Insect Succession and Decomposition Pattern on Pig Carrion During Warm and Cold Seasons in Kwazulu-Natal Province of South Africa

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Abstract

With the aim of identifying insects with potential value as indicator species in postmortem interval (PMI), the location or season of death of human or monogastric animals, two medium-sized large white pigs were used for the study during cold and warm seasons. Five stages of decomposition and their durations were observed and described in the pig carcasses during both seasons. Carcasses were first colonized by flies from seven taxa during fresh stage. Chrysomya marginalis (Wiedemann) (Diptera: Calliphoridae), Chrysomya albiceps (Wiedemann) (Diptera: Calliphoridae) and Musca domestica (Walker) (Diptera: Calliphoridae) were recorded as the overall dominant adult flies found on the carcasses in both seasons. Colonization by beetles began during the bloated stage in the warm season and active stage in the cold season. Dermestes maculatus (De Geer) (Coleoptera: Dermestidae) and Necrobia rufipes (De Geer) (Cleridae) were the most abundant beetles in both seasons. Flies and beetles were generally abundant during the warm seasons as compared to the cold season. However, the difference was only significant for beetles. The highest number of flies were recorded in the bloated stage for both seasons, however they were reduced in the active stage which coincided with the introduction of predatory beetles. The arrival time of the different arthropod species and their association with different stages of decomposition during both seasons pointed to their value in estimating the PMI in forensic investigations in the locality of KwaZulu-Natal, South Africa. Consequently, they can potentially be useful in the estimation of PMI and other cases of criminal investigations.

Key words: Diptera, coleoptera, species identification, seasonality, stages of decomposition

Forensic entomology is the scientific study of the use of insects and other arthropods associated with a carrion in forensic investigations (Wolff et al. 2001, Haskell et al. 2008). This field of study is accepted and applied in various courts of law worldwide (Amendt et al. 2007, Amendt et al. 2011), and is now recognized as an important investigative tool in many developed countries (Haskell et al. 2008, Kokdener 2016, Tembe and Mukaratirwa 2020). Medico-legal entomology deals with insect evidence collected at the crime scene, and this field has been gaining more recognition than urban and stored-product forensic entomology globally (Sukontason et al. 2007, Haskell et al. 2008).

In medico-legal entomology, insects and other arthropods are commonly used to confirm relocation of carcasses or postmortem transfer (Sumodan 2002), postmortem interval (PMI), cause and manner of death, chemical and drug verification, child abuse and neglect, and other related cases of a forensic investigation (Amendt et al. 2007, Kokdener 2016). However, its application requires accuracy and consideration of several factors, such as understanding the role of different insect species and their colonization process throughout carcass decomposition and the effect of temperature, seasons, and climatic zones on carrion associated species.

According to Parry et al. (2016), carrion-feeding insect species have been observed to vary in diverse environments. This variation may be due to factors such as habitat (Matuszewski et al. 2013), availability of food, the presence or absence of other insect species (Williams 2002), and season (Richards et al. 2009b). Therefore,
studying, recording, and comparing the different insect species found in a specific geographic area and location in different seasons can provide valuable information on the availability, abundance, and diversity of carrion-feeding insect species, subsequently improving the existing knowledge and understanding of their adaptive responses to different environmental conditions. This information is of importance in the field of forensic entomology (McIntyre 2000, Kitching 2013) and sub-Saharan African countries including South Africa are still lagging behind (Tembe and Mukaratirwa 2020).

Matuszewski et al. (2013), observed that certain insect species display a strong habitat and seasonal associations, which make them valuable as potential indicator species to determine the location and season of death. Mann et al. (1990) stated that season is one of the most important factors influencing the rate of decomposition, likely caused by changes associated with temperature, rainfall, and insect activity. Previous studies have shown that an increase in rainfall is presumed to speed up decomposition through leaching and supply of moisture for bacterial and carrion-associated insect activities (Mann et al. 1990, Lopes de Carvalho and Linhares 2001, Archer 2004). However, other authors have also stated that while there is anecdotal evidence that rainfall speeds up the rate of decomposition on large animals (Mann et al. 1990, Lopes de Carvalho and Linhares 2001), according to Archer (2004) the effect of rainfall have never been statistically studied separately from that of temperature.

According to Richard et al. (2009a,b), flies normally show fluctuations in population sizes which is strongly influenced by seasonal changes, with observed population size peaking at particular times of the year (Parry et al. 2016). The authors further stated that these peaks in population sizes are usually due to the existing climatic factors, such as humidity and temperature and strongly influence the presence and abundance of fly species (Richards et al. 2009a, Parry et al. 2016). Hence, most fly species have been observed to be abundant during the warm season compared to the cold season (Parry et al. 2016). However, other authors have demonstrated that some fly species tend to be more prevalent during the cooler seasons (Braack and De Vos 1987, de Souza and Linhares 1997, Brandage et al. 2011, Parry et al. 2016). Nonetheless, the succession pattern of insects commonly follows the same pattern at a family level, with documented variation at a genus and species level among different locations (Tabor et al. 2004).

