt	23	4.26 ± 0.45
5 - Burnt	3	5.67 ± 2.31
6 - Burnt	9	5.00 ± 1.23
ANOVA: Kruskal-Wallis test		
Post-Test: Dunn's Multiple Comparisons Test		

Table 3.37 A Mean development time in days of *Chrysomya chloropyga* from one hour old maggots until eclosion at 35°C

Treatment	n	Mean ± Std. Dev
1 - Control	13	12.00 ± 1.16
2 - Burnt	44	11.43 ± 1.28
3 - Burnt	35	10.71 ± 0.75
4 - Burnt	24	10.92 ± 1.10
5 - Burnt	5	14.20 ± 1.48
6 - Burnt	10	12.30 ± 1.77
ANOVA: Kruskal-Wallis test (p<0.0001)		

Table 3.37 B Comparison of mean development time in days of *Chrysomya chloropyga* from one hour old maggots until eclosion at 35°C (shows were the significant differences were, if any)

Comparison of treatments	Mean Rank Difference	Significance	P value
1- Control vs. 2 - Burnt	17.767	not significant	P>0.05
1- Control vs. 3 - Burnt	38.197	significant	P<0.05
1- Control vs. 4 - Burnt	33.8	not significant	P>0.05
1- Control vs. 5 - Burnt	-33.146	not significant	P>0.05
1- Control vs. 6 - Burnt	0.7038	not significant	P>0.05
2 - Burnt vs. 3 - Burnt	20.429	not significant	P>0.05
2 - Burnt vs. 4 - Burnt	16.032	not significant	P>0.05
2 - Burnt vs. 5 - Burnt	-50.914	significant	P<0.05
2 - Burnt vs. 6 - Burnt	-17.064	not significant	P>0.05
3 - Burnt vs. 4 - Burnt	-4.397	not significant	P>0.05
3 - Burnt vs. 5 - Burnt	-71.343	significant	P<0.001
3 - Burnt vs. 6 - Burnt	-37.493	not significant	P>0.05
4 - Burnt vs. 5 - Burnt	-66.946	significant	P<0.01
4 - Burnt vs. 6 - Burnt	-33.096	not significant	P>0.05
5 - Burnt vs. 6 - Burnt	33.85	not significant	P>0.05
Post-Test: Dunn's Multiple Comparisons Test			

No significant difference was found between the total development time on the control and the burnt media, except between the control treatment (#1) and burnt treatment #3, with P < 0.05 (Table 3.37B).

The maggots reared on the burnt media showed a 10.6% higher survival until eclosion. This was most likely due to many of the adult blow flies from the control treatment dying while still stuck halfway in the pupal case. It is unclear why this happened.

During this experiment, cultures of 100 maggots per container were used. It was found that the mortality of maggots was higher in some of the containers than in others. The same phenomenon occurred in another forensic entomology study where the authors did not know whether this was an artifact caused by their experimental procedure (Wells & Kurahashi, 1994).

Small cultures, each containing 15 to 25 larvae afforded maximum rearing success (Lord & Burger, 1983).

Most studies have used maggot densities of less than 100 to determine larval development rates. However, such studies can be of limited forensic application, since the application of data from baseline studies that have used lower maggot densities on maggots collected at a crime scene from high density maggot masses may result in an over estimation of maggot age (Dadour, *et al.*, 2001).

This may lead to an inaccurate estimate of PMI. Dadour *et al.* (2001) suggested that when larval age was used to estimate the PMI, observations on the size and temperature of the larval mass must be recorded at the crime scene.

This does identify a potential difficulty for the forensic entomologist. Dead eggs or pupae could easily escape detection if preserved in fluid or frozen. Obviously, the age of such individuals could not be estimated in the manner used for live eggs or pupae. Such evidence would be particularly misleading in situations where a very limited sample was available (Wells & Kurahashi, 1994).

3.6.4 Morphometrics

During a previous study, the adult dry mass and wing length of *C. chloropyga* reared on chicken liver, decreased with an increase in temperature. The exception was at 15° C.

This phenomenon could be attributed to only 1.3 percent survival found at 10°C and 5.3 percent survival found at 15°C (Kolver, 2003). The morphometric data derived from the 10°C and 15°C treatments would therefore not be statistically significant due to the small sample size. The adult dry mass and wing length at 35° C was 9.98 ± 1.63mg and 8.17 ± 0.32mm, respectively (Table 3.38).

