Decomposition and insect succession in hanging and prone carcasses, with special reference to *Chrysomya chloropyga* (Diptera: Calliphoridae)

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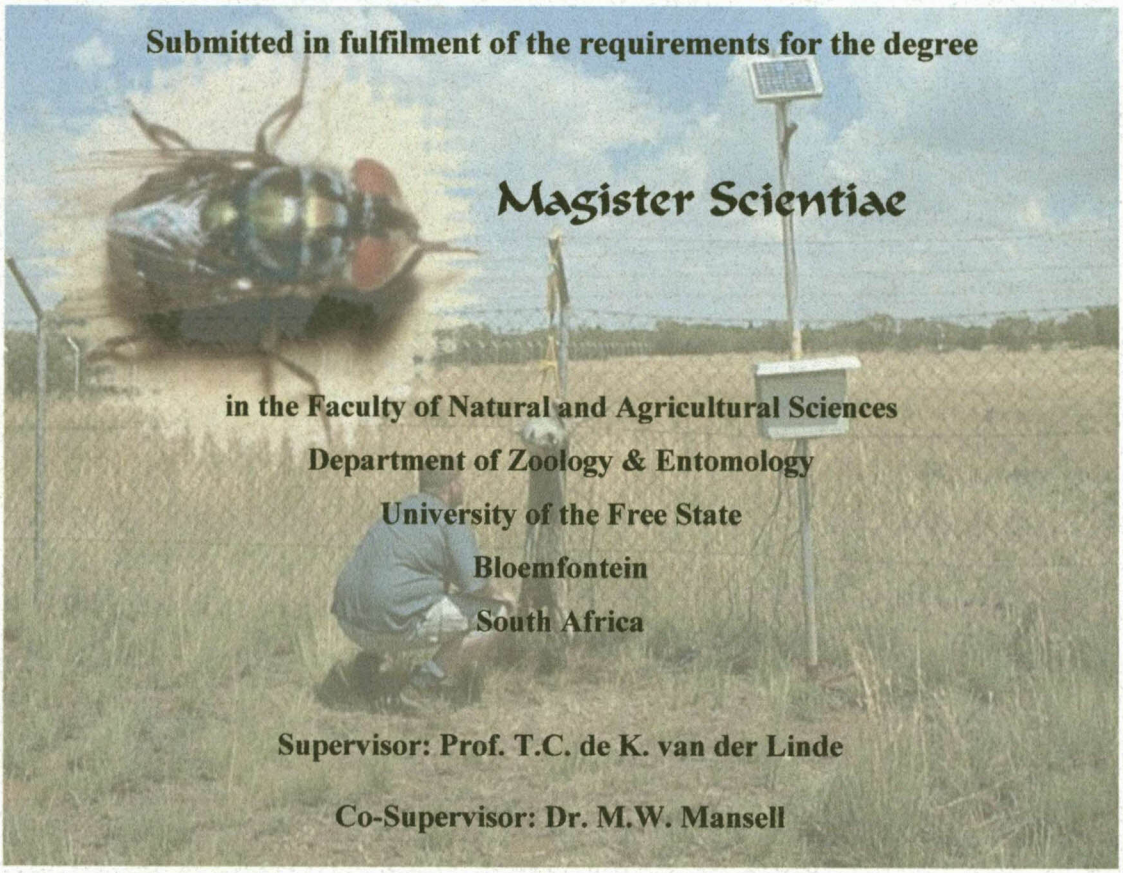

Submitted in fulfilment of the requirements for the degree

Magister Scientiae

in the Faculty of Natural and Agricultural Sciences

Department of Zoology & Entomology

University of the Free State

Bloemfontein

South Africa

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January 2003

Dedicated to my parents,

Johan & Hester du Plessis

my younger brother,

Leon

and my late grandparents,

Oupa Essie & Ouma Tina, Oupa Hendrik & Ouma Gesie.

Acknowledgements

I thank the Lord for health, strength, intellect and a sound mind to complete this project.

I am indebted to the following persons and institutions:

Prof van der Linde for being a mentor, for continual guidance, advice and for the freedom and opportunity to make my own mistakes and to learn from them.

Dr Mansell for invaluable guidance, advice and identification of Diptera.

Prof. Gerhard Prinsloo for continual interest and the identification of Hymenoptera.

Johan van Niekerk for assistance with the succession diagrams.

All the personnel and fellow students of the Department of Zoology & Entomology who showed interest in this project, some of whom helped me with various aspects of the study by helping/accompanying me with the fieldwork.

The personnel and pathologists at the government mortuary, Bloemfontein, for allowing me to attend autopsies and crime scenes.

My family and friends for their respect, interest, support and willingness to listen to long discussions on the topic of forensic entomology.

The Medical Research Council and the Department of Arts, Culture, Science and Technology for financial support during this project.

The University of the Free State for research facilities.

A very special word of thanks to the following persons:

Dr J.F. (Tromp) Els (Forensic pathologist) for continual guidance, advice and interest in this project, from the very beginning of the planning phase (December 1998). He, his wife Retha and their children, Cobus and Elizna, for being my family in Bloemfontein for the past year.

My uncle, Pieter Esterhuyse and his family for their love and support.

My mother Hester and father Johan. Without their support and unconditional love, I would not have made it this far.

"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)

CONTENTS

ABSTRACT

Persons who commit suicide sometimes hang themselves. The body is frequently only found after a few days have elapsed. In establishing the postmortem interval, it is important to know whether there are differences in the decomposition process of, and the insect succession on hanging bodies compared to bodies lying on the ground.

All field experiments were conducted at the experimental site on the western campus of the University of the Free State (29°08'S 26°10'E), Bloemfontein, South Africa. This region is a summer rainfall region with an average rainfall of 450-500 mm per annum. Hot summers and cold winters prevail, with severe frost occurring regularly during winter.

The field study was carried out by exposing prone pig carcasses to full sunlight and hanging pig carcasses to full sunlight and full shade during various seasons. Five stages of decay were identified, *viz.* fresh, bloated, active decay, advanced decay and remains. Based on mass loss, the prone carcasses decomposed further and insects removed more tissue than was the case in the hanging carcasses. The hanging carcasses probably dried out more rapidly, slowing down the decomposition process.

During this study, only the numbers of adult insects visiting the carcasses were recorded as it was impossible to make an accurate estimate of larval numbers. Significant differences were observed in the insects visiting the carcasses. On the hanging carcasses, Coleoptera were most abundant, and on the prone carcasses Diptera were most abundant. This was consistent with the hanging carcasses desiccating more quickly than the prone carcasses. Larger maggot masses formed on the prone carcasses than on the hanging carcasses. At the hanging carcasses, a "drip zone" was identified below the carcasses in which the fallen maggots developed. This area is extremely important for insect evidence analysis and is frequently overlooked in investigations by non-entomologists.

The patterns of succession of insects on the carcasses revealed that *Sarcophaga cruentata, Chrysomya albiceps, C. marginalis* and *Musca domestica* were the initial invaders of the carcasses during decomposition. Larger numbers of Calliphoridae were recorded continually on the prone carcasses, while fewer Calliphoridae were recorded on the hanging carcasses. Larger numbers of Coleoptera were recorded on the hanging carcasses while lower numbers of Coleoptera were recorded on the prone carcasses.

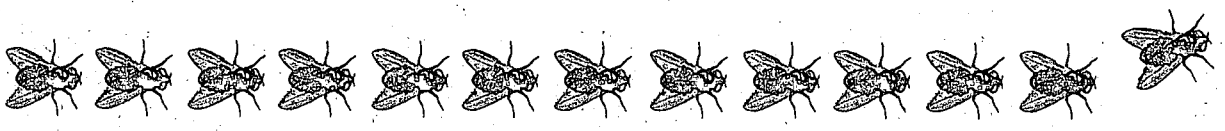
Internal carcass temperatures measured in the thorax and upper abdomen were higher than the ambient temperature owing to metabolic heat generated by large larval masses. At the hanging carcasses, the internal temperatures approximated the ambient temperature. After post-feeding larvae had migrated from the carcasses to pupate, high internal carcass temperatures were the result of sun insolation.

The high incidence of *C. chloropyga* observed on the carcasses during spring and during several case studies identified this species as an extremely important forensic indicator species in the Free State Province. Laboratory studies on *C. chloropyga* at 25°C revealed that chicken liver yielded the shortest mean development time (10.4 ± 0.71 days) with the highest mean percentage (67.11 ± 11.09) of survival of larvae to adulthood occurring with beef liver as rearing medium. Morphometric data revealed that the largest adults were produced with beef liver as feeding medium (dry mass: 0.00878 ± 0.00122 g and wing length: 8.44 ± 0.32 mm).

Temperature studies using chicken liver as feeding medium for *C. chloropyga* larvae revealed that the shortest mean development time (9.4 ± 0.66 days) occurred at 30°C. The highest mean percentage (58.22 ± 9.4) survival of larvae to adulthood occurred at 35°C. Morphometric data revealed that the heaviest adults were produced at 10°C (dry mass: 0.01123 ± 0.00015 g; percentage survival: 1.33 ± 2.0) and the longest wing lengths at 20°C (8.67 ± 0.28 mm).

CHAPTER 1

Introduction and literature review



1.1 INTRODUCTION AND LITERATURE REVIEW

One of the surprising features of the natural world is that animals continuously die around us and yet we rarely see any evidence of this. It bears testimony to the efficacy of the large variety of organisms that facilitate the decomposition of animal corpses (Turner 1991).

At the end of life, the law of entropy finally prevails (Micozzi 1986). The decomposition of plant and animal remains ensures the rapid return to the ecological system of resources bound up within them. Putman (1978) studied the flow of energy and organic matter from a carcass during decomposition, and the efficient release and recycling of this material is clearly a matter of considerable importance. In terms of their numbers and the absolute amounts of material involved, decomposers comprise one of the most significant of all trophic assemblages (Putman 1983).

Corpses pass through a series of identifiable but diffusely separated stages of decomposition. The basic pattern of decomposition is influenced, especially temporally, by three inter-related factors, climate, situation and access by insects (Turner 1991).

Immediate postmortem change may be viewed essentially as a competition between decomposition (decay and putrefaction) and desiccation. External factors, such as temperature, humidity, and sunlight, acting with internal factors such as surface area-to-volume ratio and body temperature, largely determine the outcome of this contest (Micozzi 1986).

Decomposition is a natural and necessary process responsible for the return of organic material to the ecosystem. Carrion, or dead animal matter, represents a temporary and changing food source for a varied and distinct community of organisms. Arthropods, especially insects, are the main component of this community and are the primary elements involved in the decomposition process (Richards & Goff 1997).

The term "forensic entomology" is generally applied to the study of insects and other arthropods associated with certain suspected criminal events and legal issues, for uncovering information useful to an investigation. (Keh 1985; Catts & Goff 1992).

Lord & Stevenson (1986, in Catts & Goff 1992) identified three categories of forensic entomology. These were urban, stored-product, and medicolegal forensic entomology. Urban forensic entomology includes litigation and civil law actions involving arthropods in dwellings or as house or garden pests. Lawsuits dealing with the misuse of pesticides are included in this category. Stored-product forensic entomology generally deals with arthropod infestation or contamination of a wide range of commercial products (*e.g.* beetles or their remains in candy bars, maggots in bottled food or spiders in toilet tissue). Like its urban counterpart, this category usually involves litigation. The third category, medicolegal forensic entomology, is the focus of this study and is the most popularized aspect of the science. This category deals with arthropod involvement in events surrounding felonies, usually violent crimes such as murder, suicide and rape, but also includes other violations such as physical and drug abuse. A more accurate name for this category is medicocriminal forensic entomology (Catts & Goff 1992).

One of the main functions of forensic entomology is to provide information on the time of death. This is achieved by studying the sequential arrival times of arthropods on a corpse and the developmental timetables of the offspring (Lord & Burger 1983). Smith (1986) made the important observation that it was always the insects that were being aged and this may or may not relate to the postmortem period. Considerable skill is required for the technique to be successfully applied. Accurate identification of the insect species and a detailed knowledge of their life histories and association with a cadaver are consequently fundamental to the science of forensic entomology. Effects of climate and an awareness of potential pitfalls and modifying circumstances must also be taken into account. Some of these pitfalls are well documented in the literature and include the following Insect growth rates are fundamentally influenced by temperature. Insects are exothermic,

therefore their metabolic rates and rates of growth are governed by the temperature of the immediate surroundings.

Several variables influence the decomposition process. These variables include temperature, the effect of maggot-generated heat, humidity or aridity, rainfall, soil pH, and trauma to the body. Also, 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 (Catts & Goff 1992; Anderson & VanLaerhoven 1996). Blowflies will often oviposit on carrion within the first few hours following death (Catts 1992). This starts a biological clock whereby subsequent determination of the age of the developing fly progeny is the basis for estimating the Postmortem Interval (PMI) (Catts & Goff 1992). The PMI is the time frame from the time of death to discovery of the body. A carcass, whether human or animal, undergoes a series of changes (biological, chemical and physical) 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 carcass is a temporary, rapidly changing resource that supports a large, dynamic arthropod community. When the sequence of arthropods colonising carrion is known, an analysis of the arthropod fauna on a carcass can be used to determine the time of death (Anderson & VanLaerhoven 1996).

The accurate determination of the PMI is of primary importance in any forensic investigation (Anderson & VanLaerhoven 1996; Introna *et al.* 1998). Medical parameters can be used to determine time of death in cases in which death has taken place shortly before discovery, but this becomes more difficult as time progresses. After 72 hours, forensic entomology is usually the most accurate and often the only method for determining the time of death (Anderson & VanLaerhoven 1996). The calculation of the

PMI is 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 major contribution made by a forensic entomologist in a criminal investigation is an estimate of the PMI. Some added contributions include indication of transportation of the corpse and possibly the cause of death (Catts 1992). Estimating the PMI involves the setting of the maximum and minimum probable time interval between death and corpse discovery. 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 corpse exposure. The minimum limit is determined primarily by estimating the age of developing immature insects at the time of corpse discovery. 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).

The accuracy of determining the PMI is based on the knowledge base of a research scientist. This involves a number of facts and assumptions concerning the biology, ecology and behaviour of specific insects (Dadour 2000). This is complicated because the police and the justice service demand an accurate PMI (Dadour 2000; Dadour *et al.* 2001). Correct collection, preservation and rearing of entomological specimens are of paramount importance in the accurate determination of a postmortem interval (Lord & Burger 1983).

Civilisation from a fly's perspective is the increasing proliferation of organic waste and garbage. Flies prospered with animal domestication and the rise of villages, towns and cities. Little wonder that the ubiquity of blowflies is already documented in our earliest records (Greenberg 1991).

The oldest alleged blowfly fossils known are specimens from the Late Cretaceous (about 70 million years ago) from Alberta, Canada, which were described by McAlpine (1970, in Erzinçlioğlu 1996). 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) (Kitching 1980, in Erzinçlioğlu 1996). In ancient civilisations flies appeared 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. Both the ravages and metamorphosis of flies where known to ancient Egyptians. A slip of papyrus found in the mouth of a mummy contains the 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.

The birth of forensic entomology occurred several millennia later, probably in China (Greenberg 1991). The first documented forensic entomology case was reported by the Chinese lawyer and death investigator Sung Tzu in 1235 AD in the medicolegal textbook *Hsi yuan chi lu* (The Washing Away of Wrongs). He described the case of a stabbing near a rice field. The investigator suspected that the lethal weapon was a sickle and the day after the murder he requested all the workers to lay down their sickles in front of them. Invisible traces of blood drew blowflies to a single sickle. The tool's owner confessed to his crime and "knocked his head on the floor" when confronted (Keh 1985; Greenberg 1991; Catts & Goff 1992; Benecke 2001b; Hall 2001).

Apart from the early case report from China (13th century) and later artistic contributions, the first observations on insects and other arthropods as forensic indicators were documented in Germany and France. These were reported during mass exhumations in the late 1880's by Reinhard and Hofmann, who are considered to be co-founders of the discipline (Benecke 2001b). The application of Entomology to forensic medicine was firmly established by the publication of Megnin's classic work *La Faune des Cadavres. Application de l'Entomologie à la Médicine Légale* in Paris in 1894. Prior to this

publication, Mégnin and Yovanovitch had published two shorter works on the subject in 1888. Neither of these earlier works dealt with the subject as comprehensively as Megnin's second book (Erzinçlioğlu 1983). After the French publication of Megnin's popular book on the applied aspects of forensic entomology, the concept quickly spread to Canada and the U.S.A. At the time, researchers recognized that the lack of systematic observations of forensically important insects hindered their use as indicators of postmortem interval. General advances in insect taxonomy and ecology closed this gap over the following decades. Many early case reports dealt with 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. After the World Wars, few forensic entomology cases entered the scientific literature. From the 1960's to the 1950's, Leclercq and Nuorteva were primarily responsible for maintaining the method in Central Europe, with a focus on case studies (Turner 1991; Benecke 2001b). Since then, basic research in the U.S.A., Russia and Canada has opened the way for the routine use of entomology in forensic investigations (Benecke 2001b).

Understanding the processes of postmortem change in biological systems is important to the forensic sciences (Micozzi 1986). Many decomposition studies that include a variety of animal models have been mentioned in the forensic literature. The most commonly used animals are pigs, rats and other mammals (Fuller 1934; Micozzi 1986; Tullis & Goff 1987; Hewadikaram & Goff 1991; Shean *et al.* 1993; Richards & Goff 1997; De Souza & Linhares 1997). Cornaby (1974) used lizards and toads as animal models. Rodriguez & Bass (1983, 1985) conducted studies on the insects found in association with decomposing human cadavers. These studies were conducted during different seasons and entailed the influence of mechanical damage, exposed carcasses *versus* shaded carcasses, and the effects of freezing and thawing on decomposition. In South Africa studies were conducted on the effects of scavenger mutilation on insect succession at impala carcasses (Ellison 1990). Two impala carcasses were suspended from *Acacia* trees, 2.5m above the ground, by binding all four feet together with rope. Braack (1981, 1986, 1987) also

conducted studies in South Africa on the community dynamics and visitation patterns of carrion-attendant arthropods. Payne (1965) also studied the effect of excluding insects from a carcass during decomposition. Little reference has been made to the influence of the orientation of a decomposing carcass (for example a hanging carcass *versus* a prone carcass) on the decomposition process and the succession of insects. In a recent paper, Shalaby *et al.* (2000) studied the difference in the patterns of decomposition of a hanging carcass and a carcass in contact with the soil. Payne & King (1970) conducted a comparative study of exposed pig carcasses and pig carcasses isolated from arthropods. Carcasses were suspended from trees at various heights, placed in water, buried, isolated from, partially isolated from and completely exposed to insects. In a similar study by Marchenko (2001), 23 animal carcasses were suspended at a height of about one metre from the ground. The study was conducted in Russia between 1971 and 1983, and included the placement of 180 carcasses on the soil surface, seven carcasses were buried and one carcass was placed in an unheated service room. In this study, Marchenko (2001) used the carcasses of dogs, cats, rabbits, suckling pigs, moles, pigeons and kittens. The effect of water-related deaths, burial, burning and concealment on arthropod succession patterns has also been extensively studied (Payne & King 1972; Erzinçlioğlu 1985; Rodriguez & Bass 1985; Haskell *et al.* 1989; Turner 1991; Louw & van der Linde 1993; Avila & Goff 1998; Tomberlin & Adler 1998; Hobischak & Anderson 1999; VanLaerhoven & Anderson 1999). Case studies have also revealed several important observations, including the effect of wrapping a body during the decomposition process and subsequent insect succession (Goff 1992; van der Linde 2001: unpublished data).

Diptera and Coleoptera are the two insect orders mainly used for PMI estimation, since the major forensic indicator species occur within these orders. Mites have also been identified as potential indicators of PMI (Goff 1989). PMI has also been successfully determined for a set of human remains discovered in a metal tool box by using the development time required for a Stratiomyidae fly, in combination with the time required to establish a colony of ants (Formicidae) (Goff & Win 1997). This analysis resulted in a PMI of 14 to 18 months. The victim had been missing for approximately 18 months. The

seasonal distribution of a species of fly whose dead third instar larvae were found in the mouth of a deceased woman in Norway, placed the time of death some seven months prior to discovery of the body (Stærkeby 2001). In this case, the entomological data matched the information later provided by the police. In a case study by Goff *et al.* (1991), second instar blowfly larvae 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 *et al.* 1991). Benecke & Lessig (2001) made the first report where an examination of the maggot fauna on a person illustrated neglect that had occurred prior to death. On 10 July 2000, during an enforced eviction due to a lack of rent payments, a child was found dead near its bed in the apartment of a 20-year-old woman in a city 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 (Benecke & Lessig 2001). Goff *et al.* (1991) stressed the need for caution in cases involving deaths of infants, the elderly, and individuals not capable of caring for themselves.

Rozen & Eickwort (1997) reported an interesting case of forensic melittology. Their primary task was to investigate and explain the source of blockage in an elbow and in other parts of the fuel supply unit recovered from the wreckage of a private airplane. A small clump of pollen associated with a disk-shaped gummy mass of plant fibres suggested that bees of the family Megachilidae might have been responsible for accumulating these plant materials. They concluded that contamination of the wreckage by nesting bees was obviously a post-crash phenomenon because the plant-material and

dead bees would have been destroyed by the intense fire that followed the crash (Rozen & Eickwort 1997).

Forensic entomology has also been successfully applied in the case of animals. Lockwood *et al.* (1994) reported a case where they were presented with insect remains from an unusual death. The victim was a horse that had been killed more than 400 years before. They concluded that the insects recovered from the site arrived after the horse had died, that the horse died in late spring or early summer (perhaps in late May or early June), and that Europeans had accompanied the horse. Also that the immediate habitat in which the horse died was dry and protected from the elements and that the horse may have been partially buried. Finally, their 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 *et al.* 1994). The illegal killing of two black bear cubs recently also made forensic entomology applicable to wildlife crimes (Anderson 1999). The calculated PMI was consistent with the time that the defendants were seen at the scene and was used in their conviction. This case illustrated that insect evidence can be equally as valuable in poaching cases as in homicide cases. It is extremely important for conservation officers to be educated regarding this field (Anderson 1999).

Forensic experts often disagree. These disagreements were highlighted, possible reasons for such disagreements were analyzed and avenues for resolution were suggested by Nordby (1992). The logic of interpreting scenes, and pattern injuries such as bite marks, was explained to locate potential sources for interpretive error. Observation is not interpretation. Observation involves implicit reasoning, and is instant; it is a mental experience. Interpretation, on the other hand, involves explicit reasoning and deliberate thinking (Nordby 1992). Two observers may not see the same thing, although their eyesight is normal and they are aware of the same artifact. Cases showed that both practical and theoretical investigative expectations affect what count as observations (Nordby 1992). These expectations confer evidential status on the artifact. When two observer's expectations conflict, they do not see the same thing, so are not presented with

the same evidence. If we see what we believe, we may not be warranted in believing what we see. We may suffer from inappropriate, expectation-laden observations, or we may lack appropriate true beliefs needed to supply the refined context of observation: we may not be the appropriate expert (Nordby 1992).

Exciting areas of research applicable to forensic entomology that urgently require attention include the following. A molecular method for the rapid identification of insect larvae found on a corpse was described by Sperling *et al.* (1994) whereby specific insect DNA fragments were amplified by using PCR. Direct DNA sequencing of the amplification products followed. The ability to obtain P30 and Y-STR profiles from larvae investing a cadaver, with the suspicion of sexual assault having occurred prior to death, provides a new avenue to aid in the solving of such crimes (Clery 2001).

In recent years drug-related deaths have increased throughout the world. Victims are frequently not discovered for several days or months and due to decompositional changes the PMI is estimated by using entomological techniques (Goff *et al.* 1997). The use of maggots as alternative source for toxicological analyses has been well documented (Goff *et al.* 1997; Benecke 1998). In a case dealing with mummified remains, Miller *et al.* (1994, in Goff *et al.* 1997) demonstrated the use of empty puparial cases as alternative toxicological specimens. Computer programs and algorithms have also been suggested as possible methods for the estimation of the PMI (Stinner *et al.* 1974; Lynnerup 1993; Schoenly *et al.* 1992). It is the opinion of the author that sufficient baseline studies coupled with considerable experience in the field, common sense and the ability for analytical thought should be the basis for unbiased PMI estimation.

Not all entomologists are willing to participate in an investigation at a death scene (Meek *et al.* 1983, in Keh 1985), but an experienced entomologist at the scene can make observations that may escape the uninitiated. According to Nuorteva (1977, in Keh 1985), it is an illusion that it would be possible to draw well-founded conclusions from the observation of insects collected by inexperienced laymen. To be reliable, results should

be based on a careful survey by experts of the area where the corpse was found, and on well-conducted rearing experiments, in the laboratory and under field conditions.

Since the Second World War, only a few scientists and crime scene experts have pioneered forensic entomology. All of them had the arduous task of convincing local authorities, and other scientists, of the benefits of using arthropods in criminal investigations. Judges, in numerous countries, finally decided that forensic entomology was applicable in cases ranging from complex high profile murders to wildlife violations. In recent years, the application of insect and other arthropod evidence has become routine in forensic and medicolegal investigation and research. The discipline, now 150 years old, has become sufficiently scientifically mature for practical application (Benecke 2001a). Schoenly *et al.* (1991) proposed that investigations of PMI could only be conducted by a multidisciplinary group comprised of forensic entomologists, pathologists and anthropologists.

Persons who commit suicide sometimes hang themselves. The body is frequently only discovered after a few days. In establishing the postmortem interval it is important to know whether there are differences in the decomposition process of, and the insect succession on hanging bodies compared to bodies lying on the ground. Since human bodies are not available for research projects, pig carcasses were used to address this question. The decomposition and insect succession of prone pig carcasses in full sunlight were compared to the decomposition of hanging pig carcasses, both in full sunlight and full shade.

During the decomposition studies and many case studies attended during the current study, it became clear 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 early summer. At times 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* was the most important forensic indicator

species (Mansell, pers. comm.)[1]. However, little was known about the developmental rate of this species. The need for research on the biology of this important blowfly was identified and addressed since the developmental rates derived for *C. chloropyga* will be a invaluable tool for investigators for correct PMI estimation.

[1] M.W. Mansell, Specialist Scientist, ARC-Plant Protection Research Institute, Biosystematics Division, Pretoria, South Africa.

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CHAPTER 2

Incidence of arthropods associated with decomposing carcasses

2.1. INTRODUCTION

The relationship between the ages of immature insects on a decomposing body and the postmortem interval (PMI) is determined from baseline studies, with rates adjusted by interpolation to include the influence of climate, season, weather and location (Catts 1992). Numerous decomposition studies are mentioned in the forensic literature, but little reference has been made to the influence of the orientation of the decomposing body on the decomposition process, and insect succession associated with these bodies. During the 1960's a comparative study was conducted by placing baby pig carcasses, *Sus scrofa* (Linnaeus) in water, buried and maintained free from, partially free from, and completely exposed to insects (Payne & King 1970). In addition to this, some of the baby pigs were suspended from trees at various heights.

In studies conducted in Russia between 1971 and 1983, 23 animal carcasses were hung at a height of about 1m from the ground (Marchenko 2001). These hanging carcasses were part of a larger study involving 211 carcasses that varied from dogs, cats, rabbits, suckling pigs, moles, pigeons and kittens. It is not clear which animal carcasses were used for the experiment involving hanging carcasses. The aim of the study was to determine the effect of carcass location (hung and buried) on the insects, as well as the effects of additional factors on carcass tissues, namely clothes impregnated with chemicals and the effect of burning (Marchenko 2001). During 1985, a study was conducted in South Africa on the effects of scavenger mutilation on insect succession at impala carcasses (Ellison 1990). Two impala carcasses were suspended from *Acacia* trees, 2.5m above the ground, by binding all four feet together with rope. This was done to limit the access of large vertebrate scavengers and non-flying arthropods to the carcasses. During 1997, a study was undertaken in Hawaii to determine the possible change in rates and patterns of decomposition and arthropod activity in cases where the body was hanging (Shalaby *et al.* 2000).

The present study commenced in early 1999 at the University of the Free State and was prompted by a number of cases of suspicious deaths where the body was found hanging

by the neck. In some cases it was clearly suicide, but others aroused suspicion because the victim's hands had been bound together behind their backs. The question arose as to whether there were differences between the decomposition process and insect succession on a hanging body compared to that of a body lying on the soil surface. In the current study, the differences in the decomposition process and insect succession between a prone carcass and a hanging carcass were investigated. A comparison was also made between a carcass hanging in full sunlight and a carcass hanging in partial to full shade.

2.2. MATERIAL AND METHODS

2.2.1 Study site

Field experiments were conducted at the experimental site of the University of the Free State (29°08'S 26°10'E), Bloemfontein, South Africa. This experimental site is on the western campus and consists of approximately 24 hectares of open grassveld that lies approximately 1560 m above sea level. Acocks (1988) described the vegetation as the central variation of the dry *Cymbopogon-Themeda* veld. 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 are the only trees in the area. A recent addition to the experimental site was the development of a driving range for golfers. This had an influence on the experiments, where a breach of security led to scavenger damage to one of the pig carcasses. During the experiment, the grass was cut short by the groundskeeper to reduce the risk of fire. The biodiversity of species visiting the carcasses was consequently reduced owing to many feeding niches being destroyed by lack of grass cover. A large number of tourist insects were thus excluded. 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.

2.2.2 Conditions for field experiments and carcasses

In the current study, pig carcasses were used. 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). The pigs were euthanased by lethal injection and the fresh carcasses were placed at the experimental site to simulate different conditions. Each experiment consisted of three carcasses placed under different conditions in the same area. Carcasses used by Tullis & Goff (1987) were placed 6m apart, which was regarded by the current author as being too close together for any differences in the insects visiting the carcasses to be manifest. In the current study the carcasses were consequently placed 50-100m apart.

One carcass was placed on the ground within a metal-frame cage covered with 15 mm poultry mesh (Fig. 2.1) to allow 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 northwest-southeast orientation.

Two carcasses were secured around the thorax with nylon rope and lifted to hang with the rear feet approximately 15-20 cm above the ground (Fig. 2.2). This excluded ground-dwelling insects from the carcass. One carcass was left hanging in full sunlight and the other in full to partial shade. The carcasses used in the study by Shalaby *et al.* (2000) were suspended by the neck from a tree limb, the differences being that the carcasses weighed 9.2 and 10.7 kilograms respectively, compared to the carcasses used in the current study which had masses of between 25 and 32 kilograms. Unfortunately, the masses of the carcasses used in the present study differed, although the aim was to keep the variation as small as possible. Denno & Cothram (1975, in Hewadikaram & Goff 1991) found that carcass size had a direct relationship to the numbers of individuals and species composition of the fly population. Carcass size also has a direct influence on the rate of decomposition (Hewadikaram & Goff 1991).

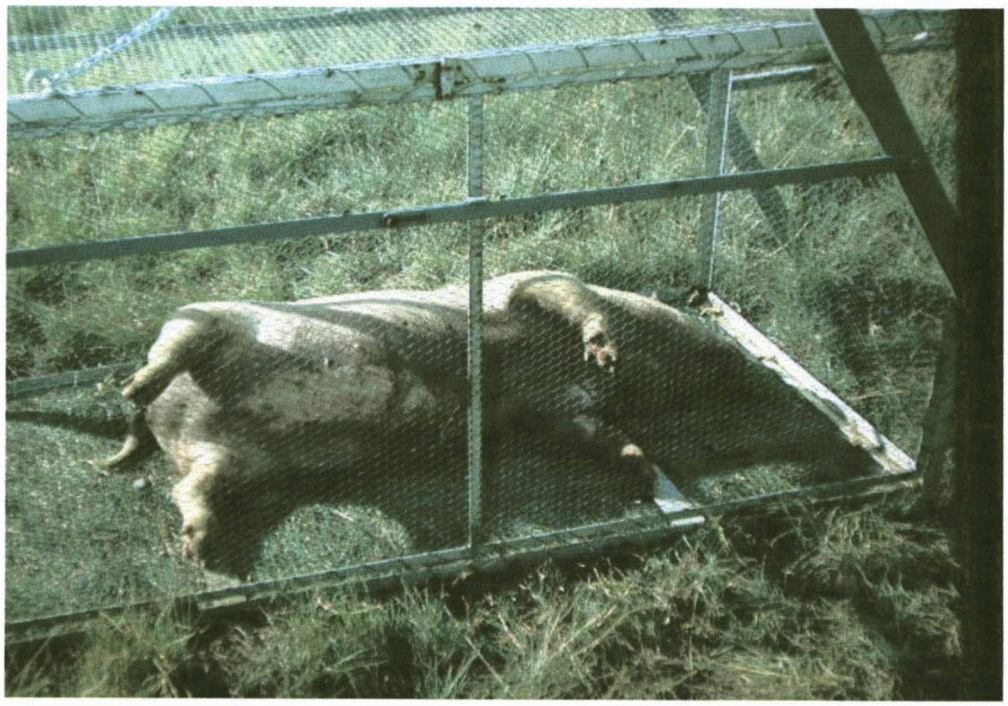

Fig. 2.1. Carcass in metal-frame cage, lying on the ground in full sunlight.

Fig. 2.2. Hanging carcass (in full sunlight)

In the present study, it was decided to exclude the immature stages from the discussion of total incidence of arthropods, as it is impossible to count or make an estimate of the number of larvae at any given time on each carcass. Immature stages collected at each carcass were reared to adulthood in the laboratory for identification. The adult blowflies obtained were used to establish laboratory colonies of different species, including: *Chrysomya albiceps* (Wiedemann), *Chrysomya marginalis* (Wiedemann), *Chrysomya chloropyga* (Wiedemann), and *Lucilia* species. This will be discussed in Chapter 5.

The experiment was conducted during summer (3 February 1999 - 15 March 1999) and repeated during autumn and winter (29 April 1999 - 1 September 1999), spring (14 September 1999 - 10 November 1999) and again during summer (1 February 2001 - 22 March 2001).

The key questions formulated for this study were:

- Are there differences between the rates of decomposition for the different carcasses (hanging *vs.* prone, and shaded hanging *vs.* exposed hanging) within each trial?
- Are there differences in the taxa that occur on each carcass within each trial?
- Are there seasonal differences in the taxa occurring on the carcasses?

2.2.3 Observations

Observations were made twice daily for the first 14 days during summer and thereafter once daily for the remainder of the study. Shalaby *et al.* (2000) adopted the same procedure during their study. During the other seasons, the carcasses were visited twice daily until insect activity ceased. Thereafter the carcasses were visited once daily, and once every 48 hours during the latter parts of the study.

Observations comprised the following procedures: The mass of each carcass was recorded by weighing it with a spring balance. The mass of each carcass was determined immediately after the pigs were killed and this mass was equated to 100% of the body's subsequent remaining mass. The actual mass loss was consequently calculated as the percentage of biomass remaining relative to the initial mass of each carcass. The subsequent mass loss during decomposition was monitored. The percentage weight loss was calculated by using the mass remaining and dividing it by the original mass. This was done in all four trials. The nylon ropes around the hanging carcasses were tied to scales so that the mass of each carcass could be recorded without disturbing the carcasses. The carcasses lying on the ground were weighed together with the metal frame cages by using a metal gantry that could be easily removed. The mass of the metal frame cages was then subtracted from the total mass. The ideal would have been to have a separate control carcass which would not be weighed, since the weighing could have disturbed the fauna found at the carcass-ground interface, but due to financial reasons this did not realise. At each visit, the numbers of adults of each family or species of insect were recorded. This was sometimes an almost impossible task. In the few instances where large numbers of insects were present (more than 200 of a single species), a small proportion was counted and an estimation of the total number was made. This method was calibrated and proved to be very effective. A similar approach was used by Braack (1981), where two of the aims of his project were to determine which insect species visit carcasses and their abundance. Braack (1981) decided to make absolute counts of the insects at the carcasses. Absolute counts have 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 (Braack 1981). Owing to the difficulties of collecting the larvae without disturbing the carcass and subsequently the decomposition process and succession of insects, it was decided to collect only small samples of larvae for identification and to establish laboratory colonies. The difficulty in collecting larvae without disturbing the carcass was mentioned by De Souza & Linhares (1997), who also did not undertake a quantitative analysis of the larvae associated with the decomposing carcasses.

2.3. RESULTS AND DISCUSSION

Payne (1965) identified six different stages of decay, namely fresh, bloated, active decay, advanced decay and dry and skeletal remains. 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. 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. The stages identified by Anderson & VanLaerhoven (1996) were applied during the current study and are discussed in Chapter 3.

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.