Although the effect of seasons and other environmental factors on the decomposition rate and succession pattern of arthropods have been previously studied in other countries including few regions of South Africa, the seasonal colonization pattern and activity of many carrion-associated arthropods to determine PMI and relocation of a carcass remains scantily studied in Africa (Parry et al. 2016). A number of studies have been previously conducted in some provinces of South Africa, on diversity of arthropods associated with animal carrion (Braack 1986) and seasonal variation related to different stages of decomposition and succession pattern of arthropods (Kelly et al. 2008, 2011, Gilbert 2014, Williams and Villet 2019) which have a different ecological niche from KwaZulu-Natal province, where this study was conducted. Therefore, this study aimed at determining the decomposition pattern of a pig carcass during the warm and cold season, and further assess the dipteran and coleopteran species associated with pig carrion and their sequence of arrival and colonization immediately after death and throughout the different stages of decomposition during the two seasons. The pig is an acceptable model for this type of study as they resemble humans and larger monogastric animals in terms of quantity of body hair and process of decomposition (Wolff et al. 2001, Tabor et al. 2004).

Materials and Methods

Study Location

The study was conducted at the University of KwaZulu-Natal Ukulinga Research and Training farm located in Pietermaritzburg, South Africa (29.6627° S, 30.4050° E) (Fig. 1). The farm is under uMgungundlovu district of KwaZulu-Natal province, South Africa. The area is characterized by warm to hot summers and mild winters, which are often accompanied by irregular frost (Kiala et al. 2017). The average monthly temperature ranges between 13.2°C and 21.4°C, with an annual mean temperature of 17°C (Mills and Frey 2004, Everson et al. 2013, Kiala et al. 2017). The area receives an annual precipitation of 680 mm in over 106 day per annum (Kiala et al. 2017) and it falls under the Southern Tall Grassveld and mainly herbaceous as a result of long-term burnings (Mills and Frey 2004, Kiala et al. 2017). The cold season is experienced from the month of May to end of August, whilst the warm season is from September to April. The cold season trial of this study was conducted from June to August 2019, with measured average temperature ranging between 18°C and 19°C, and the warm season trial was conducted from November 2019 to January 2020, with measured average temperature ranging between 21°C and 23°C.

Study Animals

Two pigs (Sus scrofa domesticus) with an average live weight of 80 kg were donated by Hmb School Trust piggery in Greytown, South Africa. After sacrifice, three knife stabs were made around the neck region to create wounds that mimic the cases of an illegally killed animal carcasses. The carcasses were immediately translocated to the University of KwaZulu-Natal Ukulinga Research and Training farm (approximately 60 km from the piggery).

Sampling Procedure

On arrival at the farm, each carcass was immediately placed in a metal cage (100 cm x 100 cm x 100 cm) which was placed in an open space in one of the farm paddocks. The cage was covered with mesh wire to protect the carcass from scavengers such as rats and other small vertebrates but allowing free movement of arthropods. The day the animals were killed was recorded as day 0 and marked the initiation of each trial which lasted 59 d.

Stages of decomposition of carcasses were determined as described by Martinez et al. (2007) and Wolff et al. (2001). The following information was collected daily during course of the study, through visual observation and recordings from the carcass and its surroundings; physical changes of the carcasses, odor, and intensity and the presence of arthropods during each stage of decomposition at given days and times as shown in Table 1. Temperature of carcass and that of the soil surrounding the carcass were measured once a day at 09:00 am using a MAC-AFRIC infrared thermometer (Adendorff machinery mart) (Temperature range -50–380°C, ± 2%). Adult arthropods found on and around the carcasses were collected using either fly traps which were hanged on the cages or by direct hand-picking for a period of two hours each day (09:00 – 11:00 am). Collected arthropods were preserved in 70% ethanol for further processing and identification. Eggs and larvae of arthropods on and around the carcass during decomposition were not collected to avoid disturbance of the maggots establishing on the carcasses, which could consequently interfere with the rate of decomposition of the carcass. Therefore, only adult arthropods were sampled during the study period.
Morphological and Molecular Identification of Collected Adult Arthropods

Prior to identification, collected adult arthropods were cleaned by immersing them in distilled water for 10–15 min to remove excess ethanol and air-dried. Arthropods were then morphologically identified under the stereo microscope following identification keys described by (Byrd and Castner 2001, Kolver 2009, Iqbal et al. 2014, Lutz et al. 2018, Lubbe et al. 2019, BugGuide 2020). Molecular techniques were used to confirm arthropod species identification using mitochondrial primers as described by Folmer et al. (1994) and Zhuang et al. (2011) Voucher specimens of arthropods were deposited in the Parasitology Lab, University of KwaZulu-Natal.

