The influence of clothing, wrapping and physical trauma on carcass decomposition and arthropod succession in central South Africa.

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Philosophae Doctor

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PREFACE

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

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J.A. Kelly

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ABSTRACT

Forensic entomology is the study of arthropods associated with bodies. Arthropod successional studies have been successfully used to estimate a postmortem interval. This research was to determine the influence of a) seasons, b) clothing, c) wrapping and d) knife wounds on carcass decomposition and arthropod succession.

The experimental site consisted of a 26 hectares grass field interspersed with trees. For the wrapped trials, six pig (Sus scrofa) carcasses were divided into three sample groups, each with a clothed carcass and an unclothed carcass wrapped in sheeting. Arthropod sampling was done (i) daily, (ii) five day intervals and (iii) ten day intervals. Two additional unwrapped carcasses, one with clothes and one without, were sampled daily as controls. For the wounds trials six carcasses were divided into three groups. Each group consisted of a carcass with clothes and one without clothes. The wounds consisted of (i) a knife wound to the throat, (ii) three deep knife wounds, in the back, in the front thoracic and in the front abdominal region. The controls, were without any wounds.

Oviposition occurred simultaneously and was not delayed or hastened by the presence of wrapping, clothing or wounds. However, during the winter wrapped trials there was a delay of four days. In winter, the carcasses remained acceptable to Diptera for oviposition over an extended period. Oviposition continued up to two months after placement, whilst in the warmer seasons oviposition occurred within the first few days. The Diptera did not select the wounds as oviposition sites. Calliphoridae and Sacrophagidae were the dominant Diptera recorded during all the trials. In the autumn and summer seasons *Chrysomya marginalis* and *Chrysomya albiceps* were the dominant species. In the spring seasons, the dominant species were *Chrysomya chloropyga* and *C. albiceps*. In the winter seasons, *Sarcophaga cruentata*, *C. chloropyga*, *Calliphora vicina,* and *Lucilia* spp. were the species breeding on the carcasses. Muscidae adults were present during all the seasons, but no maggots of this family were recorded. Due to the short oviposition time during warmer seasons, the maggots were of a similar age at any time. Due to the extended oviposition that occurred during winter, different instar groups, often the same species, were present at any time. In all seasons the Coleoptera community present on the carcasses were

dominated by *Dermestes maculatus* (adults and larvae) and *Necrobia rufipes*. In the summer *Thanatophilus micans* (adults and larvae) and Histeridae spp. were also recorded on the carcasses. There was no overall difference in arthropod succession between any of the carcasses. During the autumn seasons, noticeable predation by *C. albiceps* maggots on *C. marginalis* maggots was observed. There was limited maggot predation during the spring trials and some predation observed during the summer trials. Presence of clothing, wrapping and wounds had no influence the Coleoptera community. In the winter seasons, *D. maculatus* larvae were found while the maggots were still present on the carcasses. In summer seasons, they were only present after maggot migration. Significant maggot mortality was associated with the wrapped carcasses during the warmer seasons. The presence of the sheets or clothing did allow the maggots to move more freely on the surface of the carcasses, especially in the summer. Less skin remained on the wrapped or clothed carcasses after the maggots migrated to pupate.

Key words: Forensic entomology; arthropod succession; wrapping; clothing; stab wounds; Diptera; Coleoptera; Sarcophagidae; Calliphoridae

UITTREKSEL

Forensiese entomologie is die studie van insekte wat met dooie liggame geassosieer word. Arthropoodsuksessie studies is reeds suksesvol gebruik om die postmortem interval te bereken. Hierdie navorsing is gedoen om die invloed van a) seisoene, b) kleding, c) toedraai en d) steekwonde op karkasse m.b.t. ontbinding en inseksuksessie te bepaal. Die narvorsing is op ‘n 26 hektars grasveld perseel met verspreide bome, uitgevoer. Vir die eksperiment waarin die karkasse toegedraai is, is ses varkkarkasse (*Sus scrofa*) in drie groepe verdeel, elkeen met ‘n geklede en nie-geklede karkas toegedraai in ‘n laken. Insekversamelings is (i) daagliks, (ii) elke vyf dae en (iii) elke tien dae gedoen. Twee bykomstige nie-toegedraaide karkasse, een geklede en een nie-geklede, is daagliks as kontroles ondersoek. Vir die steekwonde-eksperiment is ses karkasse, wat in drie groepe verdeel is, gebruik. Elke groep het ‘n geklede en nie-geklede karkas ingesliut. Die wonde het bestaan uit ‘n snywond aan die keel, drie diep steekwonde, in die rug, aan die voorkant in die bors en in die abdomen. Die kontroles was sonder enige wonde.

Eierlegging het gelyktydig plaasgevind en is nie versnel of vertraag deur die aanwesigheid van ‘n laken (toegedraai), kleding of wonde nie. Gedurende die winter was daar egter ‘n vertraging van vier dae in eierlegging by die toegedraaide karkasse. Die karkasse het ook gedurende die winter lank geskik gebly vir eierlegging deur die Diptera. Eierlegging het tot twee maande na plasing in die veld voortgeduur. Gedurende die warmer seisoene het eierlegging binne die eerste paar dae plaasgevind. Die Diptera het nie die wonde vir eierlegging verkies nie. Calliphoridae en Sarcophagidae was die dominante Diptera wat gedurende al die eksperimente versamel is. Gedurende die herfs- en somerseisoene was *Chrysomya marginalis* en *Chrysomya albiceps* die volopste spesies. Gedurende die lente was *Chrysomya chloropyga* en *C. albiceps* die dominante spesies. *Sarcophaga cruentata*, *C. chloropyga*, *Calliphora vicina* en *Lucilia* spp. het gedurende die winter op die karkasses voorgekom. Muscidae volwassenes was aanwesig tydens alle seisoene, maar geen maaiers van hierdie familie is ooit waargeneem nie. As gevolg van die kort eierleggingsperiode tydens die warmer seisoene, was die maaiers op enige tydstip van dieselfde ouderdom. Weens die verlengde eierlegging tydens die winter was verskillende maaier ouderdomsgroepe, dikwels van dieselfde spesie, op dieselfde

tydstip teenwoordig. Gedurende die verskillende seisoene was Coleoptera teenwoordig op die karkasse. *Dermestes maculatus* (volwassenes en larwes) en *Necrobia rufipes* was die dominante spesies. Gedurende die somer was *Thanatophilus micans* (volwassenes en larwes) en Histeridae spp. ook teenwoordig op die karkasse. Daar was geen algemene verskil in die arthropoodsuksessie op die verskillende karkasse nie. Tydens herfs is daar aansienlike predasie deur *C. albiceps* maaiers op *C. marginalis* maaiers waargeneem. Daar was beperkte maaierpredasie tydens die lente en enkele insidente van predasie is tydens die somer waargeneem. Teenwoordigheid van kleding, die feit dat die karkasse toegedraai was en teenwoordigheid van wonde het geen invloed gehad op die Coleoptera nie. Gedurende die winter is *D. maculatus* larwes op die karkasse gevind terwyl die maaiers steeds teenwoordig was. In die somer was hulle slegs aanwesig nadat die maaiers geëmigreer het. Beduidende maaiermortaliteit is geassosieer met die toegedraaide karkasse tydens die warmer seisoene. Die teenwoordigheid van die lakens of kleding het die maaiers toegelaat om meer vrylik op die oppervlak van die karkasse te beweeg, veral in die somer. Minder vel het op die toegedraaide of geklede karkasse oorgebly nadat die maaiers geëmigreer het om papies te word.

Sleutelwoorde: Forensiese entomologie; arthropoodsuksessie; kleding; steekwonde; Diptera; Coleoptera; Sarcophagidae; Calliphoridae

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INTRODUCTION

'Who saw him die?'
'I', said the Fly
'With my little eye,
I saw him die'
Anon., 'Who killed Cock Robin?'

Forensic entomology is the study of arthropod communities as biological indicators of time elapsed since death of an organism. This is based on the principal of ecological succession of these arthropods associated with the body. These studies are usually related to suspected criminal events, for the purpose of uncovering information useful to an investigation (Smith 1986, Catts & Haskell 1990, Byrd & Castner 2001). Research in this field expands across the world.

The most commonly used and required information that can be provided by forensic entomological work is the estimated minimum time of death i.e. the postmortem interval (PMI). Normally determining the PMI estimation of a victim is the responsibility of a forensic pathologist. The pathologists use medical parameters to determine the PMI. However, the accuracy of these estimations decreases as more time elapses after death (Kashyap & Pillai 1989, Tempelman-Kluit 1993). Arthropods are used as biological indicators of PMI and have been shown to offer more reliable estimations for longer PMI intervals. From 72 hours after death, forensic entomology is usually the most accurate and in some cases the only method that can be used for making PMI estimations (Kashyap & Pillai 1989, Tempelman-Kluit 1993). Forensic entomological methods have been successfully applied in many cases, with the calculations of the PMI by entomological techniques fitting well with the time intervals established by other means (e.g. Smith 1986, Goff *et al.* 1988, Catts & Haskell 1990). In recent years it has developed and has become increasingly important.

PMI estimations are based on the body decomposition, faunal evidence analysis and the environmental influences (Fig. 1.1.) (Hall 1990).

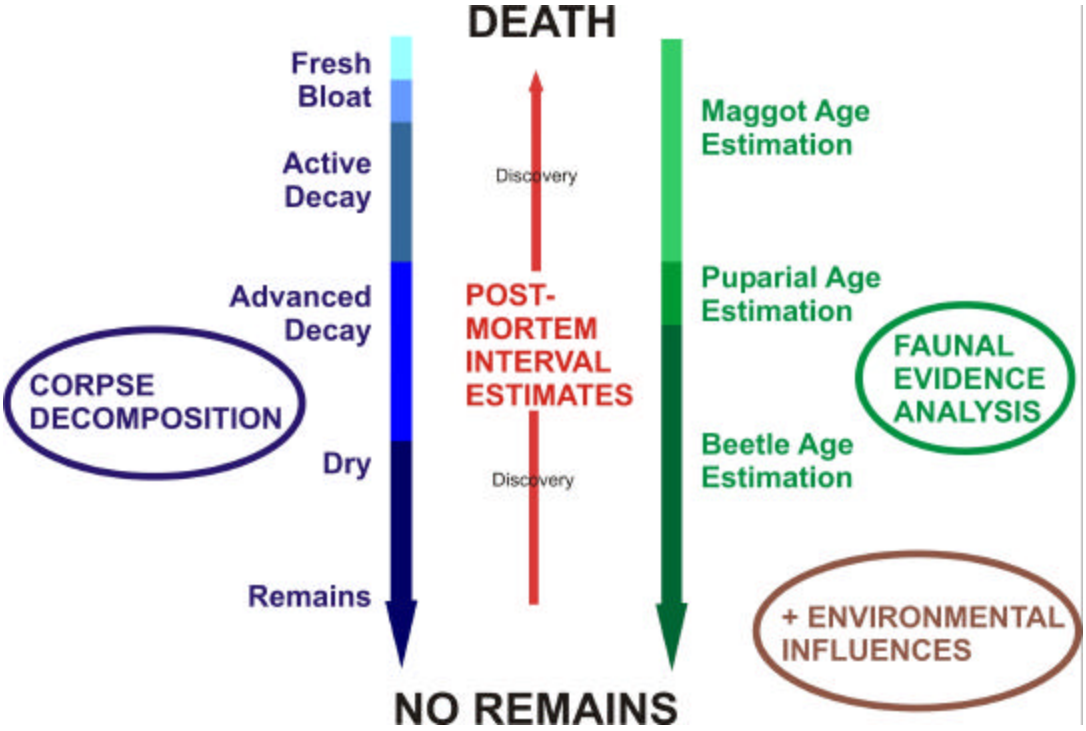


Fig. 1.1. A generalized death and decomposition scenario relating to the calculation of post mortem interval (PMI) using the analysis of faunal evidence (modified from Catts 1990).

The decomposition of the body may be influenced by a number of factors. For example, Payne (1965) showed the importance of the arthropod community in the decay of baby pigs. Pig carcasses that were free of insects, decomposed and dried very slowly, retaining their form for many months. While the carcasses that were open to insect colonization had 90% of their tissues removed in six days.

To analyze the faunal evidence found on, in or near a body, accurate identification, taxonomy and knowledge of arthropod biology, particularly their development rates and geographic distribution is essential (Hall 1990).

A definite ecological succession occurs among the arthropod community on decomposing carcasses (Payne 1965). Each stage of decay is characterized by a particular group of arthropods, each of which has a particular life strategy which allows it to occupy a particular niche (Payne 1965). These arthropods tend to be far more specifically associated with decomposing bodies than any other resource

(Putman 1983). Co-existence between the arthropods is facilitated by partitioning of the carcass resources (Wells & Greenberg 1994a) and succession within a single carcass (Schoenly & Reid 1987). The decomposers of carcasses may be divided into two groups. Those that consume the soft tissues of fresh carcasses, generally Diptera (true flies), and those that utilize the skin and hair material of decomposed carcasses, generally Coleoptera (beetles) (Payne 1965, Putman 1983).

Diptera, the primary decomposers, are known to use decomposing tissues are the Calliphoridae (blow flies), Sarcophagidae (flesh flies) and the Muscidae (filth, house flies) (Fuller 1934). These flies are considered to be the vultures of the insect world, with the ability to locate decomposing bodies over vast distances and in any landscape type (Greenberg 1991). For example, they can travel 20km in a day, although the majority of their movement is usually within a neighbourhood, especially in urban environments. In open country, these flies could probably cover greater distances (Greenberg 1991). The natural history of blow flies is thought to include severe competition for larval (maggot) food resources (Fuller 1934). In fact, almost every aspect of their biology suggests intense competition for the rapid location and consumption of decomposing bodies (Fuller 1934, Putman 1983). This resource, however, is patchy and ephemeral (Beaver 1984 in Wells & Greenberg 1994a). Blow flies are considered to be one of the most important forensic indicators during the initial decomposition of a carcass (Lord 1990), as they complete every stage of their life cycle on the carcasses (Putman 1983) (Fig. 1.2.). Knowledge of this life cycle and the duration of each of the life stages are used in PMI estimations (Fig. 1.1.). In the Diptera, the adults (Fig. 1.2A.) are morphologically different from the maggots (Fig. 1.2C.). As a result these insects undergo holometabolous metamorphosis. The maggots are vermiform larvae and have wormlike bodies with no legs (Fig. 1.2C.). The maggots undergo three larval instars before pupation into coarctate pupae (Fig. 1.2D.) (Romoser 1981).

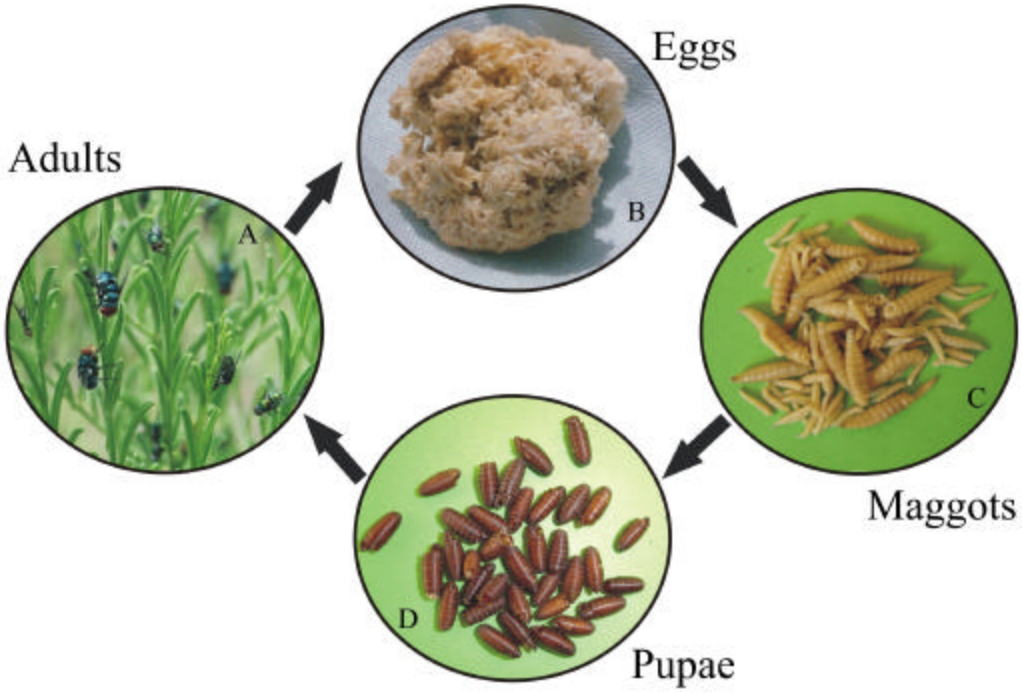


Fig. 1.2. The blow fly (Calliphoridae) life cycle.

Coleoptera, secondary decomposers, become the most important forensic indicators during later stages of decomposition, as they are known to feed upon the skin (Lord 1990, Boucher 1997). These include the Dermestidae (Fig. 1.3.) and Cleridae (Boucher 1997).

Adult Coleoptera (Fig. 1.3a) are morphologically different from their larvae (Fig. 1.3b.). As a result these insects undergo holometabolous metamorphosis. Some of the larval forms are campodeiform or platyform larvae. They have well-developed heads and thoracic legs (Fig. 1.3b.) (Romoser 1981). The life cycles of the forensically important Coleoptera may vary. For example, Dermestidae have 4-8 larval instars while Silphidae larvae moult three times and last instar (the prepupa) is dormant for a short period before pupation occurred (Boucher 1997).

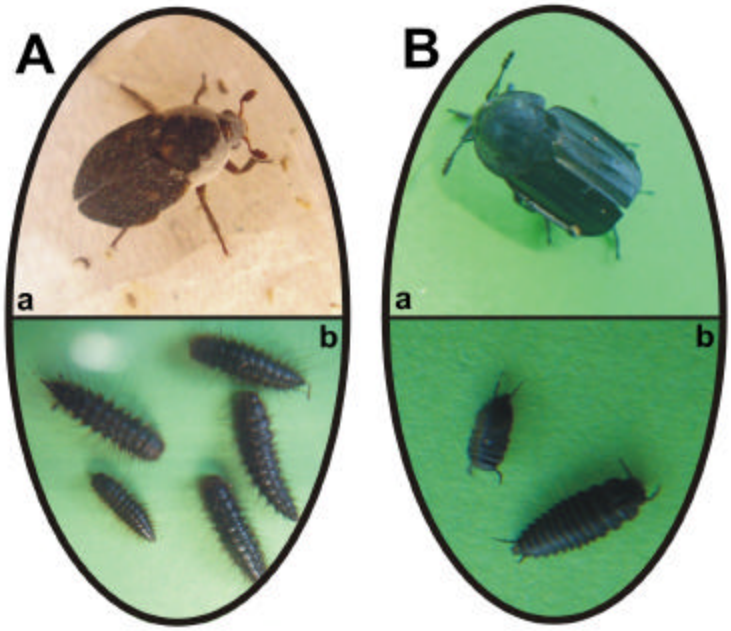


Fig. 1.3. Coleoptera adults and larvae. A – Demestidae, a) adult, b) larvae. B – Silphidae, a) adult, b) larvae.

Other arthropods from other arthropod guilds may occur on decomposing bodies. These include species that may simply be using the carcasses as shelter or occurring accidentally (incidentals or opportunists). Other species may be predators, parasitoids, parasites, opportunistic feeders and scavengers, drawn to the carcasses by prey species e.g. maggots (Putman 1983).

There are many factors that can influence the normal time sequences of carcass faunal development or succession. To allow for the most accurate PMI estimations, forensic entomological research is aimed at increasing the knowledge of these factors. This would reduce the assumptions made by a forensic entomologist (Catts 1990).

There are, however, as many factors as there are locations and circumstances in which death could occur. The environment in which a body is found is important, for example, arid environments (Galloway *et al.* 1989) or desert (Schoenly & Reid 1982, Hegazi *et al.* 1991), tropical environments (Cornaby 1974) or intertidal zones (Davis & Goff 2000). Goff *et al.* (1988) for example, showed significant differences in gross appearances of three cases of human remains found in different environments, although the postmortem intervals were similar in all cases.

In some cases forensically important arthropods may provide clues as to place of death (e.g. rural, urban, indoors or outdoors) (Greenberg 1985) or if the body has been moved. The number of taxa present on a body found indoors decreased rapidly (Early & Goff 1986, Goff & Odom 1987). Goff (1991), however, showed that there maybe a greater variety of Diptera associated with them. This contrasts with the situation outdoors, where there is an increase in the number of taxa through the first three to four weeks of decomposition (Early & Goff 1986, Goff & Odom 1987), while they may have a greater variety of Coleoptera species present (Goff 1991). Some taxa were restricted to bodies discovered inside, while others were found only associated with bodies in outdoor situations (Goff 1991).

Shean *et al.* (1993), found that a carcass exposed to full sunlight decomposed faster than a carcass that was in shaded woodland. The shaded carcass was slightly less species rich (11 species) than the exposed carcass (16 species). Graham-Smith (1916) reported that flies are usually very inactive on cloudy days.

The season may influence the co-existence or competition between the carcass arthropod communities (Denno & Cothran 1975, Hanski & Kuusela 1980, Archer & Elgar 2003). Seasonal changes of the species of blow flies (Calliphoridae) and flesh flies (Sarcophagidae) have been recorded in Maryland (USA) (Introna *et al.* 1991) and Indiana (Johnson 1975). The same trends were also recorded in Mississippi, when both fish and mammalian carcasses were used as bait (Goddard & Lago 1985). Added to this, arthropods are poikilothermic, i.e. their body temperature corresponds to the ambient temperature. This means that temperature exerts a strong influence on the reproduction and development of the arthropods (Romoser 1981). Arthropods are often reared in laboratories under constant temperature and humidity to determine the time required for their development. However, in nature, the arthropods are exposed to fluctuating temperatures, which may hasten, retard, or have no effect on the rate of development (Beck 1983). The congregation of blow fly maggots on carcasses into maggot masses may provide some form of temperature regulation or increase in the immediate areas surrounding the developing maggots (Turner & Howard 1992). Wells & Kurahashi (1994) state that blow fly maggots can delay pupation if conditions are sub-optimal e.g. the maggots have no shelter or it is very wet.

It is generally accepted that blow flies are not active and do not oviposit at night (Greenberg 1985). Typical diurnal activity curves of some blow flies show little movement in early daylight. There is a peak in activity towards early afternoon and a sharp decline in late afternoon (Nuorteva 1959, Baumgartner & Greenberg 1984, 1985). In the summer in temperate regions and in the tropics, the lack of oviposition seems to be linked to light because the temperatures can be 25^{0}C at night and the weather perfect for flying. However, Greenberg (1990) later demonstrated that blow flies may oviposit on small animal carcasses during the dark of night. Determination of ovipostion at night or not, is of forensic importance because it could change an estimation of the PMI by as much as twelve hours (Greenberg 1990).

Limitation of access by arthropod to bodies complicates analysis of specimens by forensic entomologists (Catts 1992). Access may be denied or influenced by wrapping the remains or by burying them. For example, Goff (1992) showed that wrapped pig carcasses delayed oviposition for 2.5 days following exposure. Subsequent repetitions of the experiment have yielded times of 2.4 to 3.0 days for penetration under similar temperatures (Goff 1992). Rodriquez & Bass (1985) reported that arthropod access to buried bodies is highly dependant on the depth of burial and environmental temperatures. Female Sarcophagidae deposit live larvae rather than eggs and these may be dropped when the adult cannot physically reach the body (Denno & Cothran 1975).

Another factor may be the condition of bodies, for example, Grisbaum *et al.* (1995) showed that refrigeration of pig carcasses did not alter the insect succession. Avila & Goff (1998) showed that no marked differences were noted in arthropod fauna present or the duration of the stages of decomposition between burnt and control carcasses. However, the major oviposition by flies occurred one day earlier on burnt carcasses. All of these factors are continually being researched.

Other than PMI estimates, forensic entomology can be used to provide other information related to crime scene/death investigations. This information can be vital in homicide investigations. For example, suspects have been linked to the scene of a crime as a result of them having been bitten by arthropods specific to the crime scene vicinity (Webb *et al.*1983 in Benecke 1998, Prichard *et al.*1986 in Benecke 1998).

Arthropods that live in a specific locality but are found on a body in a different area may prove that the body was moved after death (Benecke 1998). Entomotoxicology may be used to prove the use of drugs or poison by the victim. This is done by detecting the presence of the drugs or poison in the arthropods' body (Goff & Lord 1994, Catts & Haskell 1990, Introna *et al.* 2001). The presence, position and pattern of wounds may be found on a corpse due to the presence of arthropods at possible wound sites (Catts & Haskell 1990). Maggots and flies found in clean, empty or occupied rooms or crime scenes maybe explained by linking the entomological evidence to the surroundings of the scene (Nuorteva 1977 in Benecke 1998, Benecke 1996 in Benecke 1998). Nuorteva (1974) described a case where the age of a bloodstain in a decaying shirt was determined by rearing the maggots to adults. This allowed the shirt fauna to be linked to the blood in the house in question. Even in ancient times, crime instruments were reported to be identified using entomological evidence (Sung Tz'u 1981 in Benecke 1998). Forensic entomology evidence may supply information as to whether a victim was killed and/or brought outside (a) by night or by day and/or (b) while it was raining or not (Nuorteva 1977 in Benecke 1998, Smith 1986, Benecke 1996 in Benecke 1998). Late colonizing arthropods allow analysis even in badly decomposed or skeletonized corpses (Nuorteva 1977 in Benecke 1998, Lord *et al.* 1994, Beyer *et al.* 1980).

Forensic entomology can also be used to provide information on how long children had been neglected by their parents (Lord 1990) and also in cases of child abuse (Benecke & Leggig 2001). Lastly forensic entomology has also been applied to cases of wildlife poaching (Anderson 1999, Watson & Carlton 2003, 2005)

The history of forensic entomology is sporadic but the importance of flies was recognized even during ancient Egyptian times. In Egyptian Mythology, Vachit was the god of the flies. A short overview of the known milestones in forensic entomology is as follows:

1600 B.C. First published reference to blow flies in the Har-ra-Hubulla, a collection of cuneiform writings on clay. Oldest known book on zoology. First mention of "green" and "blue" fly (McKnight 1981).

907-60 Court officer hears woman's endless weeping and inquires of her troubles. Woman says her husband was killed by fire but officer sees flies clustered around the head of the corpse. An autopsy revealed a snag, which the wife later confessed to placing in the head of her husband. Reported by Cheng Ko 1890 (Greenberg & Kunich 2002).

1247 Sung Tz'u, a Chinese "death investigator". Wrote a book entitled *The Washing Away of Wrongs*. The text reports wounds inflicted by a sickle. The death investigator assembled all the farmers from the neighbourhood and had them lay their sickles on the ground. It was mid-summer, the weather was hot and the blow flies landed on only one sickle, whose owner confessed to the murder. (McKnight 1981).

1626 Francesco Redi disproves the idea of spontaneous generation based on the appearance of maggots on rotten meat (Hall 1974).

1734 History of Insects, René de Réaumur's classical work on entomology (Hellemans & Bunch 1988).

1848 Orfilia, a pathologist, listed 30 insects and other arthropods that visited a corpse to feed and oviposit. He may have been the first to systematize the knowledge of arthropod succession in human corpses (Greenberg & Kunich 2002).

1850 In Paris, Dr. Bergeret d'Arbois of Jura was the first westerner to use insects as forensic indicators. He performed an autopsy on the body of a child discovered by a plasterer while repairing a mantelpiece. He found that a flesh fly had deposited larvae in 1848 and mites had laid eggs in the dried corpse in 1849. He concluded that suspicion should fall on the occupiers of the house in 1848.

1894 J. P. Mégnin published *La Fauna des cadavers: Application de l'entomologie à la medicine légale*, making the medical and legal professions aware that entomological data could assist in forensic investigations. He established the science of Forensic Entomology (Greenberg & Kunich 2002).

1902 Niezabitowski was the first to study insects on cadavers in the Russian Empire. His observations differed from Mégnin's, casting doubt on the application of entomology in Russia (Greenberg & Kunich 2002).

1935 The Ruxton Case – 29 September 1935 police were informed of human remains discovered in a river near Edinburgh. Two bodies were re-assembled and proved to be Mrs. Ruxton and her children's nurse, Mary Rogerson. The date on which the remains were deposited was established by the presence of third instar maggots whose age was estimated at 12-14 days by Dr. A. G. Mearns. This evidence agreed with and

corroborated other evidence and led to the conviction of Dr. Ruxton (Lane & Brian 1992).

1964 England – William Brittle accused of the murder of Peter Thomas, whose decaying body was found in a wood near Bracknell, Berkshire. Brittle's alibi was destroyed when Dr. Keith Simpson testified that maggots of the common blue-bottle found on the remains had not pupated which, given the life cycle of the insect, established time of death (Lane & Brian 1992).

The Lydney Case – The prosecution's pathologist, Professor Keith Simpson, successfully used blow fly maggots to establish time of death. The case hinged on whether the testimony of three witnesses outweighed the evidence of the prosecution's witness. The defense witness claimed to have seen the victim on a certain date, but Simpson's work with maggots established that they were "at least nine or ten days old, but probably not more than 12", setting the date of death as prior to the date given by the witness. The opposing expert called by the defense agreed with Simpson, refuting the testimony of the three defense witnesses. This forensic entomology timeline is the property of Shunderson Communication Inc. SCInc.

1965 J.A. Payne published studies on the succession of insect communities found on baby pig carcasses. This began research, which has advanced forensic entomology into the science it is today. More recently field studies published have spanned the observation of early arthropod colonizers and in some cases the later stages of colonization (e.g. Reed 1958, Payne 1965, Lane 1975, Rodriguez & Bass 1983, Goddard & Lago 1985, Braak 1986, Early & Goff 1986, Braak 1987, Goff *et al.* 1988, Goff & Flynn 1991, Greenburg 1991, Anderson & VanLaerhoven 1996).

However, for forensic entomologists to use the information provided by the presence or absence of insects, an accurate interactive database of insect succession is needed. Recent reviews have summarized the current state of entomological scie nce and have pointed out areas where additional knowledge is needed (Keh 1985, Catts & Goff 1992 in Hall & Doisy 1993). In addition to this it is also suggested by Wells & Greenberg 1994b it would be useful to develop standard statistical methods for forensic entomological evidence.

It is common knowledge that South Africa unfortunately has one of the highest rates of violent crime in the world. Between the period of 1994 and 2004, there have been

an average of between 19 824 and 16 877 reported murder cases per year in South Africa (S.A.P.S 1996). There is a great need for more research to be conducted in South Africa. Most of the published articles about the work have been interest articles and a few television documentaries. We have the opportunity to de velop our research in a country where the work could be applied to solve crimes.

'Even if forensic entomology is used successfully to help solve only one murder, if the victim is someone you love you would want that tool available.' (McKeown 1991).

The project aims

This research is aimed at creating crime scene simulations (using pig carcasses), information from which will be applied to real case studies. The aims of this thesis are to determine the influence of a) season, b) clothing, c) wrapping and d) knife wounds on carcass decomposition and insect succession.

MATERIALS AND METHODS

2.1. Study site

All the trials were conducted in the Free State Province situated in the central region of South Africa. The experimental site was located in Bloemfontein (Fig. 2.1.), at the University of the Free State.

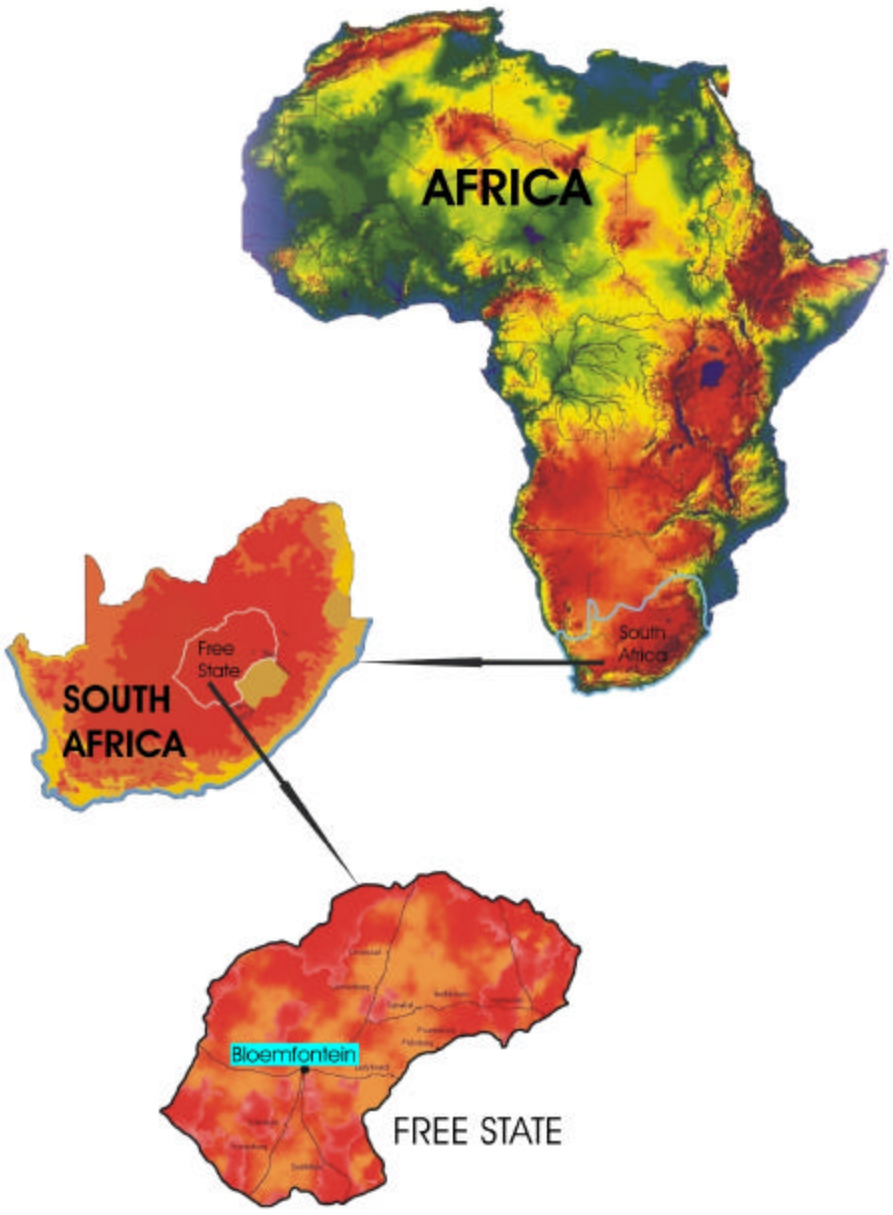


Fig. 2.1. Maps showing the location of Bloemfontein. (Map of Africa was reproduced with permission - Dr Yunlong Xia, ICIPE Insect Informatics).

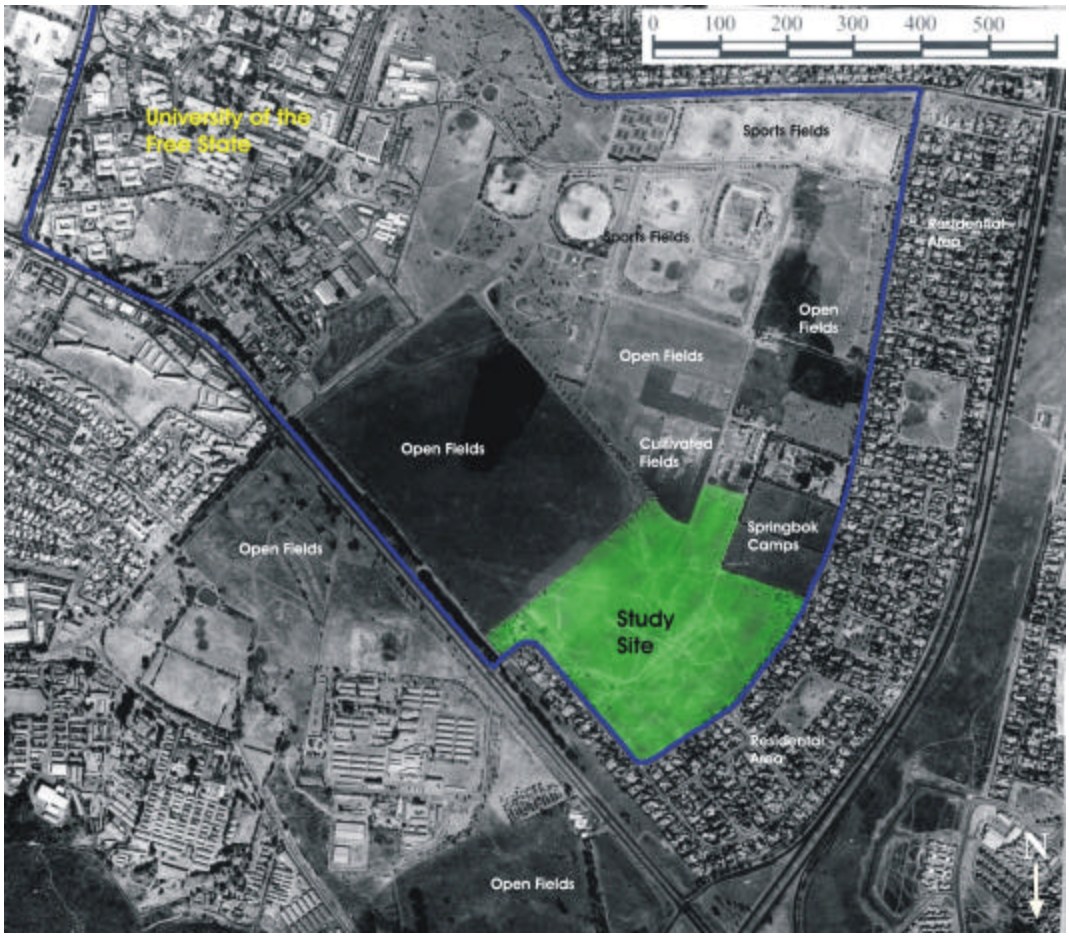


Fig. 2.2. Aerial photograph of the University of the Free State and surrounding area (Original photograph courtesy of the UFS Quantity Surveying Department).

The trials were conducted in an open field located on the western campus of the University of the Free State. ($29^0$8'S; $26^0$10'E, ± 1560 m above sea level) (Fig. 2.2.).

The field was 24 hectares of grassland with a few scattered trees. The area was subject to hot summers with estimated mid summer temperature range of 13^0C to 32^0C and cold winters with estimated mid winter temperature range of -6^0C to18^0C. The area has an annual expected rainfall of 450-500mm (Schulze 1997). The grass species were dominated by *Themeda triandra* Forsk., with scattered tufts of *Aristida congesta* Roem. *et* Schult., *Eragrostis lehmanniana* Nees, *Eragrostis capensis* (Thumb.), *Sporobolus pyramidalis* Beauv., *Heteropogon contortus* (L.) Beauv. and *Chloris virgata* Sw. The scattered trees are *Acacia karoo* Hayne and *Rhus rehmanniana* Engl.

These grass species were identified using *Chippendale Key to Grasses of Southern Africa* (Meredith 1955), while the tree species were identified using the key provided by *Trees of Southern Africa* (Coates Palgrave & Coates Palgrave 2002). These grass species were indicative of the savanna / grassland biome / veld type as described by Rutherford & Westfall (1986) and Low & Rebelo (1996). The field was mowed once a year usually in late autumn. None of the mowing coincided with any of the trials.

There were a few vertebrates present in the experimental site during the trials. These are mentioned, as they were possible predators. There were a few resident bird species throughout the year and these included two pairs of male and female black korhaans (*Eupodotis afra* (Linnaeus)), numerous francolins (*Francolinus swainsonii* (Smith)) and a small flock of crowned guineafowl (*Guttera pucherani* Hartlaub). Sporadic visitors were fiscal shrikes (*Lanius collaris* Linnaeus), who would set up territories using the data logger poles as lookout points, greater striped swallows (*Hirundo cucullata* Boddaert) and cattle egrets (*Bubulcus ibis* (Linnaeus)) These bird species were identified using *Roberts' Birds of Southern Africa* (Maclean, 1993). The last two species actively fed on the adult blow flies. The cattle egrets also perched on the cages and fed on maggots that migrated beyond the base of the cage. However, these birds were never seen in groups of larger than two. Occasionally, skinks were found hiding under the carcasses, usually only when the carcasses had begun to dry out. They would be present at the same carcass for most of that trial. They were observed to feed on the beetles and occasional flies.

2.2. Carcass layout and sampling frequency

This research was aimed at creating various crime scene simulations. Freshly killed pig carcasses (*Sus scrofa* Linnaeus), were used, as they are an internationally accepted substitute for human bodies (Catts & Goff 1992) (Fig. 2.3.). The animals were killed by a single euthanasia injection ("Euthapent" (Kyron Laboratories (Pty) Ltd) - Pentobarbitone sodium 200 mg/ml), at the animal research house located next to the field in which the trials were conducted. The carcasses were placed in the field, minutes after they were killed and the date of death was designated as day 0.

All the carcasses used were heavier than the 23 kg as recommended by Catts & Goff (1992) and thus they were in an acceptable range as not to affect the decomposition

and arthropod succession (Hewadikaram & Goff 1991). The carcasses used in each trial had the same skin colour. All clothed carcasses were simply dressed in similar coloured and quality T-shirts, shorts and underwear (male briefs) (Fig. 2.3b).

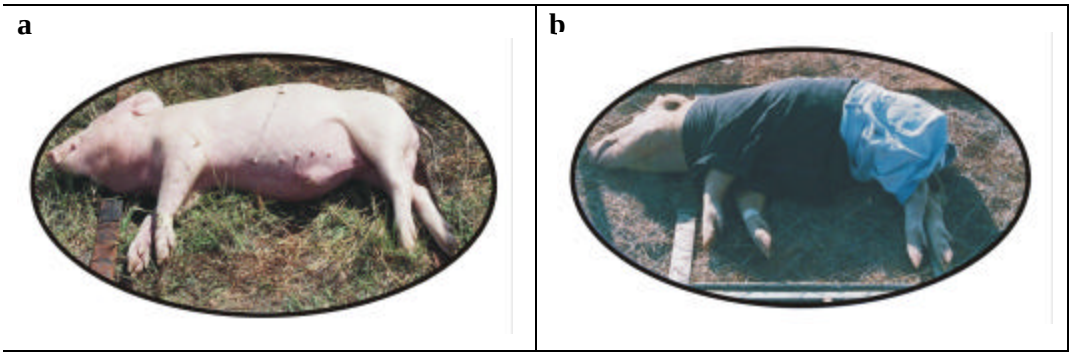


Fig. 2.3. Examples of pig (*Sus scrofa)* carcasses, without clothing (a) and clothed (b).

Each carcass was placed in a welded poultry wire mesh cage (1.6 x 0.9 x 0.9m), with a larger 5cm fence wire mesh on the base of the cage. The cages were used to facilitate weighing of the carcasses and to protect them against scavengers such as stray dogs. The carcasses were in direct contact with the ground to allow natural ground arthropod succession. All the carcasses were placed with their abdomens facing north, in a north – south orientation. They were in full sunlight, at least 50m apart and also 50m from carcass sites that were used in the previous trials. Any remains of the carcasses were removed from the field after each trial

Due to difficulty defining the beginning of the seasons in South Africa, the selection of dates of trials were based on the closest practical day to the seasonal solstices (22 June during winter and 21 December during summer) and equinoxes (22 March during autumn and 21 September during spring), in the southern Hemisphere. The trials were conducted continuously from autumn 2003 to summer 2005, with a new trial in each season (Fig. 2.4.). The trials that were conducted in the warmer seasons were 50 days in duration. The winter trials were conducted over a period of 100 days. The trials were conducted for these periods to cover as much of the decomposition changes as practically possible.

Autumn

Winter

Summer

Spring

Fig. 2.4. The study site during the different seasons.

2.2.1. Wrapped Trials

These trials were designed to simulate a criminal wrapping a dead victim to minimize the evidence left by a blood trail while moving the body to a dumpsite. They may also wrap the victim to avoid early detection of the body.

This aspect included four major scenarios:

a) a carcass without wrapping or clothes (control)

b) a clothed carcass (clothed control)

c) carcass wrapped in a sheet without clothing (wrapped no clothes)

d) clothed carcass wrapped in a sheet (wrapped clothed)

The wrapped trials dates are listed in Table 2.1. Eight carcasses were used. These eight carcasses were divided in groups according to the frequency of sampling. The first group consisted of four carcasses, containing the control carcass with no wrapping nor clothes (Fig. 2.3a.), a carcass with clothes but no wrapping (Fig. 2.3b.), and two wrapped carcasses, one with no clothes and one clothed (Fig. 2.5.). In the warmer seasons, these carcasses were sampled twice a day (morning, starting at 08:00h and midday, starting at 13:00h) for the first two weeks, then once a day (starting at 13:00h) for the next two weeks and finally three times a week for the remainder of the trial. In the winter trial, the carcasses were only sampled once a day, however, they were sampled every day at midday for the whole duration of the trials. The remaining four carcasses were divided into two sample groups. Each group consisted of a carcass wrapped with no clothes and a clothed carcass wrapped. These groups were sampled every five days and ten days respectively on the applicable day during all seasons.

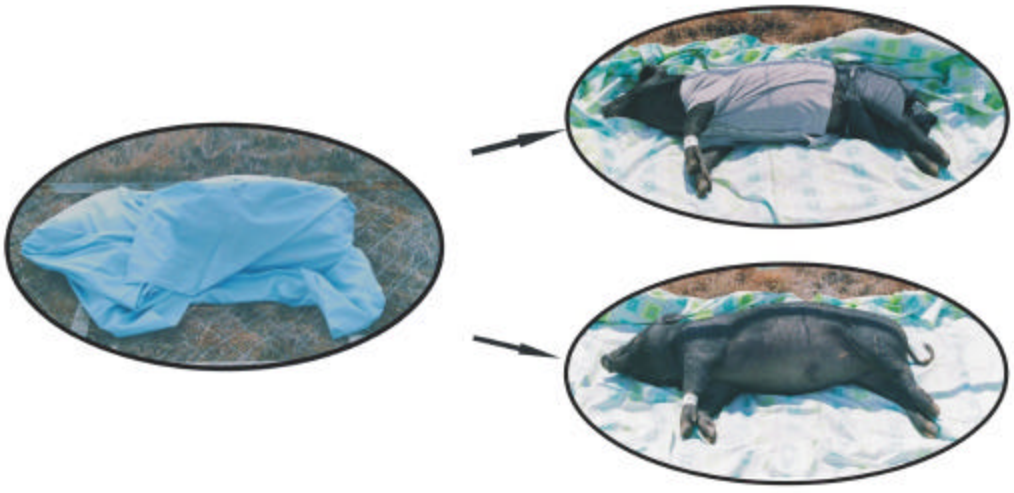


Fig. 2.5. Examples of carcasses used for the wrapped trials.

The order in which the carcasses were sampled was rotated. This was done to prevent the same carcass always being sampled first or last. The wrapping used was medium weight light blue cotton sheeting, cut to the size of a standard double bed sheet (1.5 x 2m). The sheet was not secured by pegs or rope. However, care was taken to restore the sheet to the position in which it was found, after each sampling visit.

Table 2.1. Trial dates and weight range of the carcasses used in the wrapped trials.

Season	Trial dates	Weight range of carcasses
Autumn	18th March 2003 to 7th May 2003	23.2 to 28.5 kgs
Winter	20th June 2003 to 28th September 2003	27 to 39.5 kgs
Spring	30th September 2003 to 19th November 2003	33.8 to 48.1 kgs
Summer	7th January 2004 to 26th February 2004	36.5 to 39 kgs

2.2.2. Trauma Trials

These trials were designed to simulate physical trauma caused by various knife wound scenarios. This is a common method of murder in South Africa, especially in rural areas and is often observed in human case studies.

This aspect included five major scenarios:

a) an undamaged carcass without clothes (control)

b) an undamaged carcass with clothes (clothed control)

c) stab wounds, with superficial cuts as defence wounds on a carcass without clothes (stab wounds no clothes)

d) stab wounds, with superficial cuts as defence wounds on a clothed carcass (stab wounds clothed)

e) severe trauma in the form of a deep throat wound on a carcass without clothes (severe trauma no clothes)

f) severe trauma in the form of a deep throat wound on a clothed carcass (severe trauma clothed)

The wounds trials dates are listed in Table 2.2. Six carcasses were divided into three groups. Each group consisted of a carcass with clothes and one without clothes (Fig. 2.6.). The first group had a knife wound to the throat, where the blade severed the carotid artery, trachea and terminated at the spinal column (Fig. 6A). The second

group had three deep knife wounds, i) in the back, left of the spine in the upper torso, ii) in the front, left of the sternum approximately half way down the rib cage and iii) in the front abdominal region, approximately 20cm below the sternum (Fig. 6B). There were also two superficial wounds on the fore limbs to simulate defensive wounds. The last group were the controls and therefore were free of any wounds.

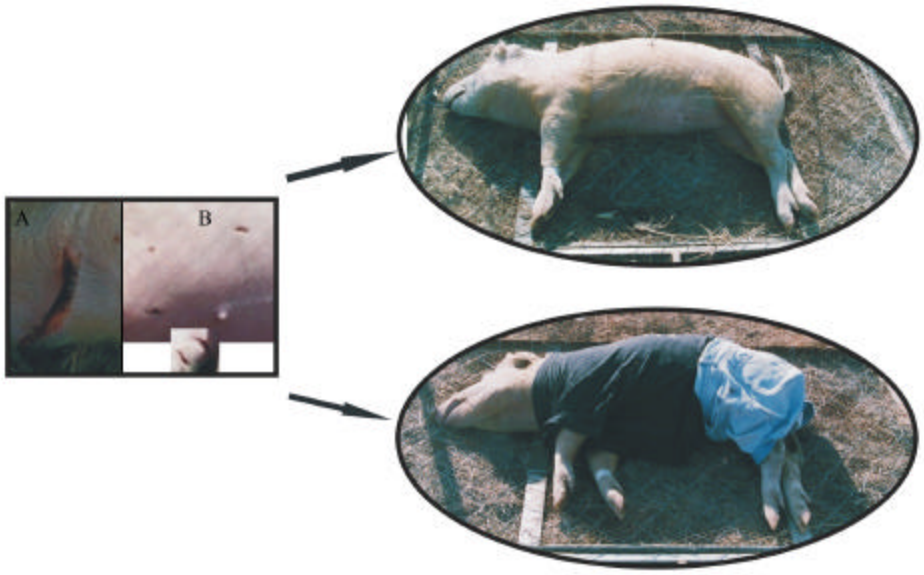


Fig. 2.6. Examples of carcasses used for the trauma trial (A - throat wound, B - knife wounds).

The sampling regime was the same as that used during the wrapped trials, the year before, i.e. during the warmer seasons, all the carcasses were sampled twice a day (morning, starting at 08:00h and midday, starting at 13:00h), for the first two weeks then once a day (starting at 13:00h) for the next two weeks and finally three times a week for the remainder of the trial. In the winter trial, the carcasses were only sampled once a day at midday however they were sampled every day for the whole duration of the trials.

Table 2.2. Trial dates and weight range of the carcasses used in the trauma trials.

Season	Trial Dates	Weight range of carcasses
Autumn	18th March 2004 to 7th May 2004	23 to 25.2 kgs
Winter	22th June 2004 to 30th September 2004	33.3 to 43.1 kgs
Spring	10th October 2004 to 1st December 2004	23 to 30.9 kgs
Summer	28th January 2005 to 15th February 2005	25.2 to 30.4 kgs

2.3. Sampling methods

2.3.1. Temperature and environmental factors

For the wrapped trials, te mperature recording devices, MCS 120-02EX (referred to as data loggers) and later iButtons (Fig. 2.7.) were used to record the ambient, external and internal temperatures of the carcasses. The data loggers were placed in waterproof casings and were attached to an upright pole placed approximately one meter from the cage (Fig. 2.7a). The specifications of the data loggers are given in Table 2.3. The data loggers were programmed to record the temperatures every 60 minutes. Temperature probes of the data loggers were placed in the head (via the mouth), thorax (through a small incision), abdomen (via the anus) and underneath the carcass. A probe was also placed in each of the cages to record the ambient temperatures.

The set up for the wrapped trials was as follows:

- *Autumn* – A data logger was placed at the control carcass, the wrapped clothed carcass sampled every day and the wrapped carcass with no clothes sampled every five days.
- *Winter* - A data logger was placed at the wrapped clothed carcass sampled every day and the wrapped carcass with clothes sampled every five days.
- *Spring* – A data logger was placed at the wrapped clothed carcass sampled every day and the wrapped carcass with clothes sampled every five days. Three iButtons were distributed in the field to record the ambient temperatures. They were hung from the branches of trees using thin insulated wire.
- *Summer* – A data logger was placed at the wrapped clothed carcass sampled every day and the wrapped carcass with clothes sampled every five days. Three iButtons recorded the ambient temperatures.

For the trauma trials only iButtons (Fig. 2.7b.) were used due to the unreliability of the data loggers. The iButtons were programmed to record the temperature every 60 minutes. The specifications are also given in Table 2.3. In the autumn trial, the buttons were placed in the head (via the mouth), thorax (through a small incision), abdomen (via the anus) and underneath the carcass. Many of these iButtons became corroded by the decomposition fluids and failed. For the trials that followed, the buttons were vacuum-sealed in a double layer of thick flexible plastic. The iButtons were placed in the head (via the mouth), thorax (through a small incision), abdomen (via the anus) and underneath the carcass. As with the wrapped trials iButtons were used to record the ambient temperatures. An additional two iButtons were hung next to each other, one in plastic and one uncovered. This was done to confirm that the plastic used to protect the iButtons did not influence the temperatures recorded.

The number and locations of the iButtons for the trauma trials is as follows;

- *Autumn* – The iButtons were placed in each carcass.
- *Winter* – The iButtons were reprogrammed to record only every 90 minutes so they could remain in the carcasses for the duration of the trial and did not need to be replaced. iButtons were placed in each carcass.
- *Spring and summer*- iButtons were placed in each carcass. The ambient temperatures were recorded by two iButtons.

Weather data were also obtained from the South African Weather Services (SAWS) Pretoria, for both the Bloemfontein city center ($29^0$07'01"S; $26^0$10'59"E, ± 1406 m above sea level) and the Bloemfontein airport ($29^0$06'00"S; $26^0$18'00"E, ±1353 m above sea level) stations.

a

b

Fig. 2.7. a) Data Loggers (MCS 120-02EX Data Logger) and b) iButtons (DS1921G) used to measure environmental and carcass temperatures during the trials.

Table 2.3. Summary of the specifications of the Data Loggers and the iButtons used in the trials.

Data Loggers (MCS 120-02EX Data Logger) Specifications	IButton (DS1921G) Specifications
• 12 analogue channels, 4 digital channels • 0 to 8000 counts per log period • Instantaneous, totalled, average, maximum and minimum, time of max/min • Storage: 2000 data points FIFO buffer • -11 ^{0}C to +50 ^{0}C (Appendix 1A)	• NV RAM • 4K bits • -40 ^{0}C to +85 ^{0}C • ±1 ^{0}C Accuracy –30 ^{0}C to 70 ^{0}C • ±1.3 ^{0}C outside this band • 0.5 ^{0}C Resolution • 2048-Byte Temperature Log • 63-bin Temperature Histogram • High/Low Temperature Alarms (Appendix 1B)

2.3.2. Carcass decomposition and arthropod composition

The recording and collection of the arthropods were based on the recommendations by Lord & Burger (1983). Collection was from on, in and beneath the carcasses. During each observation time the carcasses were described in detail in terms of

physical appearance (e.g. bloated, smell, visibility of bones and internal organs, amount of skin) and all arthropods were visually identified and their presence and numbers were recorded as per guidelines outlined by Catts & Haskell (1990). Only the few specimens that were not recorded previously were collected and added to the extensive reference collection, which was established during an introductory trial done in November 2002. These observations were done over at least a fifteen-minute time interval. This time frame was sometimes extended, especially during the active decay stage when maggots were collected.

The Diptera eggs and maggots, when present, were collected and preserved in 70% ethanol. For identification purposes, sub-samples were raised to adulthood from every second day's samples. The preserved maggots were classed into instar age and identified where possible. Drawings of the maggot mass locations were made. A photographic record of all the carcasses was maintained. Both Diptera maggots and adults were identified using keys described by Zumpt (1961, 1965a, b, 1972). During the sampling, care was taken to limit disturbance to the maggot masses and decomposition process. Only small samples of approximately 50 to 100 maggots (depending on the maggot mass size) were taken from each mass.

2.3.3. Carcasses biomass removal

The carcasses were weighed to determine biomass removal during the decomposition process. The cages were winched to a few centimetres above the ground on a large hanging scale (Fig. 2.8). This was restricted to daily weighing of the control carcass and the clothed carcass and alternate day weighing of the two wrapped carcasses sampled every day and the two wrapped carcasses sampled every five days. The alternate weighing used on the wrapped carcasses was designed to minimize the disturbance caused by lifting the cage. The other two carcasses (sampled every ten days) were only weighed on placement day and again at termination of the trial.

a

b

Fig. 2.8. a) Cages and b) device used to facilitate weighing during the trials.

2.4. Data and statistical analysis

Schoenly (1992) was the first to publish statistical methods that could apply to forensic entomological data. The similarity coefficient that Schoenly (1992) chose was the Jaccard Metric method. This method required just the absence and presence of arthropods and is not quantitative. It allowed the data to be quantified as the daily species changes of the composition of the arthropods associated with carcasses. Schoenly's methods were chosen as the results may be compared to other work (e.g. Schoenly *et al.* 1996, Boucher 1997, Tabor *et al*. 2004).

Tabor *et al.* (2004) took the analysis one step further. They tested the data from the Jaccard Metric method, using a correlation coefficient, to determine the degree of similarity in the species occurrence for the different experimental carcasses. In other words they tested the degree of consistency in the successional patterns of the composition of the arthropods associated with carcasses. To do this, they used a permutation approach based on Mantel's test.

Both of these methods were applied to the data obtained from both the wrapped trails and the trauma trials. The data were tested for i) all species found on the carcasses and also ii) those species that were of forensic importance, i.e. those which breed on the carcass and those species with a high occurrence frequency.

These analyses were first applied to data within the same trial, i.e. control carcasses versus experimental carcasses. Later the data were tested between trials, i.e. each season of successive years.

2.4.1. Analysis 1: Jaccard Metric

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

Jaccard similarity coefficients were calculated as follows:

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

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

a is the number of taxa common to both species

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

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

The Jaccard Metric ranges in value between zero, when two samples fail to match on any taxa, to unity, when they match perfectly (Ludwig & Reynolds 1988, Schoenly 1992).

To obtain the mean faunal similarity for each sample of each of the *i* samples (days) in the succession, $\mathbf{S}_i$, the average of the S_{ij}'s were taken over the n = 1 samples (*j* = S_{i1}, S_{i2}, …. S_{in}, for all $S_{i \neq j}$)

Thus: $\mathbf{S}_i = \left(\frac{\sum_{j=i}^{n} S_{ij}}{(n - 1)} \right)$, where $i \neq j$

And then the grand mean of the between-sample faunal similarities ($\overline{S}_{gmean}$) was calculated over the n samples (*i* = 1, 2, 3…, n)

Thus: $\overline{S}_{gmean} = \left(\frac{\sum_{i=j}^{n} \mathbf{S}_i}{(n)} \right)$

2.4.2. Analysis 2: Correlation coefficient

The matrices were then tested using a correlation coefficient. The coefficient was calculated using MATLAB 6.5 (The MathWorks, Natick, MA) (Appendix 1). A permutation distribution of 999 K-values was calculated by carrying out successive correlations after corresponding rows and columns of one of the similarity matrices were randomly permutated simultaneously. Statistical significance (p) of the observed correlation coefficient, K_{obs}, under the null hypothesis of similarity, between the successional patterns of arthropod species in the two similarity matrices were determined by its position in the distribution of the 1000 K values. That is, the p value is the proportion of K values = K_{obs}, with a low value ($p < 0.05$) indicating that the successional patterns of taxa in the two similarity matrices are similar. The p value was tested for significance level against the K_{obs} value (Tabor *et al.* 2004).

SECTION 3.1

The influence of wrapping and clothing on carcass decomposition and arthropod succession: an autumn study.

3.1.1. Decomposition of the carcasses

Most forensic entomological studies describe the physical characteristics of the decomposition process in context with the entomological activity on the carcasses (e.g. Payne 1965, Anderson & VanLaerhoven 1996). Descriptions can include classifications into which the various stages of decomposition can be placed (Payne 1965). These descriptions were used as a guide and five decomposition stages were used to best describe the process in this trial (Fig. 3.1.1.). The descriptive parameters of these stages are summarised in Table 3.1.1. These characteristics were found to be similar to the classes described by Anderson & VanLaerhoven (1996). However, decomposition is an ongoing process and the differences between the physical characteristics of the stages may not always be clear. For this, there are transitional stages in which characteristics from the adjoining decomposition stages can be present.

This kind of classification can be rather subjective to individual observation, but it does provide some base for comparison with other seasons and other available information. Megyesi *et al.* (2005) suggested that treating decomposition as a semi-continuous variable (by scoring it, using a point based system), together with accumulated degree-days can provide additional information for PMI estimates.

i) Fresh

The carcasses where placed in the field at approximately 15:00h on the 18th March 2003, designated day 0. The carcasses already showed the first signs of rigor mortis and slight bloating by the time the last carcass was examined and its physical parameters recorded, approximately 3 hours after death.

ii) Bloat

Early on the first morning (day 1), all the carcasses had begun to bloat (Fig. 3.1.2.). During the bloat stage no discernable body colour change was detected. This was simply because the carcasses were predominantly black, with white points. Some limited change, however, could be detected in these white areas, as they turned green.

Table 3.1.1. Summary of the decomposition stages, physical characteristics of each stage and the dominant arthropods present during the autumn wrapped trial.

FRESH (Fig. 3.1.1.) • Commenced when the animal was killed • The torso (thorax and abdomen) was soft and the limbs were flexible • No odour associated with the carcass • Very short duration	**Diptera** Muscidae: Muscidae spp. (adults)
BLOAT (Fig. 3.1.1.) • Commenced when the torso begins to harden and the abdomen in particular becomes inflated, due to the build up of gasses • Bubbles of blood formed at the **nose**, creating small puddles or saturating the sheet on the wrapped carcass • Carcass appeared 'balloon'-like • Body colour changed • Oviposition took place during this stage	**Diptera** Calliphoridae: *Chrysomya marginalis* (adults) *Chrysomya albiceps* (adults) *Lucilia* spp. (adults) Sarcophagidae: *Sarcophaga cruentata* (adults) Muscidae: *Hydrotea capensis* Muscidae spp.
ACTIVE DECAY (Fig. 3.1.1.) • Carcass deflated, as the maggots actively fed on the carcass tissues and allowed the gasses to escape • Odour of decay was prominent • The limbs collapsed back into the 'resting' position, and in some cases the clothing had moved • The skin began to peel allowing maggots to feed underneath it • The carcass mass was halved during this stage • The Diptera maggots were the most dominant insects • Areas surrounding the maggots and the tissue on which the maggots were feeding became liquefied • Plenty decomposition liquids were present	**Diptera** Calliphoridae: *Chrysomya marginalis* (adults and maggots) *Chrysomya albiceps* (adults and maggots) Chrysomya chloropyga *(maggots)* *Lucilia* spp. Sarcophagidae: *Sarcophaga cruentata* (adults and maggots) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults) Cleridae: *Necrobia rufipes* (adults) Silphidae: *Thanatophilus micans* (adults)
ADVANCED DECAY (Fig. 3.1.1.) • There was little tissue remaining on the carcass • Fewer odours were associated with the carcass • Puddles of decomposition fluids were common along the backs and upper surfaces of the carcass • The carcass was still moist but often the head was dried out. Bones of the skull, ribs and legs were often visible • The majority of the maggots moved off the carcass to pupate (i.e. less than 20 individuals remaining) • Coleoptera became the most dominant group	**Diptera** Piophilidae (adults and maggots) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults) Siphidae: *Thanatophilus micans* (larvae)
DRY REMAINS (Fig. 3.1.1.) • The carcass had little to no moisture • Gut content had dried out, although there may have been some fluids present when it was very hot • Only hair and small patches of skin remaining	**Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)

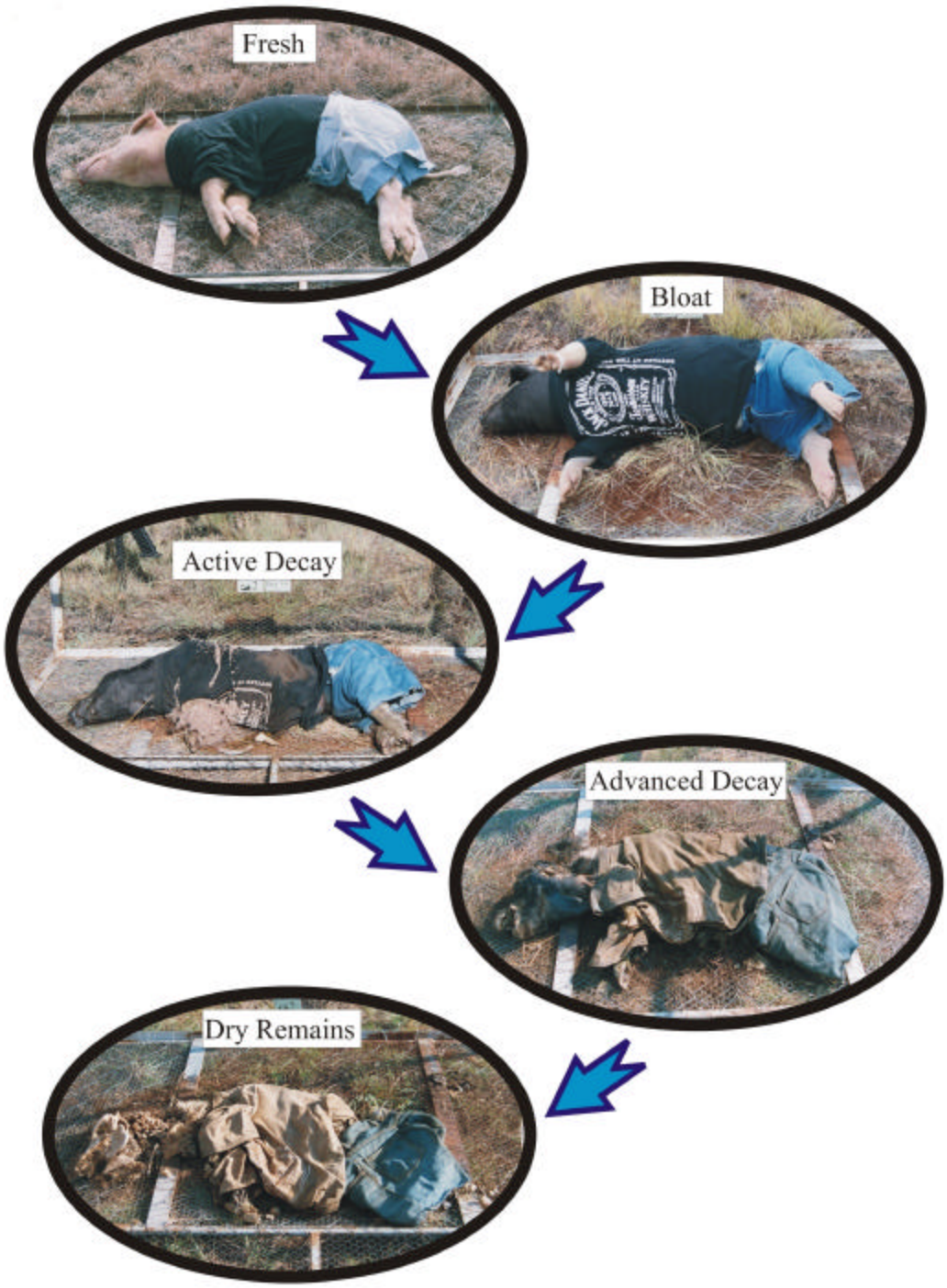


Fig. 3.1.1. Examples of the decomposition stages described in Table 3.1.1. (The carcass in the fresh stage was photographed during the preliminary wrapped trial. The other photographs are the clothed control carcass from the autumn wrapped trial. The carcasses in each trial had the same skin colour).

By the third day, all the carcasses were still bloated, only the control carcass had begun to soften. The prolonged bloating was caused by constant rainfall during the second day and the morning of the third day. There were no cases where the bloating caused any splits in the skin. No delay in oviposition by the Diptera occurred and as a result all the carcasses entered the active decay stage simultaneously.

iii) Active decay

By the fourth day, the maggots entered all the carcasses through the natural openings leading to the onset of active decay by day 5. On the wrapped carcasses, the skin became slippery and began to peel allowing the maggots additional access to the carcass tissues.

The decomposition fluids that were generated by the maggot mass, on the control and clothed control carcasses drained onto the ground. This resulted in the carcasses maintaining a high moisture content, but they were not saturated in fluid. However, the sheet around the wrapped carcasses did not allow these fluids to drain. It became clogged with decomposing matter and the carcasses were lying in a pool of decomposition fluids for an extended period. A higher level of odour emanated from these carcasses, with a strong smell of ammonia and other decomposition gasses escaping when the sheet was opened during sampling. In the control and clothed control carcasses the skin also peeled, as a result of drying out.

The control and clothed control carcasses dried out the quickest and ended the active decay stage by day 12. The wrapped clothed carcass began to dry out by day 14. The active decay stage was the longest for the wrapped carcasses with no clothes, lasting until day 19 (Fig. 3.1.2).

iii) Advanced decay

This stage was significantly longer for the wrapped carcasses, due to the sheet retaining the moisture. This delayed the transition to the dry stage for those carcasses, while the control and clothed control carcasses were able to dry out much sooner (Fig. 3.1.2.).