2.3.1 Summer trial (3 February 1999 - 15 March 1999)

2.3.1.1 Rate of decomposition

It is evident that the rate of mass loss was approximately the same for the carcasses for about the first five days (Fig. 2.3). Thereafter the carcass hanging in the sun had a slower removal of tissue. In figure 2.3 only the mass losses by the prone carcass and the carcass hanging in sun are shown. More tissue was removed from the prone carcass. The reason for this is the higher number of maggots (large maggot masses) that formed on this carcass, which was not the case with the hanging carcass. Hanged bodies were found to decompose more slowly than those lying on the ground because of higher convective heat transfer and mummification of the surface layers of the tissues (Marchenko 2001). Shalaby *et al.* (2000) also found that the rate of biomass removal from the hanging carcass was significantly slower than that of the control carcass during the bloated and decay stages of decomposition. 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).

Corpse fauna is often ignored when investigators process the death scene, and arthropods in the immediate vicinity of a corpse are often overlooked as evidence. A complex community of insects may develop in the seepage zone beneath hanging corpses and will be lost as evidence in cases where specimens are collected only from the corpse, either at the death scene or at the autopsy (Catts & Goff 1992). In the current study, this seepage zone was named the "drip zone". A large number of the maggots on the hanging carcass fell to the ground and developed in the "drip zone". This was also recorded by Shalaby *et al.* (2000). They also found that larvae feeding on the carcass lying on the ground formed dense feeding masses and were able to move freely between the carcass and the substrate.

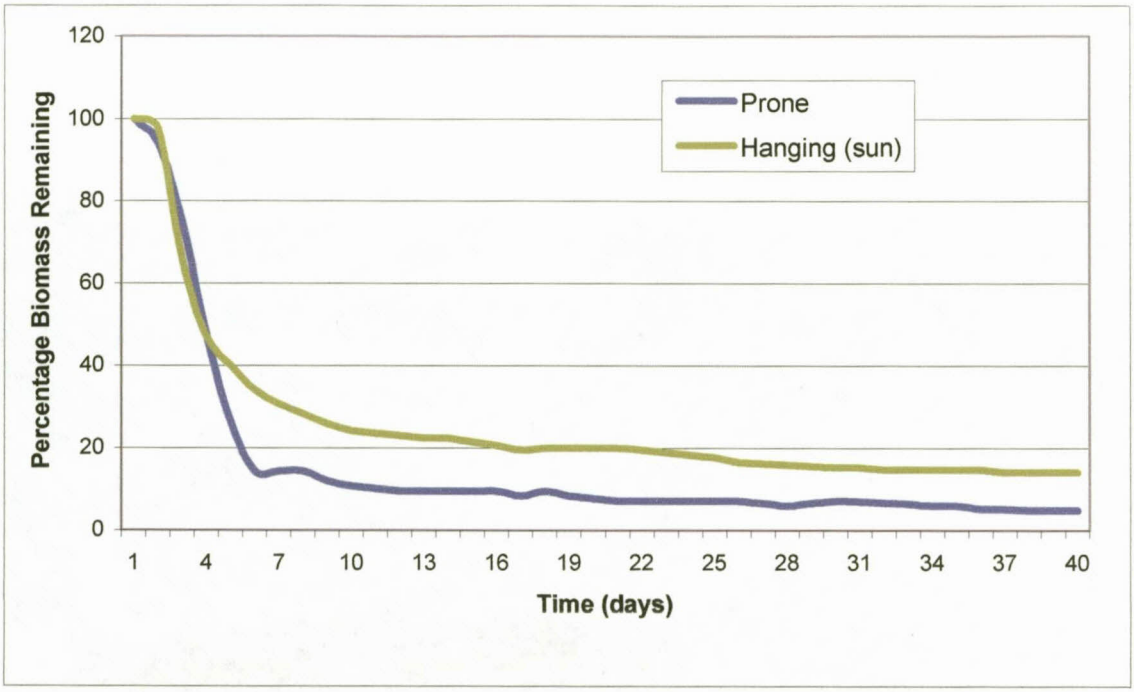

Fig. 2.3. Percentage biomass remaining of the carcasses during the summer 1999 trial.

By contrast, larvae falling from the hanging carcass were restricted to the substrate for the remainder of their development, dependent on material falling from the carcass as a food source. This resulted in a smaller population on the hanging carcass and a maggot feeding mass did not form on the carcass. A feeding mass however, was observed on the soil immediately below the hanging carcass.

Shean *et al.* (1993) found that an exposed pig carcass decomposed much faster than a shaded pig carcass, reaching a stable minimal mass two weeks before the shaded carcass. They also noted that some of the observed differences in mass loss between the shaded and exposed carcasses were probably due to the differential effects of dehydration on the two carcasses. Richards & Goff (1997) found that the greatest percentage of biomass was removed during the decay stage because of the maggot feeding masses. According to Putman (1977), the feeding activities of the maggot masses totally dominate the pattern of decomposition during the active decay stage.

Ambient temperature had a major influence on the rate of decomposition. The influence of ambient temperature on decomposition has been observed by many authors, including Micozzi (1986); Smith (1986); Mann *et al.* (1990) and Richards & Goff (1997). Temperature is the single most important environmental factor influencing the rate of carcass decay (Spitz & Fisher 1973, in Richards & Goff 1997). As was the case in the current study, Shean *et al.* (1993) found that the decomposition rates of pig carcasses were affected primarily by feeding of the calliphorid larvae and their relative rate of development, which in turn was related to ambient air temperature. The influence of temperature (ambient *vs.* internal) is discussed in Chapter 4.

2.3.1.2 Composition of arthropod orders on carcasses

It is evident from Figure 2.4 that Diptera and Coleoptera are the two major orders of insects present on the carcasses. Significantly lower numbers of Hymenoptera, Orthoptera, Mantodea and Acari were also recorded. The largest number of individual Diptera was found on the carcass hanging in the shade, while the smallest number was found on the carcass hanging in the sun. The largest number of Coleoptera was found on the carcass hanging in the sun, while the smallest numbers were found on the prone carcass (Fig. 2.4). There could be several reasons for this phenomenon. The first is the effect of convection (the flow of air currents) around the carcasses. The hanging carcasses were more exposed to the effects of convection than the carcass lying on the ground. The result was that the carcass lying on the ground did not desiccate as quickly as the hanging carcasses. The carcass on the ground was consequently far moister than the other two and this moist period lasted longer than in hanging carcasses.

Coleoptera were more prevalent during the drier stages of decomposition of the carcasses. There were consequently larger numbers of Coleoptera on the hanging carcasses than was the case with the prone carcass (Fig. 2.4).

Another difference was the discrepancy between the number of Diptera and Coleoptera on the two hanging carcasses. Nearly three times as many Diptera were found on the

carcass hanging in the shade than on the carcass hanging in the sun, while the number of Coleoptera on the carcass hanging in the shade was about half of that on the carcass hanging in the sun (Fig. 2.4). The most plausible explanation could be that the carcass hanging in the shade was under a *R. lancea* tree adjacent to an unidentified shrub, effect of creating an enclosure in which the carcass hung. This enclosure served as a buffer that regulated the temperature so that extreme heat did not reach the carcass, resulting in a milder microclimate. The enclosure also served as a physical barrier to the desiccating effects of the wind. This carcass dried out more quickly than the prone carcass, but not as quickly as the carcass hanging in the sun.

The carcasses in the sun were subject to extremes, while the carcass in the shade hung in more temperate or mild conditions. Shalaby *et al.* (2000) also found that arthropod diversity and representative species at the hanging carcass and the prone carcass were similar. However, there was a marked difference in the numbers of individuals, with the prone carcass being more heavily exploited by Diptera larvae (Shalaby *et al* 2002).

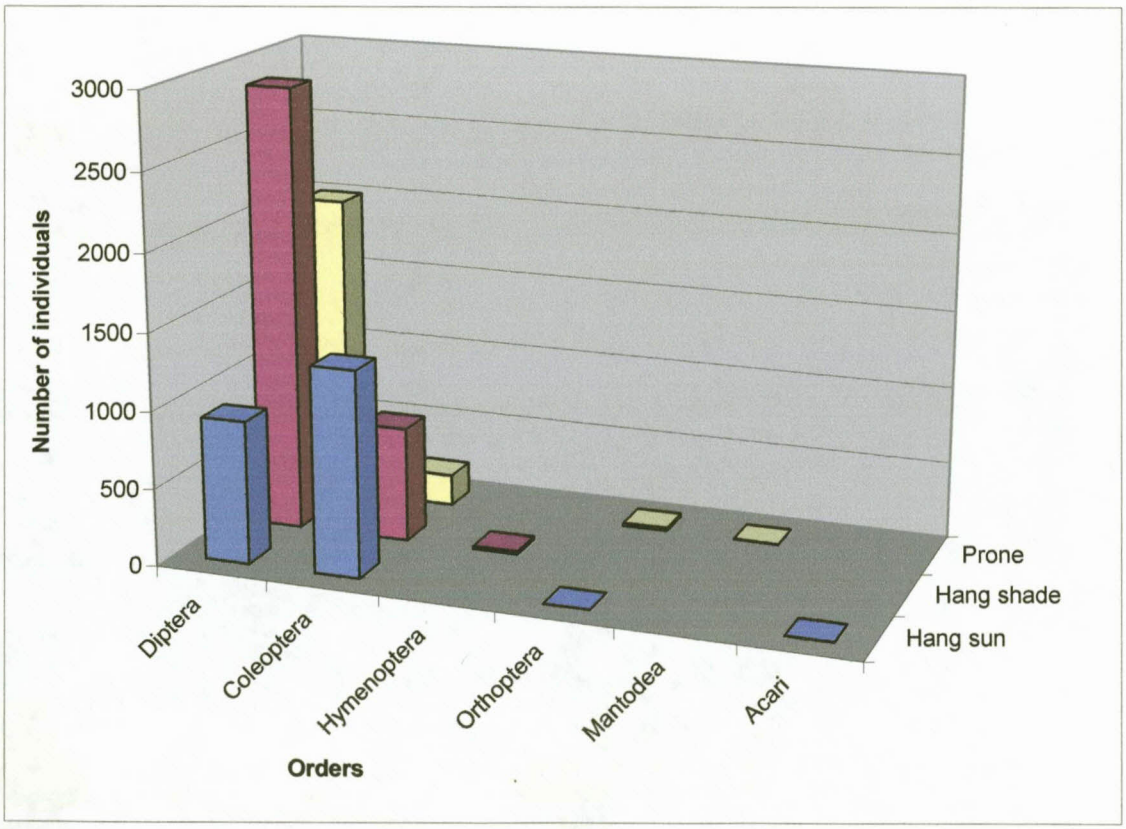

Fig. 2.4. Composition of arthropod orders recorded during the summer 1999 trial.

2.3.1.3 Diptera families

Calliphoridae and Muscidae were the dominant families of Diptera recorded during this trial (Fig. 2.5). Significantly smaller numbers of Sarcophagidae, Piophilidae, Asilidae and Drosophilidae were also found. The number of individuals of Calliphoridae on the carcass hanging in the shade and the prone carcass were similar. Significantly fewer Calliphoridae were found on the carcass hanging in the sun.

Calliphoridae larvae were responsible for removing most of the tissue from the decomposing carcasses during the initial stages of decomposition. It is thus clear that more tissue would be removed from the carcass hanging in the shade and the prone carcass than would be the case with the carcass hanging in the sun.

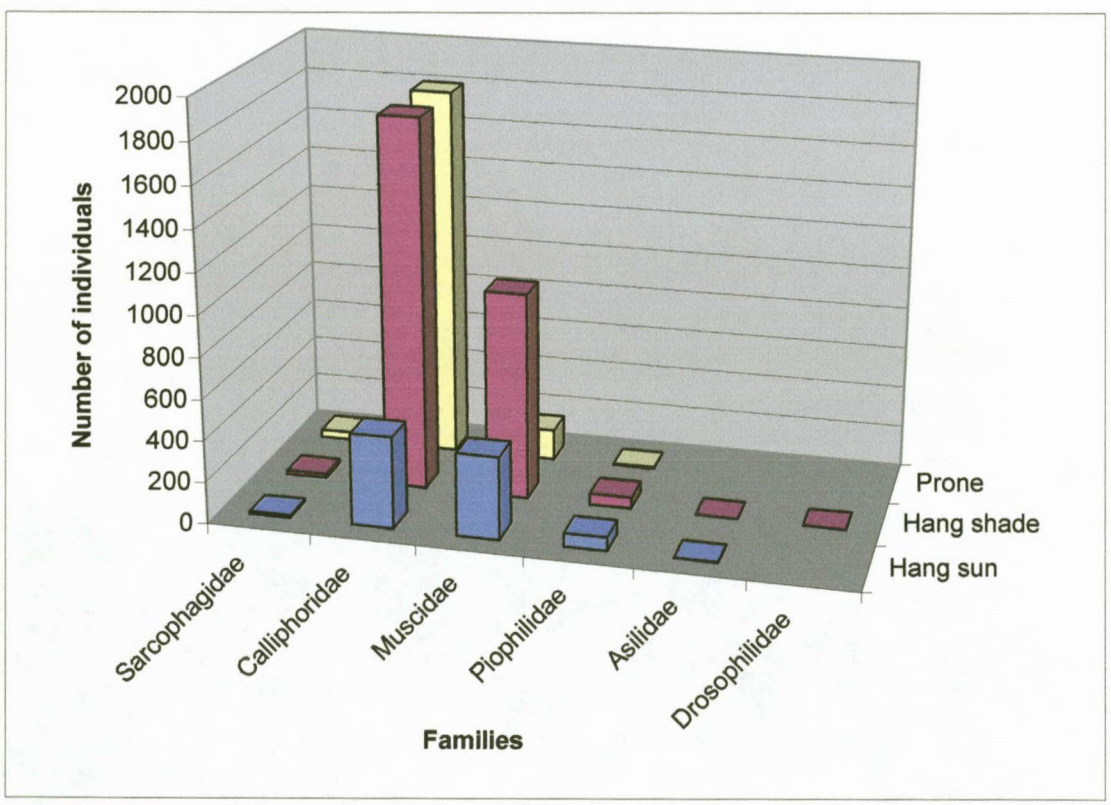

Fig. 2.5. Diptera families recorded during the summer 1999 trial.

Blowfly maggots are the initial and major consumers of carrion and the most important entomological indicator in evaluating human decomposition (Shean *et al.* 1993). The same conditions regarding the formation of large maggot masses on the prone carcass applies to this seasonal study and applied to all the replications of the experiment. This was also true for the situation at the hanging carcasses where large maggot masses did not form.

The largest number of Muscidae was found at the carcass hanging in the shade and the smallest number on the prone carcass. The reason for this could be that Muscidae prefer shady conditions. Drosophilidae were only found at the carcass hanging in the shade. No Asilidae were found at the prone carcass (Fig 2.5).

2.3.1.4 Diptera species

Chrysomya marginalis was the dominant species of Calliphoridae on all the carcasses. The second-most dominant species was *C. albiceps* (Fig 2.6). The second and third instar larvae of *C. albiceps* exhibit predaceous tendencies (Braack 1987). Braack (1986) also found *C. albiceps* and *C. marginalis* to be crucial species because of the ability of the immature stages 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. The dominant Muscidae species was *Musca domestica* Linnaeus, and the second-most dominant species was *Hydrotea capensis* (Wiedemann). Lower numbers of *Sarcophaga cruentata* (Meigen), *C. chloropyga* (Wiedemann) and *Lucilia cuprina* (Wiedemann) and/or *Lucilia sericata* (Meigen) species were also found.

2.3.1.5 Coleoptera families

The dominant Coleoptera families for all the carcasses were Dermestidae and Cleridae (Fig. 2.7). These two families were represented by one species each, *viz. Dermestes maculatus* (DeGeer) and *Necrobia rufipes* (DeGeer) respectively. The representation of Dermestidae and Cleridae by one species each was also recorded by Braack (1987). The

largest number of both these species was found at the carcass hanging in the sun and the smallest at the prone carcass (Fig. 2.7). This correlates with the theory of the effect of convection and desiccation of the hanging carcasses.

A desiccated body or carcass is more attractive to Coleoptera than a moist body. Very few, if any, insects will be observed once skeletonisation is completed. The few observed would probably be dermestid beetles, which are one of the last insect families to feed on animal remains. However, it was observed in this study, and in the numerous forensic science cases involving only skeletal remains that early succession insects could still be found upon close examination in skeletal crevices or foramen (Rodriguez & Bass 1983).

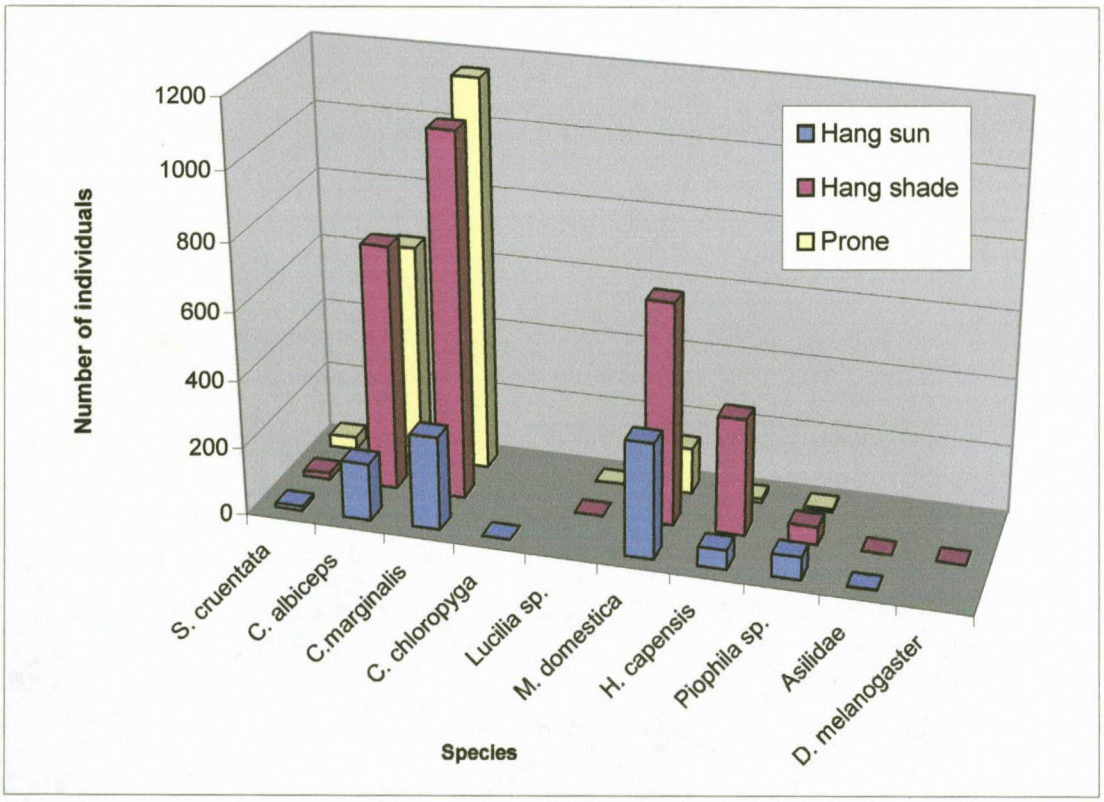

Fig. 2.6 Diptera species recorded on pig carcasses during the summer 1999 trial.

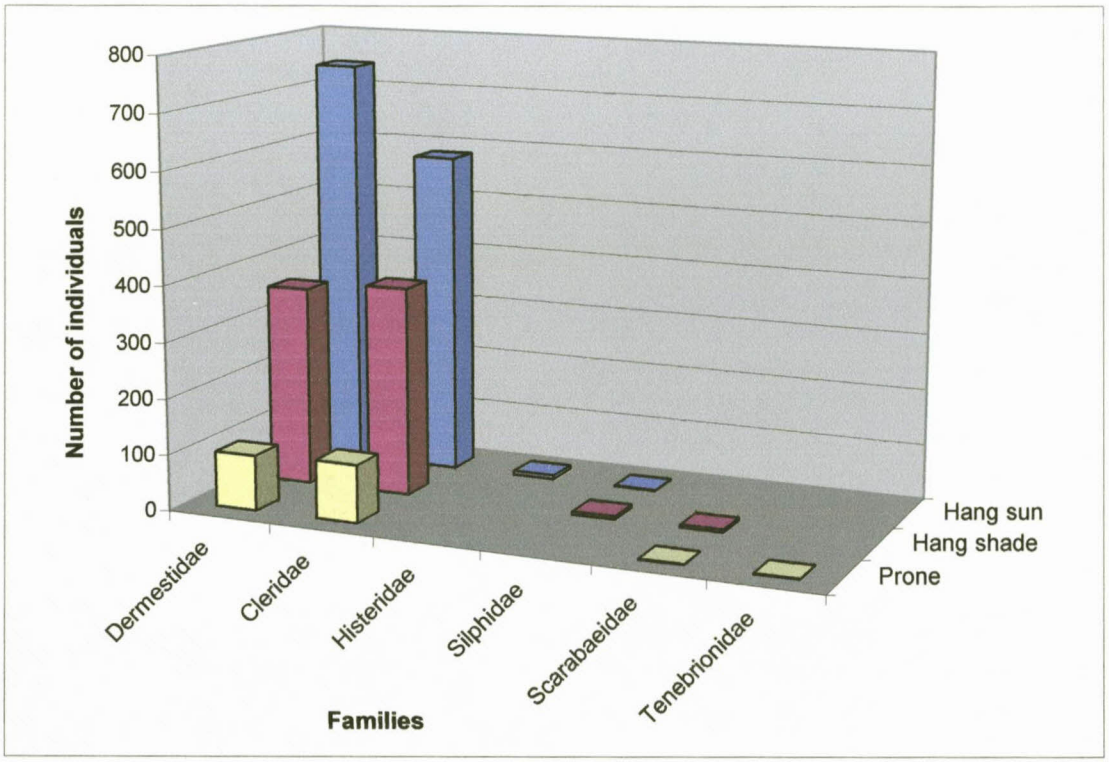

Fig. 2.7. Coleoptera families recorded on the carcasses during the 1999 summer trial.

Low numbers of Histeridae, Silphidae, Scarabaeidae and Tenebrionidae were also recorded during these experiments. Strangely, no Histeridae and Silphidae were found at the prone carcass. Histeridae and Tenebrionidae were absent from the carcass hanging in the shade, while no Scarabaeidae or Tenebrionidae were found at the carcass hanging in the sun (Fig. 2.7).

2.3.2 Winter trial (29 April 1999 - 1 September 1999)

2.3.2.1 Rate of decomposition

During the winter trial, the masses of the different carcasses also differed. The carcass lying on the ground weighed approximately 30 kg. The carcass hanging in the sun and the carcass hanging in the shade weighed 21 kg and 22,5 kg respectively. During this trial, the carcass hanging in the sun decomposed at the fastest rate (Fig. 2.8) and more tissue was removed from it than was the case in the other two carcasses. During the first 50

days of this study, the prone carcass decomposed at a faster rate than the carcass hanging in the shade. Thereafter the situation was reversed. Twenty percent more tissue was removed from the carcass hanging in the shade than the prone carcass (Fig. 2.8). The reason for this slower and relatively small mass loss of the prone carcass can be attributed to the winters in the Free State being severely cold and frost occurring regularly. This caused the prone carcass to freeze during the night and to undergo a greater degree of skin mummification. During the day, the carcass did not thaw completely, and was thus relatively unsuitable as a food source for the calliphorid larvae that remove most of the tissue during decomposition. Some of the tissue was, however, still used as a food source. The larvae that developed here did so at a very slow rate due to the low temperatures of the environment and the food source. The low ambient temperatures recorded are discussed in Chapter 4. According to Micozzi (1986), frozen-thawed animals are more susceptible to invasion by insects and microorganisms from the outside and aerobic decay of the skin and external surfaces. In contrast to this, carcasses that are not frozen-thawed are less susceptible to external decay, but putrefaction proceeds more rapidly from within.

At the other extreme was the carcass hanging in the sun. Because this carcass was not in contact with the soil, it did not freeze during the night. The maggots developing on and within this carcass were not subjected to these extremely low ground temperatures. Because this carcass did not go through the cycle of freezing and thawing, the development of the maggots was not delayed, as was the case with the prone carcass. The result was that more tissue was removed more rapidly from this carcass than from the other carcasses (Fig. 2.8). During the summer trial the effect of convection caused the hanging carcasses to desiccate more quickly. This effect was not as clear during the winter trial. All of the carcasses underwent mummification and desiccation of the skin, while the maggots fed on the inside of the carcass.

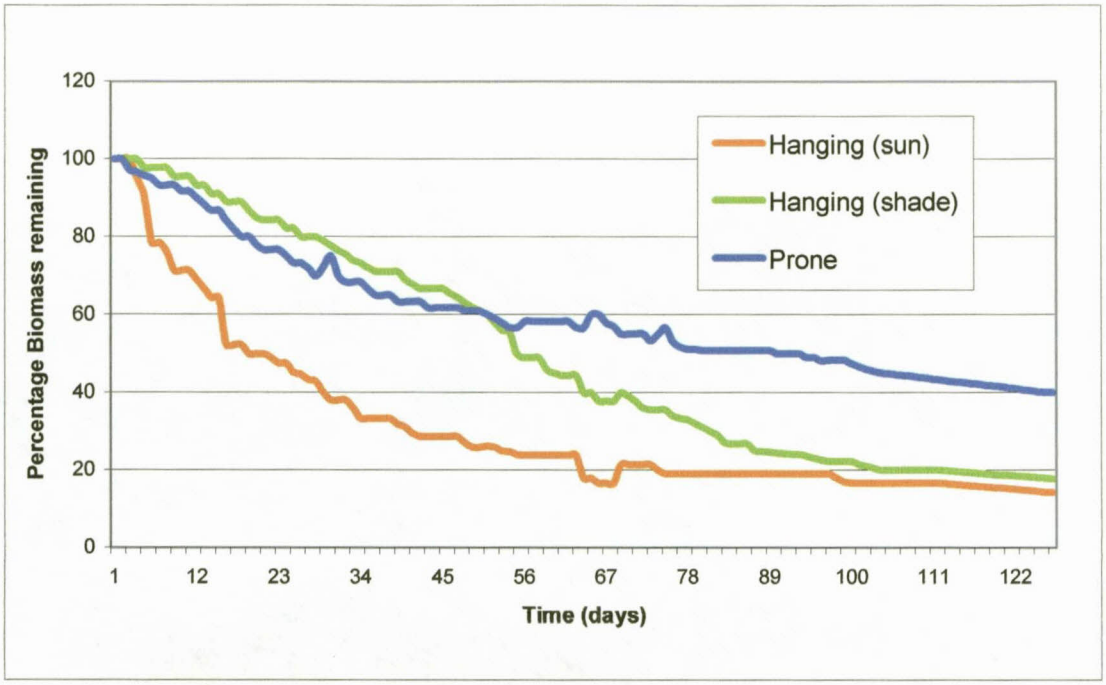

Fig. 2.8. Percentage biomass remaining of the carcasses used during the winter 1999 trial.

Initially the carcass hanging in the shade had the slowest rate of tissue removal (Fig. 2.8). After 50 days, this removal of tissue took place at a faster rate, because this carcass was not subject to the temperature extremes of the other two carcasses. This carcass too, like the carcass hanging in the shade during summer, was hanging in an enclosure of *R. lancea* and *A. karroo* trees. This enclosure served as a physical barrier and created a more stable microclimate in which these extreme temperatures were ameliorated.

Scavenger mutilation

About 2.5 kg of the original mass was removed from the lower abdomen on the dorsal side of the carcass hanging in the shade by scavenger mutilation on days 52 and 54. It is suspected that a stray dog fed on the carcass, and the dorsal lower abdomen was the only part it could reach to feed on owing to the orientation of the carcass. This was similar to the findings of Ellison (1990) where vertebrates scavenging on two impala (*Aepyceros melampus*) carcasses tore open the skin, particularly around the groin and lower abdomen, revealing substantial areas of soft body tissues. Carnivores, especially dogs, are

known scavengers of carrion and decomposing human bodies and may often remove bones (Mann *et al.* 1990). Richards & Goff (1997) found some mongoose depredation on one of their pig carcasses, despite the use of exclusion cages.

Normally one would associate scavenging by carnivores with hunger. In one documented case (Rothschild & Schneider 1997), where a 31-year-old man committed suicide through a single gunshot to the head, the victim showed extensive postmortem animal bite marks on the lower half of the face. A considerable amount of skin and subcutaneous fatty tissue was missing, especially in the areas of the nose, mouth and left cheek. This was caused by the victim's well-cared-for three-year-old Alsatian, and the scavenging occurred within 45 minutes (period between the fatal shot and discovery of the body).

Rodents have also been found to cause scavenger mutilation (Mann *et al.* 1990; Patel 1994). Rodent activity might be secondary to the mutilation initiated by household pets (rodents are frequently responsible for primary mutilation, especially at extremities of the body). It could also be an adverse modification of a pre-existing injury inflicted by human hands (Patel 1994). According to Mant (1977, in Patel 1994), insects may modify superficial antemortem skin injuries. It is consequently important to be aware of artefactual postmortem injuries to avoid misinterpretation in forensic cases. One such case was documented where the body of an elderly woman was found in her home, and aroused suspicion (Patel 1995). Blood was present on her face and hands, as well as on a table lamp. A red skin lesion on her nose resembled rodent activity. A postmortem skin biopsy revealed that the "pseudo-rodent" artefact was irritated dermatitis artefacta with no evidence of malignancy or subsequent damage by rodents.

Although blowflies oviposited in the open wound caused by scavenger mutilation on the carcass in the current study, desiccation and the low ambient temperature prevented the eggs from hatching. Oviposition behavior of blowflies in open wounds or other areas of trauma were also recorded by Mann *et al.* (1990). This consequently had no effect on the decomposition process itself. This is in direct contrast to findings by Ellison (1990). A single feeding visit by large vertebrate scavengers to an impala carcass (hanging by the

feet 2,5m above the ground from an *Acacia* tree) 10 days after death produced an extended sequence of decay. Willie & Snyder (1988, in Haglund *et al.* 1989) also pointed out that scavenging might significantly alter expected rates of insect succession. This happens when insect larvae are consumed together with body parts. This suggests that the temporal abundance of key insect families provide an inaccurate indication of PMI in circumstances where carcasses or bodies have been mutilated (Ellison 1990). Because scavengers may sometimes completely remove remains from the original site, it is crucial for forensic investigators to locate the original site (Haglund *et al.* 1989). The "drip zone" from bodies that have lain for a few days may indicate this. The PMI could also be established by means other than insect evidence, (Haglund *et al.* 1989) when scavenger damage has occurred. They propose that a sequence of carnivore scavenging and disarticulation exists in three stages whereby a postmortem interval can be established. Unfortunately, any assessment of a PMI with this method is extremely area-dependent and does not depend on a single criterion. Knowledge of the species of scavenger is also critical to any estimation of PMI when using this method.

2.3.2.2 Composition of arthropod orders

Diptera and Coleoptera were again the dominant orders at the decomposing carcasses during the winter trial (Fig 2.9). Low numbers of Hymenoptera and Aràneae were also present. The largest number of Diptera was observed at the carcass hanging in the sun and the smallest number at the carcass hanging in the shade. This corresponds to the rate of biomass removal as described above. The largest number of Coleoptera was recorded at the carcass hanging in the shade, with the smallest number at the prone carcass (Fig 2.9). The largest number of Hymenoptera was observed at the prone carcass with the numbers approximately (Fig 2.9) equal at the two hanging carcasses. Three spiders were observed at the prone carcass and one on the carcass hanging in the shade. The spiders found hunting on or around the carcasses were of the family Salticidae. Salticids are diurnal hunting spiders (Dippenaar-Schoeman & Joqué 1997). Bornemissza (1957) also found spiders of the family Salticidae associated with decomposing carcasses.

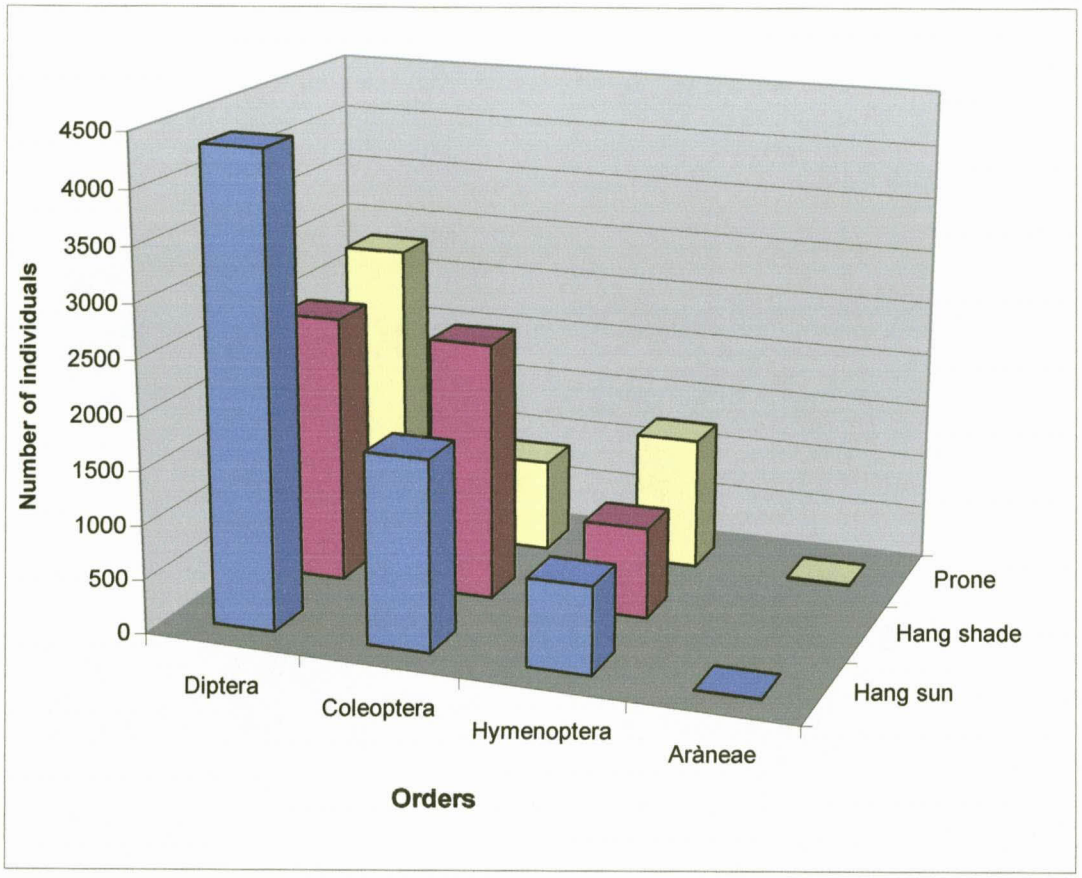

Fig. 2.9. Order composition recorded during the winter 1999 trial.

2.3.2.3 Diptera families

The dominant families of Diptera were Calliphoridae and Muscidae. Very low numbers of Sarcophagidae, Piophilidae, Asilidae and Syrphidae were recorded (Fig. 2.10). The largest and smallest numbers of Calliphoridae were found at the carcasses hanging in the sun and the shade respectively. The numbers of Muscidae did not differ significantly between carcasses. There was, however, a higher occurrence of adult Muscidae than Calliphoridae on the carcass that hung in the shade (Fig. 2.10).