Table 3.38 Morphometrics of *C. chloropyga* reared at different constant temperatures (adapted from Kolver, 2003)

Temperature	Adult dry mass (mg)	Wing length (mm)
10°C	11.22 ± 0.16	8.97 ± 0.12
15°C	7.35 ± 1.62	8.08 ± 0.54
20°C	10.53 ± 1.11	8.67 ± 0.28
25°C	10.45± 1.79	8.36 ± 0.35
30°C	10.67 ± 1.22	8.31 ± 0.29
35°C	**9.98 ± 1.63**	**8.17 ± 0.32**
40°C	not applicable	not applicable

3.6.4.1 Pupal Mass

During the previous study (Kolver, 2003), pupal mass was not recorded. It was therefore decided to determine the pupal mass once the pupal case had fully sclerotised to determine any possible significant difference.

The mean pupal mass at the control treatment and mean pupal mass calculated for all of the burnt treatments were 65.34 ± 8.15mg and 61.57 ± 5.78mg, respectively (Table 3.39 A). Significant differences in pupal mass occurred between the control treatment (#1) and burnt treatments #3, #4, and #5. No signifant differences were found between the control treatment (#1) and burnt treatments # 2 and #6. Thus there was an overall significant difference between the pupal mass of the control and the burnt treatments (Table 3.39 B).

Table 3.39 A Pupal mass (mg) of *C. chloropyga* reared from one hour old maggots at 35°C

Treatment	n	Mean ± Std. Dev
1 - Control	71	65.34 ± 8.15
2 - Burnt	64	63.06 ± 5.79
3 - Burnt	64	60.77 ± 3.85
4 - Burnt	98	68.93 ± 4.66
5 - Burnt	17	51.84 ± 7.87
6 - Burnt	55	63.27 ± 6.75
ANOVA: Kruskal-Wallis test (p<0.0001)		

Table 3.39 B Comparison of pupal mass (mg) of *C. chloropyga* reared from one hour old maggots at 35°C

Comparison	Mean Rank Difference	Significance	P value
1 - Control vs. 2 - Burnt	45.956	not significant	P>0.05
1 - Control vs. 3 - Burnt	90.816	significant	P<0.001
1 - Control vs. 4 - Burnt	-59.024	significant	P<0.01
1 - Control vs. 5 - Burnt	168.66	significant	P<0.001
1 - Control vs. 6 - Burnt	35.06	not significant	P>0.05
2 - Burnt vs. 3 - Burnt	44.859	not significant	P>0.05
2 - Burnt vs. 4 - Burnt	-104.98	significant	P<0.001
2 - Burnt vs. 5 - Burnt	122.7	significant	P<0.001
2 - Burnt vs. 6 - Burnt	-10.896	not significant	P>0.05
3 - Burnt vs. 4 - Burnt	-149.84	significant	P<0.001
3 - Burnt vs. 5 - Burnt	77.844	not significant	P>0.05
3 - Burnt vs. 6 - Burnt	-55.755	not significant	P>0.05
4 - Burnt vs. 5 - Burnt	227.68	significant	P<0.001
4 - Burnt vs. 6 - Burnt	94.085	significant	P<0.001
5 - Burnt vs. 6 - Burnt	-133.6	significant	P<0.001
Post-Test: Dunn's Multiple Comparisons Test			

3.6.4.2 Adult Dry Mass

The mean adult dry mass at the control and burnt treatments were 10.53 ± 1.89mg and 9.44 ± 1.43mg, respectively. No signifant difference was found between the control and burnt treatments (Table 3.40). This could be due to the small sample size of the control treatment (Table 3.40). The mass of only seven of the 13 individuals which survived until adulthood were recorded, since the other adults were too badly damaged to provide an accurate measurement.

Table 3.40 Adult dry mass of *C. chloropyga* reared at 35°C

Treatment	n	Mean ± Std. Dev
Control	7	10.53 ± 1.89
Burnt	48	9.44 ± 1.43
Mann-Whitney test (two-tailed P value is exact, 0.1645, considered not significant)		

3.6.4.3 Wing length

The mean wing length at the control and burnt treatments were 7.48 ± 0.35mm and 7.55 ± 0.24mm, respectively. No signifant difference was found between the control and burnt treatments (Table 3.41). This could be due to the small sample size of the control treatment (Table 3.41). Only four front wings were measured from the 13 individuals which survived until adulthood, since the other wings were too badly damaged to provide an accurate measurement.