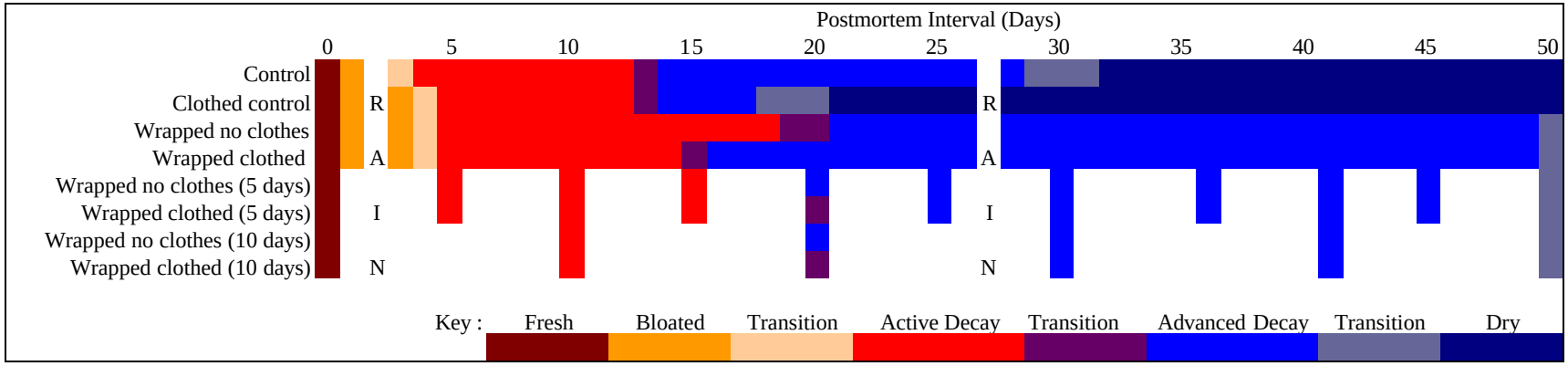


Fig. 3.1.2. Decomposition stages of the carcasses in the autumn wrapped trial.

iv) Dry remains

The control and clothed control carcasses were classified into the dry remains stage by day 32 and 21 respectively. The maggots on the clothed control carcass consumed slightly more tissue than those on the control carcass. Exactly why this happened needs further exploration. This resulted in the carcass entered the dry stage earlier than the control carcass. The wrapped carcasses remained moist and therefore remained in the advanced decay stage for the remainder of the trial (Fig. 3.1.2.). The carcasses remained fresh for one day at most, which is shorter than reported in the northern hemisphere (Anderson & VanLaerhoven 1996, Richards & Goff 1997), but similar to Payne (1965). The bloated stage lasted only three days in autumn, whereas Anderson & VanLaerhoven (1996) reported ten days. These changes in the decomposition process are to be expected due to the geographic and climatic differences of the study

sites. The advanced decay stage for both the control (control and clothed control) carcasses was considerably shorter than for any of the wrapped carcasses.

This suggested that the wrapping allowed little evaporation and/or slowed the draining of the decomposition fluids onto the ground, and thereby carcasses were kept moist. This caused the wrapped carcasses to remain in the advanced decay stage for significantly longer. This observation would need to be taken into consideration when dealing with human remains in cases where those remains are in the advanced stage of decay. The insect succession did not change although the physical appearances of the carcasses were different.

3.1.2. Arthropod succession on the carcasses

Those species that developed on the carcasses, and those that were present at the carcasses more than once, were identified and these were considered to be the most important. They included members from both the Diptera and Coleoptera. The most obvious and abundant were the Calliphoridae, *Chrysomya marginalis* (Wiedemann), *Chrysomya albiceps* (Wiedemann) and Sarcophagidae, *Sarcophaga cruentata* Meigen and the Dermestidae, *Dermestes maculatus* De Geer and Cleridae, *Necrobia rufipes* De Geer (Fig. 3.1.3.).

In most cases the first arthropods to detect the carcasses were the adult Muscidae and Callphoridae. On the morning of day 1, *Chrysomya chloropyga* (Wiedemann) was recorded on the control carcass (Fig. 3.1.4.). *Sarcophaga cruentata* and Muscidae spp. were recorded on the clothed control carcass (Fig. 3.1.5.). *Sarcophaga cruentata* was recorded on the wrapped no clothes carcass (Fig. 3.1.6.), while *C. marginalis*, *S. cruentata*, *C. albiceps* and Muscidae spp. were recorded on the wrapped clothed carcass (Fig. 3.1.7.). After the rain stopped on day 3, between five and 20 individuals of *C. marginalis* and less than five individuals of *C. albiceps* were on the wrapped no clothes (Fig. 3.1.6.) and wrapped clothed carcasses (Fig. 3.1.7.). Between 20 and 50 individuals of *C. marginalis* and between five and 20 individuals of *C. albiceps* were recorded on the clothed control carcass (Fig. 3.1.5.) Lastly, between five and 20 individuals of *C. marginalis* and *C. albiceps* were recorded on the control carcass (Fig. 3.1.4.). These numbers increased, but they generally decreased around day 7.

Diptera

Chrysomya marginalis

Chrysomya albiceps

Sarcophaga cruentata

Coleoptera

Dermestes maculatus

Necrobia rufipes

Fig. 3.1.3. Most common species occurring during autumn wrapped trial.

The numbers of *C. albiceps* were usually lower than those recorded for *C. marginalis* (Figs 3. 1.4 to 3.1.7.).

Chrysomya marginalis were not recorded on the control (Fig. 3.1.4), clothed control (Fig. 3.1.5.) and wrapped clothed (Fig. 3.1.7.) carcasses after the morning of day 11. They were recorded on the wrapped no clothes carcass until the afternoon of day 13 (Fig. 3.1.6). *Chrysomya albiceps* were recorded on the clothed control carcass until the morning of day 10 (Fig.3.1.5.), the control until the morning of day 11 (Fig. 3.1.4.), the wrapped clothed carcass until morning of day 12 (Fig. 3.1.6) and the wrapped no clothes carcass until morning of day 14 (Fig. 3.1.5). After this, single individuals were recorded sporadically on the carcasses (Figs 3.1.4. to Fig. 3.1.9).
Lucilia spp. (*Lucilia sericata* (Meigen) and *Lucilia cuprina* (Wiedemann)) and *S. cruentata* were observed at the carcasses sporadically. Muscidae, *Hydrotea capensis* (Wiedemann) and Piophillidae spp. adults were frequently recorded in low numbers (Figs 3.1.4. to 3.1.9.).

The Coleoptera community was dominated by *D. maculatus*, which successfully bred on the carcasses. Adult *D. maculatus* were recorded on the clothed control carcass from the morning of day 4 (Fig. 3.1.5.) and on the wrapped clothed carcass, from the morning of day 6 (Fig. 3.1.7.). On the control carcass, it was consistently present from morning of day 8 (Fig. 3.1.4.) and on the wrapped no clothed carcass from the morning of day 9 (Fig. 3.1.6). Generally their numbers increased as the trial progressed (Figs 3.1.4. to 3.1.9.). *Dermestes maculatus* larvae were present on the clothed control carcass from day 17 (Fig. 3.1.5). They were recorded on the wrapped clothed carcass from day 19 (Fig. 3.1.7). They were present from day 20 on the control (Fig. 3.1.4.) and wrapped no clothes (Fig. 3.1.6.) carcasses.

Necrobia rufipes adults were also present on the carcasses from the afternoon of day 9, except the control carcass where they were present from the morning of day 10 (Figs 1.3.4. to 3.1.9.). Generally their numbers increased as decomposition progressed.

Thanatophilus micans L. adults, were recorded sporadically on all the carcasses, but were not commonly seen during the advanced decay stage (Figs 3.1.4. to 3.1.9.).

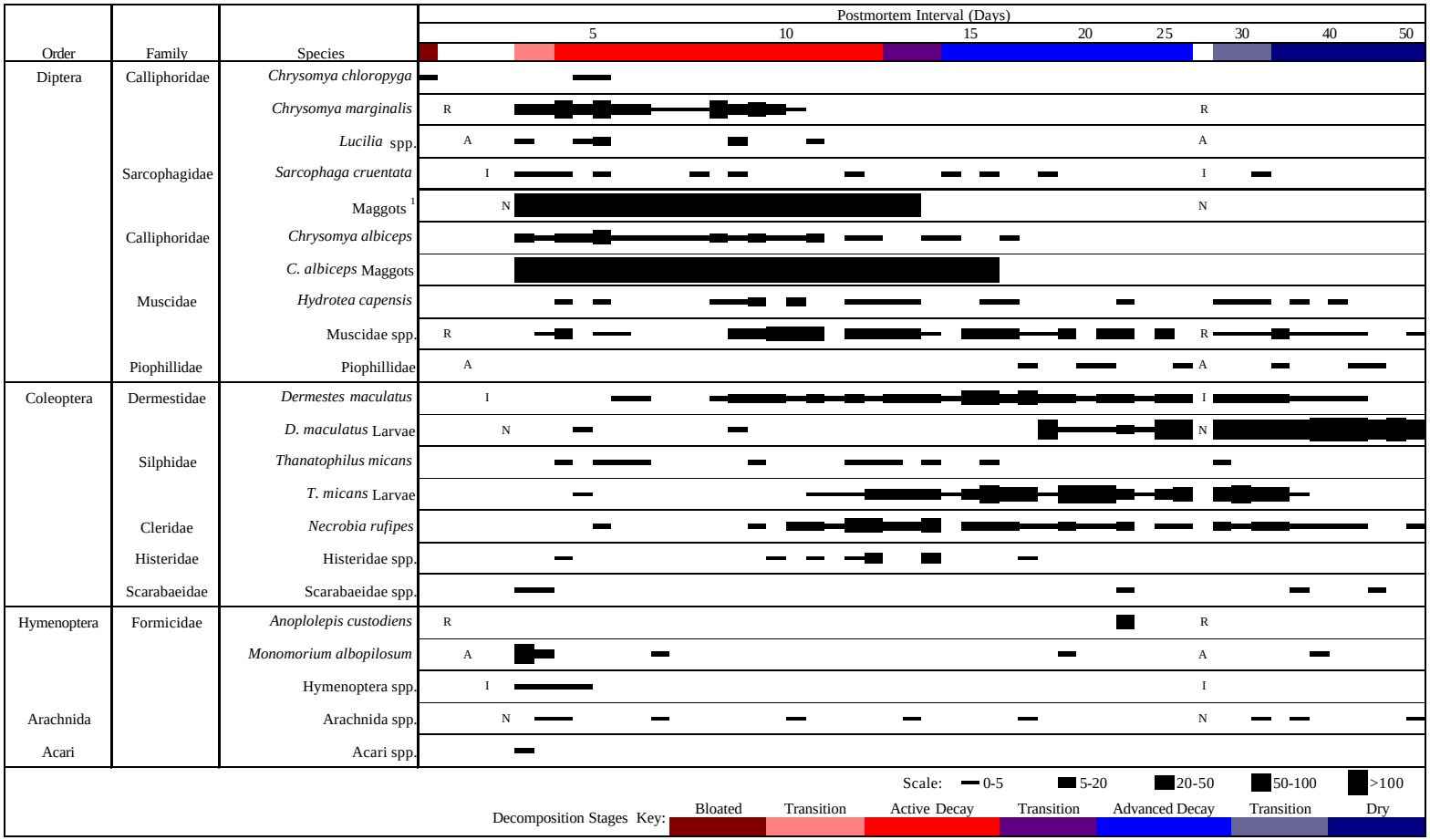


Fig. 3.1.4. Arthropod succession on the control carcass during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

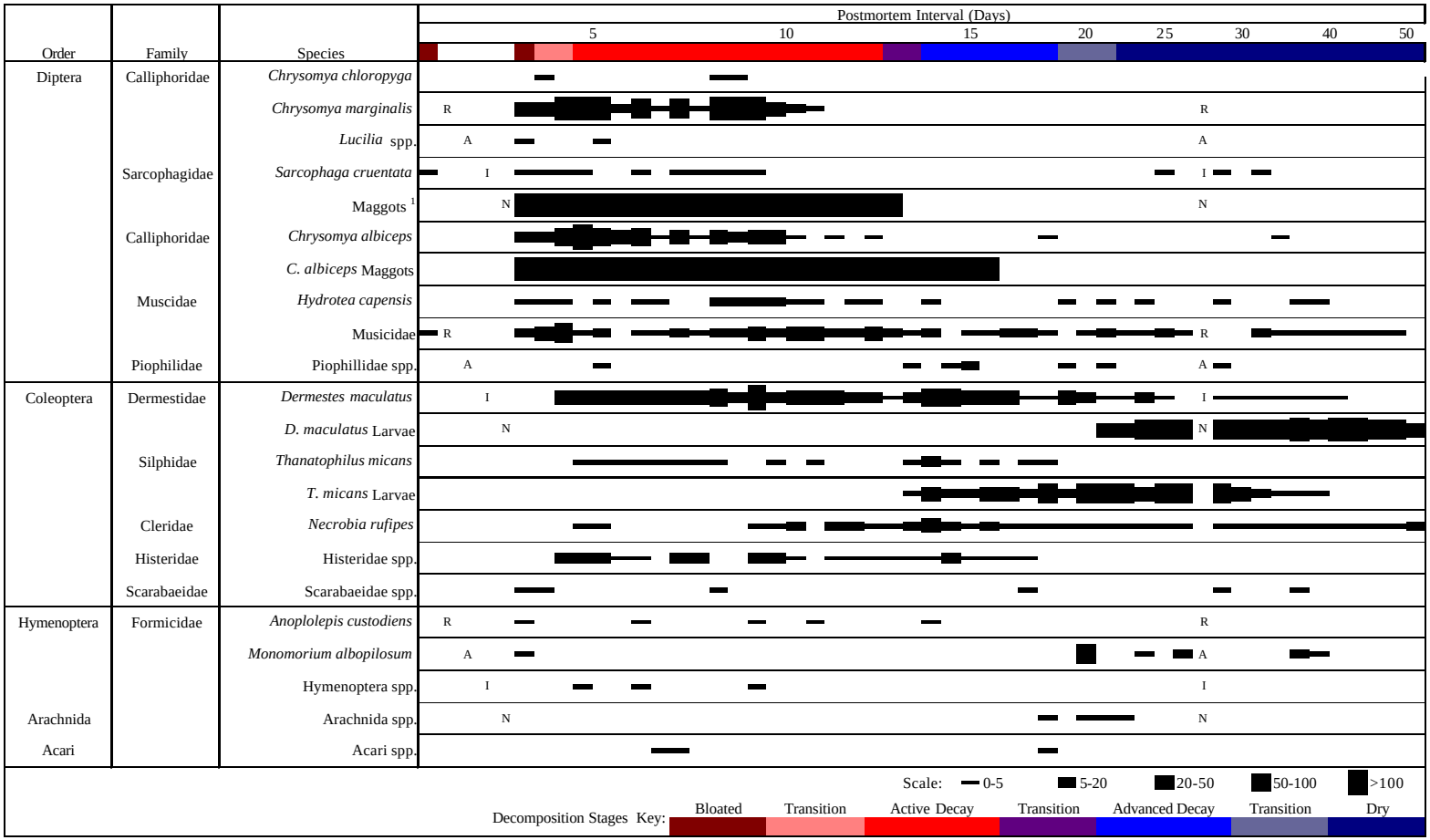


Fig. 3.1.5. Arthropod succession on the clothed control carcass during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

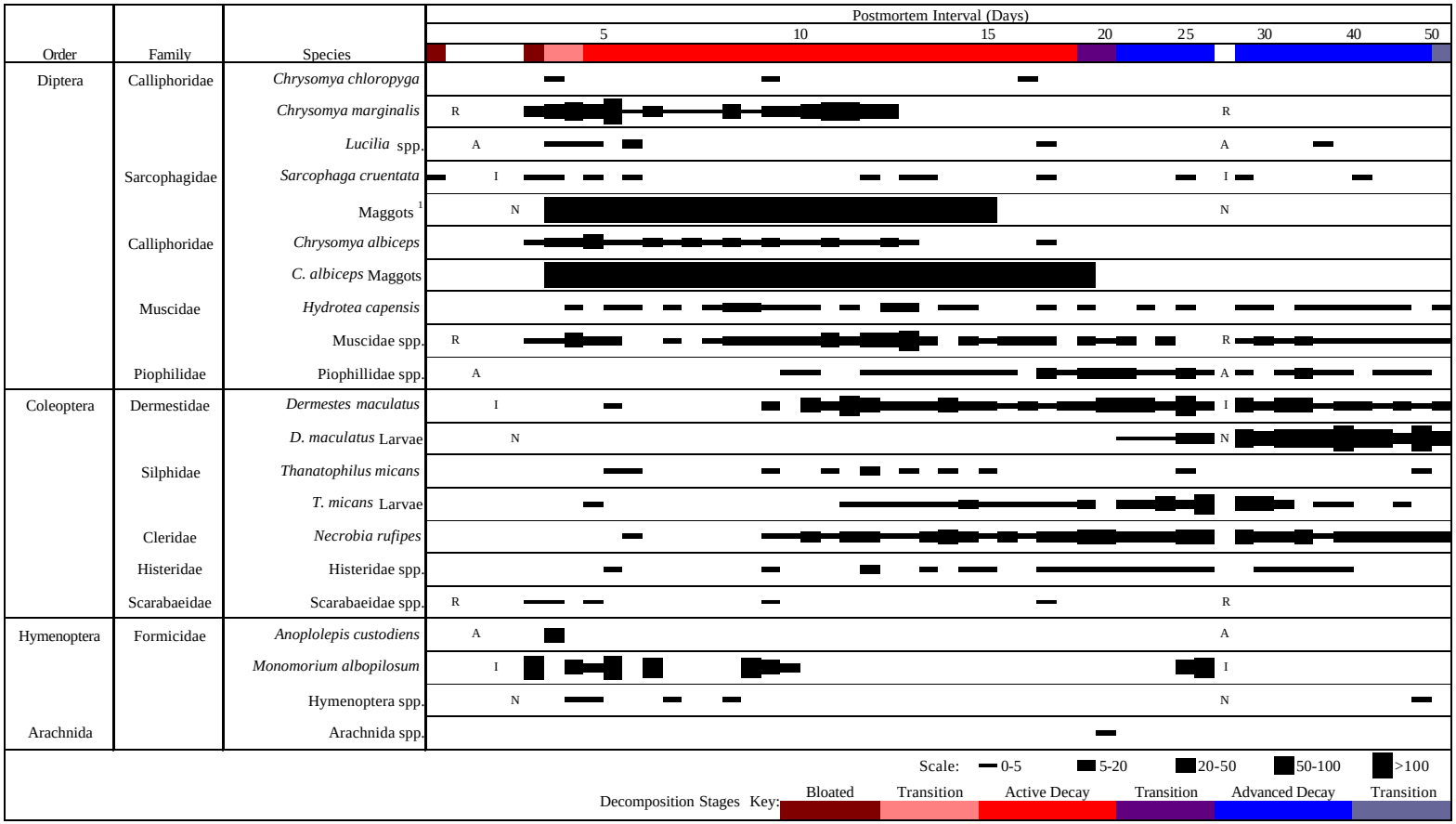


Fig. 3.1.6. Arthropod succession on the wrapped no clothes carcass during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata*.

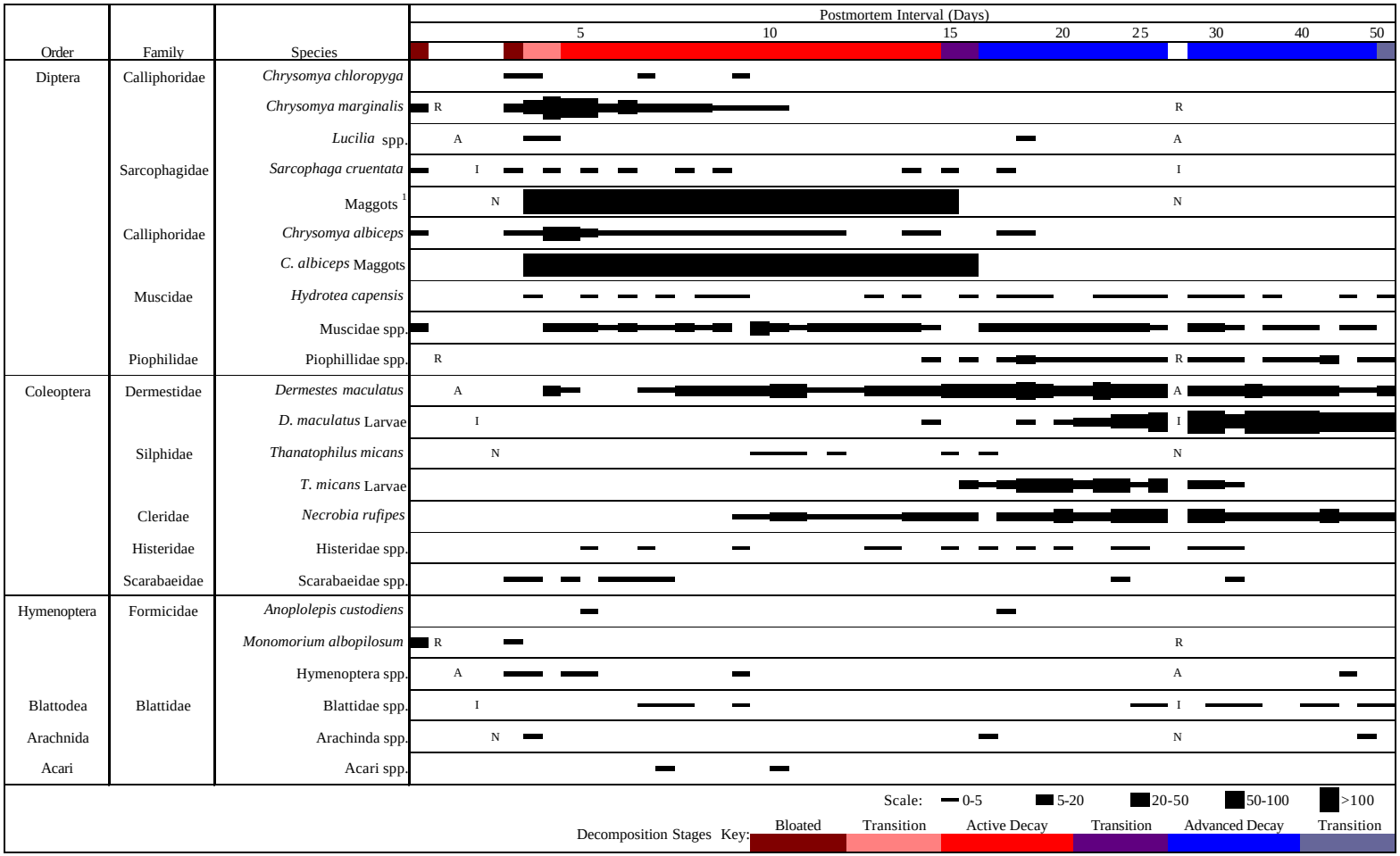


Fig. 3.1.7. Arthropod succession on the wrapped clothed carcass during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

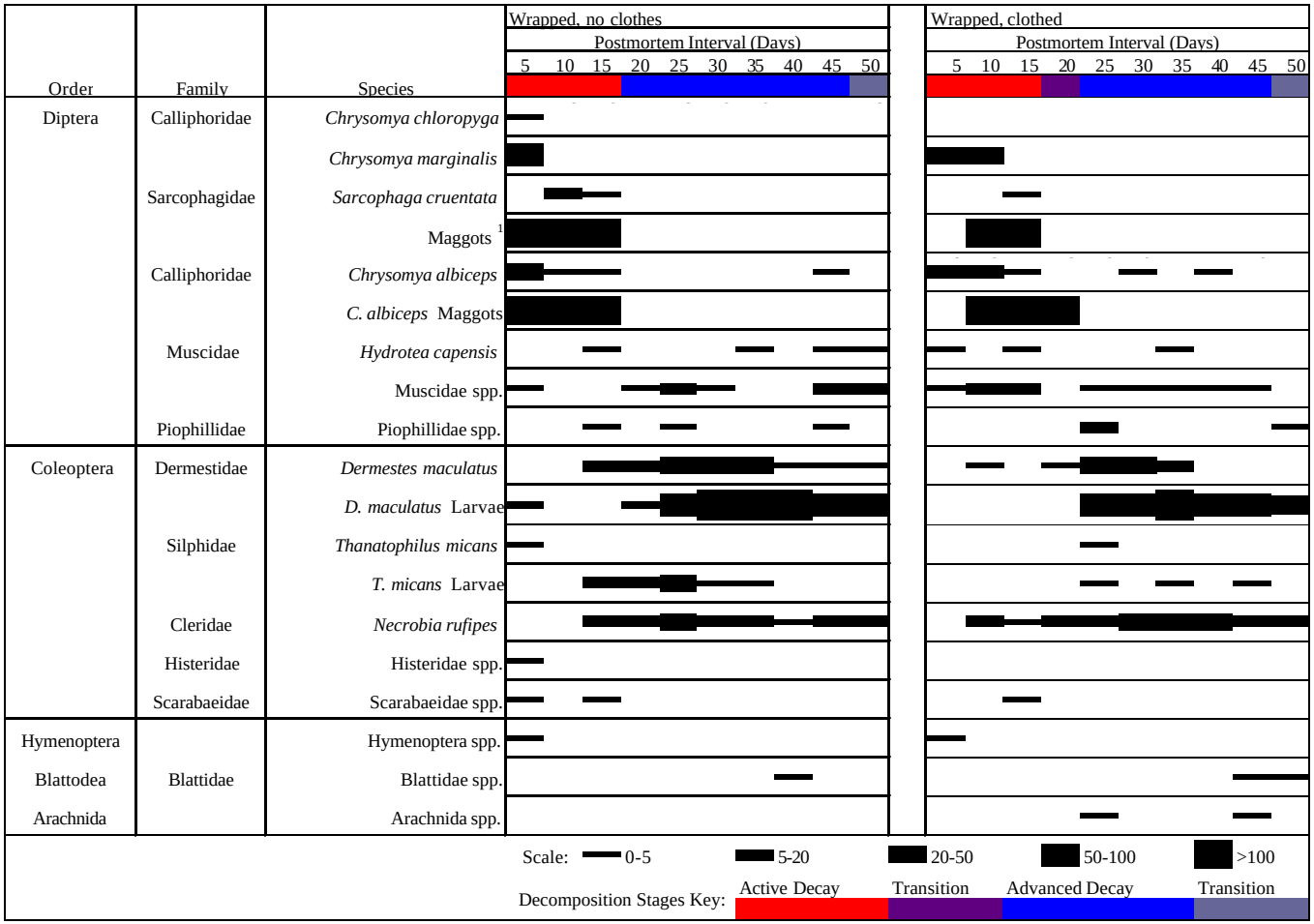


Fig. 3.1.8. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses (sampled every five days) during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

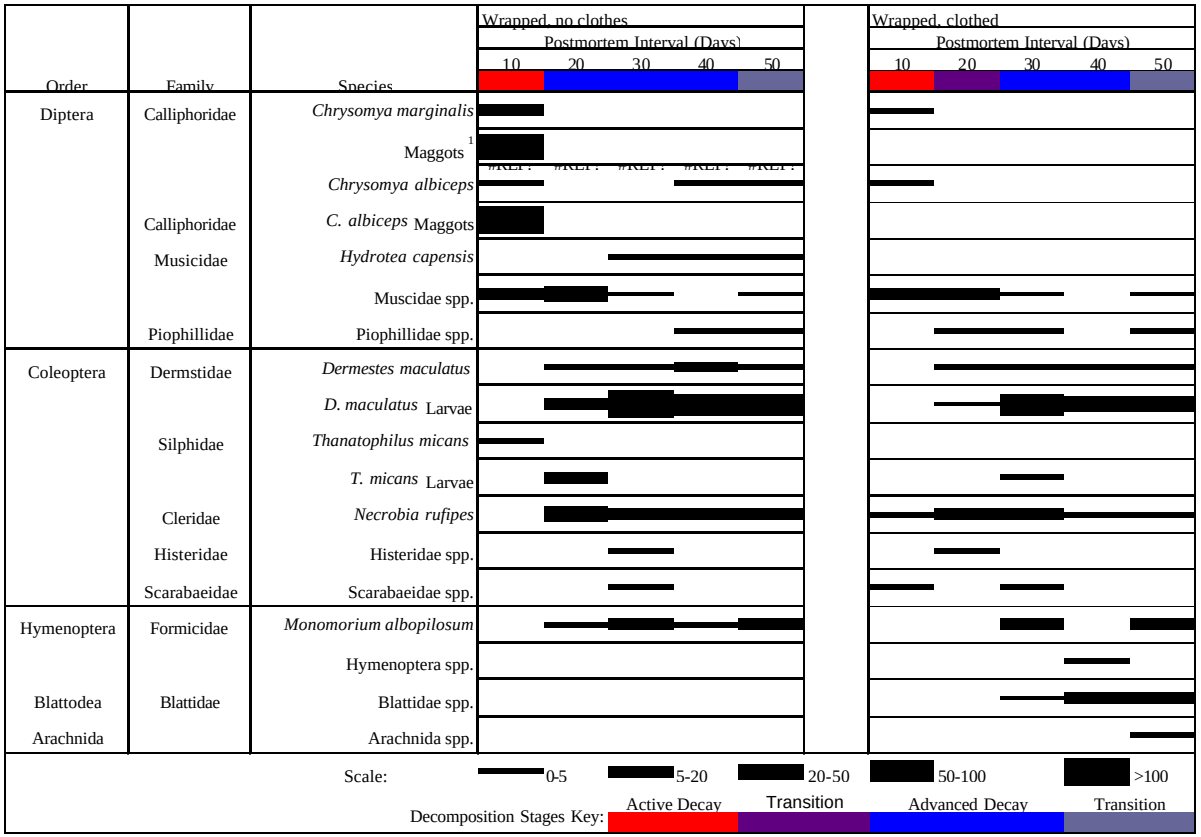


Fig. 3.1.9. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses (sampled every ten days) during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata*.

T. micans larvae were recorded consistently on the control carcass from day 11 (Fig. 3.1.4.) on the wrapped no clothes carcass from the morning of day 12 (Fig. 3.1.6.), on the clothed control carcass from the morning of day 13 (Fig. 3.1.5.) and on the wrapped clothed carcass from day 16 (Fig. 3.1.7.). They are predators.

Formicidae, *Anoplolepis custodiens* (F. Smith) and *Monomorium albopilosum* Emery were present during the entire decomposition process. *Monomorium albopilosum* sometimes occurred in high numbers (Fig. 3.1.4. to 3.1.9.). The Formicidae did not appear to have any affect on the decomposition rates. They were observed to be occasional predators on a small number of maggots, but mostly they fed on fluids on the carcass surfaces, usually during the initial stages of decomposition. They also removed small sections of the epidermis of the carcasses. The predation phenomenon has been recorded to occur on various carrion studies (Houston 1987, Wells & Greenberg 1994b). Ant nests were sometimes observed underneath the carcasses, during advanced decay, after the carcass fluids had drained away and maggots have already migrated to pupate. However, the nests did not appear to significantly decrease the number of Coleoptera species present.

On some carcasses, Scarabaeidae (dung beetles) and a few Blattodea (cockroaches) were present, usually as single individuals (Figs 3.1.4. to 3.1.9.). The Scarabaeidae spp. present at the carcasses were only recorded during the first few days after placement (Figs 3.1.4 to 3.1.9.). In a single case during, a preliminary trial run before the formal experimental trials, a dung beetle was observed rolling a ball of blow fly eggs away from a carcass. However, other egg masses remained and those that were removed were quickly replaced.

The succession of arthropods at the family level, i.e. Diptera (Calliphoridae) and the Coleoptera (Dermestidae) was similar to most references on insect succession on pig carcasses in other geographical regions (Payne 1965, Rodriques & Bass 1983, Anderson & VanLaerhoven 1996) although the species were different from those found in the northern hemisphere. The species corresponded with those found in other African and South African studies albeit the carcasses in these studies were impala *Aepyceros melampus* (Lichtenstein) (Braack 1981, Ellison 1990) or african elephants,

Loxodonta africana (Blumenbach) (Coe 1978). This suggests that the data recorded from pig (*S. scrofa*) studies may be applicable to poaching cases.

The maggot masses were comprised mostly of *C. marginalis*, with a smaller number of *C. albiceps*. *Sarcophaga cruentata* maggots were present in low numbers. Less than one percent of the samples had *C. chloropyga* maggots.

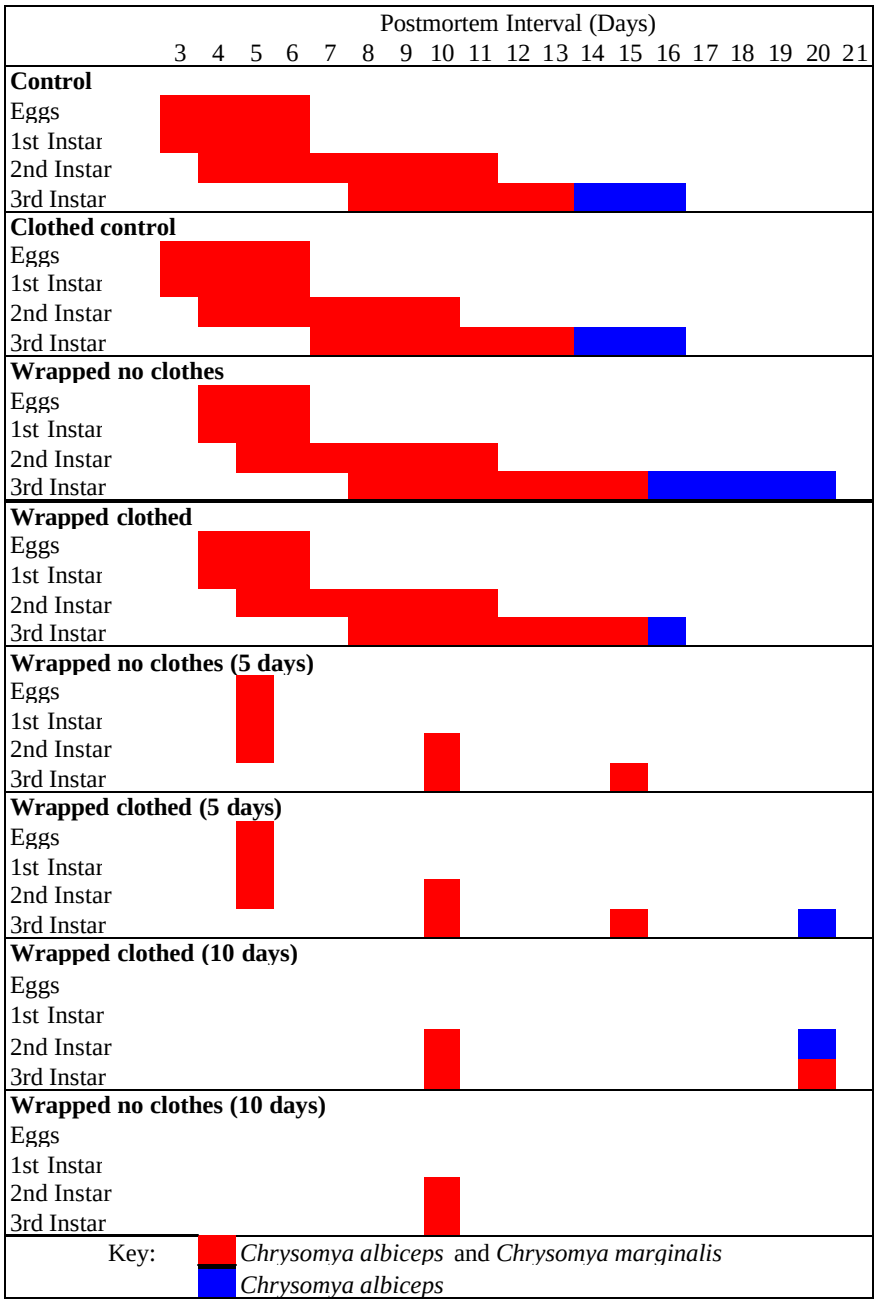


Fig. 3.1.10. Eggs and maggot succession for all carcasses during the wrapped autumn trial.

The *C. albiceps* maggots had a longer developmental time than the other Calliphoridae species, rather than a delay in oviposition by the adults of this species. This was confirmed by the presence of *C. albiceps* in the sub-samples of maggots raised to adulthood collected just after the eggs hatched. The developmental time of the maggots was similar in all the treatments, with the exception of the wrapped clothed carcass. *Chrysomya albiceps* remained on that carcass for an extra three days (Fig. 3.1.10.).

One of the most important results, in terms of a postmortem interval estimation, was that oviposition by the Diptera was recorded simultaneously at all the carcasses regardless of whether the carcasses were wrapped. There was heavy rainfall from the afternoon of day 1 until it cleared in the afternoon of day 3. Although the rain did not allow sampling on those days, flies were observed ovipositing on the afternoon of day 3, and only newly hatched maggots were observed on the carcasses. This has severely influenced this trial and could lead to a misinterpretation of the true situation. As a result all the carcasses in all the sample groups showed maggots of the same age and in most cases of the same species during the first 13 days. This is contrary to the work published by Goff (1992), who estimated a 2.5 day delay between wrapped and control carcasses. However, it should be noted that in the current trials the sheets were not secured. It is also suggested that the warm temperatures prevailing during the autumn trial might have resulted in a large number of female flies competing for access to the carcasses. In autumn, the average number of individual Calliphoridae flies counted in the afternoon sampling during the days when eggs were laid (days 4 to 6), was on average 52 to 144 per carcass. The actual number of flies to visit the carcass overall could be much higher than this estimate as sampling was done in a certain time frame and not according to the daily climatic changes which may result in more flies being attracted to the carcasses at another time.

The number of flies competing for the resource could have been enough to have caused the flies to actively pursue access to the carcass. The flies were observed pushing through the smallest gaps and folds of the sheet to the point of damaging their wings. Dead flies were sometimes found on the wrapped carcasses after they were unable to find a way out of the sheet. Thus, in a criminal investigation where a body has been wrapped and the wrapping has not been secured in any way, it may be

assumed that there would be no delay in oviposition by adult *C. marginalis* and *C. albiceps* during the autumn seasonal conditions in this region of South Africa. This assumption could be strengthened by the fact that the sheets were full double bed size and an adult human would be of a larger body size than a pig carcass. When a human body bloats, the gaps created in the folds of the sheet may even be larger allowing the flies easier access. This was also the case in an experiment done by T.C. van der Linde (2002) in January – February 2002. However, the occurrence of rain during the first two days after placement in the current trial delayed the oviposition, which would have otherwise occurred earlier.

3.1.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices and the mean faunal similarity was then calculated for each day (see 2.4.1.). These were plotted and the grand mean of all the mean faunal similarities were calculated and is represented as $\overline{S}_{gmean}$ (Fig. 3.1.11.).

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

There were significant differences in the similarity matrices between the control carcasses (control and clothed control) and the wrapped carcasses, with the exception of the wrapped carcass sampled every five days. This may be due to the low values and thus small matrices in the comparison (Table 3.1.2.).

Statistically, according to the Jaccard Metric species pairwise similarities, the graphs do not show the characteristic horseshoe-shape as described by Schoenly (1992), with the exception of control and clothed control carcasses. These carcasses dried out faster than any of the wrapped carcasses and the species turnover and richness was low and resulted in the pairwise similarities decreasing in value. These carcasses showed a more characteristic increase in similarity towards the end of the trial.

All the carcasses showed that during active decay, when there was a higher species richness, the pairwise samples were more taxonomically similar to the pairwise samples during the early and late decomposition stages. The graphs, however, do have

similar shapes. Schoenly (1992) stated that 'plots of mean similarities manifested similar shapes and the ranges seem to reflect a general property of the dynamic daily changes that occur during carrion-arthropod succession'.

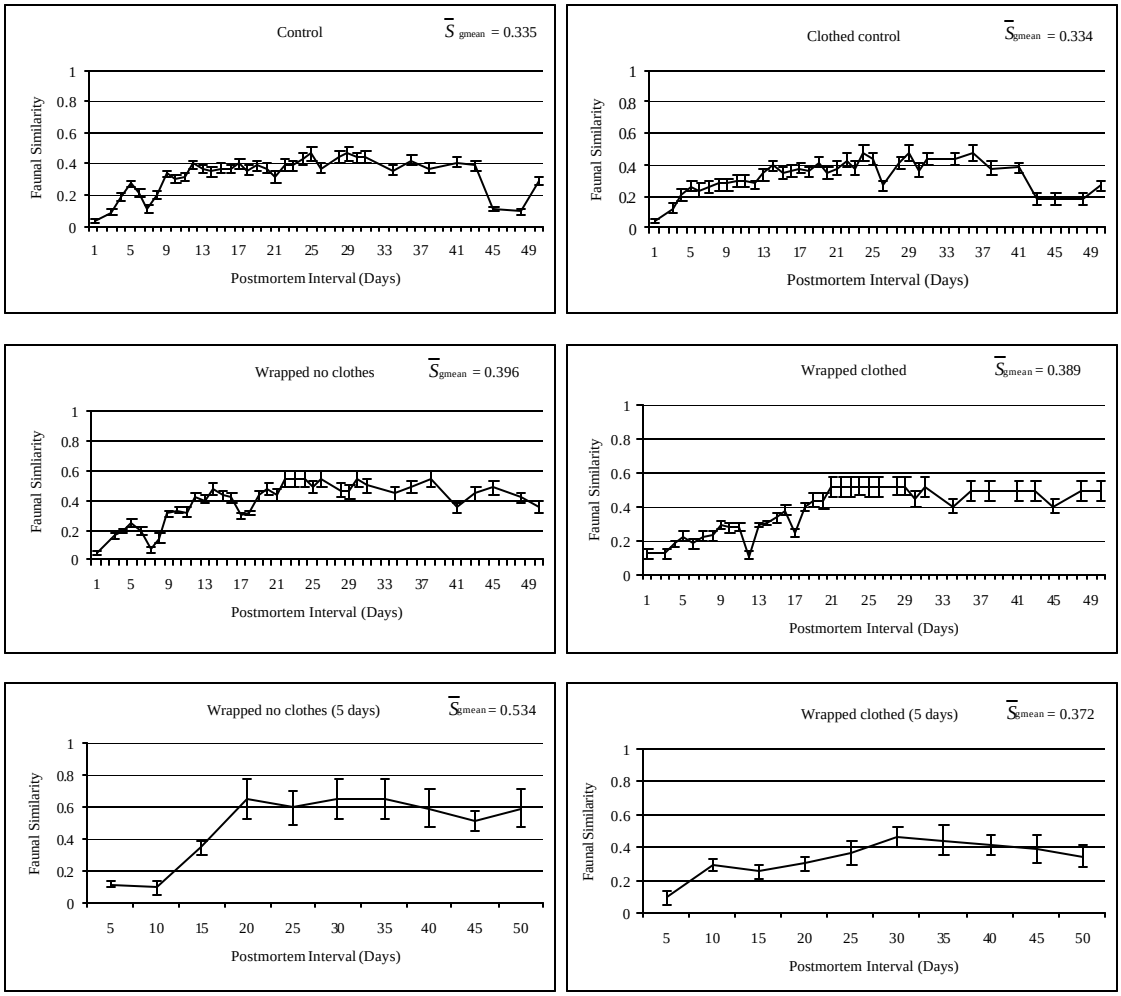


Fig. 3.1.11. Plots of mean pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors).

When Schoenly (1992) compared a variety of studies, he found the $\overline{S}_{gmean}$ values ranged from 0.218 to 0.808. The $\overline{S}_{gmean}$ values obtained from the current study, were more consistent and ranged between 0.334 and 0.396, with the exception of one value at 0.534 (wrapped no clothes (5 days) Fig. 3.1.11.). Schoenly (1992) explained that the variation for his results was due to the study using 'nonhuman carcasses of

different type'. The result obtained in the current study may be a better indication of the species similarity that could occur on pig (*S. scrofa)* carcasses in autumn.

Table 3.1.2. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values. (* - significant difference at 95% confidence interval)

Carcass (all samples)			
	Control	Clothed control	Wrapped no clothes
Clothed control	0.8274 (0.0240)		
Wrapped no clothes	0.7642 (0.0752)*	0.7320 (0.0430)	
Wrapped clothed	0.7575 (0.0520)*	0.7234 (0.0250)	0.7990 (0.0740)
Carcasses (sampled every 5 days – versus each other and relevant data from other carcass with all samples)			
	Wrapped no clothes (5 days)	Wrapped clothed (5 days)	
Control	0.8031 (0.0770)*	0.7539 (0.0)	
Clothed control	0.8687 (0.0380)	0.8010 (0.047 0)	
Wrapped no clothes	0.8025 (0.0440)	0.8299 (0.0)	
Wrapped clothed	0.7952 (0.0430)	0.8218 (0.0340)	
Wrapped and clothed (5 days)	0.7374 (0.0220)		

The Pearson's coefficient statistical analysis conducted on the carcass similarity matrices showed that there were significant differences in the overall arthropod succession between the wrapped carcasses and the control carcass. However, the clothed control did not show a significant difference when compared to the wrapped carcasses. Similarly as the wrapped carcasses showed no significant differences between the clothed carcass or the carcass without clothes (Table 3.1. 2.). This suggested that clothing on its own is significant, however, if a carcasses is wrapped in a sheet, clothing does not appear to have an influence on the similarity matrix.

3.1.4. Ambient, external and internal carcass temperatures

This trial only had two data loggers that recorded viable results. The data from the probes measuring the ambient temperature in the field were averaged to eliminate some of the variance between the readings of the various probes. The study site ambient temperatures were compared to the internal and external temperatures of the carcasses as well as data from the South African Weather Services (SAWS), recorded at the Bloemfontein city and airport stations (Fig. 3.1.12.).

The maximum temperatures provided by the SAWS for the duration of the trial were significantly lower than the temperatures recorded at the study site (Fig. 3.1.12b). This led to the conclusion that there was a malfunction with the probes used. For the

comparison of the internal and external maximum temperatures of the carcasses, the city ambient temperature data set was therefore used. The minimum ambient temperatures varied more from each other, however, the study site temperatures are in an acceptable range (Fig. 3.1.12a) and thus were used in the comparison of the internal and external temperatures of the carcasses.

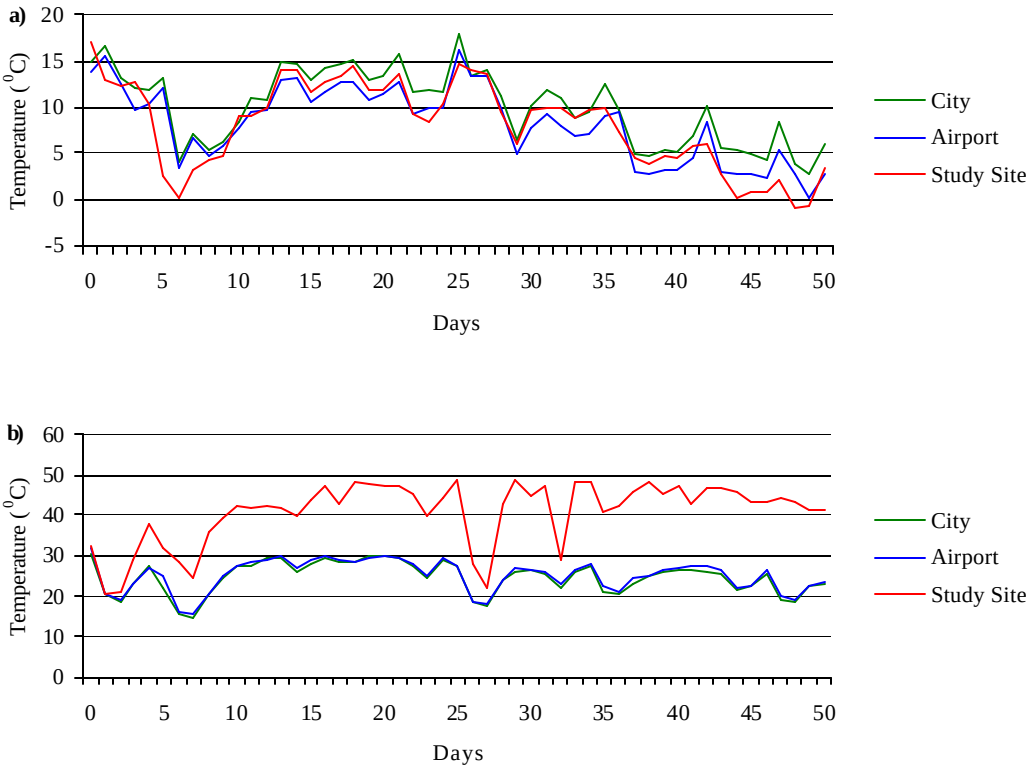


Fig. 3.1.12. Minimum (a) and maximum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the autumn wrapped trial.

The internal temperatures of the carcasses were higher than the ambient temperature most of the time. However, the minimum temperatures of the head and thorax more closely tracked the ambient temperatures after the maggots had migrated from the carcass that is, during the advanced decay stage (Figs 3.1.13 & 3.1.14).

Unfortunately, due to the uncertainty of the accuracy of the data loggers, these data sets were not analysed further. When the change was made to the iButtons during other trials, the data was further tested for trends and correlations.

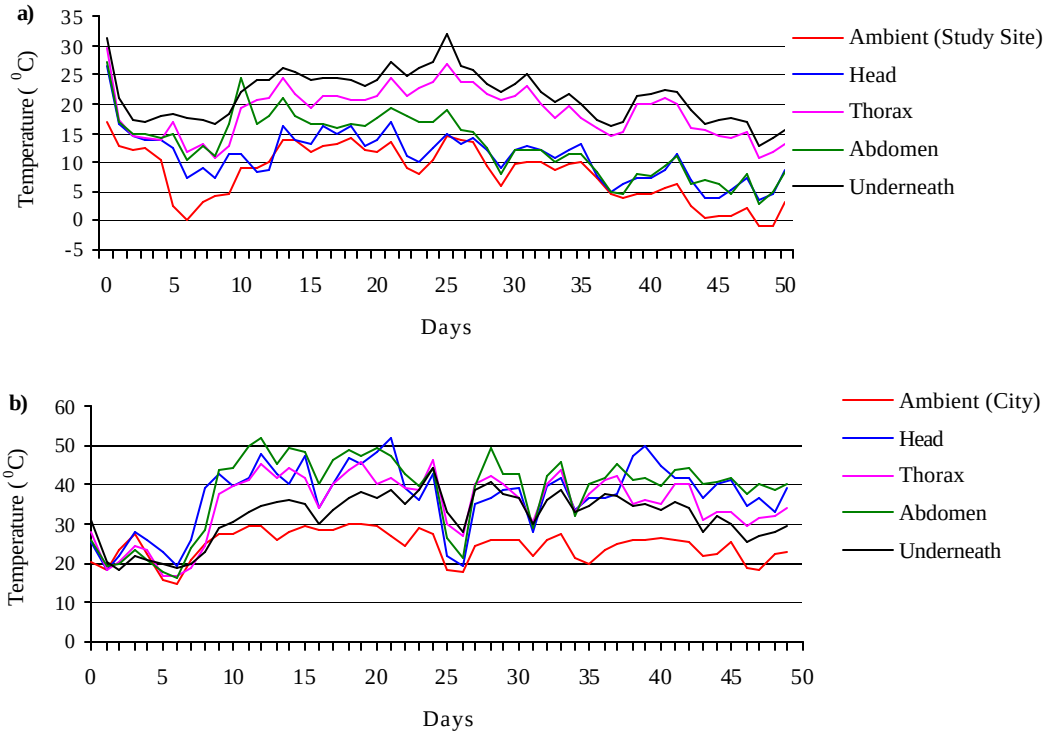


Fig. 3.1.13. Minimum (a) and maximum (b) ambient, internal and external temperatures for the wrapped clothed carcass.

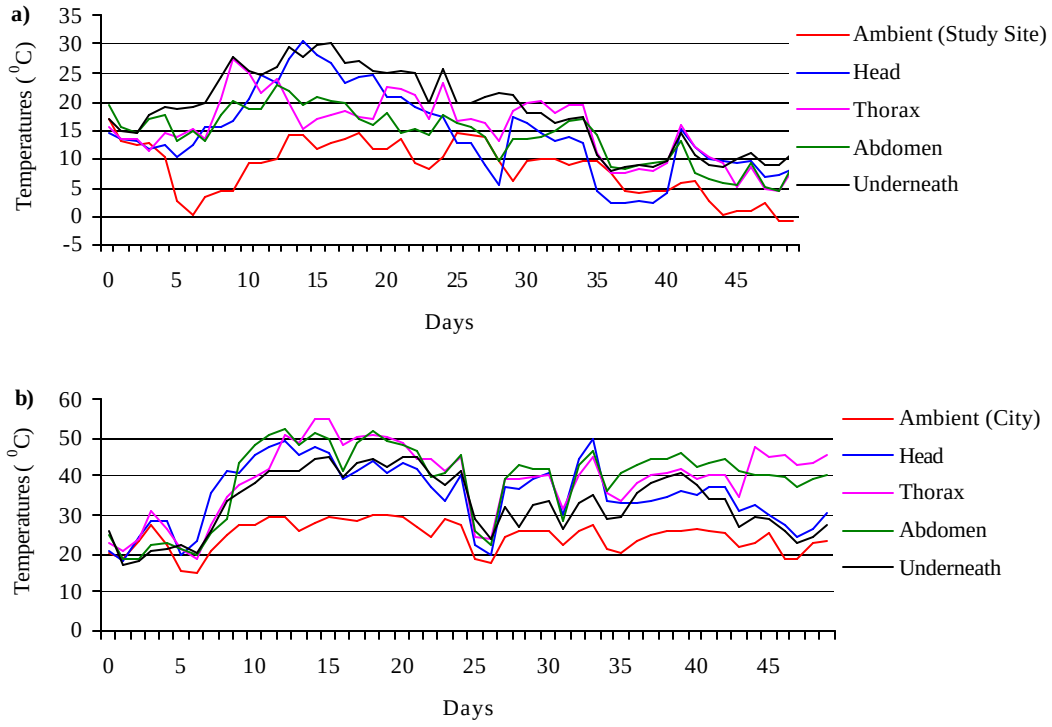


Fig. 3.1.14. Minimum (a) and maximum (b) ambient, internal and external temperatures for the wrapped and clothed carcass (5 days).

3.1.5. Mass loss

Mass loss was faster for both control and the clothed control carcasses (Fig. 3.1.15). The carcasses in the ten-day sample group were only weighed at the beginning of the trial and again at termination. Their percentage remaining tissue was similar to the other wrapped carcasses, at 37% and 32% for the carcass without clothes and the clothed carcass respectively.

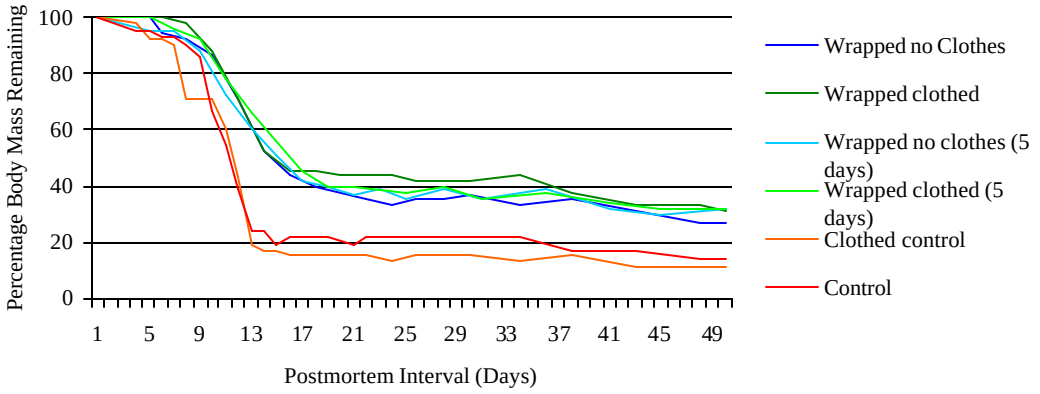


Fig. 3.1.15. Percentage body mass remaining of the carcasses during the autumn wrapped trial.

Payne (1965) showed a mass loss, which was very similar to the control and clothed control carcasses, for all his exposed carcasses. This suggests that the wrapped carcasses maintained higher moisture content for longer as the visible tissue loss was similar.

3.1.6. Maggot mass distribution

The distribution of the maggot masses was recorded daily. An example of the overall trends seen during the trial are shown in Fig. 3.1.16. These were the positions of the maggot masses on day 10 of the trial. All the carcasses showed a similar pattern, in that the maggot masses were larger in the afternoon than in the morning. In the morning, the maggots congregated in denser masses and were often found on the extremities of the carcasses. During the course of the day they spread out over the surface of the carcasses. When the maggot mass was at its largest, the carcass surface was covered almost entirely, with the exception of the control where the maggot masses were found mostly under the protection of the skin (Fig. 3.1.16.). The clothed

and wrapped carcasses also had large maggot masses on the outside of the clothing under the sheet. Whereas on the clothed control carcass, the maggot mass remained under the clothes and were seldom seen on the surface. The wrapping allowed the maggot masses great freedom of movement, and they migrated all over the carcass from tail to snout, at times covering the whole carcass surface under the protection of the sheet, compared with the control carcasses, where the visible maggot masses never completely covered the carcasses.

The movement of the maggots under the sheets could be a result of the sheet providing some protection from the surrounding environmental and climatic conditions. However, due to the possibility that the wrapping caused a build up of heat, the movement may be a result of a thermo-regulation response as insects are poikilothermic (Romoser 1981). The visual increase in the maggot mass size did not necessarily indicate that the maggot mass was any larger in overall volume, but rather that the maggots on the wrapped carcasses are more visible by their position on the surface of the carcass. The consumption of the carcass tissue was not significantly different from the control carcass, except in some cases more of the skin was consumed by the maggots on the wrapped carcasses.

The clothes, specifically the underwear and shorts, were pushed down the limbs and almost off the carcass in one case (clothed control carcass) in the autumn trial. This was similar and supported results of Komar & Beattie (1998). In the current study, the carcasses were less than 27 kgs and were protected from carnivore scavenging, both of these reasons Komar & Beattie (1998) gave for these observations not being seen in previous studies. Therefore, maggot mass movement and distribution under the sheet may explain the greater degree of clothing displacement in the current study, which may need to be considered when assumptions are made about sexual abuse in human investigations.

Some of the maggots on the wrapped carcasses completed their entire life cycle on the surface of the sheet, away from the carcass surface. They were observed in the folds of the sheet and the ground along the back of the carcasses.

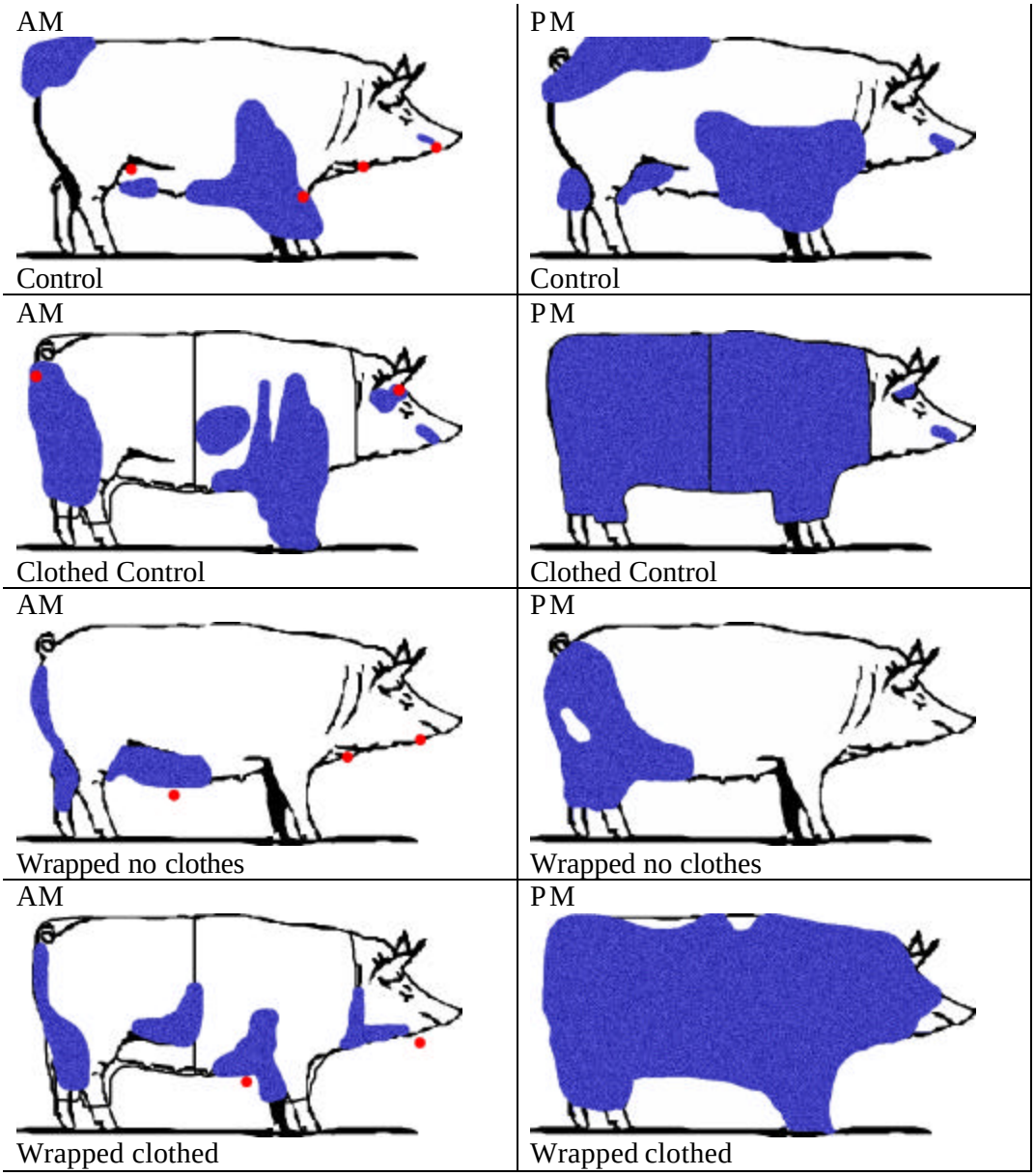


Fig. 3.1.16. Maggot mass movement recorded on day 10 of the autumn wrapped trial. Dots on the morning (AM) diagrams represent the primary oviposition sites.

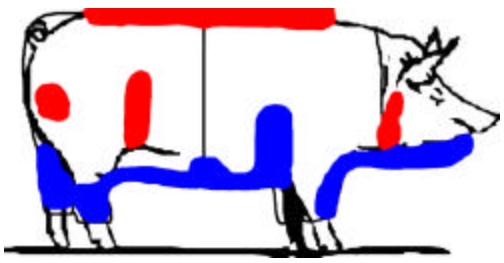
The sheet showed no signs of damage and although the maggots moved towards the head and then back towards the abdomen, their movements were restricted to the length of the fold. There was no fluid trail from maggot movement (the maggots were covered in decomposition fluids) out of the fold and no other evidence to suggest that they ever had direct access to the carcass. They fed on the tissue fluids seeping through the sheet. Nuorteva (1974) recorded a similar phenomenon of maggots

feeding on a blood stain in a decaying shirt, in the absence of a carcass. However, those species concerned were *Muscina stabulans* Fallen, *Fannia canicularis* L. and *Hydrotaea dentipes* (Fabricius) and not Calliphoridae. The observations made in the current study suggested that there is a possibility for Calliphoridae to complete their life cycles in the absence of a carcass and more detailed research would need to be conducted to confirm this.

3.1.7. Maggot mortality

On day 12 of the trial extensive maggot mortality along the back of the wrapped, clothed carcass was observed. (Fig. 3.1.17a). The maggot mass concerned was almost completely made up of third instar *C. marginalis*. The afternoon temperatures of the carcasses were high, with the carcass surface temperature at 44^{0}C, the head at 46,3^{0}C, the thorax at 38,2^{0}C and the abdomen at 42,8^{0}C. The ambient temperature was 29,4^{0}C. However, the temperature in the dead maggot mass was 56^{0}C. The other wrapped carcasses also experienced mortality, but only a few individuals were involved, also along the back of the carcass. All dead maggots were found under a sheet soaked with decomposing tissues and fluids (Fig. 3.1.17b).

a)



b)

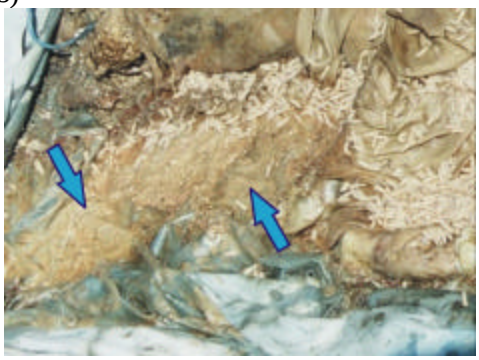


Fig. 3.1.17. a) Distribution of maggot mortality (red area along the back of the carcass) and remaining live maggots (blue), b) Sheet saturated in decomposition fluids.

When maggot mortality occurred, all dead maggots were only found on wrapped carcasses under a sheet soaked with fluids, which did not allow free flow of air. It was possible that heat generated by the sun and the maggot mass was trapped under the wet sheet which may have resulted in the mortality. It was also suggested that there

was a build up of noxious gasses, which may also have resulted in the mortality. The maggot mortality seemed to have little effect on insect succession, even in cases where large portions of the maggot mass was affected. The adult Diptera did not return to the carcass to utilize the remaining tissue or oviposit. The tissues were probably decomposed to the point where it was no longer acceptable to the adult Diptera as a food source or for oviposition. As a result the only difference observed in these cases was slightly more decomposing tissue remaining on the carcasses through the later stages of decay. This did not appear to influence the Coleoptera species either, whose numbers and community structure followed the carcasses where no mortality had occurred.

3.1.8. Maggot predation

On day 13 of the trial, *C. albiceps* maggots were observed predating on *C. marginalis* maggots (Fig. 3.1.18.). The maggots were associated with the clothed control carcass, with almost no tissue remaining. The *C. albiceps* were all third instar maggots, but about half the size of well fed maggots seen on the other carcasses and were not actively 'hunting' the *C. marginalis* maggots. They were on the ground below the carcass. The *C. marginalis* maggots moved off the carcass and they tried to move through the mass of *C. albiceps* maggots below. If a *C. albiceps* maggot made contact with a *C. marginalis* maggot, it immediately attached itself to the integument of the *C marginalis* with its powerful mouth hooks. As the *C. marginalis* maggot began to struggle the *C. albiceps* maggot would wrap itself around the *C. marginalis* body. This usually resulted in the two of them rolling around and making contact with other *C. albiceps* maggots. These other maggots would also attach themselves to the *C. marginalis* maggot. Within minutes the only remaining evidence of the *C. marginalis* maggot was a shrivelled up skin, which dried out very quickly after being released by the *C. albiceps* maggots. It was interesting to note that *C. albiceps* did not feed on each other, although they made contact. It was also observed many times that, *C. albiceps* maggots were in the same maggot mass as *C. marginalis* maggots with no predation occurring. Predation only occurred as the post-feeding *C. marginalis* maggots moved off the carcass during maggot dispersal when the remaining food source was limited.

Chrysomya albiceps is known to be a facultative predator of other Diptera maggots (Coe 1978, De Andrade *et al.* 2002). However, there were a few observations when the maggots formed distinct groups of *C. albiceps* within the *C. marginalis* dominant maggot mass, but this was not always the case. Predation was only observed in the autumn trial and occurred during maggot migration, similar to laboratory experiments done by De Andrade *et al.* (2002), where *C. albiceps* were observed to predate on *Cochliomyia macellaria* (Fabricius) during dispersal / migration. The number of *C. marginalis* observed at the carcass during emergence did not appear to be significantly impacted, however, this is only based on observations in the field and not quantified. The occurrence of predation of the *C. albiceps* on the *C. marginalis* did not affect the succession of the Coleoptera, as the predation occurred only during maggot dispersal. Predation in the current study was a result of a resource limitation, and the presence of *C. marginalis* was not completely eliminated.

Fig. 3.1.18. *Chrysomya albiceps* maggots predating on *Chrysomya marginalis* maggots during the autumn wrapped trial.

SECTION 3.2.

The influence of wrapping and clothing on carcass decomposition and arthropod succession: a winter study.

3.2.1. Decomposition of the carcasses

As with the autumn trial, the carcasses were grouped into various stages of decomposition according to Payne (1965) and Anderson & Van Laerhoven (1996). In winter the decomposition of the carcasses was very slow due to the low ambient temperatures. Thus, the characteristics used were very dependant on the absence or presence of the Diptera maggots together with physical characteristics. The decomposition stages were not as easily classified as those used in the warmer seasons. Transitional stages were used which exhibited overlapping characteristics from adjoining stages. The duration of the trial (100 days), allowed for only four decomposition stages (Table 3.2.1.).

i) Fresh

The carcasses where placed in the field at approximately 14:00h on 20th June 2003 (day 0). By the following day, the carcasses became rigid as rigor mortis caused the muscles to contract. The carcasses then slowly began to bloat. The carcasses entered the bloat stage simultaneously (Figs 3.2.1 and 3.2.2.).

ii) Bloat

The carcasses never became fully inflated or 'balloon'-like. The torsos became soft and spongy within a few days as the rigor mortis in the muscles decreased. This occurred approximately on day 4 and 5 for the control and clothed control carcasses respectively. For the wrapped carcasses it occurred approximately on day 13 and 14.

The carcasses began to show slight colour changes after three days. The skin underneath on the lower surfaces of the carcasses, changed from a dark pink to a green and in some cases black. By day 10, any visible veins were black, both underneath and on the abdomen of the carcasses. Over time the remaining pink areas of the carcasses, slowly turned white (upper surface) or green (lower parts of the

carcasses) until approximately day 19, when pink patches were restricted only to areas between the fore and hind limbs.

Table 3.2.1. Summary of the decomposition stage, physical characteristics of each stage and the dominant arthropods present during the winter wrapped trial.