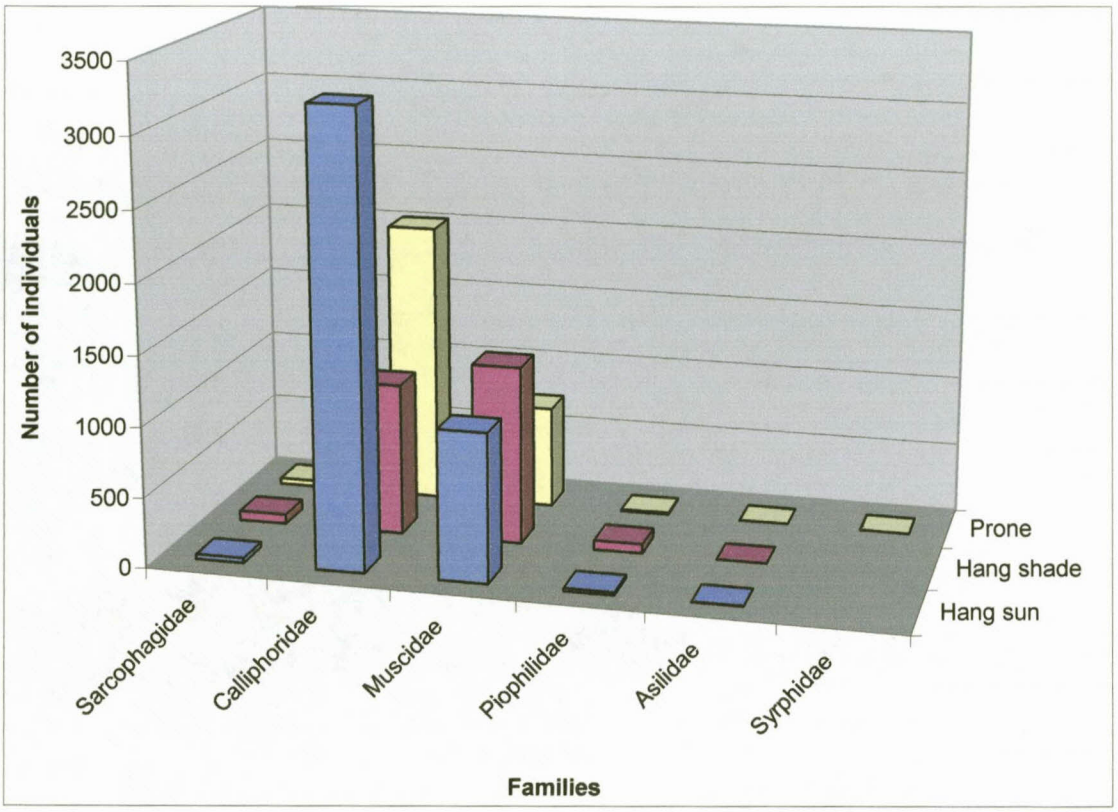

Fig. 2.10. Diptera families recorded during the winter 1999 trial.

2.3.2.4 Diptera species

The dominant Diptera species were *C. marginalis, C. albiceps* and *M. domestica* (Fig. 2.11). The largest numbers of C. *marginalis* and *C. albiceps* were recorded at the carcass hanging in the sun and the smallest numbers at the carcass hanging in the shade (Fig. 2.11). This correlated with the larvae of these two species being responsible for the removal of most tissue from the carcasses during decomposition. However, the numbers of *M. domestica* at the hanging carcasses were similar to each other, with a much lower number at the carcass lying on the soil surface (Fig. 2.11). Low numbers of *C. chloropyga* were observed at the prone carcass and the carcass hanging in the shade.

During a study by Boucher (1997), *C. chloropyga* were found in larger numbers during winter at the same experimental site.

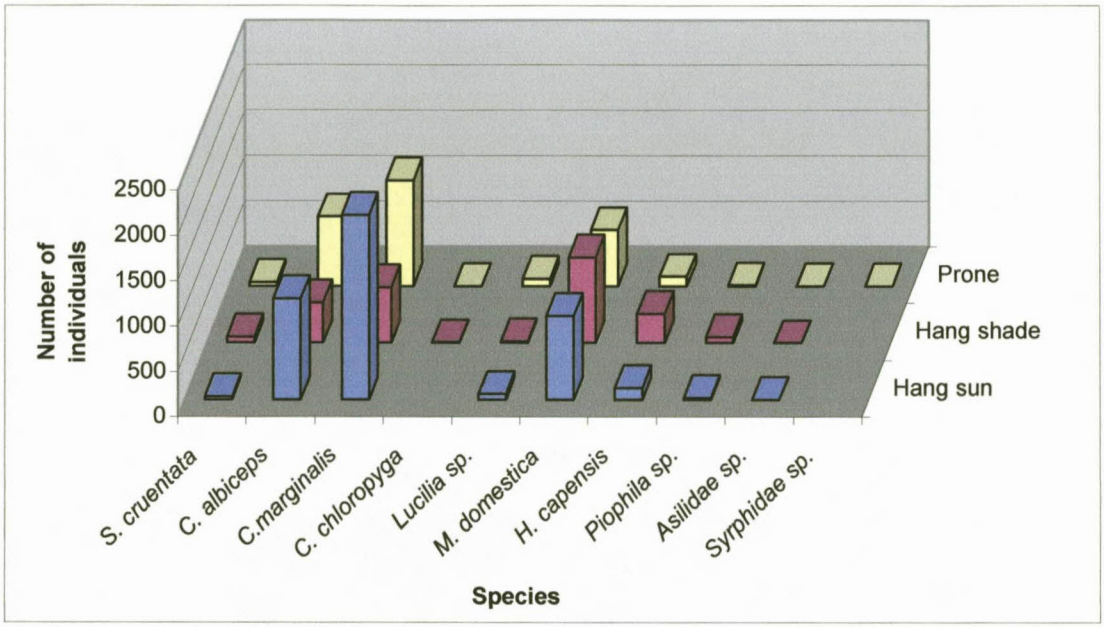

Fig. 2.11. Diptera species recorded during the winter 1999 trial.

2.3.2.5 Coleoptera families and species

As illustrated in Figure 2.12, the dominant Coleoptera families were Dermestidae and Cleridae. Each of these families was represented by only one species, *D. maculatus* and *N. rufipes* respectively. The highest occurrence of Dermestidae was observed at the carcass hanging in the shade. The lowest was at the prone carcass. The largest number of Cleridae was found at the prone carcass and the smallest at the carcass hanging in the shade. Lower numbers of unknown species of Histeridae, Scarabaeidae, Anobiidae and Coccinellidae were also observed at different carcasses (Fig. 2.12).

2.3.2.6 Hymenoptera families

One family, Formicidae, was the dominant Hymenoptera family that visited the carcasses during this study (Fig. 2.13). At the prone carcass more than 1200 individuals of this

family were recorded. At the hanging carcasses, the number of individual Formicidae were almost equal at about 800. At times the Formicidae were observed to carry away

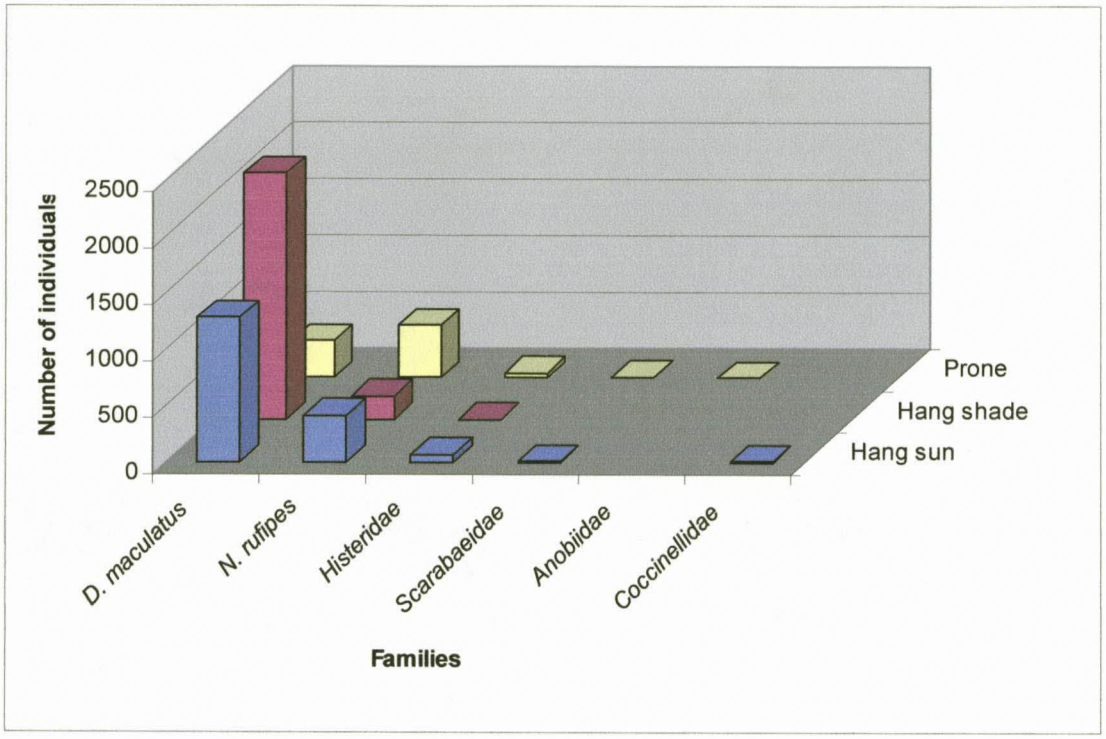

Fig. 2.12. Coleoptera families recorded during the winter 1999 trial.

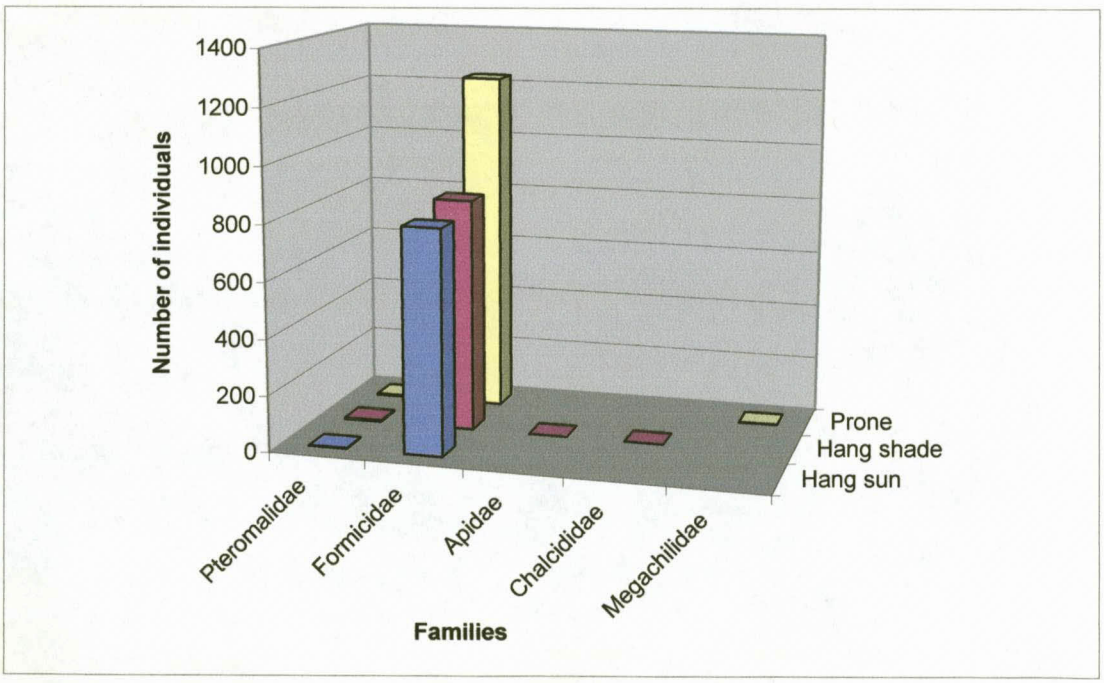

Fig. 2.13. Hymenoptera families recorded during the winter 1999 trial.

some of the eggs that were laid by blowflies. In some cases, the Formicidae also carried off living maggots that either fell from the carcasses, or were migrating to pupate. In Chapter 3 the succession of all the arthropods, including the Formicidae will be discussed, as the Formicidae were not continually observed. Low numbers (between one and six) of Pteromalidae, Apidae, Chalcididae and Megachilidae were also observed at different carcasses (Fig. 2.13).

2.3.3 Spring trial (14 September 1999 - 10 November 1999)

2.3.3.1 Rate of decomposition

During the spring 1999 trial, the mass loss of carcasses in the sun between days 9 and 17 was similar (Fig. 2.14). From day 17 to the end of the experiment, all the carcasses showed fluctuating mass loss. This could be due to the effect of rain. When it rained, the carcasses became moist and the mass increased. Of significance is the rate of mass loss for the two hanging carcasses. The curve representing the carcass hanging the sun and that representing the carcass hanging in the shade are almost identical between days 32 and 50. This shows that the rate of decomposition was the same for these two carcasses irrespective of the fact that at day 32 there was a 20 percent difference in the body mass remaining.

More tissue was removed from the carcass hanging in the sun than from the other carcasses. At the end of the experiment, the prone carcass and the carcass hanging in the shade had about 13 to 15 percent tissue remaining. The carcass hanging in the sun had 6.6 percent tissue remaining. One possible explanation could be that the carcass hanging in the sun was more desiccated than the other carcasses.

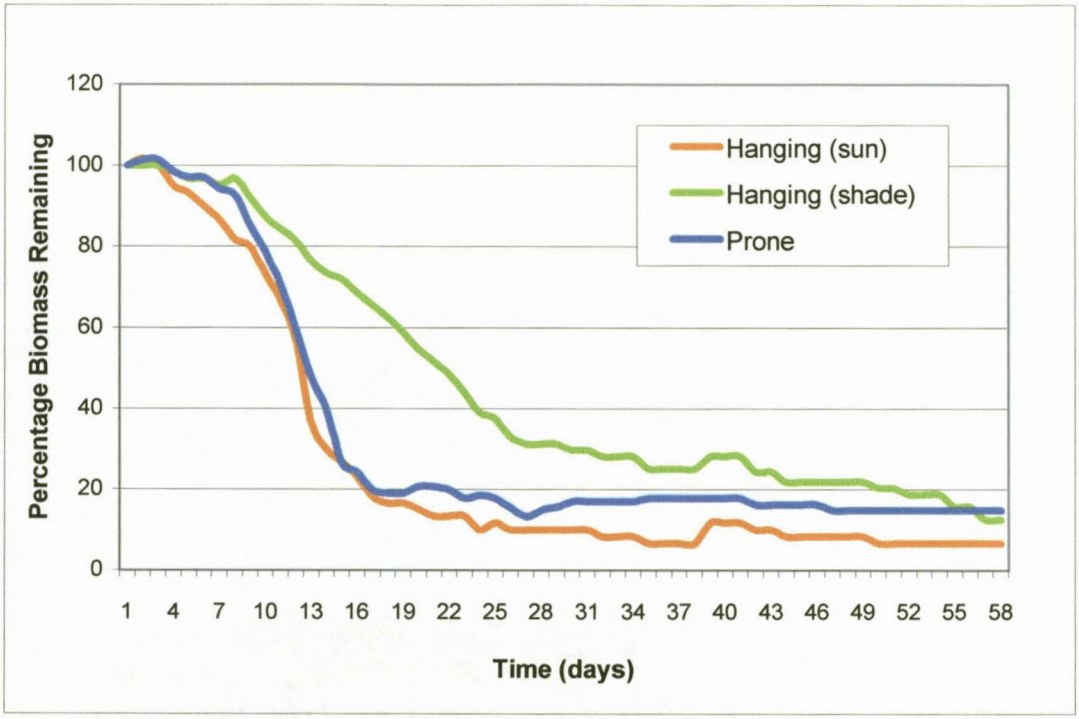

Fig. 2.14. Percentage biomass of the carcasses used during the spring 1999 trial.

2.3.3.2 Composition of arthropod orders

Diptera comprised the dominant order at all the carcasses (Fig. 2.15), while low numbers of Coleoptera and Hymenoptera were present as the second and third-most dominant orders respectively. Very low numbers (between one and seven individuals) of Orthoptera, Odonata, Lepidoptera and Blattaria were found at the prone carcass and the carcass hanging in the shade. The occurrence of 32 millipedes at the carcass hanging in the shade and three at the prone carcass is probably due to the amount of rain that preceded the experiment.

The carcass hanging in the sun was frequented by the largest number of individual Diptera, and the carcass hanging in the shade by the least. The largest number of Coleoptera was found on the carcass hanging in the shade and the least at the prone carcass. The largest number of Hymenoptera visited the carcass hanging in the sun and the smallest number visited the prone carcass (Fig. 2.15).

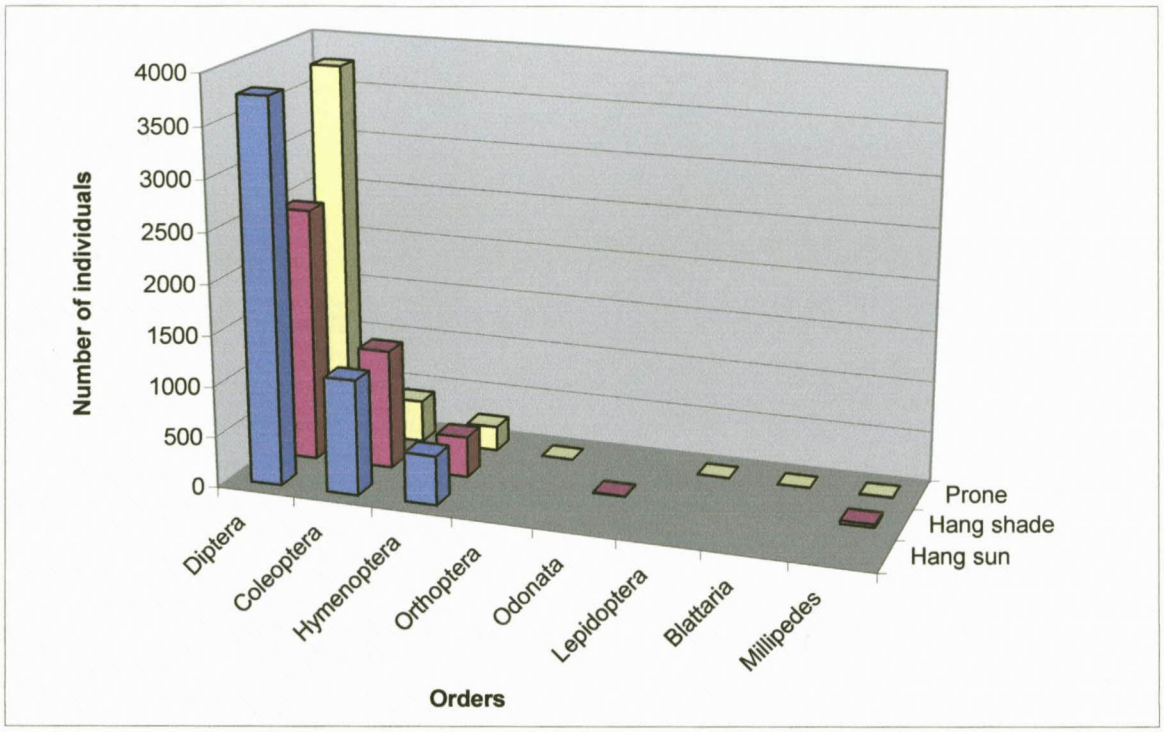

Fig. 2.15. Order composition recorded during the spring 1999 trial.

2.3.3.3 Diptera families

The dominant family of Diptera at all three carcasses was the Calliphoridae, with the largest number at the prone carcass and the smallest at the carcass hanging in the shade (Figure 2.16). The second-most dominant family was the Muscidae, with the largest number at the carcass hanging in the sun and the smallest at the prone carcass. Lower numbers of Sarcophagidae, Piophilidae and Asilidae were also observed (Fig. 2.16).

2.3.3.4 Diptera species

The dominant Diptera species during the spring trial was *C. chloropyga* (Fig. 2.17). The highest incidence of this species was found at the prone carcass (nearly 2800 adult individuals) and the lowest at the carcass hanging in the shade (1440 individuals). The second-most dominant species was *M. domestica*, with less than 500 adults at each carcass. It was evident that *C. chloropyga* was a very important forensic indicator species during spring, as the majority of tissue was removed from the carcasses by this species.

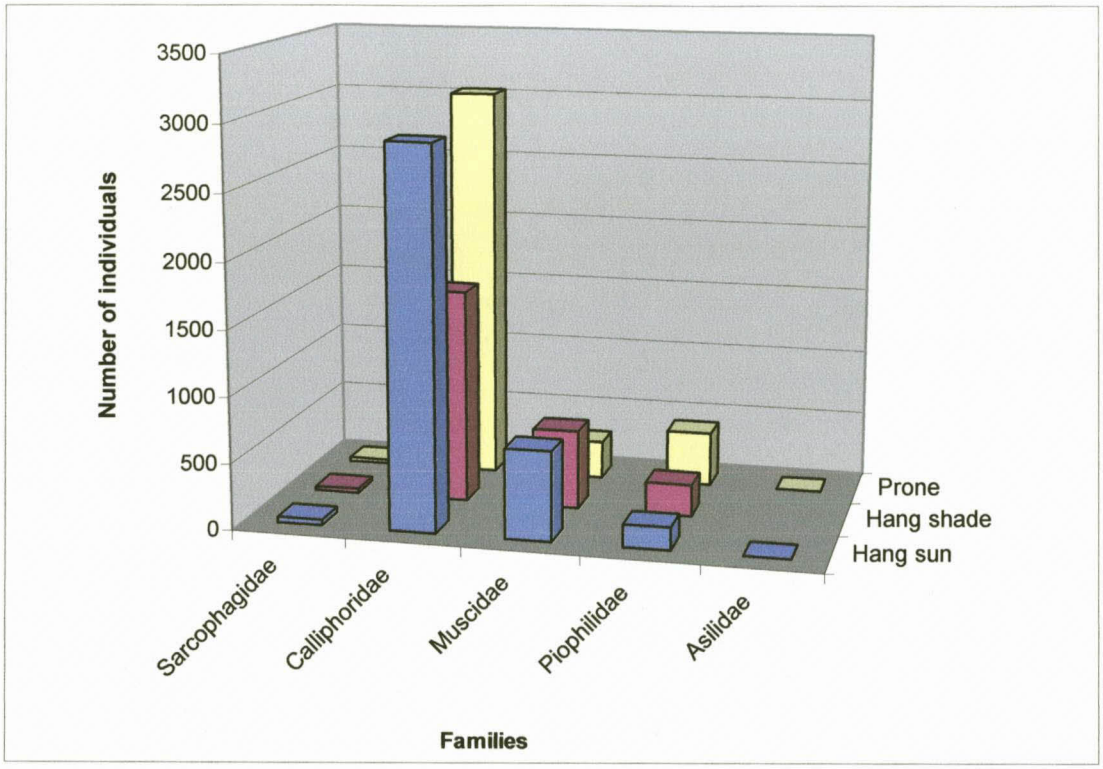

Fig. 2.16. Diptera families recorded during the spring 1999 trial.

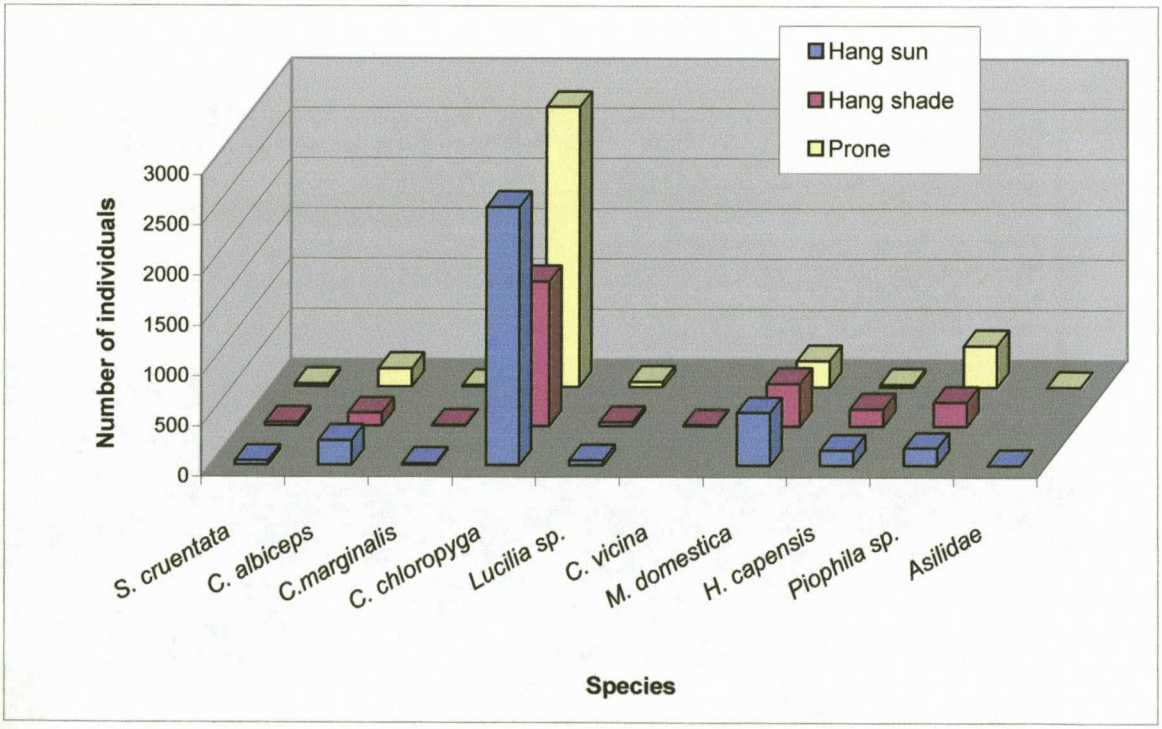

Fig. 2.17. Diptera species recorded during the spring 1999 trial.

2.3.3.5 Coleoptera families

The dominant Coleoptera family was Dermestidae, represented by *D. maculatus* (Fig. 2.18). The largest number of Dermestidae was found at the carcass hanging in the shade and the smallest at the carcass lying in sun. Lower numbers of Cleridae (represented by *N. rufipes*), Histeridae, Silphidae and Scarabaeidae were also found. The Silphidae and Scarabaeidae were only observed at the carcass hanging in the sun (Fig. 2.18).

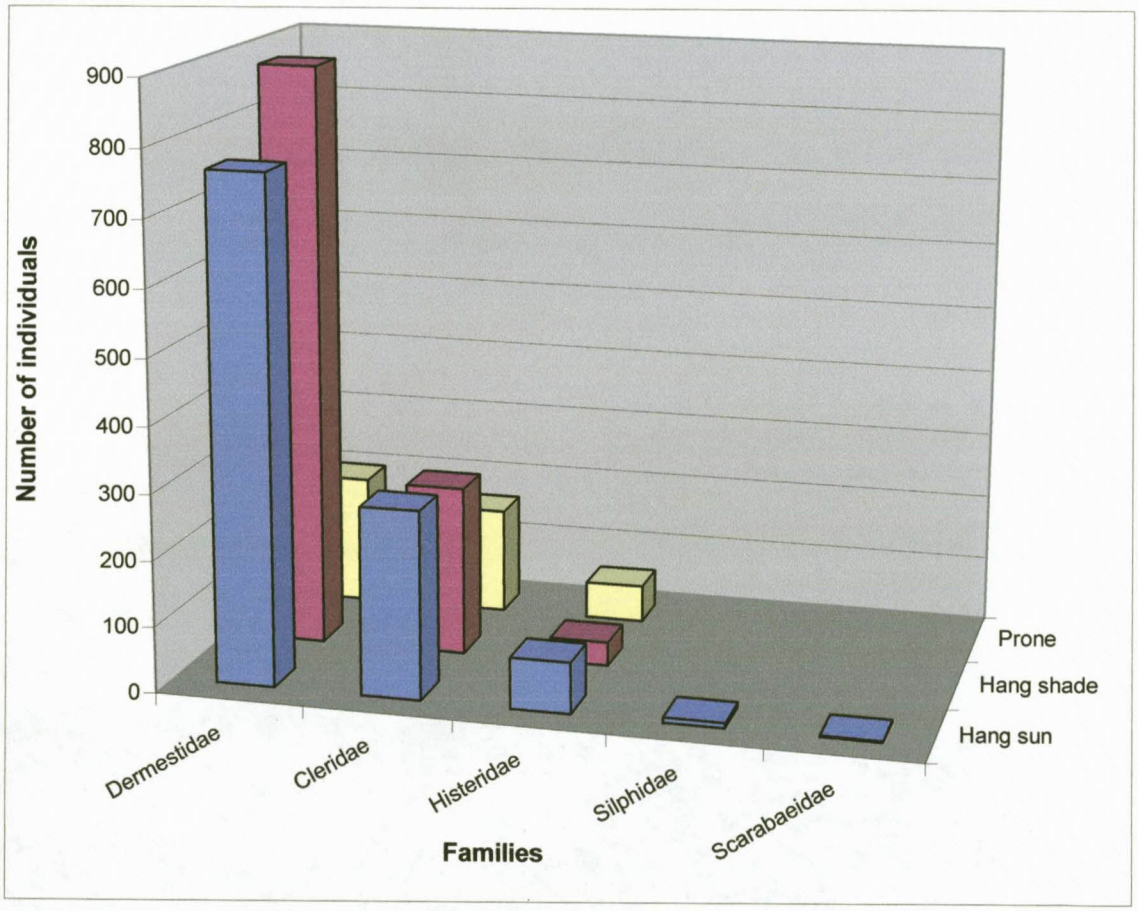

Fig. 2.18. Coleoptera families recorded during the spring 1999 trial.

2.3.3.6 Hymenoptera families

The dominant family of Hymenoptera was Formicidae with the highest incidence at the carcass hanging in the sun (at the drip zone) and the lowest at the prone carcass (Fig. 2.19). Lower numbers of Pteromalidae and Apidae were also found at some of the carcasses. Pteromalidae parasitise the blowfly pupae.

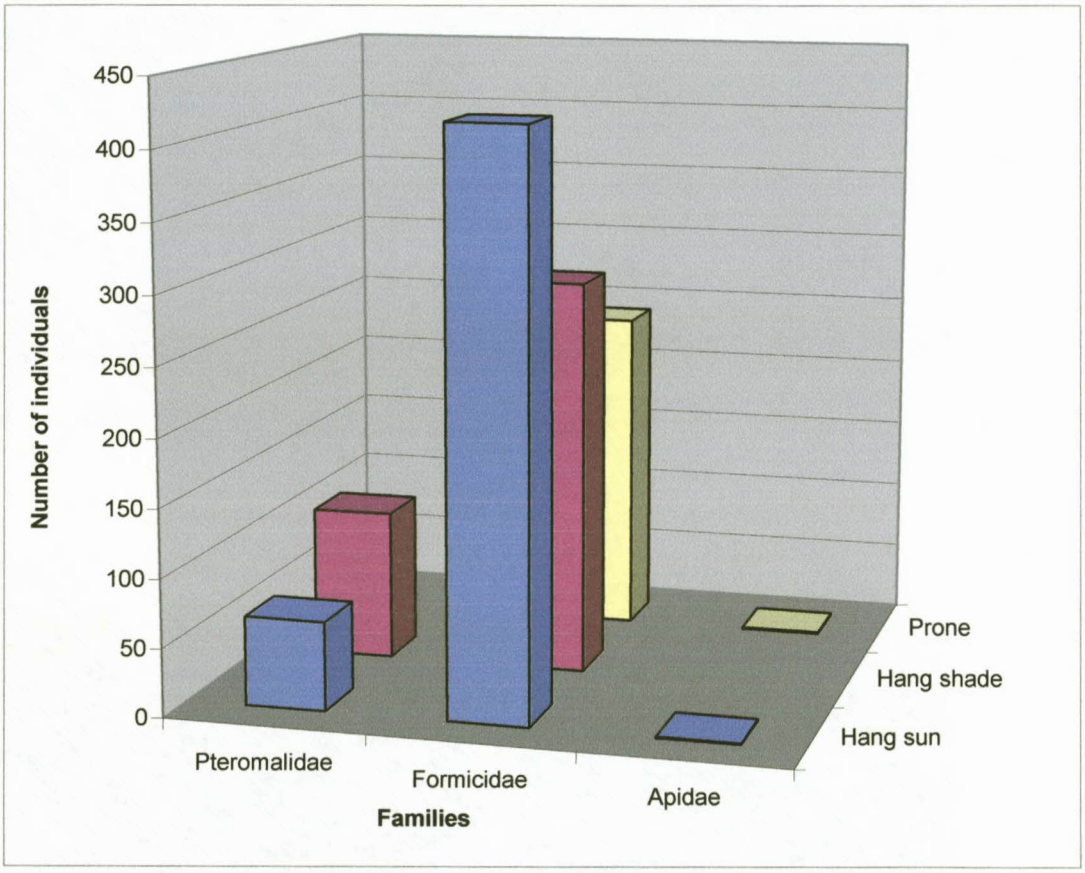

Fig. 2.19. Hymenoptera families recorded during the spring 1999 trial.

2.3.4 Summer trial (1 February 2001 - 22 March 2001)

2.3.4.1 Rate of decomposition

The biomass loss from the carcasses during this trial (Fig 2.20), was similar to that recorded during the summer of 1999 (Fig. 2.3). During this trial (summer 2001, Fig. 2.20), the prone carcass decomposed more rapidly than the other two carcasses. At the

end of the trial however, the percentage of body mass remaining was relatively similar for the three carcasses. It differed by three per cent between the carcasses hanging in the sun and the shade, while there was a three per cent difference between the carcasses hanging in the shade and lying in the sun.

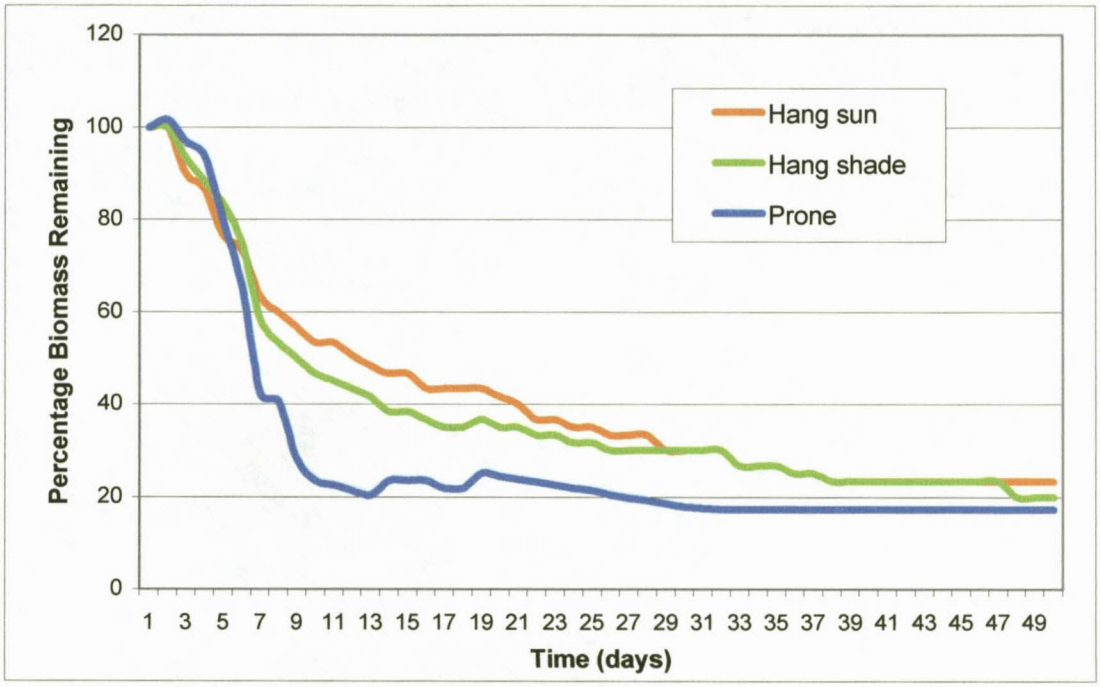

Fig. 2.20. Percentage biomass of the carcasses during the summer 2001 trial.

The greatest difference in the decomposition process was from day 6 until the end of the trial. The prone carcass reached 20 per cent of original body mass on day 13, while the carcass hanging in the shade only reached 20 per cent on day 48 (Fig. 2.20). The rate of decomposition was similar for the two hanging carcasses and followed a similar pattern. The only notable difference between the rates of decomposition of the two hanging carcasses was from day 8 to day 30, with the greatest difference in percentage body mass remaining being 8% (Fig 2.20).

2.3.4.2 Composition of arthropod orders

During this trial Diptera, Coleoptera, Hymenoptera, Hemiptera and Orthoptera represented the insects associated with the carcasses (Fig 2.21). Two spiders were also found on the carcass hanging in the sun (Family Salticidae). Hemiptera were represented by only one individual, which was found at the prone carcass. The two Orthoptera specimens were also found at this carcass. The dominant orders were Diptera and Coleoptera. The largest number of Diptera occurred at the prone carcass and the smallest at the carcass hanging in the sun. The largest number of Coleoptera was found at the carcass hanging in the shade and the smallest at the prone carcass. This corresponds to the situation found during the summer trial of 1999. Due to the large maggot masses forming on the prone carcass, most of the tissue was removed from the carcass before it became attractive to the Coleoptera. The hanging carcasses were more attractive to Coleoptera than the lying carcass due to the effect of convection, which enhanced desiccation.