Table 3.41 Wing length of *C. chloropyga* reared at 35°C

Treatment	n	Mean ± Std. Dev
Control	4	7.48 ± 0.35
Burnt	25	7.55 ± 0.24
Mann-Whitney test (two-tailed P value is exact, 0.604, considered not significant)		

Chapter 4: Conclusions

The aims of this study were to determine the influence of burning of a body during different seasons on (1) decomposition, (2) insect composition (3) insect succession and (4) the calculation of the PMI.

4.1 Influence on decomposition

The control carcasses went through the normal stages of decomposition, viz. Fresh, Bloated, Active Decay, Advanced Decay and Dry/Remains. The burnt carcasses only reached the Advanced Decay stage in the same timeframe, when the control carcasses had reached the Dry/Remains stage. This was due to large portions of the burnt carcasses not being consumed by blow fly maggots. These portions were either unattractive to the maggots or they could not digest it due to the flesh being cooked during burning.

The control and SB carcasses decomposed at a similar rate during the warmer months (spring & summer). However, during the colder months (autumn & winter), the SB carcass decomposed faster than the control carcass. The slowest decomposition occurred at the MB and HB carcasses.

The SB carcass decomposed the fastest of the burnt carcasses, due to the combined effect of skin ruptures (due to shrinkage of the skin caused by the heat of burning, combined with bloating) and more carcass tissue remaining which could be utilised by the feeding maggots. The surface of these skin ruptures provided enough moisture to attract gravid female blow flies to oviposit in these skin ruptures. These new oviposition sites had the initial effect of a more rapid decomposition on the burnt carcasses than the control carcasses due to fluid loss and maggot feeding activity. The MB and HB carcasses also had skin ruptures, but less carcass tissue remained which could be utilised by feeding maggots. This caused the MB and HB carcasses to decompose slower than the SB carcass, with more carcass tissue remaining at the end of the trial.

4.2 Influence on insect composition

Calliphoridae and Muscidae were the dominant Diptera during all trials. No Muscidae eggs or maggots were found at the carcasses. Only the Calliphoridae were of forensic importance during this study. *Chrysomya marginalis* and *Chrysomya albiceps* were the dominant Calliphoridae species during summer and autumn. During autumn, *Piophila casei* (Piophilidae) also dominated with low numbers of *Chrysomya chloropyga* recorded. *Chrysomya chloropyga* and *P. casei* were dominant during the winter trials, although the winter trial where *C. chloropyga* was dominant was during late winter/early spring. The spring trials were dominated by *C. chloropyga* and *C. albiceps*, with fewer *P. casei* observed.

The maggot mass species were not necessarily represented by the adult Diptera present on the carcasses. Sampling of the maggots revealed the preference of *C. chloropyga* and *C. marginalis* maggots to feed on and inside the carcasses, whilst *C. albiceps* maggots preferred to feed inside and underneath the carcasses. This partitioning of the food resource occurred due to interspecific competition. *Chrysomya albiceps* maggots predated on *C. marginalis* and *C. chloropyga* maggots during times when the carcass tissues were rapidly consumed before the maggots migrated from the carcasses to pupate.

Coleoptera were dominated by Dermestidae (*Dermestes maculatus* adults and larvae) and Cleridae (*Necrobia rufipes*). Silphidae (*Thanatophilus micans* adults) and Histeridae spp. adults were also recorded on the carcasses. *Thanatophilus micans* and Histeridae spp. larvae were only recorded during autumn. Coleoptera dominance increased with the level of burning, most notably the dominance of *D. maculatus*.

4.3 Influence on insect succession

Faunal similarity values were calculated by means of the Jaccard metric which was compiled from the succession diagrammes. Variations found in the faunal similarity values could be ascribed to the different treatments in the study. In effect, the different degrees of burning influenced the insect colonisation and thus also influenced the insect succession. According to the correlation coefficients calculated from the Jaccard metric, burning had no effect on the colonisation of *Chrysomya chloropyga* during summer and autumn. This could be due to *C. chloropyga* being less abundant during summer and autumn. However, burning had an effect on the colonisation of *C. chloropyga* during winter and spring. *Chrysomya chloropyga* is most abundant from late winter until late spring. Burning had an effect on the colonisation of *Chrysomya marginalis* and *Chrysomya albiceps* during all seasons. The exception occurred during the first spring trial, where burning had no influence on the colonisation of *C. marginalis*. This phenomenon could be due to the fact that there was no HB carcass during the first spring trial.