FRESH (Fig. 3.2.1.) • Commenced when the animal was killed • The torso (thorax and abdomen) was soft and the limbs were flexible	
BLOAT (Fig. 3.2.1.) • Commences when the torso slowly began to harden and the abdomen in particular became inflated, due to the build up of gasses • Bubbles of blood formed at the nose, creating small puddles or saturating the sheet on the wrapped carcasses • Carcass became spongy rather than 'ballon'-like • Oviposition took place during this stage	**Diptera** Calliphoridae: *Lucilia* spp. (adults) *Chrysomya albiceps* (adults) *Chrysomya chloropyga* (adults) *Chrysomya marginalis* (adults) *Calliphora vicina* (adults) Muscidae: *Hydrotea capensis* (adults)
ACTIVE DECAY (Fig. 3.2.1.) • Carcass slowly deflated, as the maggots actively fed on the carcass tissues and allowing some of the gasses to escape • Slight odour of decay was present • The limbs collapsed back into the 'resting' position and were soft and flexible • The skin dried out causing it to peel and allowing maggots to feed underneath it • The Diptera maggots were the most dominant insects • The thorax and abdominal tissues softened and were spongy to touch • Some decomposition fluids were present • Oviposition continued during this stage	**Diptera** Calliphoridae: *Chrysomya marginalis* (adults) *Chrysomya. albiceps* (adults) Chrysomya chloropyga *(adults and maggots)* *Calliphora vicina* (adults and maggots) *Lucilia* spp. (adults) Sarcophagidae: *Sarcophaga cruentata* (adults and maggots) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)
ADVANCED DECAY (Fig. 3.2.1.) • Similar in appearance to active decay • The exposed skin was still soft at the beginning and dried out towards the end of this stage • The head was dried out • Gut contents started to dry out • Clothing, when present, was mostly dry • Small parts of the skeleton were visible, usually the skull, ribs and spinal column	**Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)

The presence of maggots, actively feeding on the carcass tissues, resulted in the onset of active decay (Fig. 3.2.2). The maggots, even though there were often less than 50 individuals present, were easily detected. The physical appearance of the carcasses during the winter remained similar to the bloated stage even during most of the active decay stage (Fig. 3.2.1).

iii) Active Decay

The active decay stage dominated the decomposition process for most of the trial. The control and clothed control carcasses entered the active decay stage by day 6 and 4 respectively. The wrapped carcasses only entered active decay on days 14 and 15. As active decay in this season was indicative of maggot mass presence, this delay indicated a delay in oviposition by the adult Diptera.

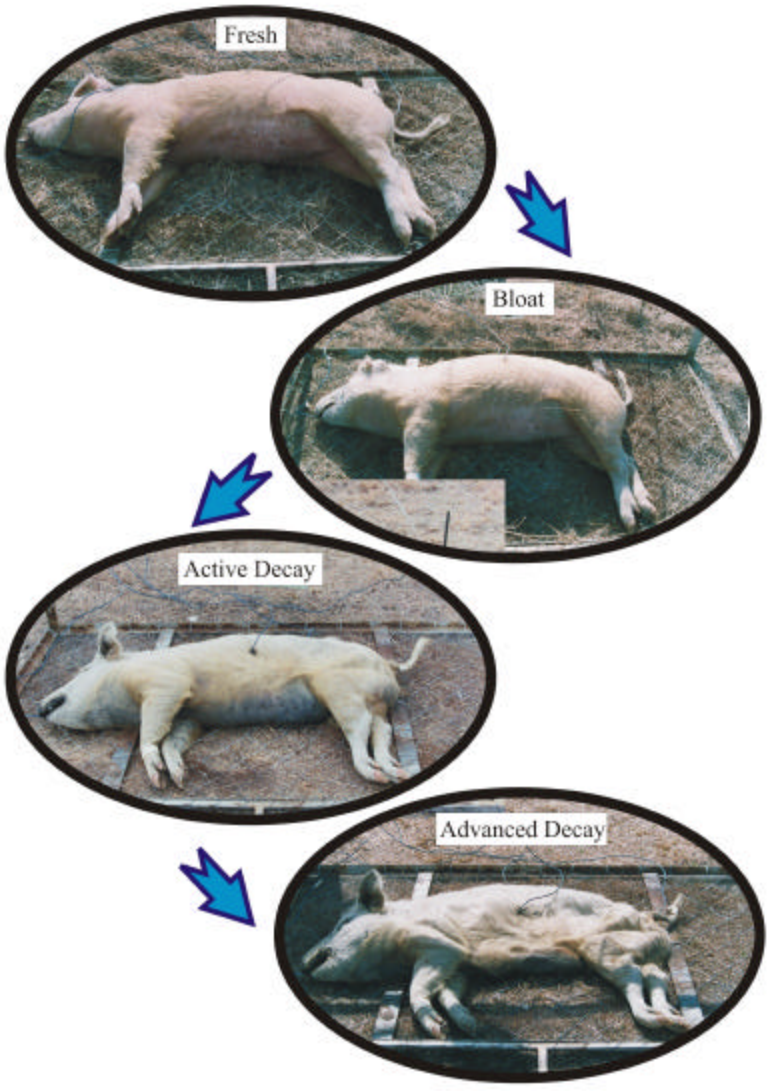


Fig. 3.2.1. Examples of the decomposition stages described in Table 3.2.1.

Decomposition fluids often collected in the mouths of the carcasses and drained on the ground of the exposed carcasses or saturated the sheets of the wrapped carcasses. As active decay advanced, more fluids collected underneath the carcasses, usually along the abdomen and/or back. Overall the amount of decomposition fluid was noticeably less than that which occurred in the autumn wrapped trial.

Active decay was characterised by a slight gaseous smell, which continued until the carcasses began to collapse. This happened very slowly. The hips and surrounding areas of the exposed carcasses (control and clothed control) collapsed first (from day 38). The wrapped carcasses only showed similar collapse from day 42. Collapse of the shoulders and finally the torso followed.

As the maggots fed on the tissues, a more prominent smell of decay became apparent. On some of the carcasses small blisters formed on the skin within the first few days after placement. Only one carcass had a small section where the gut broke through the skin on day 29. However, all the carcasses had sections of skin slippage mostly on the abdomen. This occurred from after day 26 for some carcasses and after day 36 for others. Sometimes skin slippage occurred along the backs of the carcasses. The skin slippage allowed more decomposition fluids to drain from the carcasses. Sections of the skin continued to slip and form small slits until the end of the trial. Towards the end of this stage some of the tissues started to liquefy and became 'creamy' in appearance. The remaining tissue on the torso continued to be soft and spongy. The tissue on the limbs became hardened and dried out.

iv) Advanced Decay

This stage was very similar to active decay. The transition from the active decay stage to advanced decay stage was characterised by less than ten post feeding or migrating maggots remaining on the carcass. Little decomposition fluids were seeping from the carcasses. The maggots on the control carcass were the first to migrate from day 75 to 80. They were followed by those on the clothed control and the wrapped carcass no clothes, 7 days later. The last maggots on the wrapped clothed carcass only began to migrate towards the end of the trial (Fig. 3.2.2.). *Dermestes maculatus* larval feeding damage was visible, mostly on the remaining skin of the head.

When the carcasses were dissected at the end of the trial, it was found that the internal organs, with the exception of the brain were intact. The digestive track was perforated in some sections in some carcasses.

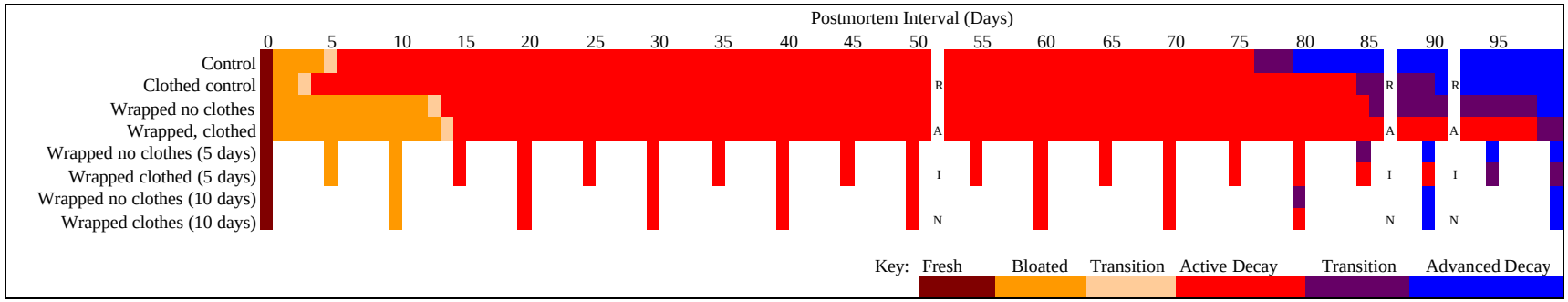


Fig. 3.2.2. Decomposition stages of the carcasses in the wrapped winter trial.

3.2.2. Arthropod succession on the carcasses

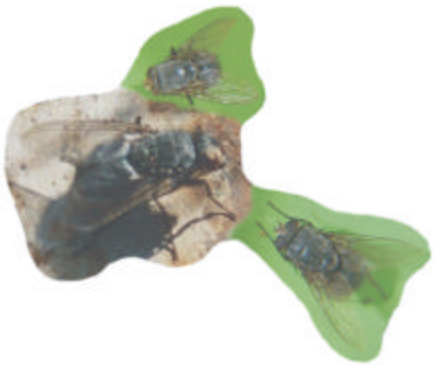
The overall insect succession, with Diptera and Coleoptera species dominating the fauna, was consistent with other studies from around the world (Anderson & VanLaerhoven 1996, Campobasso *et al.* 2001, Wolff *et al.* 2001). As expected, the most dominant Diptera recorded to visit and breed on the carcasses during winter were the Calliphoridae and Sarcophagidae (Fig. 3.2.3). The adult Calliphoridae were represented by *Calliphoria vicina* Robineau-Devoidy (Fig. 3.2.3.), *C. chloropyga* (Fig. 3.2.3.), and *Lucilia* spp. (Fig.3.2.3.) (Table 3.2.2.). The numbers of adult Diptera were noticeably higher for the control carcass (Fig. 3.2.4.) and the clothed control (Fig. 3.2.5.), when compared with the wrapped carcasses (Figs 3.2.6. and 3.2.7.). There was an unexpected low number of Muscidae spp. attracted to the carcasses during this trial, with the exception of *H. capensis*, which were present throughout the trial (Figs. 3.2.4 to 3.2.9).

It is generally accepted that Diptera numbers increase to a peak during the initial stages of decomposition and Coleoptera numbers only increase significantly during the advanced stages of decomposition (Campobasso *et al*. 2001). However, throughout the winter trial, the adult Diptera species appeared to be consistently present with Coleoptera species, i.e. Diptera adults and maggots as well as Coleoptera adults and larvae were observed at the carcasses at the same time.

The Diptera that successfully bred on the carcasses were *Lucilia* spp., *C. chloropyga*, *C. vicina* and *S. cruentata* (Table 3.2.2.). *Chrysomya marginalis* and *C. albiceps* adults were recorded at the carcasses, however, no maggots from these two species were recorded in the sub-samples that were collected and raised to adulthood (Table 3.2.2.). There was a higher species diversity than recorded during the autumn trial, where *C. marginalis*, *C. albiceps* and *S. cruentata* were the dominant successful breeding species.

The Coleoptera present were Dermestidae and Cleridae. The Dermestidae were represented by *D. maculatus* (Fig. 3.2.3.), the Cleridae by *N. rufipes* (Fig. 3.2.3.) and Histeridae, *Saprinus* sp. Cleridae and Dermestidae also represented the major Coleoptera species in most other insect successional studies done elsewhere (Goff & Flynn 1991, Anderson & VanLaerhoven 1996, Kulshrestha & Satpathy 2001). *Dermestes maculatus* larvae were recorded for the first time on day 31 on the clothed control and the wrapped clothed carcasses (Figs 3.2.5. and 3.2.7.). They were only recorded on the wrapped carcass no clothes from day 43 (Fig. 3.2.6.). Lastly, they were recorded on the control carcass only from day 53 (Fig. 3.2.4.). This suggested that the presence of the clothing and wrapping may have allowed the *D. maculatus* to breed sooner, as the larvae have been observed to feed on the fibres of clothing, often the underwear, or the sheets. The early presence of the *D. maculatus* and *N. rufipes* adults was unexpected as the carcasses were still in active decay, moist and intact with large percentage of the body mass remaining. The *D. maculatus* adults were recorded within 14 days after carcass placement. *Necrobia rufipes* adults were recorded from approximately day 21 onwards on the control and clothed control carcasses and day 28 on the wrapped carcasses. Both species numbers steadily increased, especially towards the end of the trial (Figs 3.2.4. to 3.2.9.).

Diptera



Calliphoria vicina

Chrysomya chloropyga

Lucilia spp.

Sarcophaga cruentata

Coleoptera

Dermestes maculatus

Necrobia rufipes

Fig. 3.2.3. Most common forensically important species occurring during the winter wrapped trial.

Table 3 .2.2. Diptera species reared from maggot masses for each carcass during the wrapped winter trial.

	Postmortem Interval (Days)																
	11	12	15	20	25	30	35	40	45	50	55	60	65	69	75	80	85
Control																	
Chrysomya chloropyga						X		X	X	X	X	X	X	X			
Lucilia spp.					X	X	X				X	X		X	X	X	X
Calliphora vicina				X	X	X	X	X	X	X	X	X	X	X	X		
Sarcophaga cruentata		X			X			X	X	X	X	X	X	X			X
Clothed control																	
Chrysomya chloropyga		X			X	X	X	X	X	X	X	X	X	X	X	X	
Lucilia spp.	X	X		X	X	X	X										
Calliphora vicina				X				X	X	X	X			X			
Sarcophaga cruentata	X	X		X	X									X		X	
Wrapped, no clothes																	
Chrysomya chloropyga			X						X	X	X	X	X	X	X		
Lucilia spp.			X						X	X	X	X	X	X	X		
Calliphora vicina			X	X	X	X	X		X	X	X						
Sarcophaga cruentata			X		X	X	X		X	X	X	X	X	X		X	
Wrapped, clothed																	
Chrysomya chloropyga					X	X	X	X	X	X	X	X					
Lucilia spp.							X	X	X		X	X		X	X		
Calliphora vicina					X	X			X	X	X	X					
Sarcophaga cruentata			X			X			X	X	X	X		X			
Wrapped, no clothes (5 days)					X			X			X	X	X		X		
Chrysomya chloropyga								X	X	X	X	X	X	X			
Lucilia spp.				X	X	X	X	X	X	X	X	X					
Calliphora vicina						X	X		X	X	X	X	X	X			
Sarcophaga cruentata																	
Wrapped, clothed (5 days)																	
Chrysomya chloropyga							X	X		X		X	X	X			
Lucilia spp.			X	X	X	X	X	X		X	X	X	X	X	X	X	X
Calliphora vicina						X	X	X	X	X	X	X	X	X		X	
Sarcophaga cruentata				X	X	X	X	X			X	X			X		X
Wrapped, no clothes (10 days)								X		X		X		X			
Chrysomya chloropyga						X		X		X							
Lucilia spp.				X		X		X		X							
Calliphora vicina										X		X					
Sarcophaga cruentata																	
Wrapped, clothed (10 days)						X		X		X		X		X			
Chrysomya chloropyga						X		X				X					
Lucilia spp.						X		X									
Calliphora vicina						X						X					
Sarcophaga cruentata																	

Saprinus sp. (Histeridae) were not recorded on the carcasses constantly. Their numbers also appeared to vary between carcasses.

The co-existence of these insects may be a result of the low numbers of individuals present. It may also be as a result of the small areas which the maggots were utilizing,

allowing the other arthropods to utilize the remainder of the carcass. Competition can be the principal cause of succession in carrion (Fuller 1934) and is a well known driving factor in other ecological systems (Samways 1994). In winter, when the carrion arthropod numbers were low and resources provided by the carcass were plentiful, the competition factor maybe almost non-existent. Thus, allowing all the species to co-exist successfully on the carcasses. Added to this, the Diptera and Coleoptera use different resources. The Diptera favouring the wet decomposing tissues, while the Coleoptera utilize the skin and drying tissue of the carcasses.

It has been reported that during the warmer seasons, the number and type of carrion insects increase (Campobasso *et al.* 2001). In the current winter trial, the numbers of individuals of each species recorded at the carcasses were certainly considerably lower than in warmer seasons. However, the number of species i.e. the species diversity, utilizing the carcasses for breeding was higher.

During research done on carrion insect succession in the northern region of South Africa during May/June 1979 (Braack, 1986, 1987) and July/August 1985 (Ellison 1989), on Impala (*A. melampus*) carcasses, *C. albiceps* and *C. marginalis* were observed breeding on the carcasses. This contrasts with the presence and breeding of *C. chloropyga* and *C. vicina* in the central region of South Africa, with *Lucilia* spp. common to both. This suggests that there was a seasonal, environmental or geographical separation of the Calliphoridae. The first trials had a maximum average daily temperature of 29.4 ^{0}C and average daily minimum of 16.3^{0}C (Braack 1987).

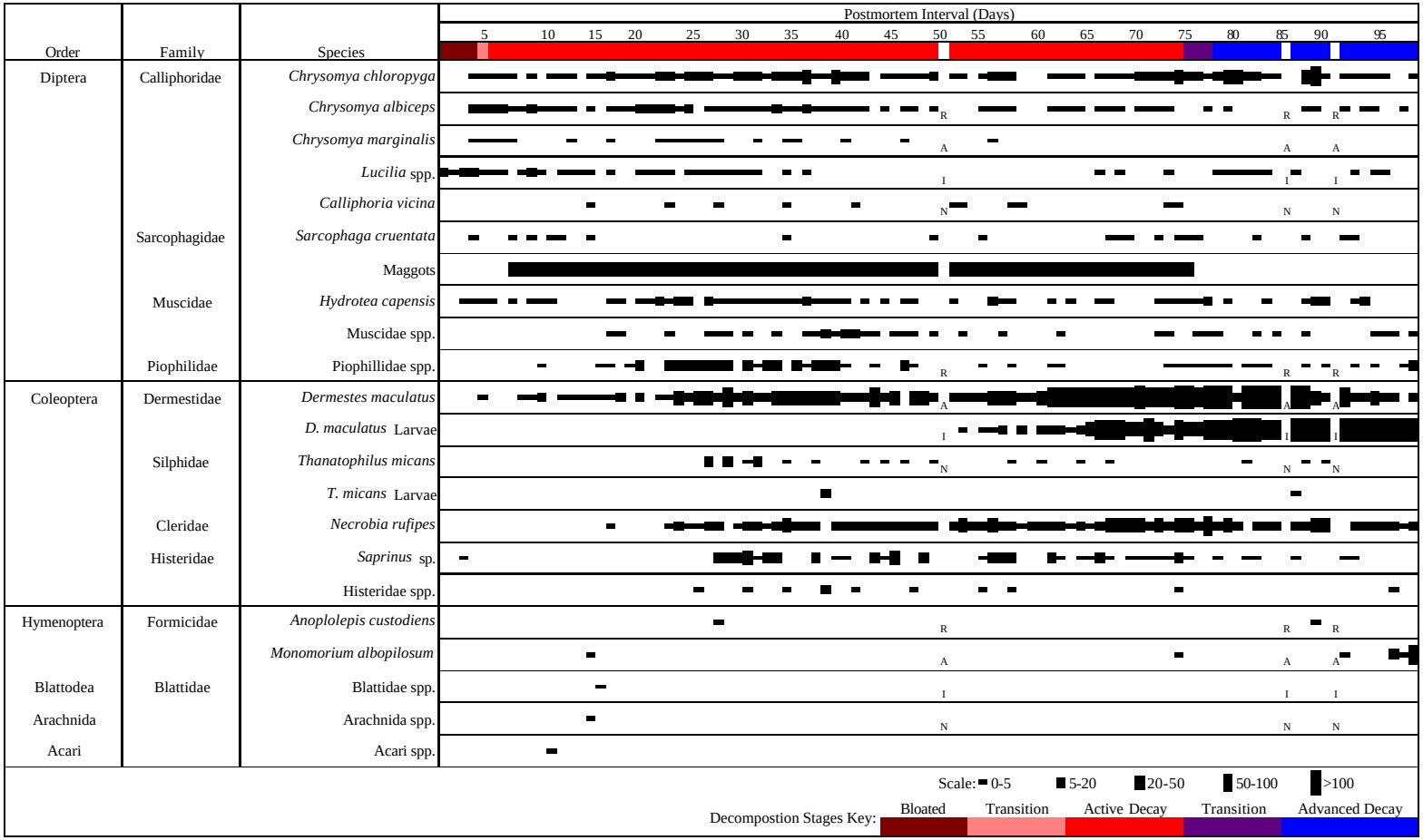


Fig. 3.2.4. Arthropod succession on the control carcass during the winter wrapped trial. Maggots – all species.

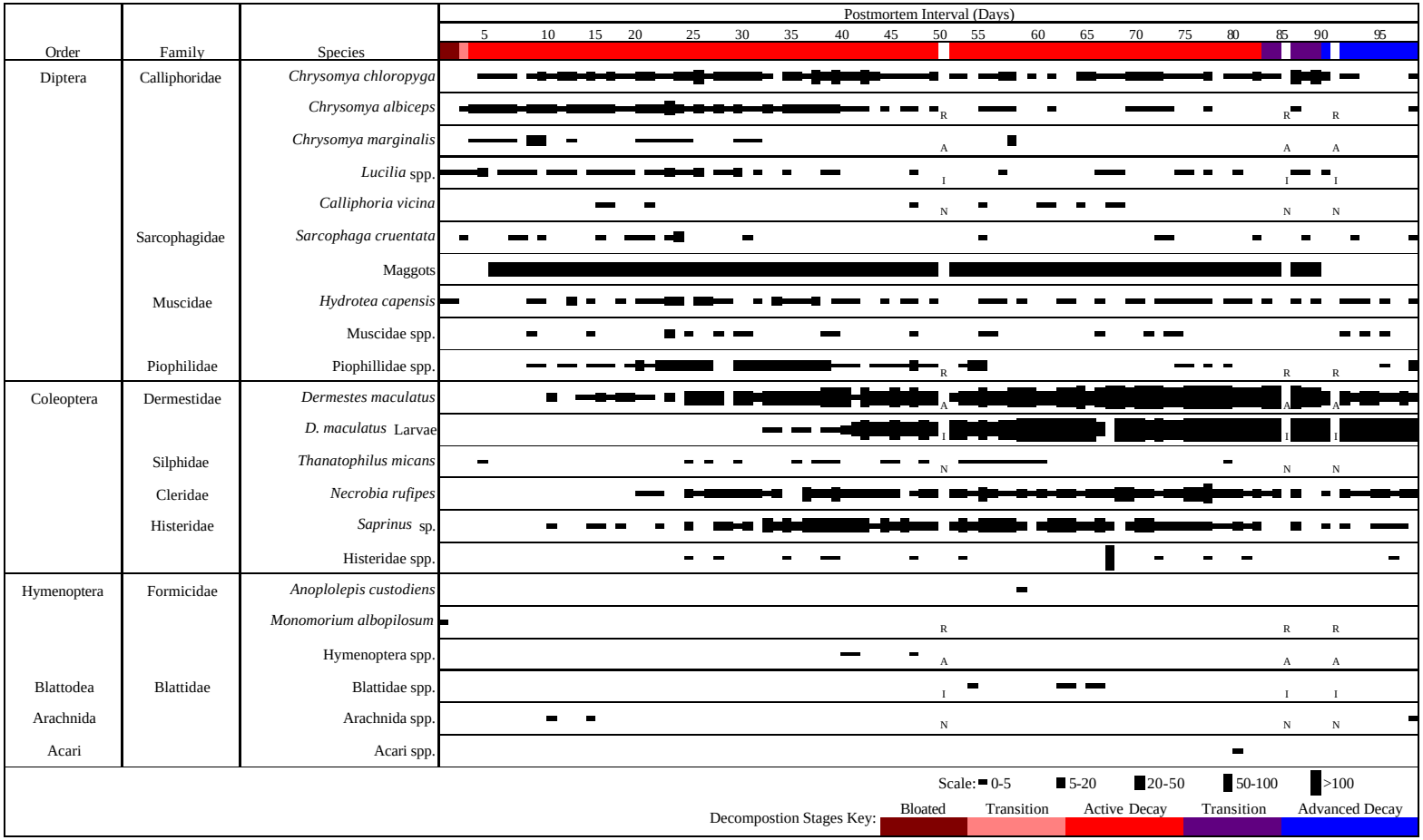


Fig. 3.2.5. Arthropod succession on the clothed control carcass during the winter wrapped trial. Maggots – all species.

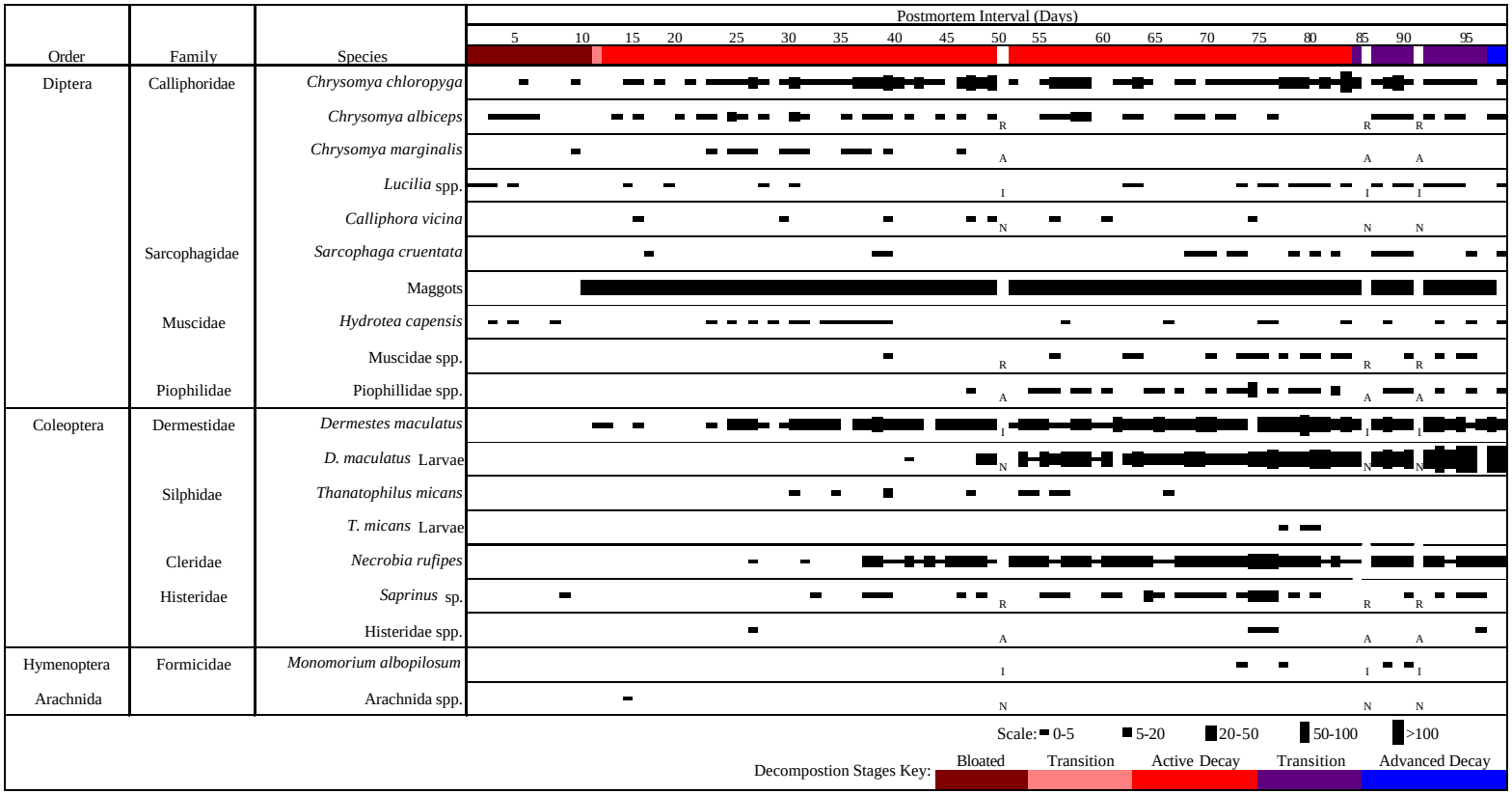


Fig. 3.2.6. Arthropod succession on the wrapped no clothes carcass during the winter wrapped trial. Maggots – all species.

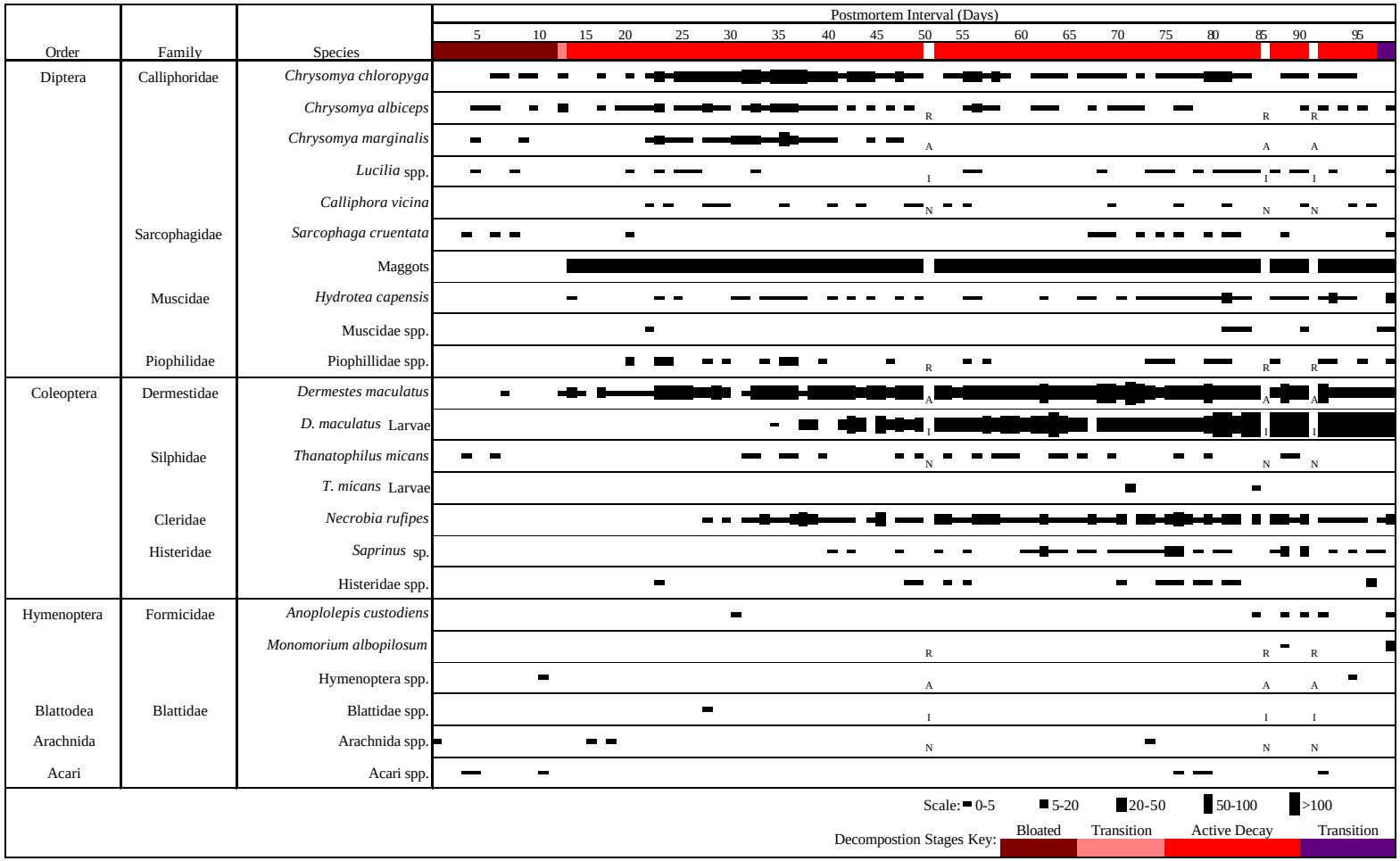


Fig. 3.2.7. Arthropod succession on the wrapped clothed carcass during the winter wrapped trial. Maggots – all species.

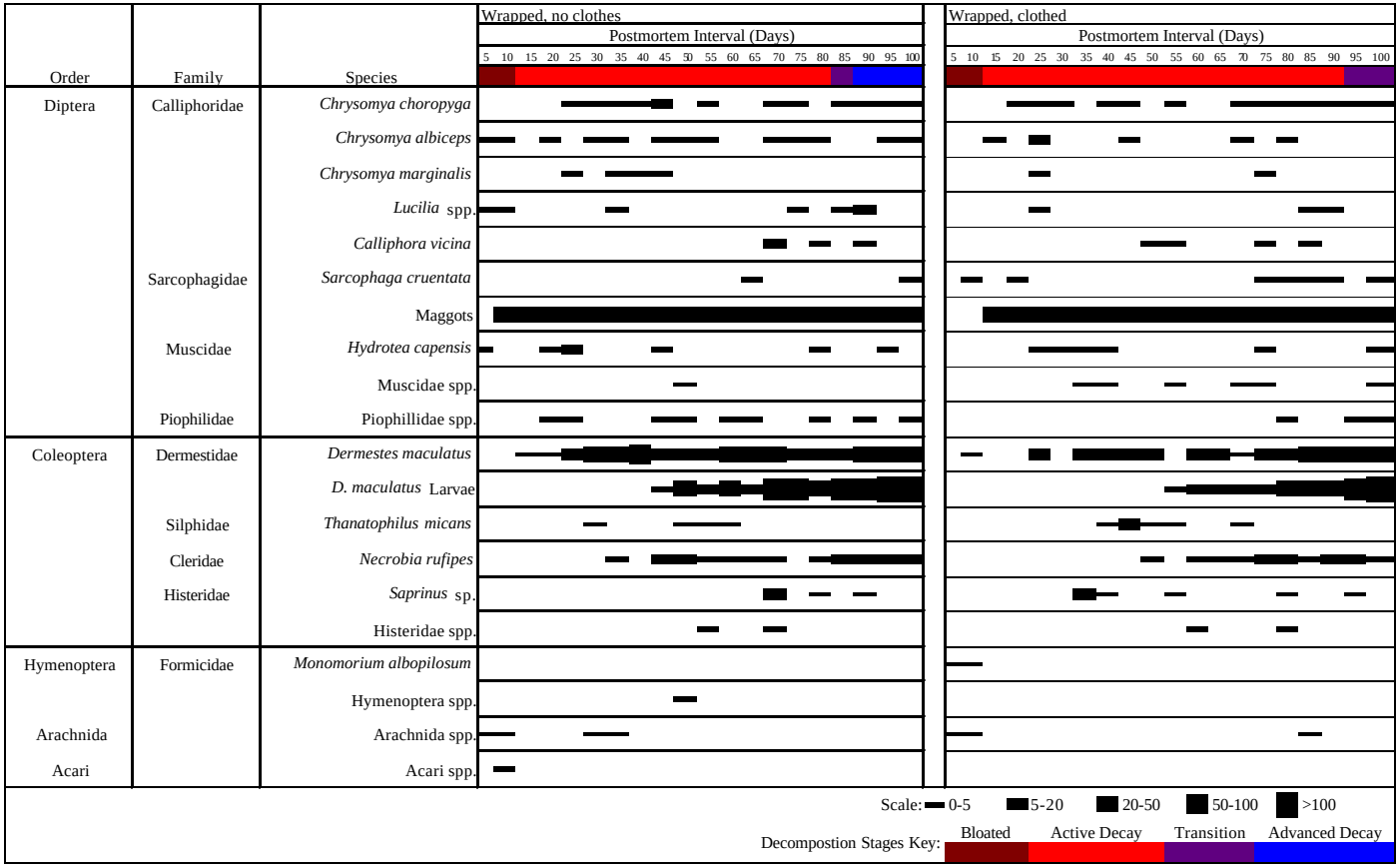


Fig. 3.2.8. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses, sampled every five days during the winter wrapped trial. Maggots – all species.

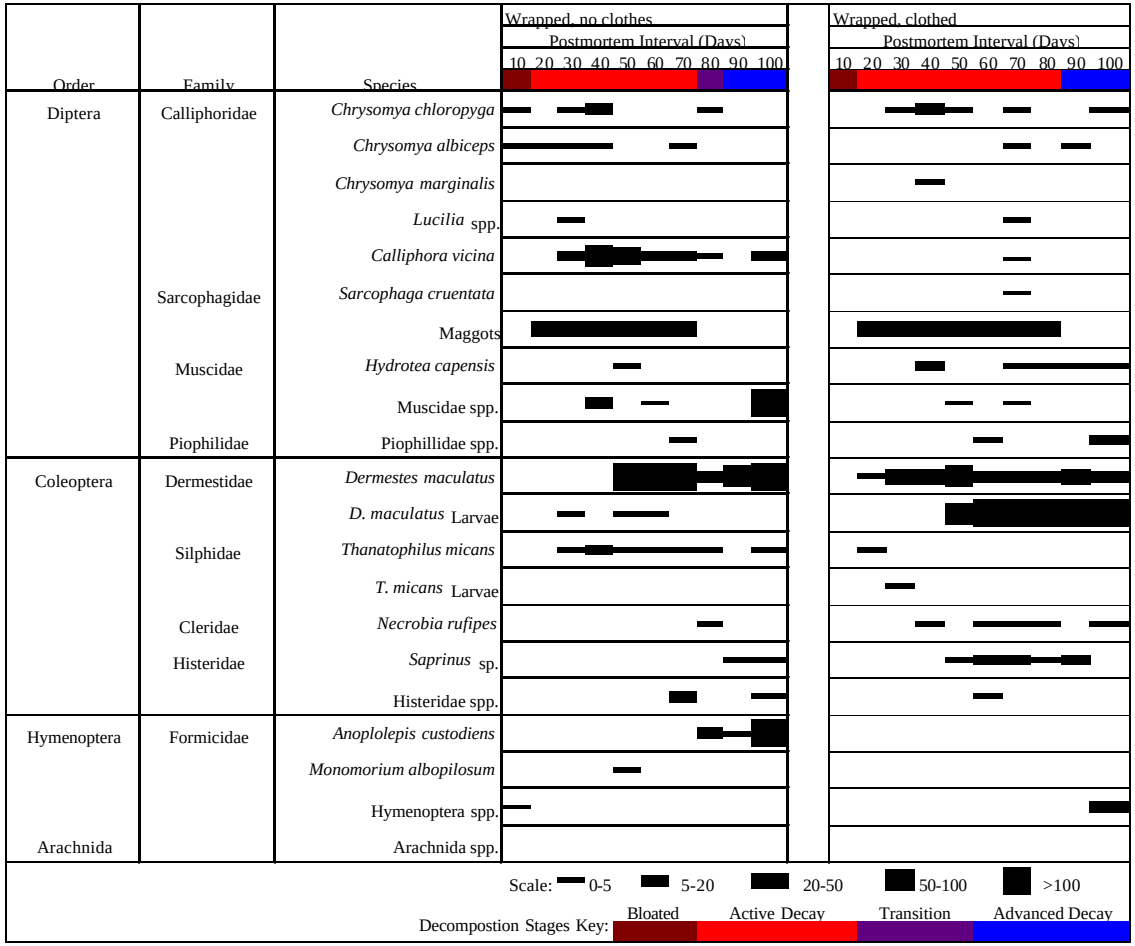


Fig. 3.2.9. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses, sampled every ten days during the winter wrapped trial. Maggots – all species.

The average daily temperatures given for the second trial in the northern region were a maximum 27.4 ± 3.2 ^{0}C and a minimum of 11.4 ± 2.5 ^{0}C (Ellison 1989). The central region, where the current trial was conducted, had a colder average daily maximum of 19.8 ± 4.3^{0}C and a colder average daily minimum of 2.1 ± 4.5^{0}C. This would rather suggest that the differences in species utilizing carrion was a direct consequence of temperature rather than different environmental or geographic factors. This is supported by the fact that *C. vicina* has been documented as seasonally driven elsewhere (Oliva 2001).

Viable Diptera eggs were deposited on the control and clothed control carcasses for the first time on day 5. On the control carcass, eggs were seen early on day 3, however, the egg mass was not viable and did not hatch. Oviposition occurred later on the wrapped carcasses, from day 11 onwards and continued up to day 55 (Fig. 3.2.10.). A study in Hawaii, U.S.A. (Goff 1992) showed a 2.5 day delay in oviposition of *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifaces* (Macquart) on a wrapped carcass in December (winter) with an average temperature given as 20.5^{0}C to 23.8 ^{0}C.

The delay in oviposition or larviposition on the wrapped carcasses during the current trial could be a consequence of the relatively low number of adult Calliphoridae and Sarcophagidae present during the initial decomposition of the carcasses (Figs 3.2.4. to 3.2.9.). The gravid females may have selected the exposed and more easily accessible carcasses first, thus moving to the wrapped carcasses only when the primary oviposition sites were utilized. However, the females are often attracted to the same oviposition sites on a carcass (Greenberg 1991). This suggested that competition for these oviposition sites was very low. In situations where there is more competition, the females may more actively seek out concealed carcasses. Another factor may be that the Diptera utilise decomposition odour to find suitable substrates (Fuller 1934, Marchenko 2001). It is also possible that the wrapping resulted in a slower release of these odours, which would have otherwise attracted them. However, the wrapping was cotton sheeting and should not have restricted the odours.

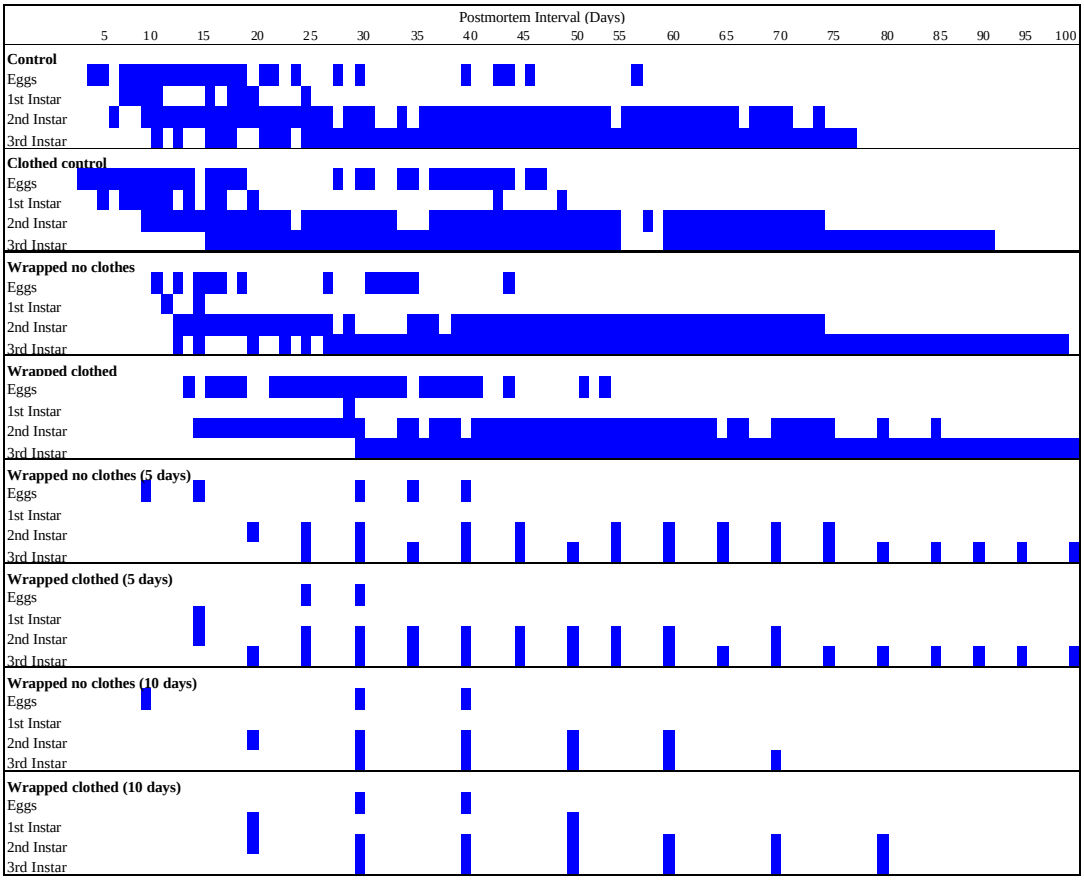


Fig. 3.2.10. Eggs and maggot succession for all carcasses during the winter wrapped trial.

The presence of clothes did not affect the oviposition site. A point to note is that one of the species present in this trial was *C. vicina,* which has been documented to oviposit during the night (Singh & Bharti 2001), however, the low temperatures recorded at night during the current trial may have prohibited this. Further research should be done to determine if these Diptera oviposit during the night in South Africa. The maggots on the control carcass were the first to migrate (day 75 to 77), followed by the clothed (12 days later) and the wrapped carcases no clothes 7 days after that (day 98). The maggots on the other carcasses only began to migrate sometime after the trial was terminated (Fig. 3.2.10.).

Because the carcasses were still acceptable to adult Diptera for oviposition for such an extended period, it resulted in maggots of different age groups of the same species being present on the carcasses at any time. The succession of the Diptera species was

not always clear. The co-existence and persistence of the species suggested that there was no intra- nor inter-specific competition.

3.2.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices (See 2.4.1.). The mean faunal similarity was calculated for each day. These were plotted and the grand mean of the between mean faunal similarity was calculated and is represented as $\overline{S}_{gmean}$. The $\overline{S}_{gmean}$ values for this trial ranged from 0.333 to 0.442 with the control and the clothed control carcasses with values between 0.421 and 0.442 (Fig. 3.2.11.). All the carcasses showed an overall increase in the beginning of the trials. The trend tended to level off for the remainder of the trial (fluctuating around the $\overline{S}_{gmean}$ values) (Fig. 3.2.11.). The fluctuations between the samples may be a result of the species not always being recorded in successive days due to the low numbers of individuals present at the carcasses.

When Schoenly (1992) compared a variety of studies, he found the $\overline{S}_{gmean}$ values ranged from 0.218 to 0.808. As with the autumn trials the $\overline{S}_{gmean}$ values obtained from the current study were more consistent with a smaller range. Therefore, the result obtained in the current study may be a good indication as to the species similarity found on the carcasses.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

There were significant differences in the similarity matrices between all the carcasses that were sampled on a daily basis. There were, however, no significant differences between the carcasses that were sampled every five days and the relevant data from the other carcasses. This suggested that the sample size may have been too small for an accurate comparison (Table 3.2.3.). The significant differences found in the carcasses (all samples) were expected due to the differences in oviposition, together with the low numbers of individuals present. Although there was no change in species richness between the carcasses, the species were often recorded at different times. This was probably due to the cryptic nature and daily movement of the individual arthropods and low numbers recorded on each carcass.

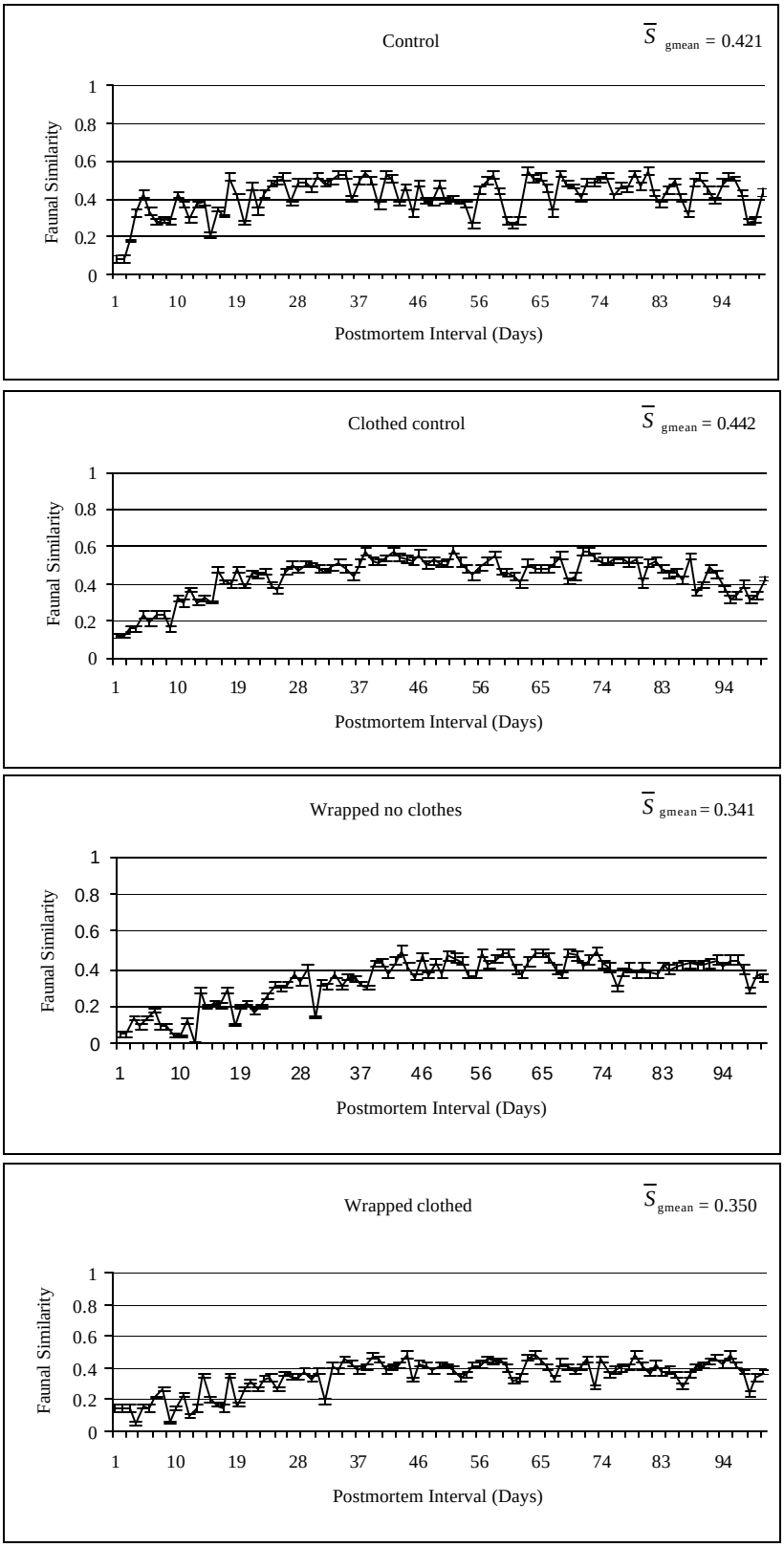


Fig. 3.2.11. Plots of mean pairwise similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors) during the winter wrapped trial.

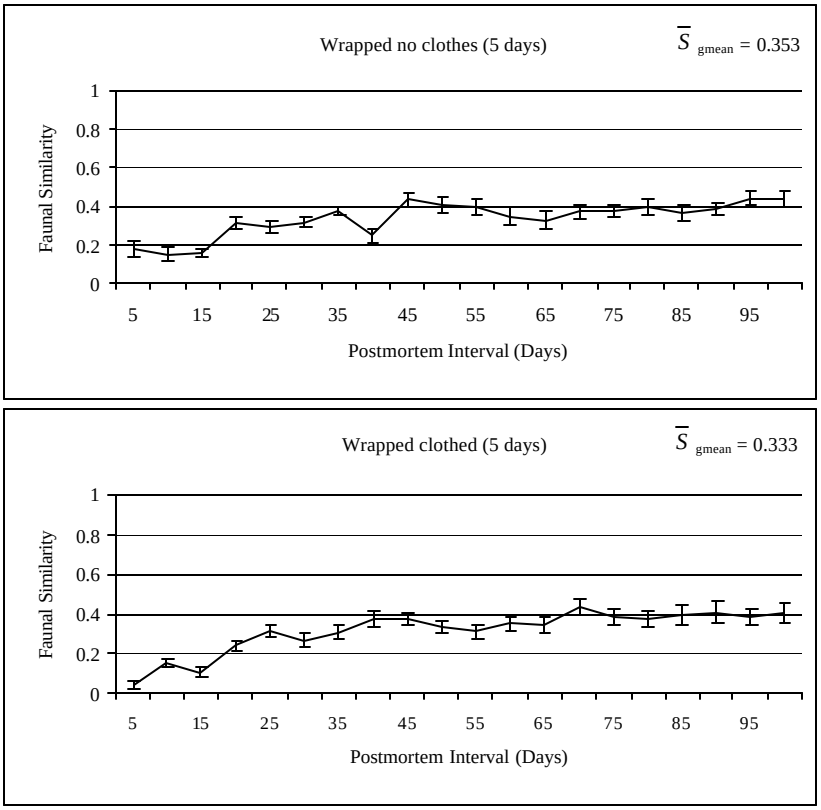


Fig. 3.2.11. Cont. Plots of mean pairwise similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors) during winter wrapped trial.

Table 3.2.3. Results from similarity matrices analysis, $K_{obs\ value}$ and p-values. (* - significant difference at 95% confidence interval)

Carcass (all samples)			
	Control	Clothed control	Wrapped no clothes
Control			
Clothed control	0.5712 (0.1180)*		
Wrapped no clothes	0.5114 (0.1030)*	0.6493 (0.0910)*	
Wrapped clothed	0.5488 (0.0810)*	0.6159 (0.0870)*	0.6637 (0.0730)*
Carcasses (sampled every 5 days) – versus each other and relevant data from other carcass with all samples)			
	Wrapped no clothes (5 days)	Wrapped clothed (5 days)	
Control	0.5957 (0.0130)	0.6243 (0.0100)	
Clothed control	0.6937 (0.0120)	0.7532 (0.0080)	
Wrapped no clothes	0.6920 (0.0440)	0.8089 (0.0050)	
Wrapped clothed	0.7258 (0.0120)	0.7865 (0)	
Wrapped clothed (5 days)	0.7628 (0.0060)		

3.2.4. Ambient, external and internal carcass temperatures

As mentioned (see 2.2.2.) this trial only had two data loggers that recorded viable results. These were placed at the wrapped clothed carcass and the wrapped clothed carcass sampled every five days. The data from the probes measuring the ambient temperature in the field were averaged to eliminate some of the variance in the data recorded. These data (field ambient temperature) were then compared to the internal and external temperatures of the carcasses as well as data from the South African Weather Services (SAWS), recorded at the Bloemfontein city and airport stations. The temperatures provided by the SAWS for the duration of the trial are slightly lower than the temperatures recorded at the study site by the data loggers (Fig. 3.2.12.). Thus, for the comparison of the internal and external maximum temperatures of the carcasses, the temperatures recorded in the field were used, as they are in an acceptable range (Fig. 3.2.12.).

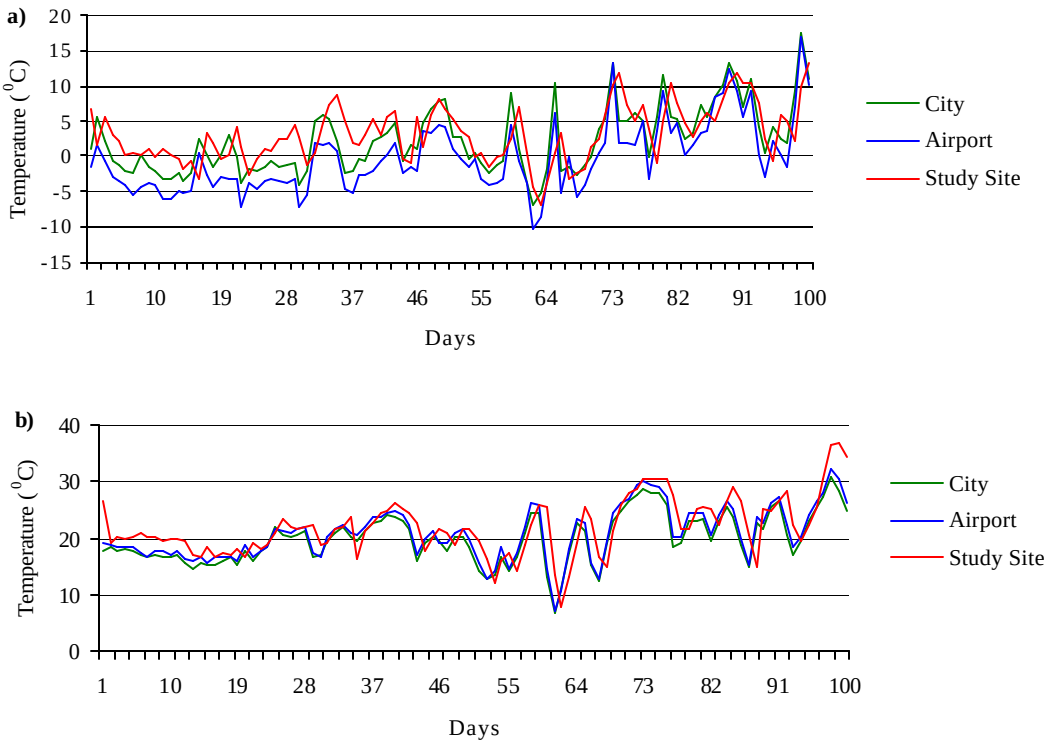


Fig. 3.2.12. Minimum (a) and maximum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the winter wrapped trial.

The internal temperatures of the wrapped and clothed carcass (Fig. 3.2.13.) closely followed the trends of the ambient temperature. The maximum internal temperatures were slightly lower than the ambient temperatures for the first 80 days. After that the internal temperatures were almost identical to the ambient temperature (Fig. 3.2.13a). The minimum internal temperatures were consistently slightly higher than the ambient temperatures. This was due to the presence of the maggot masses and remaining carcass material. The thorax minimum temperature was the most stable and the highest of the internal minimum temperatures (Fig. 3.2.13b). These trends were expected as the carcass tissues, would not cool or heat as quickly as the surrounding ambient air temperature.

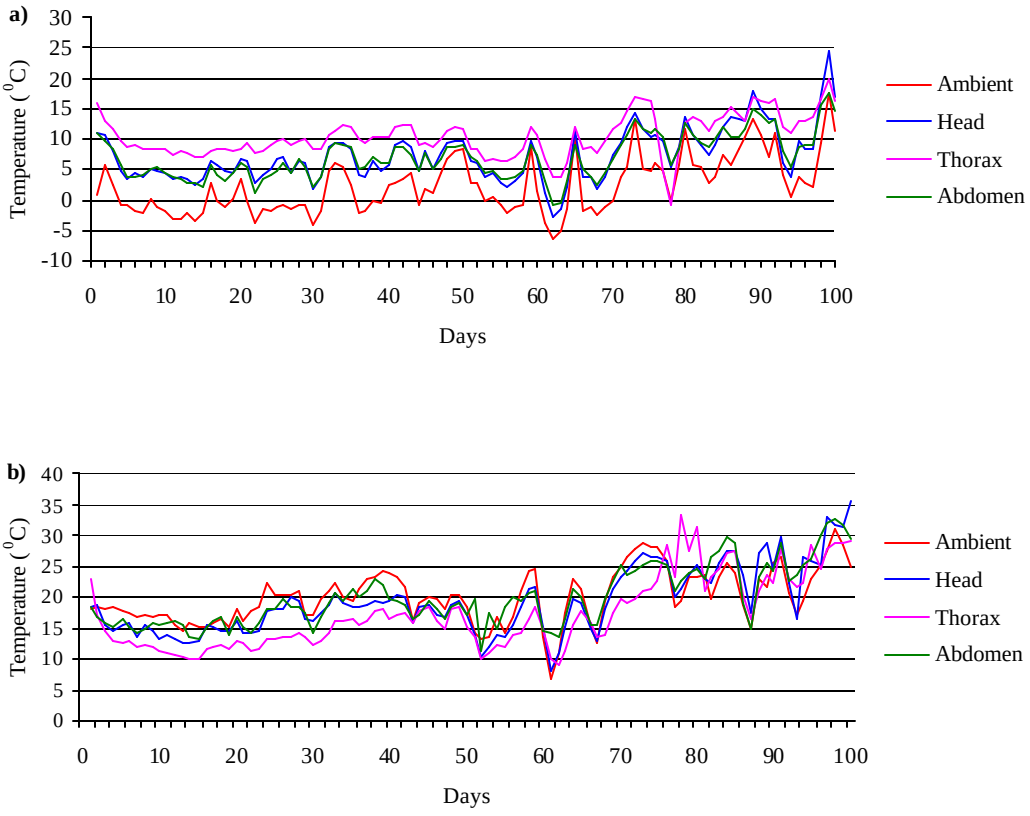


Fig. 3.2.13. Minimum (a) and maximum (b) daily ambient, internal and external temperatures for the wrapped and clothed carcass.

The wrapped clothed carcass sampled every five days did not show the trends as clearly as the wrapped clothed carcass. The internal minimum temperatures fluctuated

around the ambient temperatures (Fig. 3.2.14a). The minimum internal temperatures, however, were more consistently slightly higher than the ambient temperatures (Fig. 3.2.14b).

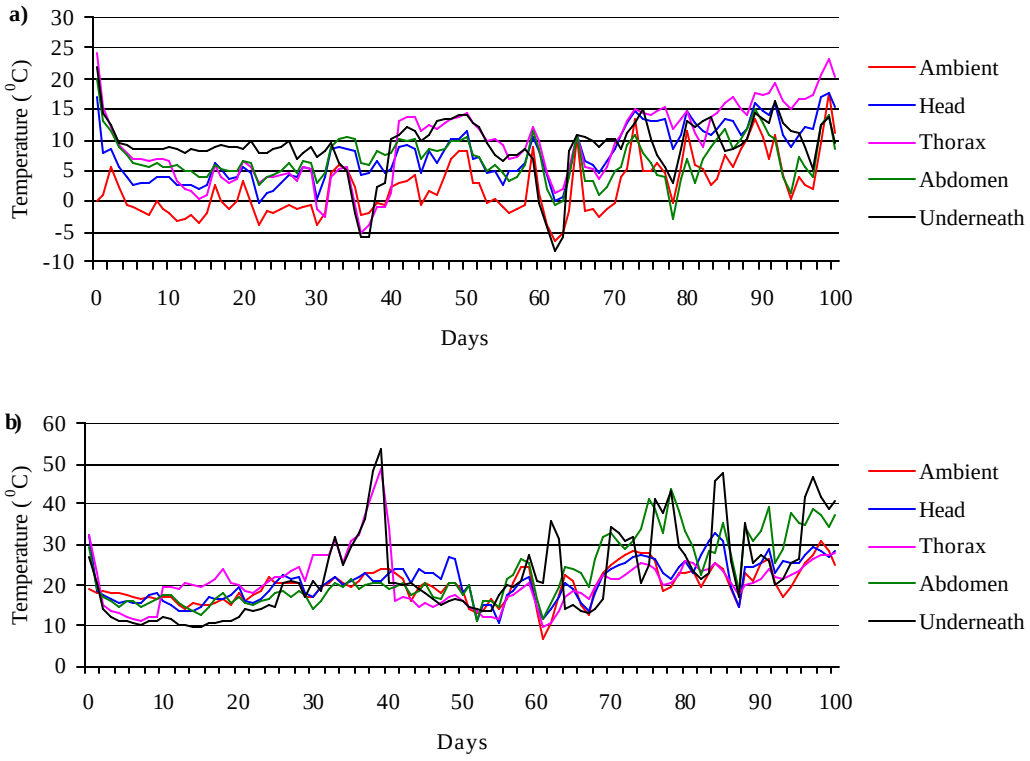


Fig. 3.2.14. Minimum (a) and maximum (b) daily ambient, internal and external temperatures for the wrapped and clothed carcass (5 days)

It is well known that temperature influences insect development (Cloudsley-Thompson 1953, Nayar *et al.* 1976, Beck 1983, Higley & Haskell 2001). Calliphoridae development is slowed down at lower temperatures, with almost no growth occurring at very low temperatures (below 10 ^{0}C) (Davies & Ratcliff 1994, Ames & Turner 2003). Thus, the prolonged presence of the maggots was probably due to these lower temperatures and certainly not to a lack of resources. *Calliphora vicina* has been reported to be more cold tolerant and this was supported during the winter trial (1-3 ^{0}C halts development, but resumes from 10-26^{0}C) (Mariluis & Schnack 1989, Davies & Ratcliff 1994, Anderson 2000). The presence of clothing and

wrapping may have helped to stabilise the internal temperatures of the carcasses with the thoracic temperatures showing only a slight variance.

3.2.5. Mass loss

The mass loss for all the carcasses was similar during the first 30 days of the trial. After this the control and clothed control carcasses began to lose mass slightly faster than the wrapped carcasses. The wrapped carcasses at the end of the trial still had between 50 and 60% mass remaining, while the control and clothed control carcasses had between 45 and 35% mass remaining (Fig. 3.2.15.).

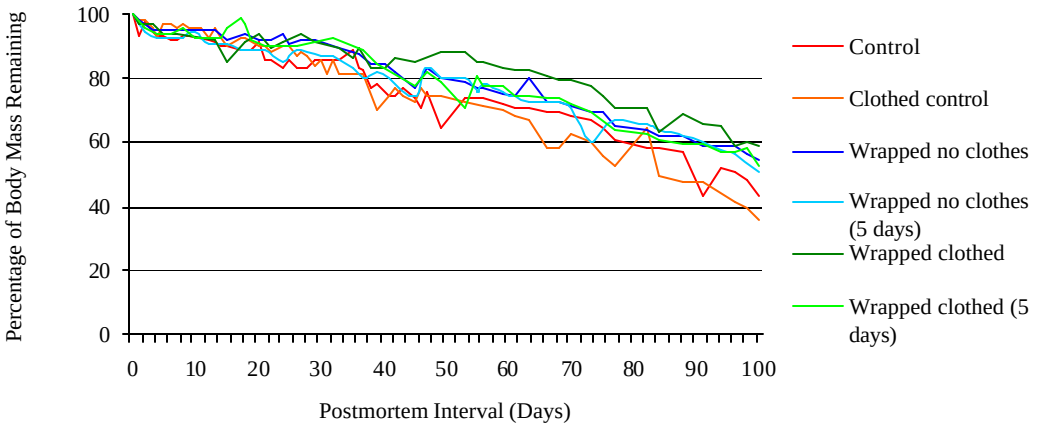


Fig. 3.2.15. Percentage body mass remaining for the carcasses during the winter wrapped trial.

The carcasses in the ten-day sample group, were only weighed at the beginning of the trial and again at termination. Their remaining end mass was lower than the other wrapped carcasses, at 27 and 34% for the no clothes and the clothed carcasses respectively. In the cooler months the carcass tissues decomposed and dried out very slowly and thus the mass loss was also limited. The control and clothed control carcasses lost more mass overall, due to the drying out of the limbs and the head.

3.2.6. Maggot mass distribution

The oviposition sites were consistent, regardless of clothing or wrapping. The primary site for oviposition was the mouth, followed by the nostrils and pinna of the ears, then the eyes and lastly in the folds of the sheets or clothes, under the thorax along the

ground. The maggot masses were restricted to the head and then between the fore and hind legs. The maggot masses were small in size, never exceeding more than a few hundred individuals. It is suggested that these lower number may be species related. Neither clothing nor wrapping affected maggot mass distributions or size. Maggot masses did not cause any displacement of clothing during this trial. This was due to limited bloating and small maggot masses. Therefore, displacement of clothes in criminal investigations under these conditions and in the absence of vertebrate scavengers, may suggest sexual assault, rather than insect activity (Komar & Beatie 1998).

SECTION 3.3.

The influence of wrapping and clothing on carcass decomposition and arthropod succession: a spring study.

3.3.1. Decomposition of the carcasses

The same set of criteria that were used in the wrapped autumn trial were used for this trial (Table 3.3.1.).

i) Fresh

The carcasses where placed out at approximately 13:00h on 30th September 2003 (day 0). The carcasses remained in the fresh stage only until the middle of day 1, which is shorter than reported elsewhere (Anderson & VanLaerhoven 1996, Richards & Goff 1997), but similar to Payne (1965).

ii) Bloat

By the afternoon sampling on day 1, all the carcasses had begun to bloat (Fig. 3.3.1.). As with the wrapped autumn trial, no discernable change in body colour was detected, because the carcasses were predominantly black. The carcasses bloated quickly and by day 3, their limbs had collapsed back into the resting position. Only the control carcass had skin split and the gut distended (day 2). This occurred through the anus and the split was on the lower abdomen near the ground. There was no delay in oviposition by the Diptera and as a result all the carcasses entered the active decay stage simultaneously. The bloat stage lasted only two days in spring, one day shorter than in the autumn wrapped trial.

iii) Active decay

Diptera maggots penetrated all the carcasses through the natural openings and began feeding by day 2. The active decay process and release of decomposition fluids, followed very closely to the description given in the wrapped autumn trial (see 3.1.1). As recorded in the autumn wrapped trial, the active decay stage was the longest for the wrapped carcases, up to day 17, while the control and clothed control carcasses began to dry out after day 11 (Fig. 3.3.1.). These observations were also of a similar time frame to those recorded during the autumn wrapped trial, where active decay

Table 3.3.1. Summary of the decomposition stages, physical characteristics of each stage and the dominant arthropods present during the autumn wrapped trial.

FRESH (Fig. 3.1.1.) • Commenced when the animal was killed • The torso (thorax and abdomen) was soft and the limbs were flexible • No odour associated with the carcasses • Very short duration	**Diptera** Calliphoridae: *Chrysomya chloropyga* (adults) *Chrysomya albiceps* (adults)
BLOAT (Fig. 3.1.1.) • Commences when the torso began to harden and the abdomen in particular becomes inflated, due to the build up of gasses • Bubbles of blood formed at the nose, creating small puddles or saturating the sheet on the wrapped carcass • Carcass appears 'balloon'-like • Body colour changed • Oviposition took place during this stage	**Diptera** Calliphoridae: *Chrysomya chloropyga* (adults) *Chrysomya albiceps* (adults) *Lucilia* spp. (adults) Sarcophagidae: *Sarcophaga cruentata* (adults) Muscidae: *Hydrotea capensis* (adults) Muscidae spp.
ACTIVE DECAY (Fig. 3.1.1.) • Carcass deflated, as the maggots actively fed on the carcass tissues and allowed the gasses to escape • Odour of decay was prominent • The limbs collapsed back into the 'resting' position, and in some cases the clothing had moved • The skin began to peel allowing maggots to feed underneath it • The carcass mass was halved during this stage • The Diptera maggots were the most dominant insects • Areas surrounding the maggots and the tissue on which the maggots were feeding became liquefied • Plenty of decomposition liquids were present	**Diptera** Calliphoridae: *Chrysomya chloropyga* (adults and maggots) *Chrysomya albiceps* (adults and maggots) *Lucilia* spp. (adults) *Sarcophagidae:* *Sarcophaga cruentata* (adults and maggots) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults) Cleridae: *Necrobia rufipes* (adults)
ADVANCED DECAY • There was little tissue remaining on the carcass • Fewer odours were associated with the carcass • Puddles of decomposition fluids were common along the backs and upper surfaces of the carcasses • The carcass was still moist but often the head was dried out. Bones of the skull, ribs and legs were often visible • The majority of the maggots moved off the carcass to pupate (i.e. less than 20 individuals remaining) • Coleoptera became the most dominant group	**Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)

lasted until day 15 and 19 for the wrapped carcasses and until day 12 for the control carcasses (Fig. 3.1.1.). The duration of active decay was significantly shorter on the control and clothed control carcasses. This may be due to more eggs being laid on these carcasses. As a result, more tissue was consumed by the Diptera maggots, forcing them to migrate sooner as their food resource was depleted and the carcasses dried out.