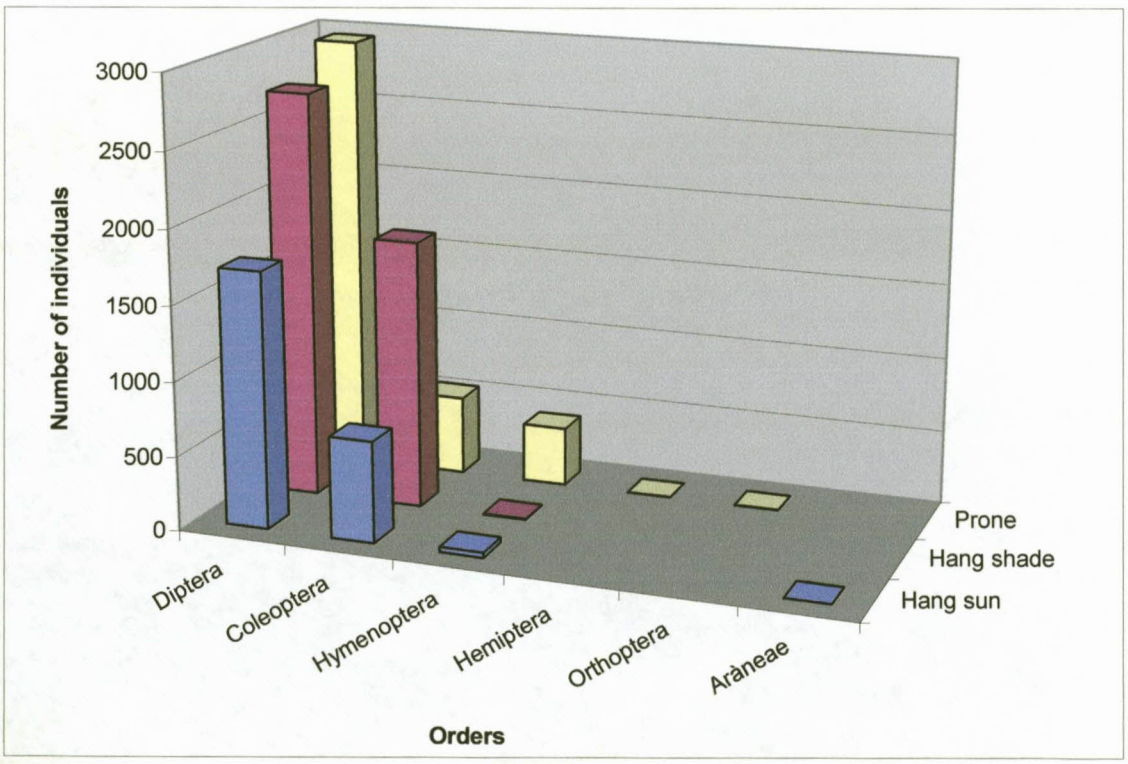

Fig. 2.21. Order composition recorded during the summer 2001 trial.

2.3.4.3 Diptera families

Calliphoridae and Muscidae were the two dominant families of Diptera during this trial. The largest aggregation of Calliphoridae was at the prone carcass and the smallest at the carcass hanging in the sun (Fig. 2.22). This corresponds to the order composition. The largest number of Muscidae was at the carcass hanging in the shade and the smallest at the prone carcass. Lower numbers of Sarcophagidae, Piophilidae and Asilidae were observed at the carcasses (Fig. 2.22).

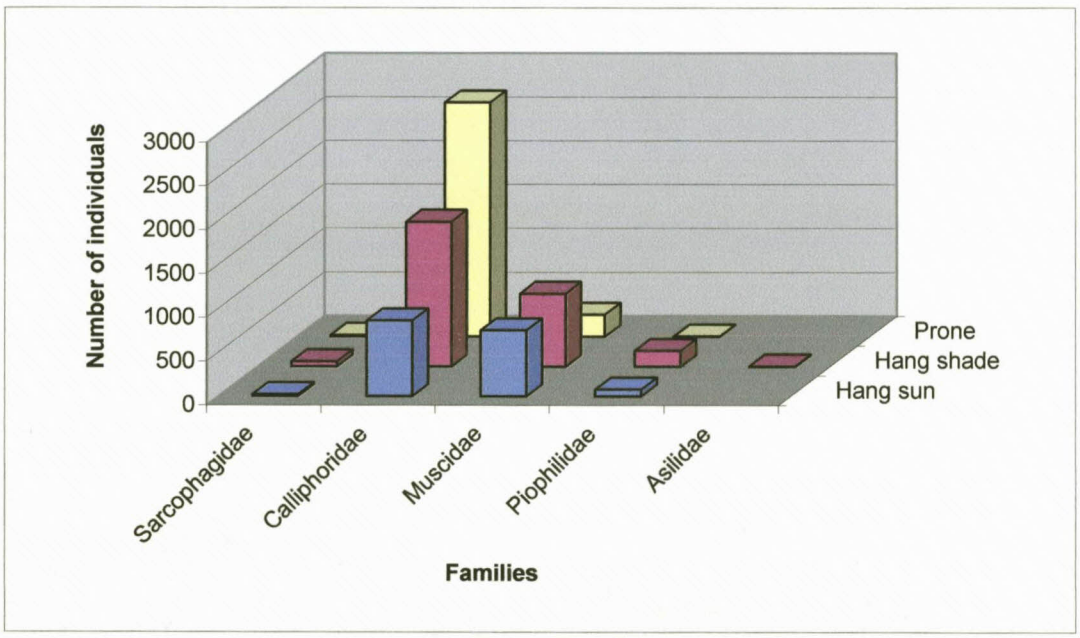

Fig. 2.22. Diptera families recorded during the summer 2001 trial.

2.3.4.4 Diptera species

The dominant Diptera species were *C. marginalis, C. albiceps* and *M. domestica* (Fig. 2.23). Small numbers of *S. cruentata*, *Lucilia sp.*, *H. capensis*, *Piophila sp.* and Asilidae were recorded (Fig. 2.23).

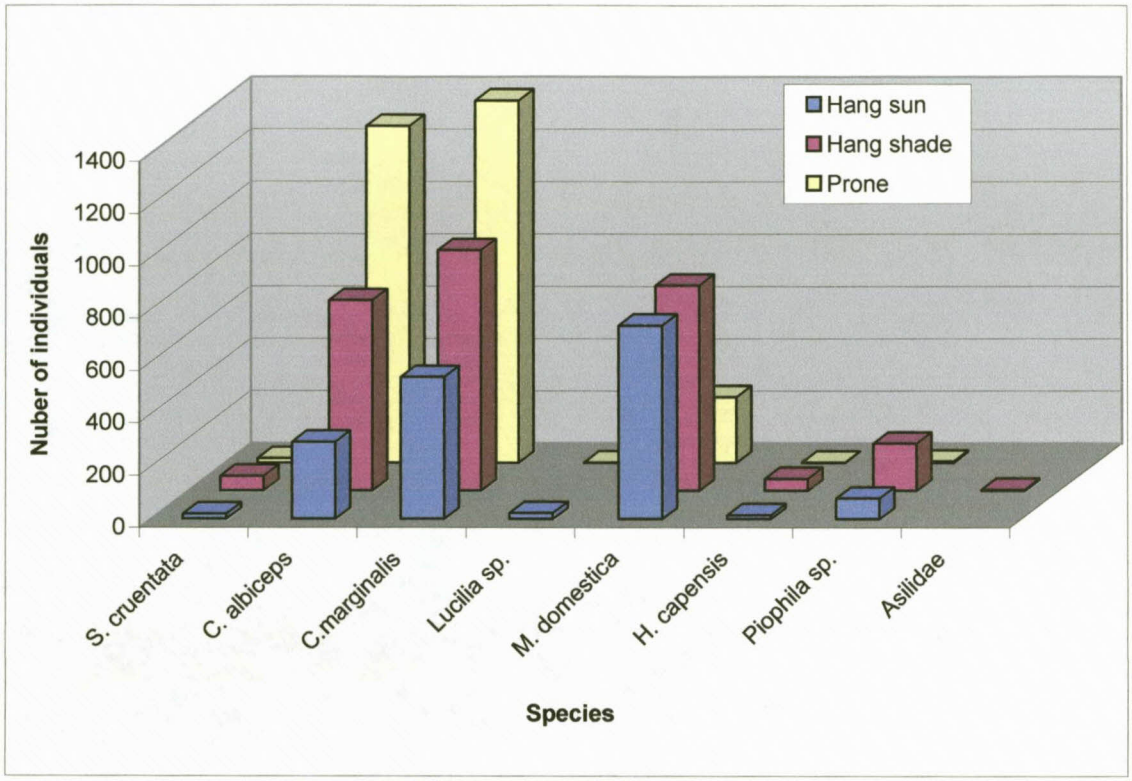

Fig. 2.23. Diptera species recorded during the summer 2001 trial.

2.3.4.5 Coleoptera families and species

Similar to previous field trials, Dermestidae and Cleridae were represented by one species each, namely *D. maculatus* and *N. rufipes.* The carcass hanging in the shade had the most Dermestidae and Cleridae and the prone carcass the least (Fig. 2.24). Unidentified specimens of Histeridae and Silphidae were also observed. The Scarabaeidae specimen that was found was identified as belonging to the subfamily Cetoniinae.

2.3.4.6 Hymenoptera families

The dominant family of Hymenoptera was again the Formicidae. No Formicidae were observed at the carcass hanging in the shade (Fig. 2.25). At the carcass hanging in the sun approximately 30 individuals were seen, but at the prone carcass this number reached

approximately 400. Low numbers of Pteromalidae were observed at all three carcasses. A single honeybee (Apidae) was observed at the carcass hanging in the shade.

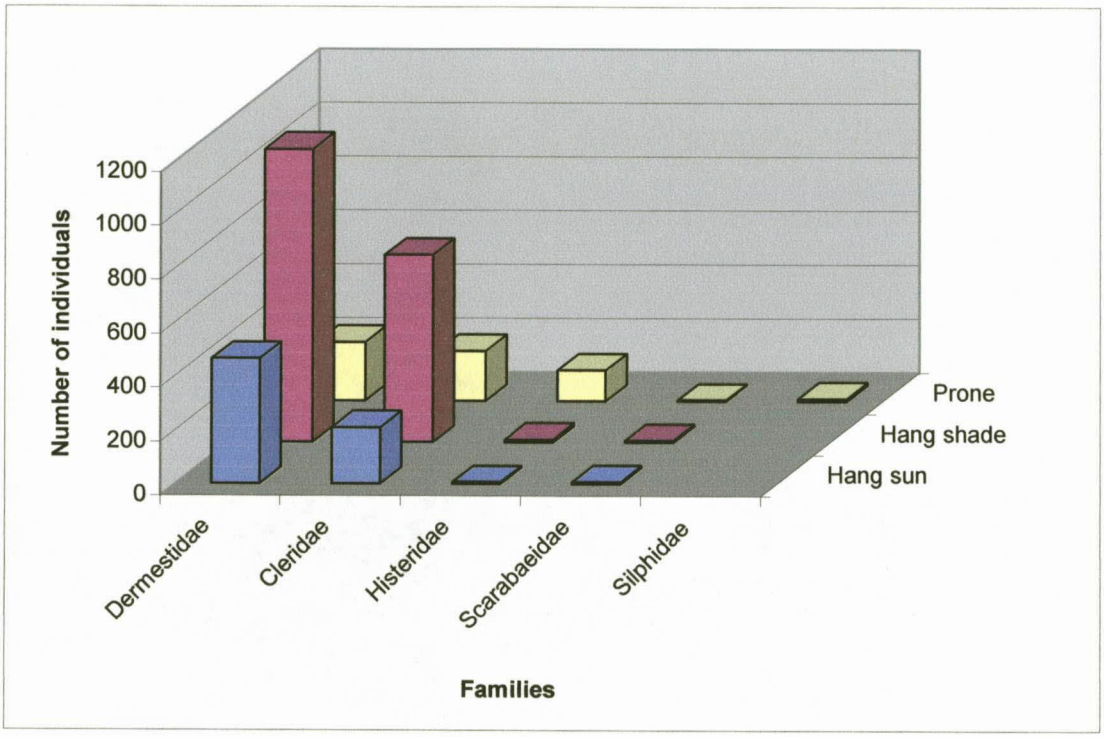

Fig. 2.24. Coleoptera families recorded during the summer 2001 trial.

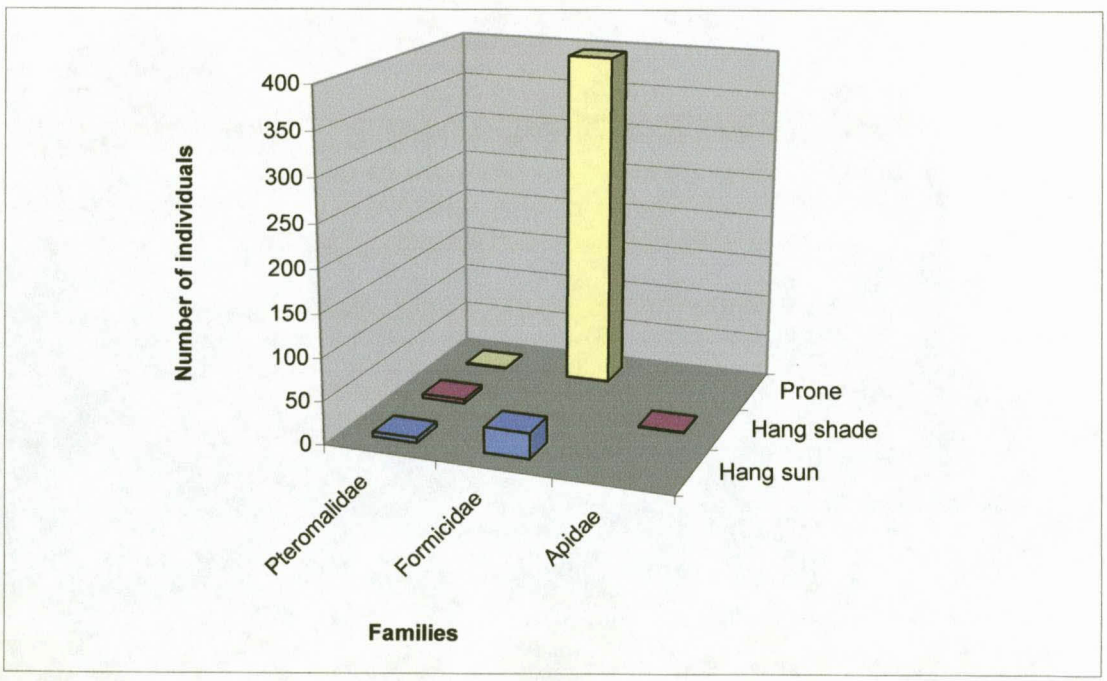

Fig. 2.25. Hymenoptera families recorded during the summer 2001 trial.

2.4. CONCLUSION

The greatest percentage of biomass removal occurred because of maggot feeding masses, an outflow of body fluids and desiccation. The rapid loss of carcass mass during the decay stage was the result of conversion of carcass biomass to dipteran larval biomass and the subsequent departure of these larvae from the remains to pupate. Differences in the rate of decomposition between study sites was also observed and attributed to the variations in temperatures between sites. The hanging carcasses decomposed at a slower rate than the carcasses lying on the ground. Some of the observed differences in mass loss between the exposed and shaded carcasses were probably due to the differential effects of dehydration on the different carcasses. The greatest difference in seasonal occurrence of insects found at the carcasses was observed for *C. chloropyga*. Large numbers of this species dominated the Diptera during the spring 1999 trial, while this species were rarely observed during the other trials.

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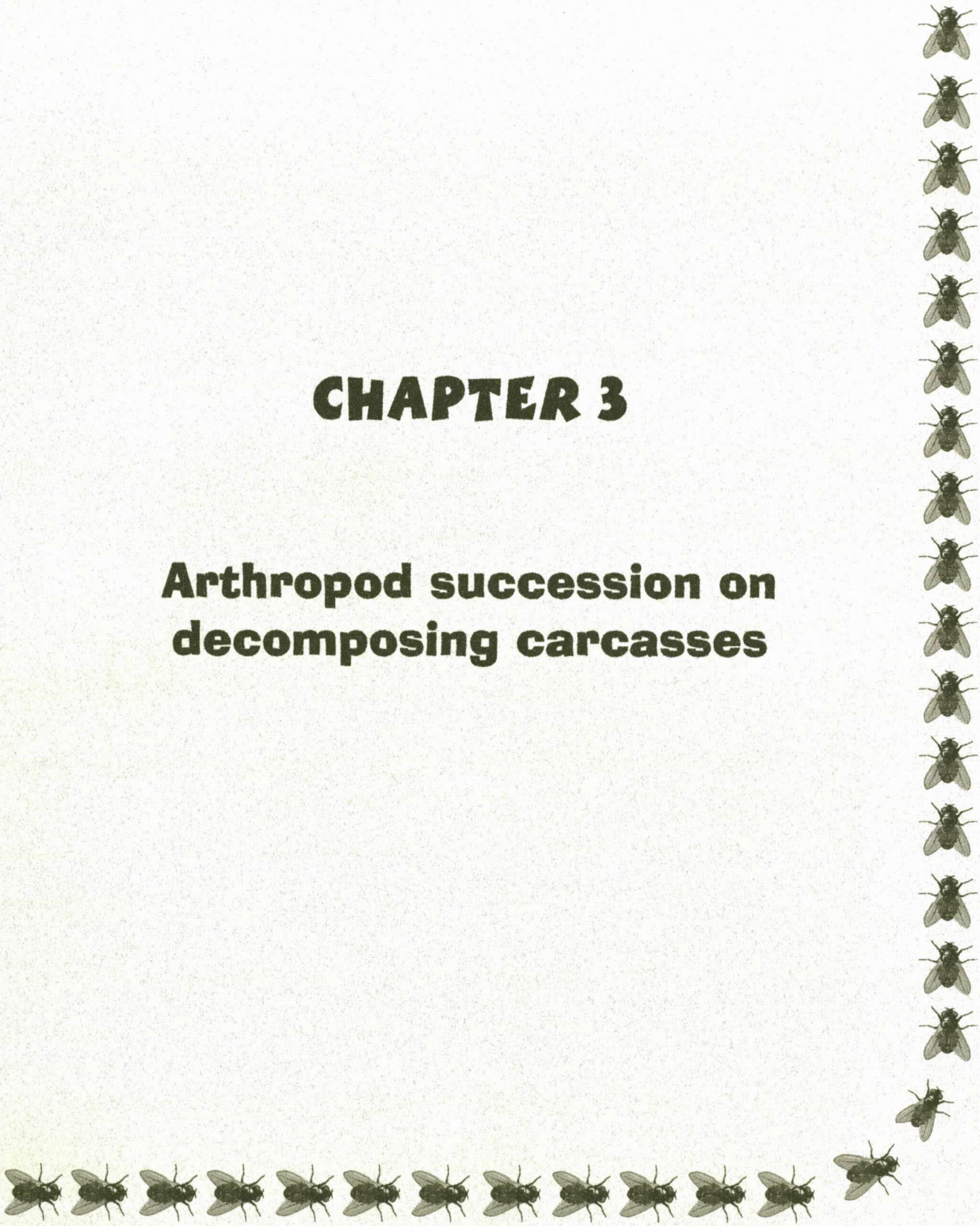
CHAPTER 3

Arthropod succession on decomposing carcasses



3.1. INTRODUCTION

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).

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, in Braack 1981) is generally regarded as the classic work in the field of carcass-inhabiting insects (Payne 1965; Tullis & Goff 1987).

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).

The key questions in this aspect of the study were:

- What is the general pattern of succession manifest by insects visiting the carcasses?
- Are there differences in the succession of arthropods on a hanging body versus a prone body?
- Are there differences in the succession of arthropods on a body hanging exposed in full sunlight compared to a body hanging in partial to full shade?

3.2. MATERIAL AND METHODS

3.2.1. Study site

The study site was the same as described in Chapter 2 (2.2.1.).

3.2.2. Conditions for field experiments and carcasses

The conditions for field experiments and carcasses were the same as those described in Chapter 2 (2.2.2.). Fuller (1934) also carried out experiments during all seasons to include insects that were present at different times of the year. In the study conducted by Braack (1981), none of the collecting methods proved effective for efficient collection of species that were present in very low numbers or which were rarely present at the carcasses. Rather than produce doubtful numerical results, Braack recorded insects as present, common or abundant. In the present study, it was decided to provide numerical results for all the insects observed.

3.2.3. Observations

The observations were the same as those described in Chapter 2 (2.2.3.).

3.3. RESULTS AND DISCUSSION

Since it was virtually impossible to estimate of the number of immatures, the following discussion refers to adult insects only.

3.3.1. Stages of decomposition

It was decided to use the following stages of decomposition during this study (see Chapter 2): fresh, bloated, active decay, advanced decay, and remains. These 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 following stages of decomposition were applied during this study:

- **Fresh:** From the time the carcass was placed in the field until bloating commenced (Payne 1965; Anderson & VanLaerhoven 1996).

  During the fresh stage of decay, blowflies and muscid flies were the primary insects observed. Their activities comprised feeding and reproduction. Oviposition by adult flies first occurred in the area of the face, eggs being deposited 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.

- **Bloated:** From the time that bloating commenced due to the accumulation of gases until the carcass starts to deflate due to loss of decompositional gases.

  Rodriguez & Bass (1983) observed carrion beetles at the onset of the bloated stage in association with cadavers.

- **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).

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

- **Advanced decay:** From the time that the maggot masses migrated from the carcass to pupate until only skin and bone remained (less than 15 percent of the original mass remaining). 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 large amount of weight comprising viscous material that constitutes by-products of decay (BOD).

- **Remains:** Only skin and bone remained (less than 15 percent of the original mass). 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.

  Dermestid beetles were observed feeding on the remaining dry tissue, hair, and fungus on cadavers during the onset of the dry stage (Rodriguez & Bass 1983).

According to Bornemissza (1957) the number of stages of decomposition that can be observed in a decaying vertebrate carcass is not only dependent on climatic and seasonal conditions, but also the type of soil may also have a modifying influence that should be taken into account.

3.3.2. Summer trial (3 February 1999 - 15 March 1999)

3.3.2.1 Hanging in sun:

The initial invaders of this carcass were *S. cruentata, M. domestica and C. albiceps* on day 1 (Fig. 3.1). *C. marginalis* and a single *C. chloropyga* arrived on the carcass on day 2. For the first week to one-and-a-half weeks, the numbers of C. *albiceps* and *C. marginalis* were high. During this time, the same pattern was applicable to both species. Initially the numbers were low, reaching a peak one or two days after the first arrivals.

This peak was maintained for one day and the numbers started to decline on the following day. In the case of *C. albiceps* there was a period of five days when no adults of this species were observed. The immatures were still feeding on the carcass or pupating in the soil. When this species was next observed, it was the newly emerged adults from the pupae. In the case of *C. marginalis* no adults were observed over a period of seven days. It appears that this time represented the pupal stage duration of the two species. *M. domestica* was only periodically observed during the second half of this study (from week 4 onwards).

Dermestidae and Cleridae were continually observed in high numbers from day 4 onwards. During the second half of this study (from week 4 onwards), these two families were only periodically observed (Fig. 3.1). The reason for this could be that observations were not carried out every day at this stage of the experiment, but every second to third day. Hewadikaram & Goff (1991) first observed beetles of the families Dermestidae, Histeridae, Staphylinidae and Tenebrionidae associated with carcasses between days 3 and 5 after death.

Adult and larval Dermestidae both occur in a variety of situations, with *D. maculatus* 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 *et al.* 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 *et al.* 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.

According to Clausen (1940, in Payne & King 1970) the genus *Necrobia* departs from the general food habits of Cleridae and the various species subsist largely as scavengers. In 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).

3.3.2.2 Hanging in shade:

The pattern of arthropod succession on this carcass (Fig. 3.2) was similar to the carcass hanging in the sun, the only difference was that higher numbers of Calliphoridae were observed at this carcass than at the carcass hanging in the sun. Higher numbers of Muscidae were also present.

3.3.2.3 Prone carcass:

The basic pattern of arthropod succession was also seen at this carcass (Fig. 3.3). *Sarcophaga cruentata* was observed more continually on this carcass than was the case with the other two. Lower numbers of Muscidae were observed. During the second generation of blowflies, extremely high numbers of *C. albiceps* and *C. marginalis* were observed. Cleridae and Dermestidae only occurred periodically and in low numbers on this carcass.

3.3.3. Winter trial (29 April 1999 - 1 September 1999)

Smit (1931) found that *C. albiceps* females do not lay eggs in winter in South Africa. This was not the case in the present study. *Chrysomya albiceps* did breed in the carcasses during winter, but in smaller numbers than during summer. Adults emerged in low numbers and were not as synchronised as during summer. Ullyett (1950) mentioned that in South Africa, *C. albiceps* is a summer carrion breeder, and maximum adult abundance occurs and coincides with breeding in carrion during this season. During winter, the metabolic rates of maggots are slowed down and the life cycle is extended owing to lower temperatures. This causes the succession pattern to be more easily observed (Braack 1987).

3.3.3.1 Hanging in sun:

During this trial, species of Calliphoridae and Muscidae occurred in large numbers continually for the first two-and-a-half weeks (Fig. 3.4). Thereafter the numbers declined gradually. Sarcophagidae occurred periodically in low numbers throughout this trial. Cleridae arrived on the carcass on the second day and Dermestidae only two days later. Both these families were continually present thereafter. Observations were not carried out every day at this stage of decomposition, hence the gaps between data.

Large numbers of ants (Formicidae) were periodically present on or around the carcass. 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). Ants are omnivorous, feeding both as necrovores and as predators. At the onset of carcass availability, ants are necrophilous and also prey on fly eggs (Schoenly & Reid 1983; Anderson & VanLaerhoven 1996). Ants use blood or moisture oozing from body cavities, prey on blow fly eggs and later prey on larvae or feed on organic detritus at the carcass (Braack 1987).

Formicidae also fed on the soft tissue of the carcass and caused feeding damage in the form of a characteristic pitted appearance formed by the removal of minute portions of tissue (Braack 1987). This generally occurs in winter when blow fly activity is reduced. In summer, ants only rarely have the opportunity to gain access to the carcass because the carcass is covered in a dense, highly active layer of maggots (Braack 1987). The current study has, however, shown that ants were present at the carcasses during early decomposition. Ants usually arrive long before maggots have developed (Mansell, pers. comm.)[1]. Ants were also predacious, carrying off blowfly eggs and sometimes may even carry away maggots over distances of 20 metres from large maggot masses (Van der Linde, pers. comm)[2]. Previous research has demonstrated that ants can dramatically

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retard dipteran larval activities and extend the time necessary for decomposition, or cause carcasses to remain untouched by maggots and available to vertebrate scavengers (Early & Goff 1986; Houston 1987, in Richards & Goff 1997).

3.3.3.2 Hanging in shade:

The insect succession followed the same pattern as on the carcass hanging in the sun (Fig. 3.5). Calliphoridae and Muscidae did not occur initially in such high numbers, as was the case for the carcass hanging in the sun. *Chrysomya marginalis* was observed until week 10 (Fig. 3.5), in contrast to week 5 for the carcass hanging in full sunlight (Fig. 3.4). Dermestidae were present in larger numbers than Cleridae and were more continually present than Cleridae. Formicidae, as in the carcass hanging in the sun, occurred periodically in large numbers. Piophilidae occurred more often on this carcass than on the other two.

3.3.3.3 Prone carcass:

Calliphoridae occurred in large numbers for the first two weeks (Fig. 3.6), thereafter only periodically in lower numbers. Formicidae were present more regularly in large numbers than on the other two carcasses. Cleridae occurred more continually on this carcass. Low numbers of *C. chloropyga* were observed during weeks 9, 10 and 12 (Fig. 3.6). Contrary to the observations made, *C. chloropyga* was expected to be observed early in the decomposition process.

3.3.4. Spring trial (14 September 1999 - 10 November 1999)

3.3.4.1 Hanging in sun:

The greatest difference in the insect succession between the winter and spring trial, was the large numbers of *C. chloropyga* that were observed (Fig 3.7). *Chrysomya chloropyga* arrived on this carcass on day one. *Lucilia* species were also observed in greater)

numbers than during the other trials. *Chrysomya marginalis* occurred in extremely low numbers. A higher incidence of Piophilidae was also found.

3.3.4.2 Hanging in shade:

Chrysomya chloropyga arrived on this carcass on day three (Fig. 3.8). Again, *C. marginalis* only occurred in extremely low numbers. *Calliphora vicina* was observed in small numbers for the first time during the study. In their work on the insect infestations of small corpses, Blackith & Blackith (1990) found that one of the calliphorids emerging in large numbers from small corpses was *C. vicina*. This species apparently did not breed in the carcasses in the current study. Tantawi *et al.* (1996) found that *C. vicina* was well represented in carrion (in Egypt) in winter only, indicative of a preference for cooler temperatures. In a study by Lane (1975), the majority of *Calliphora* caught in traps on corpses were females visiting the corpse for oviposition and feeding, while the males (nectar feeders only) visited the corpse only for copulation with the females. Blackith & Blackith (1990) mentioned that the fate of the vast numbers of small corpses, that become available to carrion feeders in some habitats, remains almost entirely undocumented. In effect, this means that a huge biomass of potential breeding sites of insects of potential forensic importance has been overlooked.

Piophilidae were present for a longer period than at the carcass hanging in the sun. Several millipedes (Class Diplopoda) were observed near the decomposing carcass later in the study, after heavy rains. These millipedes may have been predators (Borror *et al.* 1989), or may have fed on the decomposing remains (Hoffman & Payne 1969, in Catts & Haskell 1990). Upon closer examination it was found that the millipedes was scattered over the entire experimental site.

3.3.4.3 Prone carcass:

Muscidae were not present as continuously as on the other two carcasses (Fig. 3.9). Dermestidae occurred much later than Cleridae on this carcass. The number of

Dermestidae was lower on this carcass relative to the other two carcasses. Histeridae were also periodically present during weeks 2 and 3 (Fig. 3.9).

3.3.5. Summer trial (1 February 2001 - 22 March 2001)

3.3.5.1 Hanging in sun:

During the other seasonal trials, the carcasses were placed in the field by midday or late afternoon and the first observations were made the following morning. During this trial, the carcasses were placed in the field early in the morning and the first observations were made 2 to 3 hours later. For this reason the first adult insects were observed on the carcasses on day 0, compared to previous trials (Fig. 3.10). The first arrivals on this particular carcass were *S. cruentata, C. marginalis, Lucilia* spp. and *M. domestica. Chrysomya marginalis* was observed in large numbers during the first five days and again in low numbers in the middle of week three (second generation). Piophilidae were present throughout the study in low numbers. Cleridae and Dermestidae were recorded from day 3 until late in week 7. In comparison with other carcasses during previous seasonal studies, the numbers and occurrence of *H. capensis* were extremely low. Formicidae were only recorded twice.

3.3.5.2 Hanging in shade:

The basic pattern of succession was similar for this carcass with *S. cruentata* and *M. domestica* arriving on the carcass on day 0 (Fig. 3.11), whereas *C. albiceps, C. marginalis* and *H. capensis* did so on day 1. *Sarcophaga cruentata* also occurred more frequently than on the carcass hanging in the sun. The patterns of succession of Dermestidae and Cleridae (Fig. 3.11) were similar to those on the carcass hanging in the sun (Fig. 3.10).

3.3.5.3 Prone carcass:

The succession pattern of insects on the prone carcass differed from the pattern of succession on the hanging carcasses (Fig 3.12). Lower numbers of adult *C. albiceps* and *C. marginalis* were observed on this carcass during the first week, with the newly emerged adults from the second generation recorded late during the second week. Calliphoridae were not recorded later than the middle of week 3, in contrast to the situation on the hanging carcasses (Figs 3.10 – 3.12). Two species of Formicidae were observed at the beginning of the study and near the end. Histeridae occurred in larger numbers and more frequently at this carcass than on the two hanging carcasses. The occurrence of Dermestidae and Cleridae was similar to the other two carcasses, the difference being the smaller numbers of individuals observed (Fig. 3.12).

3.4. GENERAL DISCUSSION

It was found that the dominant insect orders were Diptera and Coleoptera. The carcasses were initially invaded by Diptera that were gradually replaced by Coleoptera at each of the carcasses. These two orders were the main contributors to decomposition of the carcasses. The only other order that had a major impact during the study was Hymenoptera. Diptera were attracted to the decomposing carcasses during the early and middle stages of decomposition, while Coleoptera were attracted to the carcasses during the middle and late stages of decomposition.

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).

Adult Calliphoridae and Sarcophagidae were the first insects to arrive, within five minutes of exposure. These observations were in agreement with research done by Hall & Doisy (1993). *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart) were observed by Tullis & Goff (1987), and by Hewadikaram & Goff (1991), as the first adult insects to arrive on pig carcasses in Hawaii. In the study by Richards & Goff (1997), it was 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. 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). Braack (1987) also noted partitioning of the carcass resource. In Braack's study, conducted in Africa, two species in the family Calliphoridae, *C. regalis* (*C. marginalis*) and *C. albiceps* mimic the pattern observed in Hawaii in *C. megacephala* and *C. rufifacies*, respectively. 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*. Braack (1987) noted that *C. regalis* (*C. marginalis*) would oviposit first, followed by the predatory *C. albiceps*. This also occurred during the current study.

A gravid female blowfly can detect the presence of a carcass over great distances (Erzinçlioğlu 1996). With the use of radioactively labelled blowflies and baited traps, Braack & Retief (1986) found that *C. albiceps* and *C. marginalis* dispersed from a central release point over distances of 37.5 kilometres and 63.5 kilometres respectively (incorrectly cited as Braack 1981, in Anderson 2001). Shean *et al.* (1993) observed that blowflies were attracted to carcasses within 20 minutes of death and oviposition was observed 2 to 3 hours later. In the study by Schoenly & Reid (1983), adult Diptera were collected in high 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.

The start of arthropod succession on a carcass is marked by oviposition (Avila & Goff 1998). 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. In the present study blowflies oviposited in the natural openings such as the mouth, nose, eyes, ears and anus. Large numbers of eggs were also laid where the carcass touched the ground (at the prone carcasses). According to Anderson & VanLaerhoven (1996), this is caused by the possible attraction to the areas of body fluid accumulation and seepage.

The presence of blowflies is of utmost importance to the carrion insect complex. Blowflies are a crucial determinant of community structure, despite the large array of resources available at the carcass habitat. A large number of species depend either directly on the blowfly larvae as a food source or indirectly on the influence of maggots on the carcass. The activity of maggots not only has a tremendous accelerating effect on carcass decomposition, but their effects additionally provide a wide range of different species with their particular food and space requirements (Braack 1987). Calliphoridae larvae are important to the onset of decay in the carcasses. This was evident from a study by Johnson (1975) where carcasses were placed in an area during the time of year when the flies were not breeding. The carcasses went through a different decomposition process that skipped the decay stage. Braack (1986) found that *C. albiceps* and *C. marginalis* were important determinants of community structure due to the larvae dominating in the use of carcass soft tissues, and in serving as an abundant prey item. Schoenly & Reid (1987) suggested that the establishment of later arrivals on a carcass is dependent upon the modifying actions of early colonists. The presence of maggots and their duration of tenure is a prime factor in determining succession at carcasses (Braack, 1987).

It was only after advanced carcass decay had commenced that a rapid decline in numbers of adult flies was observed. Braack (1987) also observed that pupation marked the end of the most intense period of activity at the carcass, resulting in a steep decline in the number of species present at the carcass. No synchronised departure of larvae occurred during Braack's study (1981), but pupation was rather indicated by the gradual diminishing in larval numbers over a period of approximately four days, commencing on the sixth day after death. The present study yielded similar results.

The difference in the succession of arthropods was most remarkable between:

- the same families on different carcasses (*e.g.* Calliphoridae on prone and hanging carcasses)
- between different families on the same carcass (*e.g.* Calliphoridae and Dermestidae on prone carcasses)

Several other orders and families of insects were also observed at the carcasses, but these insects did not play a major role in the decomposition process.

Based on the analysis of cases from the island of Oahu, Hawaii (Shalaby *et al.* 2000) the number of arthropod taxa invading a hanging body is far less than that encountered in a body decomposing in contact with the soil, regardless of habitat. This was not as clear during the present study.

Arthropod succession, as observed by Tantawi *et al.* (1996), followed the same general pattern in both tropical and temperate areas. Adult Diptera (especially Calliphoridae and Sarcophagidae) rapidly invaded carcasses and the arthropod diversity reached its maximum with the presence of dipterous larvae and adult Coleoptera. Thereafter a distinct decline occurred in arthropod richness and dispersal from the carcasses.