Differences in arthropod succession between the carcasses occurred due to the effect of burning on the time of oviposition. Skin ruptures appeared on the burnt carcasses due to shrinkage caused by burning, a loss of elasticity and bloating. These skin ruptures created new oviposition sites that were not available on the control carcasses, resulting in earlier oviposition.

Oviposition occurred simultaneously at all carcasses (autumn, spring & during heavy prolonged rainfall in summer), at the burnt carcasses one day prior to the control carcass (spring & summer) and at the burnt carcasses three to five days prior to the control carcass (autumn & winter). An exception occurred during a single winter trial when oviposition occurred at the burnt carcasses five days after oviposition at the control carcass, due to bloating not being rapid enough or sufficient to create skin ruptures (fewer new oviposition sites & less olfactory stimulant for attracting blow flies) before oviposition occurred at the control carcass.

4.4 Influence on the calculation of the PMI

The pattern of insect succession was the same at all of the carcasses in the sense that Diptera initially invaded the carcasses, followed by Coleoptera. The time of invasion/oviposition was the key difference between the control and burnt carcasses and this could have a major influence on the PMI estimation.

This would result in the PMI being one to five days shorter, depending on the extent of bloating of the burnt body, the season and ambient temperature. During warmer months the PMI of a CGS level 2 or 3 burnt body and an unburnt body would essentially be the same due to the simultaneous oviposition.

During the warmer seasons oviposition time was shorter, resulting in maggots of similar age at all of the carcasses. During the colder seasons oviposition time was extended, resulting in maggots of different ages and instars on the same carcass and between carcasses. Estimating the PMI correctly during colder seasons may prove to be difficult due to smaller numbers of adults and smaller maggot masses at the carcasses. This results in a lack of competition. PMI estimation can, however, still be accurate due to a larger diversity of species at the carcasses and by using the largest maggots as indicators of PMI.

Laboratory trials revealed that feeding on burnt media caused *C. chloropyga* maggots to reach pupation one day faster than the control. No significant difference was found between the treatments for the development time from pupation until adult eclosion. No significant difference was found between the treatments for the mean total development time for *C. chloropyga*. This implies that if the PMI estimate is based solely on pupae, the PMI would be one day shorter than is normally the case, in addition to the effect of burning on the time of oviposition. This could be due to the feeding medium becoming unsuitable for utilisation by the maggots. The maggots then develop into the next life stage, which is the pupal stage. This was not true for the laboratory experiments due to the fact that the unutilised feeding medium was still moist. This could, however, be a factor when dealing with field experiments and/or case studies.

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Appendices

Jaccard Metric – Computer Program

```
function j = jaccard(a, b)
% Computes Jaccard metric between vectors a and b.
% By Sean van der Merwe, University of the Free State.
j = sum(a & b)/sum(a | b);
end
%% Introduction
% Jaccard Metric Calculations for J.H. Kolver - October 2009
% Program by Sean van der Merwe, University of the Free State
%% Spring 2003
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','1');
d = d0(2:end,:);
t = {t0{2:3:end,2}}';
n = length(t);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jmb(i) = jaccard(d(3*i-2,:),d(3*i-1,:));
    jsb(i) = jaccard(d(3*i-2,:),d(3*i,:));
end
out = cell(n+1,3);
out{1,1} = 'Species'; out{1,2} = 'MB vs Control'; out{1,3} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jmb(i);
    out{i+1,3} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n1Spr2003');
```

```
%% Summer 2004
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','2');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n2Sum2004');
```

```
%% Autumn 2004
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','3')
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n3Aut2004');
```

```matlab
%% Winter 2004
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','4');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n4Win2004');
```

```matlab
%% Spring 2004
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','5');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n5Spr2004');
```

```matlab
%% Summer 2005
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','6');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n6Sum2005');
```

```matlab
%% Autumn 2005
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','7');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n7Aut2005');
```

```matlab
%% Winter 2005
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','8');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n8Win2005');
```

```matlab
%% Summer 2006
clear;
clc;
[d0,t0] = xlsread('Kolver.xlsx','9');
d = d0(2:end,:);
t = {t0{2:4:end,2}}';
n = length(t);
jhb = zeros(n,1);
jmb = zeros(n,1);
jsb = zeros(n,1);
for i = 1:n
    jhb(i) = jaccard(d(4*i-3,:),d(4*i-2,:));
    jmb(i) = jaccard(d(4*i-3,:),d(4*i-1,:));
    jsb(i) = jaccard(d(4*i-3,:),d(4*i,:));
end
out = cell(n+1,4);
out{1,1} = 'Species'; out{1,2} = 'HB vs Control';
out{1,3} = 'MB vs Control'; out{1,4} = 'SB vs Control';
for i = 1:n
    out{i+1,1} = t{i};
    out{i+1,2} = jhb(i);
    out{i+1,3} = jmb(i);
    out{i+1,4} = jsb(i);
end
xlswrite('Jaccard.xlsx',out,'n9Sum2006');
```