Data Analysis

To determine the statistical differences in the abundance of Dipteran and Coleopteran groups between the different seasons, a $\chi^2$ and Fisher exact test was performed and $P < 0.05$ was considered statistically significant.
Results

Five decomposition stages were observed in the pig carcasses during the warm and cold season based on the postmortem changes of the carcass and categorized as shown in Fig. 2A and B. The results also showed a marked relationship between soil temperature and carcass temperature regardless of season and decomposition stage. The carcass temperature followed the temperature trend of that of the soil (Fig. 3A and B). There were no observed differences in the duration decomposition stages between the two seasons.

Fig. 2. Photographs showing decomposition stages of pig carcass during the warm season (A) and cold season (B). Images (a–e) represents different stages of decomposition: (a) fresh stage, (b) bloated stage, (c) active stage, (d) advanced stage, (e) dry stage.
Fresh Stage (0–1 d)
The fresh stage for both seasons, commenced directly after the animals were humanely killed, and it was associated with soft torsos and flexible limbs and no foul odor (Table 1; Fig. 2A and B). There was an observed decrease in the body temperature from 25.4°C to 15.4°C during the cold season and 37°C to 24°C during the warm season (Fig. 3A and B). Seven arthropod taxa (Dipteran) were recorded in this stage during both warm and cold seasons (Fig. 4), and comprised of the following species; *Chrysomya marginalis* (Wiedemann) (Diptera: Calliphoridae), *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae), *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae), *Chrysomya chloropyaga* (Wiedemann) (Diptera: Calliphoridae), *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), *Musca domestica* (Walker) (Diptera: Muscidae), and *Sarcophaga calicifera* (Boettcher, 1912) (Diptera: Sarcophagidae). The species were the first to arrive and colonize on the pig carcasses within few hours of death in both warm and cold seasons (Table 2). Higher numbers of necrophagous flies were collected during the warm season (*n* = 76) as compared to the cold season (*n* = 34, Table 3). *M. domestica* (*n* = 22), and *Ch. albiceps* (*n* = 16) were more abundant during the warm season, whereas, *M. domestica* was
the most abundant species in the cold season (n = 11, Table 3). There was no significance difference in the abundance of necrophagous species collected during different seasons (P = 0.082) (Table 3).

Bloated Stage (2–6 d)
At this stage, the body color of both carcasses changed from white and became darkened, a foul odor was being emitted from the carcasses (Table 1; Fig. 2Ab and Bb). In the warm season, the carcass temperature at the beginning of the bloated stage was 32.5°C, an increase from 24°C recorded on the last day of the fresh stage (Fig. 3A). On the second day of this stage, the temperature then decreased to 21°C, followed up by an increase to 38.6°C until the fourth day of the stage. On the last day of the bloated stage, the carcass temperature decreased to 28.7°C (Fig. 3A). A different trend was observed during the cold season, where the body showed an increase in temperature in the first two days (26.5°C and 28.7°C) of the bloated stage, then decreased on the third day to 22.7°C (Fig. 3B). The body temperature then increased to 24.8°C until the last day of this stage (Fig. 3B). The body temperature was slightly higher than that of soil temperature, during both seasons (Fig. 3A and B). The number of arthropod taxa increased during both warm (n = 11) and cold seasons (n = 8, Fig. 4). The Dipteran species observed and recorded during the fresh stage persisted on the carcasses to the bloated stage (Ch. marginalis, Ch. putoria, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, S. calcifera) for both seasons (Table 2). However, there was an additional new species Antherigona soccata Rondani (Diptera: Muscidae), found on the carcass during the cold season. A. soccata and Ch. putoria were last collected and recorded during this stage (Table 2).

There was an increase in the total number of necrophagous flies collected on the pig carcasses during the warm season (n = 152) and cold season (n = 92) (Table 3), however, the difference was not statistically significant (P = 0.150, Table 3). The results also show that the recorded numbers of M. domestica (n = 38), Ch. albiceps (n = 38), and Ch. marginalis (n = 35) were higher during warm season than in the cold season (M. domestica [n = 26], Ch. albiceps [n = 14] and Ch. marginalis [n = 22]) (Table 3). In addition to the dipteran species collected during this stage, 24 beetles were collected comprising four species, namely Dermestes maculatus (n = 11) (De Geer) (Coleoptera: Dermestidae), Thanatophilus micans (n = 4) (Fabricius) (Coleoptera: Silphidae), Onthophagus crassicollis (n = 5) (Boucomont, 1913) (Coleoptera: Scarabaeidae), and Hycleus lunatus (n = 4) (Pallas, 1782) (Coleoptera: Meloidae) during the warm season. These four species persisted on the carcass until the last stage of decomposition (dry stage) except H. lunatus which was recorded only during this stage of decomposition.