The arrival times of different species of insects is influenced by geographical region. In some cases, time of carrion colonisation may be more closely related to peak seasonal appearance of species, rather than the state of decomposition of the remains. Data

generated in one region or biogeoclimatic zone should therefore not be used to determine the PMI in a different region. Databases should be developed in which insects are used to determine the PMI for every biogeoclimatic zone (Anderson & VanLaerhoven 1996; Anderson 2001).

The seasonality of certain insects and the potentially differing times of colonisation of the remains in different seasons are important for several reasons (Anderson 2001):

- Carrion studies should be performed throughout the year to develop a valid database for an area.
- Insects may be valuable in determining the season of death, since some species only occur during certain seasons (for example when remains are discovered several years after death, even if the insects are of little use in determining a precise PMI).

3.5. CONCLUSION

The carcasses were initially invaded by Diptera during the onset of decomposition and were gradually replaced by Coleoptera. Diptera preferred the carcasses during the moist stages of decomposition while Coleoptera preferred the carcasses during the drier stages.

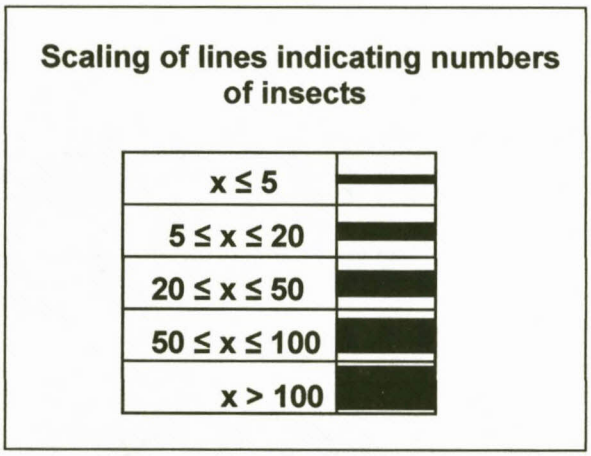
Dermestidae and Cleridae occurred in larger numbers on the hanging carcasses compared to the prone carcasses during all seasons. More Muscidae were observed on the hanging carcasses than on the prone carcasses during the warmer seasons.

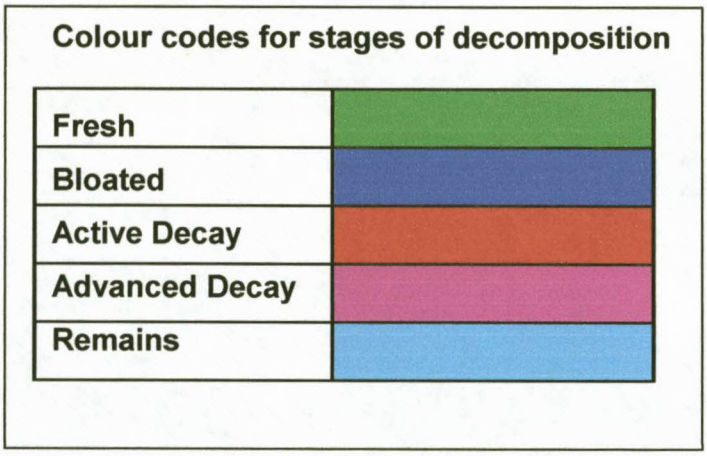
The differences in the succession of arthropods on carcasses/decomposing bodies in different orientations may lead to an incorrect estimation of the PMI. Studies that highlight these differences are of utmost importance.

3.6. SUCCESSION DIAGRAMS

Fig. 3.1 – 3.12 Black lines depict arrival, occupation and departure times for adult arthropods at the carcasses, while the height of the lines indicate the relative abundance of the individuals.

The following scale was used:





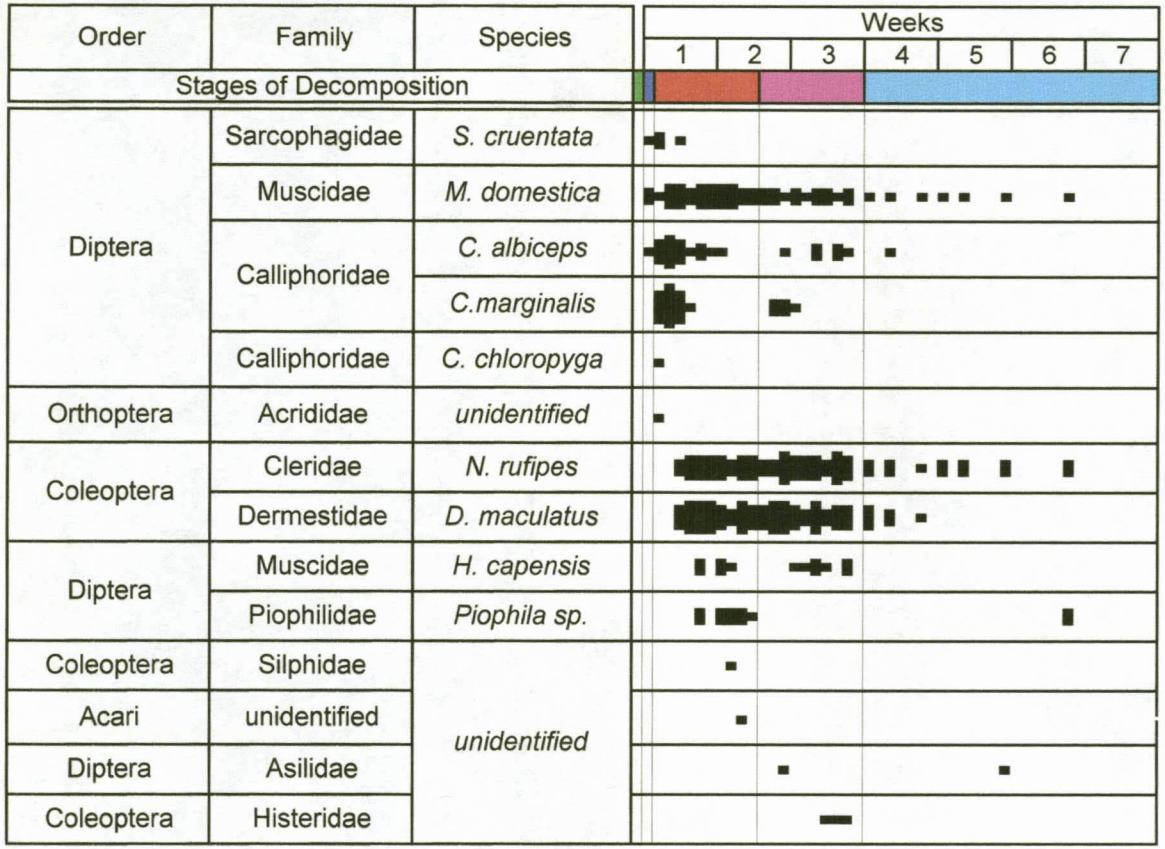


Fig. 3.1. Succession diagramme of arthropods associated with a pig carcass hanging in full sunlight during summer (3 February 1999 - 15 March 1999)

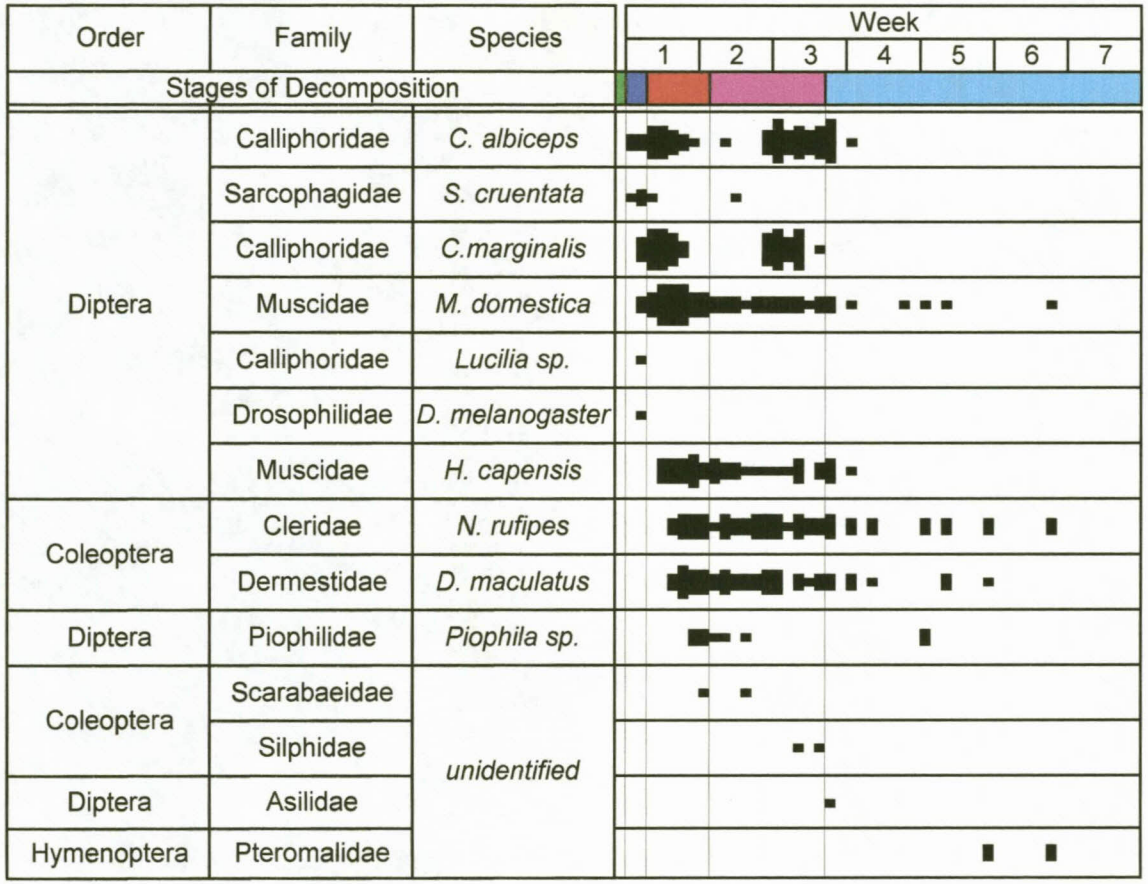


Fig. 3.2. Succession diagramme of arthropods associated with a pig carcass hanging in shade during summer (3 February 1999 - 15 March 1999)

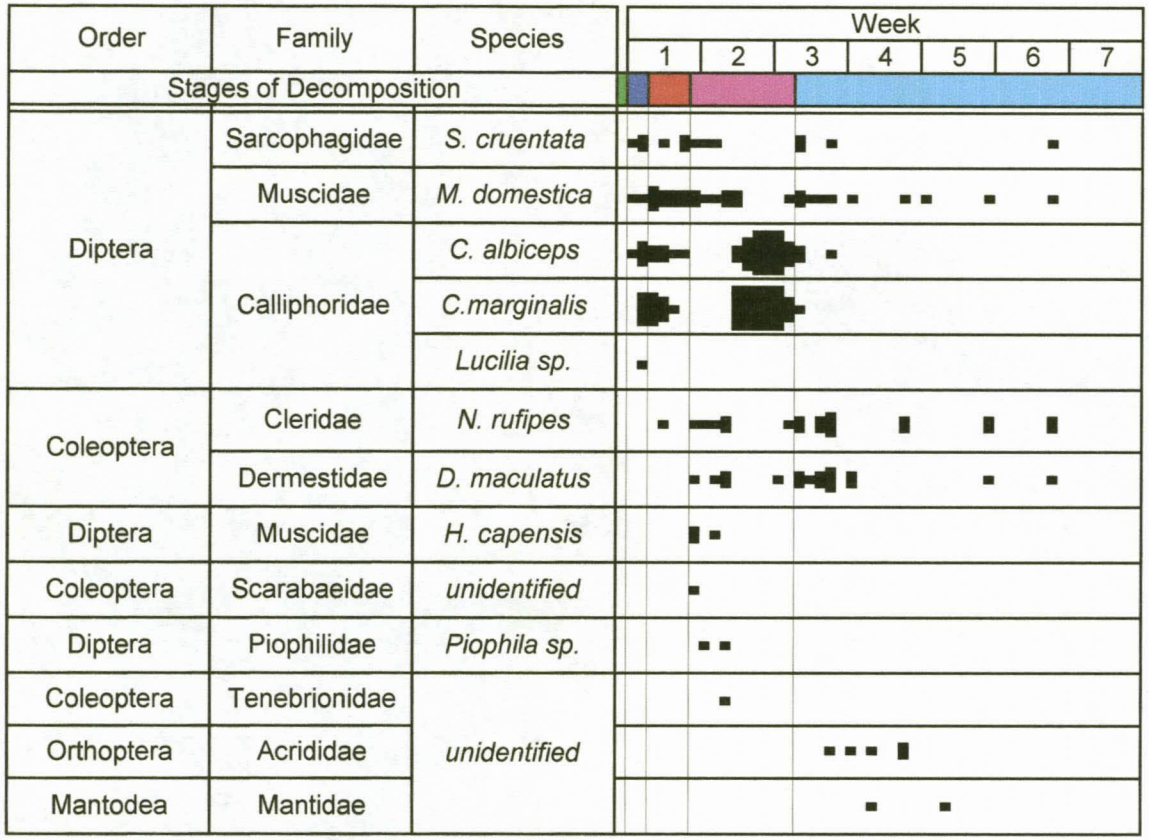


Fig. 3.3. Succession diagram of Arthropoda associated with a pig carcass lying in full sunlight during summer (3 February 1999 - 15 March 1999)

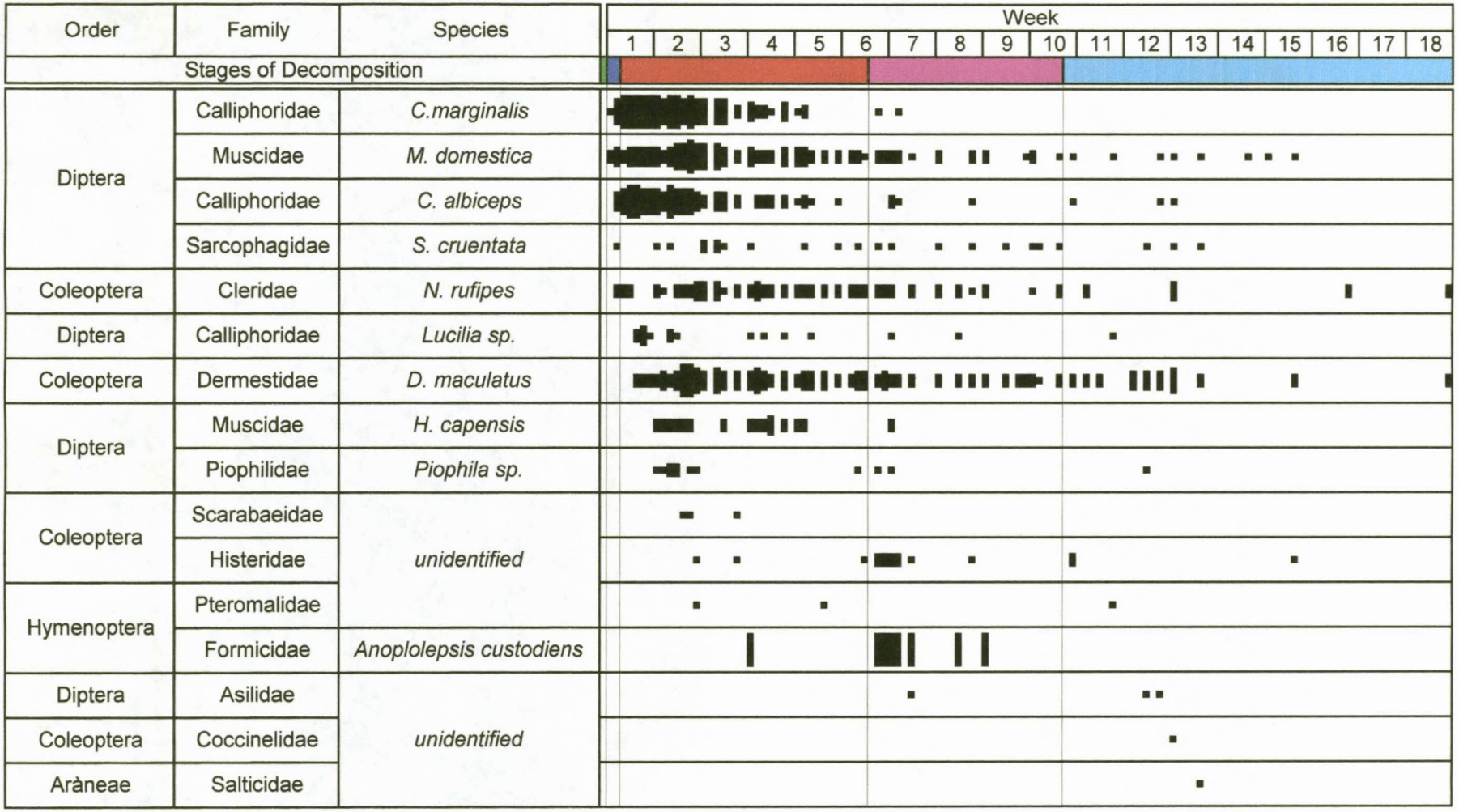


Fig. 3.4. Succession diagram of Arthropoda associated with a pig carcass hanging in full sunlight during winter (29 April 1999 - 1 September 1999)

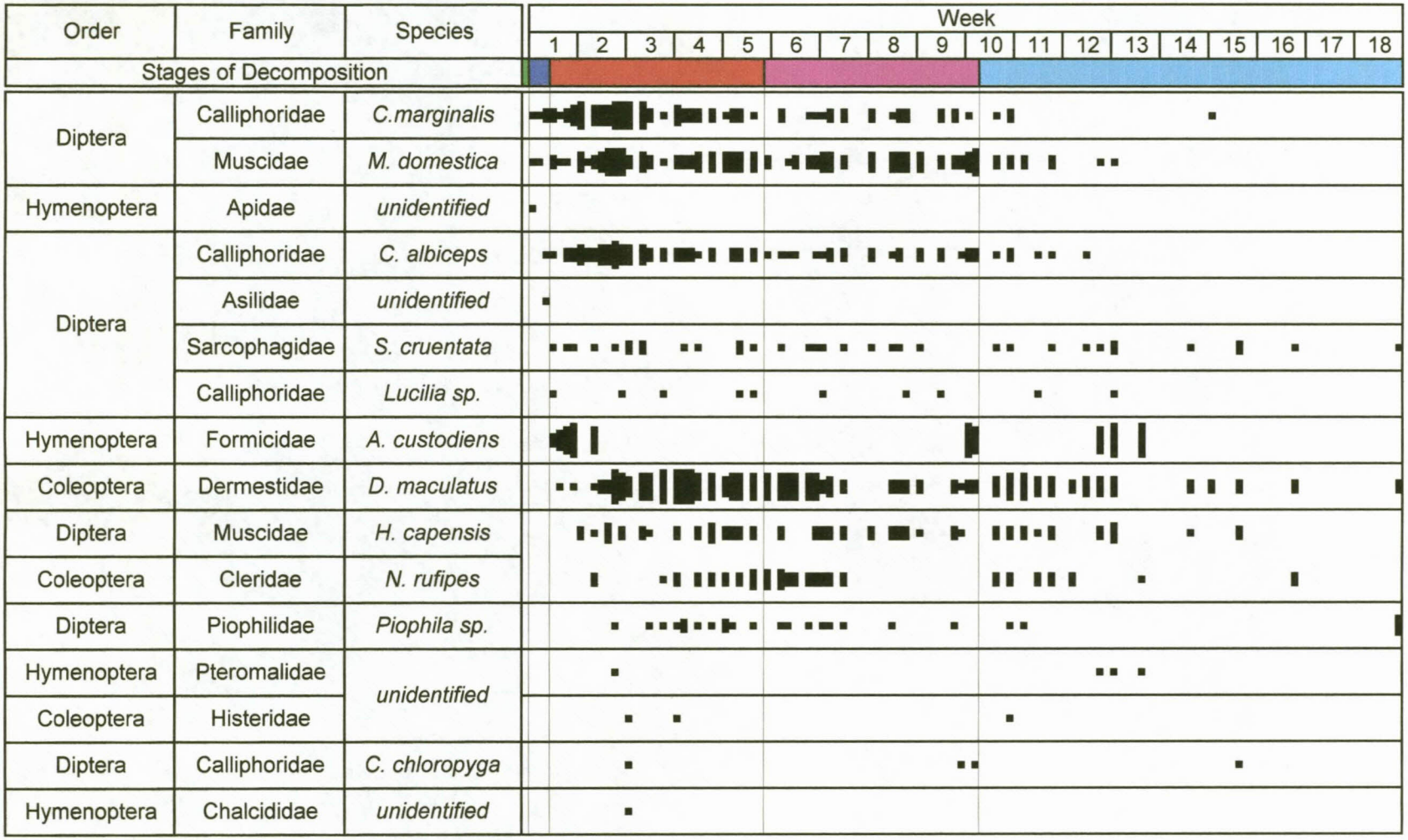


Fig. 3.5. Succession diagramme of arthropods associated with a pig carcass hanging in shade during winter (29 April 1999 - 1 September 1999)

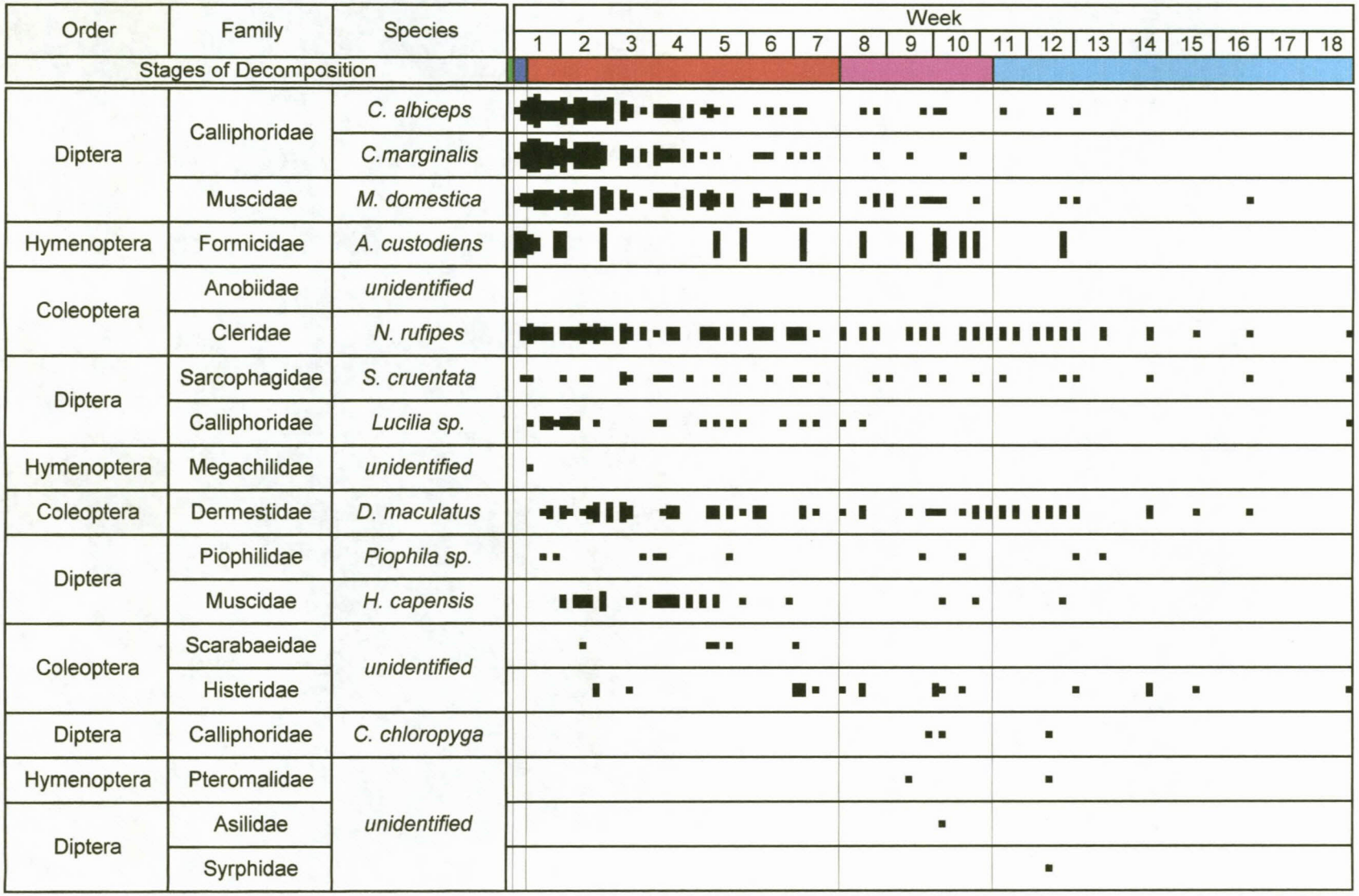


Fig. 3.6. Succession diagramme of arthropods associated with a pig carcass lying in full sunlight during winter (29 April 1999 - 1 September 1999)

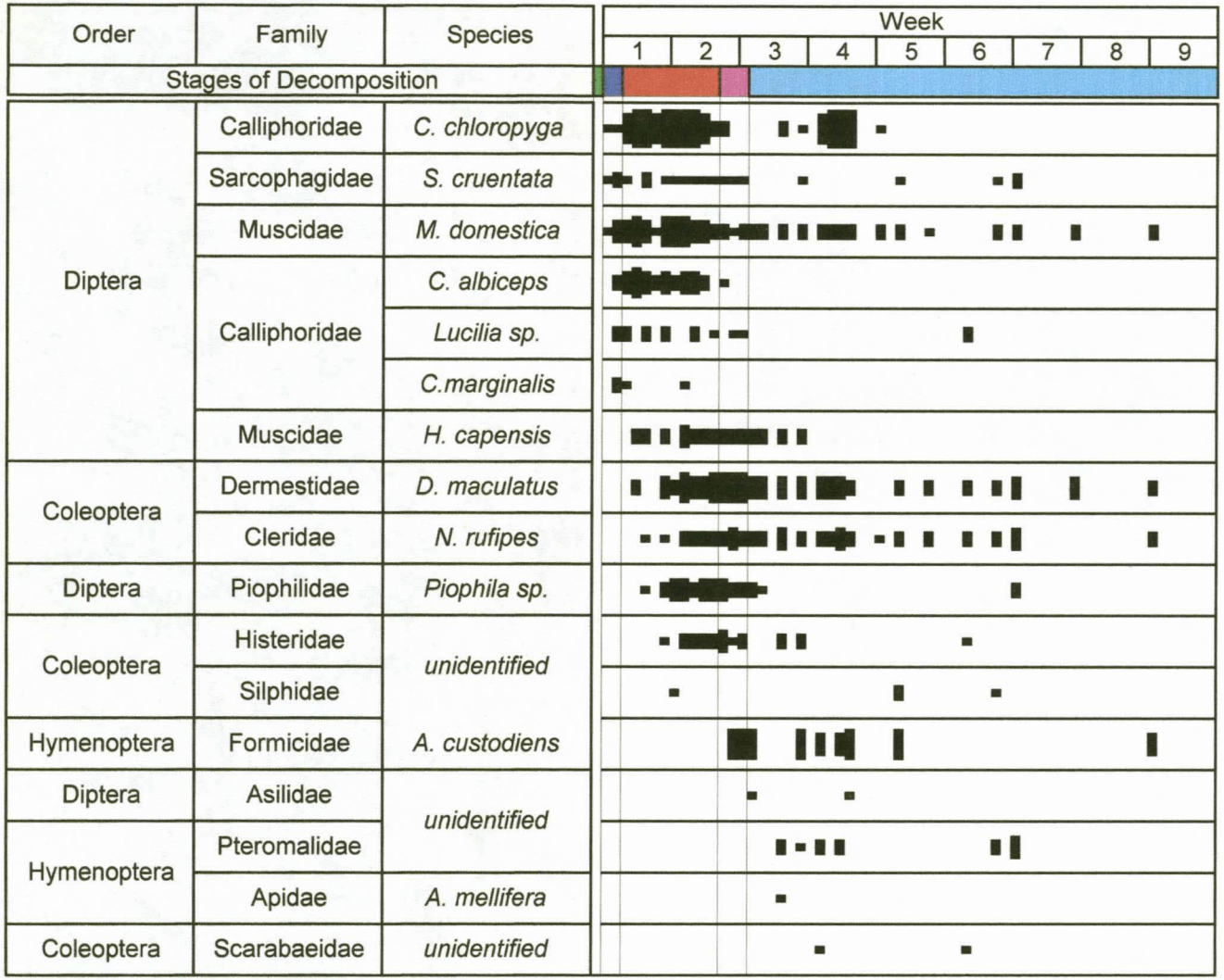


Fig. 3.7. Succession diagram of Arthropoda associated with a pig carcass hanging in full sunlight during spring (14 September 1999 - 10 November 1999)

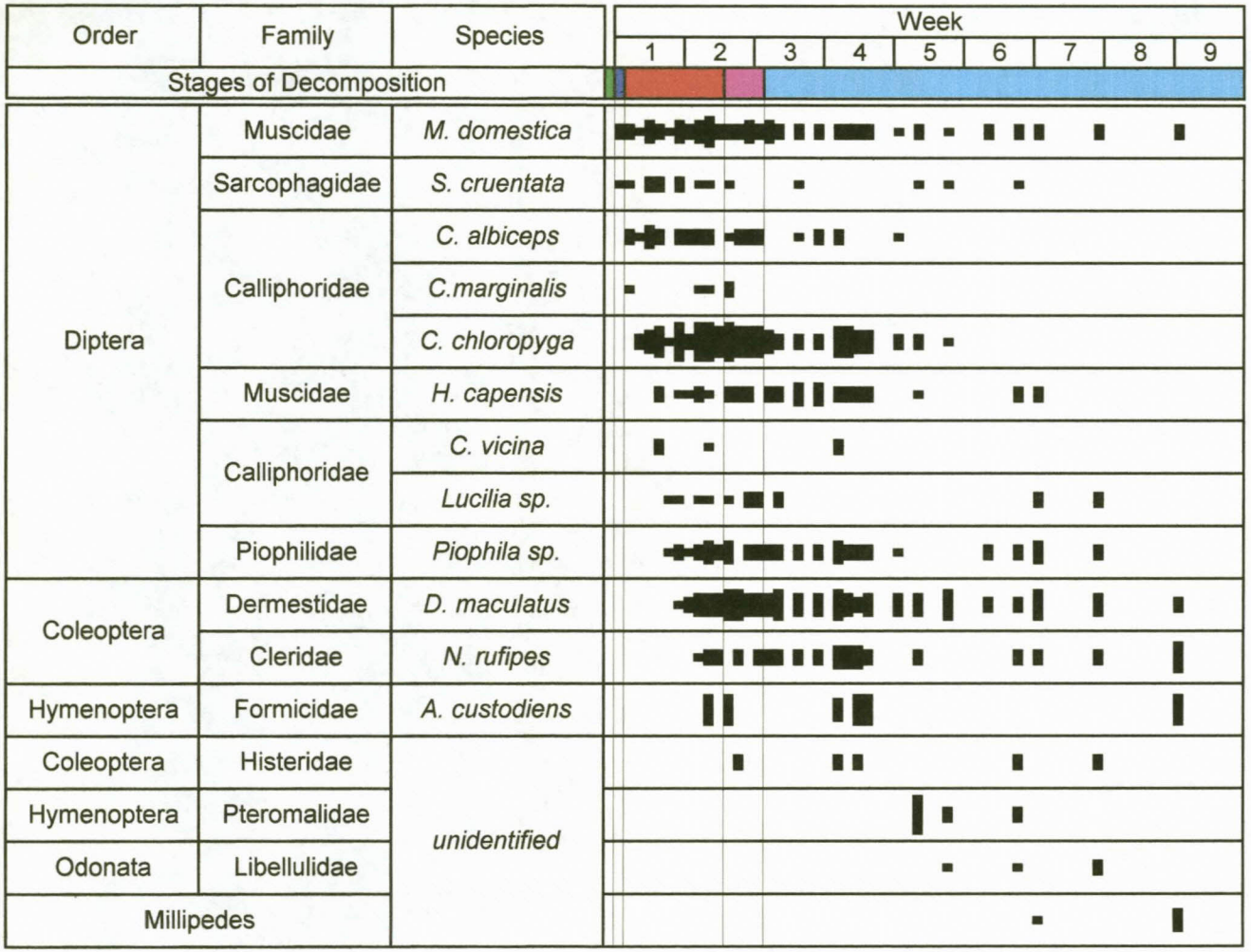


Fig. 3.8. Succession diagramme of arthropods associated with a pig carcass hanging in shade during spring (14 September 1999 - 10 November 1999)

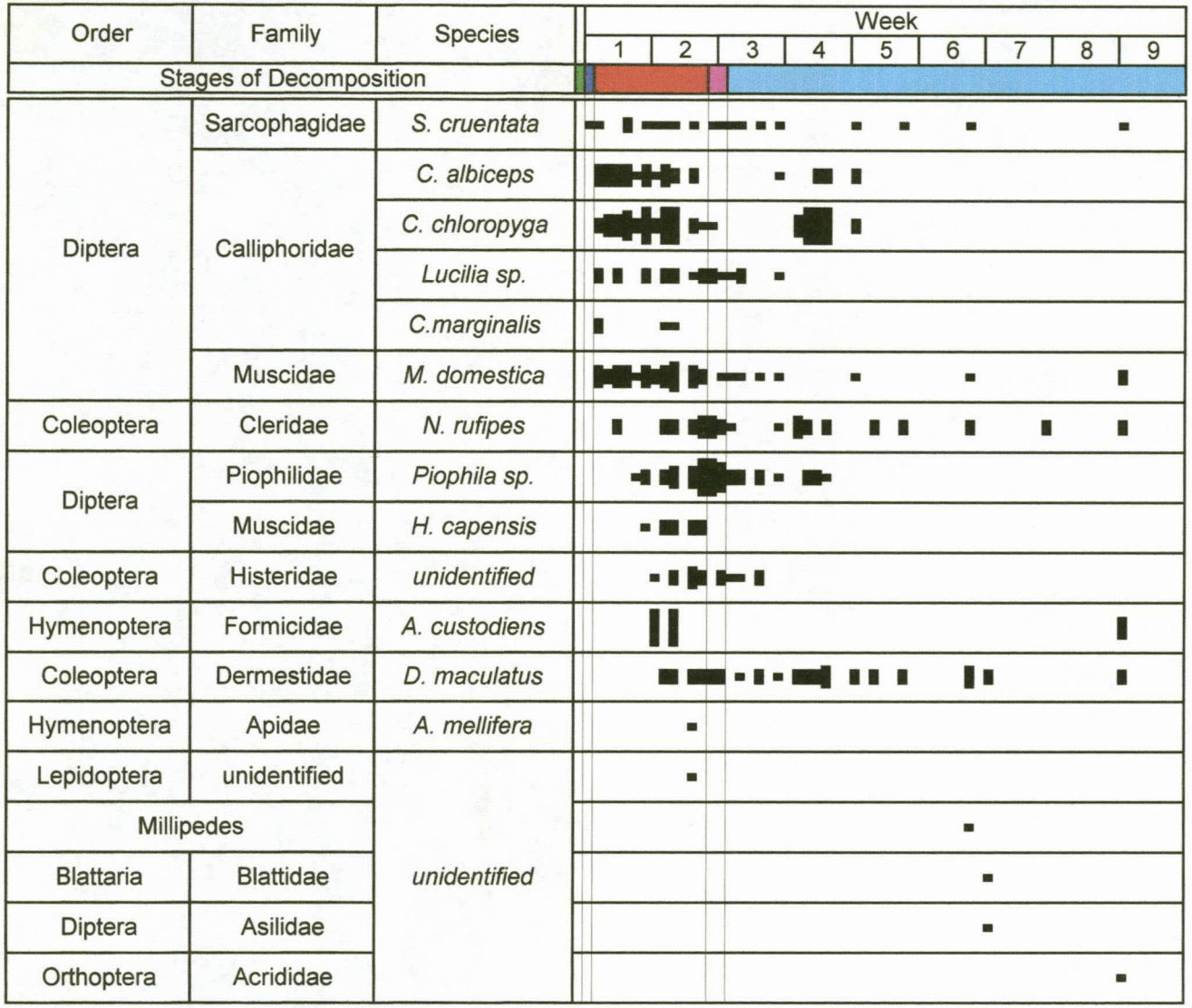


Fig. 3.9. Succession diagramme of arthropods associated with a pig carcass lying in full sunlight during spring (14 September 1999 - 10 November 1999)