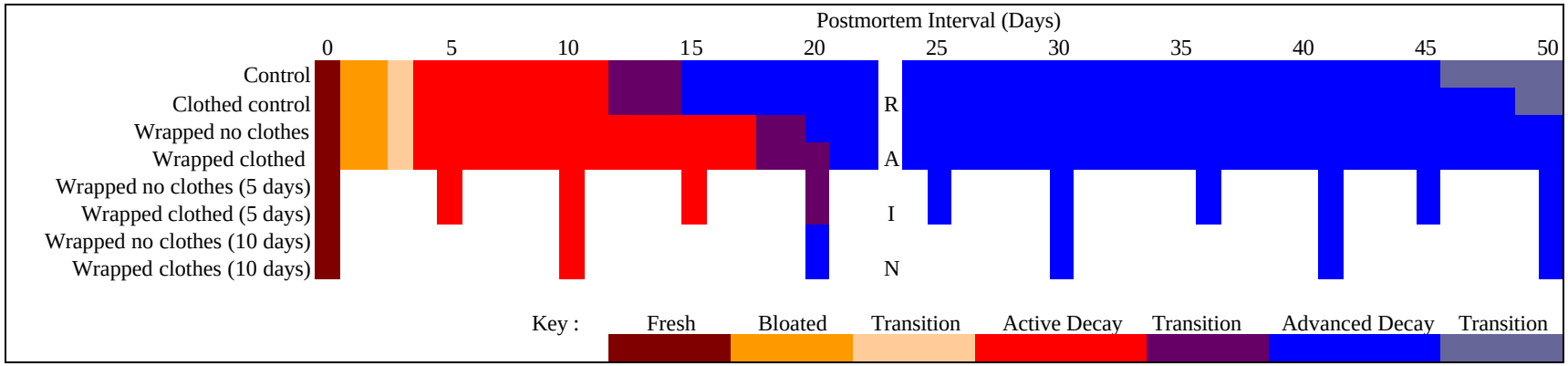


Fig. 3.3.1. Decomposition stages of the carcasses in the spring trial.

iii) Advanced decay

The control and clothed control carcasses entered the advanced decay stage simultaneously (day 14). The wrapped carcasses, however, entered the advanced stage approximately six days later. This stage was longer for the wrapped carcasses (Fig. 3.3.1), due to the sheet retaining moisture. As a consequence of the lower temperatures, the carcasses did not dry out as rapidly as they may have in higher temperatures that occurred for example in the autumn trial (Fig. 3.3.1.).

iv) Dry remains

Both the control and the clothed control carcasses were classified as being in the transitional stage before the dry remains stage after day 45. The wrapped carcasses remained in the advanced decay stage for the remainder of the trial (Fig. 3.3.1.).

3.3.2. Arthropod succession on the carcasses

The arthropod succession on all carcasses followed the same pattern as seen in the autumn trial. The adult Diptera detected the carcasses first. These were the Muscidae and the Calliphoridae. By the morning of day 1, between five and 20 individuals of *C. chloropyga* (Fig. 3.3.2.) were recorded on control (Fig. 3.3.3) and wrapped clothed carcasses (Fig. 3.3.6.). Between 50 and 100 individuals were recorded on the wrapped no clothes carcass (Fig. 3.3.5.) and over 100 individuals were recorded on the clothed control (Fig. 3.3.4.). By day 2, the number of *C. chloropyga* had increased to over 100 on the control (Fig. 3.3.3.) and wrapped no clothes carcasses (Fig. 3.3.5.). These number remained high even after oviposition ceased on day 3. Their numbers began to decrease after day 5, when less than five individuals were recorded on the clothed control (Fig. 3.3.4.) by the afternoon. By the morning of day 6 on the wrapped no clothes (Fig. 3.3.5) carcass, there were less than five individuals recorded. Less than five individuals were recorded by the afternoon of day 6 and morning of day 7, on the wrapped clothed carcass (Fig. 3.3.6.) and control (Fig. 3.3.3.) carcasses respectively. Single individuals of *C. chloropyga* were recorded on the carcasses sporadically during the remainder of the trial.

Chrysomya albiceps (Fig. 3.3.2.) were often recorded with *C. chloropyga*. However, their numbers were only between one and 20 individuals. Single *Lucilia* spp., *C. vicina* and *S. cruentata* (Fig. 3.3.2.) individuals were recorded sporadically throughout the trial. Groups of between three and five individuals of *H. capensis* were also recorded sporadically on the carcasses. Muscidae spp. were recorded on the carcasses. During the first part of the trial, their numbers were often between five and 20, sometimes increasing to 50 individuals (Figs 3.3.3. to 3.3.8.).

Diptera

Chrysomya chloropyga

Chrysomya albiceps

Lucilia spp.

Sarcophaga cruentata

Coleoptera

Dermestes maculatus

Necrobia rufipes

Fig. 3.3.2. Most common forensically important species occurring in spring wrapped trial.

The Coleoptera community was dominated by *D. maculatus* (Fig. 3.3.2.), which appeared to be present throughout the year so far. *Dermestes maculatus* adults occurred sporadically on the carcasses during the early part of the trial. They became established on the clothed control carcass from the morning of day 10 (Fig. 3.3.4.). By the afternoon of day 12 and morning of day 13 they were recorded on the wrapped carcasses no clothes (Fig. 3.3.5.) and clothed respectively (Fig. 3.3.6.). They were only recorded on control carcass from day 12 (Fig. 3.3.3.). They successfully bred on the carcasses from the end of the second week to the end of the trial. *D. maculatus* larvae were present first on the control and clothed control carcasses, by day 14 and 15 respectively (Fig. 3.3.3. and 3.3.4.). They were present from day 18 and 20 on the wrapped no clothes and wrapped clothed carcasses (Fig. 3.3.5. and 3.3.6.) respectively.

Necrobia rufipes (Fig. 3.3.2.) were consistently present from day 9 on the control, clothed control and the wrapped clothed carcasses (Figs 3.3.3., 3.3.4. and 3.3.6.). They were recorded on the wrapped no clothes (Fig. 3.3.5.) carcass from day 10. Their numbers increased as decomposition progressed, however, never more than 50 individuals were recorded on the carcasses.

A single adult *T. micans,* was recorded once on two carcasses, the control (Fig. 3.3.3.) and the wrapped no clothes (5 days) carcasses (Fig. 3.3.7.). Histeridae spp. were also sometimes present but were not commonly seen. The *Saprinus* sp., was also present but not in the same number or frequency as recorded in the wrapped winter trial (Figs 3.3.3. to 3.3.8.).

Formicidae were recorded on four carcasses. Less than five individuals of *M. albopilosum* were recorded on the control carcass on day 1 (Fig. 3.3.3.) and on the wrapped clothed carcass (10 days) (Fig. 3.3.8.). They were recorded in higher numbers and more frequently on the wrapped no clothes carcass (Fig. 3.3.5.). They did not appear to influence the other arthropods present on the carcass. However, they were observed predating on a few of the Diptera maggots. A few individuals of *A. custodiens* were recorded on day 30 on the wrapped no clothes carcass (Fig. 3.3.7.).

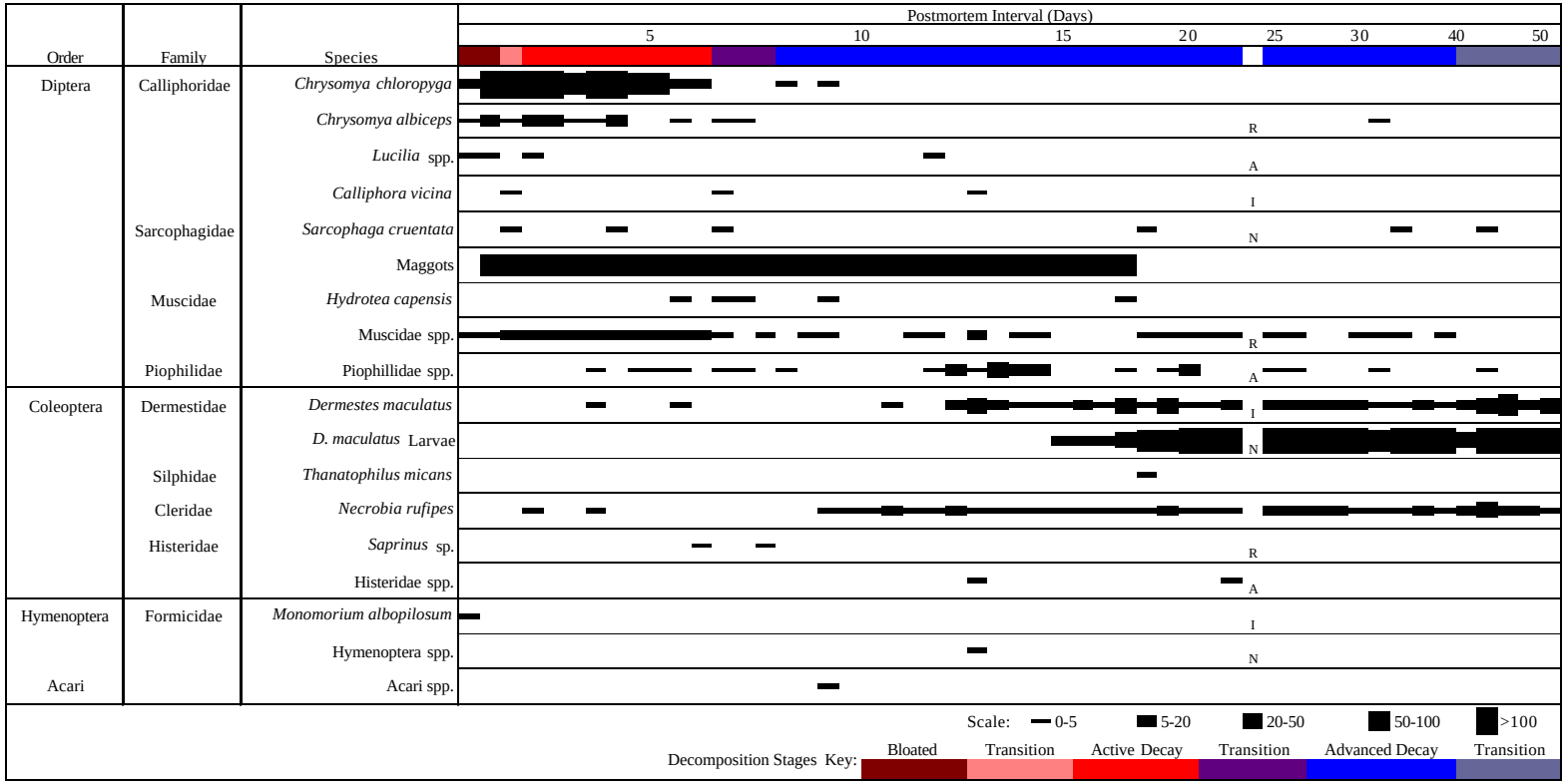


Fig. 3.3.3. Arthropod succession on the control carcass during the spring wrapped trial. Maggots – all species.

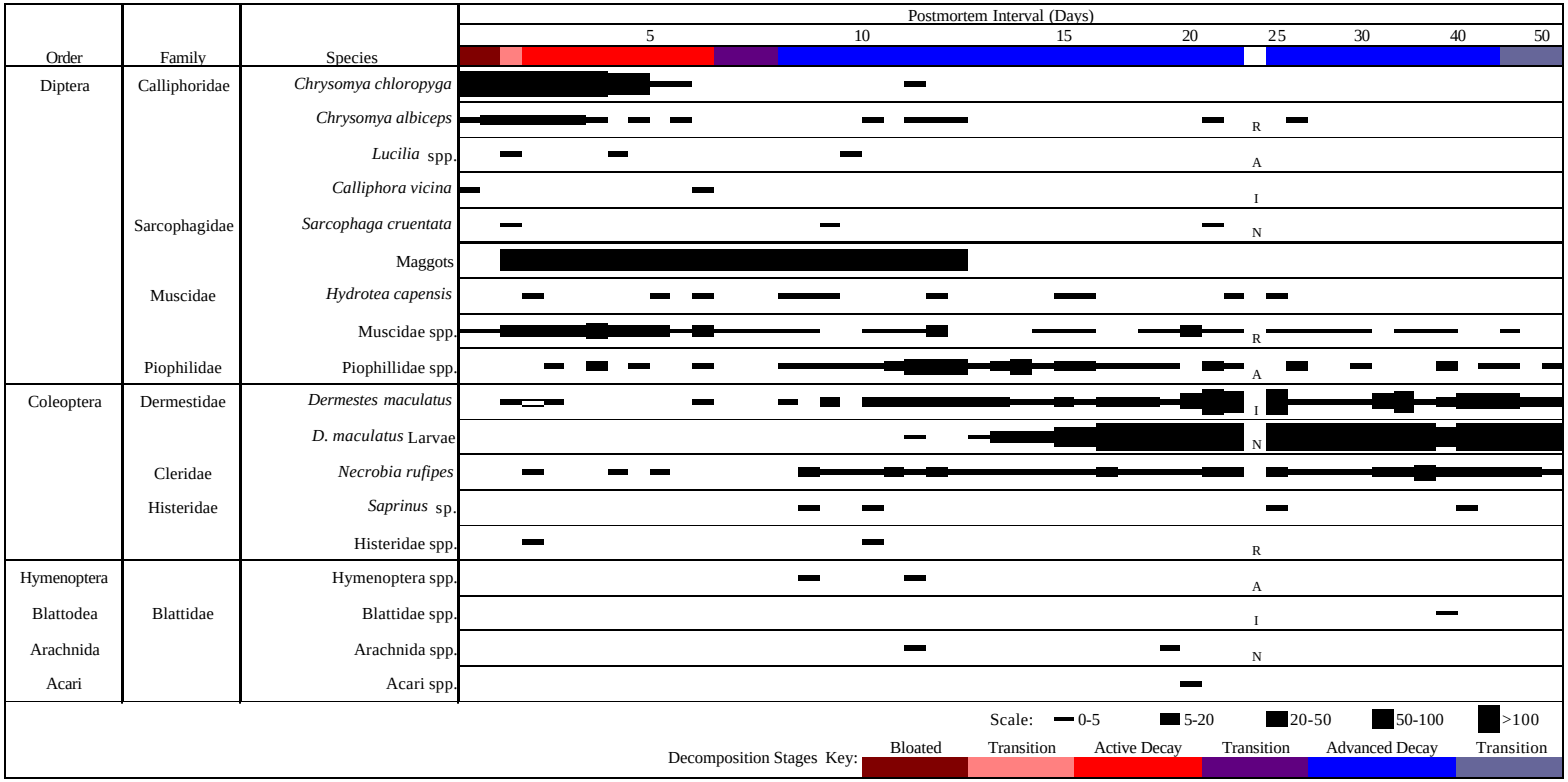


Fig. 3.3.4. Arthropod succession on the clothed control during the spring wrapped trial. Maggots – all species.

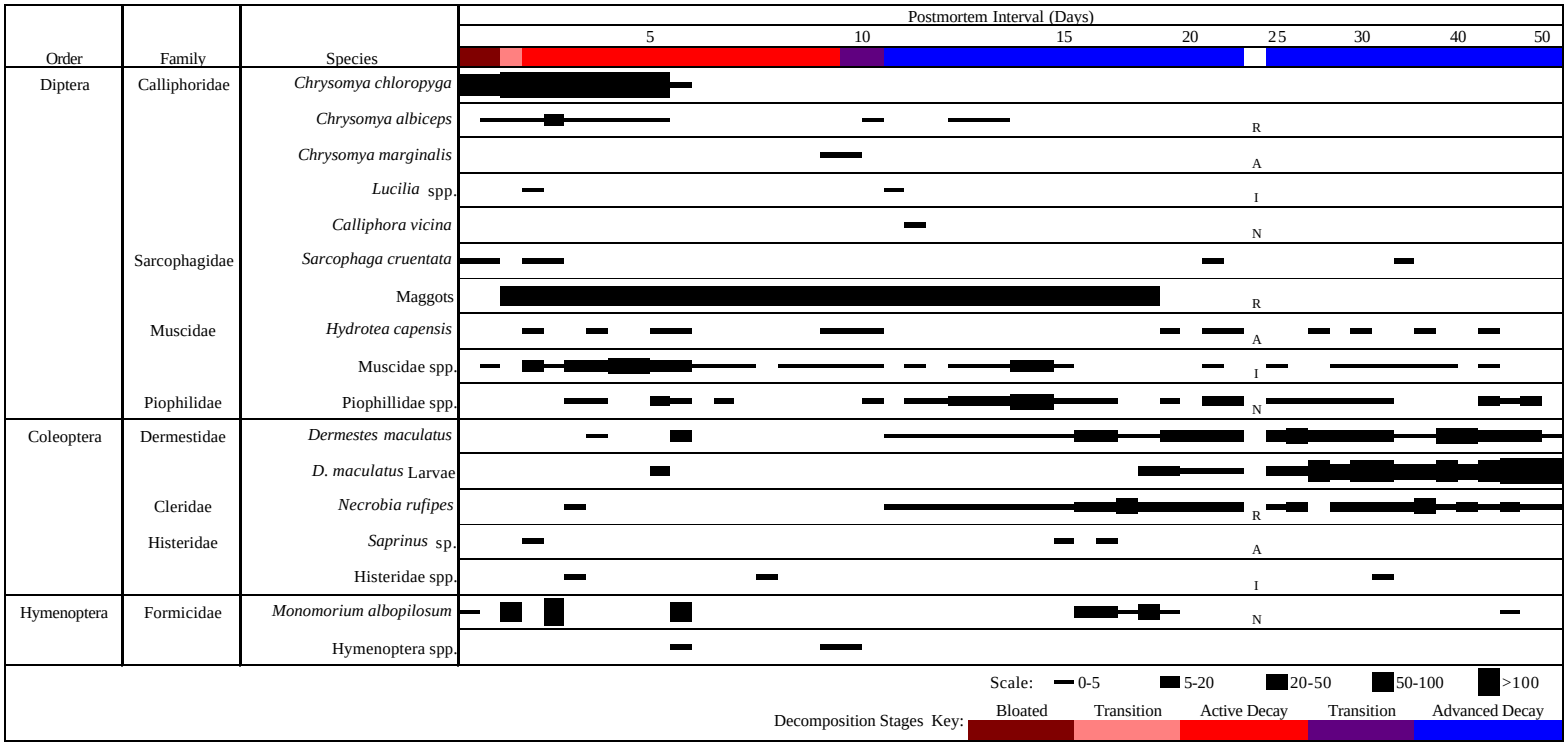


Fig. 3.3.5. Arthropod succession on the wrapped no clothes carcass during the spring wrapped trial. Maggots – all species

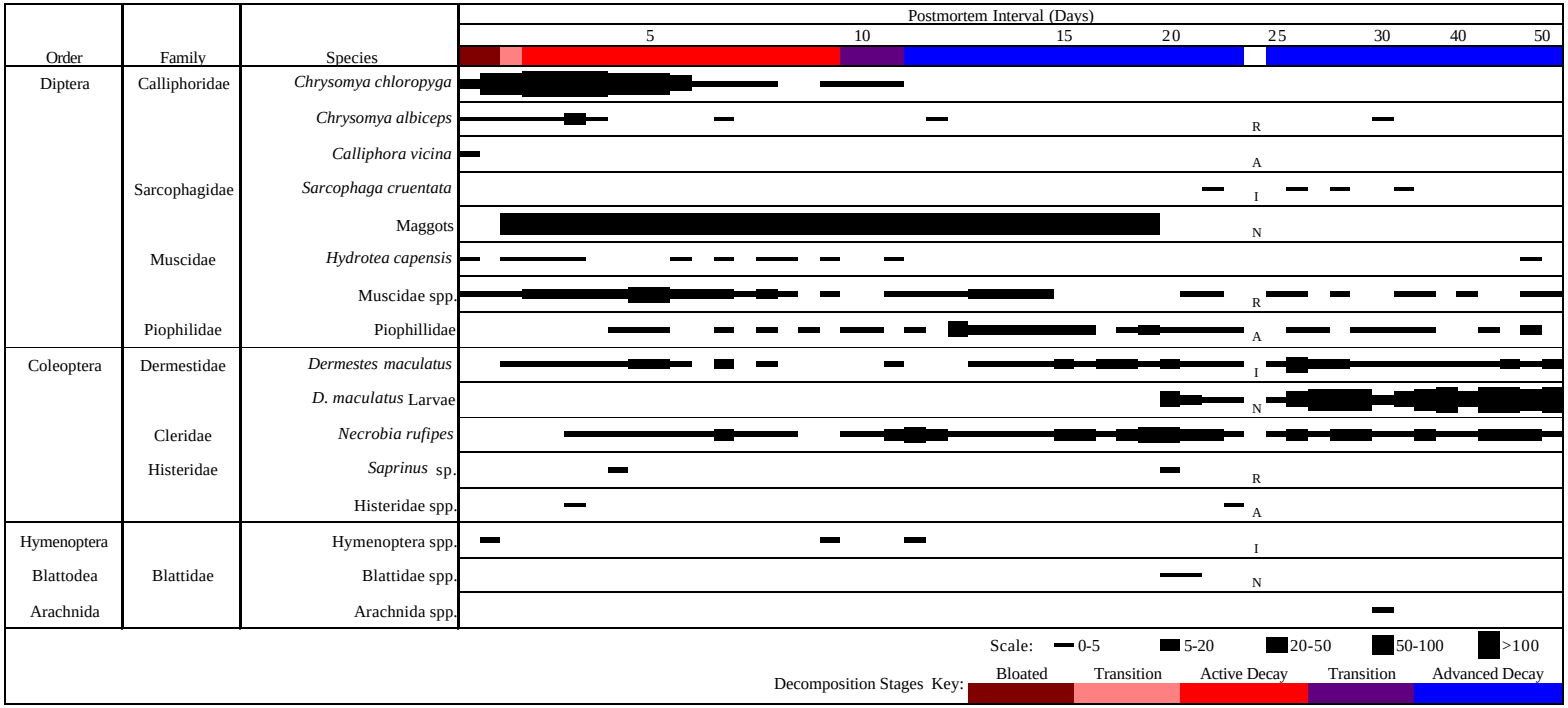


Fig. 3.3.6. Arthropod succession on the wrapped clothed carcass during the spring wrapped trial. Maggots – all species.

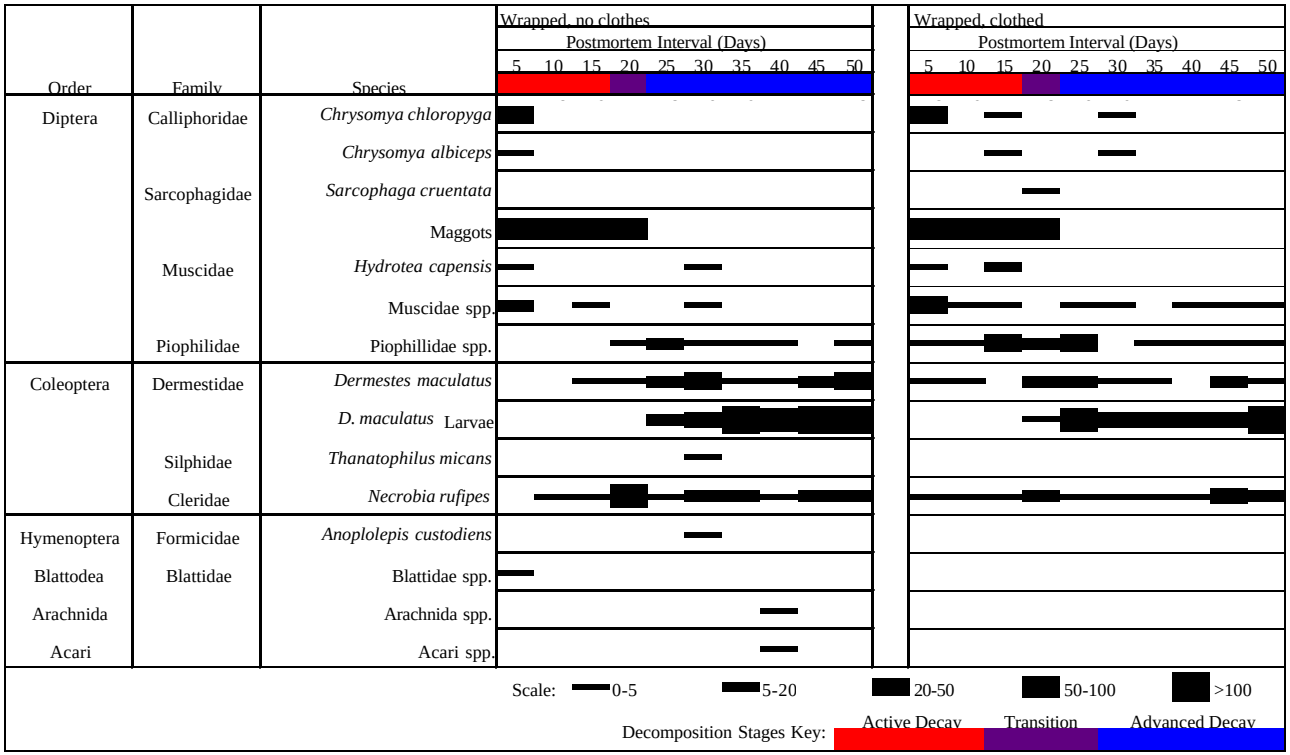


Fig. 3.3.7. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses (sampled every 5 days) during the spring wrapped trial. Maggots – all species.

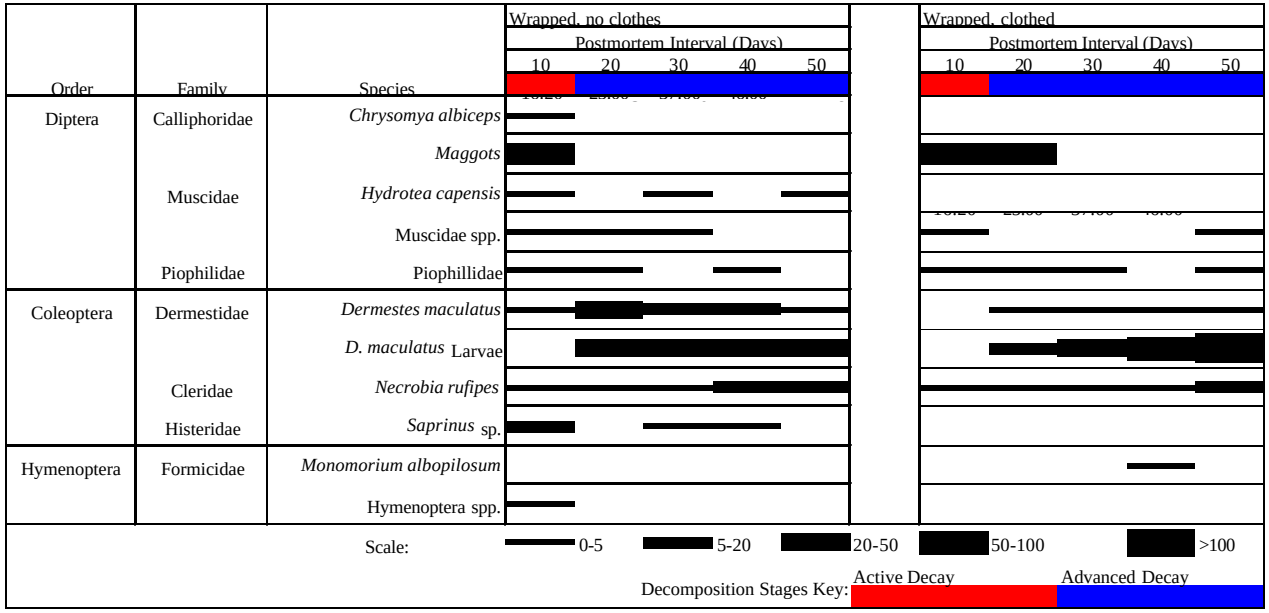


Fig. 3.3.8. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses (sampled every 10 days) during the spring wrapped trial. Maggots – all species.

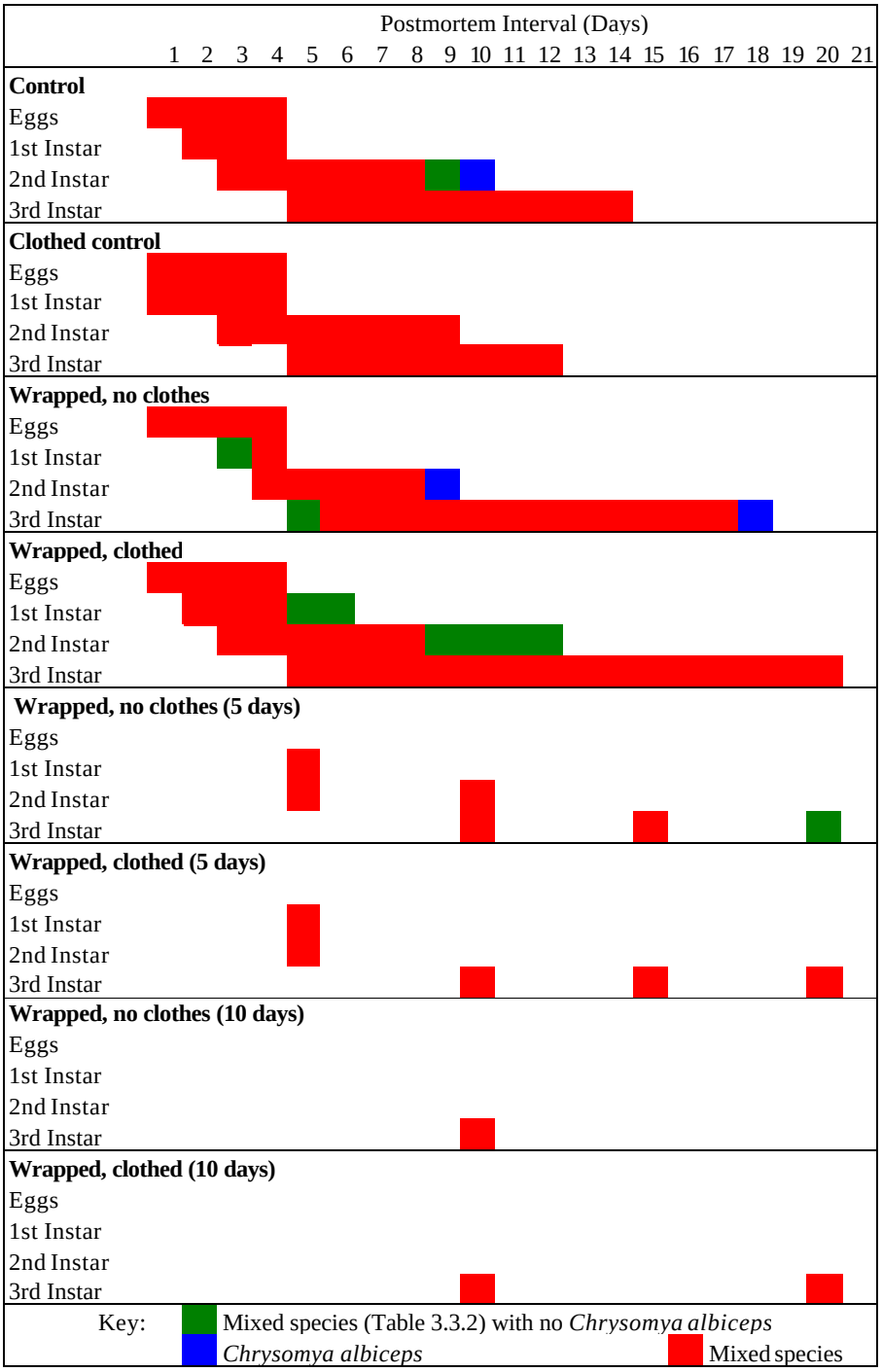


Fig. 3.3.9. Eggs and maggot succession for all carcasses during the spring wrapped trial.

The succession of insects at the family level, i.e. Diptera (Calliphoridae) and the Coleoptera (Dermestidae) was similar to references on insect succession on pig carcasses in other geographical regions (Payne 1965, Rodriques & Bass 1983,

Anderson & VanLaerhoven 1996) although the local species were different from those found in the northern hemisphere.

One of the most important results, in terms of a postmortem interval estimation, was the fact that oviposition by the Diptera occurred simultaneously on all the carcasses regardless of whether the carcasses were wrapped or not. The oviposition began on day 1. Similar to the autumn wrapped trial, the numbers of Calliphoridae were very high during the oviposition period. Competition might have resulted in the active pursuit of the carcasses. As a result all the carcasses in all the sample groups showed maggots of the same age and of the same species during the first 14 days (Fig. 3.3.9).

Table 3.3.2. Diptera species reared from maggot masses for each carcass during the wrapped spring trial.

	Postmortem Interval (Days)								
	1	3	5	7	10	13	15	17	20
Control									
Chrysomya chloropyga			X	X	X	X			
Chrysomya albiceps		X			X	X	X		
Sarcophaga cruentata			X						
Clothed control									
Chrysomya chloropyga		X	X	X	X				
Lucilia spp.					X				
Chrysomya albiceps			X	X	X				
Sarcophaga cruentata		X							
Wrapped, no clothes									
Chrysomya chloropyga		X	X	X	X	X	X		
Chrysomya marginalis.			X						
Chrysomya albiceps			X	X	X	X	X	X	
Sarcophaga cruentata			X	X					
Wrapped, clothed									
Chrysomya chloropyga		X	X	X	X	X	X		X
Lucilia spp.		X		X					
Chrysomya albiceps		X	X	X	X	X	X	X	X
Wrapped, no clothes (5 days)									
Chrysomya chloropyga			X		X		X		X
Lucilia spp.			X						
Chrysomya albiceps			X		X		X		
Sarcophaga cruentata					X				
Wrapped, clothed (5 days)									
Chrysomya chloropyga			X		X		X		X
Chrysomya marginalis			X						
Chrysomya albiceps			X		X		X		X
Wrapped, no clothes (10 days)									
Chrysomya chloropyga					X				
Lucilia spp.					X				
Chrysomya albiceps					X				
Wrapped, clothed (10 days)									
Chrysomya chloropyga					X				X
Chrysomya albiceps					X				X

The maggot masses were comprised mostly of *C. chloropyga*, with a smaller number of *C. albiceps*. *Lucilia* spp. and *S. cruentata* maggots were present in low numbers. Less than one percent of the samples consisted of *C. marginalis* maggots (Table 3.3.2.). The *C. albiceps* maggots during this season did not appear to have a longer developmental time than the other Calliphoridae species. There were cases where *C. albiceps* maggots were present in the preserved sample but not recorded in the corresponding sub-sample from which the adults were reared and *visa versa*. It is possible that *C. albiceps* may have a development time similar to *C. chloropyga*. The maggots remained on the wrapped carcasses (Fig. 3.3.9.) for longer than the control carcass. They were still present on the carcasses five days after the last maggots had migrated from the control carcasses.

3.3.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices and the mean faunal similarity was then calculated for each day (see 2.2.3.). These were plotted and the grand mean of all the mean faunal similarities were calculated and is represented as $\overline{S}_{gmean}$ (Fig. 3.3.10.).

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

There were significant differences in the similarity matrices between the control carcasses (control and clothed control) and the wrapped carcasses, with the exception of the wrapped carcass sampled every 5 days. This may be due to the low values and thus small matrices in the comparison (Table 3.3.2.). The results of the spring trial were similar to those obtained during the autumn trail for the carcasses (all samples). However, there were more significant differences between the carcasses sampled every 5 days in spring than in the autumn.

Statistically, according to the Jaccard metric species pairwise similarities, the graphs showed the characteristic horseshoe-shape as described by Schoenly (1992). When Schoenly (1992) compared a variety of studies, he found the $\overline{S}_{gmean}$ values ranged from 0.218 to 0.808. The $\overline{S}_{gmean}$ values obtained from the current study were more consistent and ranged between 0.335 and 0.490 (Fig. 3.3.10.). These results showed a

higher range than those recorded in the wrapped autumn trial. However, the range was still well within the range recorded by Schoenly (1992). Therefore the results were a good indication of species similarity on the carcasses.

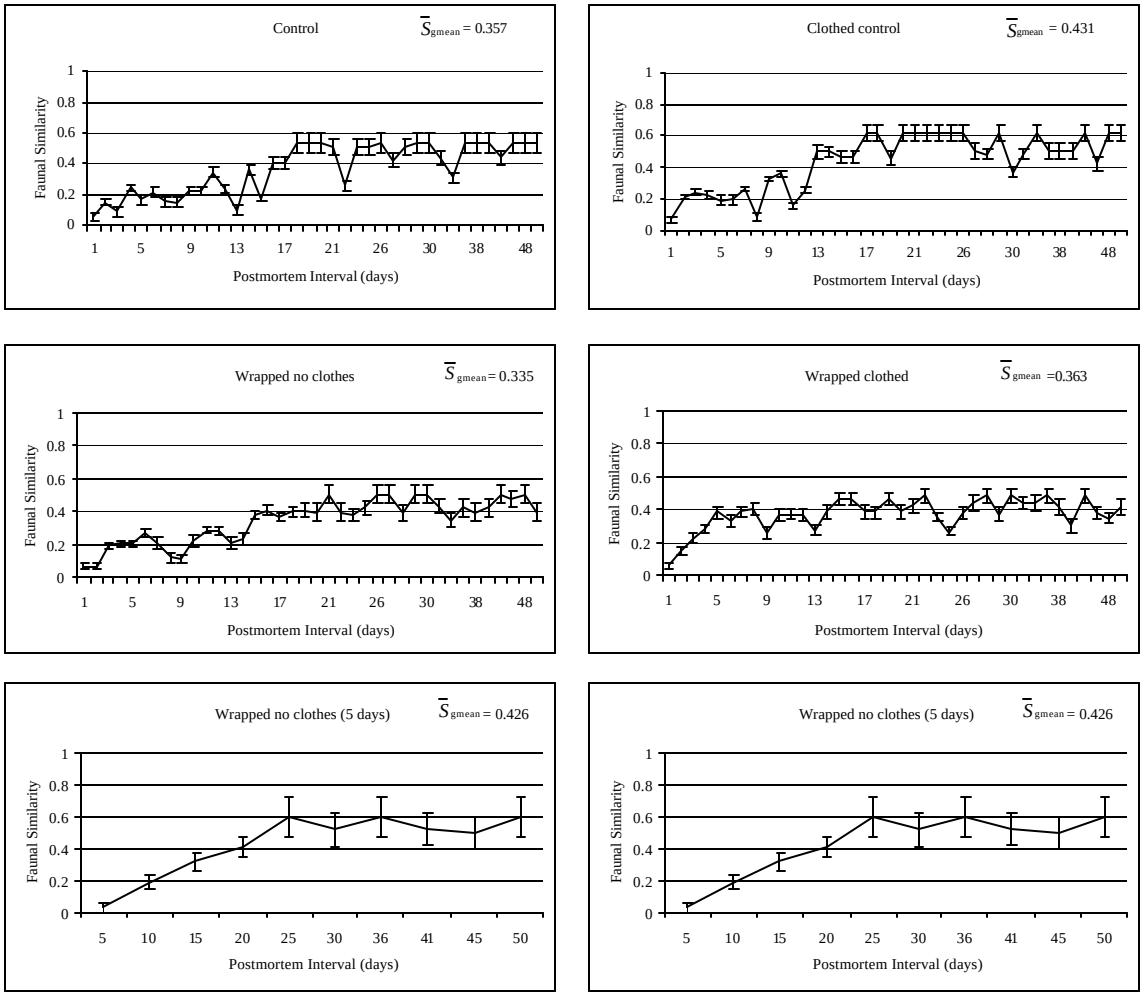


Fig. 3.3.10. Plots of mean pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors).

The Pearson's coefficient statistical analysis conduced on the carcass similarity matrices concluded that there were significant differences between all the carcasses. This was unexpected as the succession on the control carcasses (clothed contr ol and control) should have been similar to each other, just as the wrapped carcasses should have been similar to each other. The lack of similarity between all the carcasses may have been a result of the lower species diversity.

Table 3.2. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values. (* - significant difference at 95% confidence interval)

Carcass (all samples)			
	Control	Clothed control	Wrapped no clothes
Control			
Clothed control	0.7179 (0.0830)*		
Wrapped no clothes	0.8041 (0.0680)*	0.6906 (0.1200)*	
Wrapped clothed	0.5577 (0.1560)*	0.5008 (0.1710)*	0.7067 (0.0750)*

Carcasses (sampled every 5 days – versus each other and relevant data from other carcass with all samples)		
	Wrapped no clothes (5 days)	Wrapped clothed (5 days)
Control	0.8385 (0.2590)*	0.6656 (0.2130)*
Clothed control	0.8552 (0.0310)	0.7350 (0.0300)
Wrapped no clothes	0.8345 (0.1460)*	0.6561 (0.1550)*
Wrapped clothed	0.6982 (0.0830)*	0.6127 (0.1060)*
Wrapped clothed (5days)	0.8217 (0.0340)	

3.3.4. Ambient, external and internal carcass temperatures

The data loggers were repaired after the previous trial, however, during this trial only two data loggers recorded reliable results. The data from the probes measuring the ambient temperature in the field were averaged to eliminate some of the variance between the readings of the various probes. The study site ambient temperatures were compared to the internal and external temperatures of the carcasses as well as data from the South African Weather Services (SAWS) recorded at the Bloemfontein city and airport stations.

The minimum ambient temperatures varied more from each other, with the study site temperatures slightly higher than the SAWS data for the first 17 days. After that the study site minimum temperatures were between those recorded at the city and airport weather stations (Fig. 3.3.11a). These minimum ambient temperatures varied considerably during the trial (between 2 and 18^0C). The maximum temperatures provided by the SAWS for the duration of the trial were almost identical to the temperatures recorded at the study site. (Fig. 3.3.11b). These temperatures showed less variability than the minimum temperatures and mostly ranged between 22 and 32^0C. The internal temperatures of the carcass were higher than the ambient temperature most of the time (Figs 3.3.12. and 3.3.13.). The trends were similar for both carcasses. In both cases the maximum temperature recorded underneath carcasses were the highest. The head temperatures (both the maximum and minimum) were only slightly above the ambient temperature and followed the same pattern.

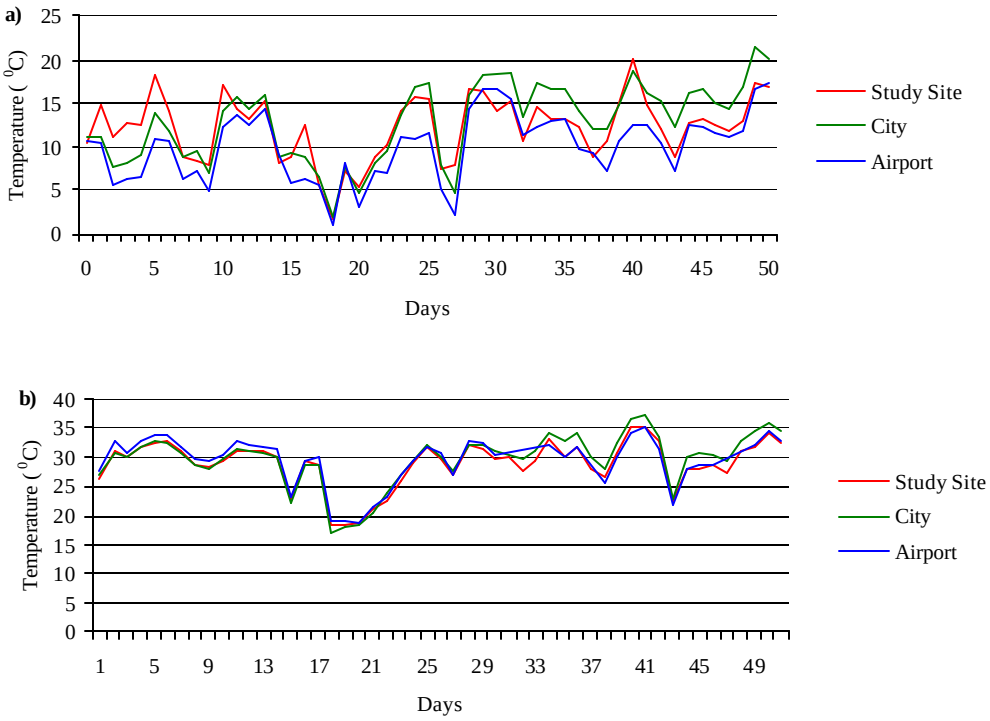


Fig. 3.3.11. Minimum (a) and maximum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the spring wrapped trial.

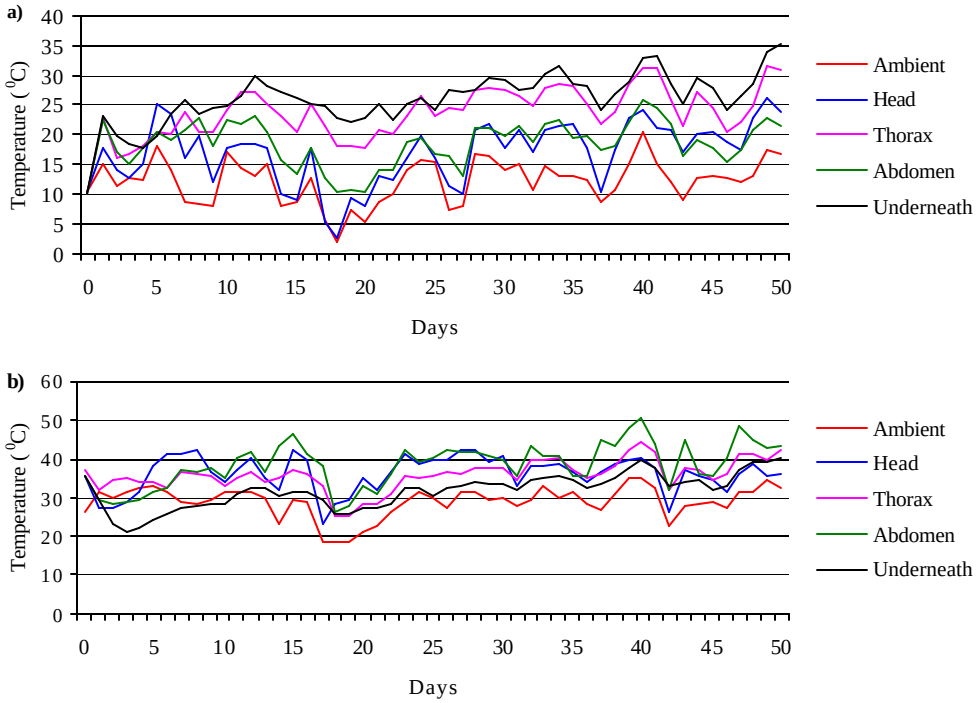


Fig. 3.3.12. Minimum (a) and maximum (b) ambient, internal and external temperatures for the wrapped and clothed carcass.

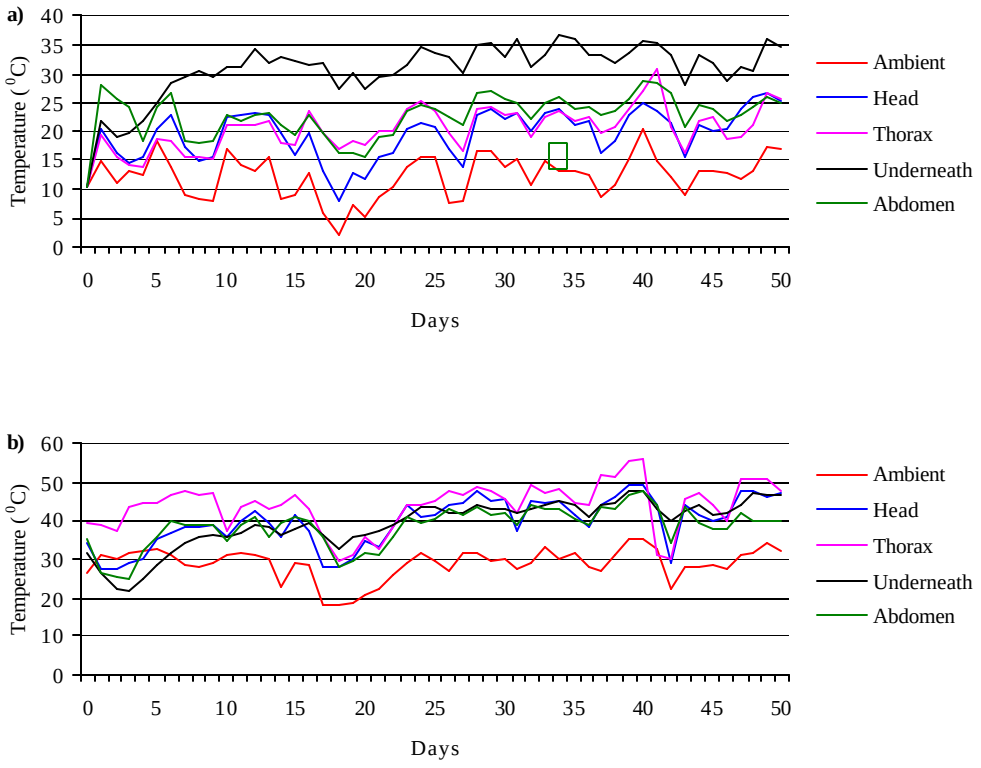


Fig. 3.3.13. Minimum (a) and maximum (b) ambient, internal and external temperatures for the wrapped and clothed carcass (5 days).

This was expected as the head had the least amount of tissue and no clothing that could have provided some insulation. Unfortunately, due to the uncertainty of the accuracy of the data loggers, these data sets were not analysed further. When the change was made to the iButtons during other trials, the data were further tested for trends and correlations.

3.3.5. Mass loss

Mass loss was faster for both control and the clothed control carcasses (Fig. 33.14.). The carcasses in the 10 days sample group were only weighed at the beginning of the trial and again at termination. Their percentage remaining tissue was similar to the other wrapped carcasses, at 33 and 45% for the carcass no clothes and the clothed carcass respectively.

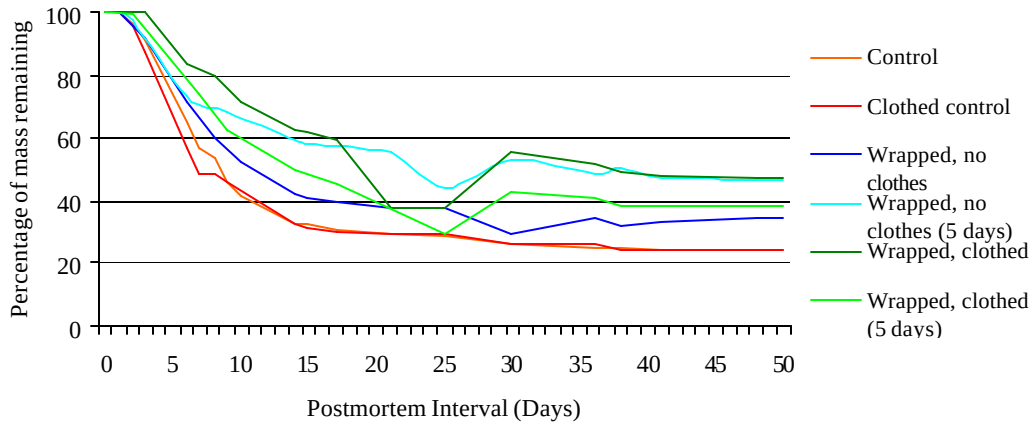


Fig. 3.3.14. Percentage of body mass remaining of the carcasses during the spring wrapped trial.

The trends in this trial are very similar to those found in the autumn wrapped trial. This suggested that the wrapped carcasses maintained higher moisture content and thus mass for longer as the visible tissue loss was somewhat similar.

3.3.6. Maggot mass distribution

The distribution of the maggot masses was recorded daily. This showed the same trends as those recorded in the autumn wrapped trial. The maggot masses were more widely spread in the afternoon than in the morning. In the morning, the maggots appeared to congregate in denser masses and were often found on the extremities of the carcasses. When the maggot mass was at its largest, the carcass surface underneath the clothing or wrapping was covered almost entirely, with the exception of the control carcass where maggot masses were found mostly under the protection of the skin. The wrapped clothed carcasses also had large maggot masses on the outside of the clothing under the sheet.

On the clothed control carcass, the maggot mass remained under the clothes where they fed and were seldom seen on the surface. The clothing and wrapping appeared to allow the maggot masses greater freedom of movement, compared with the control carcasses, where the visible maggot masses never completely covered the carcasses. In this trial there was no displacement of clothing on any of the carcasses i.e. pushed down the limbs or off the carcass, as was found on one carcass during the autumn

wrapped trial. The shorts were forced open on the wrapped clothed carcass. There was also no evidence of any Diptera maggots completing their life cycles on the surface of the sheets as was seen in the autumn wrapped trial. The maggots in this trial were all in direct contact with the carcasses inside the sheets or clothing when present. No observations were made of *C. albiceps* predation on any other maggots.

SECTION 3.4.

The influence of wrapping and clothing on carcass decomposition and arthropod succession: a summer study.

3.4.1. Decomposition of the carcasses

The same characteristics that were used for the autumn wrapped trial were used for the current trial (Table 3.4.1.). Overall, the carcasses in the current trial decomposed faster than those in the autumn or the spring trials. Because of this, the classification of the carcasses into each decomposition stage was more obvious and easier, although the classifications still included transitional stages.

i) Fresh

The carcasses remained in the fresh stage for a very short time. They were placed in the field at approximately 10:00h on 7th January 2004 (day 0) and began to bloat within the hour. By 14:00h on day 0, the torsos of the carcasses were already firm and the limbs were becoming distended. By the morning of day 1, the carcasses were fully bloated (Fig. 3.4.1.).

ii) Bloat

The carcasses did not stay bloated for long (Fig. 3.4.1.). By the morning of day 2, the torsos of the carcasses had begun to soften. Blood seeped through the nose, mouth and around the eyes from day 1. Gasses were continually being released through all the natural openings of the carcasses. The gut had distended through the anus (clothed wrapped carcass) on day 2. Blisters formed on the skin of the carcasses (including the wrapped carcasses). These blisters were filled with fluids and blood. As the carcasses began to soften, skin slippage occurred (from day 3). This was the shortest period recorded for skin slippage.

iii) Active decay

Similar to the autumn and spring trials there was no delay in oviposition by the Diptera during this trial. The carcasses entered active decay simultaneously with the exception of the control carcass.

Table 3.4.1. Summary of the decomposition stages, physical characteristics of each stage and the dominant arthropods present during the summer wrapped trial.

FRESH (Fig. 3.1.1.) • Commenced when the animal was killed • The torso (thorax and abdomen) was soft and the limbs were flexible • No odour associated with the carcass • Very short duration	**Diptera** Calliphoridae: *Chrysomya maginalis* (adults) *Chrysomya albiceps* (adults) Muscidae: Muscidae spp. (adults)
BLOAT (Fig. 3.1.1.) • Commenced when the torso began to harden and the abdomen in particular became inflated, due to the build up of gasses • Bubbles of blood formed at the nose, creating small puddles or saturating the sheet on the wrapped carcass • Carcass appeared 'balloon'-like • Body colour changed • Oviposition took p lace during this stage	**Diptera** Calliphoridae: *Chrysomya marginalis* (adults) *Chrysomya albiceps* (adults) Sarcophagidae: *Sarcophaga cruentata* (adults) *Muscidae:* *Hydrotea capensis* (adults) Muscidae spp. (adults)
ACTIVE DECAY (Fig. 3.1.1) • Carcass deflated, as the maggots actively fed on the carcass tissues and allowed the gasses to escape • Odour of decay was prominent • The limbs collapsed back into the 'resting' position, and in some cases the clothing had moved • The skin began to peel allowing maggots to feed underneath it • The carcass mass was halved during this stage • The Diptera maggots were the most dominant insects • Areas surrounding the maggots and the tissue on which the maggots were feeding became liquefied • Plenty of decomposition liquids were present	**Diptera** Calliphoridae: *Chrysomya marginalis* (adults and maggots) *Chrysomya albiceps* (adults and maggots) Sarcophagidae: *Sarcophaga cruentata* (adults and maggots) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults) Cleridae: *Necrobia rufipes* (adults) Silphidae: *Thanatophilus micans* (adults)
ADVANCED DECAY (Fig. 3.1.1.) • There was little tissue remaining on the carcass • Fewer odours were associated with the carcasses • Puddles of decomposition fluids were common along the backs and upper surfaces of the carcass • The carcass was still moist but often the head was dried out. Bones of the skull, ribs and legs were often visible • The majority of the maggots moved off the carcass to pupate (i.e. less than 20 individuals remaining) • Coleoptera became the most dominate group	**Diptera** Piophillidae: Piophilidae spp. (adults and larvae) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)
DRY REMAINS (Fig. 3.1.1) • The carcass had little to no moisture • Gut content had dried out, although there may have been some fluids present when it was very hot • Only hair and small patches of skin remained	**Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) Cleridae: *Necrobia rufipes* (adults)

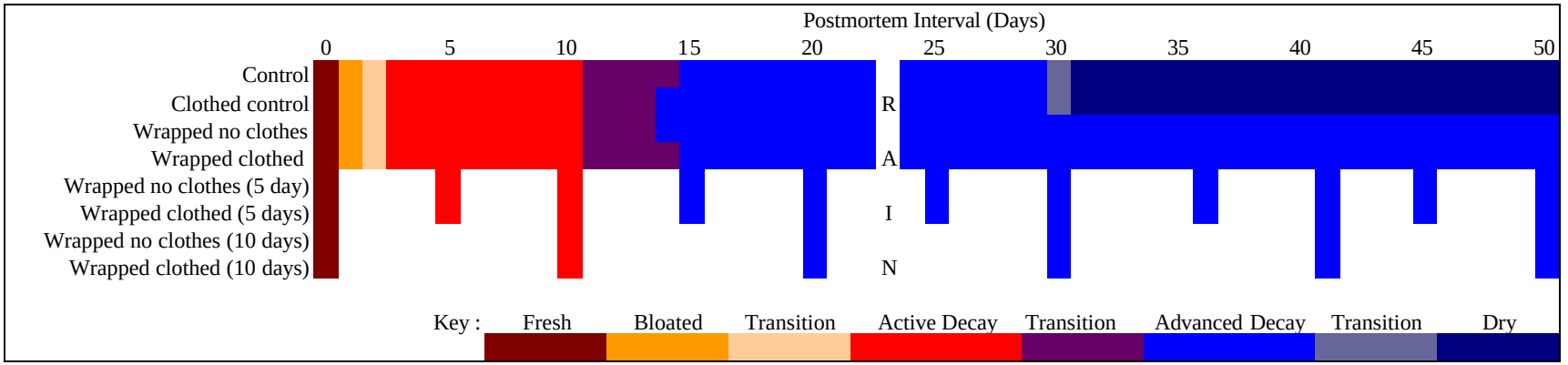


Fig. 3.4.1. Decomposition stages of the carcasses during the summer wrapped trial.

The maggots on the control carcass moved into the head and were not visible on day 3. However, by day 4, they began feeding between the fore-limbs and the carcass entered the active decay stage. Active decay was shorter in this trial than the autumn and spring trials with almost all the maggots migrating by day 9 and 10 and allowing the carcasses to dry out and enter the advanced decay stage. Unlike the previous trials, all the carcasses entered the transition into advanced decay simultaneously (Fig. 3.4.1.).

iii) Advanced decay

The control and clothed control carcasses remained in the advanced decay stage for a similar period of time (up to day 29) (Fig. 3.4.1.). During this time, the remaining oily skin dried out. The wrapped carcasses however, had decomposition fluids trapped inside the sheets, keeping them moist for an extended period, up to the end of the trial.

iv) Dry remains

Only the control and clothed control carcasses dried out enough to be classified into the dry remains stage (Fig. 3.4.1.). The arthropods consumed most of the remaining skin, specifically the *D. maculatus* larvae. There was minimal tissue of any type remaining on the bones of these carcasses.

3.4.2. *Arthropod succession on the carcasses*

The arthropod succession on all carcasses followed the same pattern as seen in the autumn and spring wrapped trials. The adult Diptera arrived at the carcasses within minutes after death. A few individuals of *C. marginalis* (Fig. 3.4.2.) and *C. albiceps* (Fig. 3.4.2.) were recorded on morning of day 1. By the afternoon between 50 and 100 individuals of *C. marginalis* and between 20 and 50 individuals of *C. albiceps* were recorded on all the carcasses (Figs 3.4.3. to 3.4.8.). By the afternoon of day 4, no *C. marginalis* individuals were recorded on the clothed control (Fig. 3.4.4.) and wrapped no clothes (Fig. 3.4.5.) and wrapped clothed carcasses (Fig. 3.4.6.). They were not recorded on the control after the morning of day 4 (Fig. 3.4.3.).

The number of *C. albiceps* decreased to between one and five individuals by the morning of day 4 on the clothed control carcass (Fig. 3.4.4.). By the afternoon of the same day they had decreased on the wrapped no clothes (Fig. 3.4.5.) and wrapped clothed (Fig. 3.4.6.) carcasses. By the morning of day 5, between one and five individuals were recorded on the control carcass (Fig. 3.4.3.). There were single individuals of *C. albiceps* recorded on the carcasses sporadically during the rest of the trial, with the exception of the control carcass (Figs 3.4.3. to 3.4.6.).

Hydrotea capensis were recorded sporadically on the carcasses during the trail. They were mostly recorded in groups of four to five, sometimes in higher number of individuals (Figs 3.4.3. to 3.4.6.). Muscidae spp. were present at all the carcasses for the duration of the trial. Their numbers were higher during the first part of the trial (Figs 3.4.3. to 3.4.6.). However, as with the other trials conducted so far the Muscidae spp. did not utilize the carcasses for breeding purposes and merely fed on the decomposition fluids.

Diptera

Chrysomya marginalis

Chrysomya albiceps

Sarcophaga cruentata

Cole optera

Dermestes maculatus

Necrobia rufipes

Fig. 3.4.2. Most common species occurring in wrapped summer trial.

Dermestes maculatus (Fig. 3.4.2) adults were observed on the carcasses very early during the decomposition process (Figs 3.4.4. to 3.4.8.). Their numbers increased and they were consistently recorded on the carcasses from the morning of day 9 on the clothed control carcass (Fig. 3.4.4.), by the afternoon of day 9 on the control (Fig. 3.4.3.) and wrapped no clothes (Fig. 3.4.5.), and by the morning of day 10 on the wrapped clothed carcass (Fig. 3.4.6). The adult *D. maculatus* successfully bred on the carcasses and by day 11 they were recorded on the wrapped clothed carcass (Fig. 3.4.6.). The larvae were recorded on the control carcass (Fig. 3.4.3.) on day 12 after the rain and finally they were recorded on day 14 on the clothed control (Fig. 3.4.4.) and wrapped no clothes (Fig. 3.4.5.) carcasses. Their numbers increased quickly and they became the most dominant species (in terms of number of individuals) found on the carcasses during the advanced decay stage.

Necrobia rufipes (Fig. 3.4.2.) were observed early in the arthropod succession, simultaneously on day 5 on all the carcasses (Figs 3.4.3 to 3.4.6.). Their numbers increased as the carcasses began to dry out. The numbers of *N. rufipes* recorded during this trial were noticeably higher than those recorded during the autumn and spring wrapped trials. Histeridae spp. were present sporadically almost from the beginning of the trial. They were most commonly observed between day 5 and day 20 (Figs 3.4.3. to 3.4.6.).

Formicidae, *A. custodiens*, *Camponotus petersii* Emery, *Crematogaster* sp. and *M. albopilosum*, were not recorded on the carcasses very often, although they were observed frequently in the surrounding areas. This was the first time that *C. petersii* was recorded (Fig. 3.4.6.).

The most important result, in terms of a postmortem interval estimation was that oviposition by the Diptera was recorded simultaneously at all the carcasses regardless of the wrapping (Fig. 3.4.9). This result was the same as the results recorded during the autumn and spring wrapped trials.