Conferences

Parts of this study were presented at the following conferences:

i.   The 15th Entomological Congress of Entomological Society of Southern Africa, held in Grahamstown, 10-12 July 2005.

Kolver, J.H. & Van der Linde, T.C. *The effect of burning on decomposition and arthropod assemblages on burnt bodies.*
(Oral Presentation)

ii.   The XXIII International Congress of Entomology, held in Durban, South Africa, 6-12 July 2008.

The author also acted as Chairman of the Forensic Entomology Session.

Kolver, J.H. & Van der Linde, T.C. *A Forensic Anomaly: Decomposition in the fast lane.*
(Oral Presentation)

Kolver, J.H. & Van der Linde, T.C. *A Forensic Conflict: What happened to Sarcophaga cruentata?*
(Oral Presentation)

Forensic Entomology Case Studies

More than 100 case studies have been conducted during this study and the previous study (Decomposition and Insect Succession in Hanging and Prone Carcasses, with special reference to *Chrysomya chloropyga*).

These case studies were conducted both alone and in association with Prof. T.C. Van der Linde, in conjunction with the S.A. Police Services, the State Pathologists and the Department Of Microbial, Biochemical and Food Biotechnology, University of the Free State.

The purpose of the bulk of these case studies was to correctly determine the postmortem interval in homicide and suicide cases whilst a small part consisted of blow flies and blow fly eggs found in food.

3 Crime scenes were also visited.

Junior students were also trained in forensic techniques during these case studies.

Sadly, only one of these cases was presented in court where the author testified as expert witness for the state in a serial killer case.

Teaching

During the course of this study, the author was involved as demonstrator and lecturer in the following courses at the University of the Free State, Bloemfontein, South Africa.

Undergraduate:

Demonstrator: First Year Zoology practicals (2003)

Demonstrator: Entomology 114 practicals (2006 - 2007)

Lecturer: BLG144 (Entomology Division) (2006 - 2007)

Lecturer: FOV126 (2006 - 2007)
(A short course in Forensic Nursing, Faculty of Health Sciences, UFS)

Lecturer: ENT114 (2007)
(Agricultural Service Module)

Lecturer: ENT314 (2007)
(Advanced Medical, Veterinary and Forensic Entomology)

Postgraduate:

Lecturer: ENT674 (2007)
(Forensic Entomology Honours)

Popular Lecture & Seminars

The following popular lecture and seminars originated from this study and were presented by the author during the course of this study.

Kolver, J.H. & Van der Linde, T.C. The influence of burning on the decomposition and insect succession of pig carcasses, with reference to a proposed study of Diptera larvae as potential alternative toxicological indicators.
(Seminar, 2003)

Kolver, J.H. Maggot Detectives: The history, evolution and application of Forensic Entomology.
(Popular lecture, 2004)

Kolver, J.H. & Van der Linde, T.C. The influence of burning on the decomposition and insect succession of pig carcasses.
(Seminar, 2004)

Kolver, J.H. & Van der Linde, T.C. The effect of burning on decomposition and arthropod assemblages on burnt bodies during autumn.
(Seminar, 2005)

Kolver, J.H. & Van der Linde, T.C. Decomposition in the fast lane.
(Seminar, 2006)

Kolver, J.H. & Van der Linde, T.C. A forensic conflict: What happened to the _Sarcophaga cruentata_?
(Seminar, 2007)