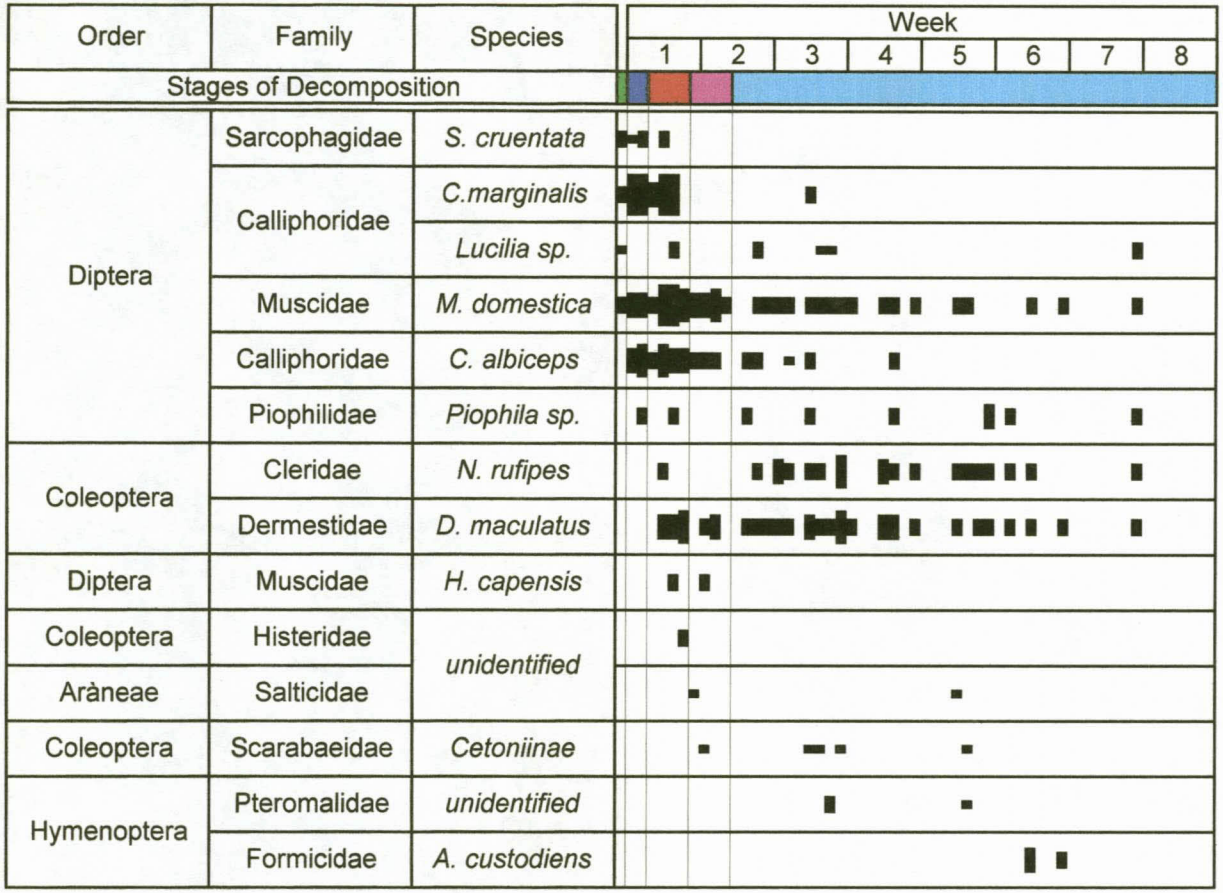


Fig. 3.10. Succession diagram of Arthropoda associated with a pig carcass hanging in full sunlight during summer (1 February 2001 - 22 March 2001)

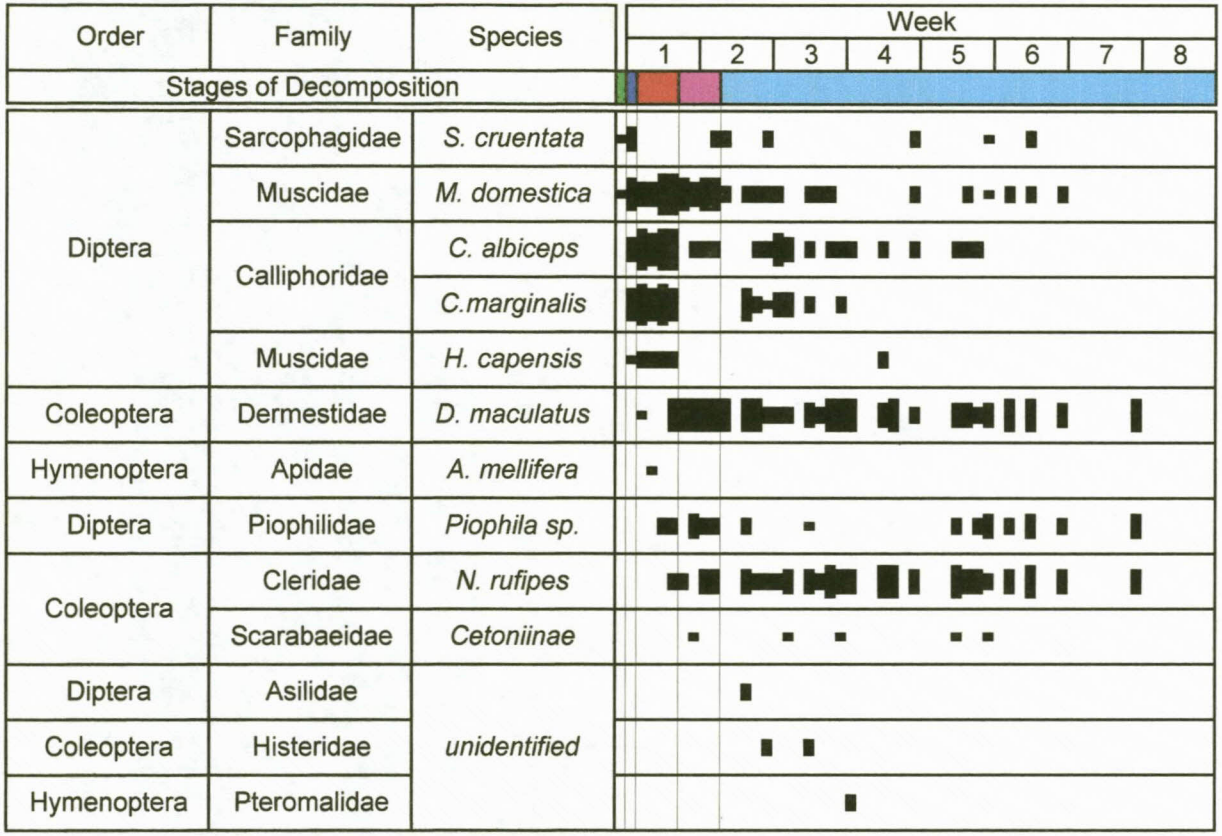


Fig. 3.11. Succession diagramme of arthropods associated with a pig carcass hanging in shade during summer (1 February 2001 - 22 March 2001)

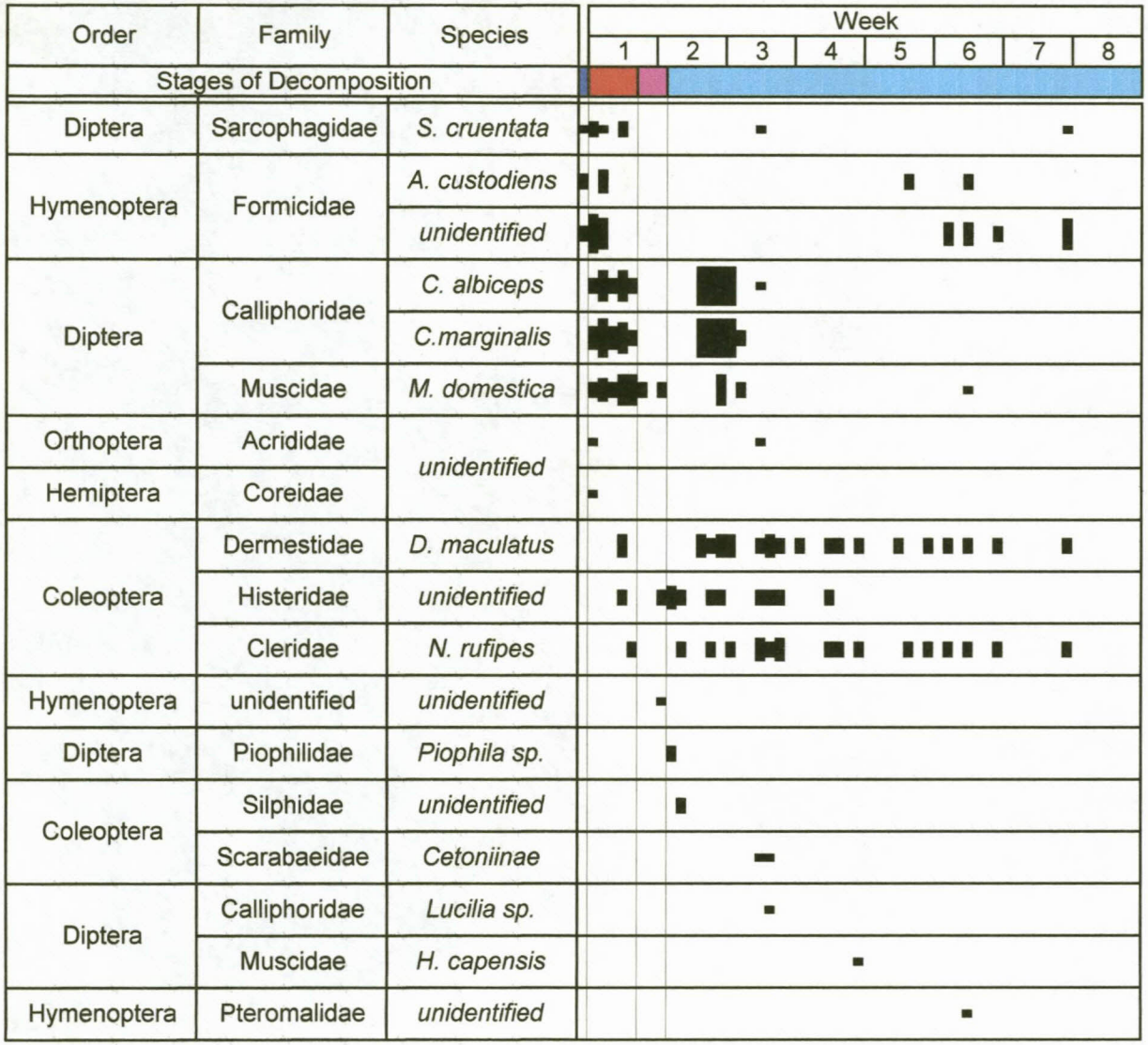


Fig. 3.12. Succession diagramme of arthropods associated with a pig carcass lying in full sunlight during summer (1 February 2001 - 22 March 2001)

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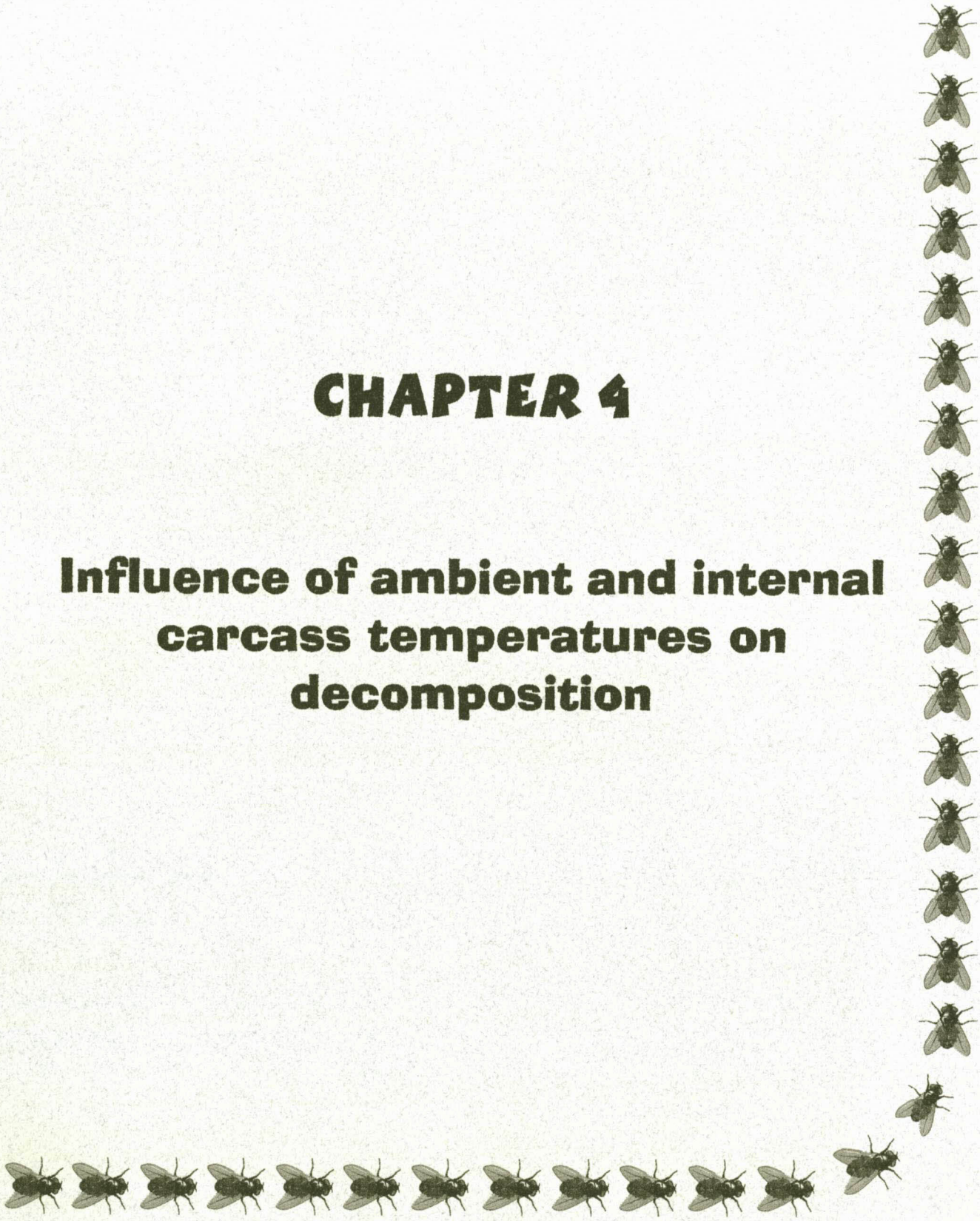
CHAPTER 4

Influence of ambient and internal carcass temperatures on decomposition



4.1. INTRODUCTION

The temperature at which maggots develop in a decomposing body is often much higher than the ambient temperature, and varies according to the state of decomposition and location within the decomposing body (Deonier 1940; O'Flynn 1983). Ambient temperature appears to have the greatest effect on the rate of decay of the human body. 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). Temperature plays a significant role in the development of carrion flies, with maggot-mass temperatures often becoming elevated above ambient temperatures (Joy *et al.* 2002). 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). Ambient temperature and the internal temperature (of the body and maggot mass) are the temperature measurements required when investigating the insects associated with a decomposing body (West 1951, in Dadour *et al.* 2001). 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 determination of the PMI.

The key questions in this aspect of the study were:

- What is the influence of ambient temperature on the decomposition process?
- How does the temperature of a maggot mass differ from the ambient temperature?
- What effect does this difference between ambient and maggot-mass temperature have on maggot development rate?

4.2. MATERIAL AND METHODS

4.2.1. Study site

The study site was the same as was described in Chapter 2 (2.2.1.).

4.2.2. Conditions for field experiments and carcasses

The conditions for field experiments and carcasses were the same as those described in Chapter 2 (2.2.2.).

4.2.3. Observations

In addition to the observations that were described in Chapter 2 (2.2.3.), the internal carcass temperatures were recorded during the 1999 trials by means of an *AMA-Digit* handheld digital thermometer. The probe was inserted into five regions of the carcass, *viz.* the head (mouth), neck, thorax, upper abdomen and lower abdomen. The holes made by the probe was covered with tape immediately after temperatures were recorded to prevent blowflies from ovipositing in these holes. For the purpose of this study, only the temperatures of the thorax and upper abdomen will be discussed, as the largest maggot masses were concentrated there. Ambient temperature was recorded with the same thermometer by shielding it from the sun and wind at a height of one metre above the soil surface. Ambient temperature records for the nearest meteorological station (± 2km from experimental site) were also obtained from of the South African National Weather Bureau in Pretoria for comparison. 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.

During the 2001 trial, three *MC Systems* dataloggers were used to record the internal and ambient temperatures. The internal body temperatures were measured by inserting thermocoupled wires into openings in the carcass at the same positions where temperatures were measured during previous trials. These data were recorded hourly. For the purpose of consistency with previous trials where dataloggers were not available, it was decided to only use the temperatures recorded during the actual times of observation. However, during the 2001 trial the use of dataloggers provided a complete set of continuous records since one of the dataloggers was also equipped as a weather station and recorded ambient temperature, wind speed and direction, humidity and solar insolence. Internal carcass temperatures were also measured with the handheld 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.

4.3. RESULTS

4.3.1. Summer trial (3 February 1999 - 15 March 1999)

Ambient temperatures measured at the different carcasses during the time of observations were higher than the average temperature (for the whole day) that was obtained from the Weather Bureau, although a similar pattern was observed (Fig. 4.1). The temperatures at the prone carcass and the carcass hanging in the sun were not measured beyond day 11 and day 20 respectively, since the remains stage had been reached and this part of the experiment was concluded (Fig. 4.1).

The temperatures measured at the thorax and upper abdomen approximated the ambient temperature (Fig. 4.2). This was due to the carcass being heated by direct insolation. The large maggot masses that produced metabolic heat were responsible for the higher temperatures between days 5 and 10.

The thorax and upper abdomen temperatures of the carcass hanging in the shade were higher than the ambient temperature between days 5 and 8 (Fig. 4.3), corresponding to

larval activity. Thereafter the temperatures were similar to the ambient temperature. This was due to the effect of shade from the trees surrounding the area where the carcass was hanging. This created a more stable microclimate with smaller temperature fluctuations.

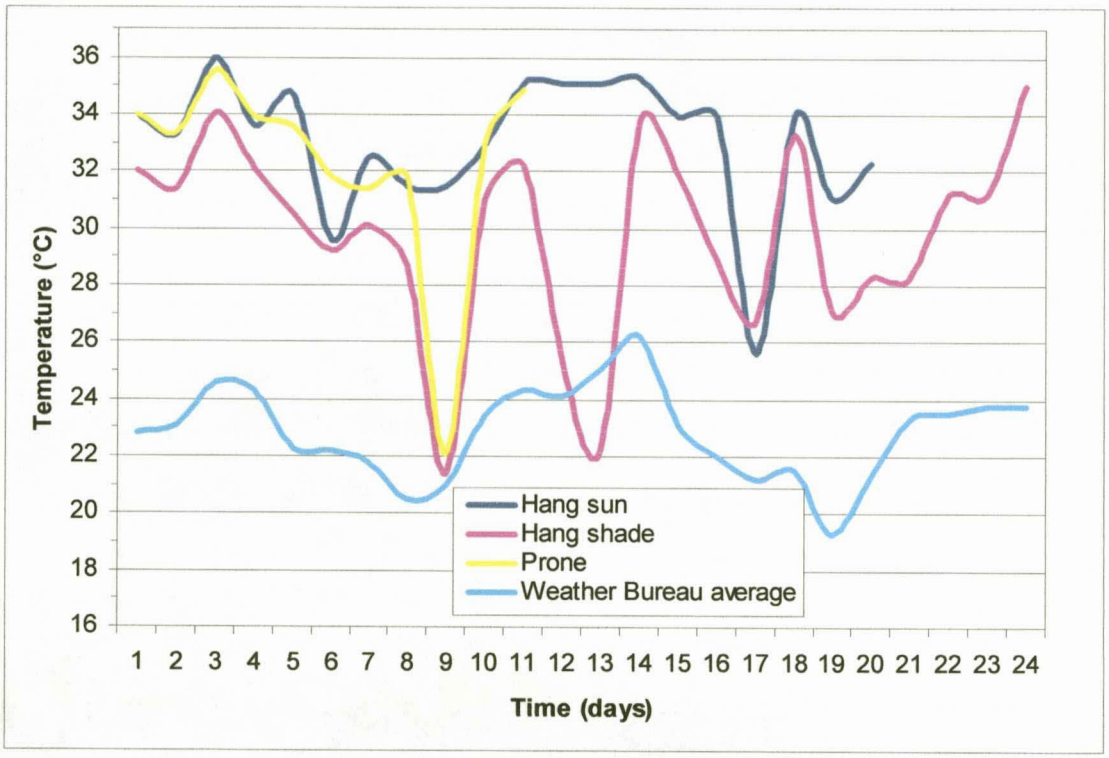


Fig. 4.1. Ambient temperatures recorded at the differently placed carcasses, compared to the average temperature data received from the Weather Bureau during the summer 1999 trial (3 February 1999 - 15 March 1999)

The temperatures measured at the thorax and upper abdomen of the prone carcass were higher than the ambient temperature between days 3 and 8 (Fig. 4.4), owing to the activity of the large maggot masses associated with the carcass. From day 8 onwards the difference between internal carcass temperatures and ambient temperature was due to heating caused by insolation from the sun, since the maggot masses moved away from the carcass to pupate.

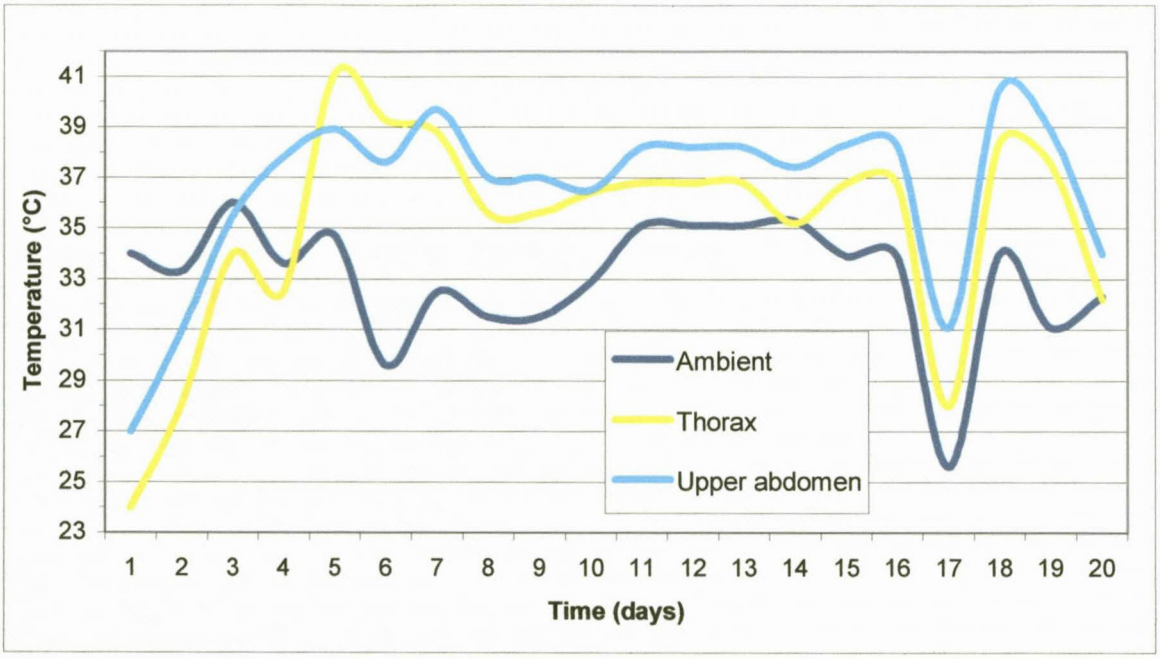


Fig. 4.2. Temperatures measured at the carcass hanging in the sun during the summer 1999 trial (3 February 1999 - 15 March 1999).

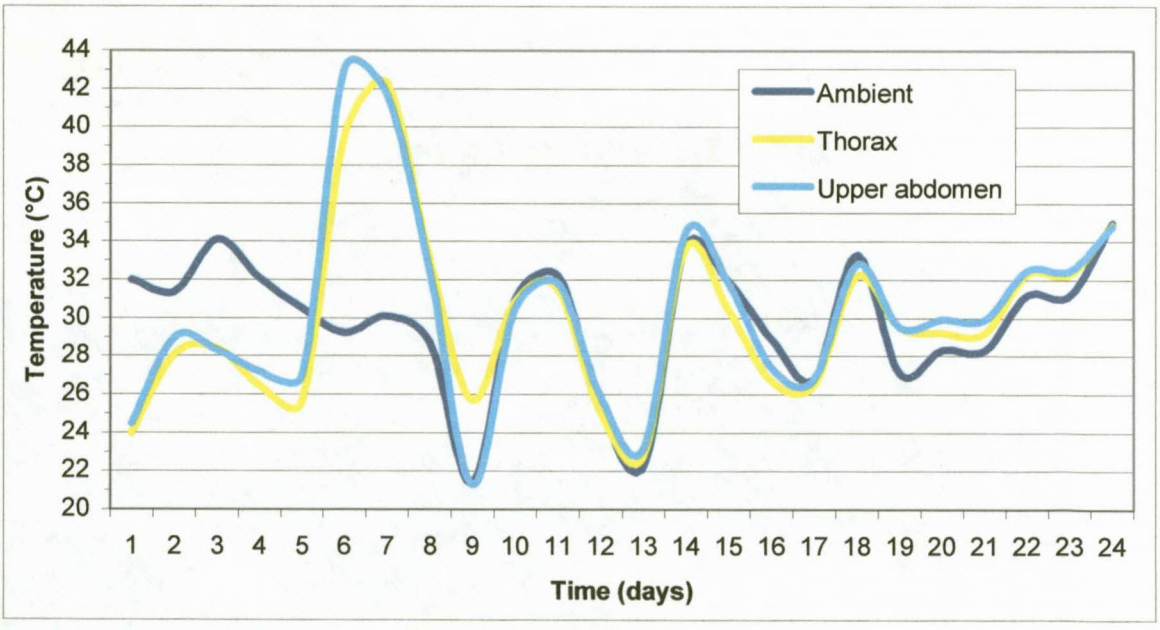


Fig. 4.3. Temperatures measured at the carcass hanging in shade during the summer 1999 trial (3 February 1999 - 15 March 1999).

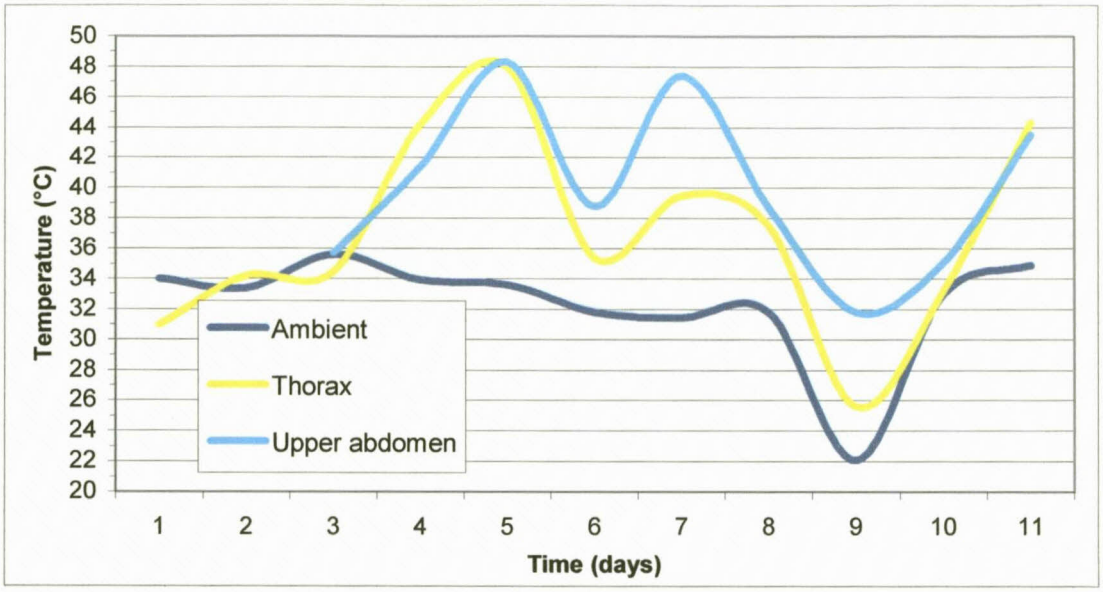


Fig. 4.4. Temperatures measured at the prone carcass during the summer 1999 trial (3 February 1999 - 15 March 1999).

4.3.2. Winter trial (29 April 1999 - 1 September 1999)

Ambient temperatures measured at each carcass during the winter trial were much higher than the Weather Bureau average (Fig. 4.5).

The temperatures measured at the differently treated carcasses compared to the ambient temperature revealed the same pattern as was previously observed (Figs 4.6 – 4.8). At the carcass hanging in the sun (Fig. 4.6), temperatures were higher than the ambient temperature owing to the activity of the larval masses and sun insolation. At the carcass hanging in the shade (Fig. 4.7) the difference between the measured temperatures and the ambient temperature was not as marked. Temperatures measured at the carcass hanging in the shade approximated the ambient temperature more closely owing to the enclosure causing a more stable microclimate. The temperatures of the prone carcass were lower than the ambient temperature (Fig. 4.8). This carcass froze at night and did not completely thaw during the day. The maggot masses consequently developed very slowly because of the lower temperatures.

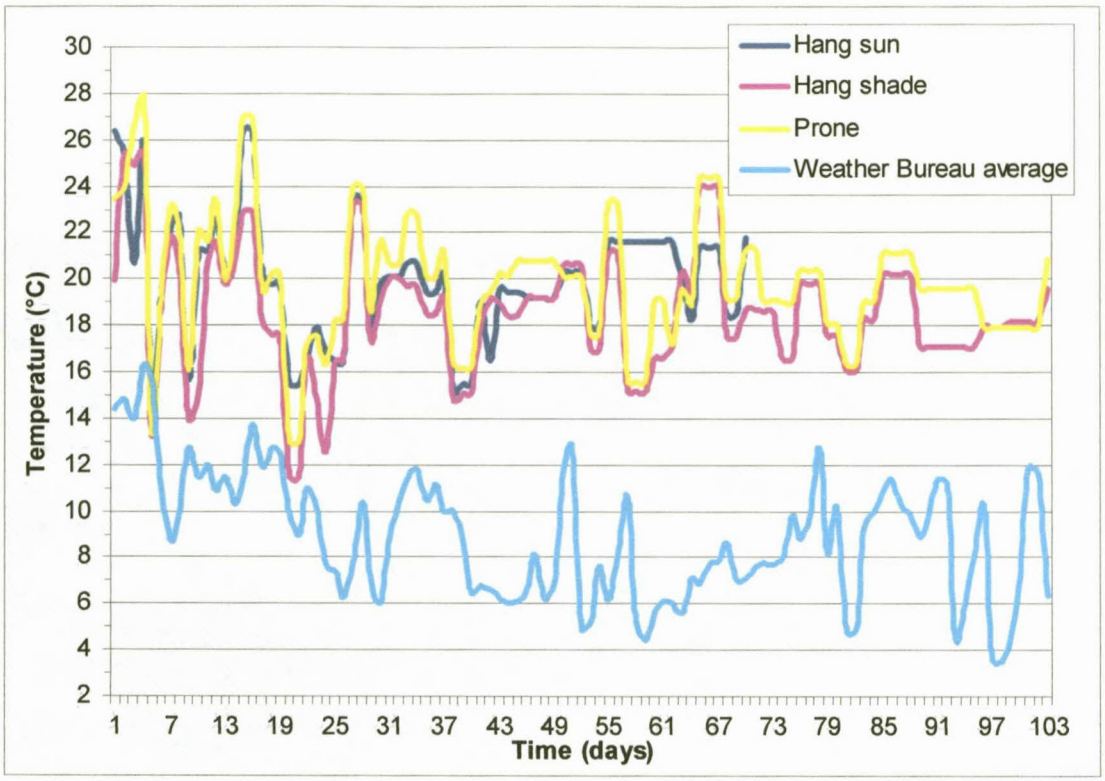


Fig. 4.5. Ambient temperatures during the winter 1999 trial (29 April 1999 - 1 September 1999)

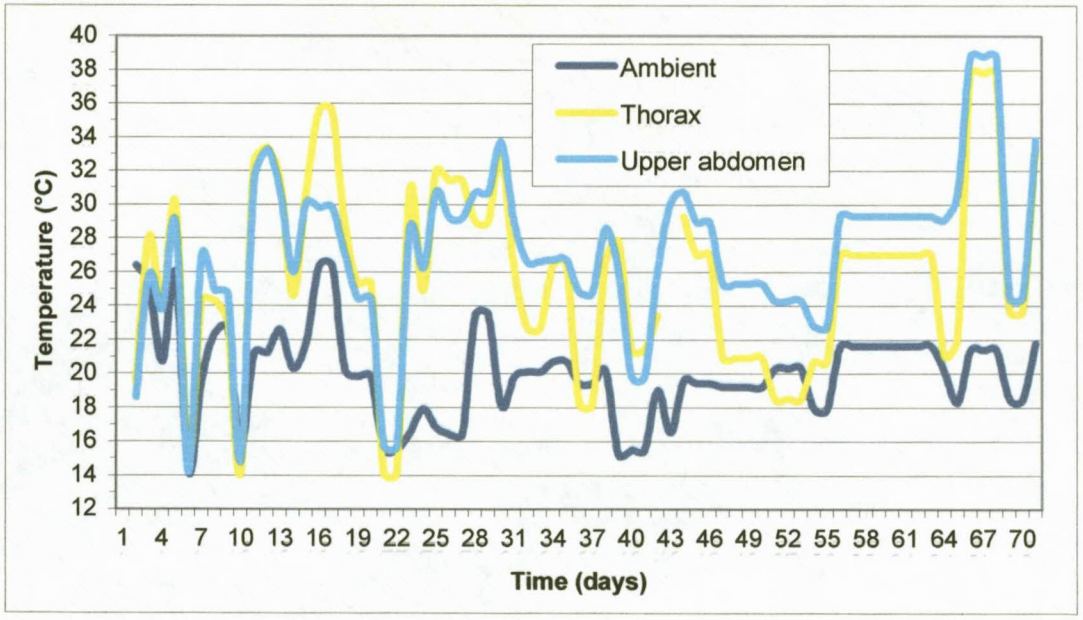


Fig. 4.6. Temperatures measured at the carcass hanging in the sun during the winter 1999 trial (29 April 1999 - 1 September 1999)

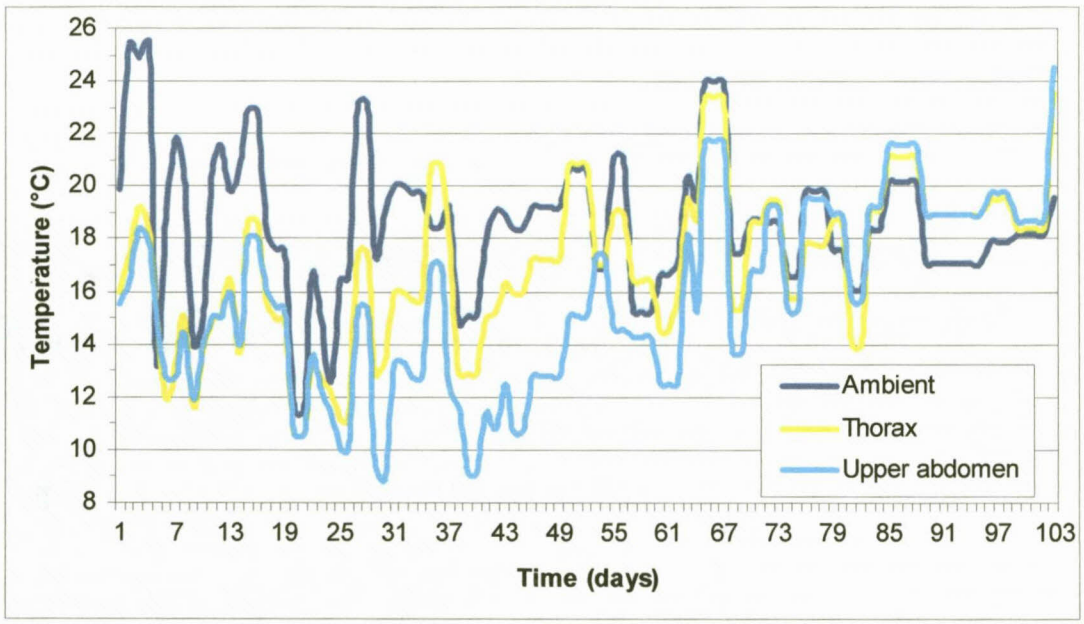


Fig. 4.7. Temperatures measured at the carcass hanging in the shade during the winter 1999 trial (29 April 1999 - 1 September 1999)

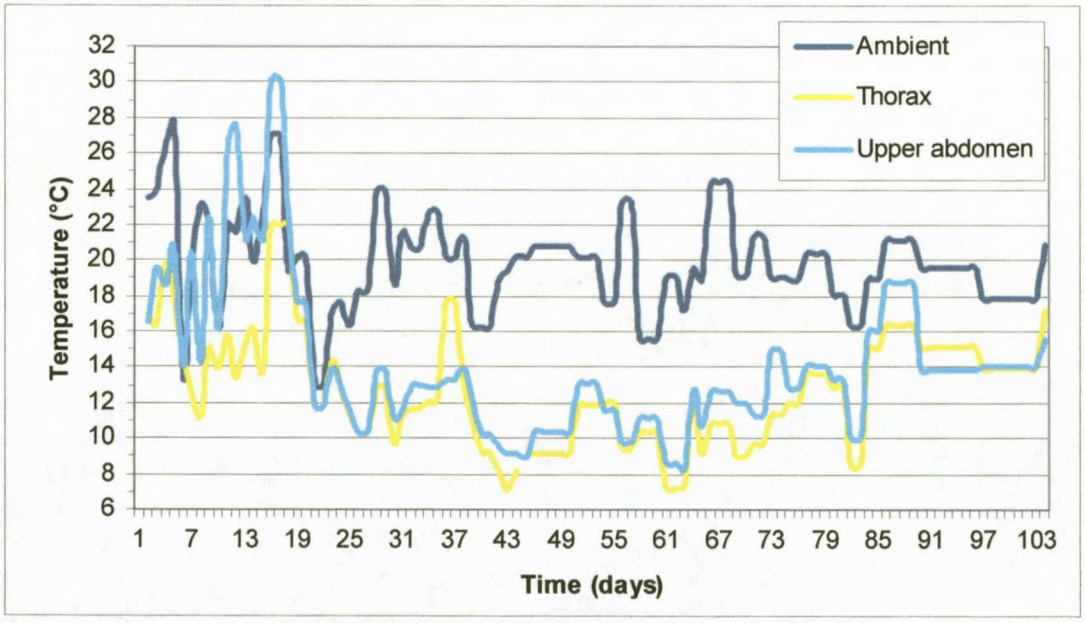


Fig. 4.8. Temperatures measured at the prone carcass during the winter 1999 trial (29 April 1999 - 1 September 1999)

4.3.3. Spring trial (14 September 1999 - 10 November 1999)

The ambient temperatures for the spring trial at the different carcasses were similar and showed the same pattern as the ambient temperature provided by the Weather Bureau (Fig. 4.9). The temperatures at the prone carcass and the carcass hanging in the sun were not measured beyond day 16, since the remains stage had been reached and this part of the experiment was concluded.