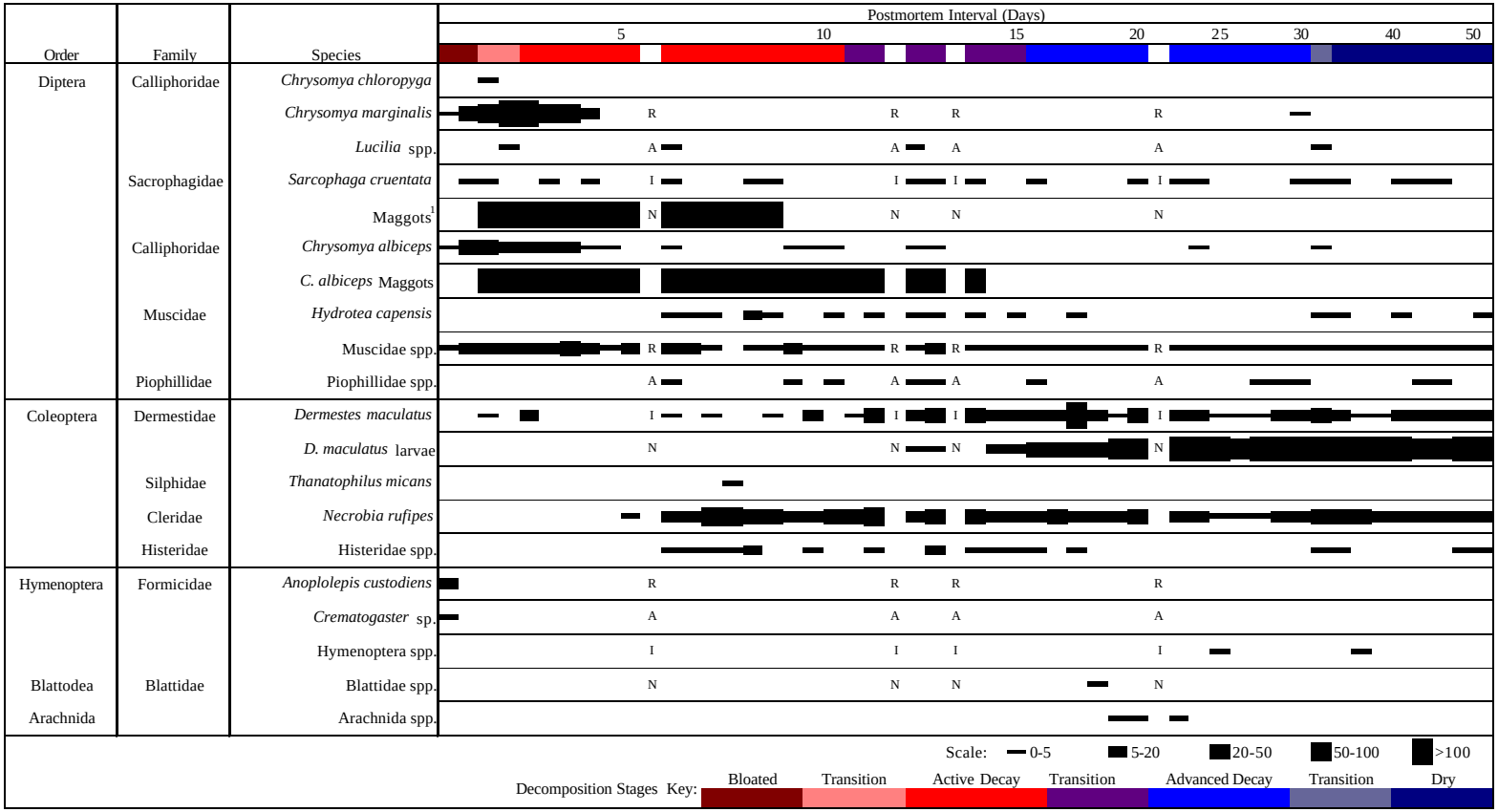


Fig. 3.4.3. Arthropod succession on the control carcass during the summer wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

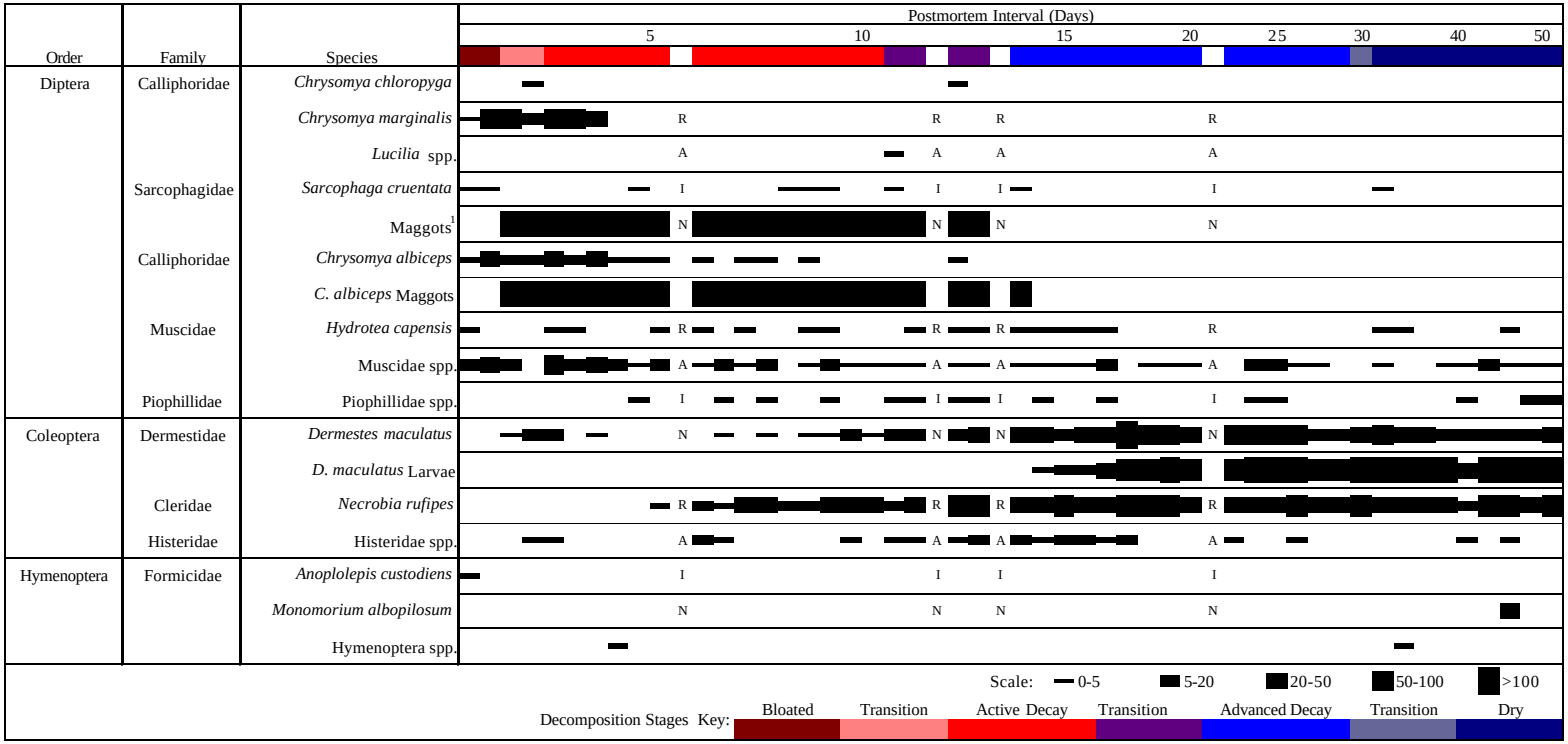


Fig. 3.4.4. Arthropod succession on the clothed control during the summer wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

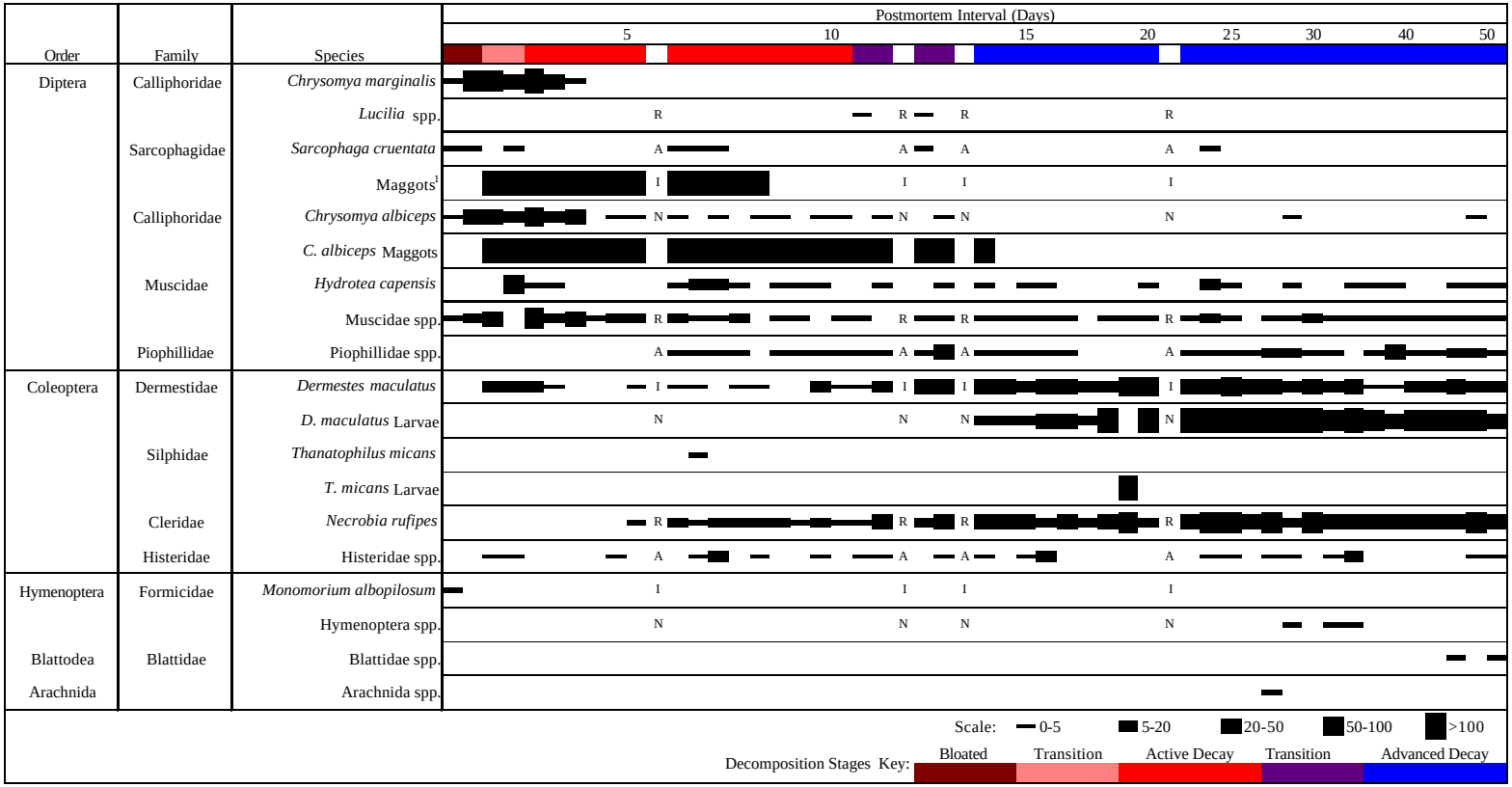


Fig. 3.4.5. Arthropod succession on the wrapped, no clothes carcass during the summer wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

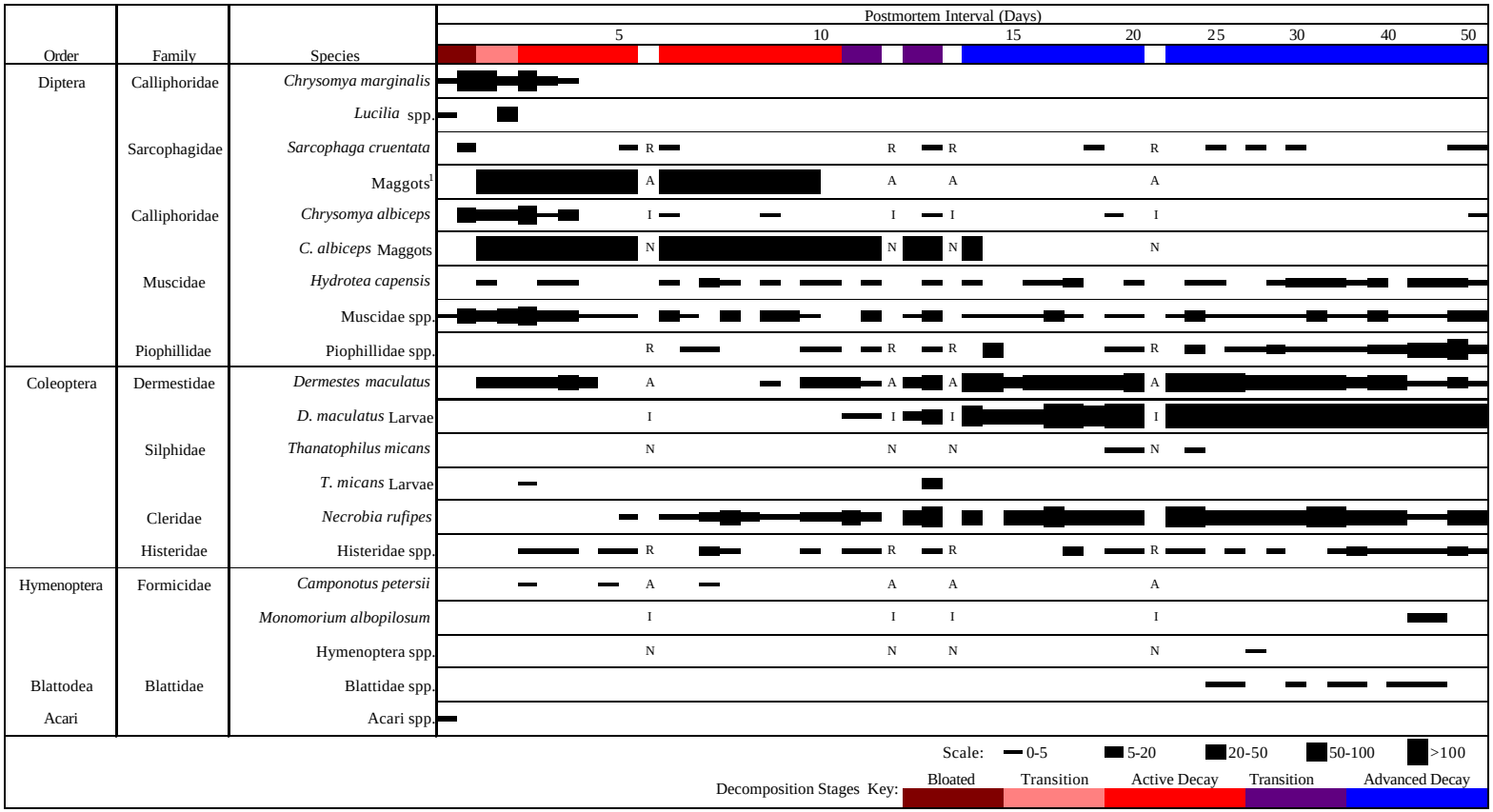


Fig. 3.4.6. Arthropod succession on the wrapped, clothed carcass during the summer wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

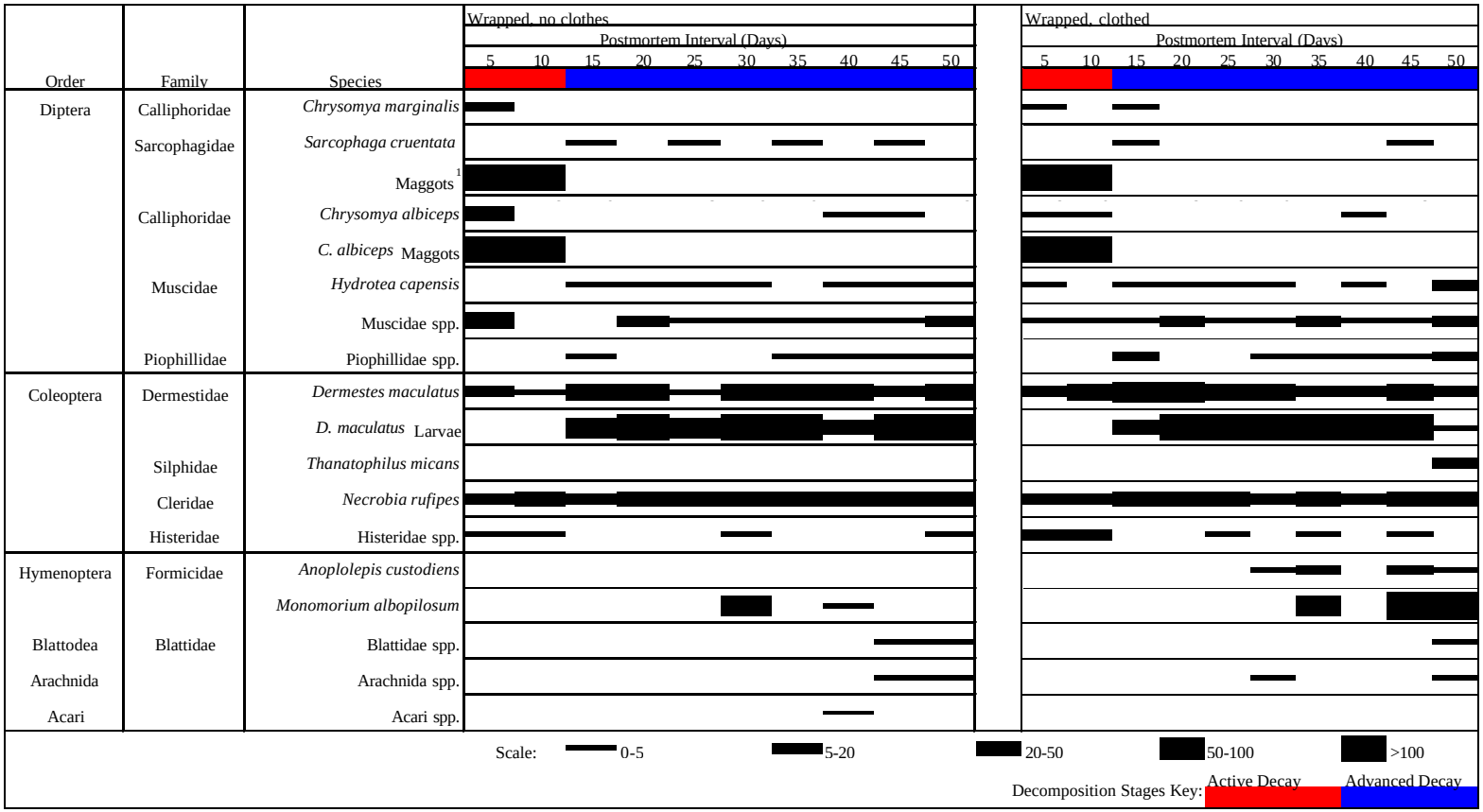


Fig. 3.4.7. Arthropod succession on the wrapped no clothes and wrapped clothed carcasses (sampled every 5 days) during the wrapped summer trial. Maggots[1] – *Chrysomya margin alis* and *Sarcophaga cruentata* .

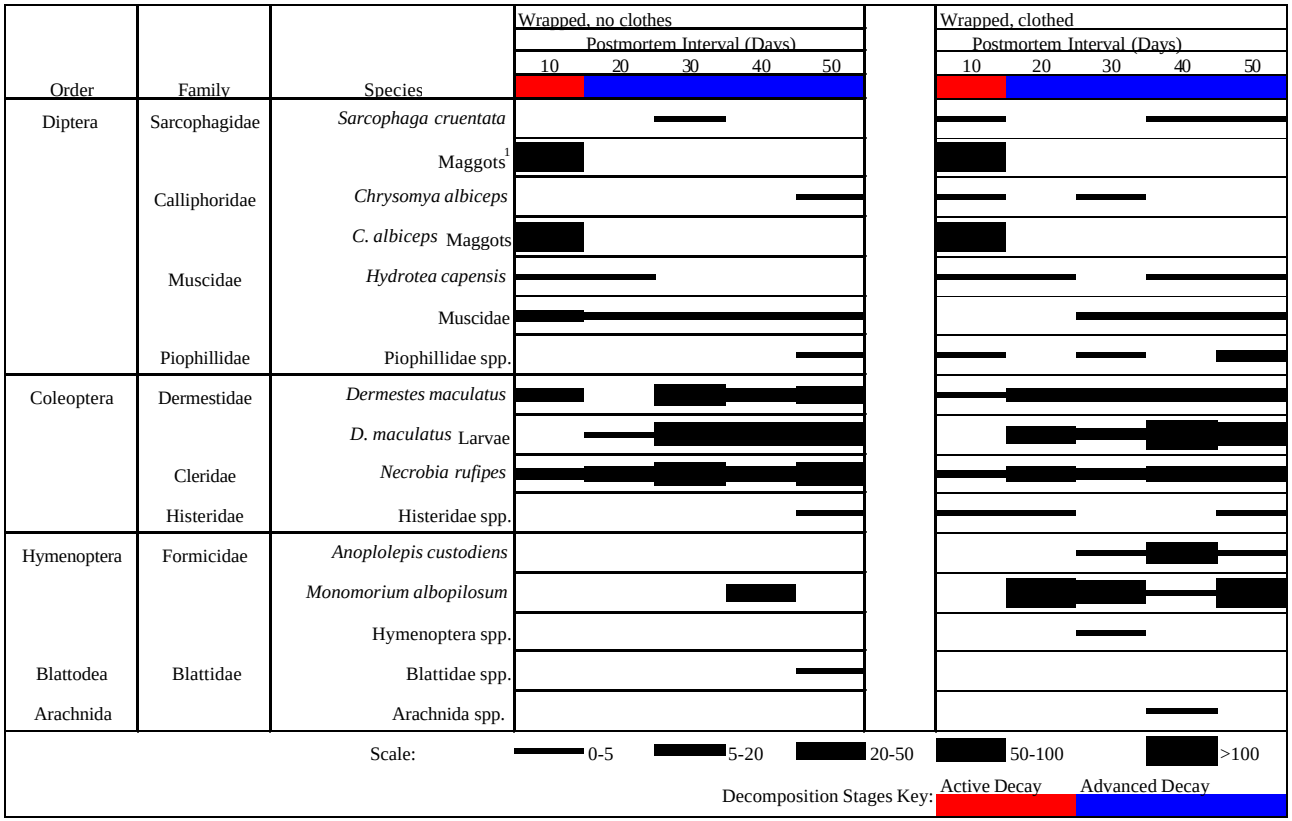


Fig. 3.4.8. Arthropod succession on the wrapped, no clothes and wrapped, clothed carcasses (sampled every 10 days) during the wrapped summer trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata*.

Eggs were deposited on all the carcasses on day 1 (Fig. 3.4.9.). The egg masses were large, with many females ovipositing next to each other adding to already existing egg masses. The eggs that were deposited, hatched quickly. Oviposition only took place for only two da ys and no new eggs were observed on the carcasses by the afternoon of day 3 (Fig. 3.4.9.).

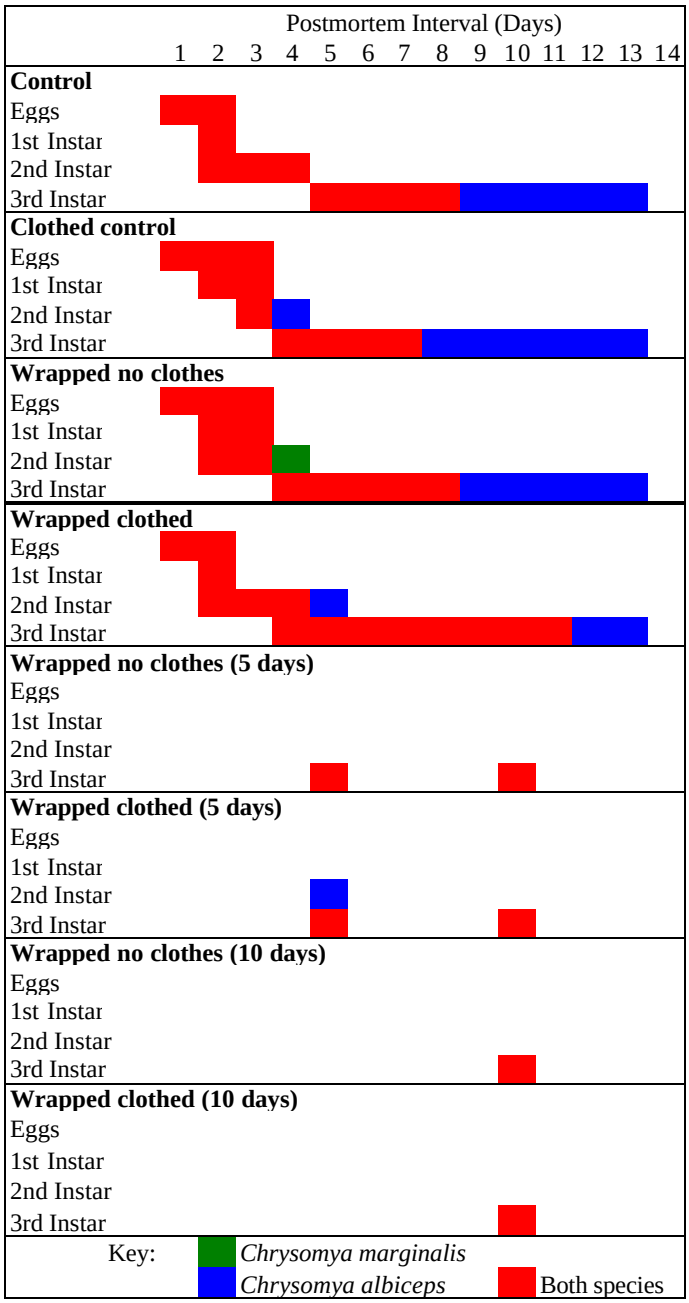


Fig. 3.4.9. Eggs and maggot succession for all carcasses during the summer wrapped trial.

By the afternoon of day 2 actively feeding of second instar maggot masses were observed (Fig. 3.4.9). Third instar maggots were only recorded on the control carcass by day 5. However, the initial maggot mass was in the mouth of the carcass and it may be possible that the third instar maggots migrated into the head and were not easily seen. The shortened period of oviposition resulted in second and third instar maggots only being recorded together on the carcasses for a day or two (Fig. 3.4.9.).

The maggot masses were dominated by *C. marginalis* and *C. albiceps*. Both species were reared from newly hatched maggot masses, suggesting that both species oviposited simultaneously. This was confirmed by personal observations made at the study site where adults of both species were ovipositing eggs next to each other. *Chrysomya albiceps* maggots stayed on the carcasses longer than *C. marginalis* maggots (Fig. 3.4.9.). *Chrysomya albiceps* maggots also migrated in smaller groups with their numbers decreasing over a period of approximately three days. *Chrysomya marginalis* migrated *en masse* and their numbers decreased rapidly over a period of approximately 48 hours.

3.4.3 *Statistical analysis of arthropod succession*

i) Jaccard Metric species pairwise similarities

The $\overline{S}_{gmean}$ values during the summer were between 0.457 and 0.530 (Fig. 3.4.10.). The spring trial had values of between 0.335 and 0.490 (Fig. 3.3.10). The autumn trial had values between 0.334 and 0.396 (and one value of 0.534) (Fig. 3.1.11.) and the winter trial had values 0.333 to 0.442 (Fig. 3.2.11.).

The $\overline{S}_{gmean}$ values of the summer trial are higher than any of the other wrapped trials $\overline{S}_{gmean}$ values. In the summer trial the mean pairwise faunal similarities generally levelled off from day 17 (Fig. 3.4.10), showing more stability than was seen in the spring or autumn wrapped trials. This stability is reflected in the decomposition and arthropod succession, as the carcasses decomposed very quickly and tissue was consumed allowing the Coleoptera community to establish and became stabilised.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

The significant differences found between the similarity matrices of almost all the carcasses, with the exception of the wrapped clothed and the wrapped no clothes (5 days) carcasses, and the clothed control and the wrapped clothed (5 days) carcasses (Table 3.4.2.).

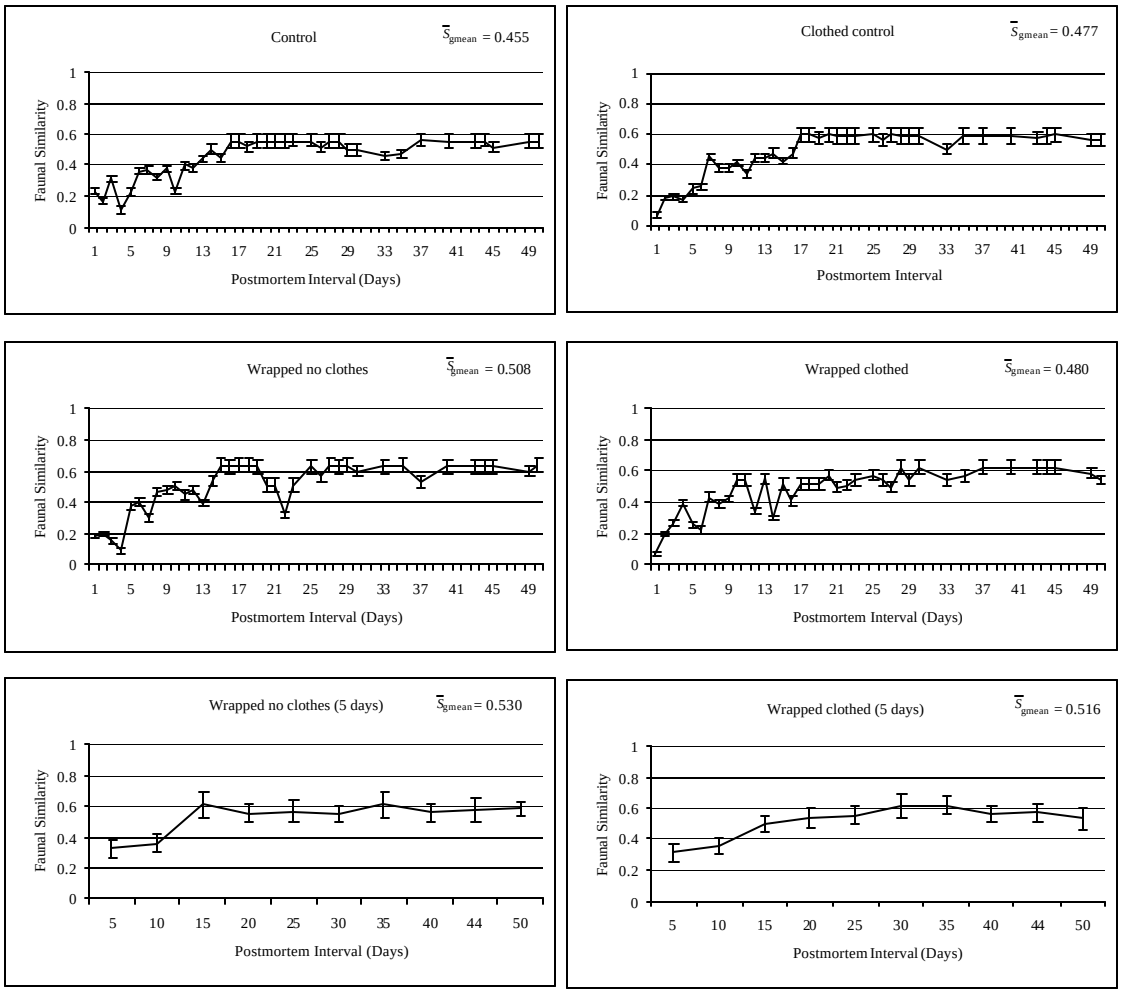


Fig. 3.4.10. Plots of mean pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors).

Table 3.4.2. Results from similarity matrices analysis, $K_{obs\ value}$ and p-values. (* - significant difference at 95% confidence interval).

Carcass (all samples)			
	Control	Clothed Control	Wrapped, No Clothes
Control			
Clothed Control	0.7578 (0.1020)*		
Wrapped, No clothes	0.7201 (0.1380)*	0.7467 (0.1620)*	
Wrapped, Clothed	0.6292 (0.1430)*	0.7469 (0.0790)*	0.6756 (0.1110)*

Carcasses (sampled every 5 days – versus each other and relevant data from other carcass with all samples)		
	Wrapped, No Clothes (5 days)	Wrapped, Clothed (5 days)
Control	0.7986 (0.0660)*	0.8348 (0.0870)*
Clothed Control	0.6702 (0.2040)*	0.8109 (0.0400)
Wrapped, No Clothes	0.8121 (0.0830)*	0.7353 (0.2250)*
Wrapped, Clothed	0.6758 (0.0130)	0.7346 (0.1020)*
Wrapped and Clothed (5days)	0.8363 (0.0560)*	

The maggot masses were on the carcasses for a similar time frame. Although the decomposition was delayed on the wrapped carcasses due to their higher moisture content, the Coleoptera populations appeared to colonise the carcasses at the same rate as the control carcasses (Figs 3.4.3. to 3.4.7.). The significant differences thus can not be easily explained. However, the differences may have been a result of the rapid turnover of species during the current trial.

3.4.4. Ambient, external and internal carcass temperatures

The minimum ambient temperatures recorded at the study site were very similar to the temperatures recorded at the airport according to the SAWS Data. The minimum ambient temperatures ranged between 11 and 20^0C (Fig. 3.4.11a). The maximum ambient temperatures recorded during the summer trial were also similar to those recorded at the airport. The maximum ambient temperatures varied from 25 to 32^0C (Fig. 3.4.11b). There was less variability in these temperatures than recorded in the autumn and the spring trials.

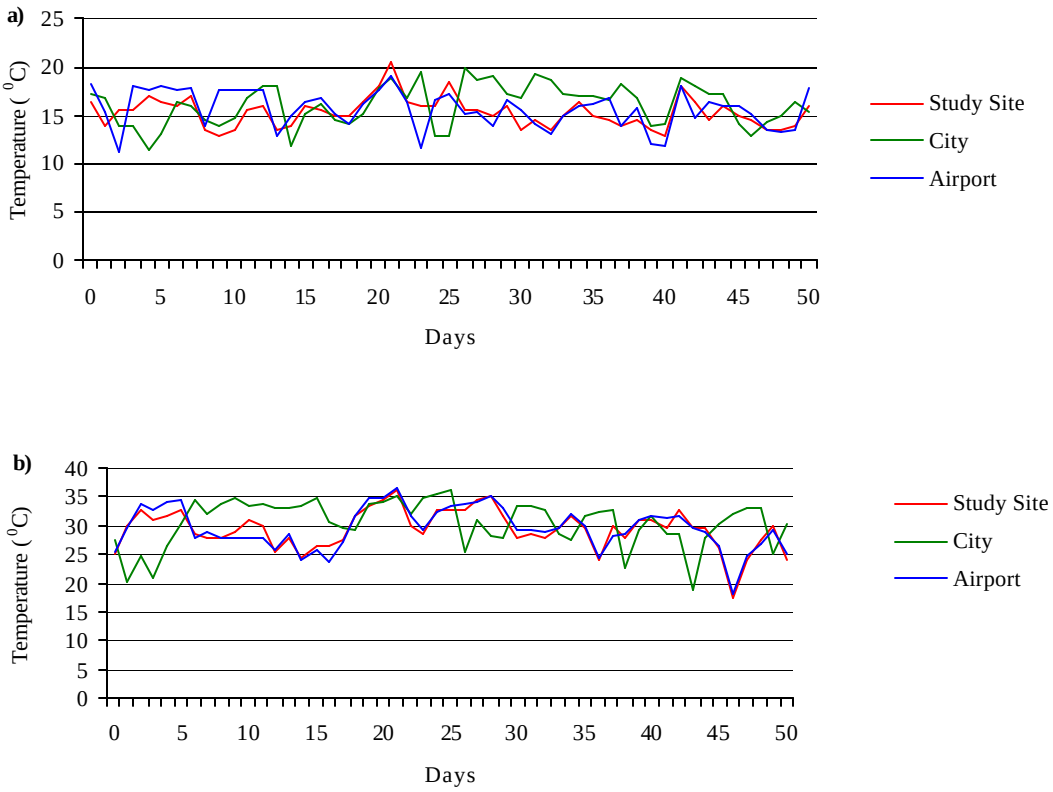


Fig. 3.4.11. Minimum (a) and maximum (b) ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the summer wrapped trial.

The maximum and minimum internal carcass temperatures were higher than the ambient temperatures (Fig. 3.4.12). The temperatures recorded underneath the carcasses and abdomen temperatures fluctuated the least. These regions were the least sensitive to the ambient temperature changes.

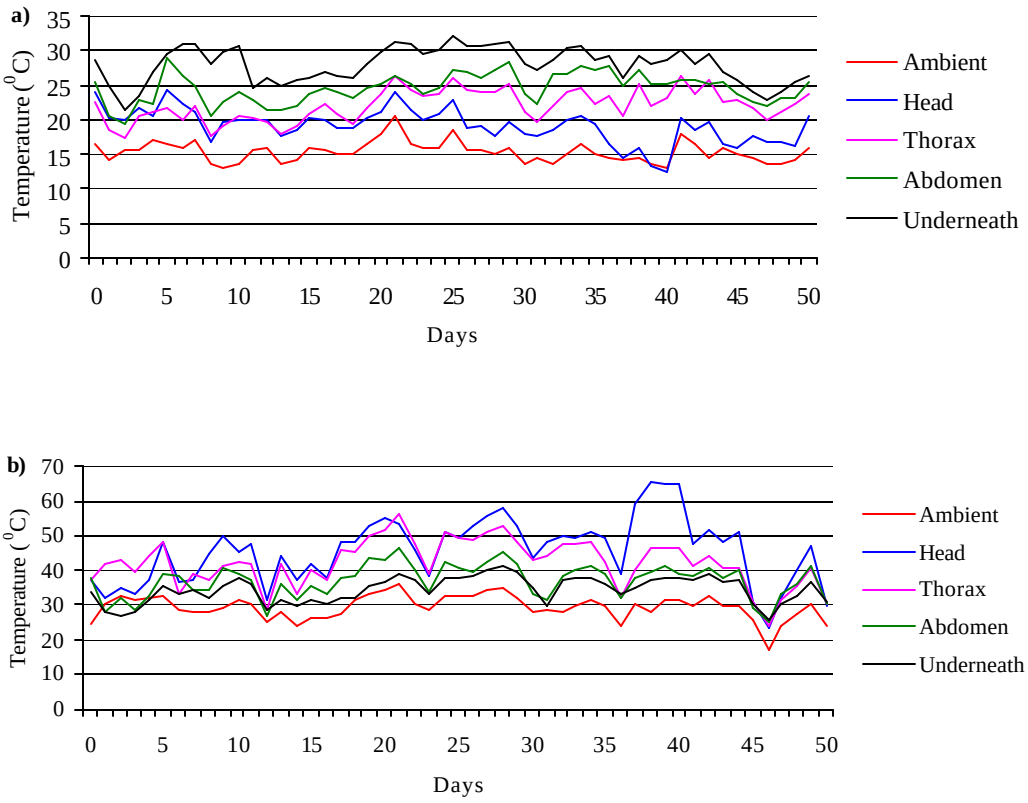


Fig. 3.4.12. Minimum (a) and maximum (b) ambient, internal and external temperatures for the wrapped and clothed carcass (5 days).

The maximum head temperatures were the least similar to maximum ambient temperatures suggesting that the head absorbed the most heat. However, the head region also cooled the most, as the minimum temperatures were the closest to the ambient temperature. Interestingly, there was not a noticeable peak in the internal temperatures when the maggot masses were present. This suggested that the maggot masses were not in one region of the carcasses to cause a spike in the daily temperatures. The maggot mass effect on the internal temperatures may be reflected in a finer time scale. It is also suggested that the maggot mass may have regulated the internal temperatures, as the maggot masses were very large during this trial and were distributed throughout the carcass.

3.4.5. Mass loss

The body mass loss followed the same trends as previously recorded in the autumn and spring trials (Fig. 3.4.13.). The controls (control and clothed control) both dried out, thus losing more body mass than the wrapped carcasses. The wrapping again slowed the moisture loss, hence the higher percentage body mass remaining.

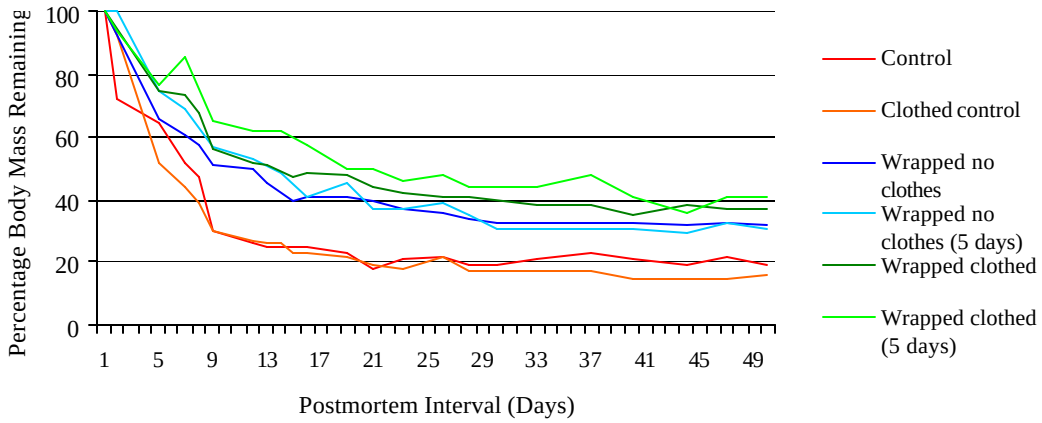


Fig. 3.4.13. Percentage body mass remaining of the carcasses during the wrapped summer trial.

3.4.6. Maggot mass distribution

The movement of the maggot masses was different to those recorded in autumn and spring trials. In those trials, the maggot masses tended to be more widely spread in the afternoons. In the summer however, the maggot masses tended to be more widely spread in the mornings (Fig. 3.4.14). In the heat of the afternoons, the maggots tended to move off the carcasses into the folds of the sheets, especially in front of the carcasses. They moved away from the direct sunlight on the backs of the carcasses into the shaded areas underneath the carcasses. This change in movement by the maggot masses could be attributed to the much higher ambient temperatures recorded during the summer. To maintain some sort of thermoregulation, the maggots would have to move away from the hotter sections of the carcasses. The regions that had very high temperatures appeared to be where the most oily decomposition fluids collected. This was not always in the same region for each carcass.

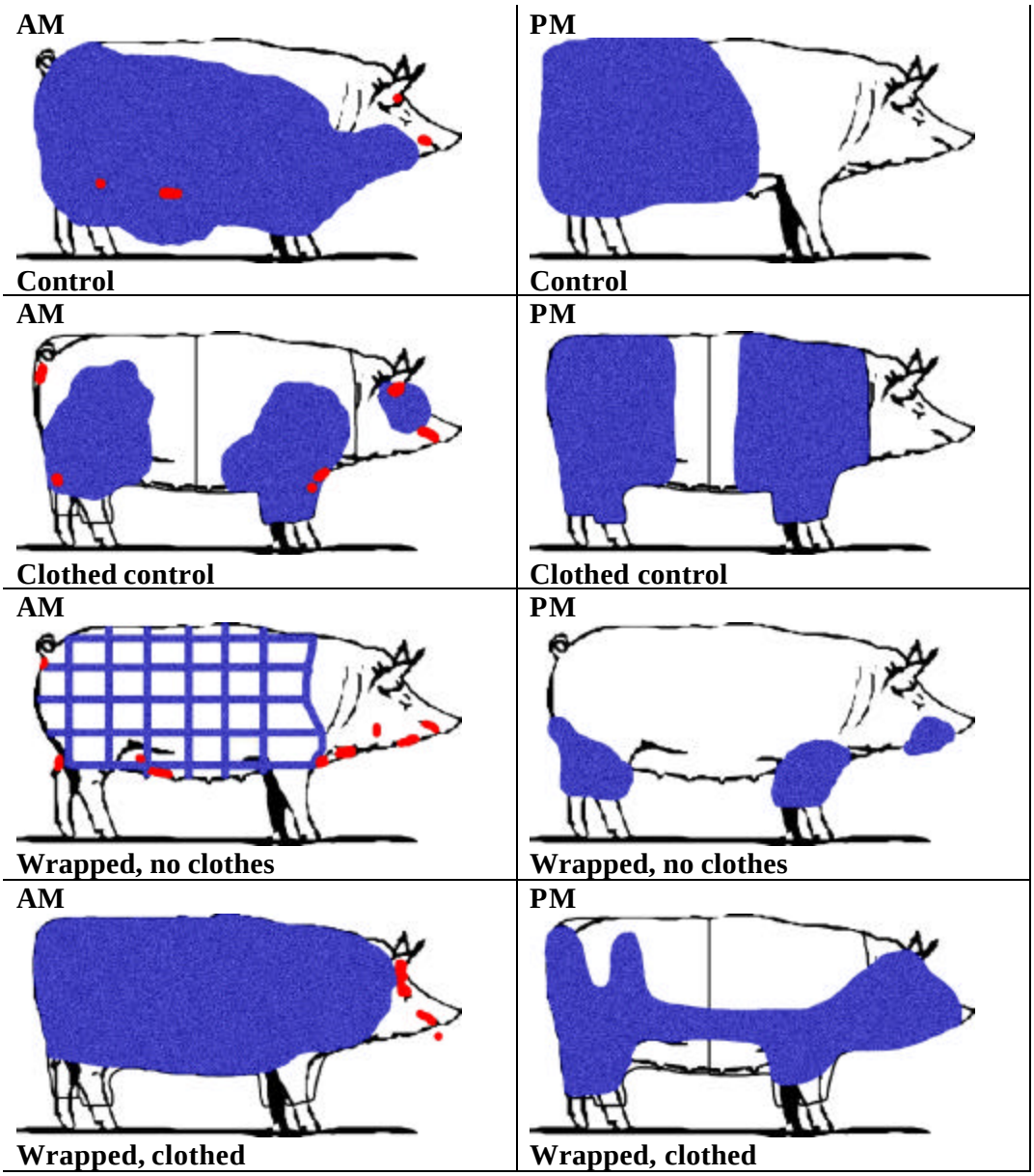


Fig. 3.4.14. Maggot mass movement recorded on day 7 of the summer wrapped trial. Red dots on the morning (AM) diagrams represent the primary oviposition sites (# - scattered maggots).

3.4.7. Maggot mortality

On day 4 of the trial there was maggot mortality along the abdomen of the wrapped no clothes carcass (Fig. 3.4.15). The next day there were further mortalities along the back of the carcass. There was also maggot mortality along the back of the wrapped clothed carcass (Fig. 3.4.15). As with the autumn trial, the maggot masses affected

were almost completely made up of third instar *C. marginalis*. The afternoon temperatures of the carcasses were high on day 4, with the following temperatures on the different areas e.g. body surface of 43^{0}C, the head 46,8^{0}C, the thorax 43,3^{0}C and the abdomen 42^{0}C. The ambient temperature was 32,9^{0}C. The afternoon temperatures of the carcasses were even higher on day 5, with a surface temperature of 44^{0}C and 47^{0}C, the head of 50,8^{0}C and 46.9^{0}C, the thorax of 44,7 and 45,2^{0}C and the abdomen of 46,5^{0}C and 44,9^{0}C, for the wrapped, no clothes and wrapped clothed carcasses respectively. The ambient temperature was 33,2^{0}C. All dead maggots were found under a sheet soaked with decomposing tissues and fluids.

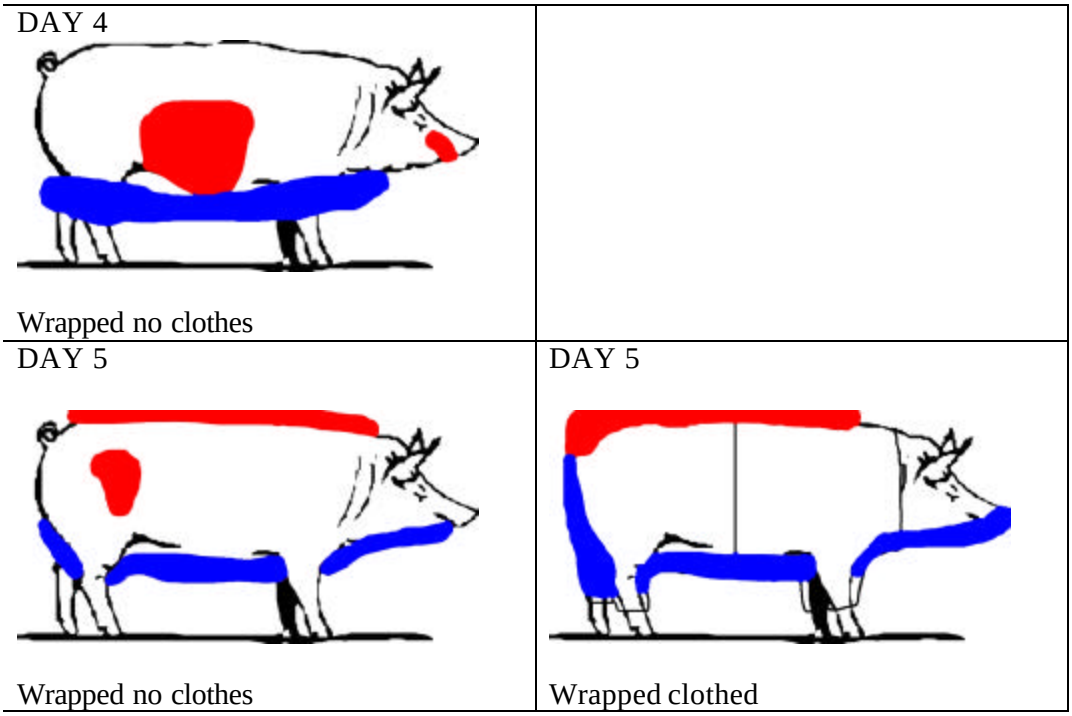


Fig. 3.4.15. Distribution of maggot mortality (red) and remaining live maggots (blue).

3.4.8. Maggot predation

There was some predation by *C. albiceps* maggots on *C. marginalis* recorded during this trial. However, the predation appeared to be limited. At times the maggot masses were predominantly one species or the other. As long as there was enough tissue on the carcasses, *C. albiceps* did not predate on the other maggots.

SECTION 3.5

The influence of stab wounds, severe trauma and clothing on carcass decomposition and arthropod succession: an autumn study.

3.5.1. Decomposition of the carcasses

The same physical characteristics used for the autumn wrapped trial were used for this trial. The carcasses remained in the fresh stage for less than a day and began to bloat within hours of placement. The carcasses were placed in the field at approximately 11:00h on the 18th March 2004 (day 0). The carcasses bloated quickly and by day 2, their limbs had collapsed back into the resting position (Fig. 3.5.1.). The bloat stage in this trial was shorter than the autumn wrapped trial. This observation reinforces the impact rain had on decomposition during the initial placement of the carcasses during the wrapped trial, as it may delay the oviposition by the adult blow flies and the decomposition process.

The carcasses in the current trial entered into active decay by day 3 and 4 (Fig. 3.5.1.). The active decay stage lasted for approximately 10 days both autumn seasons (with the exception of the two wrapped carcasses). The control and clothed control carcasses in the current trial dried out quickly. Unlike the sheet used in the wrapped trials, the clothing did not appear to delay the drying out of the carcasses. Two clothed carcasses and two carcasses without clothes reached the dry remains stage during the trial. There was also no clear indication that the presence of wounds, had any affect on the overall carcass decomposition (Fig. 3.5.1.).

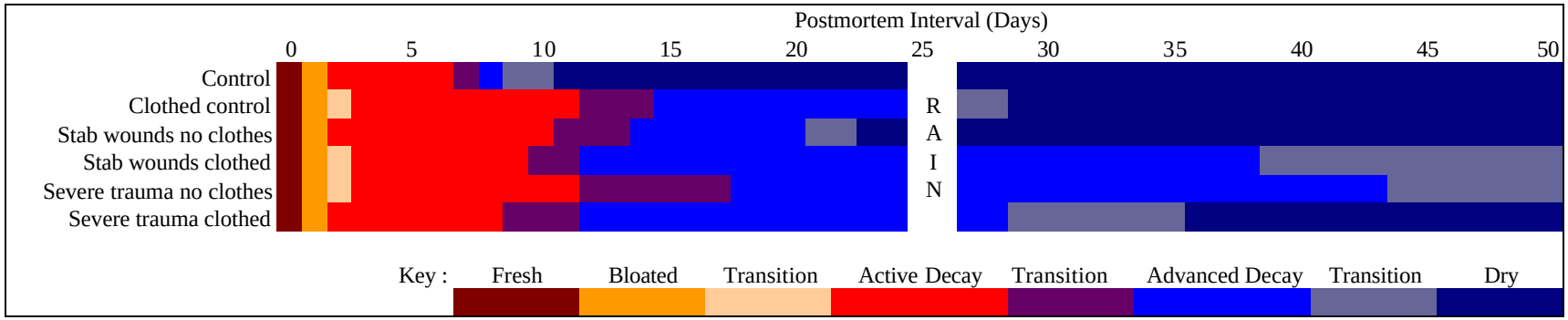


Fig. 3.5.1. Decomposition stages of the carcasses in the autumn wounds trial.

3.5.2. Arthropod succession on the carcasses

The most common species occurring the current trial were the same species as those found in the previous autumn wrapped trial (Table 3.5.1.).

Table 3.5.1. Most common species occurring in wounds autumn trial.

Diptera	Coleoptera
Chrysomya marginalis	*Dermestes maculatus*
Chrysomya albiceps	*Necrobia rufipes*
Sarcophaga cruentata	

The Calliphoridae occurred in very high numbers during the first four days after placement. *Chrysomya marginalis* was recorded in numbers of between 50 and 100 individuals by the morning of day 1 on all the carcasses and increased to over 100 by day 2 (Figs 3.5.2. to 3.5.7.). *Chrysomya albiceps* was also recorded at all the carcasses by the morning of day 1, in numbers between five and 20 individuals (Figs 3.5.2. to 3.5.7.).

Muscidae spp. were also present in numbers between five and 20 individuals on day 1. Their numbers increased during the first part of the trial, after which they occurred in lower numbers (Figs 3.5.2. to 3.5.7.).

Chrysomya marginalis were no longer recorded on the control (Fig. 3.5.2.) and the severe trauma clothed (Fig. 3.5.7.) carcasses after the afternoon of day 5. They were recorded until the morning of day 8 on the clothed control (Fig. 3.5.3.), stab wounds no clothes (Fig. 3.5.4.), the stab wounds clothed (Fig. 3.5.5.) carcasses and until the morning of day 9 on the severe trauma no clothes carcass (Fig. 3.5.6.). *Chrysomya albiceps* was recorded on the control carcass only until after the rain on day 6 (Fig. 3.5.2.). They were recorded on the stab wounds no clothes (Fig. 3.5.4.), stab wounds clothed (Fig. 3.5.5.) and severe trauma clothed carcasses (Fig. 3.3.7.) until the morning of day 14. They were recorded on the severe trauma no clothes carcass until day 16 (Fig. 3.5.6). *Sarcophaga cruentata,* as with the previous trials, were recorded sporadically in low numbers. *Hydrotea capensis* was also recorded throughout the trial (Figs 3.5.2. to 3.5.7.).

Dermestes maculatus adult individuals were recorded on the carcasses even while the Diptera maggots were still present. They were recorded on the clothed control (Fig. 3.5. 3.), stab wounds no clothes (Fig. 3.5.4.) and severe trauma no clothes (Fig. 3.5.6.) carcasses by the morning of day 6. They were recorded from the morning of day 7 on the control (Fig. 3.5.2.) and severe trauma clothed (Fig. 3.5.7.) carcasses. Lastly, the y were recorded on the stab wounds clothed (Fig. 3.5.5.) carcass from the morning of day 8 (Fig.3.5.5.). However, their numbers only slightly increased as the maggots migrated and as the carcasses dried out (Figs 3.5.2. to 3.5.7.). *Dermestes maculatus* larvae were sporadically recorded on some of the carcasses during the initial part of the trial. They were consistently present from day 18 on the control (Fig. 3.5.2.) and severe trauma clothed (Fig. 3.5.7.) carcasses. They were present from day 19 on the clothed control (Fig. 3.5.3.), stab wounds no clothes (Fig. 3.5.4.) and severe trauma no clothes (Fig. 3.5.6.) carcasses. They were recorded on stab wounds clothed carcass by day 20 (Fig. 3.5.5.).

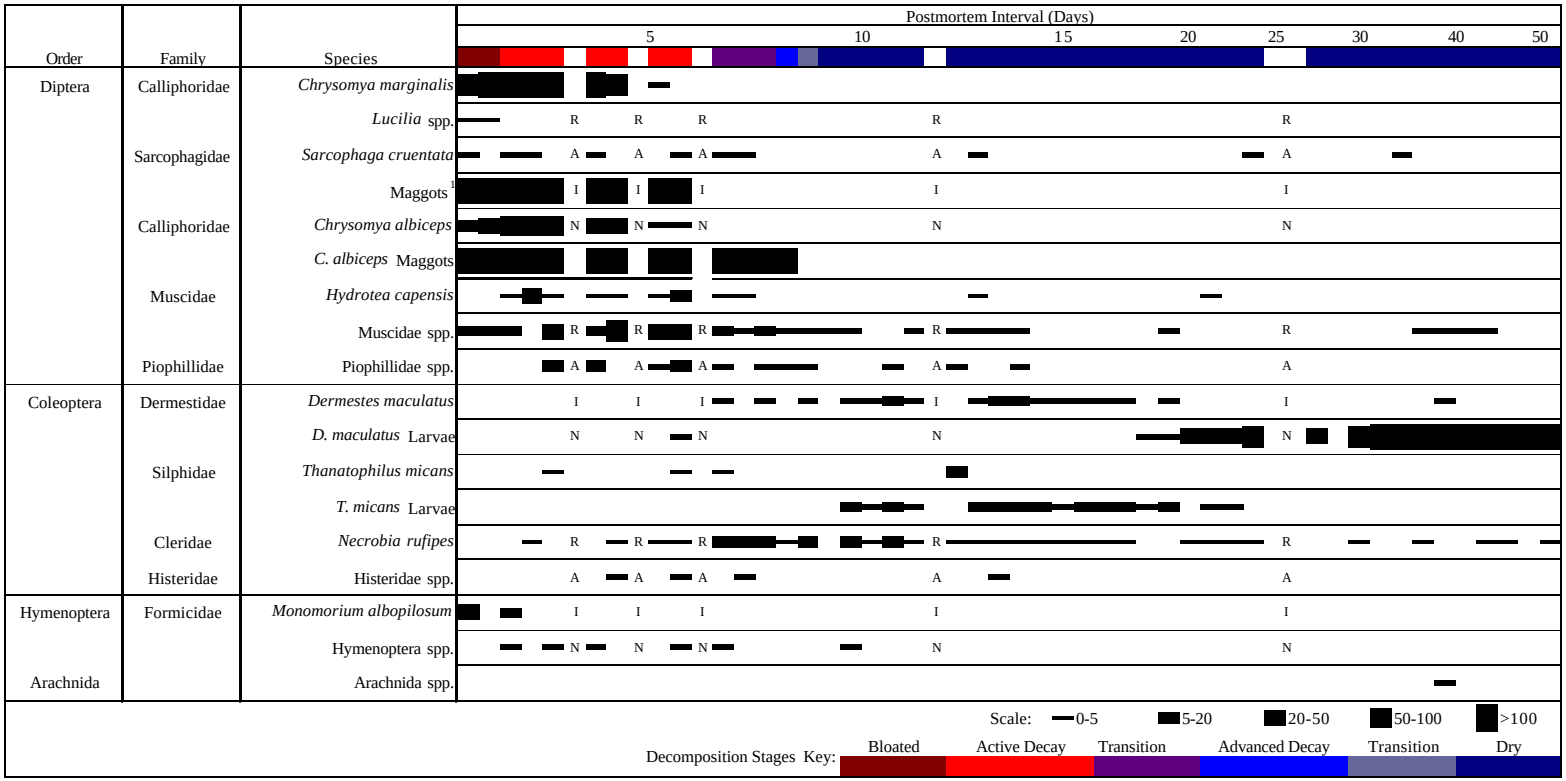


Fig. 3.5.2. Arthropod succession on the control carcass during the autumn wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

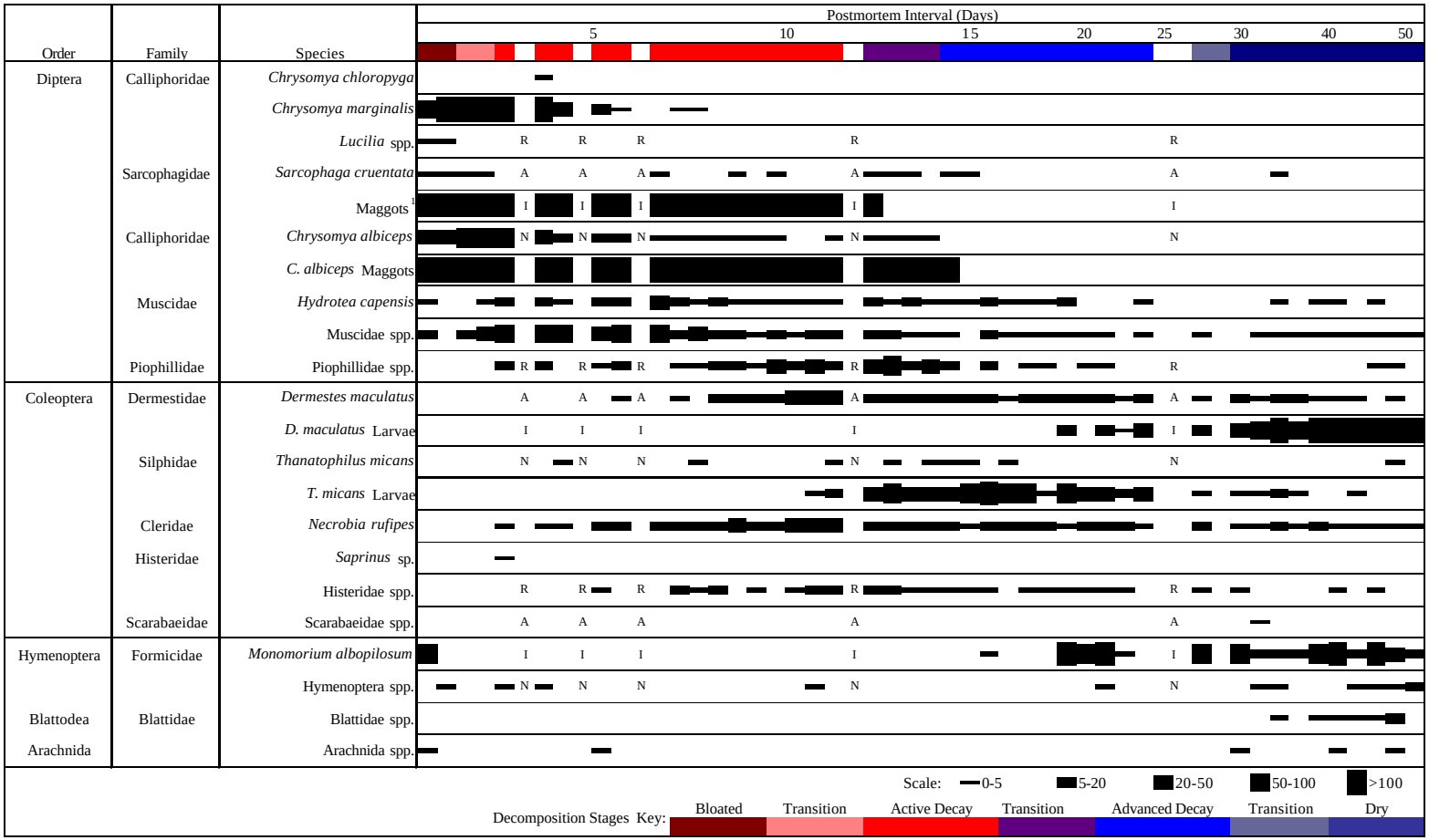


Fig. 3.5.3. Arthropod succession on the clothed control during the autumn wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

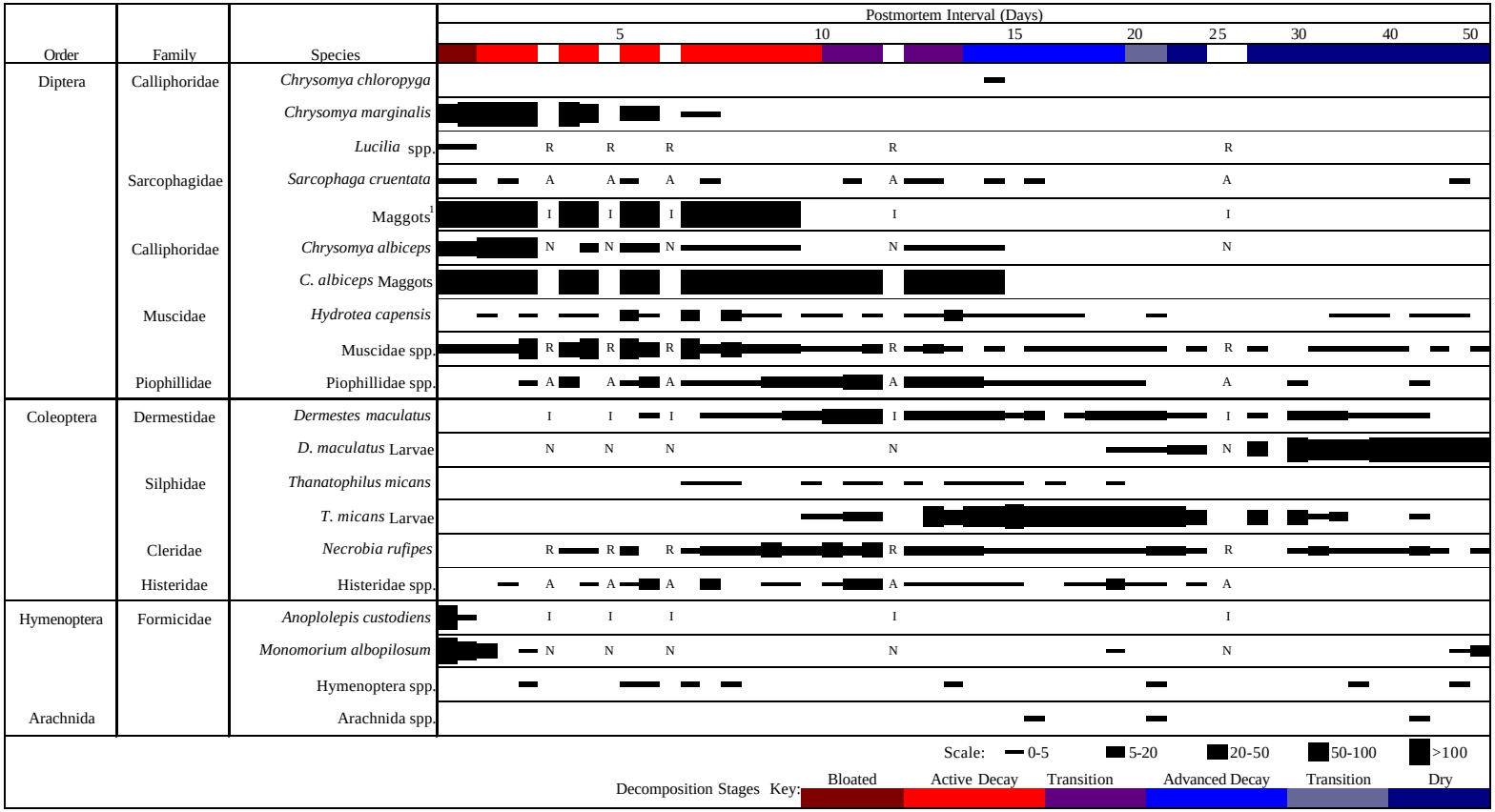


Fig. 3.5.4. Arthropod succession on the stab wounds no clothes carcass during the autumn wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

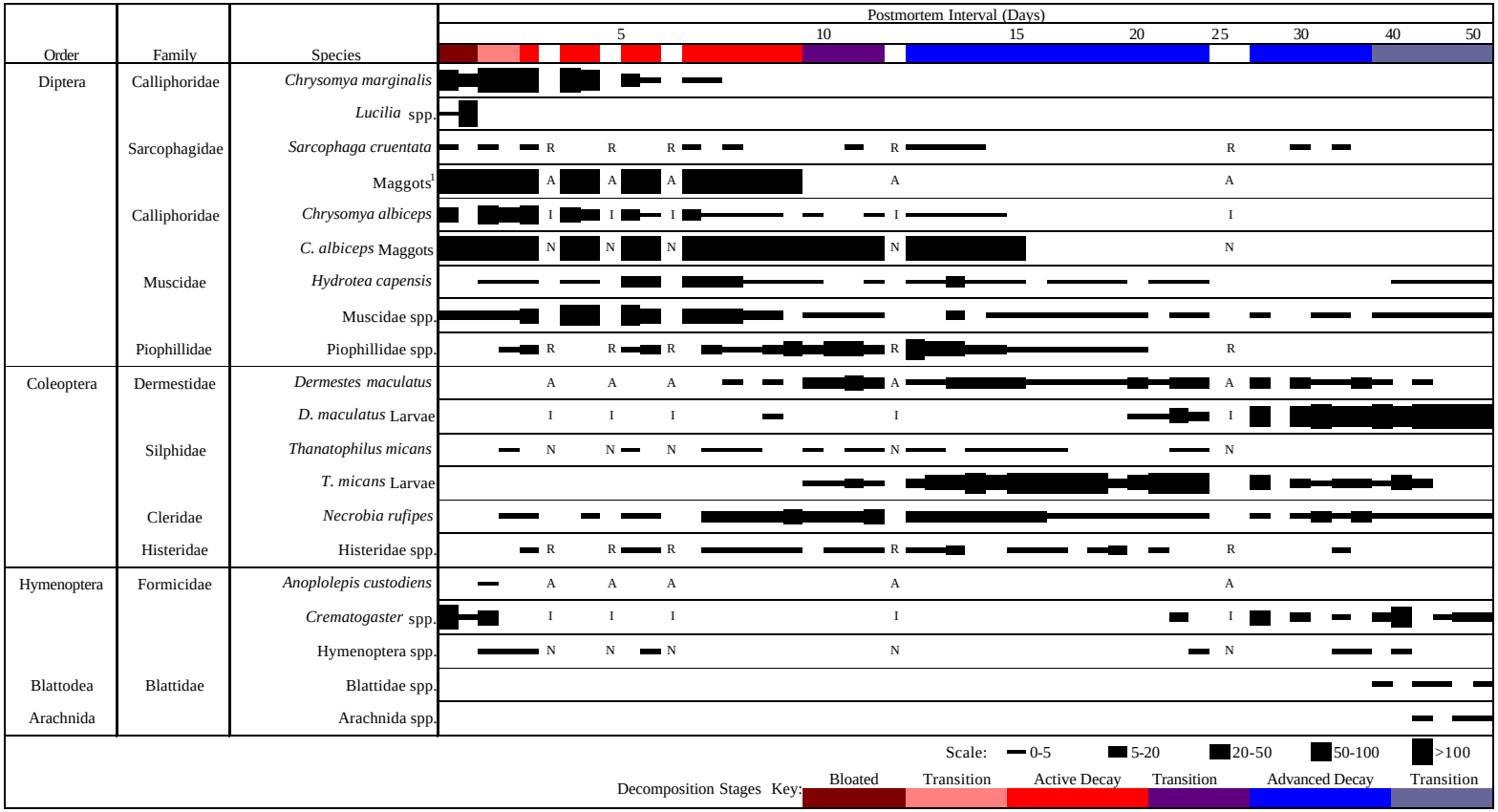


Fig. 3.5.5. Arthropod succession on the stab wounds clothed carcass during the autumn wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

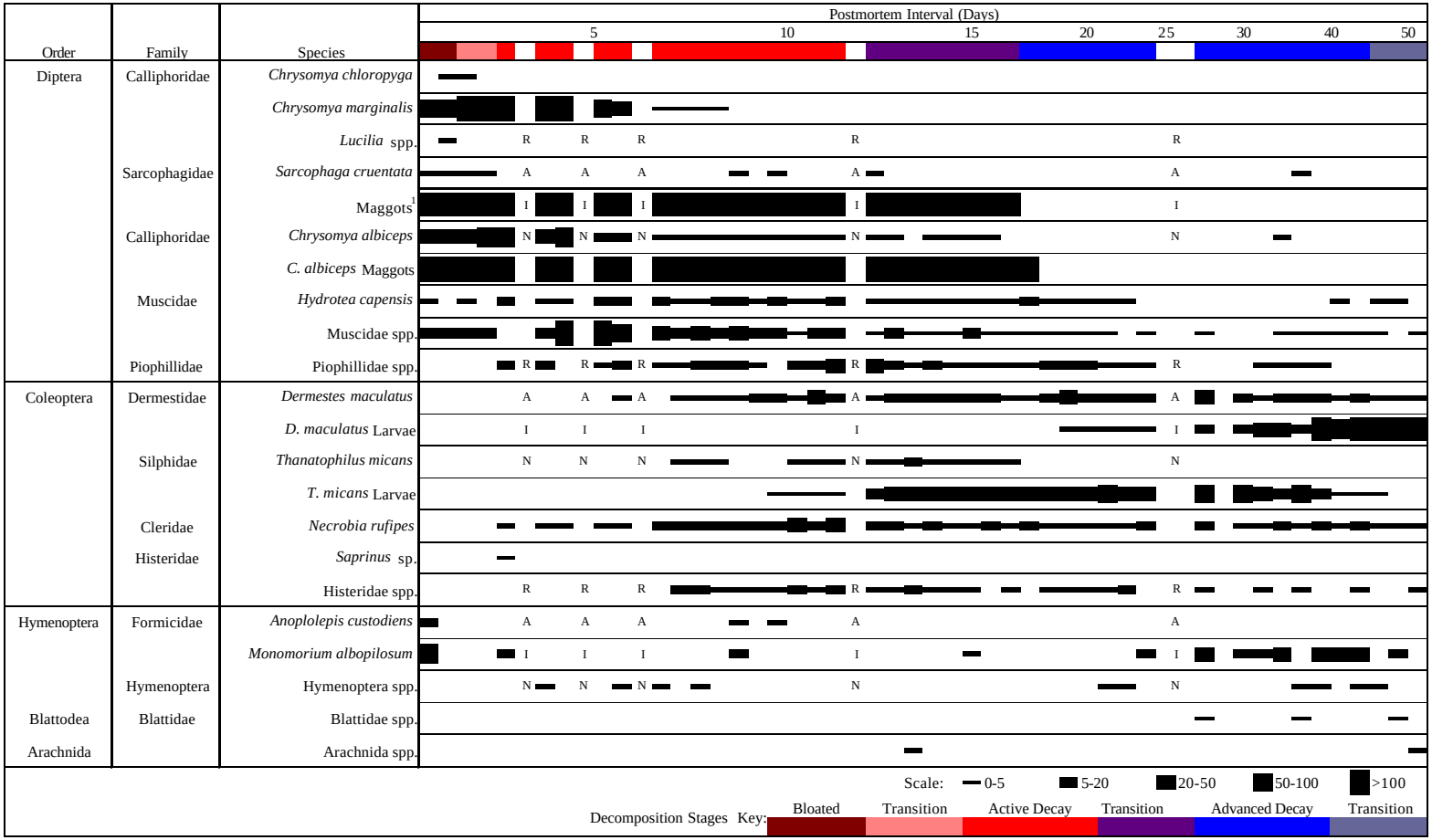


Fig. 3.5.6. Arthropod succession on the severe trauma no clothes carcass during the autumn wrapped trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

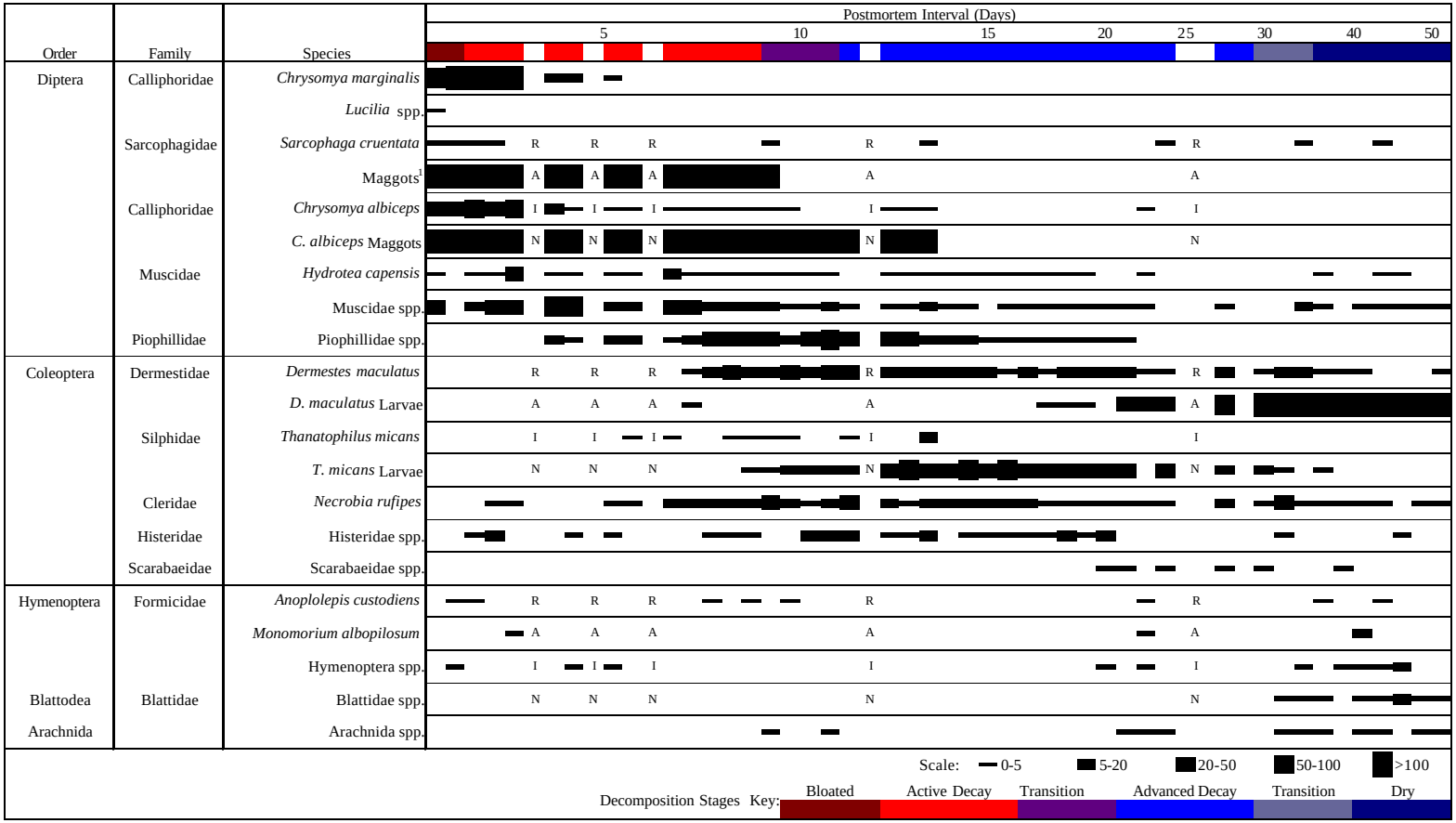


Fig. 3.5.7. Arthropod succession on the severe trauma clothed carcass during the autumn wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

Their numbers rapidly increased as the carcasses dried out. This was also the case in the autumn wrapped trial when *D. maculatus* larvae were recorded from day 15.