The same principles applied regarding the temperatures measured at the thorax and upper abdomen, as was the case during the first summer trial (Figs 4.10 – 4.12).

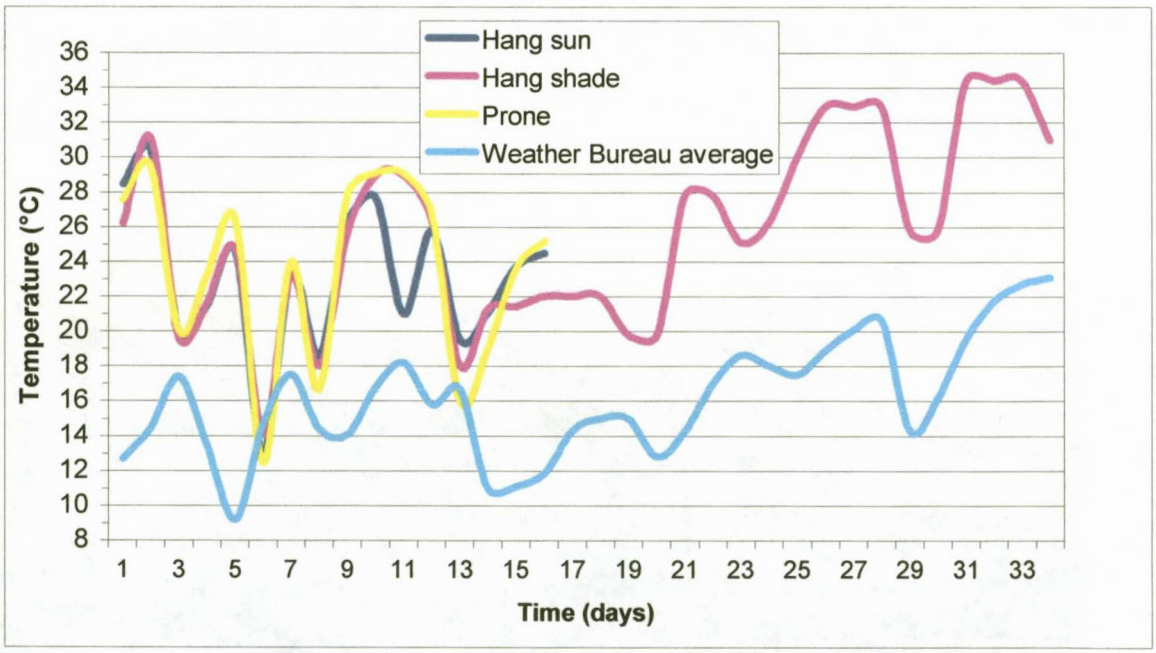


Fig. 4.9. Ambient temperatures during the spring 1999 trial (14 September 1999 - 10 November 1999)

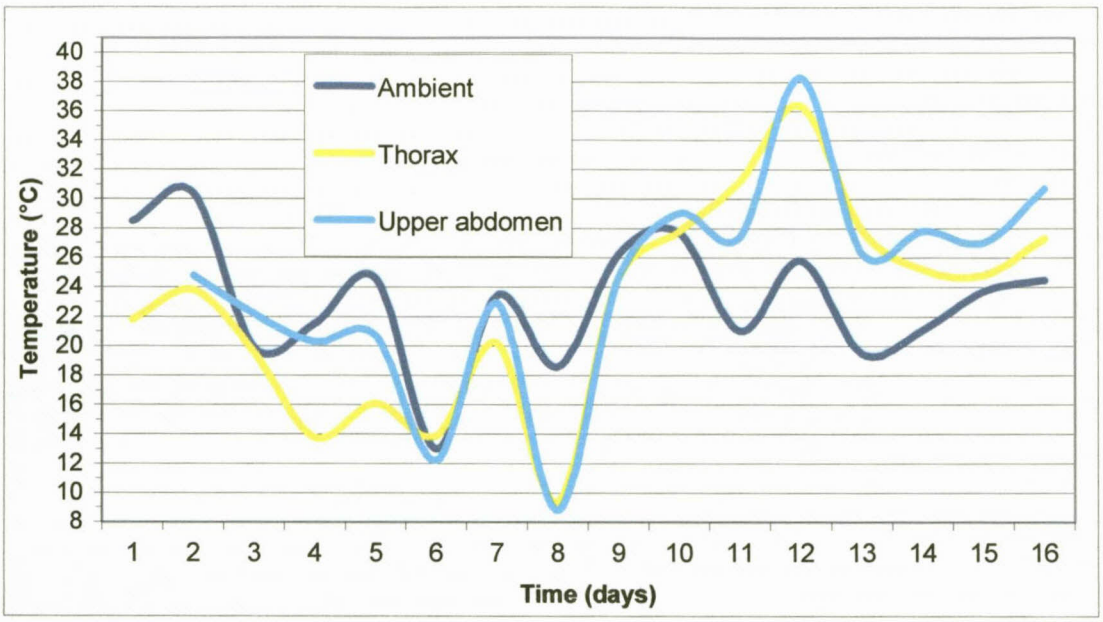


Fig. 4.10. Temperatures measured at the carcass hanging in the sun during the spring 1999 trial (14 September 1999 - 10 November 1999)

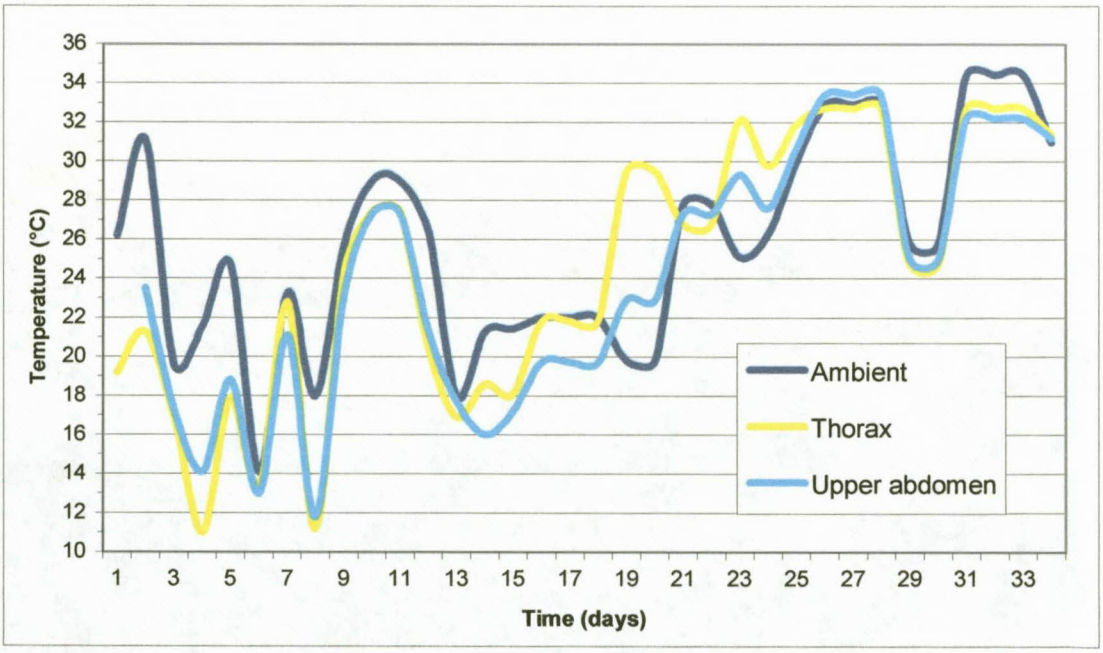


Fig. 4.11. Temperatures measured at the carcass hanging in the shade during the spring 1999 trial (14 September 1999 - 10 November 1999)

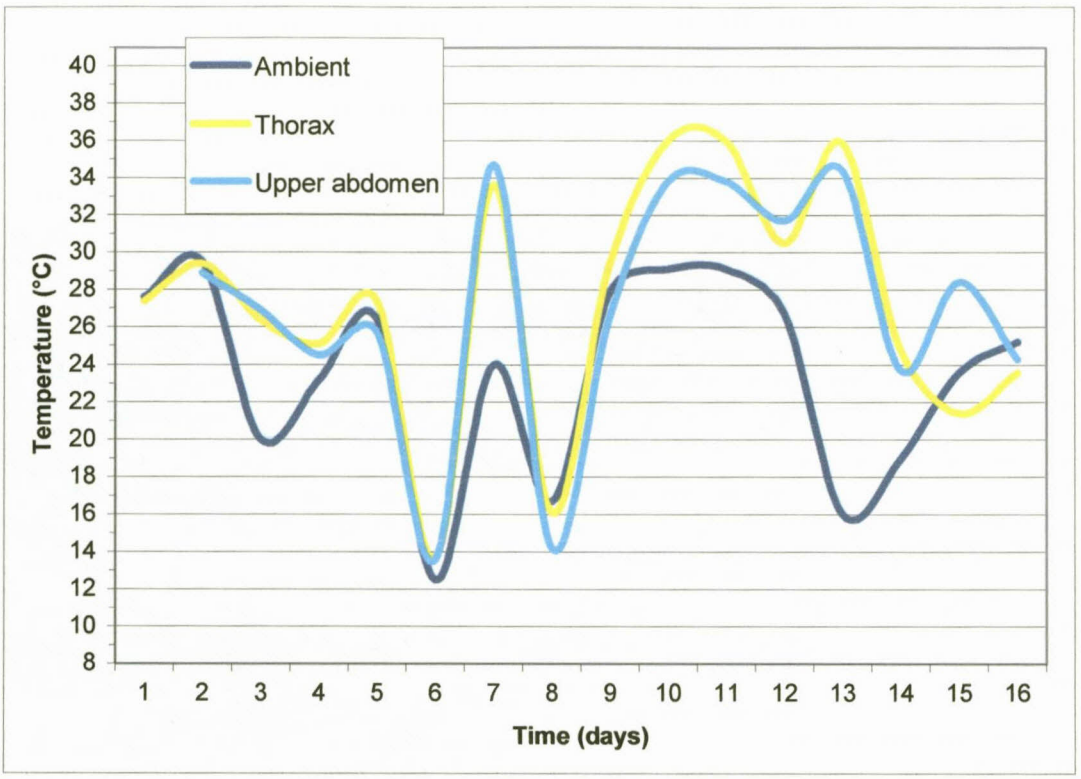


Fig. 4.12. Temperatures measured at the carcass lying in the sun during the spring 1999 trial (14 September 1999 - 10 November 1999)

4.3.4. Summer trial (1 February 2001 - 22 March 2001)

During the summer 2001 trial, the average ambient temperature measured by the data logger was lower than the ambient temperature measured at the time of the observations, although the same general pattern was observed.

The temperatures measured in the thorax and the upper abdomen of the carcass hanging in the sun followed the same pattern, although higher, than the ambient temperature (Fig. 4.14). Between days 5 and 13, the higher internal temperatures were caused by maggot-mass activity. Sun insolation was the major cause of higher internal temperatures after day 13, because all the maggots had migrated to pupate.

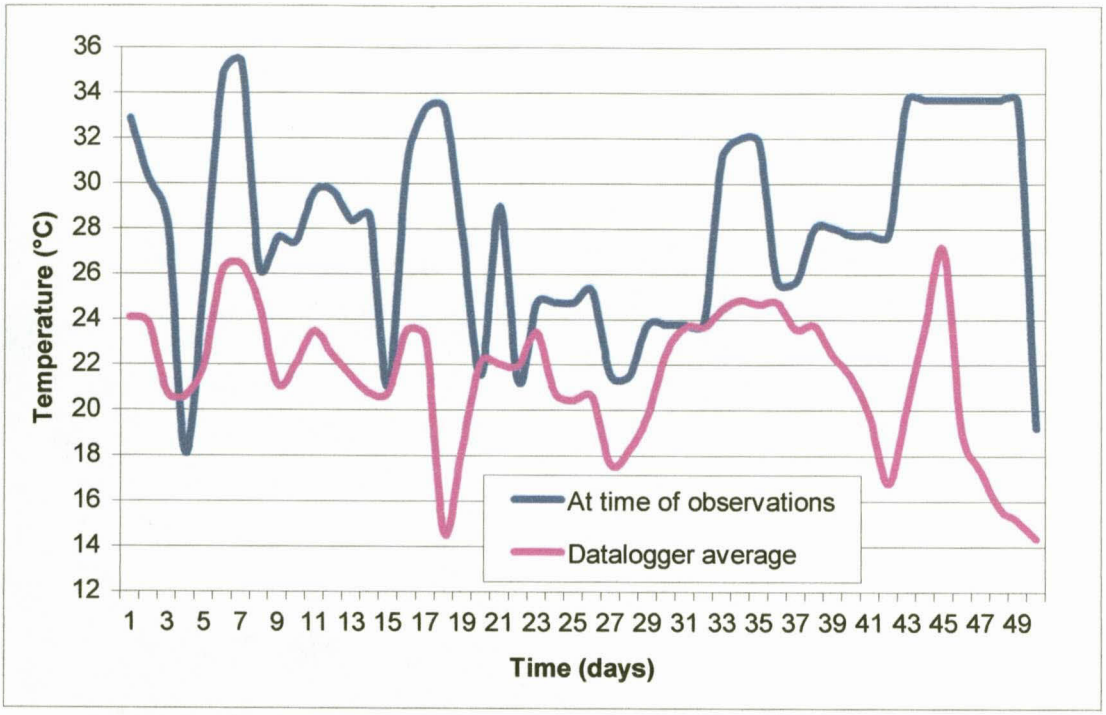


Fig. 4.13. Ambient temperatures during the summer 2001 trial (1 February 2001 - 22 March 2001)

Metabolic heat generated by maggot masses was reflected by the higher internal temperatures measured between days 8 and 15 at the carcass hanging in the shade (Fig. 4.15). The maggots migrated for pupation by day 17 and sun insolation caused the high internal carcass temperatures measured after day 17.

The higher internal temperatures measured between days 5 and 15 (Figure 4.16) at the prone carcass were due to metabolic heat generated by the maggot masses. The higher internal temperatures measured after this time were caused by sun insolation. By this time, all the maggots had migrated for pupation.

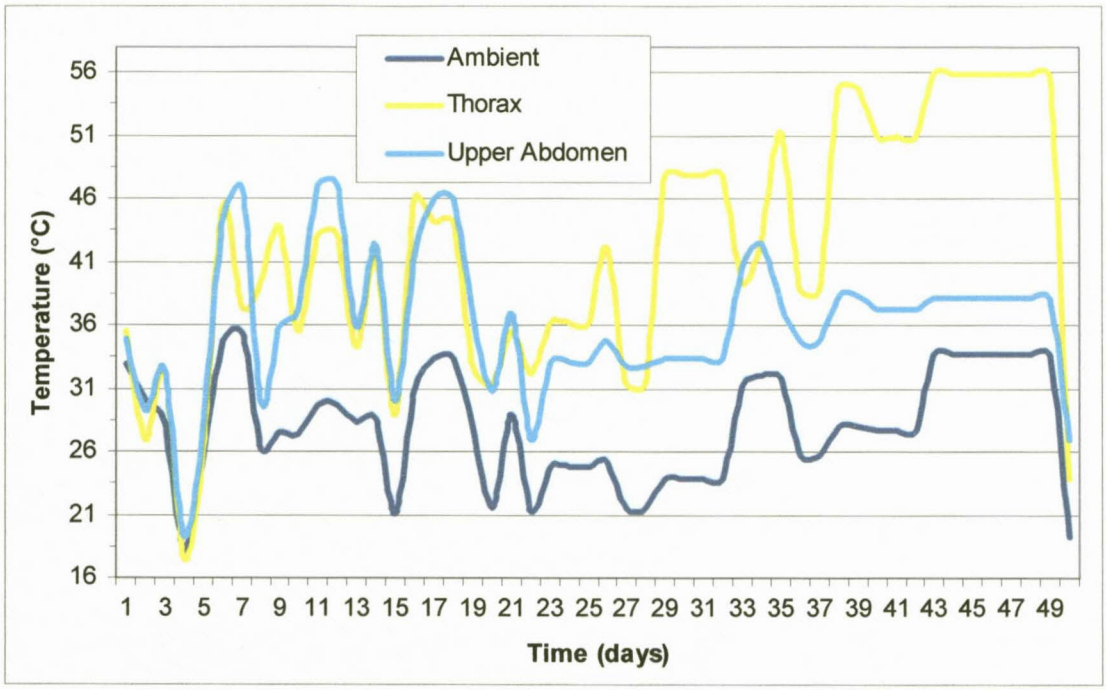


Fig. 4.14. Temperatures measured at the carcass hanging in the sun during the summer 2001 trial (1 February 2001 - 22 March 2001)

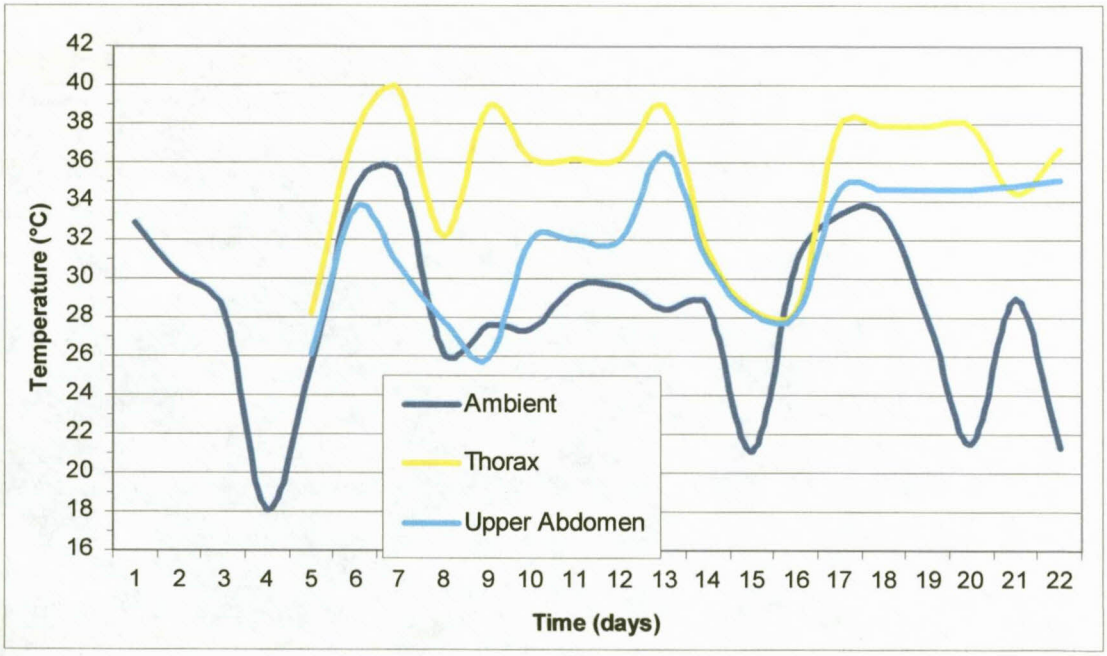


Fig. 4.15. Temperatures measured at the carcass hanging in the shade during the summer 2001 trial (1 February 2001 - 22 March 2001)

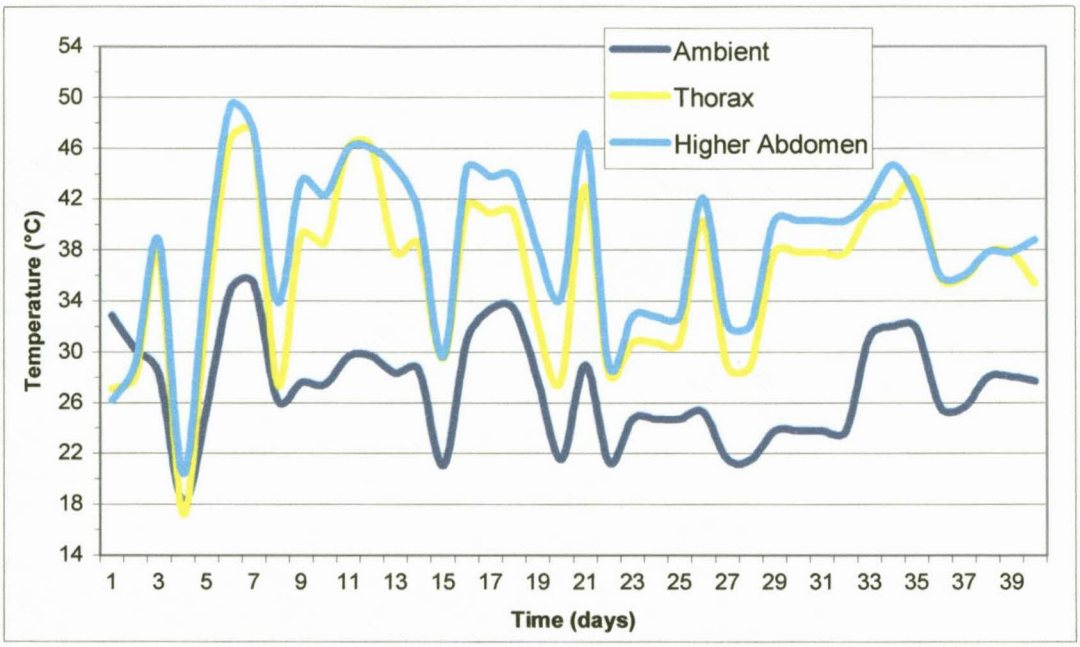


Fig. 4.16. Temperatures measured at the prone carcass during the summer 2001 trial (1 February 2001 - 22 March 2001)

Little is mentioned in the literature about the correlation between internal carcass temperatures and ambient temperatures at times when the observations were not made, for example at night. At night the ambient temperature may drop substantially and this may affect the maggot mass in such a way that the development of larvae may slow down, which could possibly affect the correct determination of the PMI. Joy *et al.* (2002) increased the frequency (every three hours) of sampling throughout their experimental period to provide equal emphasis to nocturnal and diurnal collections. De Jong & Chadwick (1999) also only recorded internal temperatures during the warmest part of the day. They noted that nocturnal internal temperatures might have been considerably lower at their experimental sites, retarding decomposition rates. The practice by investigators to observe a few times during the day in a field study is a practice that may yield an incomplete picture of larval development and fluctuations in the ambient and the maggot mass temperatures.

The use of the data loggers during the 2001 trial made it possible to access nocturnal data for the prone carcass (Fig. 4.17). Further research is needed before any conclusions can be drawn. The internal carcass temperatures measured between days 5 and 15 at 04:00 (coldest time during the night for the 2001 trial) in the prone carcass were higher than the ambient temperature (Fig. 4.17). The difference was between 1°C and 12°C. During the remainder of the study, the ambient temperature was also lower than internal carcass temperatures, but this could be due to radiation from the soil surface warming up the carcass.

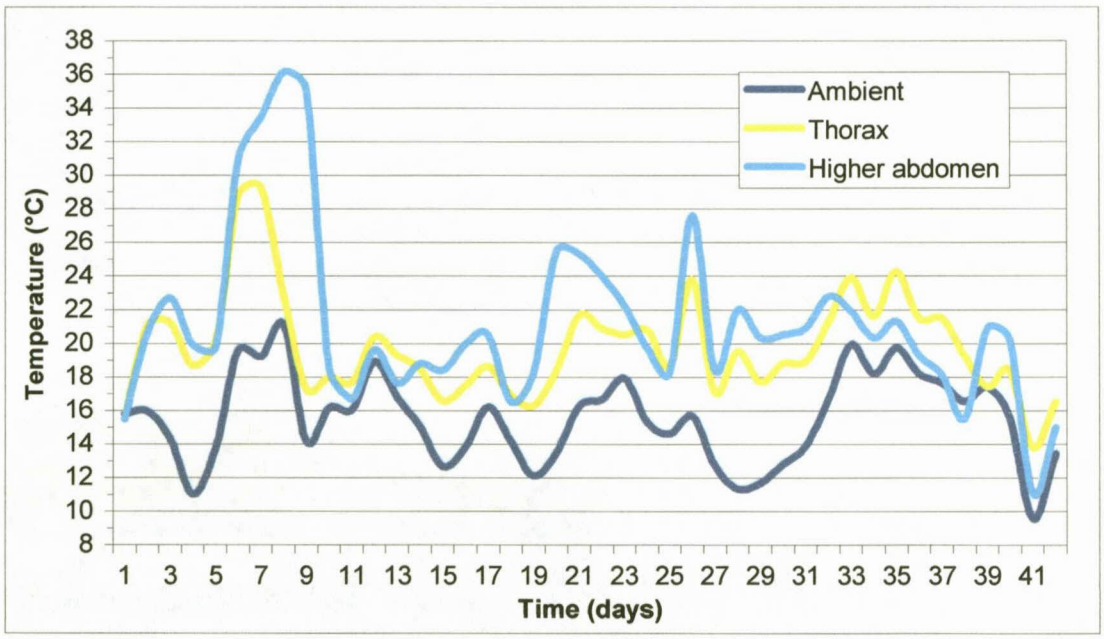


Fig. 4.17. Temperatures measured at 04:00 at the carcass lying in the sun during the summer 2001 trial (1 February 2001 - 22 March 2001)

4.4. DISCUSSION

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). Consistent with earlier reports (Payne 1965; Goodbrod & Goff 1990; Richards & Goff 1997), the present study showed that maggot-mass temperatures exceeded ambient temperatures in carcasses where large numbers of maggots were active. Shean *et al.* (1993) reported that maggot-mass temperatures were not consistently higher than ambient temperatures until six days after death. They also noted that the mean maggot-mass temperature was higher at the sunlit carcasses than at the shaded carcasses. De Jong & Chadwick (1999) reported elevated maggot mass temperatures in rabbit carcasses subjected to relatively cool temperatures at high elevation sites in Colorado. Joy *et al.* (2002) found that temperatures generated by aggregations of active third instar larvae were essentially the same at both sunlit and shaded raccoon carcasses. They also found that maggot-mass temperatures remained well above ambient temperatures, coincidental with third instar activity.

Temperatures higher than ambient were also recorded during times when there was no maggot activity. This was believed to be due to insolation from the sun (Deonier 1940) and/or absorption of heat from the soil surface. Internal carcass temperature will be affected by both air temperature and exposure to sunlight (Wells & Lamotte 2001). This effect of direct sunlight on a decomposing body acts as a catalyst and stimulates maggot growth and activity (Shean *et al.* 1993).

Hewadikaram & Goff (1991) found that during the fresh and bloated stages, the internal temperatures of a small carcass (8.4 kg) were more responsive to changes in ambient temperature. During the decay stage, they found no detectable influence of ambient temperature on the internal carcass temperature. The internal temperature decreased to approximately the ambient temperature by the remains stage. Internal temperatures of a large carcass (15.1 kg) were less influenced by changes in external ambient temperatures. Internal temperature of this carcass increased slowly through day 4 and peaked on day 5 at 53°C. Following this, coinciding with the end of the decay stage and the onset of the

post-decay stage, the temperature declined rapidly. Temperatures above ambient were recorded for the large carcass into the later stages of decomposition, including the post-decay and remains stages (Hewadikaram & Goff 1991).

Many other variables also affect the relationship between the decay rates of human cadavers and insect activity. The presence of clothing on the remains is one variable that can affect this relationship. According to Rodriguez & Bass (1983), other important variables are the physical size and physique of an individual and the type of environment in which the body is situated.

During the current study, it was found that carcasses hanging in the shade did not manifest such temperature extremes, as was the case in the other carcasses. This was due to the enclosure formed by the trees surrounding the carcasses. During a study by Shean *et al.* (1993), it was found that temperatures were typically higher at the exposed site during the day but often fell below those of the shaded site during the night and just before dawn. They also found that temperatures at the shaded site fluctuated less than those at the exposed site. Shean *et al.* (1993) concluded that ambient air temperatures were extremely important in influencing carrion decomposition primarily through the activities of calliphorid larvae. They showed that air temperatures can vary significantly between areas as little as 300 m apart, and tend to show more extreme maximum and minimum temperatures at exposed locations than do areas that are more shaded/protected. These differences in temperature patterns may have a profound effect upon the decomposition rate of a corpse. Galloway *et al.* (1989) concluded that confinement within closed structures presents a different pattern of decay since these circumstances may prevent onset of mummification and accelerate decomposition. 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.

During a study of hanging carcasses as opposed to a prone carcass (Shalaby *et al.* 2000), it was found that the maximum internal temperatures were recorded during the decay

stage. The primary site for the activity of Diptera larvae was in the area immediately under the carcass. The hanging carcass was exposed to the cooling effects of the surrounding air and internal temperatures more closely approximated the ambient air temperatures. The prone carcass was less subject to cooling effects of the air and temperatures were elevated above ambient air temperature through the end of the decay stage. The internal temperatures recorded in each carcass were also directly related to the level of colonisation of the carcass by Diptera larvae (Shalaby *et al.* 2000). The current study supports these findings. Marchenko (2001) also observed larval mass self-heating through metabolic heat release. His results revealed a greater stability in internal temperatures of prone cadavers compared to hanging cadavers where heat exchange was intensified by turbulence effects. The self-heating effect was recorded during autumn from 16 cadavers lying on the ground, but this process was not evident in two hanging cadavers during the same period.

During the study by Shean *et al.* (1993), it was found that maggot-mass temperatures were partly a function of their size. Unfortunately no outdoor study indicates how many larvae constitute a maggot mass or the thermal contribution of each instar (Greenberg 1991). There is an increase in temperature even in small maggot feeding-masses, *i.e.* more than four larvae per gram of substrate (Goodbrod & Goff 1990). As was described in Chapter 2, large maggot masses did not form on the hanging carcasses. According to Shalaby *et al.* (2000), this results in slower biomass removal and lower internal temperatures. By contrast, maggot masses formed on the carcasses lying on the ground resulted in higher internal temperatures.

Insect development is accelerated as temperature increases (within a certain range of temperatures), but this is not true at temperature extremes that may be lethal to the insect. Richards & Goff (1997) suggested that there might be a maximum number of degrees above ambient or a maximum temperature a maggot mass can reach or tolerate. An individual maggot is unlikely to survive at such high temperatures for any length of time. Maggots in a mass constantly move down to the feeding site and back out to the exterior of the mass (Anderson & VanLaerhoven 1996). The actual temperature at which an

individual maggot develops is consequently unknown. Temperature is the key to estimating time of death based on maggot development. The internal temperature of the carcass can only have an effect on certain immature stages in blowfly development, as the prepupa and pupa develop away from the carcass (Anderson & VanLaerhoven 1996).

The current study has shown that there is little evidence to indicate a direct relationship between ambient environmental temperatures and the actual temperatures experienced by large masses of developing maggots. Environmental temperatures undergo daily cycles (thermoperiods) in which daytime temperatures tend to be higher (thermophase) than night temperatures (cryophase) (Beck 1983). Anderson & VanLaerhoven (1996) found that this was also the case in internal carcass temperatures. 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). Estimates of the postmortem interval must also take into account the accuracy of estimates derived from maximum - minimum daily temperatures compared with hourly readings, the effect of the sun's insolation on a body's temperature and developmental rates based on constant *vs.* fluctuating (natural) temperatures (Greenberg 1991).

Anderson & VanLaerhoven (1996) related the internal carcass temperature as a maximum and a minimum range. In most decomposition studies only a single internal temperature is given. This was also adopted during the current study, but a continuous record of temperature data for the 2001 trial exists. Anderson & VanLaerhoven (1996) showed that there was a greater fluctuation in internal temperature than in ambient temperature, with diel differences of more than 35°C.

4.5. CONCLUSION

Ambient temperature has a direct influence on the decomposition process. A higher ambient temperature results in a more rapid decomposition rate. During summer, a body decomposes more rapidly than a body in similar circumstances during colder times of the year. At higher temperatures maggots develop more rapidly than at lower temperatures. At low temperatures, the total development time of blowflies (from egg to adult) will be much longer than at higher temperatures (This aspect is discussed in Chapter 6). At higher temperatures, the feeding behaviour of blowflies is more voracious than at lower temperatures, resulting in greater removal of tissue from the carcass in less time. Decomposition will consequently be faster and more complete in instances where a body is subject to higher ambient temperatures (*e.g.* during summer as opposed to winter).

The internal temperature of a prone body exceeds the ambient temperature during warmer seasons, mostly due to the metabolic heat generated by large maggot masses. During winter the internal temperatures of a prone body approximates the ambient temperature. The internal temperature of a body hanging in full sunlight exceeds the ambient temperature. This is due to insolation by sunlight, as well as the metabolic heat generated by maggot masses (smaller maggot masses than on a prone body). The internal temperature of a body hanging in the shade approximates the ambient temperature more closely than a prone body or sunlit hanging body.

Maggot mass temperatures often exceed the ambient temperature (when the maggot mass is sufficiently large). During cold conditions large maggot masses will remain warm and continue feeding and developing, even when the ambient temperature is below the minimum threshold of development for the specific species of larvae. This has serious implications for the correct estimation of the PMI. It is of utmost importance to understand and interpret the microclimate in which the larvae develop.

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CHAPTER 5

Evaluation of rearing media for *Chrysomya chloropyga* immatures

5.1 INTRODUCTION

The females of a number of species of blowflies require a protein meal for the development of the first and subsequent batches of eggs (Mackerras 1933; Roberts & Kitching 1974). There are no known physiological reasons for the uptake of protein by males (Roberts & Kitching 1974). Protein feeding increases the receptivity of female blowflies to mating attempts (Barton Browne *et al.* 1976; Barton Browne *et al.* 1980; Barton Browne *et al.* 1990). Mating in the field may occur at or near a protein source when females are obtaining additional meals. This conclusion is based on the possibility that females need to visit protein sources more than once to obtain sufficient nutrients to develop their first batch of eggs. The findings by Barton Browne *et al.* (1976) that the ingestion of a relatively small amount of protein renders females highly receptive, further supports this assumption. It may be unusual for female blowflies in the field to locate and feed on a protein source as early as the day after emergence (Barton Browne *et al.* 1976)

The larval rearing medium used in the laboratory is extremely important in forensic investigations since larval food can alter insect growth rates. For this reason, insects whose growth data are to be used in legal investigations should be reared only on animal tissue (Byrd 2001). Essential qualities in a food source should include ease of preparation, readily available materials, low cost, be minimally offensive and lead to efficient production of healthy flies. Depending upon the investigator's goals, rearing media should be developed as a simple, inexpensive, sterile, homogenous, tissue-based medium (Sherman & Tran 1995). It was decided to evaluate the efficacy of four different rearing media, namely beef liver, chicken liver, canned dog food and pets mince.

5.2 MATERIAL AND METHODS

5.2.1 Rearing of larvae and maintenance of colonies

Procedures for the establishment and maintenance of laboratory colonies of blowflies were similar to techniques described by Spiller (1963), Smith (1986), Catts & Haskell (1990), Erzinçlioglu (1996), Sherman & Wyle (1996) and Byrd (2001).

The colonies were maintained in electronically controlled insectaries, at a temperature of 25°C ± 1°C and a relative humidity of 65% ± 5%. A day-night cycle of 12L:12D, including a dawn and dusk period of 30 minutes was regulated by a dawn-dusk simulator.

Diptera larvae were collected during the field experiments as described in Chapter 2. The larvae were taken to the laboratory and placed in "maggot motels" for rearing. These "maggot motels" comprised of five-litre plastic buckets with ventilation holes cut through the lids. The holes were covered with fine gauze to prevent the larvae from escaping. Another aperture was cut through the side of the bucket. This hole was fitted with a section of plastic pipe that was covered at one end with gauze. The pipe was connected to an extraction fan system in the rearing chamber.

The bucket was filled to one quarter with sawdust. A sheet of absorbent paper towel was placed on the sawdust. Chicken liver placed on the absorbent towel paper served as feeding medium for the developing larvae. The chicken liver and larvae were covered with a piece of aluminium foil to prevent desiccation. The chicken liver was replaced when the larvae had consumed it. When the larvae reached the post feeding stage, they burrowed into the sawdust. The paper, remaining liver and aluminium foil were then discarded.

The sawdust containing the pupae was transferred to rectangular plastic containers and placed in gauze cages. These cages were suspended within cuboid metal frames. A

container filled with sugar and another with moist cotton wool was placed in the cages as a food source for the adults. Two to three days after the adults had emerged from the pupae, a portion of fresh chicken liver was placed in the cage. The adult flies fed on this protein source. At the end of the day the liver was removed. Three to four days later another portion of liver was placed in the cage to serve as a substrate for oviposition, as well as an additional protein meal. The section of liver with eggs was then removed and placed in a "maggot motel", repeating the process.

5.2.2 Rearing media

Beef liver first had to be minced or cut (a time-consuming process) because larvae had desiccated when they could not penetrate the beef liver in earlier trials.

Twenty-five one-hour-old larvae were used for each of the rearing media trials. Small cultures, each containing 15 to 25 larvae afforded maximum rearing success (Lord & Burger 1983). They were placed in "maggot motels" containing 25g of the medium. The medium was replaced when necessary. The experiment was replicated nine times. Observations were made daily and the time required for the larvae to reach pupation was recorded. Once the pupae were removed from the sawdust, they were placed in emergence containers and monitored closely for the emergence of adults. This procedure provided information on both the duration and rate of emergence, which are essential data in accurate PMI estimations based on entomological evidence (Byrd 2001).

Pupal mass, adult dry mass, forewing length and forewing width were also recorded to determine the growth rate and success of each rearing medium. The mass was determined to five decimal places by means of a microbalance. The wing length was measured from the point of attachment to the thorax to the wing apex. The wing width was taken as the broadest section across the wing.

All statistical analyses used the Kruskal-Walis test with Dunn's Multiple Comparisons Test (post-test performed if $p < 0.05$), except for the survival analysis, where the One-

way Analysis of Variance (ANOVA) and the Tukey-Kramer Multiple Comparisons Test (post-test performed if $p < 0.05$) were used.

5.3 RESULTS

5.3.1 Development time

Since only a small number of the larvae that fed on dog food pupated, no development data are shown in Tables 5.1 –5.3. Desiccation of the medium was the greatest constraint. Larvae fed on chicken liver had the shortest mean development time (in days) from one-hour-old larvae until pupation and those feeding on pets mince the longest (Table 5.1). With Kruskal-Wallis test and Dunn's Multiple Comparisons Test extremely significant differences ($p<0.0001$) in the development time of beef liver, chicken liver and pets mince occurred.

Table 5.1: Development time (days) of *Chrysomya chloropyga* from one-hour-old larvae to pupation, fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance* (p<0.0001)
Beef liver (a)	168	5.45 ± 0.64	a
Chicken liver (b)	83	5.19 ± 0.45	b
Pets mince (c)	96	6.63 ± 0.68	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

Pupae that formed from larvae fed on beef liver had the shortest mean and pets mince the longest mean development time (in days) from pupation to adulthood (Table 5.2).

Table 5.2: Development time (days) of *Chrysomya chloropyga* from pupation to adulthood, fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance* (p = 0.1252)
Beef liver (a)	151	5.12 ± 0.95	abc
Chicken liver (b)	78	5.21 ± 0.87	abc
Pets mince (c)	86	5.27 ± 0.9	abc

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

With the Kruskal-Wallis test and Dunn's Multiple Comparisons test no significant differences ($p = 0.1252$) were evident between the different treatments.

Chicken liver yielded the shortest total development time (Table 5.3) and pets mince the longest. The total mean development time of larvae that fed on the beef liver did not differ significantly from the total mean development time of larvae that fed on chicken liver. The total mean development time of larvae fed on pets mince, however, differed significantly from the total mean development time of larvae fed on beef liver, as well as those fed on chicken liver ($p < 0.0001$).

Table 5.3: Total development time (days) of *Chrysomya chloropyga* from one-hour-old larvae to adulthood, fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	151	10.56 ±0.7	ab
Chicken liver (b)	78	10.40 ± 0.71	ab
Pets mince (c)	86	11.86 ± 0.6	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

5.3.2 Survival

The percentage survival of each replication was calculated, and these percentages were used for statistical analyses, hence n = 9 in Table 5.4 and 5.5.

The highest percentage of survival of larvae reaching the pupal stage occurred with beef liver as a feeding medium, while the survival on chicken liver and pets mince did not differ significantly ($p < 0.0001$) (Table 5.4).

The highest percentage of survival of larvae reaching adulthood also occurred with beef liver. With chicken liver and pets mince, survival did not differ significantly ($p < 0.0001$) (Table 5.5).

Table 5.4: Mean percentage survival of one-hour-old *Chrysomya chloropyga* larvae to pupation, fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	9	74.67 ± 10.39	a
Chicken liver (b)	9	36.89 ± 23.48	bc
Pets mince (c)	9	42.67 ± 21.91	bc
Dog food (d)	9	0.44 ± 1.33	d

* Means with different letters differ significantly from each other
(One-way ANOVA + Tukey-Kramer Multiple Comparisons Test)

Table 5.5: Mean percentage survival of one-hour-old *Chrysomya chloropyga* larvae to adulthood, fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	9	67.11 ± 11.09	a
Chicken liver (b)	9	34.67 ± 22.36	bc
Pets mince (c)	9	38.22 ± 19.81	bc
Dog food (d)	9	0.00 ± 0.00	d

* Means with different letters differ significantly from each other
(One-way ANOVA + Tukey-Kramer Multiple Comparisons Test)

5.3.3 Morphometric data

The largest mean pupae mass resulted from larvae that fed on beef liver. The mass of pupae that originated from larvae fed on beef liver and chicken liver did not differ significantly from each other ($p < 0.0001$), but differed significantly from the mass of pupae that originated from larvae fed on pets mince ($p < 0.0001$) (Table 5.6).

Table 5.6: Mean mass (g) of *Chrysomya chloropyga* pupae from larvae fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	168	0.062 ± 0.007	ab
Chicken liver (b)	83	0.059 ± 0.005	ab
Pets mince (c)	95	0.048 ± 0.009	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The mean dry mass of adults was the highest for individuals that originated from larvae fed on beef liver. The mean mass of adults that originated from larvae fed on beef liver and those that fed on chicken liver respectively did not differ significantly from each other ($p < 0.0001$), but differed significantly from the mass of adults that originated from larvae fed on pets mince ($p < 0.0001$) (Table 5.7).

Table 5.7: Mean dry mass (g) of *Chrysomya chloropyga* adults that developed from larvae fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	135	0.00878 ± 0.00122	ab
Chicken liver (b)	72	0.00876 ± 0.00132	ab
Pets mince (c)	78	0.00678 ± 0.00125	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The broadest wings were present on adults that originated from larvae fed on beef liver. Significant differences ($p < 0.0001$) were found in the widths of the wings of adults that originated from larvae fed on different feeding media (Table 5.8).

Table 5.8: Mean wing width (mm) of *Chrysomya chloropyga* adults from larvae fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	128	3.13 ± 0.146	a
Chicken liver (b)	68	3.058 ± 0.12	b
Pets mince (c)	74	2.959 ± 0.138	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The longest wings were manifest on adults that originated from larvae fed on beef liver. Significant differences ($p < 0.0001$) were found in the lengths of the wings of adults that originated from larvae fed on different feeding media (Table 5.9).

Table 5.9: Mean wing length (mm) of *Chrysomya chloropyga* adults from larvae fed on different rearing media.

Food type	n	Mean ± SD	Statistical significance * (p<0.0001)
Beef liver (a)	128	8.443 ± 0.316	a
Chicken liver (b)	68	8.253 ± 0.303	b
Pets mince (c)	74	8.05 ± 0.397	c

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

5.4 DISCUSSION

Chrysomya chloropyga was only one of six species of blowflies that were reared during this study. Other species were *C. marginalis, C. albiceps, C. vicina, L. cuprina* and *S. cruentata*. Oviposition occurred mostly during the day, even at times when the insectary was dark. Erzinçlioğlu (1996) stated that some blowflies will enter very dark places, such as cellars and chimneys, in search of carcasses during the daytime when they are normally active. Conventional belief holds that blowflies are neither active, nor do they lay eggs during night. This method of estimating the time of oviposition was modified when Greenberg (1990) reported nocturnal oviposition by three calliphorid species that are occasionally used as forensic indicators. Singh & Barti (2001) criticised this work because the placement of the bait was on the ground among bushes. They maintained that this could have made it possible for the flies already resting near the bait to crawl over the piece of meat and lay eggs. They, however, recognised that Greenberg had proved that blowflies could lay eggs both at night, and during the day. They replicated his experiments and placed bait on raised platforms, where oviposition occurred during the night. In determining the age of maggots, the possibility of nocturnal oviposition by blowflies should consequently always be taken into consideration.

An interesting observation was the strong odour of ammonia that emanated from the containers in which the maggots were reared. Blowfly larvae produce ammonia in large amounts as an excretory product (Erzinçlioğlu 1996). Another significant observation was the late presence of first instar maggots in the rearing culture, when all the eggs had been laid at approximately the same time. When blowflies mate, sperm passes through the spermathecal ducts into the spermathecae. When the eggs are being laid, they are passed singly down the oviduct and are fertilised by sperm in the spermathecal ducts (Erzinçlioğlu 1996). If a carcass or suitable substrate is available, the fly deposits its eggs immediately and they will continue their development on the carcass. If a carcass is not immediately available, the first egg that passes down the oviduct is fertilised and held in the vagina until the fly finds a carcass. Since it has been fertilised, the egg continues to

develop and when laid it will be at a more advanced stage of development than the eggs laid later. This is why a single first instar larva is sometimes present amongst a mass of unhatched eggs (Erzinçlioğlu 1996). This has serious implications for PMI estimations as it means that some eggs could hatch immediately after oviposition. If the PMI estimation is based only on the largest larvae found during a case study, the result may be an erroneous PMI.

Watson *et al.* (1993) found that only 4% of *M. domestica* larvae survived without bacteria in their feeding medium. Okorie & Okeke (1990) also stated that blowfly larvae feed on decaying organic matter, with bacteria being the principal food of the larvae. Desiccation could have led to bacteria not flourishing, but desiccation was probably the primary cause of first instar mortality.

Pets mince produced the longest development time with low percentages surviving. Larvae reared on pets mince yielded the smallest individuals. Desiccation was the major obstacle in the use of pets mince as rearing medium. Beef liver and chicken liver yielded the best results. The shortest development time occurred with larvae feeding on chicken liver, but the survival of larvae was greater with beef liver as rearing medium. According to Prins (1982) the total larval life span of *C. chloropyga* is 162 to 230 hours, depending on food availability, temperature and humidity. The mean development time of larvae fed on beef liver was 253.4 hours, and with chicken liver 249.6 hours. Greenberg & Szyka (1984) found that the minimal egg-to-adult interval of *C. chloropyga putoria* was 228 hours. No significant differences were found in the development times of pupae that originated from larvae fed on beef liver, chicken liver or pets mince. The developmental rate of the pupae, like that of larvae, is influenced by temperature (Erzinçlioğlu 1996).

Byrd (2001) stated that pork or beef tissue should be the preferred rearing medium in the forensic laboratory. He maintained that successful development would also occur on chicken, but that chicken produced excess liquid during decomposition. It was found in the current study that this liquid-producing characteristic of chicken liver was essential to prevent desiccation of the media and the larvae.

Although technically simple, the rearing of necrophagous flies can be an expensive, unpleasant, time consuming and space-wasting endeavour. Personnel time remained the major expense for the maggot laboratory, requiring six to eight hours per week in maintenance, as was indicated by Sherman & Wyle (1996).

5.5 CONCLUSION

Beef liver produced satisfactory results in the rearing of *C. chloropyga* larvae. However, due to the preparation time involved, it was decided to use chicken liver as the rearing medium of choice, because of its convenience and efficacy as a rearing medium.

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CHAPTER 6

Influence of different constant temperatures on the development of *Chrysomya chloropyga* immatures

6.1 INTRODUCTION

The effects of climate must be taken into account and potential pitfalls and modifying circumstances should be considered to determine the correct PMI (Turner 1991). Weather, including measurable factors such as temperature, rainfall and sunshine may affect blowfly populations. Prevailing weather may either affect the insects directly or it may influence their immediate environment, or both (Fuller 1934). Rates of development of eggs, larvae and pupae of necrophagous calliphorid flies (Diptera: Calliphoridae) at known temperatures, have long formed the basis for producing minimum estimates of PMI or time since exposure to fly oviposition in forensic and allied entomological studies (Davies & Ratcliffe 1994). Insect growth rates are fundamentally influenced by temperature. Insects are exothermic, and their metabolic rate and rate of growth is consequently influenced by the temperature of their immediate surroundings (Turner 1991). Greenberg (1991) pointed out that the development times from oviposition to adult emergence might possibly differ in various regions of the world. This raised the question as to whether it was valid to assume that the thermal constant of a species from the Holarctic Region was the same elsewhere (Greenberg 1991; Grassberger & Reiter 2002). It was consequently decided to study the development of *C. chloropyga* in the Central Free State region at different temperatures.

The key questions in this aspect of the study were:

- How is the development rate of *C. chloropyga* larvae affected at different temperatures?
- What is the optimal temperature for the development of *C. chloropyga*?
- Is the size of adult flies influenced by different rearing temperatures?

6.2 MATERIAL AND METHODS

The larvae used in this experiment were obtained from an established laboratory colony (Chapter 5). The effect of seven different constant temperatures on the development of *C. chloropyga*, namely 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C was studied. Twenty-five one-hour-old larvae were exposed to each of the temperatures. Small cultures, each containing 15 to 25 larvae afford maximum rearing success (Lord & Burger 1983). At the low density of 25 larvae there was no effect of heat building up due to the aluminium foil covering the maggots and the feeding media, but at higher densities this could have a greater influence. The larvae were placed in one litre "maggot motels" directly onto 25g of chicken liver. The chicken liver was replaced when necessary. The "maggot motels" were placed in incubators that had previously been set to the appropriate temperatures (± 1°C). The time required for the larvae to reach pupation was recorded. Once the pupae were sifted from the wood shavings, they were transferred to emergence containers, returned to the appropriate incubators and monitored for the emergence of adults. This procedure provided information on both the duration of the pupal stage and rate of emergence, which are essential and invaluable data in accurate PMI estimation from entomological evidence (Byrd 2001). Adult dry mass, forewing length and forewing width were also recorded and statistical analysis was done as outlined in Chapter 5. The experiment was replicated nine times.

6.3 RESULTS

6.3.1 Development time

The shortest development time for one-hour-old *C. chloropyga* larvae to pupation occurred at 30°C (Table 6.1), and from pupae to eclosion it was at 35°C (Table 6.2). The shortest total development time of *C. chloropyga* occurred at 30°C (Table 6.3). The longest development time was at 10°C with only three individuals reaching pupation (Table 6.2) and adulthood (Table 6.3).

The development times of larvae reared to pupation at 10°C, 15°C and 20°C did not differ significantly ($p < 0.0001$) (Table 6.1). No significant differences were found in the development time of larvae reared to pupation at 10°C (due to small sample size caused by high mortality), 25°C and 35°C ($p < 0.0001$) (Table 6.1). The pupal development time of larvae reared at 10°C and 25°C, and at 30°C and 35°C did not differ significantly ($p < 0.0001$) (Table 6.2).

Total development times did not differ significantly at 10°C, 15°C and 20°C ($p < 0.0001$) (Table 6.3). No significant difference was found in the total development time at 10°C and 25°C ($p < 0.0001$) (Table 6.3). The total development time of larvae reared at 30°C and 35°C also did not differ significantly ($p < 0.0001$) (Table 6.3).

Table 6.1: Mean development time (days) of *Chrysomya chloropyga* from one-hour-old larvae to pupation at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	33 ± 0.00	abcdefg
15 (b)	22	28.41 ± 3.71	abc
20 (c)	118	9.58 ± 1.16	abc
25 (d)	130	6.11 ± 1.10	adf
30 (e)	141	5.41 ± 0.64	ae
35 (f)	151	5.80 ± 0.81	adf
40 (g)	94	6.55 ± 0.91	ag

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

Table 6.2: Mean development time (days) of *Chrysomya chloropyga* from pupation to eclosion at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	37.00 ± 2.65	abcd
15 (b)	12	22.25 ± 2.18	abc
20 (c)	117	8.03 ± 1.18	abc
25 (d)	125	5.10 ± 1.27	ad
30 (e)	127	3.98 ± 0.84	ef
35 (f)	130	3.77 ± 0.89	ef

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

Table 6.3: Mean development time (days) of *Chrysomya chloropyga* from one-hour-old larvae to eclosion at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	72.67 ± 4.16	abcd
15 (b)	12	48.17 ± 1.53	abc
20 (c)	117	17.59 ± 0.82	abc
25 (d)	125	11.11 ± 0.95	ad
30 (e)	127	9.43 ± 0.66	ef
35 (f)	130	9.55 ± 0.86	ef

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The reciprocal of the total development time of *C. chloropyga* at different constant temperatures is shown in Fig. 6.1. The linear regression line of these data points shows that the developmental threshold of *C. chloropyga* occurs at 7.05°C.

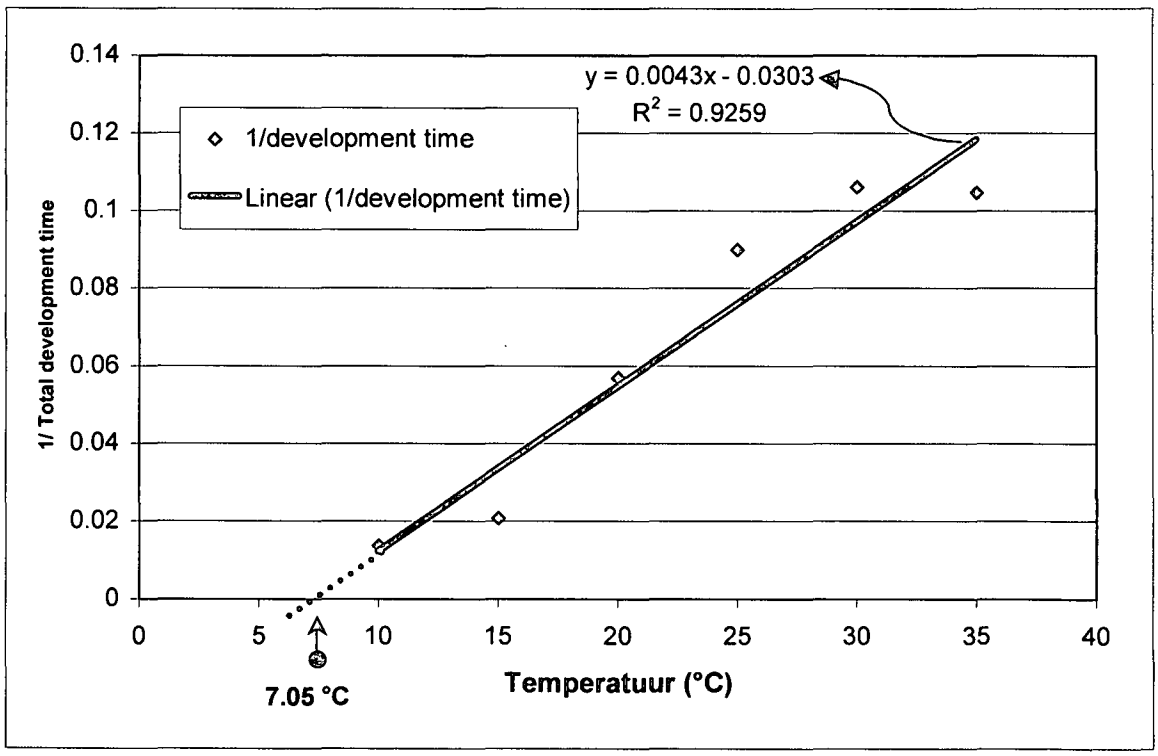

Fig. 6.1. Reciprocal (1/development time) and linear regression for development time against temperature for *Chrysomya chloropyga*, from one-hour-old larvae to eclosion.

6.3.2 Survival

The percentage survival of each replication was calculated and these percentages were used for statistical analyses, hence n = 9 in Table 6.4 and 6.5.

The highest mean percentage survival was recorded at 35°C for both one-hour-old *C. chloropyga* larvae to pupation (Table 6.4) and for one-hour-old *C. chloropyga* larvae to eclosion (Table 6.5). The lowest percentage survival was recorded at 10°C for both one-hour-old *C. chloropyga* larvae to pupation (Table 6.4) and for one-hour-old *C. chloropyga* larvae to eclosion (Table 6.5). The percentage survival of larvae reared to pupation at 10°C and 15°C; reared at 20°C, 25°C and 40°C, and reared at 30°C and 35°C showed no significant difference ($p < 0.0001$) (Table 6.4). No significant difference was found between larvae reared to eclosion at 10°C and 15°C, and at 20°C, 25°C, 30°C and 35°C ($p < 0.0001$) (Table 6.5).

Table 6.4: Mean percentage survival of one-hour-old *Chrysomya chloropyga* larvae to pupation exposed to different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	9	1.33 ± 2.00	ab
15 (b)	9	16.89 ± 9.96	ab
20 (c)	9	52.44 ± 12.07	cdefg
25 (d)	9	57.77 ± 21.46	cdefg
30 (e)	9	62.67 ± 10.58	cdef
35 (f)	9	67.11 ± 5.93	cdef
40 (g)	9	41.78 ± 10.60	cdg

* Means with different letters differ significantly from each other
(One-way ANOVA + Tukey-Kramer Multiple Comparisons Test)

Table 6.5: Mean percentage survival of one-hour-old *Chrysomya chloropyga* larvae to eclosion exposed to different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	9	1.33 ± 2.00	ab
15 (b)	9	5.33 ± 9.59	ab
20 (c)	9	52.00 ± 12.17	cdef
25 (d)	9	55.56 ± 20.24	cdef
30 (e)	9	56.44 ± 10.67	cdef
35 (f)	9	58.22 ± 9.40	cdef

* Means with different letters differ significantly from each other
(One-way ANOVA + Tukey-Kramer Multiple Comparisons Test)

6.3.3 Morphometric data

The highest mean dry mass of adult *C. chloropyga* was recorded at 10°C (Table 6.6). The lowest mean dry mass was recorded at 15°C. The mean adult dry mass of individuals reared at 10°C, 20°C, 25°C, 30°C and 35°C did not differ significantly from each other ($p < 0.0001$) (Table 6.6).

Table 6.6: Mean dry mass (g) of *Chrysomya chloropyga* adults that originated from larvae reared at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	0.01122 ± 0.00016	acdef
15 (b)	11	0.00735 ± 0.00162	b
20 (c)	111	0.01053 ± 0.00111	acdef
25 (d)	110	0.01045 ± 0.00179	acdef
30 (e)	111	0.01067 ± 0.00122	acdef
35 (f)	118	0.00998 ± 0.00163	acdef

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The highest mean wing width of adult *C. chloropyga* was at 20°C (Table 6.7). The smallest mean wing width was at 15°C. Wing widths of adults from larvae reared at 10°C, 20°C, 25°C, 30°C & 35°C did not differ significantly ($p < 0.0001$) (Table 6.7).

Table 6.7: Mean wing width (mm) of *Chrysomya chloropyga* adults that originated from larvae reared at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	3.17 ± 0.12	abcdef
15 (b)	10	2.85 ± 0.20	abef
20 (c)	110	3.21 ± 0.55	ac
25 (d)	119	3.06 ± 0.15	ade
30 (e)	100	3.04 ± 0.13	abdef
35 (f)	119	3.00 ± 0.14	abef

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

The longest mean wing length of adult *C. chloropyga* was at 10°C (Table 6.8). The shortest mean wing length was at 15°C. Wing lengths of adults from larvae reared at 10°C, 20°C, 25°C and 30°C, and at 15°C, 25°C, 30° and 35°C did not differ significantly ($p < 0.0001$) (Table 6.8).

Table 6.8: Mean wing length (mm) of *Chrysomya chloropyga* adults that originated from larvae reared at different constant temperatures.

Temperature (°C)	n	Mean ± SD	Statistical significance * (p<0.0001)
10 (a)	3	8.97 ± 0.12	acde
15 (b)	10	8.08 ± 0.54	bdef
20 (c)	110	8.67 ± 0.28	ac
25 (d)	119	8.36 ± 0.35	abde
30 (e)	100	8.31 ± 0.29	abdef
35 (f)	119	8.17 ± 0.32	bef

* Means with different letters differ significantly from each other
(Kruskal-Wallis test + Dunn's Multiple Comparisons Test)

6.4 DISCUSSION

Most studies have used larval densities of less than 100 to determine larval development rates. Dadour *et al.* (2001a), however, considered such studies to be of limited forensic application. They argued that determining the age of larvae collected at a crime scene from high density larval masses and then applying data from baseline studies that have used lower larval densities may result in an over estimation of larval age. This may lead to an inaccurate estimate of PMI. Dadour *et al.* (2001a) 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.

Insect development is temperature dependent. Metabolic rate increases with increased temperature (Chapman 1998), which results in a faster rate of development. The duration of development consequently decreases with increased temperature, and *vice versa* within optimum temperatures for that species. Entomological data can be used to estimate the

PMI in two basic ways. During the earlier progress of decomposition, the estimate is based on the period needed for each represented species to develop to the growth stage collected at the death scene. Most often these are fly maggots, primarily blowflies and flesh flies (Catts & Goff 1992). Generally, within a certain range of temperatures, development is accelerated as temperature is increased, but this does not hold true at temperature extremes that may prove lethal to the insect. The current study also showed that development is retarded at high temperatures. Both air temperature and exposure to sunlight will affect corpse temperature (Wells & Lamotte 2001). The effect of maggot-mass temperature may also differ seasonally. In temperate regions during spring with gradual warming trends, the rate of egg and early immature maggot development is governed largely by changes in ambient temperatures. This is reversed in autumn and the seasonal cooling trends probably have much less of an effect on maggot development rates because of maggot-mass generated metabolic heat (Catts 1992).

Morris (1994, in Dadour *et al.* 2001b) stated that the higher the temperature the faster the rate of development of the insect. This was also observed in the current study up to 30°C (total development time). At 35°C the total development time increased slightly from the development time at 30°C.

Comparisons between development rate under fluctuating and constant temperatures, indicated that development rates are species specific (Dadour *et al.* 2001a). In some species development is accelerated, in others it is retarded, and in some there are no quantifiable differences (Dadour *et al.* 2001a).

The effects of temperature on insect growth, behavior, general physiology and ecology have been comprehensively studied. Such studies have, however, been devoted almost exclusively to the elucidation of the effects of different constant temperatures, or to the determination of the insect's responses to day-degree accumulations of daily means above a development threshold. Little attention has been paid to the effects of thermoperiod on insect biology (Beck 1983). Scrutiny of fluctuating temperature curves with the

corresponding constant temperature curves reveals important differences in rates of growth at an equivalent thermal sum (Davies & Ratcliffe 1994).

When insects are reared under constant temperature regimes, their growth rates tend to be directly proportional to the temperatures applied to a specific species. Such proportionality tends to be linear over much of the physiological temperature range but may deviate from linearity as either maximum or minimum temperatures are approached. A development threshold temperature (or development zero) can be estimated by extrapolation from the linear portion of the regression of development rate on temperature to the baseline (Beck 1983). The development threshold temperature for *C. chloropyga* was established to be 7.05°C.

A study on *C. vicina* was carried out by Johl & Anderson (1996) to simulate the influence of chilling on insects at 3°C before their collection from cadavers in a mortuary. In their study, eggs, larvae and pupae reared at 24°C were held for 24 hours at an ambient temperature of 3.0 ± 0.14°C. Such treatment of any stage induced a 24-hour delay in adult emergence because the insects did not appear to develop while chilled. No mortality occurred in any stage, including eggs and first instar larvae, during chilling (Johl & Anderson 1996). Unfortunately no information was given on what happens after 24 hours and how long the larvae can survive chilling.

Chilling at 3°C arrested development, which resumed when the insects were returned to room temperature. A time-lag equivalent to the time spent under chilling was evident throughout development up to adult emergence (Johl & Anderson 1996). Recent work has shown that *C. vicina* can continue to develop at an extremely slow rate, under very cool conditions (103-115 days spent in the pupal stage at 5°C) (Davies & Ratcliffe 1994). Johl & Anderson (1996), however, found that chilling significantly retarded development. The delay in development approximates the chilled time (Johl & Anderson 1996). This information is important for, and should improve the accuracy of forensic investigations.

In the current study it was found that the mortality of larvae was higher in some of the "maggot motels" than in others. Wells & Kurahashi (1994) made similar observations in a previous study. Their sample sizes and direct observation of dead eggs and pupae indicated that *C. megacephala* suffered high mortality in some jars. As in the current study, they did not know whether this was an artifact caused by their experimental procedure. This does, however, 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).

Studies on the effect of low temperatures on development were performed by Leopold (2000) and entailed the effect of short-term cold storage of Muscidae embryos. Chilling tolerance was influenced by the length of the storage period and by the age of the embryo at the time of exposure to low temperature. Three-hour-old embryos placed in cold storage at 5°C were able to survive for three days with little reduction in larval emergence or adult eclosion and vitality. One-hour-old embryos were the least tolerant of chilling and could not survive for even one day at 5°C (Leopold 2000). Three patterns of chilling injury were expressed. They were characterized as immediate, accumulative, and latent. Expression of immediate and accumulative injury was linked to the age of the embryo at the time of chilling, and the latent type of injury was expressed during the postembryonic stages of development and was related to length of cold exposure (Leopold 2000). In the current study none of the embryos was subjected to cold storage, since one-hour-old larvae were used. However, only three individuals reared at 10°C reached adulthood (1.33 ± 2.00%). The greatest survival of *C. chloropyga* occurred at 35°C (58.22 ±9.40%), making 35°C the optimum temperature for rearing of *C. chloropyga* larvae. The mortality of larvae reared at a constant 40°C was 100%.

6.5 CONCLUSION

"*Physical factors influence the biology of the blowfly and they exercise a more profound effect on the population through the medium of the environment. The direct effect of physical factors, however, may play some small part, since they are known to affect the rate of development and activity of the insects. This part could not be measured, but it is certainly small.*" (Fuller 1934).

Temperature has the most significant influence on the development of blowfly larvae, and this effect is most certainly measurable. Different constant temperatures provide an indication of the temperature tolerance of blowfly larvae, but fluctuating temperatures exist in nature, and may have a more profound effect on blowfly development. It would be advisable to conduct fluctuating temperature studies by using actual temperature data from the crime scene in any case study to correctly determine the PMI.

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CHAPTER 7

Summary

SUMMARY AND GENERAL CONCLUSIONS

- Prone pig carcasses were exposed to full sunlight, while others were hung in full sunlight and full shade during various seasons. Five stages of decay were recognised, fresh, bloated, active decay, advanced decay and remains. Prone carcasses decomposed further (based on mass loss) and insects removed more tissue from prone carcasses than at the hanging carcasses. This was due to the greater effect of desiccation observed at the hanging carcasses. The greatest percentage of biomass removal occurred as a result of maggot feeding masses, an outflow of body fluids and desiccation. Differences in the rate of decomposition between study sites was also observed and attributed to the variations in temperatures between sites.

- The carcasses were initially invaded by Diptera during the onset of decomposition and were gradually replaced by Coleoptera. Diptera preferred the carcasses during the moist stages of decomposition while Coleoptera preferred the carcasses during the drier stages. Dermestidae and Cleridae occurred in larger numbers on hanging carcasses compared to the prone carcasses during all seasons. A higher occurrence of Muscidae was observed on the hanging carcasses than on the prone carcasses during the warmer seasons.

- Internal carcass temperatures measured in the thorax and upper abdomen were higher than the ambient temperature owing to metabolic heat generated by large larval masses. At the hanging carcasses the internal temperatures approximated the ambient temperature more closely. After post-feeding larvae had migrated from the carcasses to pupate, high internal carcass temperatures were the result of sun insolation.

- The greatest difference in seasonal occurrence of insects found at the carcasses was observed for *C. chloropyga*. Large numbers of this species dominated the Diptera during the spring 1999 trial, while this species was rarely observed during the other trials. Several case studies identified this species as an extremely important forensic indicator species in the Free State region.

- If two decomposing bodies, one prone and one hanging, are found at the same time in the same stage of decomposition, and are occupied by the same insects, the prone body will have a shorter PMI than the hanging body. This is due to the effect of orientation of the body on the factors driving decomposition, namely desiccation and temperature.

- Laboratory studies with *C. chloropyga* at 25°C revealed that chicken liver yielded the shortest mean development time (10.4 ± 0.71 days) with the highest mean percentage (67.11 ± 11.09) of survival of larvae to adulthood occurring with beef liver as rearing medium. Morphometric data showed that the largest adults were produced with beef liver as feeding medium (dry mass: 0.00878 ± 0.00122 grams and wing length: 8.44 ± 0.32 mm).

- Beef liver produced satisfactory results in the rearing of *C. chloropyga* larvae. However, due to the preparation time involved, it was decided to use chicken liver as the rearing medium, because of its convenience and efficacy as a rearing medium.

- Laboratory studies in which chicken liver was used as feeding medium for *C. chloropyga* larvae revealed that the shortest mean development time (9.4 ± 0.66 days) occurred at 30°C with the highest mean percentage (58.22 ± 9.4) of survival of larvae to adulthood occurring at 35°C. Morphometric data showed that the heaviest adults were produced at 10°C (dry mass: 0.01123 ± 0.00015 grams; percentage survival: 1.33 ± 2.0) and the longest wing lengths at 20°C (8.67 ± 0.28 mm).

- Different constant temperatures provide an indication of the temperature tolerance of blowfly larvae, but fluctuating temperatures exist in nature, and may have a profound effect on blowfly development. It would be advisable to conduct fluctuating temperature studies by using actual temperature data from the crime scene in any case study to correctly determine the PMI.