Necrobia rufipes were recorded on the carcasses as early as the afternoon on day 2 on the control (Fig. 3.5.2.), stab wounds clothed (Fig. 3.5.5.) and severe trauma clothed (Fig. 3.5.7.) carcasses. They were recorded on the morning of day 3 on the clothed control (Fig. 3.5.3.) and severe trauma no clothes carcasses (Fig. 3.5.6.). They were only recorded on the stab wounds no clothes carcass by the morning of day 4 (Fig. 3.5.4). *Necrobia rufipes* were also recorded this early in the autumn wrapped trial. However, their numbers were not as high or their presence as constant as recorded during the current trial.

Thanatophilus micans, adults were sporadically on all the carcasses (Figs 3.5.2. to 3.5.7.) *Thanatophilus micans* larvae were recorded on the carcasses from the afternoon of day 9, except the clothed control, where it was recorded on the morning of day 10 (Figs 3.5.2. to 3.5.7.). This was similar to the autumn wrapped trial where *T. micans* larvae were recorded from day 10.

Formicidae, *A. custodiens* and *M. albopilosum* were present during the entire decomposition process. *Monomorium albopilosum* sometimes occurred in high numbers especially during the first part of the trial (Figs 3.5.2. to 3.5.7.). They did not appear to have any influence on the arthropod succession during this trial.

An important result observed was that there was no delay in oviposition by the adult Calliphoridae (Fig. 3.5.8.). Eggs were found on all the carcasses by the morning of day 1 regardless of the experimental status of the carcasses i.e. presence of clothing or wounds. It was expected that the female Diptera would have been attracted to the carcasses due to the presence of blood or the wounds (Catts & Haskell 1990). However, not even the severe trauma carcasses, with pools of blood around the wounds, attracted more female Diptera than the control carcasses.

As with the autumn wrapped trial, the maggot masses consisted almost exclusively of *C. marginalis* and *C. albiceps*. Some *S. cruentata* maggots were also present in low numbers. *Chrysomya albiceps* appeared to have a longer development time than the

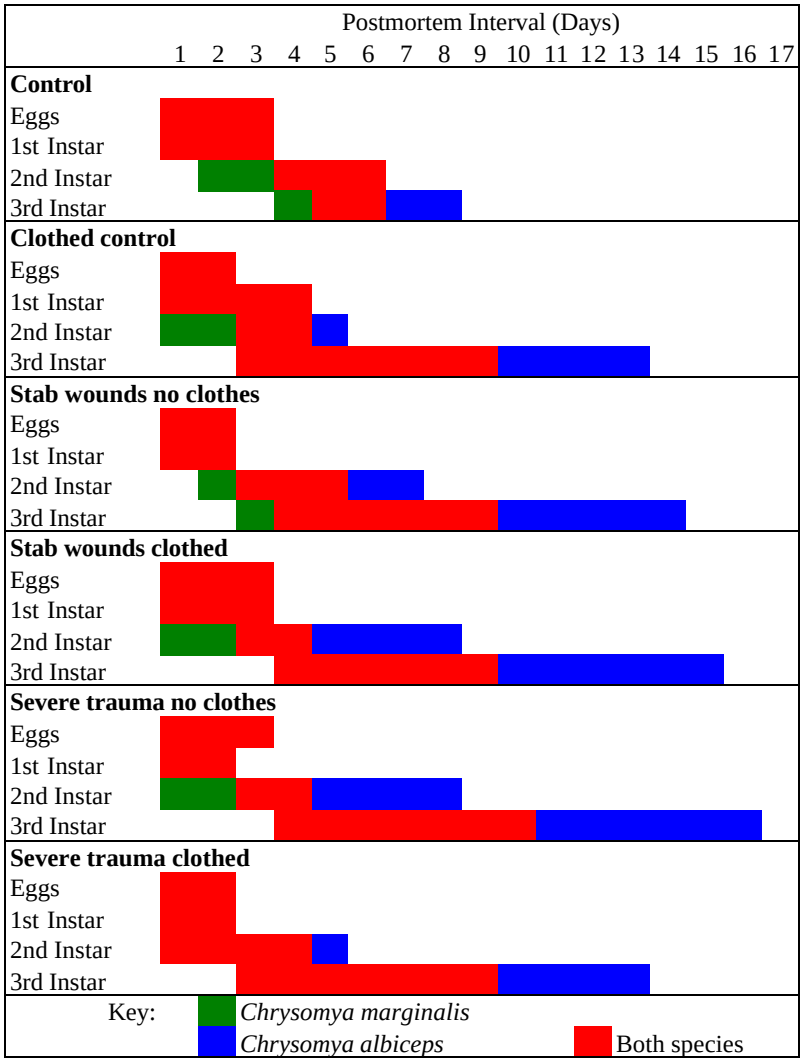


Fig. 3.5.8. Maggot succession for all carcasses during the autumn wounds trail.

C. marginalis. Chrysomya marginalis and *C. albiceps* were recorded to oviposit at the same time. This was confirmed by the presence of *C. albiceps* in the early sub-samples that were reared to adulthood. These results were the same as those recorded in the autumn wrapped trial.

The maggots were present on the control carcass only until day 9. The carcass was consumed quickly and there was little tissue remaining on whic h the maggots could feed, thus forcing them to move off the carcass to seek food or pupate sooner than those on the other carcasses. It is unclear if those maggots developed into pupae that were viable and able to develop into breeding adults.

The overall maggot development time on the carcasses was similar to the control and clothed control carcasses of the autumn wrapped trial. The eggs hatched within hours and maggots were recorded from day 1. There was no evidence that the presence of clothing influenced the development time of the maggot mass. Other than the control carcass, the developmental time of the maggot masses was similar. *Chrysomya marginalis* maggots migrated within a day of each other. *Chrysomya albiceps*, however, migrated over a longer period and some carcasses may have had maggots present up to four days longer.

3.5.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices and the mean faunal similarity was then calculated for each day (see 2.2.3.). These were plotted and the grand mean of all the mean faunal similarities were calculated and is represented as $\overline{S}_{gmean}$ (Fig. 3.5.9.). The graphs were characteristically horse-shoe shaped as described by Schoenly (1992). The $\overline{S}_{gmean}$ values for the current trial ranged from 0.374 and 0.426, except the control carcass with a value of 0.290 (Fig. 3.5.9.). Similar to the autumn wrapped trial, this range was less than the 0.218 to 0.808, which Schoenly (1992) recorded.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

There were significant differences recorded between the control carcass and all the other carcasses except the stab wounds clothed carcass (Table 3.5.2.). This may be due to the very short time the maggots were on this particular carcass. The other carcasses that showed a significant difference in Jaccard similarity matrices were the stab wounds, no clothes versus the severe trauma clothed carcass and the severe trauma, no clothes carcass versus the severe trauma clothed carcass (Table 3.5.2.). Although these differences exist are not easily explained, it should be noted that the p-values are not far from the 0.05 confidence interval. The results did not show an overall clear trend that clothing showed a significant difference, however, as both of these differences were between clothed versus no clothes carcasses. This may warrant further investigation.

As expected there was a significant difference between the control and clothed control carcasses from the previous year wrapped trial and the current trial. This could be due to the rain during the initial stages of the wrapped trials (Table 3.5.3.).

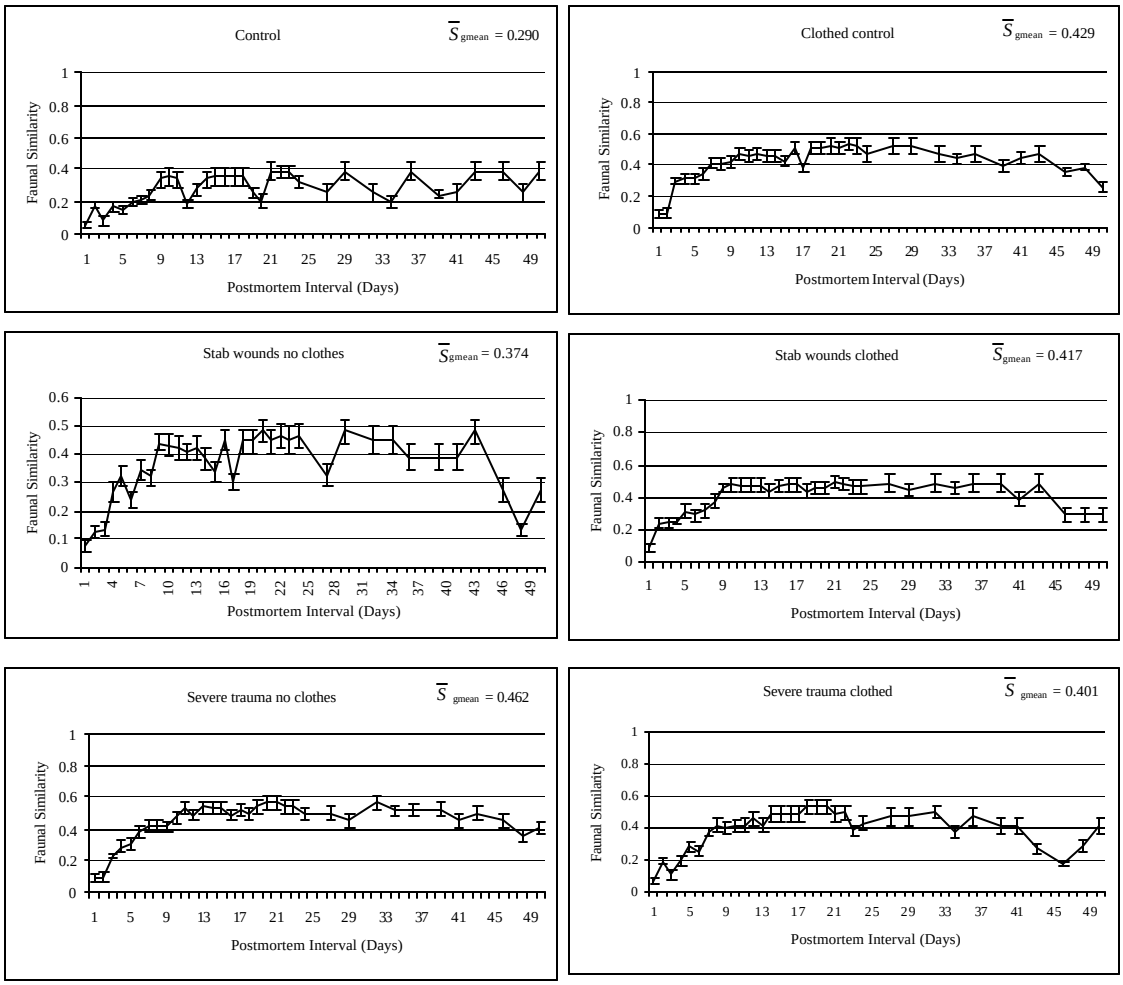


Fig. 3.5.9. Plots of mean pairwise faunal similarities (Jaccard Metric) for each sampling period in the succession during the autumn trial (vertical bars represent the standard errors).

Table 3.5.2. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values. * - significant difference (at 95% confidence interval)

	Control	**Clothed control**	**Stab wounds no clothes**
Clothed control	**0.5767 (0.0940)***		
Stab wounds no clothes	**0.6149 (0.0740)***	**0.8472 (0.0320)**	
Stab wounds clothed	**0.6925 (0.0470)**	**0.8242 (0.0260)**	**0.8312 (0.0570)**
Severe trauma no clothes	**0.6475 (0.0670)***	**0.8160 (0.0500)**	**0.7682 (0.0440)**
Severe trauma clothed	**0.6061 (0.0970)***	**0.7743 (0.0280)**	**0.7561 (0.0520)***
	Stab wounds Clothed	**Severe trauma no clothes**	
Clothed control			
Stab wounds no clothes			
Stab wounds clothed			
Severe trauma no clothes	**0.8337 (0.0290)**		
Severe trauma clothed	**0.7903 (0.0320)**	**0.7579 (0.0710)***	

Table 3.5.3. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the control and clothed control carcasses in the autumn trials of 2003 and 2004. (* - significant difference at 95% confidence interval)

	Control (2004)	Clothed control (2004)
Control (2003)	0.5544 (0.1100)*	0.7530 (0.0630)*
Clothed control (2003)	0.6098 (0.0510)*	0.7805 (0.0530)*

3.5.4. Ambient, external and internal carcass temperatures

The ambient temperature data recorded by the iButtons at the study site closely followed the data obtained from the South African Weather Services (SAWS) of the Bloemfontein city and airport stations. During the beginning of the current trial, the minimum temperatures recorded were mostly above 10^0C (Fig. 3.5.10a), while during the previous year the minimum temperatures were lower during the same period. The maximum temperatures did not vary as much as the minimum temperatures. These temperatures mostly varied between 20 and 30^0C (Fig. 3.5.10b). Towards the end of the trial there was a larger difference between the minimum and maximum temperatures (Fig. 3.5.10.).

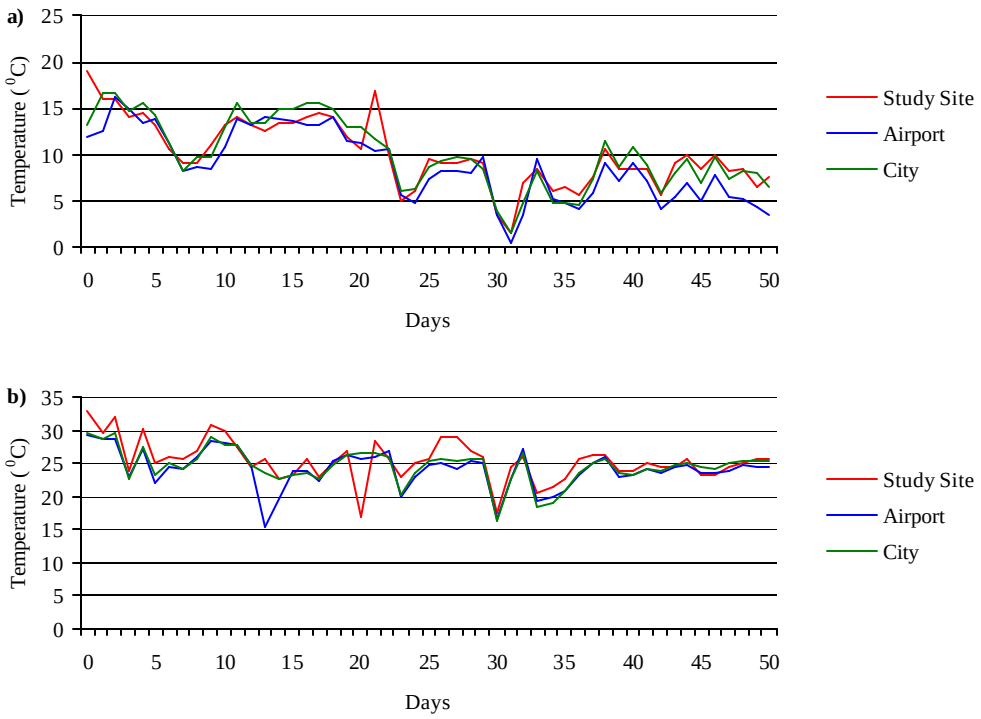


Fig. 3.5.10. Minimum (a) and maximum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the autumn wounds trial.

Data recorded from the data logger showed that the minimum internal temperatures for the stab wounds no clothes carcass were higher than the minimum ambient temperatures (Fig. 3.5.11a). However, there were no clear trends between the regions of the carcass. Unfortunately, the accuracy of the data logger were doubtful.

Many of the iButtons used in this trial to record internal temperature failed. The data that were obtained from the remaining iButtons were pooled (Fig. 3.5.12.). The iButton data showed similar trends to the data obtained from the data logger. However, the range of the temperatures appeared to be smaller.

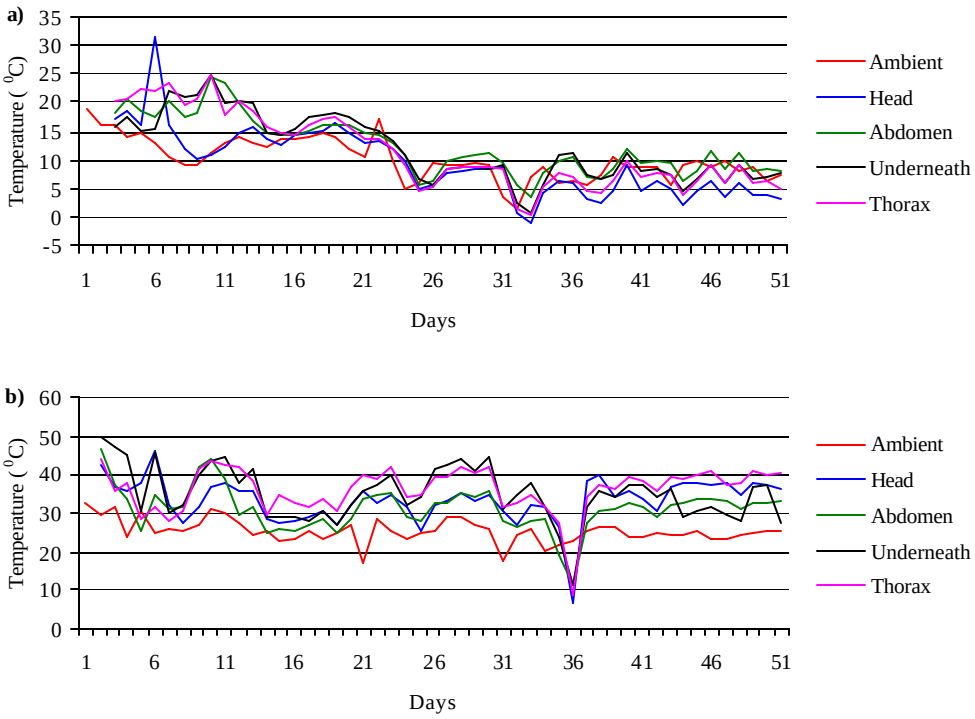


Fig. 3.5.11. Minimum (a) and maximum (b) ambient, internal and external temperatures for the stab wounds no clothes carcass during the autumn wounds trial.

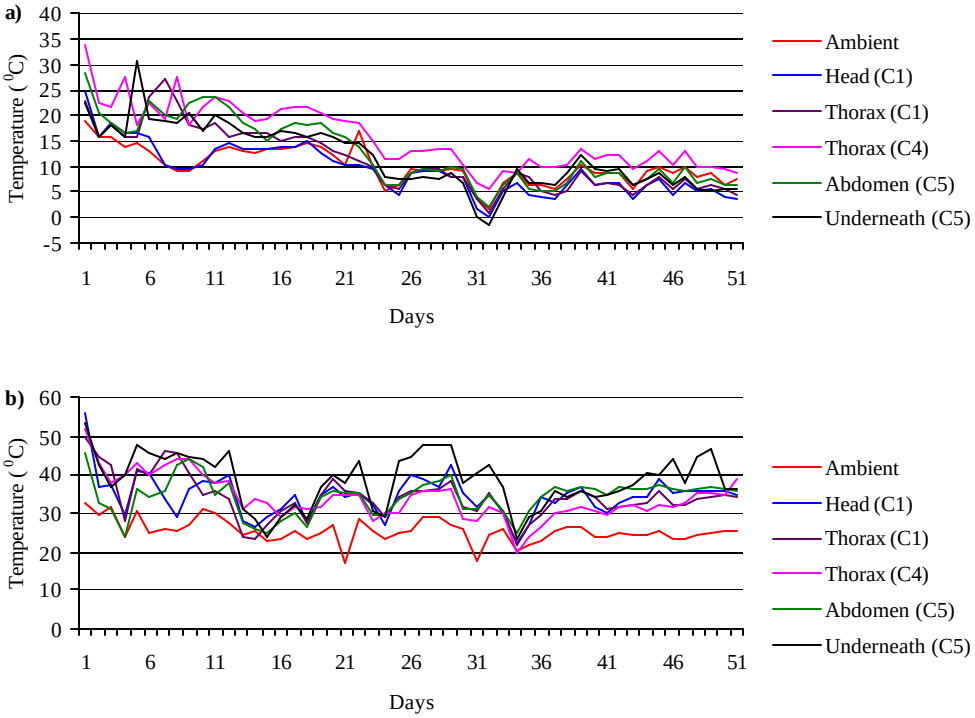


Fig. 3.5.12. Minimum (a) and maximum (b) ambient, internal and external temperatures for the pooled iButton data (five days) during the autumn wounds trial.

3.5.5. Maggot mass distribution

The adult Calliphoridae did not oviposit in or near the wounds. The females selected the natural openings of the carcasses, a trend that has been consistently recorded for all the trials. The mouth, nostrils, and pinna of the ears were the primary oviposition sites. When clothes were present, they appeared to allow more oviposition sites as eggs were occasionally found in the folds of the clothing. On the carcasses without clothing, eggs were sometimes deposited underneath the carcass in the shade.

The wounds were observed to continually seep body fluids, especially during the fresh and bloat stages of the decomposition. Since the wounds were saturated with fluids and the eggs might have drowned or been smothered by the fluids, oviposition did not take place in or near the wounds. The overall trends in the maggot mass movement are shown in Fig. 3.5.13. The maggots on the clothed carcasses tended to remain underneath the protective layer of the clothing. As the temperatures increased during the day, the maggot mass covered the surface of the carcass, although remaining under the protection of the clothing. On the carcasses without clothing, the maggots remained mostly on the ground or around the edges of the carcasses. This occurred only where the remaining skin provided some sort of protection.

The maggots more readily consumed the skin of the clothed carcasses. This was probably due to the clothing allowing the skin to remain moist and thus an acceptable substrate on which the maggots could feed. There was a slight displacement of the clothing due to maggot mass movement. There were no cases where the clothing was completely pushed off the carcasses. Although in one case the shorts barely covered the hindquarters of the carcass by the time the maggots migrated.

3.5.6. Maggot predation

There were numerous observations of *C. albiceps* maggot predation on the other maggots. This was especially noticeable on the control carcass when all the tissue on the carcass was consumed. This phenomenon was the same as described in the autumn wrapped trial.

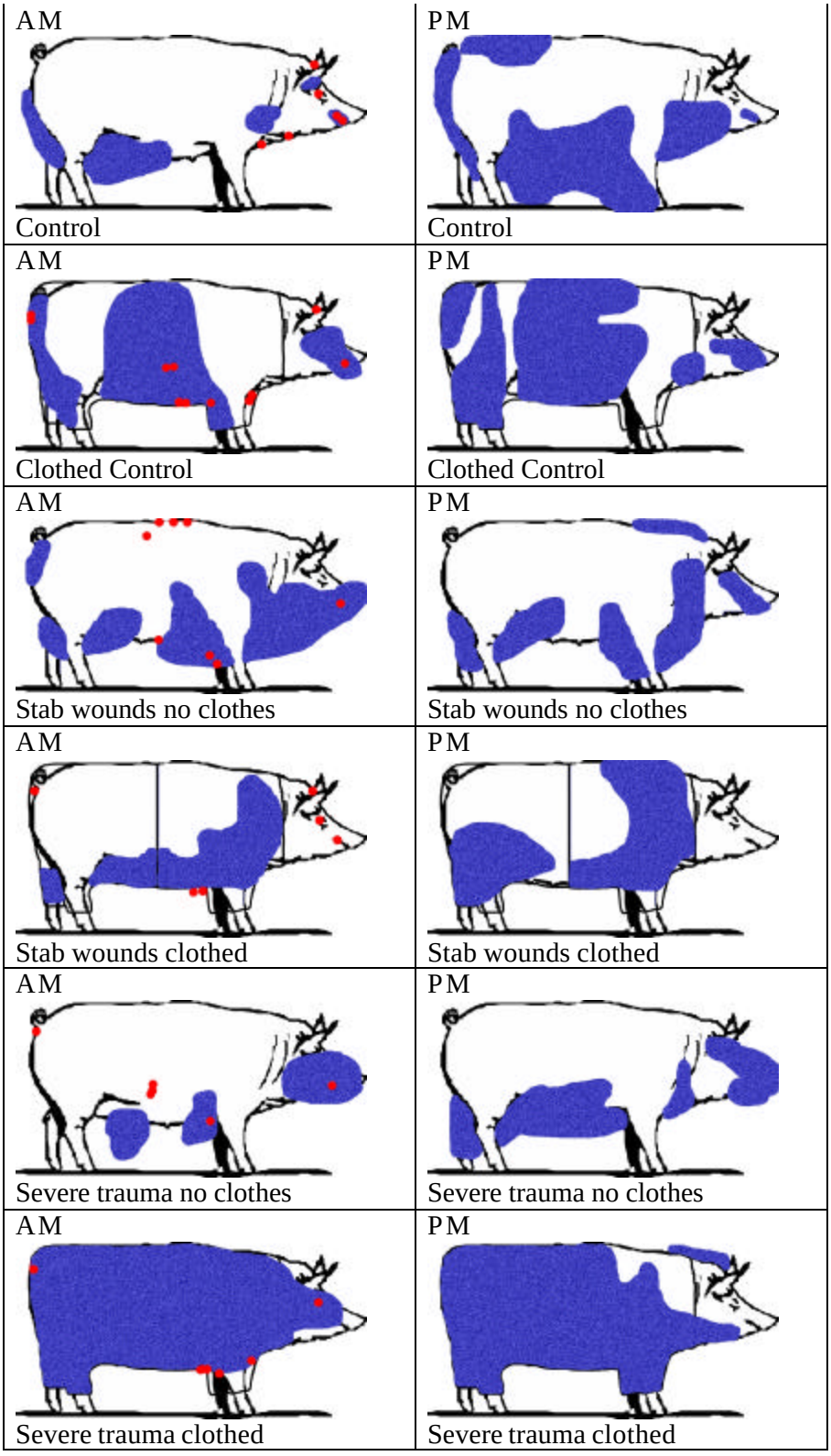


Fig. 3.5.13. Maggot mass movement recorded on day 10 of the autumn wounds trial. Dots on the morning (AM) diagrams represent the primary oviposition sites.

SECTION 3.6.

The influence of stab wounds, severe trauma and clothing on carcass decomposition and arthropod succession: a winter study.

3.6.1. Decomposition of the carcasses

The same characteristics that were used in the winter wrapped trial were used for this trial. In winter the decomposition of the carcasses was very slow due to the low ambient temperatures and characteristics differed from those used in the warmer season trials.

The carcasses were placed in the field at approximately 15:00h on the 22nd June 2004 (day 0). They began to bloat during day 1. By day 4 all but two of the carcasses had begun to soften and their limbs had collapsed back into the resting position. The two carcasses, stab wounds no clothes and the severe trauma no clothes carcasses remained in the bloated stage i.e. their torsos were still hard to the touch, inflated and had inflexible limbs, until day 9. Although eggs were laid on all the carcases simultaneously, these two carcasses only showed the characteristics associated with active decay after day 10 (Fig. 3.6.1.).

The carcasses in this trial remained in the active decay stage for almost the entire trial. Only the clothed carcasses began to show some characteristics of advanced decay stage during the final days of the trial (Fig. 3.6.1.). This contrasts with the winter wrapped trial where all the carcasses showed characteristics of advanced decay towards the end of that trial.

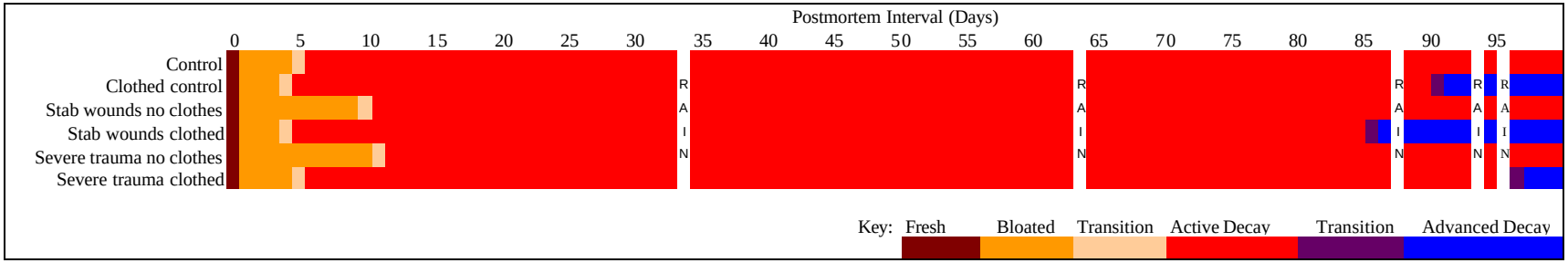


Fig. 3.6.1. Decomposition stages of the carcasses in the winter wounds trial.

3.6.2. Arthropod succession on the carcasses

The most dominant species recorded during this trial (Table 3.6.1.) were the same as those recorded during the winter wrapped trial.

Table 3.6.1. Most common forensically important species occurring in winter wounds trial.

Diptera	Coleoptera
Calliphoria vicina	*Dermestes maculatus*
Chrysomya chloropyga	*Necrobia rufipes*
Lucilia spp.	*Saprinus* sp.
Sarcophaga cruentata	

As with the previous winter trial, the Diptera, both adults and maggots were present on the carcasses at the same time as the Coleoptera adults and larvae. This again emphasizes the importance of the season when determining an estimated minimum postmortem interval using insect succession.

The presence of wounds and blood did not appear to influence the numbers or frequency of Calliphoridae recorded on the carcasses. This was unexpected as the numbers of Diptera utilizing the carcasses during winter was low. Thus, the competition for the carcasses was correspondingly low. This should have allowed the Diptera to be more selective as regards the presence of wounds and blood. However, this was not the case. *Chrysomya chloropyga* were recorded on the all carcasses from day 6, except the stab wounds no clothes carcass where they were recorded from day 8 (Figs 3.6.3. to 3.6.8.). On some carcasses their number increased between day 60 and 70.

Lucilia spp were recorded on the carcass from day 1 to day 7 on all the carcasses (Figs 3.6.3. to 3.6.8.). *Chrysomya albiceps* individuals were recorded on the control carcass on day 2 (Fig. 3.6.3.). They were recorded on the stab wounds no clothes (Fig. 3.6.5.) and severe trauma clothed (Fig. 3.6.8.) carcasses from day 6. They were recorded from day 7 on the stab wounds clothed (Fig. 3.6.6.) carcass and by the following day on the clothed control (Fig. 3.6.4.) and severe trauma clothed (Fig. 3.6.8.) carcasses. *Sarcophaga cruentata* were recorded from day 2 on the stab wounds clothed carcass (Fig. 3.6.6.). They were recorded on the severe trauma no clothes (Fig. 3.6.7.) and the severe trauma clothed carcasses (Fig. 3.6.8.) from day 4. By day 6, *S. cruentata* were recorded on the remaining carcasses i.e. the control (Fig. 3.6.3.), clothed control (Fig. 3.6.4.) and stab wounds clothed (Fig. 3.6.6.) carcasses. All these Diptera were recorded sporadically but occurred throughout the trial (Figs 3.6.3. to 3.6.8.).

Adult *D. maculatus*, were recorded on the control (Fig. 3.6.3) and severe trauma clothed (Fig. 3.6.8.) carcasses from day 15. They were recorded on the remaining carcasses from day 14 (Figs 3.6.4. to 3.6.7.). This was comparable to the previous winter trial when they were recorded on the carcasses within 14 days. Their numbers increased as the trial progressed, however, they appeared to increase to a certain level and then were recorded in constant numbers for the remainder of the trial. Overall, the numbers of *D. maculatus* adults recorded during this trial were lower then the previous winter trial.

Scattered individuals of *D. maculatus* larvae were recorded on the carcasses from approximately day 30. They were recorded consistently on the clothed control carcass

from day 29 and on the control (Fig. 3.6.3.) and stab wounds clothed carcasses (Fig. 3.6.6.) from day 30. And the larvae were recorded on the stab wounds no clothes (Fig. 3.6.5.), severe trauma no clothes (Fig. 3.6.7.) and severe trauma clothed (Fig. 3.6.8.) carcasses from day 33. Their presence only became established and their numbers increased from between day 45 and 50. They became the most dominant arthropods recorded on the carcasses from approximately day 65. This trend was similar to the previous winter trial where *D. maculatus* larvae were recorded for the first time on day 31 on the clothed control and the wrapped clothed carcasses. They were only recorded on the wrapped carcass with no clothes from day 43. Lastly, they were recorded on the control carcass only from day 53.

Necrobia rufipes generally occurred in lower numbers during the trial. They were recorded consistently on each carcass from day 14 on the control (Fig. 3.6.3.), stab wounds no clothes (Fig. 3.6.5.), severe trauma no clothes (fig. 3.6.7.) and severe trauma clothed carcasses (Fig. 3.6.8.). They were recorded a day later on the clothed control (Fig. 3.6.4.) and stab wounds clothed (Fig. 3.6.6.) carcasses. Their numbers increased as the trial progressed, but not nearly to the extent as the *D. maculatus* larvae. During the previous winter wrapped trial, *N. rufipes* adults were only recorded from approximately day 21 onwards on the control and clothed control carcasses.

The Histeridae, *Saprinus* sp. were also present on the carcasses for most of the trial. This species appears to be a seasonally driven species as it has only been recorded in the cooler seasons. Their numbers varied between carcasses similar to the *N. rufipes*, but their presence was consistent between the different carcasses. The number of *Saprinus* sp. recorded also varied between carcasses during the previous winter trial. However, their presence was not as consistent.

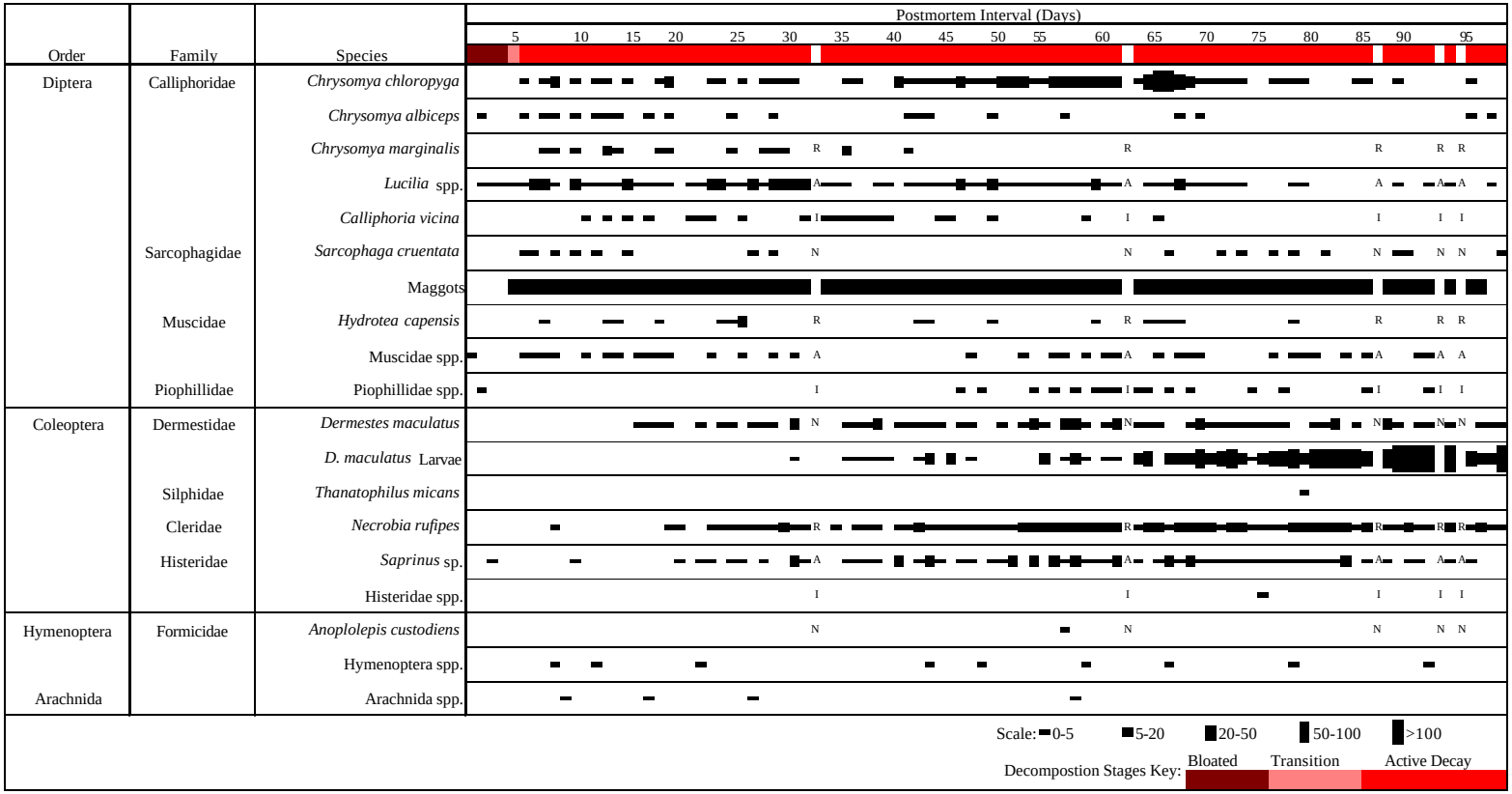


Fig. 3.6.3. Arthropod succession on the control carcass during the winter wounds trial. Maggots – all species.

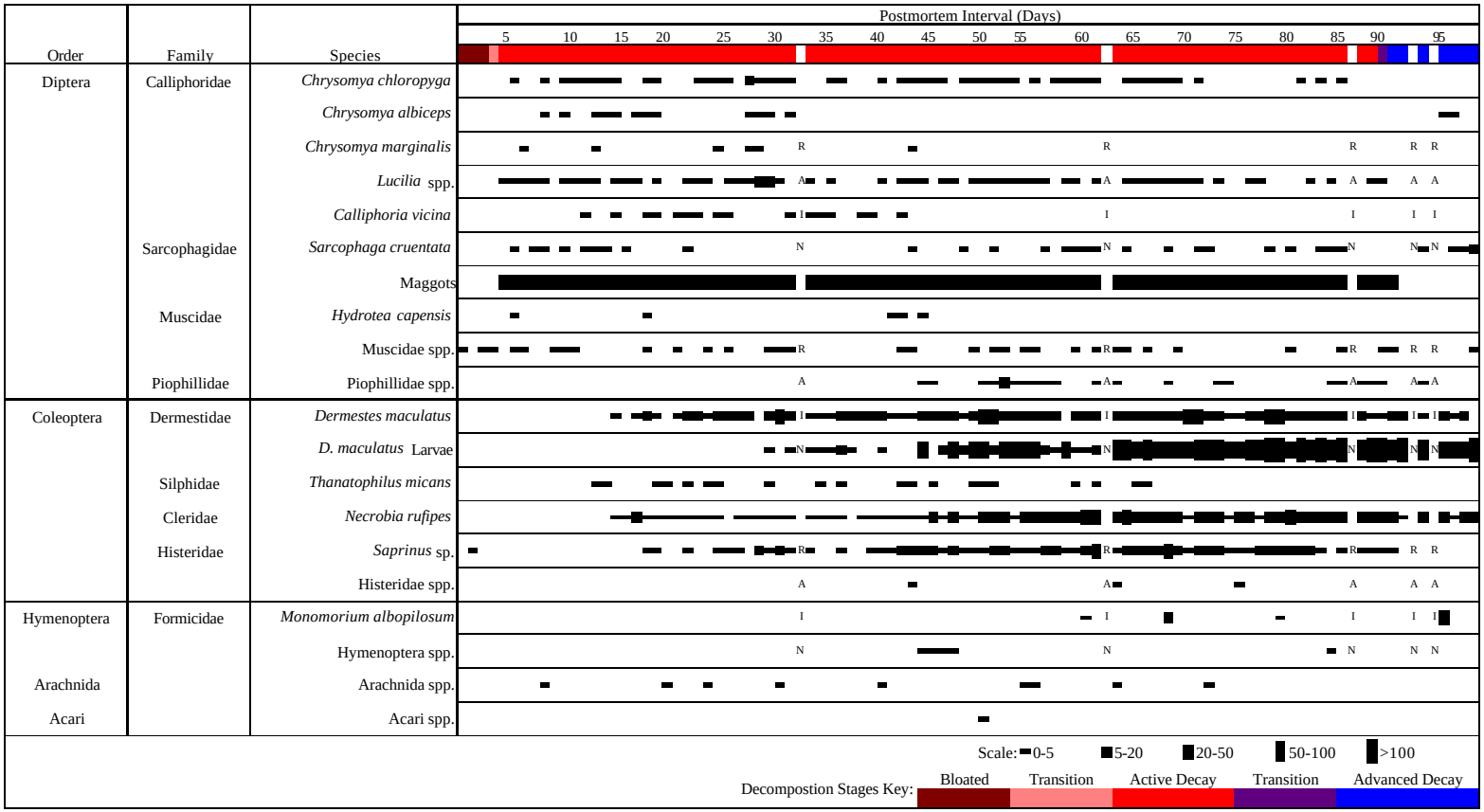


Fig. 3.6.4. Arthropod succession on the clothed control carcass during the winter wounds trial. Maggots – all species.

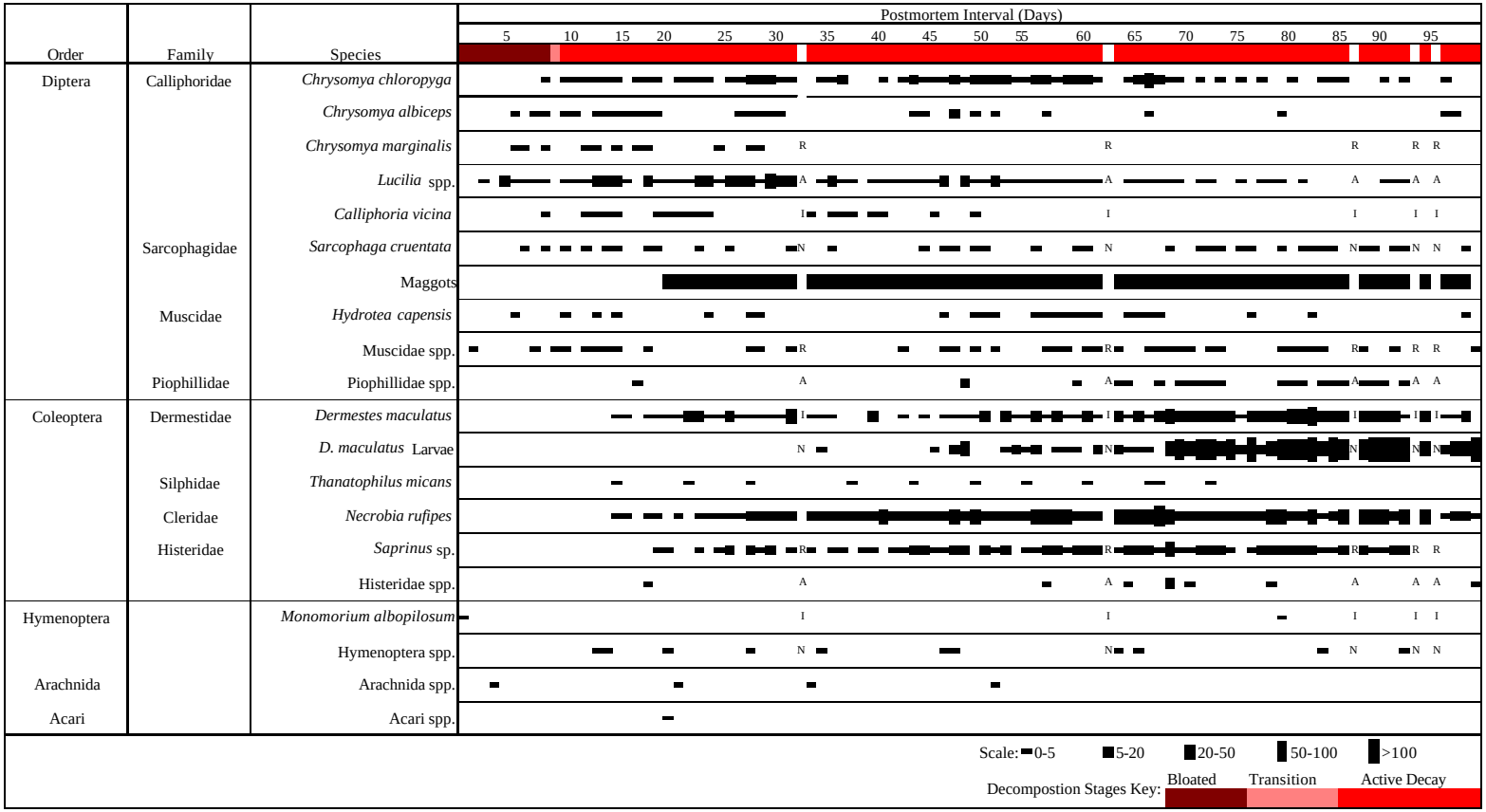


Fig. 3.6.5. Arthropod succession on the stab wounds no clothes carcass during the winter wounds trial. Maggots – all species.

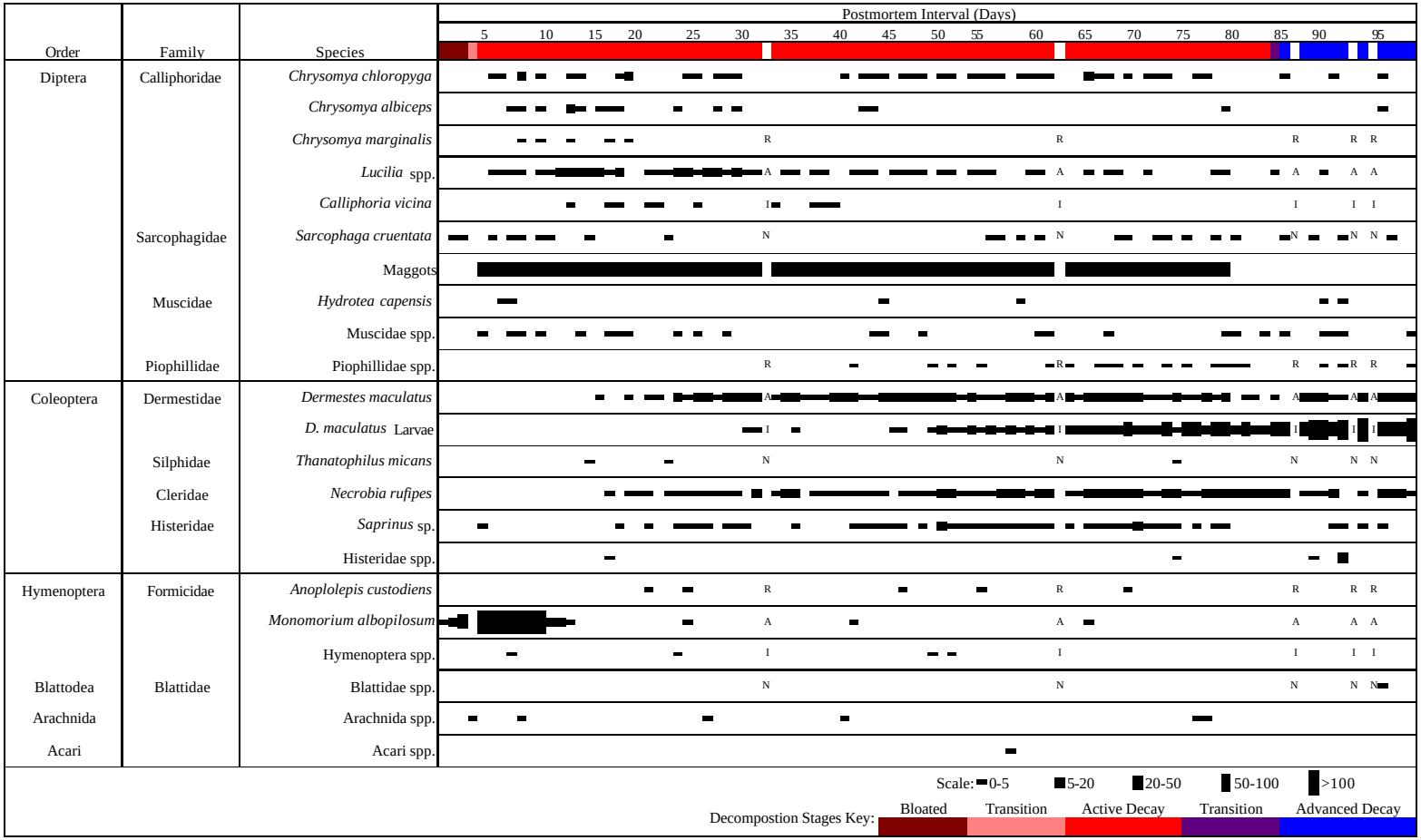


Fig. 3.6.6. Arthropod succession on the stab wounds clothed carcass during the winter wounds trial. Maggots – all species.

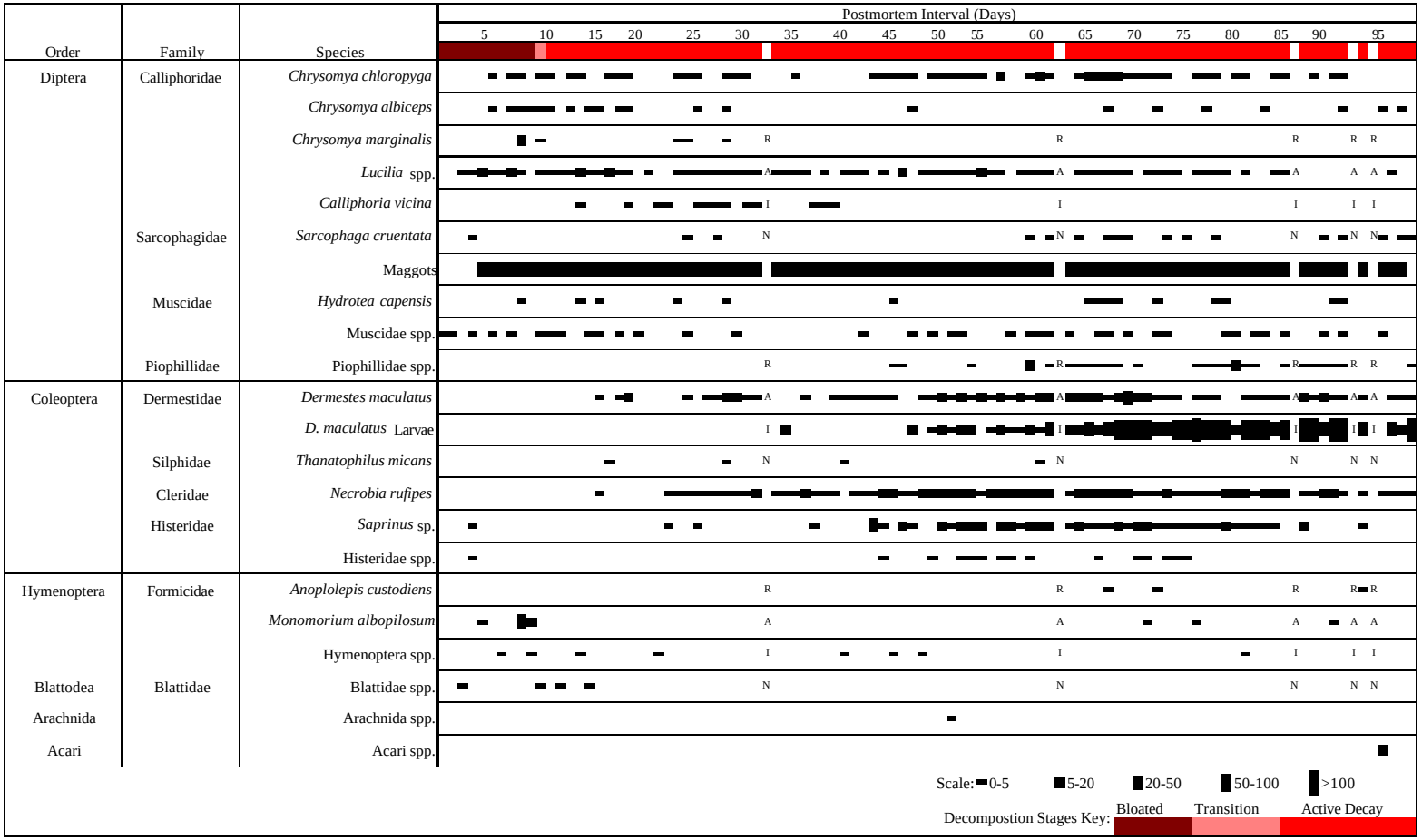


Fig. 3.6.7. Arthropod succession on the severe trauma no clothes carcass during the winter wounds trial. Maggots – all species.

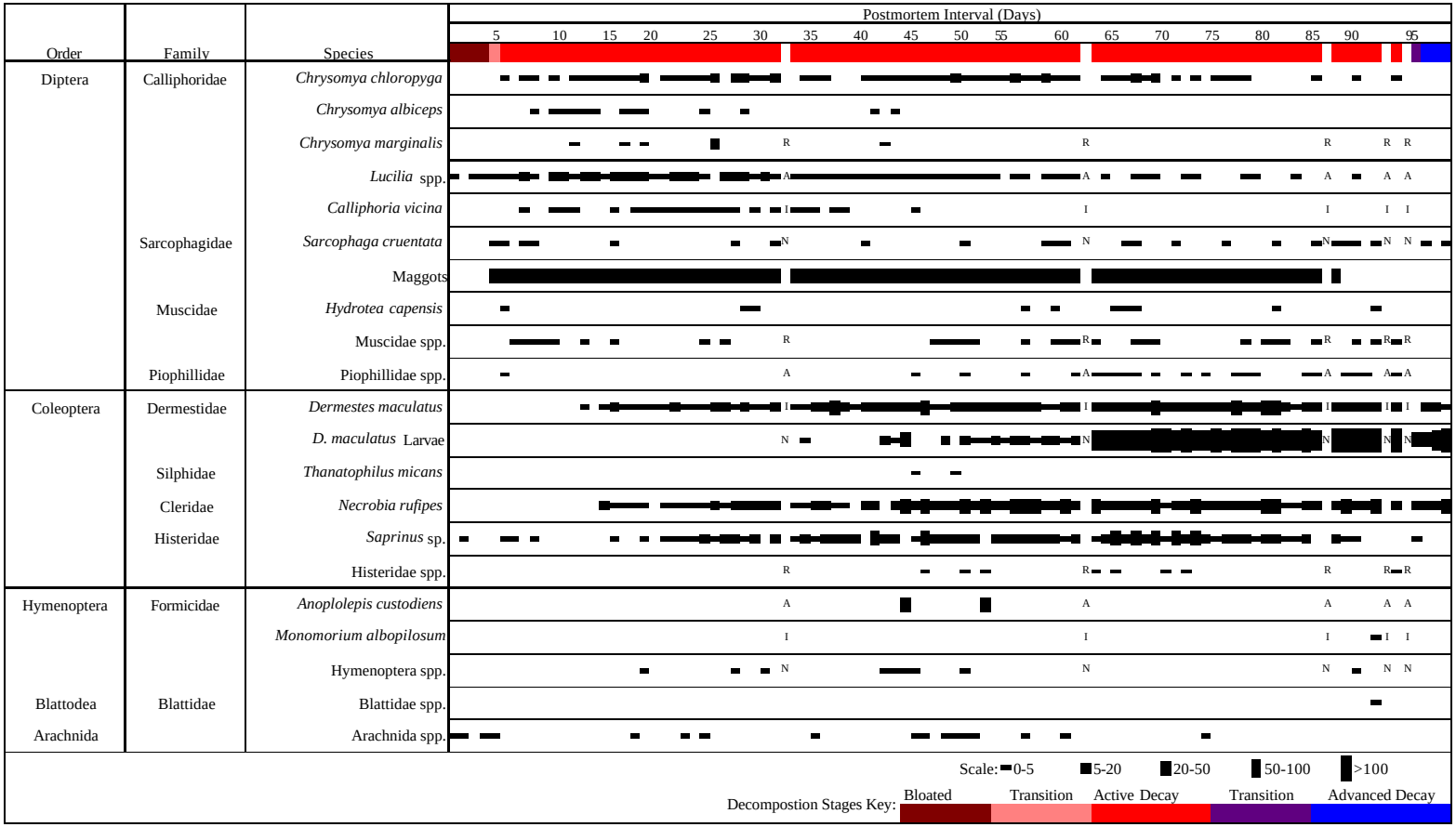


Fig. 3.6.8. Arthropod succession on the severe trauma clothed carcass during the winter wounds trial. Maggots – all species.

Unfortunately, the success rate in rearing the maggot samples during this trial was very low. Exactly why this happened was unknown as they were reared in the laboratory under the same conditions as the winter wrapped trial. As a result all the data collected were pooled (Table 3.6.2.). However, as with the winter wrapped trial, *S. cruentata* , *C. vicina* and *Lucilia* spp. appeared to have the highest number of maggots on the carcasses with some *C. chloropyga* maggots.

Table 3.6.2. Diptera species raised from maggot masses from all carcasses during the winter wounds trial.

	Postmortem Interval (Days)													
	15	20	25	30	35	40	45	50	55	60	65	70	75	80
Chrysomya chloropyga			X		X					X		X		
Lucilia spp.		X	X	X	X	X		X	X	X	X	X	X	
Calliphora vicina				X	X	X	X	X	X	X		X	X	X
Sarcophaga cruentata	X	X	X	X	X	X	X	X	X	X			X	X

There was an extended period over which the eggs were deposited on the carcasses. The maggots found in the maggot masses were of mixed age and consisted of second and third instar maggots. Often eggs were found on the carcasses at the same time as the maggots (Fig. 3.6.9.). The maggots developed slowly due to the low ambient temperature experienced during this trial.

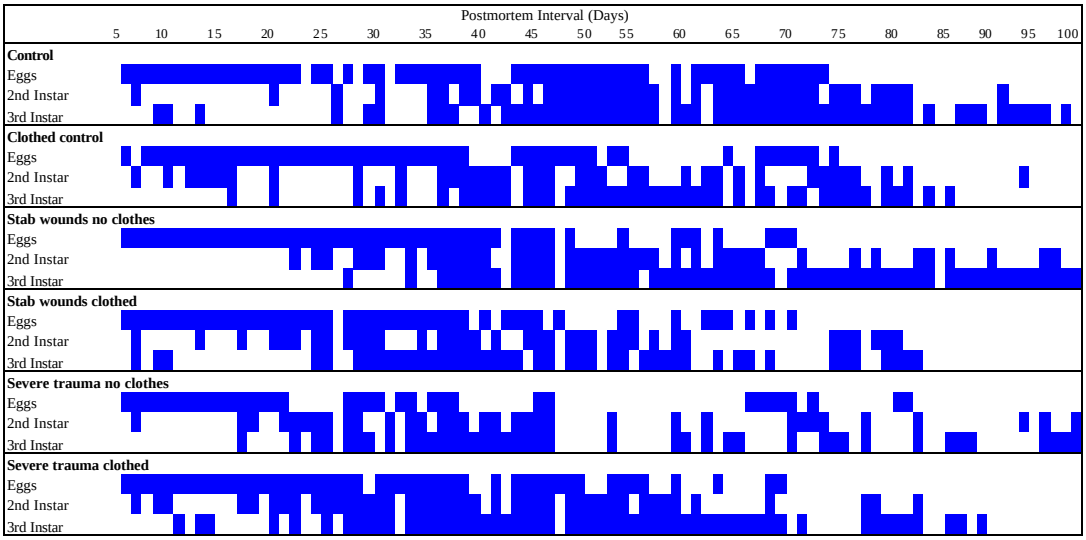


Fig. 3.6.9. Egg and maggot succession for all carcasses for the winter wounds trial.

3.6.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices (see 2.4.1.). The mean faunal similarity was then calculated for each day. These were plotted and the grand mean of the between mean faunal similarity was calculated and is represented as $\overline{S}_{gmean}$. The $\overline{S}_{gmean}$ values for this trial ranged from 0.326 to 0.425. All the carcasses showed an overall increase in faunal similarity in the beginning of the trials. The trend tended to level off for the remainder of the trial (fluctuating around the $\overline{S}_{gmean}$ values) (Fig. 3.6.10.). The fluctuations between the samples may be a result of the species not always being recorded in successive days due to the low numbers of individuals present at the carcasses. The $\overline{S}_{gmean}$ values for the winter wrapped trial ranged from 0.333 to 0.442.

When Schoenly (1992) compared a variety of studies, he found the $\overline{S}_{gmean}$ values ranged from 0.218 to 0.808. As with the previous trials the $\overline{S}_{gmean}$ values obtained from the current study, were more consistent with a smaller range. The result obtained in this study may be a good indication as to the species similarity found on the carcasses.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

All carcasses showed a significant difference between their matrices (Table 3.6.3.). This was a similar result to that found in the previous winter trial (the daily sampled carcasses). This suggests that the sample size and low number of individuals may have been too small for an accurate comparison. Although there was no change in species richness between the carcasses, the species were often recorded at different times. This was probably due to the cryptic nature and daily movement of the individual insects.

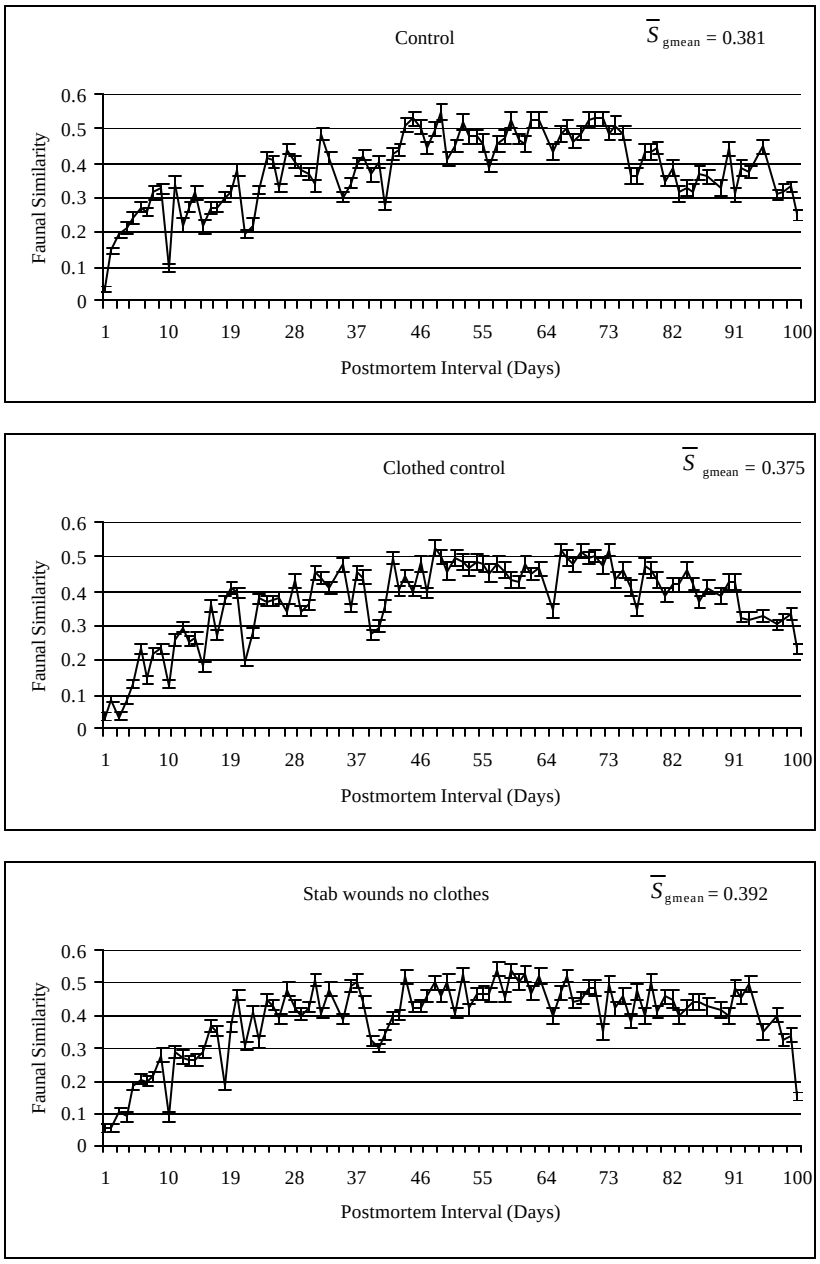


Fig. 3.6.10. Plots of mean pairwise similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors) during the winter wounds trial.

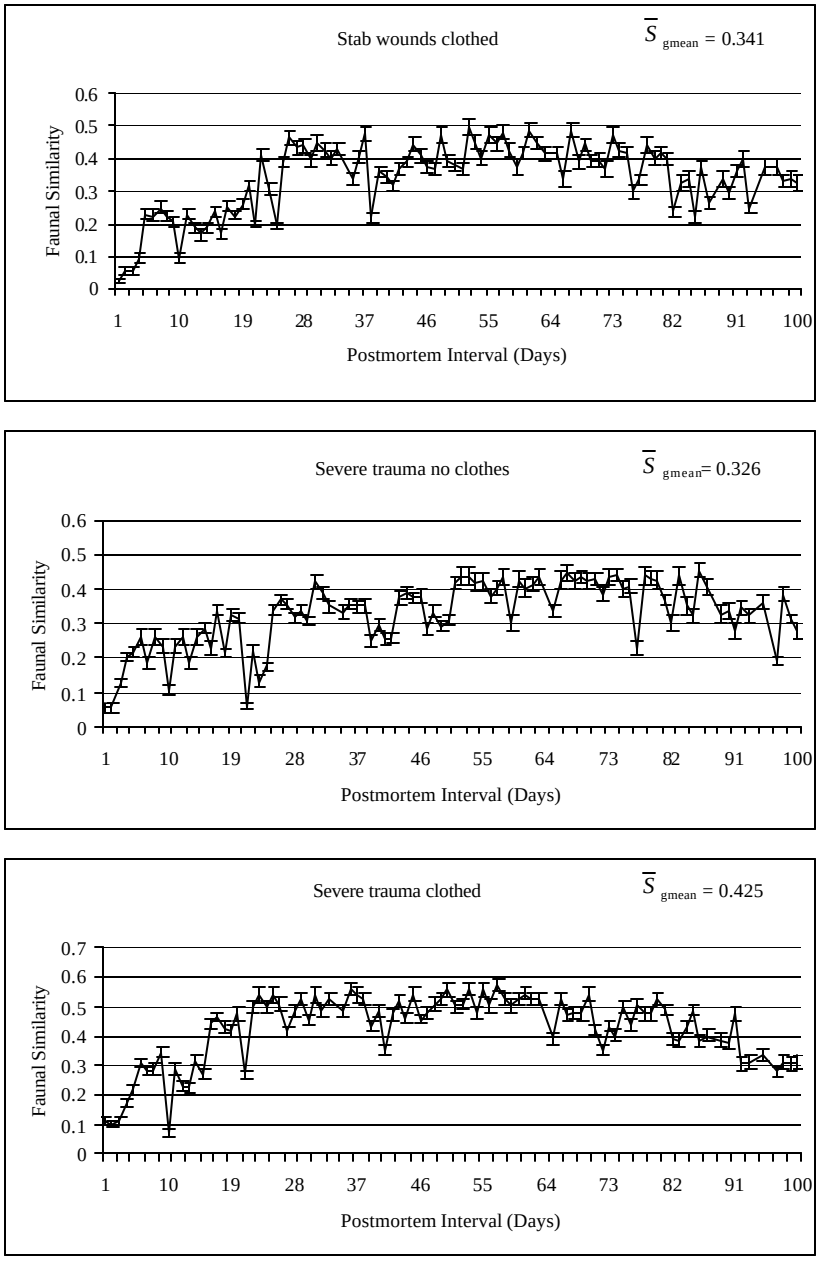


Fig. 3.6.10. cont. Plots of mean pairwise similarities (Jaccard Metric) for each sampling period in the succession (vertical bars represent the standard errors) during the winter wounds trial.

Table 3.6.3. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the winter wounds trials (* - significant difference at 95% confidence interval)

	Control	**Clothed control**	**Stab wounds no clothes**
Clothed control	**0.6816 (0.0740)***		
Stab wounds no clothes	**0.6570 (0.1040)***	**0.7326 (0.0620)***	
Stab wounds clothed	**0.6369 (0.0970)***	**0.6457 (0.0950)***	**0.6881 (0.0850)***
Severe trauma no clothes	**0.6256 (0.0760)***	**0.6354 (0.0870)***	**0.6428 (0.0980)***
Severe trauma clothed	**0.6378 (0.0670)***	**0.7127 (0.0710)***	**0.7359 (0.0570)***
	Stab wounds clothed	**Severe trauma no clothes**	
Clothed control			
Stab wounds no clothes			
Stab wounds clothed			
Severe trauma no clothes	**0.6439 (0.0690)***		
Severe trauma clothed	**0.6789 (0.0750)***	**0.5913 (0.0930)***	

Table 3.6.4. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the control and clothed control carcasses in the spring of 2003 and 2004 (* - significant difference at 95% confidence interval).

	Control (2004)	Clothed control (2004)
Control (2003)	0.6800 (0.1130)*	0.6982 (0.1250)*
Clothed control (2003)	0.7566 (0.1150)*	0.7911 (0.0560)*

3.6.4. Ambient, external and internal carcass temperatures

The minimum temperatures recorded at the study site were higher than the data obtained from the South African Weather Services (SAWS) at the Bloemfontein airport and city stations (Fig. 3.6.11a). The minimum ambient temperature range was high and they varied from -7 to 14^0C. The maximum ambient temperatures increased as the trial progressed, and were not as variable as the minimum temperatures (Fig. 3.6.11b.). The iButtons at the study site recorded maximum temperatures that were very similar to the data obtained from the South African Weather Services (SAWS, airport and city)

The problems with iButtons that were experienced during the autumn wounds trial were overcome. The iButtons were sealed in a double layer of plastic which protected them. iButtons in plastic were placed side by side with unsealed iButtons at the study site and it was found that the plastic had no affect on the data recorded.

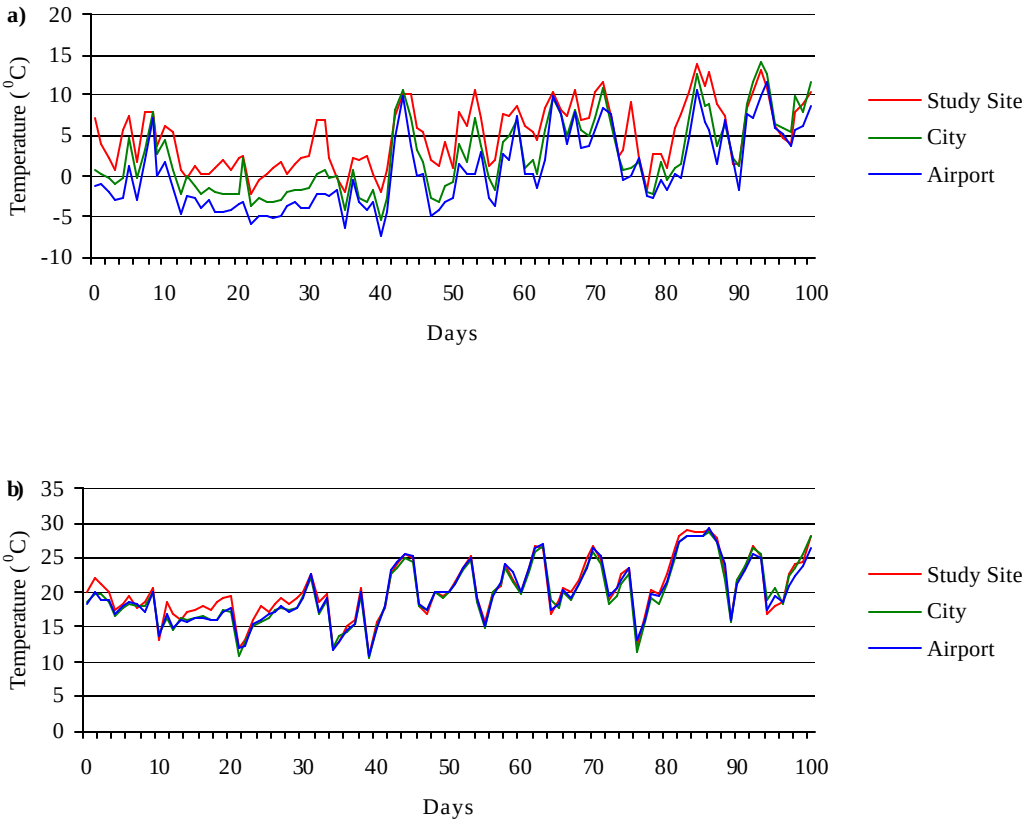


Fig. 3.6.11. Minimum (a) and maximum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the winter wounds trial.

The average for minimum and maximum temperatures obtained for all the heads of the carcasses and the ambient minimum and maximum temperatures are shown in Figure 3.6.12. Overall, the minimum and maximum averages had very low standard errors, showing that there was little variance between the carcasses. The minimum average head temperatures were almost the same as the minimum ambient temperatures, indicating that the heads do not retain any heat. The maximum average head temperatures were slightly higher than the maximum ambient temperatures. (Fig. 3.6.12).

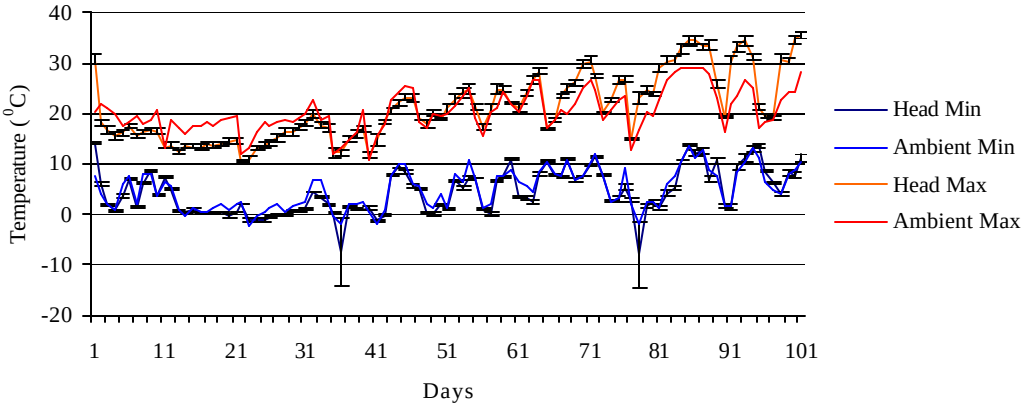


Fig. 3.6.12. Maximum and minimum ambient and internal carcass head temperatures during the winter wounds trial.

The average minimum and maximum temperatures obtained for all the thoraxes from the carcasses and the ambient minimum and maximum temperatures are shown in Figure 3.6.13. The minimum average thorax temperatures had low standard errors, indicating that the data between the carcasses had little variance. The minimum average thorax temperatures also did not show much difference from the ambient temperatures (Fig. 3.6.13.). The difference between the maximum average thorax temperatures and the ambient maximum temperatures increased as the trial progressed (Fig. 3.6.13.). While this may seem to indicate maggot mass activity, maggots were not recorded in this region of the carcass. Thus, this increase in temperatures is probably a result of decomposition.

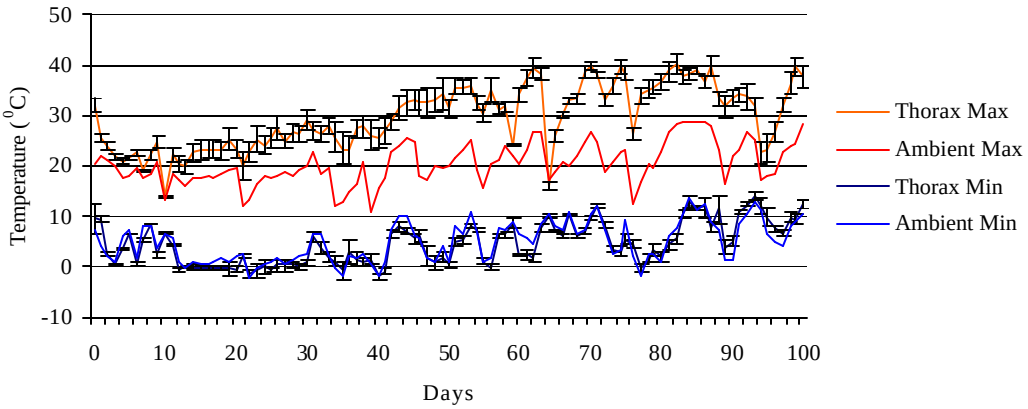


Fig. 3.6.13. Maximum and minimum ambient and internal thorax carcass temperatures during the winter wounds trial.

The average minimum and maximum temperatures obtained from the abdomens of the various carcasses and the ambient minimum and maximum temperatures are shown in Fig. 3.7.14. The trends recorded for the abdomen temperatures correlated very closely with those observed for the thorax (Fig. 3.6.14.).

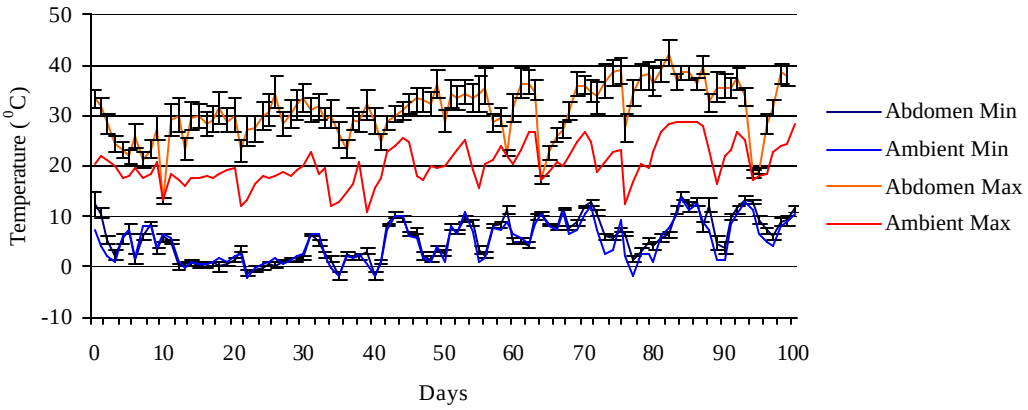


Fig. 3.6.14. Maximum and minimum ambient and internal abdomen carcass temperatures during the winter wounds trial.

The average minimum and maximum temperatures obtained from the surface of all the carcasses and the ambient minimum and maximum temperatures are shown in Figure 3.6.15. The trends recorded for the surface temperatures correlated closely with those observed for the thorax and abdomen (Fig. 3.6.15.). However, the differences between the average maximum surface temperatures and the ambient maximum temperatures may be due to the carcasses maintaining a high body mass and solid matter which absorbed more heat than the ambient temperatures.

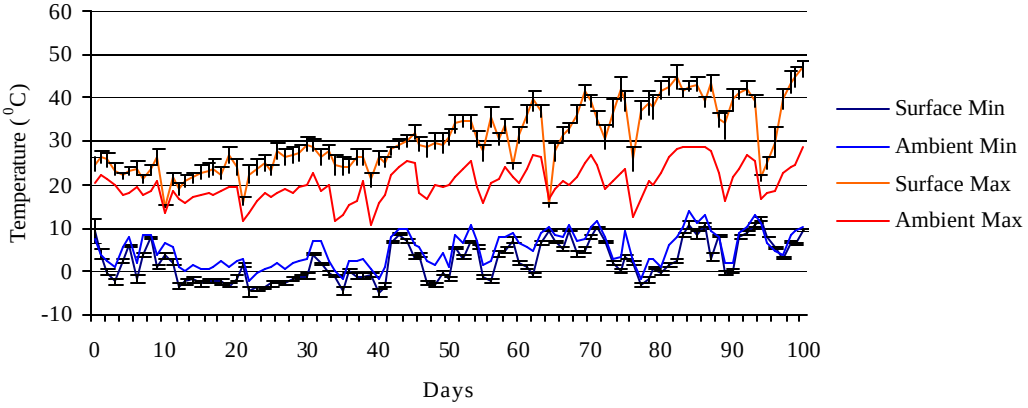


Fig. 3.6.15. Maximum and minimum ambient and carcass surface temperatures during the winter wounds trial.

The average minimum and maximum temperatures obtained from underneath all the carcasses and the ambient minimum and maximum temperatures are shown in Fig. 3.6.16. The average minimum temperatures underneath the carcasses were higher than the ambient minimum temperatures (Fig. 3.6.16.). The average maximum temperatures underneath the carcasses were higher than the ambient maximum temperatures. The carcasses may have provided some insulation and retained the heat. However, the difference was not as large as seen in other regions of the carcasses.

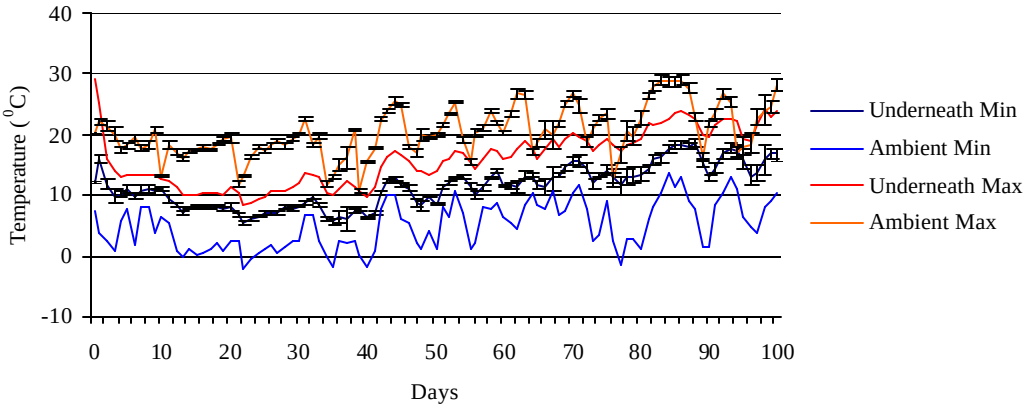


Fig. 3.6.16. Maximum and minimum ambient and underneath of the carcasses temperatures during the winter wounds trial.

3.6.5. Maggot mass distribution

The oviposition sites were consistent on all the carcasses. The mouth, nostrils and pinna of the ears were the primary oviposition sites. The eyes, inside the clothing, when present and underneath the carcasses on the ground were also utilised. This pattern was similar to the winter wrapped trial. The maggot masses were restricted to the head and between the fore and hind limbs of the carcasses. The maggot masses were generally smaller, consisting of fewer individuals, than those recorded during the winter wrapped trial. The presence of wounds or clothing did not appear to affect the maggot mass distribution. There was no displacement of clothing.

SECTION 3.7.

The influence of stab wounds, severe trauma and clothing on carcass decomposition and arthropod succession: a spring study.

3.7.1. Decomposition of the carcasses

The same set of characteristics that were used for the autumn wrapped trial was applied to the current study. The carcasses were placed on the 12th September 2004 (day 0). As expected and recorded in the wrapped spring trial, the carcasses did not stay in the fresh stage for longer than a few hours. The carcasses began to bloat within hours of placement (Fig. 3.7.1.).

The bloat stage passed quickly and by day 3 the carcass limbs had collapsed back into the resting position and the torsos had begun to soften (Fig. 3.7.1.). This was almost identical to the observations recorded for the control and clothed control carcasses during the spring wrapped trial. However, the transition into active decay was not as clearly defined as seen in the autumn wrapped trial. After the rainfall on day 5, the carcasses entered the active decay stage simultaneously (Fig. 3. 7.1.).

The characteristics of active decay were seen until day 12 (Fig. 3.7.1.). Similarly, during the spring wrapped trial, active decay was recorded on the control and clothed control carcasses until day 11. After this time, the first signs of advanced decay were observed. All the carcasses, with the exception of the control carcass, entered the advanced decay stage between days 16 and 17 (Fig. 3.7.1.). During the spring wrapped trial the control carcass and clothed control carcass reached this stage by day 15.

The advanced decay stage persisted until the end of the trial (Fig. 3.7.1.). This was different from the control and clothed control carcasses in the spring wrapped trial, where the carcasses showed characteristics of the dry remains stage by the end of the trial. The delay of this stage recorded during the current trial was probably due to the moist conditions due to higher rainfall than the previous year. However, the similarities between this trial and the previous trial show that there was a seasonal trend in the decomposition of the carcasses.

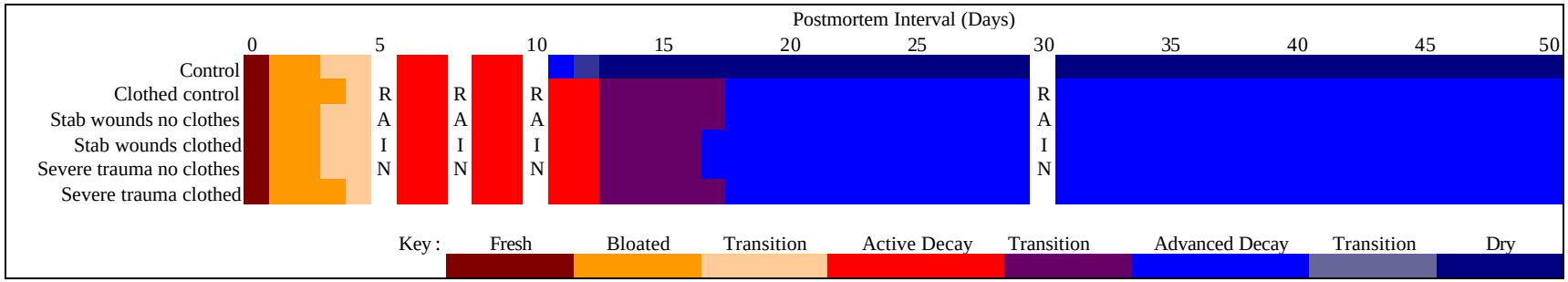


Fig. 3.7.1. Decomposition stages of the carcasses in the wounds spring trial.

The control carcass, however, dried out very quickly after the maggots consumed almost all of the tissues and skin of the carcass. The bony remains dried out quickly (Fig. 3.7.1). Why the maggots consumed all the tissues of this particular carcass is unknown.

3.7.2. Arthropod succession on the carcasses

The most common species recorded during this trial (Table 3.7.1.) were the same species recorded during the spring wrapped trial.

Table 3.7.1. Most common species occurring in spring wounds trial.

Diptera	Coleoptera
Chrysomya chloropyga *Chrysomya albiceps* *Lucilia* spp. *Sarcophaga cruentata*	*Dermestes maculatus* *Necrobia rufipes*

Chrysomya chloropyga adults were the most abundant of the Calliphoridae, recorded on the carcasses. Between two and 25 individuals were recorded on all carcasses on morning of day 1. Their numbers increased until by the afternoon of day 2 when more than 100 individuals were recorded on the all the carcasses (Figs 3.7.2. to 3.7.7.). After the rain on day 5, their numbers decreased to below five individuals with the exception of the stab wounds clothed (Fig. 3.7.5.) and severe trauma no clothes (Fig. 3.7.6.) carcasses where between five and 20 individuals were recorded. Single individuals of *C. chloropyga* were recorded sporadically on the carcasses after the afternoon of day 6. Despite the rain on day 5 this pattern was similar to the spring wrapped trial.

A few individuals of adult *C. albiceps* were recorded on the carcasses on the afternoon of day 1 (Figs 3.7.2 to 3.7.7.). A single individual was recorded on the severe trauma clothed carcass on the morning of day 1 (Fig. 3.7.7.). *Chrysomya albiceps* numbers increased from the morning of day 3, but were not recorded in numbers higher than 100. There were between one and five individuals recorded on the carcasses on day 6 after the rain on day 5 and single individuals were sporadically recorded on the carcasses after the rain on day 7 (Figs 3.7.2. to 3.7.7.).

The Muscidae spp. numbers during the first part of this trial were noticeably higher than those recorded during all the previous trails. Although their numbers decreased, they were recorded on the carcasses until the end of the trial (Figs 3.7.2 to 3.7.7.). This may have been due to the carcasses remaining moist and the adult Muscidae spp. were able to continue to feed on the fluids.

Dermestes maculatus adults recorded on the carcasses as early as the afternoon of day 2 on the control (Fig. 3.7.2.), clothed control (Fig. 3.7.3.) and the severe trauma clothed carcasses (Fig. 3.3.7.). They were recorded on the morning of day 3 on the stab wounds no clothes (Fig. 3.7.4.), stab wounds clothed (Fig. 3.7.5.) and severe trauma no clothes (Fig. 3.7.6.) carcasses. The number of *D. maculatus* adults increased as the trial continued, except the control carcass where only between one and 20 individuals were recorded throughout the trial (Figs 3.7.2. to 3.7.7.). This was different from the spring wrapped trial when the first *D. maculatus* adults were recorded on the carcasses from day 10.

Dermestes maculatus larvae were recorded on the severe trauma clothed carcass (Fig. 3.7.7.) from day 17. A day later they were recorded on the clothed control (Fig. 3.7.3.), stab wounds clothed (Fig. 3.7.5.) and the severe trauma no clothes (Fig. 3.7.6.) carcasses. Their numbers steadily increased until there were over 100 individuals. *Dermestes maculatus* larvae were only recorded on the control carcass by day 22 (Fig. 3.7.2.).

Necrobia rufipes were present on all the carcasses by the afternoon of day 2 with the exception of the clothed control, when they were present by the following morning. This was earlier than the spring wrapped trial when their presence was recorded by day 10. Their numbers slowly increased as the trial progressed (Figs 3.7.2 to 3.7.7.).

Saprinus sp., which was common during the winter trials, were recorded in numbers less than five, on the carcasses during the beginning of this trial. They were not recorded later in the trial, which suggests they were more abundant in the colder months.

Thanatophilus micans, was recorded on all the carcasses sporadically. They were only recorded on two carcasses during the spring wrapped trial. They successfully bred and *T. micans* larvae were recorded very sporadically between day 9 and day 19 on all the carcasses except the severe trauma no clothes carcass (Figs. 3.7.2 to 3.7.7).

Formicidae, *A. custodiens* and *Crematogaster* sp. were recorded on four of the carcasses (Figs 3.7.2. to 3.7.4, 3.7.7.). Generally, these Formicidae were recorded early in the trial. They did not appear to remove any eggs, but were observed feeding on the fluids from the carcasses.

Oviposition took place on all the carcasses simultaneously over the first four days, which was similar to the spring wrapped trial. The first eggs were found on all the carcasses by the afternoon of day 1 (Fig. 3.7.8.).

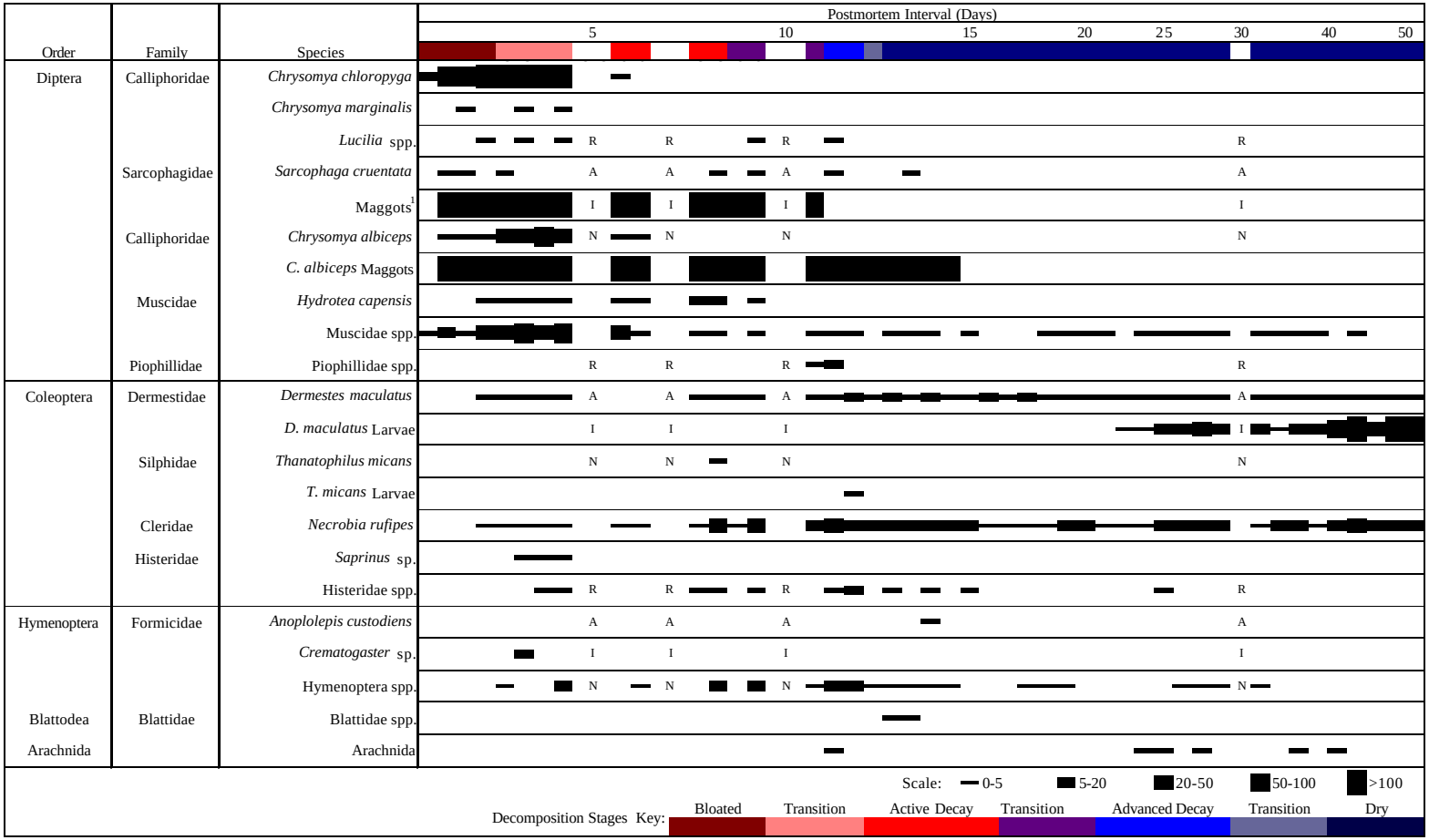


Fig. 3.7.2. Arthropod succession on the control carcass during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp. and *Sarcophaga cruentata* .

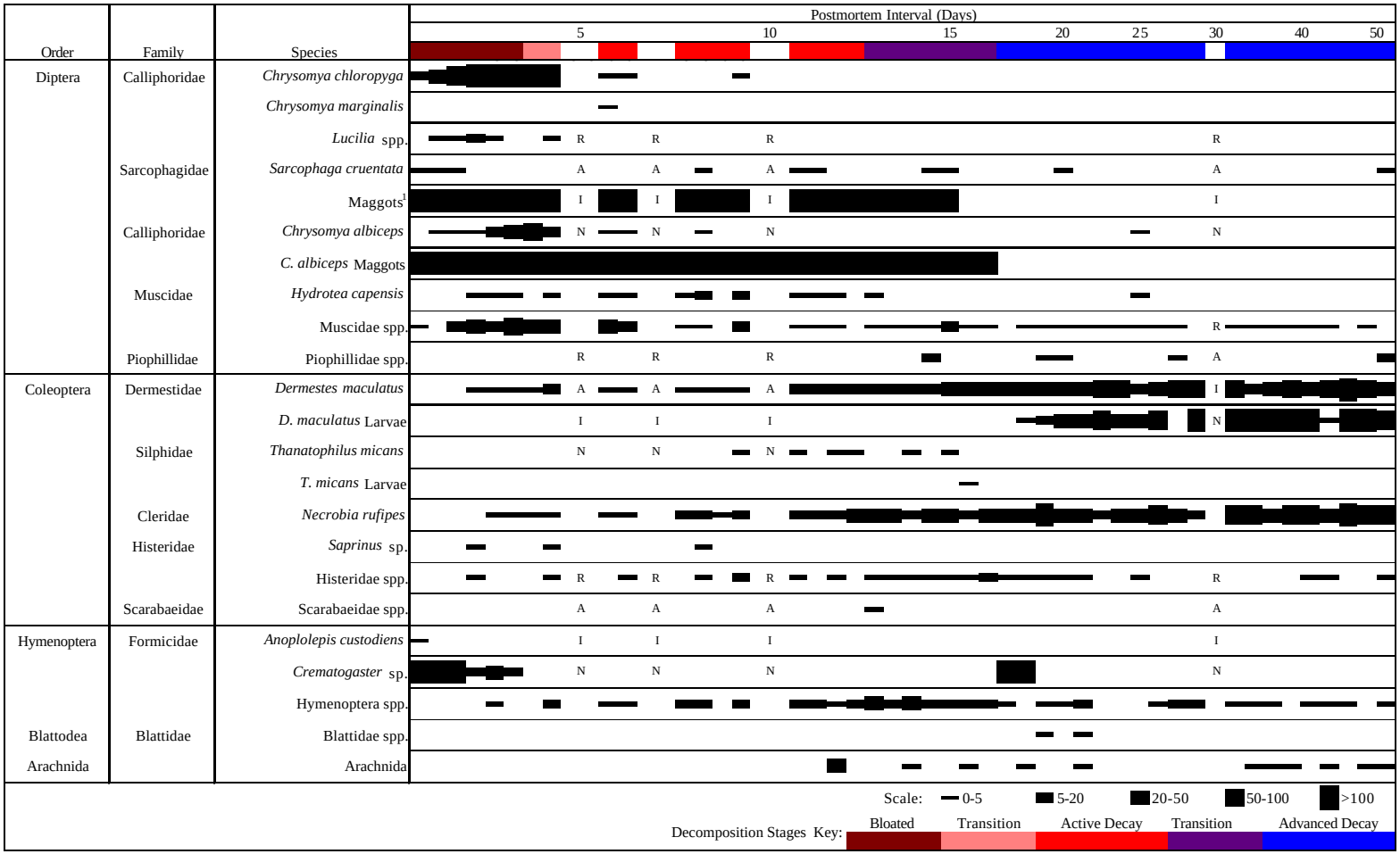


Fig. 3.7.3. Arthropod succession on the clothed control during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp. *Chrysomya marginalis.*

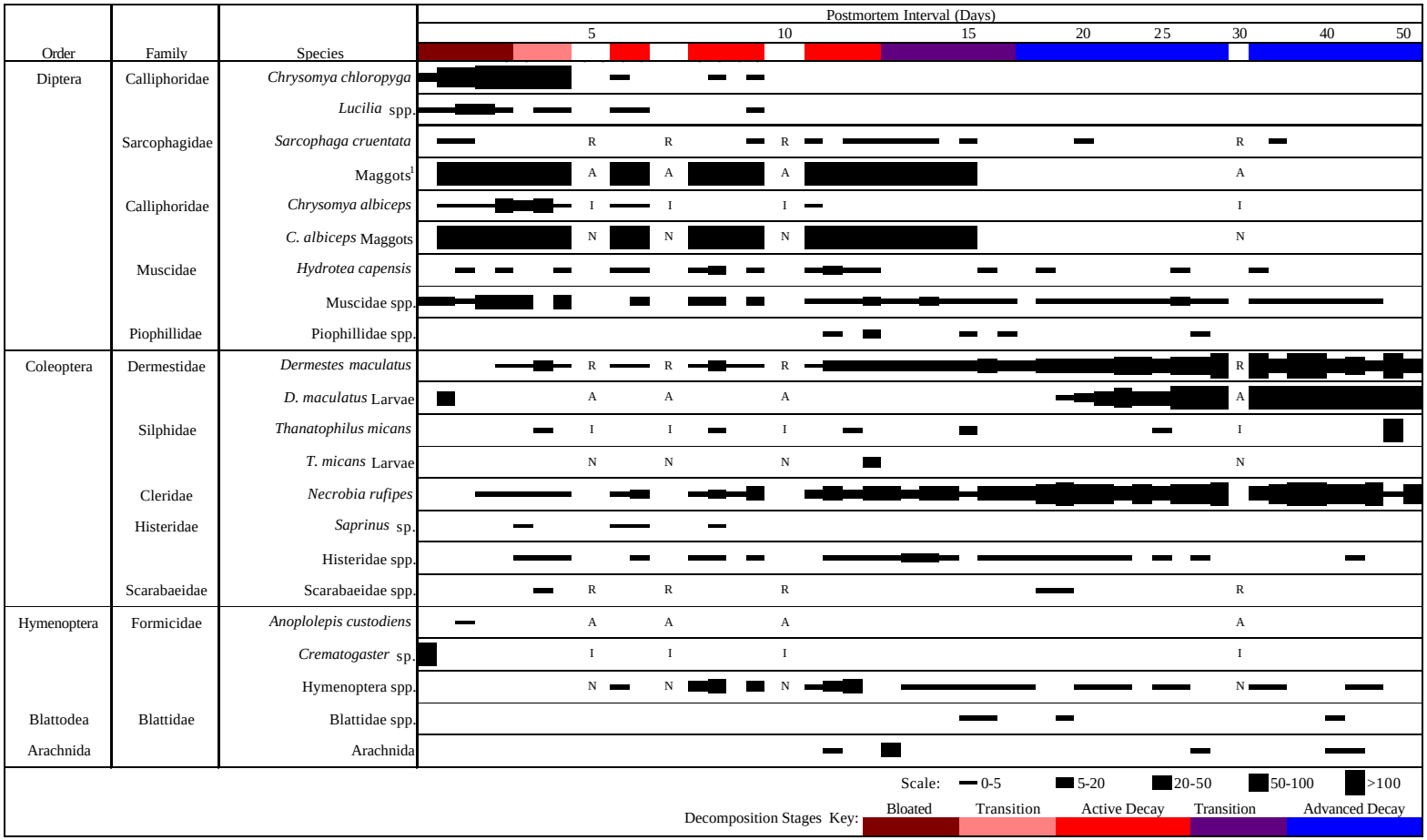


Fig. 3.7.4. Arthropod succession on the stab wounds no clothes carcass during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp., *Chrysomya marginalis*, *Calliphora vicina* and *Sarcophaga cruentata* .

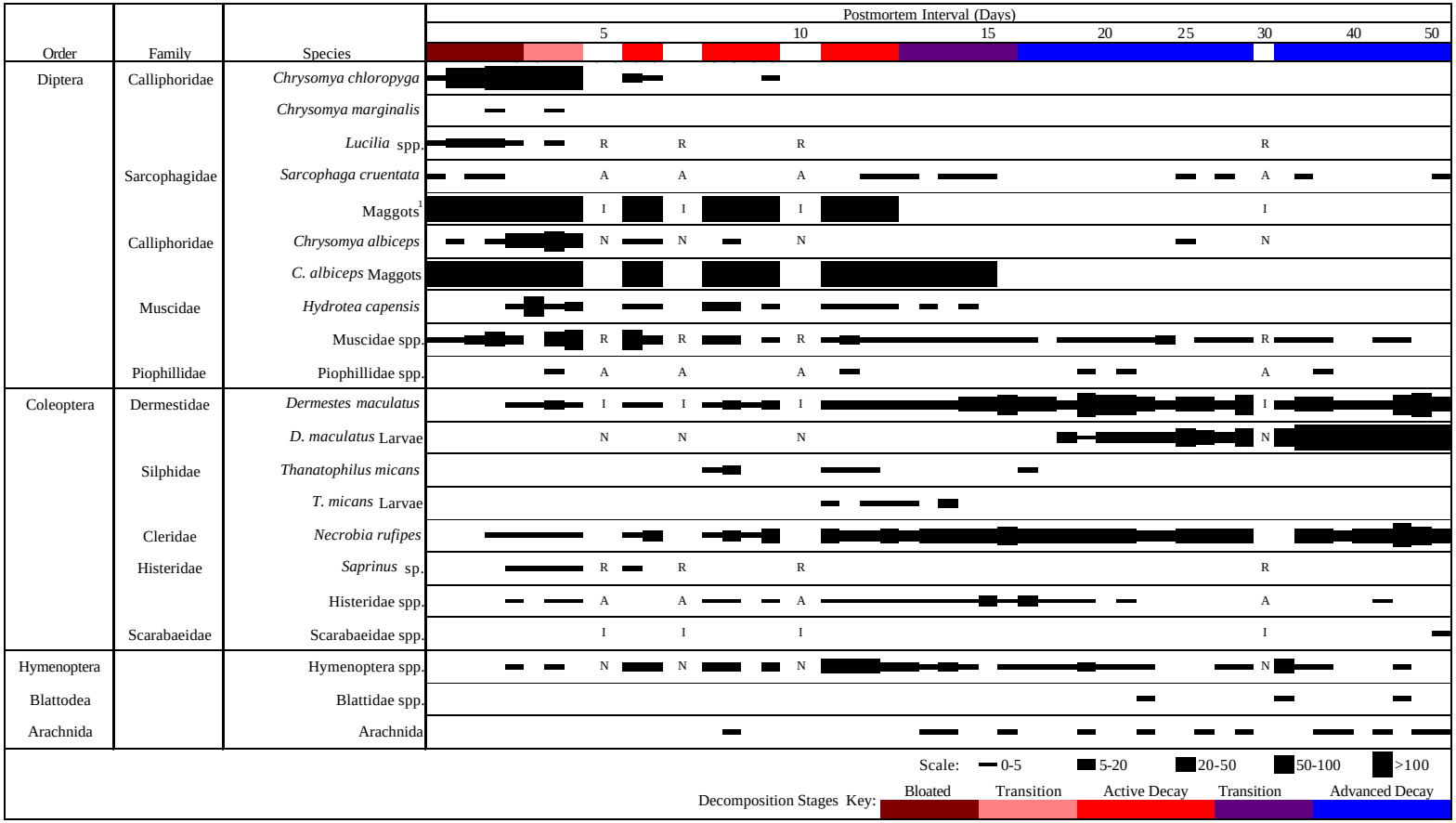


Fig. 3.7.5. Arthropod succession on the stab wounds clothed carcass during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp., *Chrysomya marginalis* and *Sarcophaga cruentata* .

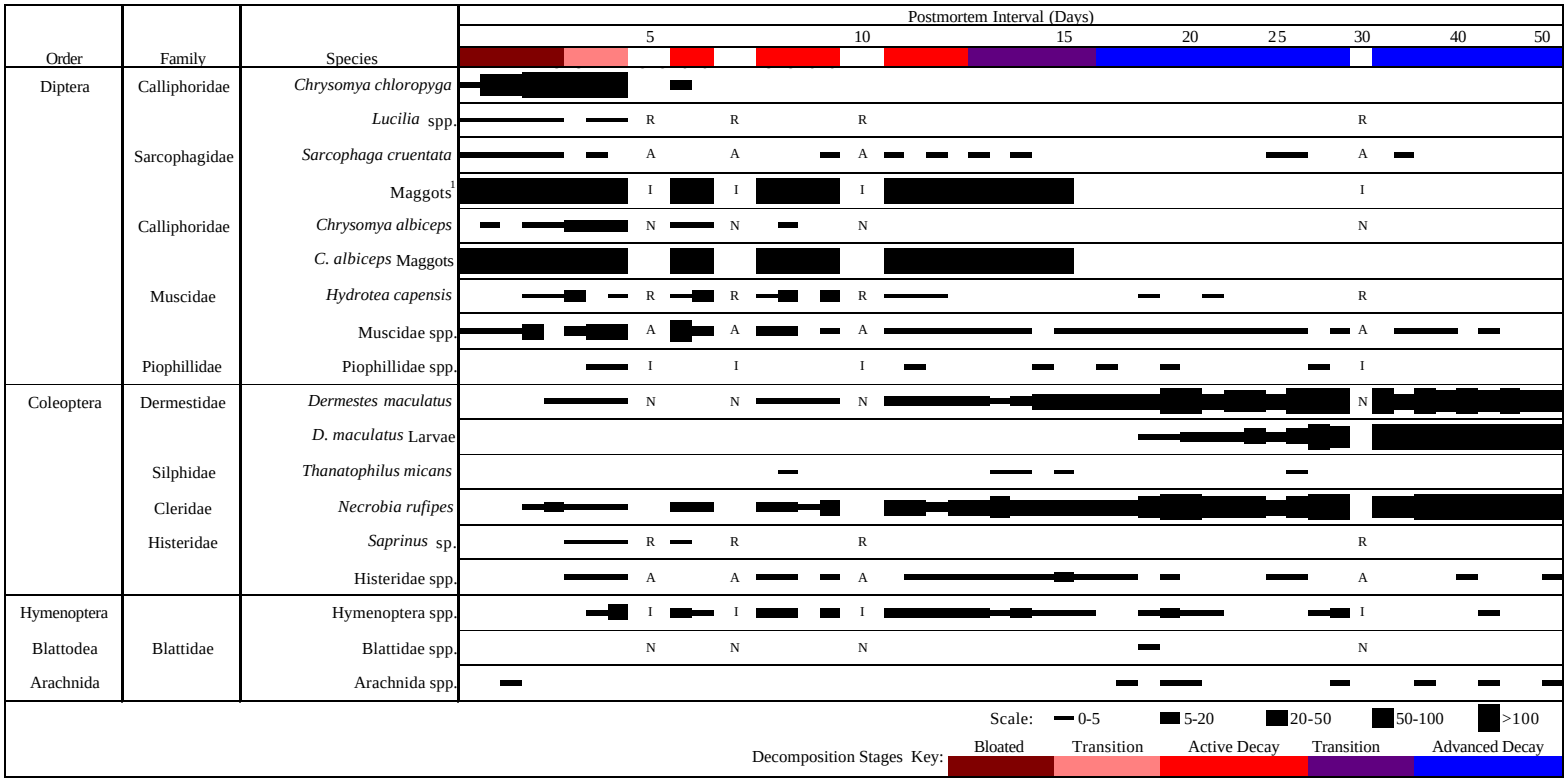


Fig. 3.7.6. Arthropod succession on the severe trauma no clothes carcass during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp., *Sarcophaga cruentata*.

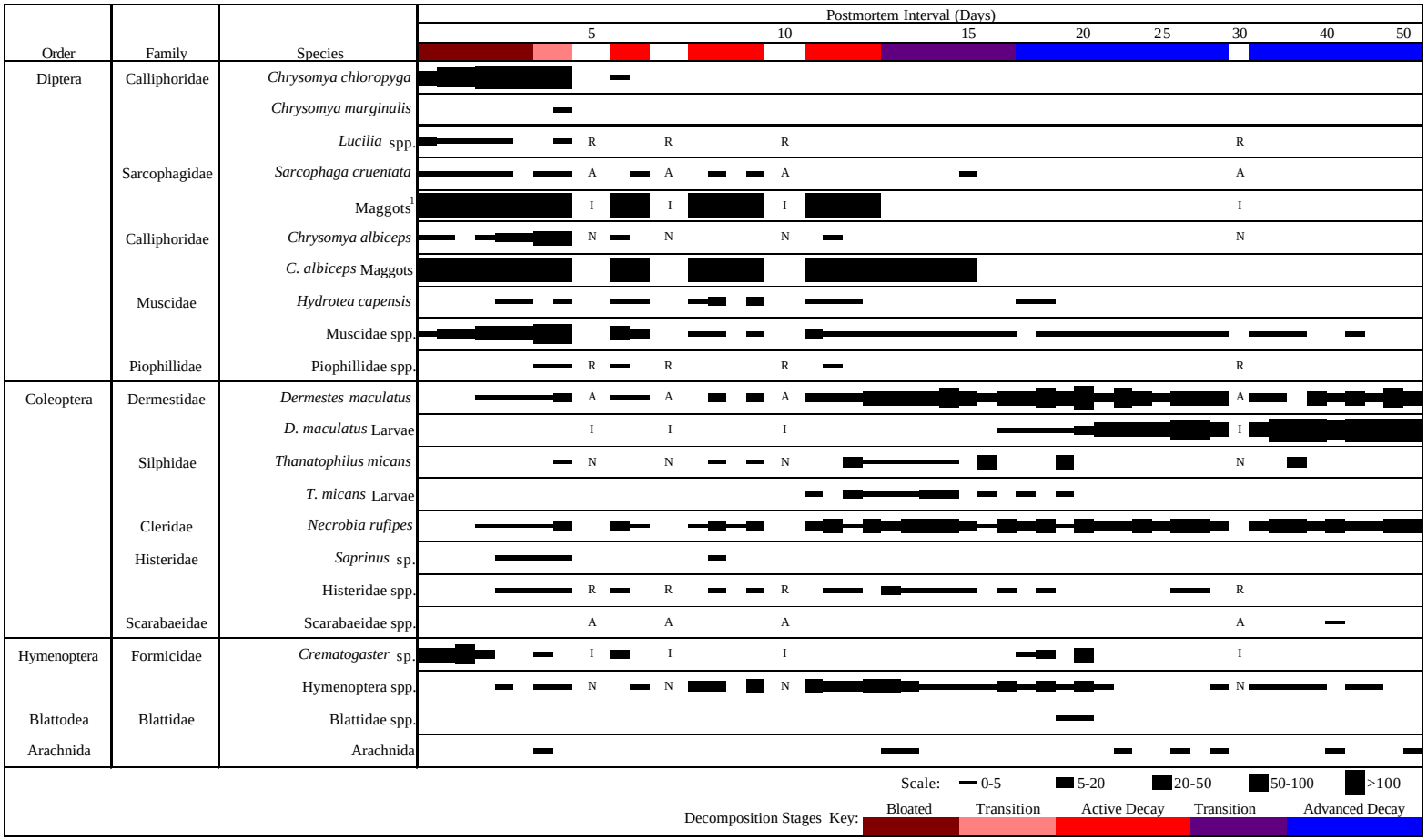


Fig. 3.7.7. Arthropod succession on the severe trauma clothed carcass during the spring wounds trial. Maggots[1] – *Chrysomya chloropyga*, *Lucilia* spp. *Chrysomya marginalis* and *Calliphora vicina*.

The composition of the maggot masses was similar to the spring wrapped trial. They were predominately *C. chloropyga* and *C. albiceps* (Table 3.7.2.). *Sarcophaga cruentata* bred on some of the carcasses. A few individuals of *C. marginalis* and *C. vicina* were also recorded (Table 3.7.2.). *Lucilia* spp. were also recorded frequently in the maggot masses, however, their overall occurrence was less than *C. chloropyga* and *C. albiceps*.

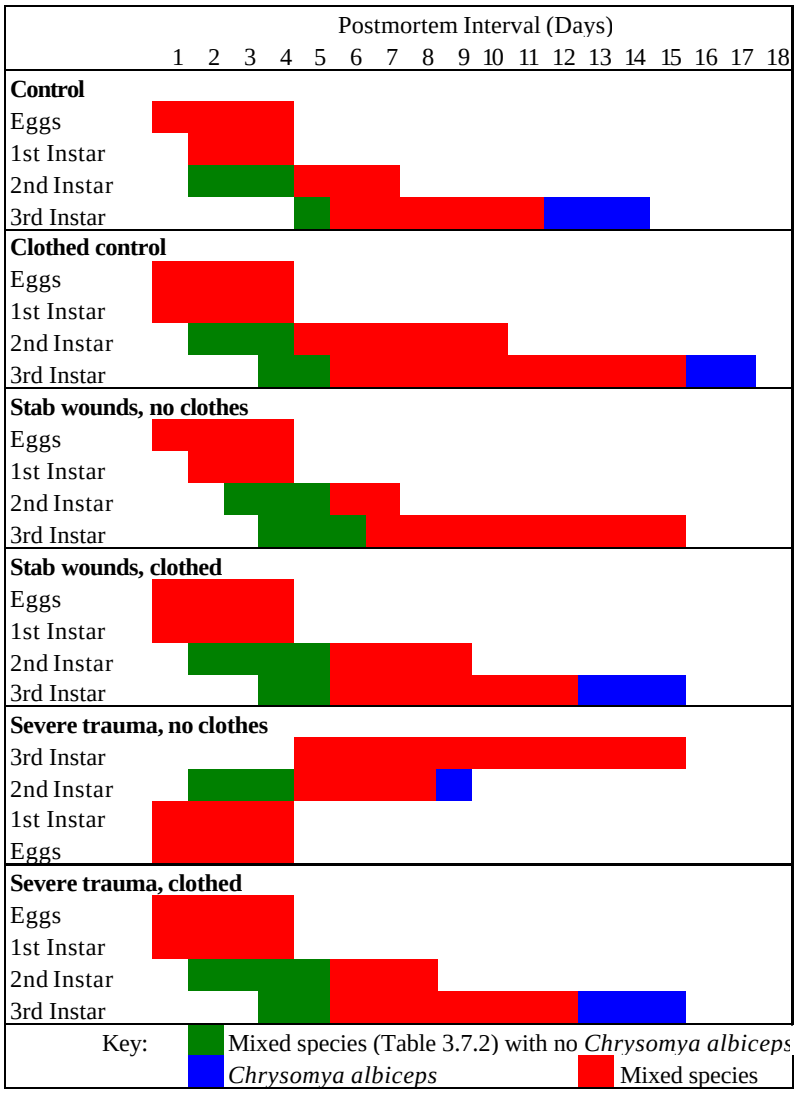


Fig. 3.7.8. Maggot succession for all carcasses during the spring wounds trial.

Table 3.7.2. Diptera species reared from maggot masses for each carcass during the spring wounds trial.

Days	2	3	5	8	10	14	15	17	20
Control									
Chrysomya chloropyga		X	X	X	X				
Lucilia spp.			X						
Chrysomya albiceps			X	X	X	X			
Sarcophaga cruentata	X	X	X						
Clothed control									
Chrysomya chloropyga	X	X	X	X	X	X	X		
Lucilia spp.			X						
Chrysomya albiceps			X	X	X		X		
Chrysomya marginalis			X						
Stab wounds no clothes									
Chrysomya chloropyga	X	X	X	X	X	X			
Lucilia spp.	X	X	X						
Chrysomya albiceps		X	X	X	X	X			
Chrysomya marginalis			X						
Calliphora vicina				X					
Sarcophaga cruentata			X						
Stab wounds clothed									
Chrysomya chloropyga	X	X	X	X	X				
Lucilia spp.	X	X	X	X					
Chrysomya albiceps			X	X	X	X			
Chrysomya marginalis				X					
Sarcophaga cruentata		X							
Severe trauma no clothes									
Chrysomya chloropyga		X	X	X	X	X			
Lucilia spp.	X	X	X						
Chrysomya albiceps			X	X	X	X			
Sarcophaga cruentata									
Severe trauma clothed									
Chrysomya chloropyga	X	X	X	X	X		X		
Lucilia spp.	X	X	X		X	X			
Chrysomya albiceps		X	X	X	X	X			
Chrysomya marginalis			X						
Calliphora vicina		X	X						

Interestingly, during the current trial, second instar maggots of mixed species were recorded before *C. albiceps* second instar maggots were found (Fig. 3. 7. 8.). This differed from the spring wrapped trial. In the current trial, second instar *C. albiceps* maggots were only recorded from days 5 and 6. The eggs and initial maggot masses, however, all contained *C. albiceps* when the sub samples were reared to adulthood. This may have been a result of collection bias.

The maggot masses were present on the carcasses until days 14 and 15. However, the clothed control had *C. albiceps* maggots present up to day 17 (Fig. 3.7.8.). The maggot masses on the control and clothed control carcasses of the spring wrapped

trial were present until days 13 and 14 (Fig. 3.7.8.). Again, neither the presence of the wounds or clothing appeared to have had any affect on the maggot mass development.

3.7.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices and the mean faunal similarity was calculated for each day (see 2.4.1.). These were plotted and the grand mean of all the mean faunal similarities were calculated and is represented as $\overline{S}_{gmean}$ (Fig. 3.7.9.). Statistically, according to the Jaccard metric species pairwise similarities, the graphs showed the characteristic horseshoe-shape as described by Schoenly (1992). The $\overline{S}_{gmean}$ values were between 0.403 and 0.463 (Fig. 3.7.9.). This range was even smaller than the results obtained from the spring wrapped trial (0.335 and 0.490). The range was well within the range recorded by Schoenly (1992). Therefore, the results were a good indication of species similarity on the carcasses.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

The Pearson's coefficient statistical analysis conduced on the carcass similarity matrices concluded that there were significant differences between some of the carcasses (Table 3.7.3.). The succession on the control carcasses (control and clothed control) was similar to each other. However, some of the experimental carcasses showed a lack of similarity between all the carcasses (Table 3.7.3.). The reason for this is unknown, as the overall succession appears to be similar. The coefficient, however, may be sensitive to very small changes.

There was no similarity between the two seasons (Table 3.7.4.), suggesting that there was a change in the arthropod communities between the two seasons. There were slight changes discussed in the arthropod succession section.

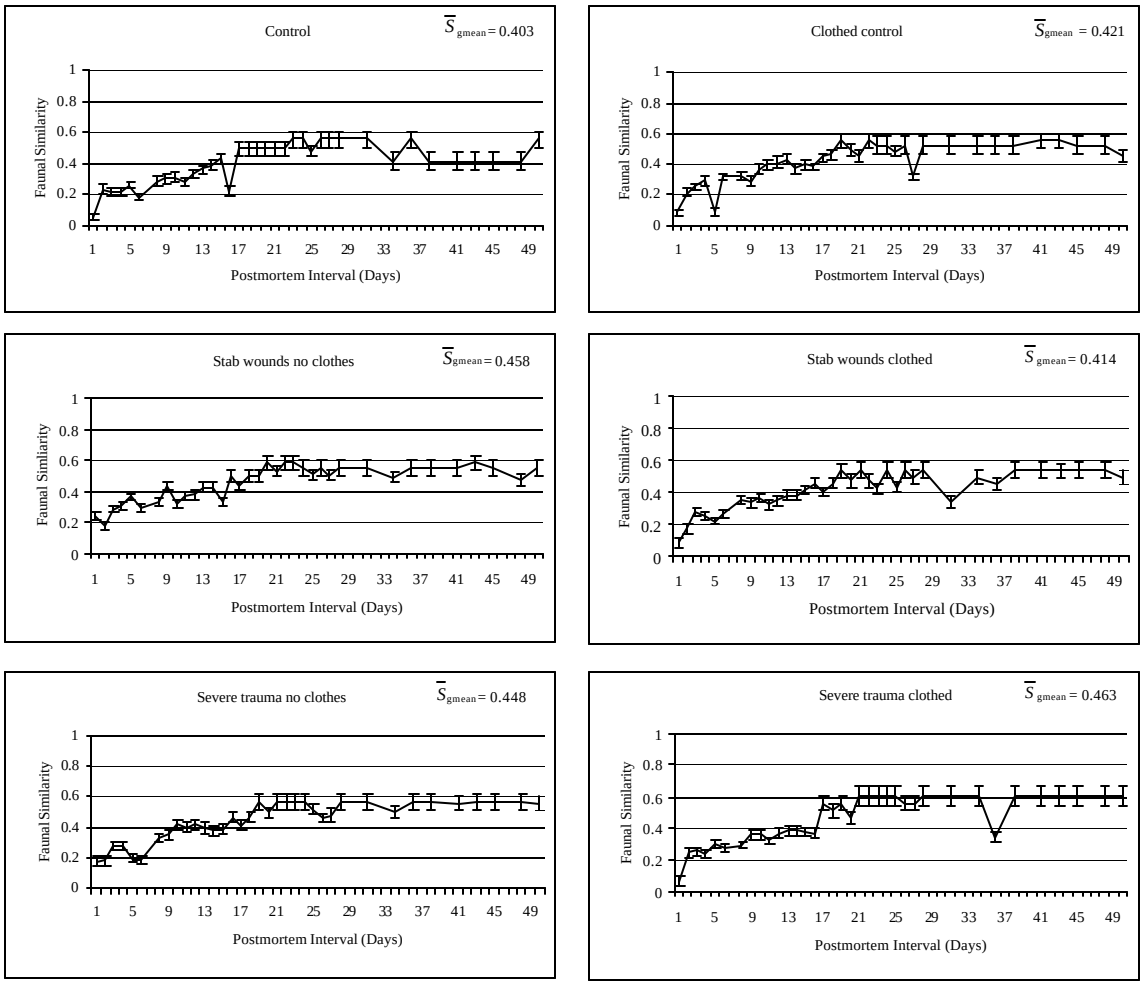


Fig. 7.9. Plots of mean pairwise faunal similarities (Jaccard metric) for each sampling period in the succession (vertical bars represent the standard errors).

Table 3.7.3. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the spring wounds trials. (* - significant difference at 95% confidence interval).

	Control	Clothed control	Stab wounds no clothes
Clothed control	0.7597 (0.0460)		
Stab wounds no clothes	0.7904 (0.0570)*	0.8226 (0.0490)	
Stab wounds clothed	0.7705 (0.0880)*	0.8334 (0.0360)	0.8383 (0.0550)*
Severe trauma no clothes	0.7582 (0.0830)*	0.8514 (0.0670)*	0.8209 (0.0830)*
Severe trauma clothed	0.7869 (0.0500)	0.8048 (0.0930)*	0.8094 (0.0910)*
	Stab wounds clothed	Severe trauma no clothes	
Clothed control			
Stab wounds no clothes			
Stab wounds clothed			
Severe trauma no clothes	0.8375 (0.0600)*		
Severe trauma clothed	0.8263 (0.0540)*	0.8463 (0.0500)	

Table 3.7.4. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the control and clothed control carcasses in the spring of 2003 and 2004.

	Control (2004)	Clothed control (2004)
Control (2003)	0.7521 (0.1320)*	0.7497 (0.0740)*
Clothed control (2003)	0.7250 (0.0710)*	0.7405 (0.1100)*

3.7.4. Ambient, external and internal carcass temperatures

The iButtons at the study site recorded temperatures that were very similar to the data obtained from the South African Weather Services (SAWS) recorded at the Bloemfontein airport and city stations (Fig. 3. 7.10.). The minimum ambient temperature range was high. During the initial part of the trial the minimum ambient temperatures were between 8 and 16^0C. These temperatures then increased to between 12 and 22^0C (Fig. 3.7.10a). This high variability was similar to the spring wrapped trial. The maximum ambient temperatures increased as the trial progressed and were not as variable as the minimum temperatures. It varied between 20 and 36^0C over the entire trial (Fig. 3.7.10b).

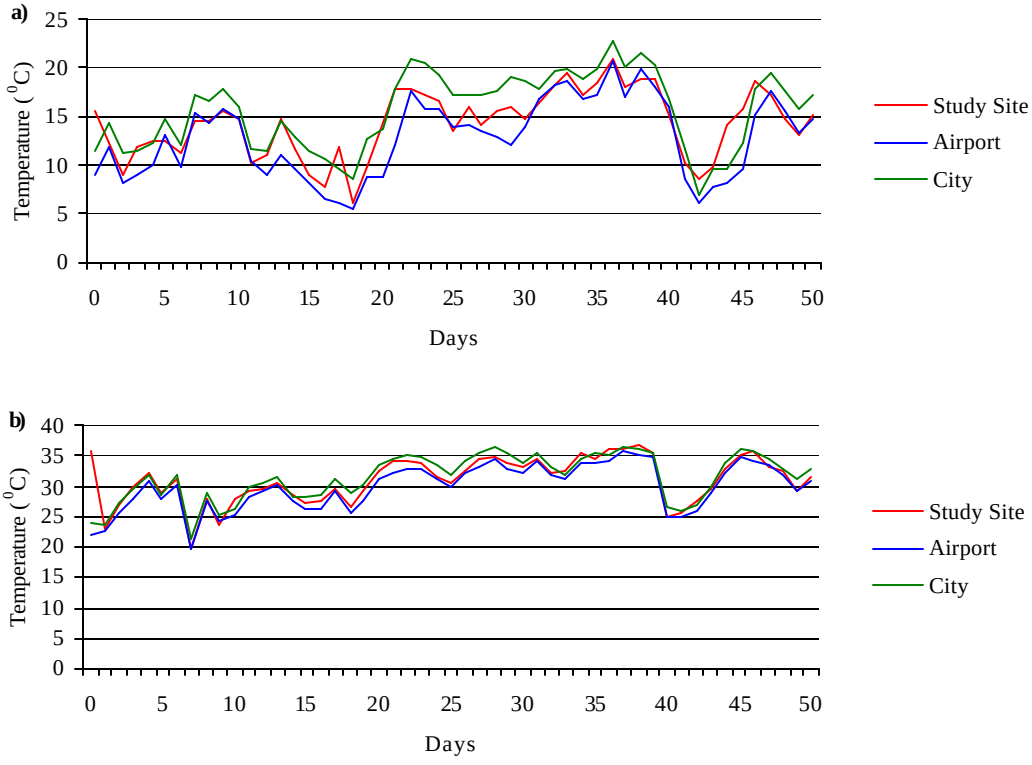


Fig. 3.7.10. Maximum (a) and minimum (b) daily ambient temperatures recorded at the Bloemfontein city and airport SAWS stations and at the study site during the spring wounds trial.

Overall the minimum and maximum average head temperatures had very low standard errors, indicating that the data between the carcasses did not vary significantly. The minimum average head temperatures followed the same trends than the ambient temperatures. However, between days 5 and 10, the minimum head temperatures were slightly higher than the ambient temperatures (Fig 3.7.11.). This was when the maggot masses were present on the carcasses. The maggot masses are known to generate metabolic heat (Cianci & Sheldon 1990, Goodbrod & Goff 1990, Greenberg 1991, Turner & Howard 1992). This deviation from the ambient temperatures would indicate that the maggots were present in the head during this time.

The maximum average head temperatures were consistently higher than the ambient temperatures. However, because the minimum average head temperatures closely track the ambient, this would indicate that the heads do not retain any heat (Fig. 3.7.11).

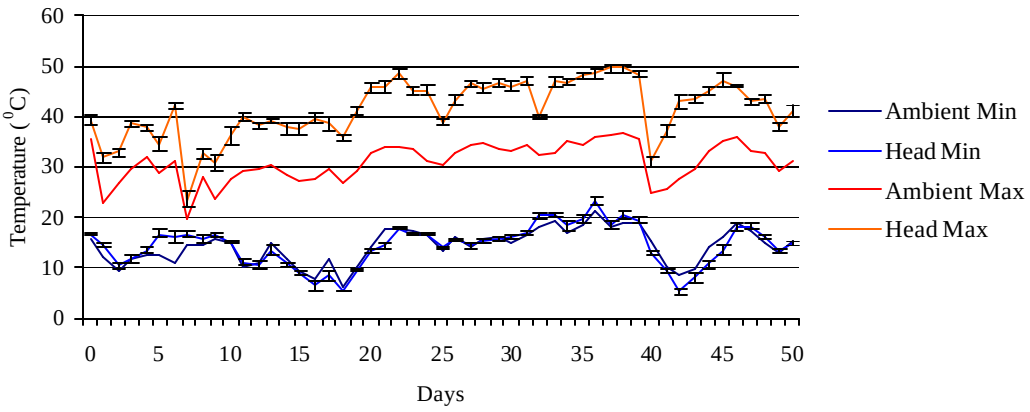


Fig. 3.7.11. Maximum and minimum ambient and internal carcass head temperatures during the spring wounds trial.

There was a noticeable period where the minimum average thorax temperatures were higher than the ambient temperatures from approximately days 5 to day 15 (Fig. 3.7.12). This suggested maggot mass activity in the thorax during this period. For the rest of the trial, the minimum average thorax temperatures were only slightly higher than minimum ambient temperatures (Fig. 3.7.12.). The maximum average thorax temperatures were also constantly higher than the maximum ambient temperatures. However, there was a high standard error, indicating that there was some variability between the carcasses (Fig. 3.7.12.).

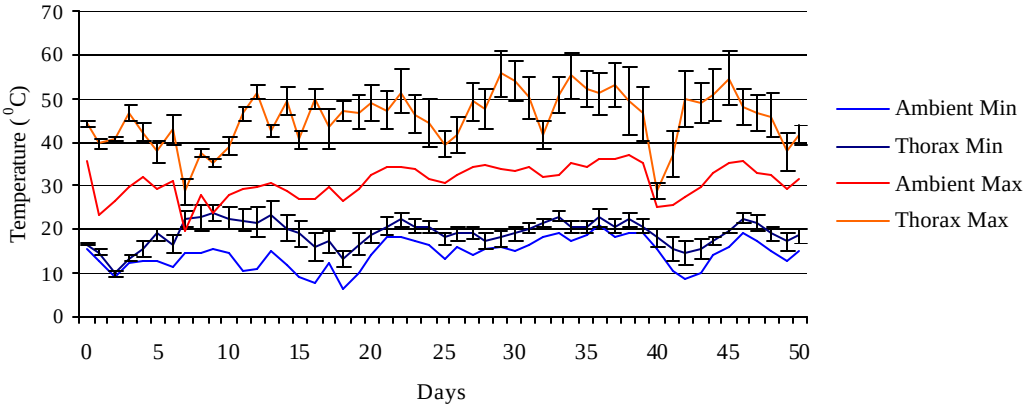


Fig. 3.7.12. Maximum and minimum ambient and internal carcass thorax temperatures during the spring wounds trial.

The minimum average abdomen temperatures, similar to the minimum average head temperatures, showed a small increase from the minimum ambient temperatures. Increase in temperature was seen between days 4 and 9 indicating maggot mass activity (Fig. 3.7.13.). The maximum average abdomen temperatures showed a similar trend to the thorax average maximum temperatures. However, the standard errors were not as high as in the thorax, indicating a smaller variance between the carcasses (Fig. 3.7.13.).

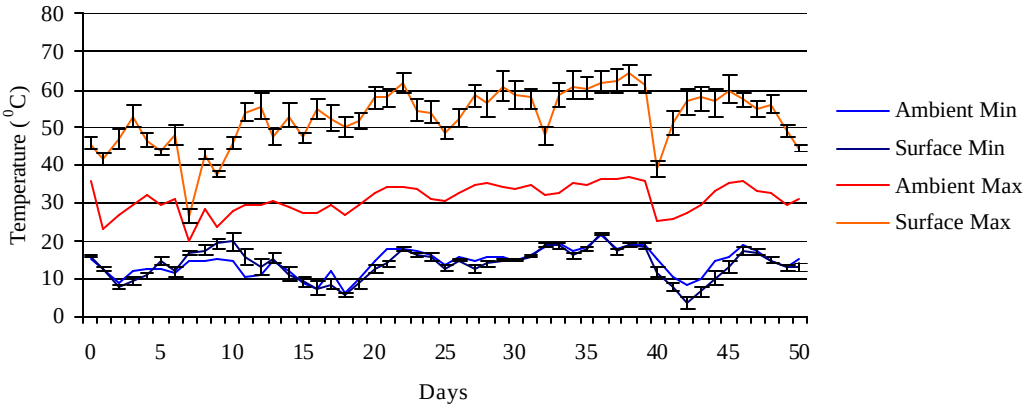


Fig. 3.7.13. Maximum and minimum ambient and internal carcass abdomen temperatures during the spring wounds trial.

The minimum average temperatures taken on the surfaces of the carcasses were very similar to the minimum ambient temperatures (Fig. 3.7.14.). The average maximum temperatures were noticeably higher than the maximum ambient temperatures (Fig. 3.7.14.). The range between the average minimum and the average maximum temperature was much larger than recorded in any other region of the carcasses (Fig. 3.7.14.).

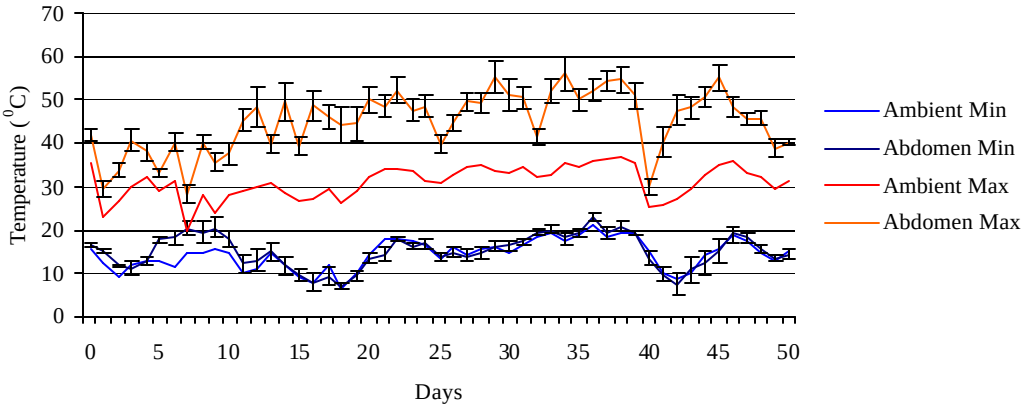


Fig. 3.7.14. Maximum and minimum ambient and surface temperatures of the carcasses during the spring wounds trial.

The average minimum temperatures recorded underneath the carcasses were noticeably higher than the minimum ambient temperatures. The carcasses may have provided some insulation and retained the heat. The difference was slightly higher between days 5 and 11, indicating maggot mass activity (Fig. 3.7.15.). The average maximum temperatures underneath the carcasses were higher than the ambient maximum temperatures. However, this difference was not as great as seen in other regions of the carcasses, indicating the carcasses were providing some insulation.

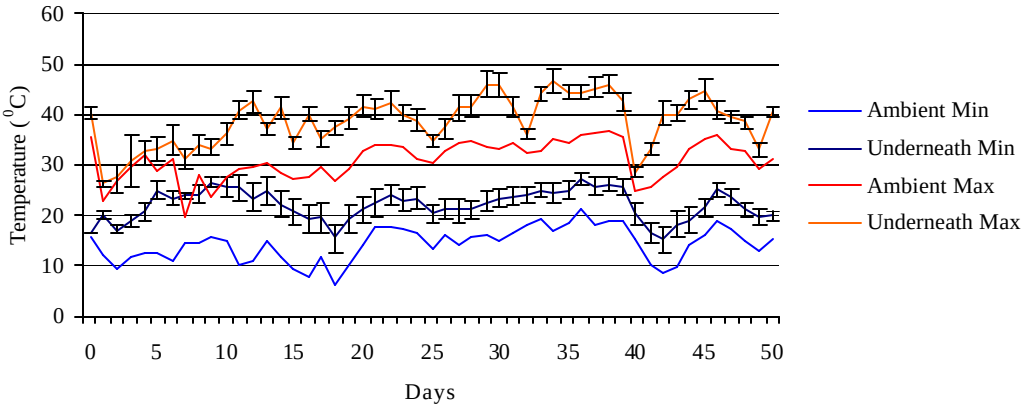


Fig. 3.7.15. Maximum and minimum ambient and underneath temperatures of the carcasses during the spring wounds trial.

3.7.5. Maggot mass distribution

The primary oviposition sites recorded were similar to those recorded in all the other trials (Fig. 3.7.16.). The maggot masses in this trial did not show the movement under the clothing as clearly as recorded in wrapped trials. However, the trend could still be distinguished (Fig. 3.7.16.). The maggot masses during this trial appeared to be more densely packed rather than containing fewer individuals. There was no displacement of clothing due to maggot movement on any of the carcasses.

3.7.6. Maggot predation

Predation by *C. albiceps* maggots on other maggots was recorded. However, it appeared to occur infrequently. The predation mostly occurred on the control carcass where most of the tissues had been consumed by the maggots. It dd not appear to involve the large numbers that were recorded during the autumn trails.

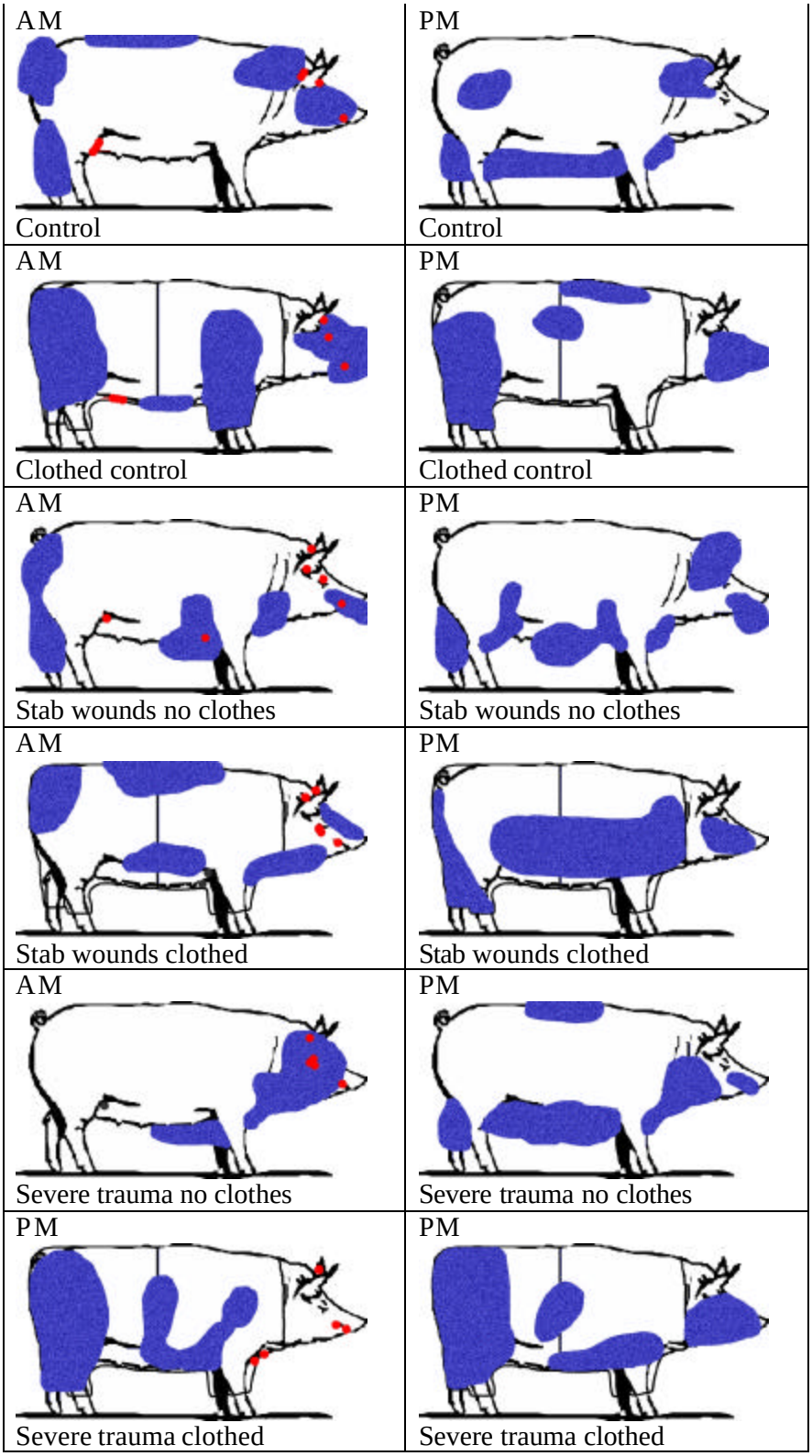


Fig. 3.7.16. Maggot mass movement recorded on day 6 of the spring wounds trial. Dots on the morning (AM) diagrams represent the primary oviposition sites.

SECTION 3.8.

The influence of stab wounds, severe trauma and clothing on carcass decomposition and arthropod succession: a summer study.

3.8.1. Decomposition of the carcasses

The same characteristics that were used for the autumn wrapped trial were used for the current trial. The carcasses were placed in the field at approximately 10:00h on the 28th December 2004 (day 0). The succession of the carcasses through the fresh and bloated stages followed almost exactly the same pattern as the summer wrapped trial. The carcasses remained in the fresh stage for a very short time (Fig. 3.8.1.). The carcasses began to bloat within an hour after death. By 14:00h on day 0, the torsos of the carcasses were already firm and the limbs were already becoming distended. By the morning of day 1, the carcasses were fully bloated (Fig. 3.8.1.). The carcasses did not stay bloated for long and by the morning of day 2, the torsos had begun to soften (Fig. 3.8.1.).

On day 2, three of the carcasses entered into the active decay stage (Fig. 3.8.1.). The other three carcasses also had maggots present, but they moved into the head and were not visible. Skin slippage occurred from day 3, the same as recorded in the summer wrapped trial. Active decay was also shortened in this trial. The clothed carcasses remained in active decay for longer than the carcasses without clothes (Fig. 3.8.1.). The clothing may have allowed the moisture to retained for longer, allowing the maggot mass to feed on the remaining tissues. However, almost all the maggots began to migrate by days 6 and 7. This allowed the carcasses to dry out and enter the advanced decay stage (between days 8 and 10) (Fig. 3.8.1.). This happened noticeably quicker than the wrapped trial when the carcasses entered the advanced decay stage between day 14 and 15. The carcasses entered advanced decay within a day of each other (Fig. 3.8.1.).

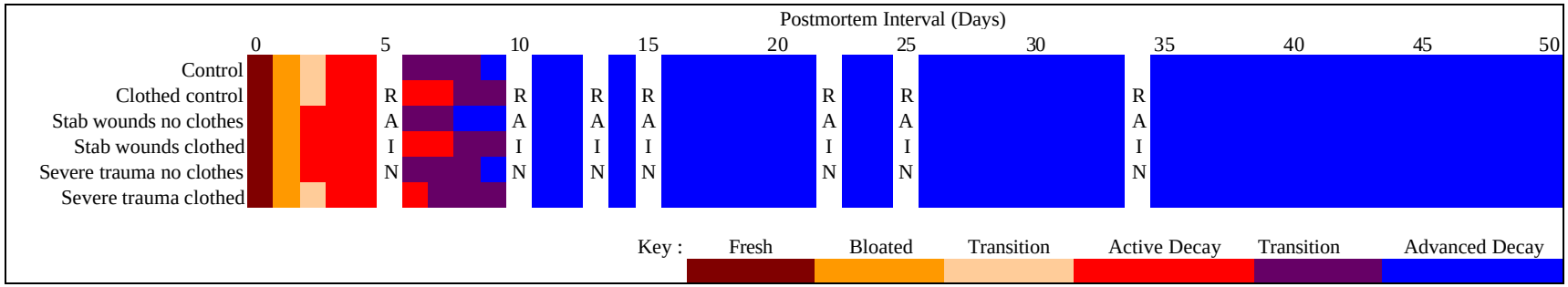


Fig. 3.8.1. Decomposition stages of the carcasses in the summer wounds trial.

The carcasses remained in advanced decay until the end of the trail. In the previous summer trial the exposed carcasses (control and clothed control) entered the dry remains stage after day 31. However, during the current trail there was very high rainfall, preventing the carcasses from drying out.

3.8.2. Arthropod succession on the carcasses

The most common species recorded during this trial (Table 3.8.1.) were the same as those recorded in the summer wrapped trial.

Table 3.8.1. Most common species occurring in wounds summer trial

Diptera	Coleoptera
Chrysomya marginalis *Chrysomya albiceps* *Sarcophaga cruentata*	*Dermestes maculatus* *Necrobia rufipes*

The insect succession on all carcasses followed the same pattern as seen in all the warm season trials. The adult Diptera arrived at the carcasses within minutes after death. By the morning of day 1, high numbers of *C. marginalis*, (50 to 100 individuals) were recorded on the control (Fig. 3.8.2.) and stab wounds no clothes (Fig. 3.8.4.), and between 20 to 50 individuals were recorded on rest of the carcasses (Fig. 3.8.3, 3.8.5 to 3.8.7.).

Between five and 20 individuals of *C. albiceps* were recorded on the clothed control (Fig. 3.8.3.), severe trauma no clothes (Fig. 3.8.6.) and the severe trauma clothed (Fig. 3.8.7.). Between 20 and 50 individuals were recorded on the control (Fig. 3.8.2.), and stab wounds clothed (Fig. 3.8.5.) carcasses. By the afternoon of day 3, *C. marginalis* were no longer recorded on the control (Fig. 3.8.2.) and stab wounds no clothes (Fig. 3.8.4.). By the morning of day 4 they were also no longer recorded on the remaining carcasses (Figs 3.8.3, 3.8.5. to 3.8.7.). No *C. albiceps* were recorded on the carcasses after the morning of day 4 (Fig. 3.8.2. to 3.8.7.). These trends were similar to the summer wrapped trial

Muscidae spp. were present on all the carcasses for the duration of the trial. As with all the other trials conducted so far, the Muscidae spp. numbers were higher during the first part of the trial (Figs 3.8.2. to 3.8.7.). They also did not utilize the carcasses for breeding purposes but merely fed on the decomposition fluids. *Hydrotea capensis* were recorded on all the carcasses sporadically in groups usually between three and five individuals (Figs 3.8.2. to 3.8.7.).

Dermestes maculatus adults were observed on the carcasses very early during the decomposition process. They were recorded on the morning of day 1 on the stab wounds no clothes (Fig. 3.8.4.), stab wounds clothed (Fig. 3.8.5.) and the severe trauma clothed (Fig. 3.8.7.) carcasses. They were recorded by the morning of day 2 on the clothed control (Fig. 3.8.3.) and the severe no clothes (Fig. 3.8.6.) carcasses. Lastly they were recorded on the control carcass on the morning of day 5 (Fig. 3.8.2.). A similar result was recorded during the summer wrapped trial, with *D. maculatus* adults recorded on days 2 and 3. Their numbers increased quickly after the maggots had migrated from day 6.

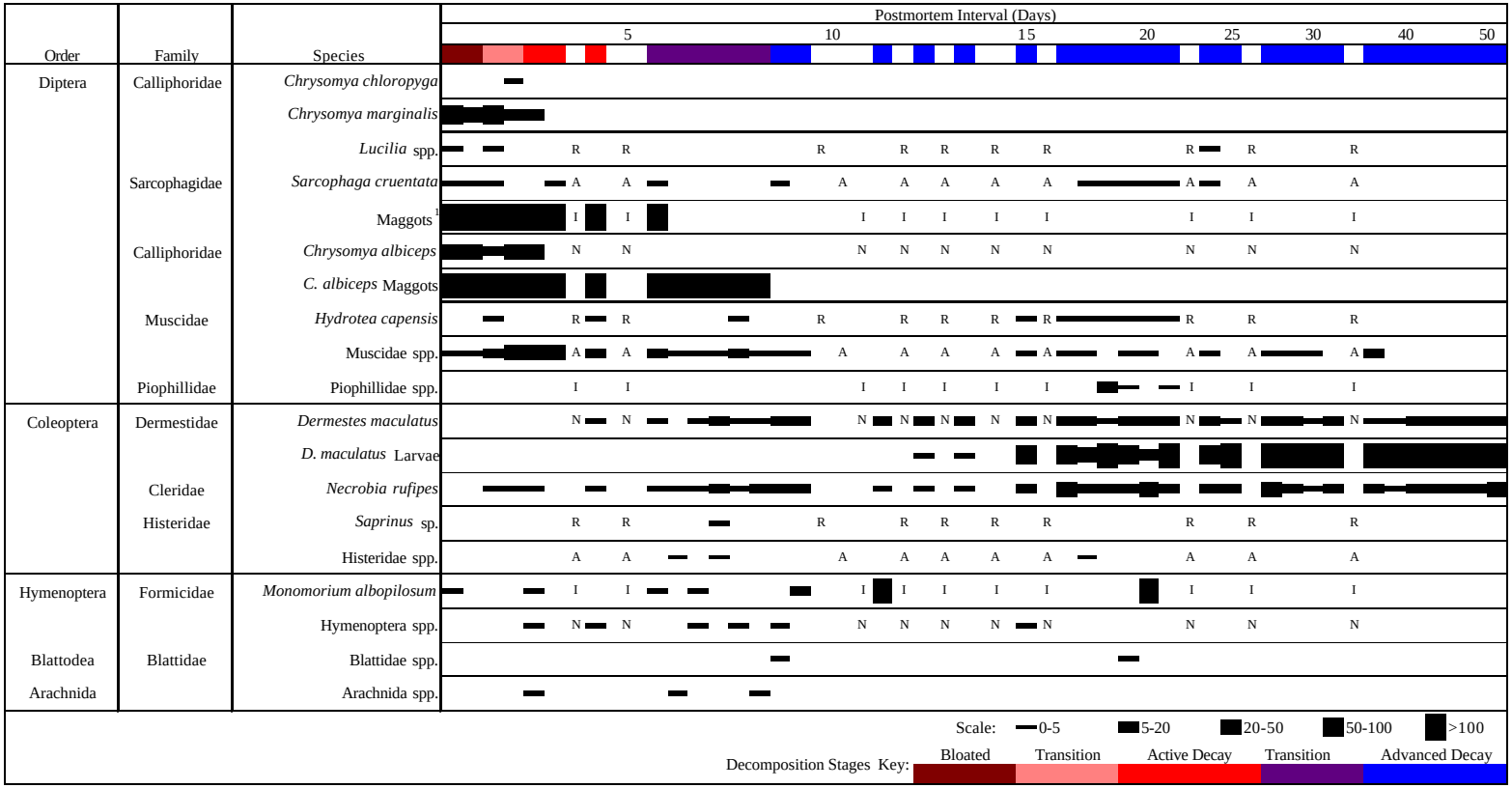


Fig. 3.8.2. Arthropod succession on the control carcass during summer wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

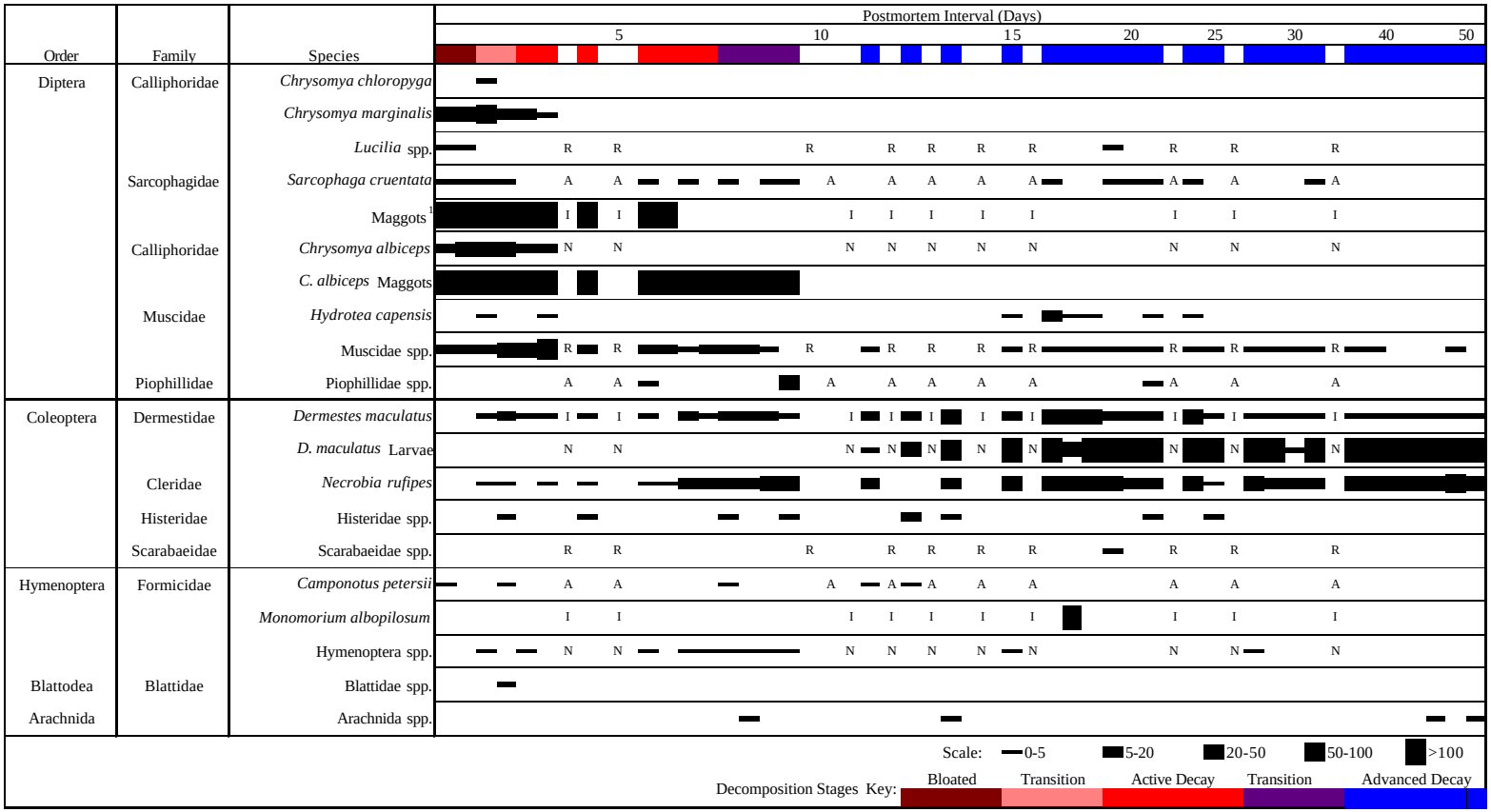


Fig. 3.8.3. Arthropod succession on the clothed control during summer wounds trial. Maggots [1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

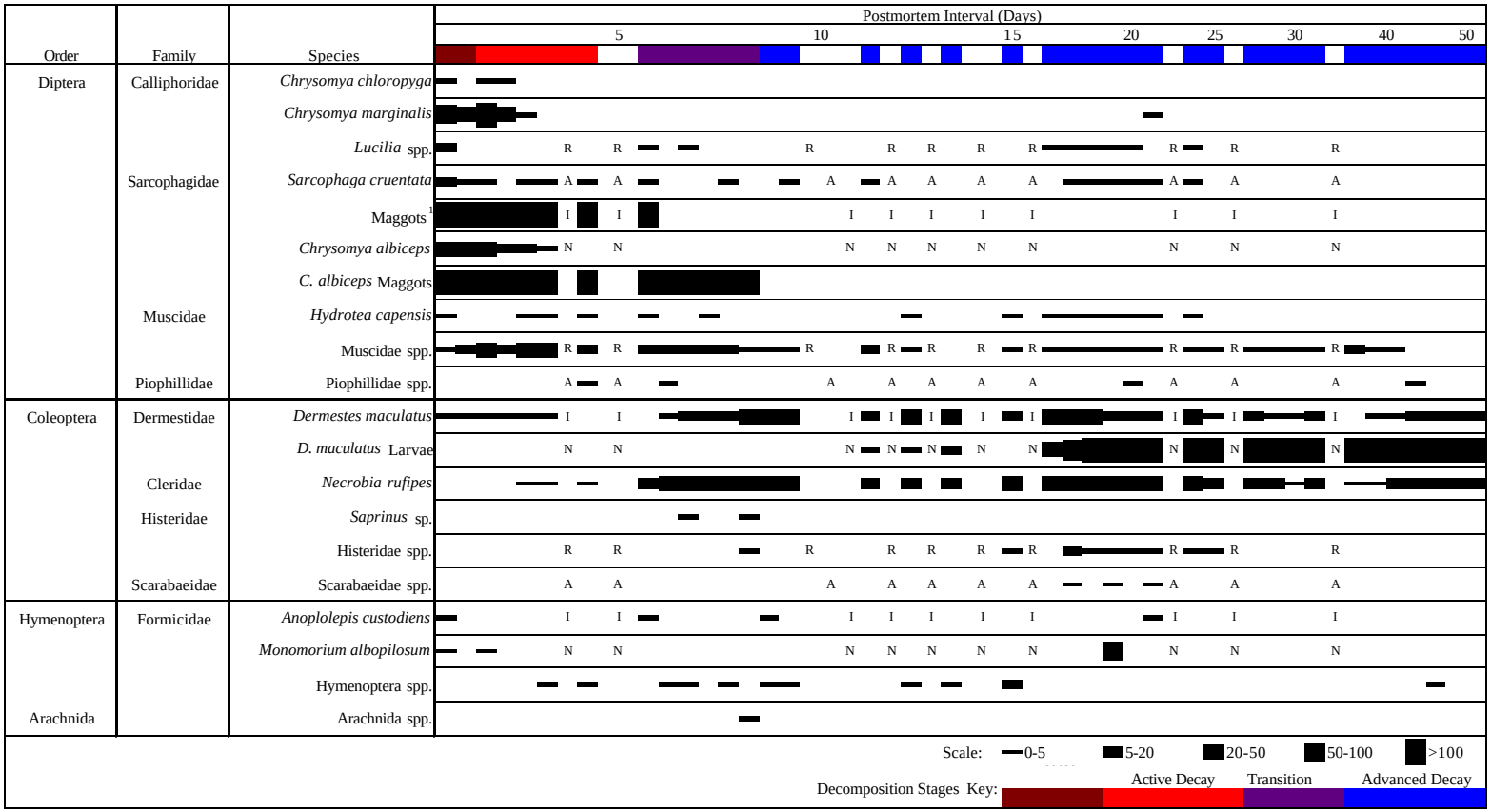


Fig. 3.8.4. Arthropod succession on the stab wounds no clothes carcass during summer wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

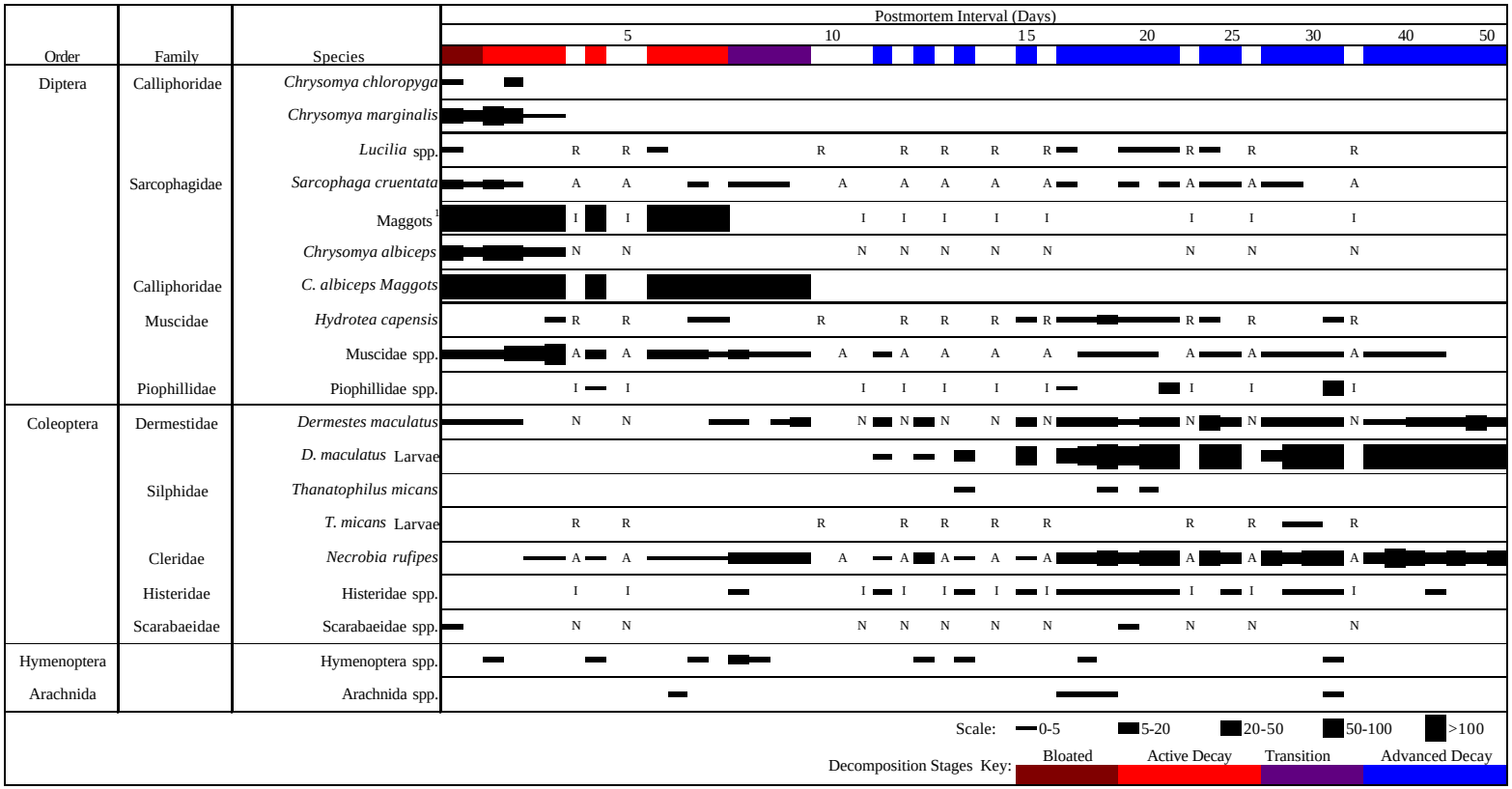


Fig. 3.8.5. Arthropod succession on the stab wounds clothed carcass during summer wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

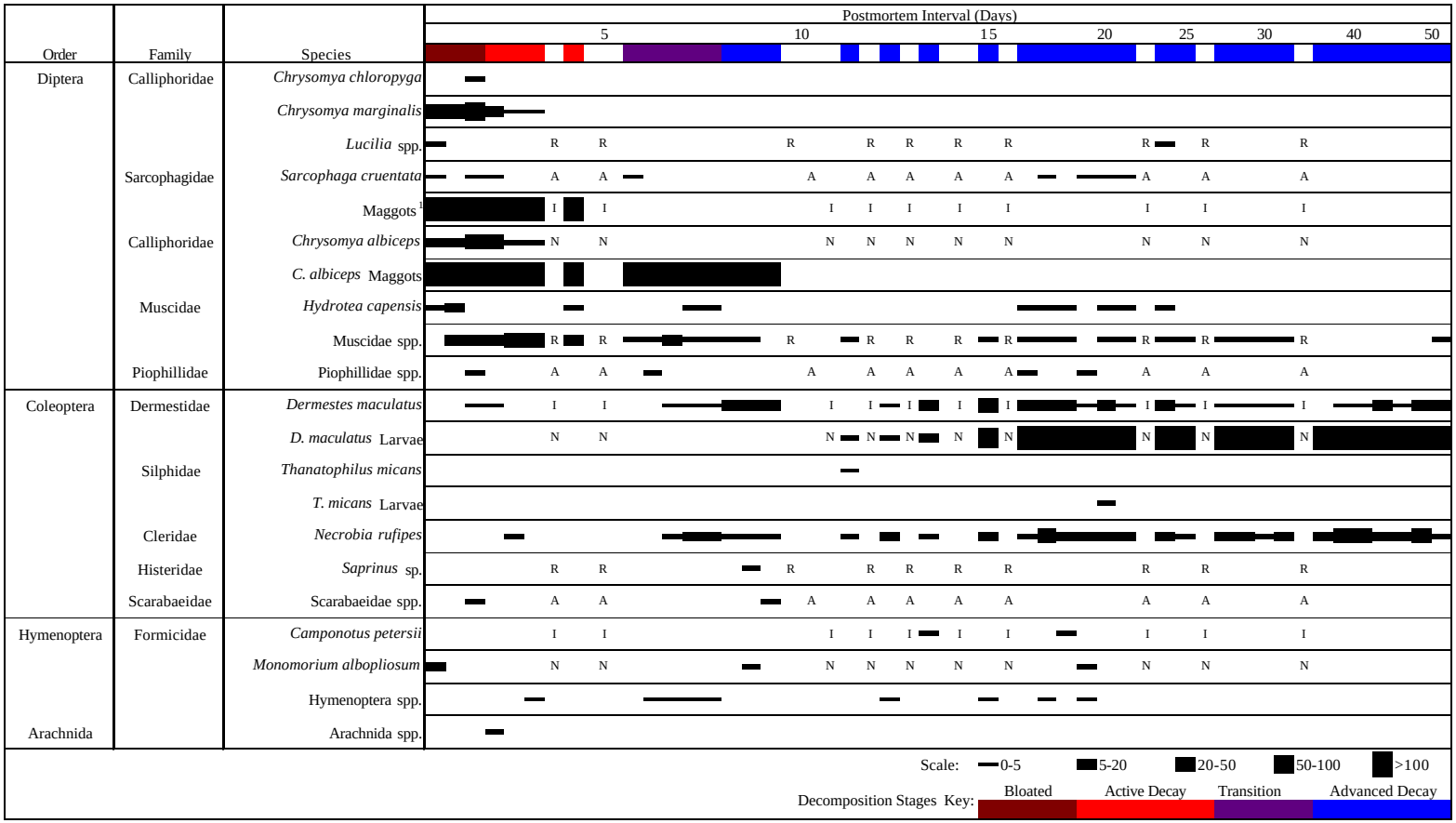


Fig. 3.8.6. Arthropod succession on the severe trauma no clothes carcass during summer wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

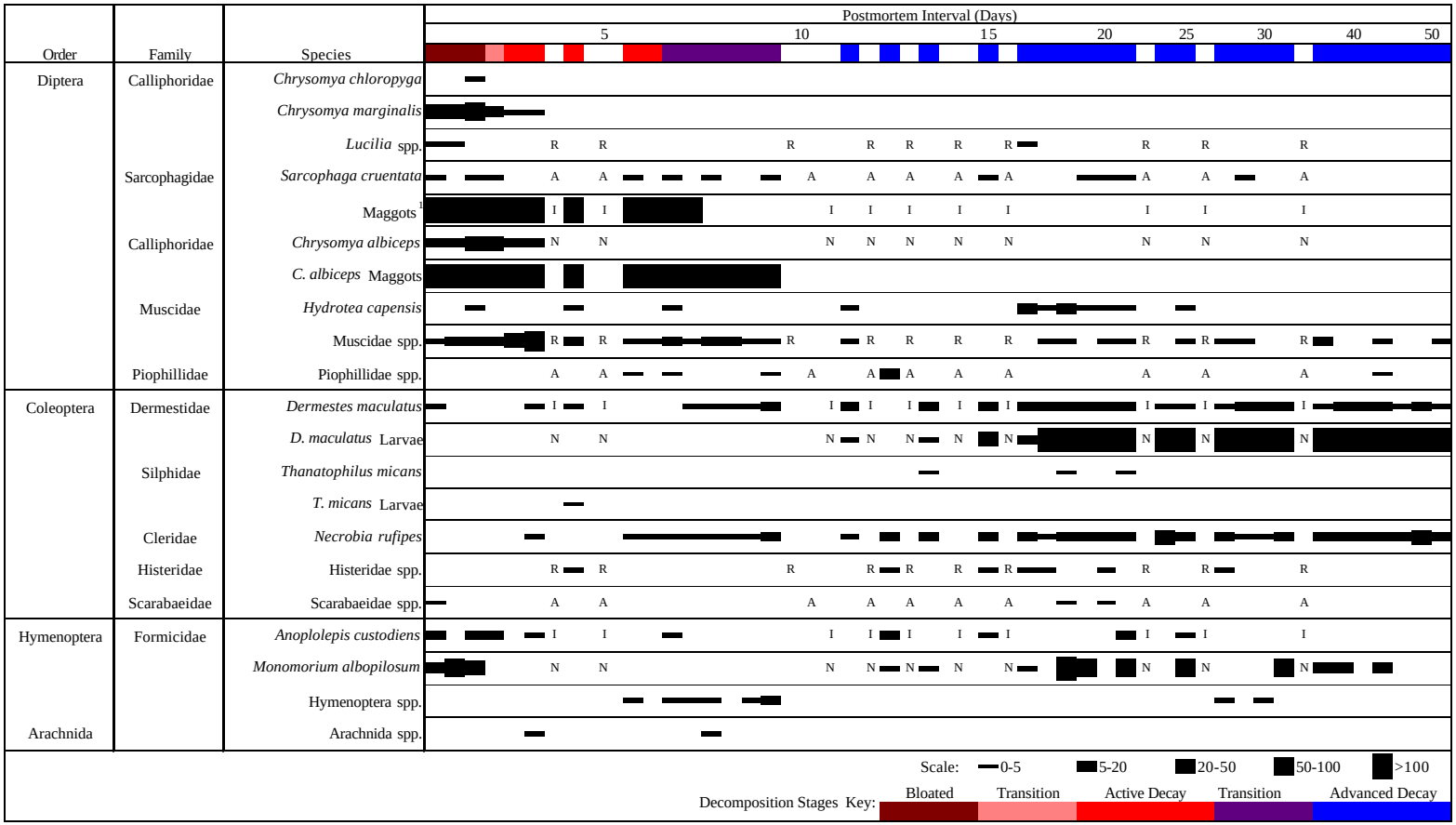


Fig. 3.8.7. Arthropod succession on the severe trauma clothed carcass during summer wounds trial. Maggots[1] – *Chrysomya marginalis* and *Sarcophaga cruentata* .

Dermestes maculatus larvae were recorded on all the carcasses by the morning of day 11 except the control carcass where they were recorded from the morning of day 12 (Figs 3.8.2. to 3.8.7). Their numbers increased quickly from approximately day 13, after which they were the most dominant species, in terms of number of individuals, on the carcasses. Heavy rainfall during this period made sampling difficult.

Necrobia rufipes were also observed early in the arthropod succession. They were recorded on the control and clothed control carcasses (Fig. 3.8.2. and 3.8.3.) from day 2. They were recorded on the remaining carcasses by the morning of day 3 (Figs 3.8.4. and 3.8.7). Their numbers increased from approximately day 6. The numbers of *N. rufipes* were recorded during this trial were noticeably higher than those recorded during the autumn and spring wrapped trials and similar to those numbers recorded in the summer wrapped trial.

Histeridae spp. were also present sporadically almost from the beginning of the trial. They were most commonly observed between day 3 and day 20 (Figs 3.8.2., 3.8.3. and 3.8.7.).

Diptera oviposition was recorded simultaneously from day 0 on all the carcasses regardless of whether the carcasses had wounds or were clothed (Fig. 3.8.8.). This result was the same as those recorded during all the autumn, spring wrapped, wounds and summer wrapped trials.

As recorded in the summer wrapped trial, the eggs that were deposited hatched quickly. Oviposition only took place for two days and no new eggs were observed on the carcasses by the afternoon of day 2. By the afternoon of day 2 actively feeding maggot masses of second instar maggots were observed (Fig 3.8.8.). Third instar maggots were recorded on all the carcasses by day 3. Because of the shortened period of oviposition, second and third instar maggots were recorded together on the carcasses for a day (Fig. 3.8.8.).

The maggots that were observed on the carcasses were dominated by *C. marginalis* and *C. albiceps*. Both species were reared from newly hatched maggot masses, indicating that both species oviposited simultaneously. *Chrysomya albiceps* maggots

stayed on the carcasses longer than *C. marginalis* maggots (Fig. 3.8.8.). These results were all very similar to those recorded in the summer wrapped trial. However, there was one difference, the maggots during this trial were only recorded on the carcasses until days 9 and 10 (Fig. 3.8.8.). The maggots during the summer wrapped trail were recorded until day 13.

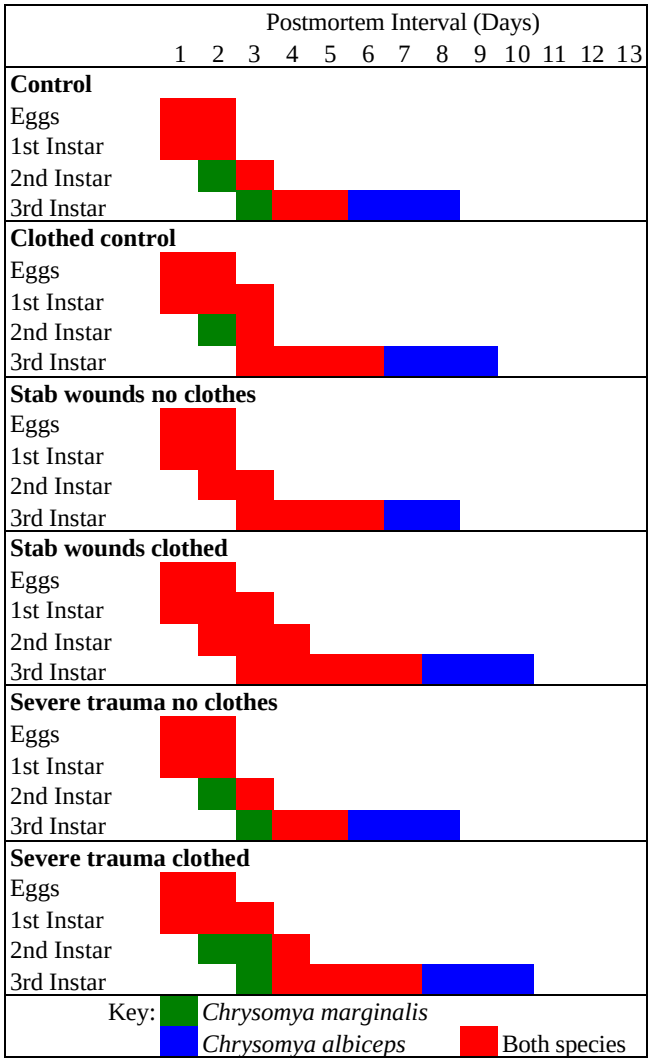


Fig. 3.8.8. Egg and maggot succession for all carcasses during the summer wounds trial.

3.8.3. Statistical analysis of arthropod succession

i) Jaccard Metric species pairwise similarities

Data from the succession diagrams were used to create the Jaccard occurrence matrices and the mean faunal similarity was then calculated for each day (see **2.4.1).**

These were plotted and the grand mean of all the mean faunal similarities were calculated and are represented as $\overline{S}_{gmean}$ (Fig. 3.8.9.). The $\overline{S}_{gmean}$ values during the summer were between 0.427 and 0.537 (Fig. 3.8.9.). This was almost identical to the summer wrapped trials with values of between 0.477 and 0.530. The $\overline{S}_{gmean}$ values were higher than any other trial $\overline{S}_{gmean}$ values. In the current summer trial the mean pairwise faunal similarities generally levelled off from day 15 (Fig. 3.8.9). This showed more stability than seen in the spring or autumn trials. As stated in the summer wrapped trial, this stability was reflected in the decomposition and arthropod succession, as the carcasses decomposed very quickly and tissue was consumed allowing the Coleoptera community to establish and become stabilised.

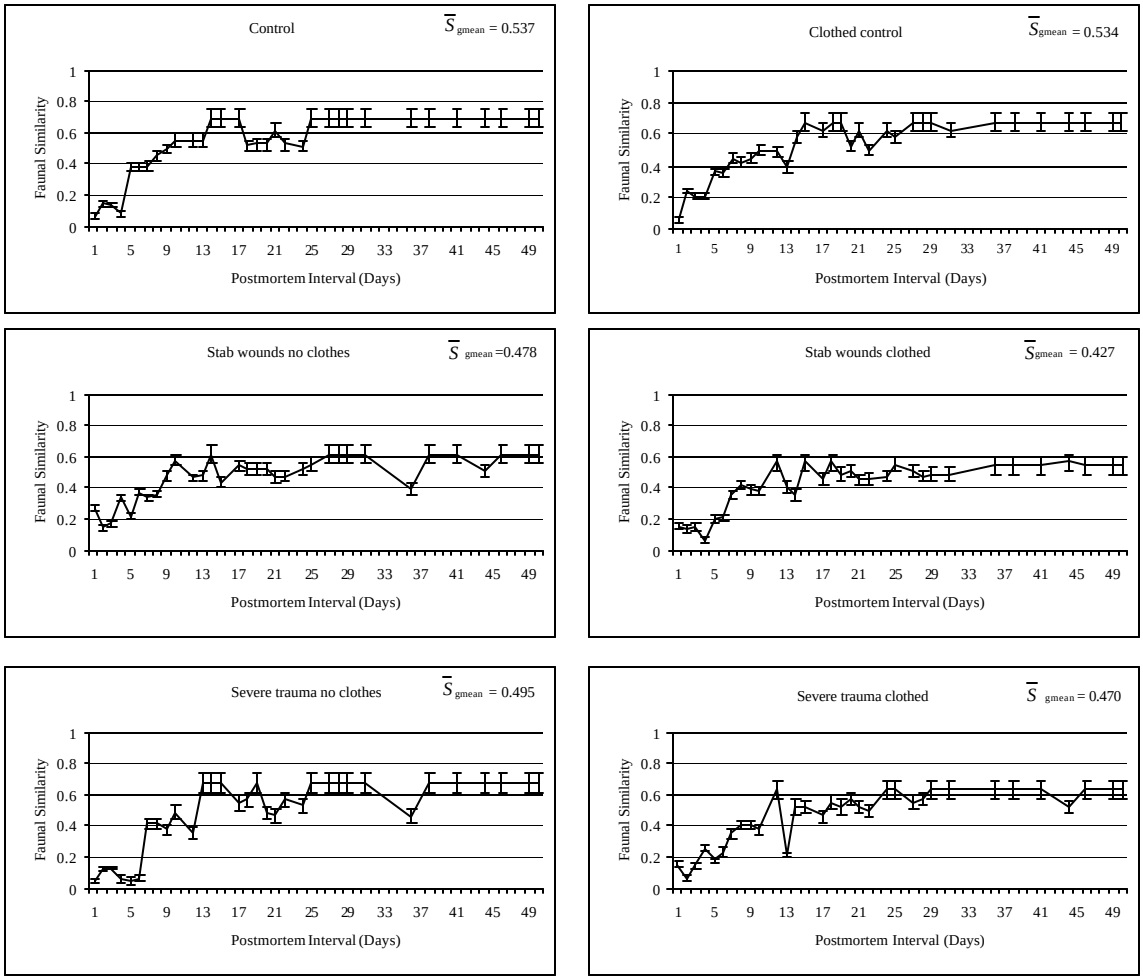


Fig. 3.8.9. Plots of mean pairwise faunal similarities (Jaccard metric) for each sampling period in the succession (vertical bars represent the standard errors) for the summer wounds trial.

ii) Pearson's Correlation Coefficient on Jaccard similarity matrices

All the results showed significant differences between the carcases with the exception of the control versus clothed control carcasses and the severe trauma no clothes versus stab wounds no clothes carcasses (Table 3.8.2.). There was also significant differences between the two seasons (Table 3.8.3). This may be due to the high rainfall experienced during the current trial.

Table 3.8.2. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the summer wounds trials (* - significant difference at 95% confidence interval).

	Control	**Clothed control**	**Stab wounds no clothes**
Clothed control	**0.8493 (0.0360)**		
Stab wounds no clothes	**0.8082 (0.0860)***	**0.7200 (0.1300)***	
Stab wounds clothed	**0.7905 (0.1180)***	**0.8266 (0.0660)***	**0.6831 (0.1510)***
Severe trauma no clothes	**0.8536 (0.0540)***	**0.8300 (0.0970)***	**0.7887 (0.0300)**
Severe trauma clothed	**0.7798 (0.0630)***	**0.8150 (0.1300)***	**0.7191 (0.1260)***
	Stab wounds clothed	**Severe trauma no clothes**	
Clothed control			
Stab wounds no clothes			
Stab wounds clothed			
Severe trauma no clothes	**0.7921 (0.0920)***		
Severe trauma clothed	**0.8242 (0.0660)***	**0.7547 (0.1840)***	

Table 3.8.3. Results from similarity matrices analysis, $K_{obs\ value}$ and *p*-values for the control and clothed control carcasses in the spring of 2004 and 2005 (* - significant difference at 95% confidence interval).

	Control (2005)	Clothed control (2005)
Control (2004)	0.6800 (0.1130)*	0.6982 (0.1250)*
Clothed control (2004)	0.7566 (0.1150)*	0.7911 (0.0560)*

3.8.4. Ambient, external and internal carcass temperatures

The iButtons at the study site recorded temperatures that were very similar to the data obtained from the South African Weather Services (SAWS) recorded at the Bloemfontein airport and city stations (Fig. 3. 8.10.). The minimum ambient temperature range was high. The minimum ambient temperatures recorded at the study site were between 11 and 22^0C (Fig. 3.8.10a). These were similar to the summer wrapped trial. The maximum ambient temperatures recorded at the study site varied between 20 and 38^0C over the entire trial (Fig. 3.7.10b), and were generally higher than those recorded in the summer wrapped trial.

As in the spring wounds trials, the iButtons were sealed in a double layer of plastic which protected them. However, some of the iButtons did record errors and a few failed.

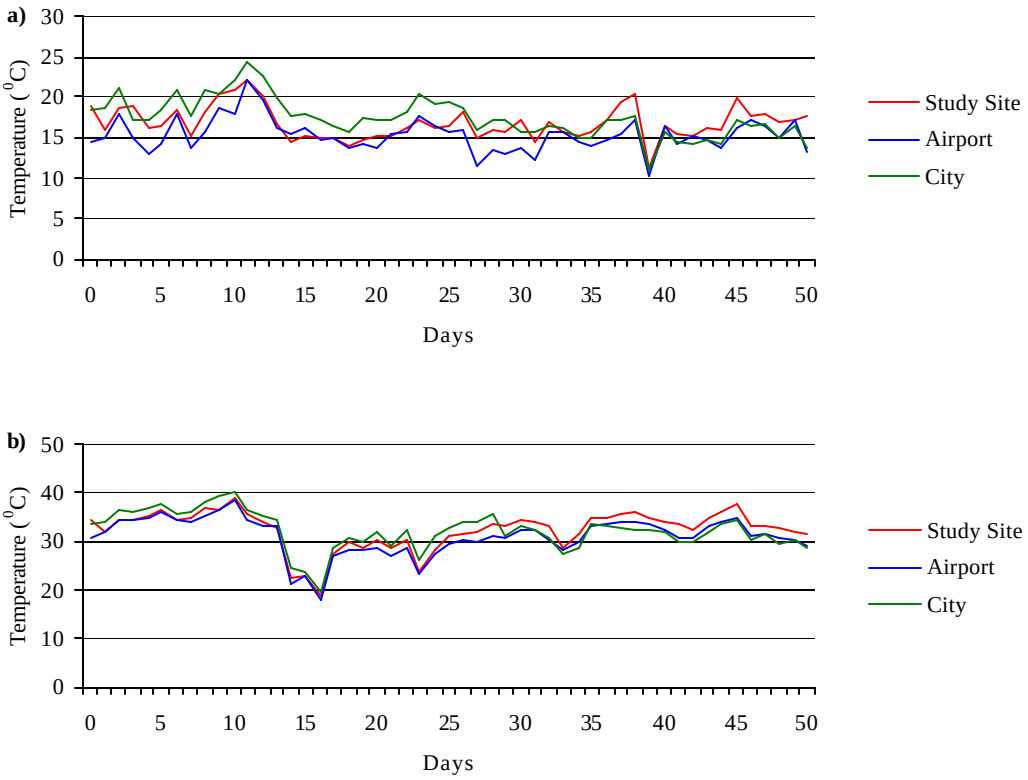


Fig. 3.8.10. Maximum (a) and minimum (b) daily ambient temperatures recorded at he Bloemfontein city and airport SAWS stations and at the study site during the summer wounds trial.

In general the maximum and minimum head averages had very low standard errors, proving that the data between the carcasses did not vary much. The minimum average head temperatures showed nearly no difference from the ambient temperatures. However, between days 3 and 4, the minimum head temperatures were slightly higher than the ambient temperatures (Fig. 3.8.11.). In the summer wrapped trial this occurred between days 5 and 10. This was when the maggot masses were present on the carcasses. The maximum average head temperatures were steadily higher than the ambient temperatures. However, because the minimum average head temperatures

closely track the ambient, this would indicate that the heads do not maintain the heat (Fig. 3.8.11).

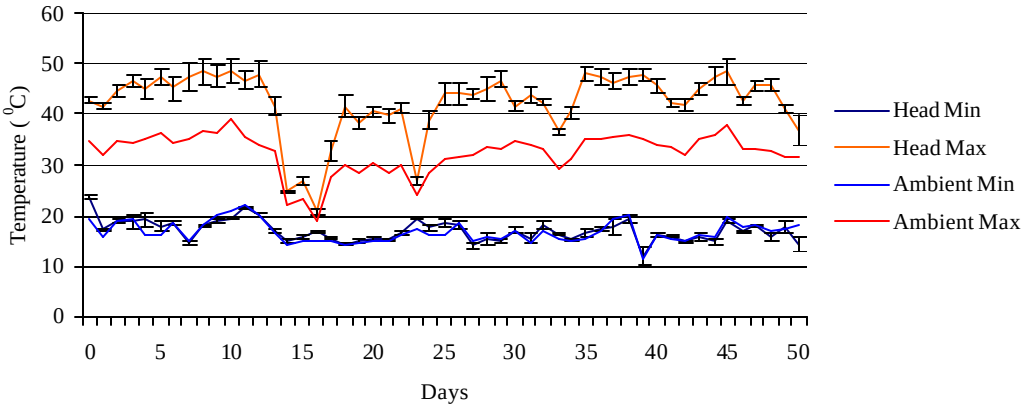


Fig. 3.8.11. Maximum and minimum ambient and internal carcass head temperatures during the summer wounds trial.

The minimum average thorax temperatures had low standard errors, indicating that the data between the carcasses varied little. The minimum thorax temperatures were noticeably higher than the ambient temperatures between days 4 to 9 (Fig. 3. 8.12.). This occurred in the summer wrapped trial between days 5 and 15. The maximum average thorax temperatures were also constantly higher than the maximum ambient temperatures (Fig. 3.8.12.). However, there was a high standard error, indicating that there was some variability between the carcasses (Fig. 3.8.12.). These results were similar to those recorded during the spring wounds trial.

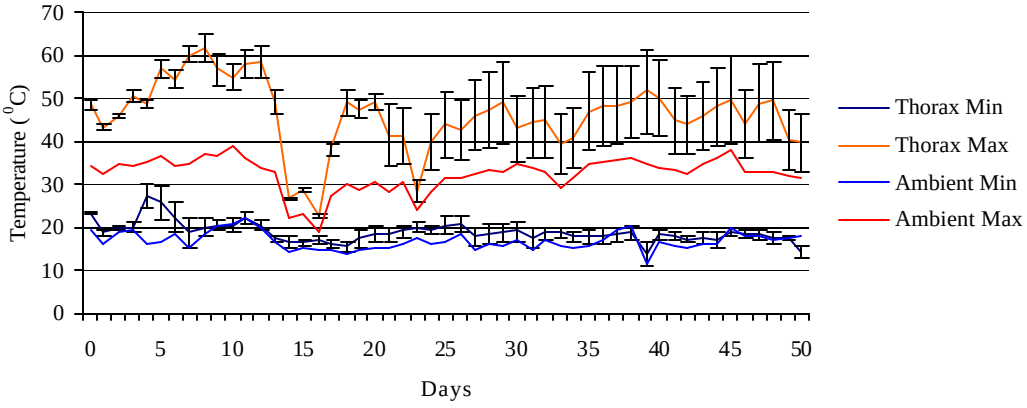


Fig. 3.8.12. Maximum and minimum ambient and internal carcass thorax temperatures during the summer wounds trial.

The average minimum abdomen temperatures, similar to the average minimum head temperatures, were slightly higher than the ambient minimum temperatures. The increase seen between days 4 and 9 (Fig. 3.8.13), which probably indicated maggot mass activity, was identical to the summer wrapped trial. The average maximum abdomen temperatures showed a comparable trend to the average thorax maximum temperatures. However, the standard errors were not as high showing less variance between the carcasses (Fig. 3.8.13).

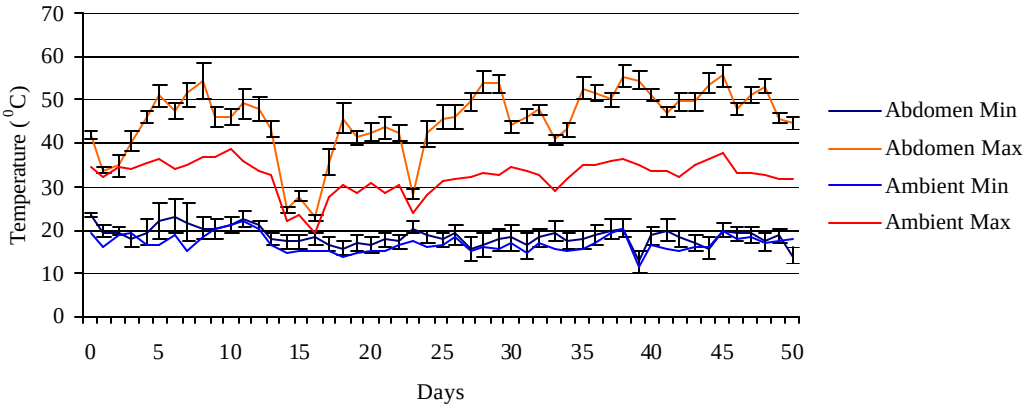


Fig. 3.8.13. Maximum and minimum ambient and internal carcass abdomen temperatures during the summer wounds trial.

The average minimum temperatures taken on the surfaces of the carcasses were generally very similar to the minimum ambient temperatures. The average minimum surface temperatures showed a small increase from the ambient minimum temperatures. This increase was seen between days 4 and 9 indicating maggot mass activity. The similarity between the regions of the carcasses was due to collapsing of the carcasses and the wide spread activity of the maggot mass. The average maximum temperatures were higher than the maximum ambient temperatures. The range between the average minimum and the average maximum temperatures were considerably larger than recorded in any other region of the carcasses, which was similar to the spring trial.

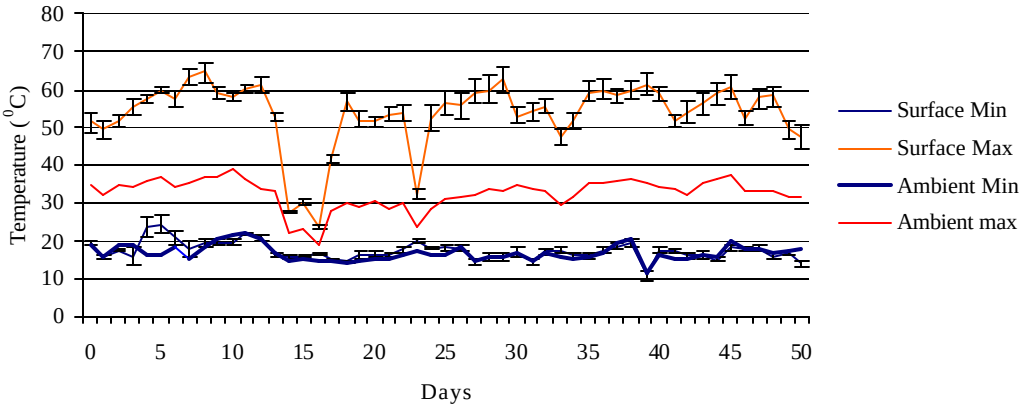


Fig. 3.8.14. Maximum and minimum ambient and surface temperatures of the carcasses during the summer wounds trial.

The average minimum underneath temperatures were noticeably higher than the minimum ambient temperatures (Fig. 3.8.15.). This is the only region of the carcass that showed this trend. The difference was slightly higher between days 5 and 11, indicating maggot mass activity identical to those recorded during the spring wounds trail. The carcasses may have offered some insulation and retained the heat. The average maximum temperatures underneath the carcasses were higher than the maximum ambient temperatures. However, this difference was not as great as seen in other regions of the carcasses, which was an indication that the carcasses provided some insulation.

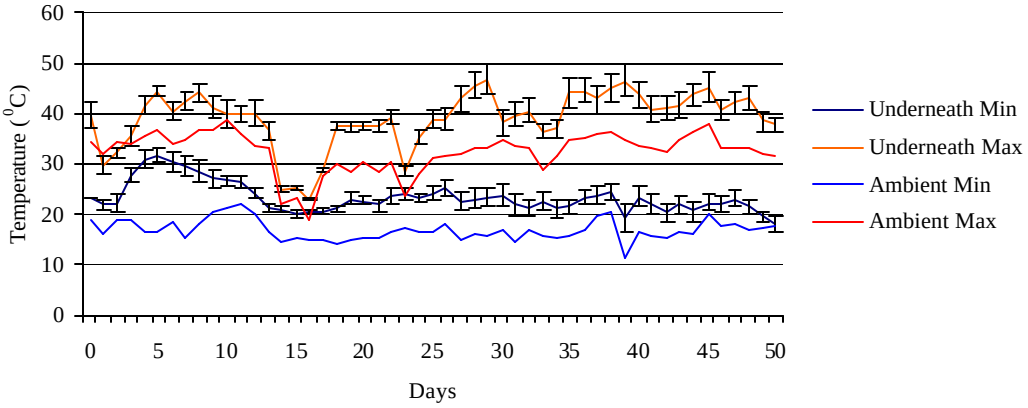


Fig. 3.8.15. Maximum and minimum ambient and the underneath temperatures of the carcasses during the summer wounds trial.

3.8.5. Maggot mass distribution

The primary oviposition sites did not change from the autumn and spring wounds and wrapped and summer wrapped trials. The movement of the maggot masses was similar to those recorded in the autumn and the spring wounds trials. However, these trends were not always obvious. The maggot masses tended to be more widely spread in the afternoons. In the heat of the afternoons, the maggots tended to be more off the carcasses and utilize the edges of the carcasses (Fig. 3.8. 16.).

3.8.6. Maggot predation

Chrysomya albiceps predation was recorded in both the autumn trials but there was minimal predation during the current trial. Similar to the summer wrapped trial, the predation appeared to be limited. The maggot masses were predominantly *C. marginalis* or *C. albiceps* and did not interact with each other.

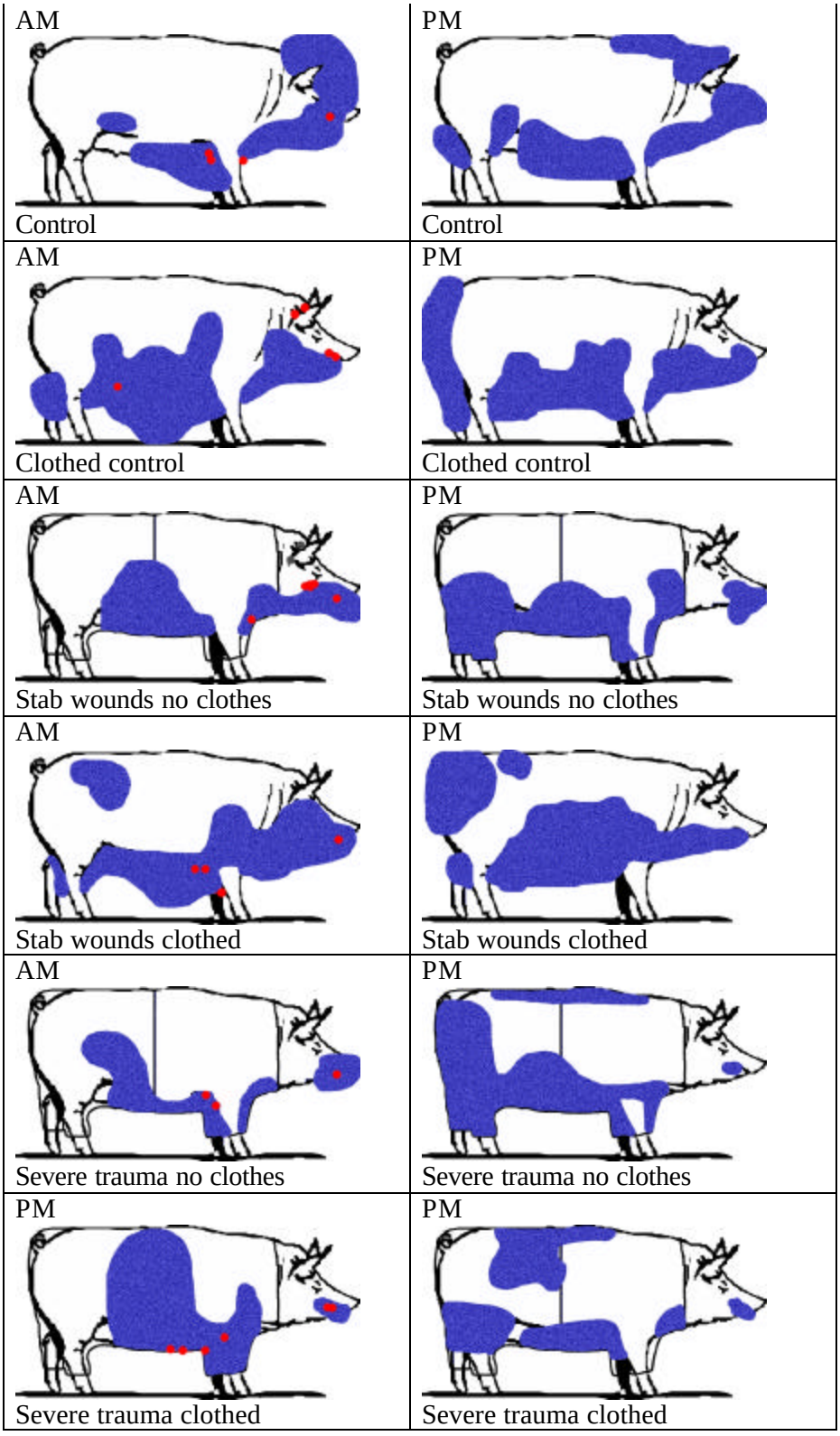


Fig. 3.8.16. Maggot mass movement recorded on day 3 of the summer wounds trial. Dots on the morning (AM) diagrams represent the primary oviposition sites.

CONCLUSIONS

The aims of this study were to determine the influence of a) season, b) clothing, c) wrapping and d) knife wounds on carcass decomposition and insect succession.

Influence of season

Diptera recorded during all the trials were dominated by the Calliphoridae and Sarcophagidae. In the autumn and summer seasons, the Diptera species were dominated by *C. marginalis* and *C. albiceps* (Table 4.1). In the spring, the Diptera species were dominated by *C. chloropyga* and *C. albiceps* (Table 4.1). This species composition was reflected in the maggot masses. In the winter seasons, *S. cruentata*, *C. chloropyga*, *C. vicina*, and *Lucilia* spp. were the species breeding on carcasses (Table 4.1). However, the adult Diptera species present at the carcasses did not necessarily represent accurately the maggot mass species composition. In the warmer seasons (autumn, spring and summer) the maggot mass tended to consist of maggots of a similar age as oviposition by all the blow fly species only took place over a few days. Also it was recorded that *C. marginalis* and *C. albiceps* oviposited at the same time and sometimes adding to the egg mass of the other species. In the winter the maggot masses when present on the carcasses consisted of maggots of different age groups as oviposition took place over an extended period of a few weeks.

During the autumn seasons there was predation by *C. albiceps* maggots on *C. marginalis* maggots was observed. Predation only occurred when all the carcass tissues were consumed rapidly before the maggots migrated. There was limited maggot predation during the spring trials and some predation observed during the summer trials. The lack of competition resulted in winter being a difficult time of the year to accurately estimate PMI, however, the presence of such a diversity of the blow fly species still showed that an estimation of PMI can be made.

In all seasons the Coleoptera community present and breeding on the carcasses were dominated by Dermestidae, *D. maculatus* (adults and larvae) and Cleridae, *N. rufipes*. In the summer Silphidae, *T. micans* (adults and larvae) were also recorded on the carcasses.

Table 4.1. Diptera species composition and abundance during each season.

	Autumn	Winter	Spring	Summer
Abundance 1 Most dominant	 *C. marginalis*	 *C. vicina*	 *C. chloropyga*	 *C. marginalis*
2 Most dominant	 *C. albiceps*	 *C. chloropyga*	 *C. albiceps*	 *C. albiceps*
3 Few individuals	 *S. cruentata*	 *Lucilia* spp.	 *S. cruentata*	 *S. cruentata*
4 Few individuals	 *Lucilia spp.*	 *S. cruentata*		 *C. chloropyga*

During the autumn seasons, noticeable predation by *C. albiceps* maggots on *C. marginalis* maggots was observed. There was limited maggot predation during the spring trials and some predation observed during the summer trials.

Influence of clothing

Clothing had no influence on the decomposition or arthropod succession on the carcasses. Unlike the sheet used in the wrapped trials, the clothing did not delay the drying out of the carcasses. In the warmer seasons (autumn, spring and summer) the presence of clothing altered the maggot mass distribution. The clothing allowed for greater maggot mass movement. This also resulted in more skin tissue being consumed by the maggot mass. The difference in maggot mass distribution was the most pronounced during the autumn trials. In winter the clothing may restrict the loss of heat during the colder times of the day, but because of the very small maggot masses the clothing did not appear to have any effect on the distribution of the maggot masses. The small maggot masses found in winter was probably due to species

composition as well as low temperatures. There was occasionally clothing displacement due to maggot mass activity during the autumn and summer trials. This observation would need to be considered when sexual abuse in a human investigation is suspected.

Influence of wrapping

The wrapping of the carcasses caused differences in the decomposition and there was a significant delay in drying out of the carcasses tissues during all the warmer seasons. In the autumn and spring trials, the control and clothed control carcasses dried out the faster than wrapped carcasses. They entered advanced decay simultaneously. In the autumn, the wrapped carcasses only entered advanced decay approximately three to seven days after the controls. In the spring this time delay was approximately a week. In summer all the carcasses entered advanced decay simultaneously. However, only the control and clothed control carcasses dried out and entered dry remains after day 30. Generally only the control and clothed control carcasses entered the transition stage or dry remains stage, as the wrapped carcasses remained moist. This suggested that the wrapping allowed little evaporation and slowed the draining of the decomposition fluids onto the ground. During the winter trial the carcasses stayed in active decay for almost the entire trial and there was little change in decomposition.

The wrapping of the carcasses caused no delay in oviposition by the Diptera in the warmer seasons (autumn, summer and spring). The blow fly eggs were oviposited on all the carcasses simultaneously. In the autumn trials there was a delay in oviposition on all the carcasses due to rainfall during in the first two days after carcass placement. In the winter, wrapping of the carcasses delayed the oviposition of the Diptera by four days. Because of the delay in oviposition, maggots were present on the wrapped carcasses until the end of the trial. However, there was no overall change in arthropod succession due to the presence of wrapping.

During the warmer seasons (autumn, spring and summer), the distribution of the maggot masses was altered by the presence of wrapping as it allowed more maggot movement. In winter, as with the presence of clothing, the maggot mass distribution was not affected by the presence of wrapping. In the summer and autumn seasons,

extremely high internal temperatures and trapped metabolic gasses caused significant maggot mortality on some of the wrapped carcasses.

Influence of knife wounds

The decomposition process was not influenced by the presence or absence of wounds. In all the trials there was no selection by the female Diptera for carcasses with wounds. The presence of the wounds and blood did not affect the oviposition by the Diptera or the overall insect succession.

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APPENDIX 1

1. Correlation coefficient

Details of the correlation coefficient used by MATLAB 6.5 (The MathWorks, Natick, MA).

R = *correlation coefficient*[1] (X) returns a matrix R of correlation coefficients calculated from an input matrix (*X*) whose rows are observations and whose columns are variables. The matrix R = *correlation coefficient*[1] (X) related to the covariance matrix C = *covariance matrix*[2] (X) by

$$R(i,j) = \frac{C(i,j)}{\sqrt{C(i,i)\,C(j,j)}}$$

Where:
C = *covariance matrix*[2] (x) where x is a vector returns the variance of the vector elements. For matrices where each row is an observation and each column a variable, *covariance matrix*[2] (x) is the covariance matrix. *Diagonal matrices*[3] (*covariance matrix*[2] (x)) is a vector of variances for each column, and square root(*diagonal matrices*[3] (*covariance matrix*[2] (x)) is a vector of standard deviations.

C = *covariance matrix*[2] (x,y), where x and y are column vectors of equal length, is equivalent to *covariance matrix*[2] ([x y]). C*ovariance matrix*[2] removes the mean from each column before calculating the result. The covariance function is defined as *covariance matrix*[2] $(x_1,x_2) = E[(x_1-\Phi_1)(x_2-\Phi_2)]$ where *E* is the mathematical expectation and $\Phi_1 = Ex_1$.

Diagonal matrices[3] (v,k) = when v is a vector of n components, returns a square matrix X of order n+ *absolute value*[4] (k), with the elements of v on the kth diagonal. k = 0 represents the main diagonal, k > 0 above the main diagonal, and k < 0 below the main diagonal. X = *diagonal matrices*[3] (v) puts v on the main diagonal, same as above with k = 0. v = *diagonal matrices*[3] (X,k) for matrix X, returns a column vector

v formed from the elements of the kth diagonal of X. v = *diagonal matrices*[3] (X) returns the main diagonal of X, same as above with k = 0.

Absolute value[4] (X) = returns an array Y such that each element of Y is the absolute value of the corresponding element of X. If X is complex, *absolute value*[4] (X) returns the complex modulus (magnitude), which is the same as sqrt(real part of complex number (X).^2 + imaginary part of the elements of array X.^2)

R = *correlation coefficient*[1] (x,y) where x and y are column vectors is the same as *correlation coefficient*[1] ([x y]).

The K_{obs} is computed by transforming the correlation to create a t statistic having n-2 degrees of freedom, where n is the number of rows of X. The confidence bounds are based on an asymptotic normal distribution of 0.5*log((1+R)/(1-R)), with an approximate variance equal to 1/(n-3). These bounds are accurate for large samples when X has a multivariate normal distribution. The 'pairwise' option can produce an R matrix that is not positive definite (MATLAB 6.5 Equation and Help files).

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