Active Stage (7–12 d)
The foul odor from decomposing tissues from the carcasses was more intense, exudates were discharged by the body, and the peeling of skin commenced was observed in carcasses during the two seasons (Table 1; Fig. 2Ac and Bc). During the warm season, the carcass temperature at the beginning of the active stage was 17.5°C, which subsequently increased until the last day to 38.9°C (Fig. 3A). During the cold season, the active stage began with the carcass body temperature of 22.8°C, which then slightly declined to 22.2°C on the second day of the active stage, increasing to 31°C until the last day of the stage (Fig. 3B). There was a slight decrease in the total number of arthropods observed and collected during the warm season (n = 10), however in the cold season, the total number of arthropods taxa remained the same (n = 8, Fig. 4). The total number of necrophagous flies collected during this

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**Table 2. Succession pattern of different arthropods (Diptera and Coleoptera) species attracted during the five different stages of decomposition of pig carcass during the warm and cold season at Ukulinga research and training farm in the province of KwaZulu-Natal, South Africa.**

| Ecological category | Order    | Family        | Species                        |
|---------------------|----------|---------------|--------------------------------|
| Necrophagous        | Diptera  | Calliphoridae | Chrysomya marginalis           |
| Necrophagous        | Diptera  | Calliphoridae | Chrysomya putoria              |
| Necrophagous        | Diptera  | Calliphoridae | Chrysomya albiceps             |
| Necrophagous        | Diptera  | Calliphoridae | Chrysomya chloropyga           |
| Necrophagous        | Diptera  | Calliphoridae | Lucilia cuprina                |
| Necrophagous        | Diptera  | Muscidae      | Musca domestica                |
| Necrophagous        | Diptera  | Muscidae      | Atherigona soccata             |
| Necrophagous        | Diptera  | Sarcophagidae | Sarcophaga calcifera           |
| Predators           | Coleoptera| Cleridae      | Necrobia rufipes               |
| Necrophagous        | Coleoptera| Dermestidae   | Dermestes maculatus            |
| Predators           | Coleoptera| Silphidae     | Thanatophilus micans           |
| Coprophagous        | Coleoptera| Scarabaeidae  | Onthophagus crassicollis       |
| Incidental          | Coleoptera| Meloidae      | Hycleus lunatus                |

Black fill arrow – represents warm season; black dotted arrow – represents cold season.
stage decreased from 152 to 131 during the warm season, and from 92 to 62 during the cold seasons (Table 3). During both seasons, Ch. marginalis, Ch. albiceps, Ch. chloropyga, L. cuprina, M. domestica, and S. calcifera continued to persist on the carcasses (Table 2). However, Ch. chloropyga did not persist from this stage during the cold season and L. cuprina during both seasons (Table 2). There was an increase in the total number of beetles collected at this stage during the warm season (Table 3). The number of beetle individuals collected on the carcasses decreased (Table 3). The total number of necrophagous flies collected from both carcasses declined at this stage in comparison to the previous stages in both warm and cold seasons (Table 3). The total number of necrophagous flies collected from both carcasses declined at this stage in comparison to the previous stages in both warm and cold seasons (Table 3). The total number of beetle species remained constant during the warm season (Table 3). Additionally, T. micans (n = 9) and O. crassicollis (n = 4) appeared on the pig carcass for the first time during the cold season (Tables 2 and 3).

### Dry Stage (52–58 d)

Both carcasses had dry skin during this stage of decomposition (Table 1; Fig. 2Ad and Bd). A similar pattern of body temperature was observed as in the advanced stage, where body temperature was similar to soil temperature (Fig. 3A and B). M. domestica and S. calcifera were the only Diptera species which persisted on pig carcasses to this stage during both warm and cold seasons (Table 2). There was a decrease in the total number of arthropod taxa recorded during the warm season (n = 6) and cold season (n = 5, Fig. 4). The number of beetle species remained constant during the warm season (n = 5) and decreased during the cold season (n = 4). However, the total

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### Table 3. Abundance of adult Dipteran and Coleopteran species associated with different stages of pig carcasses during different stages of decomposition during the cold and warm season

| Ecological category | Order | Family | Genus/ species | Fresh (0–1 d) | Bloated (2–6 d) | Active (7–12 d) | Advanced (13–51 d) | Dry (52–58 d) | Total |
|---------------------|-------|--------|----------------|---------------|----------------|----------------|-------------------|--------------|-------|
| Necrophagous Diptera| Calliphoridae | Chrysomya | marginalis | 6 | 22 | 18 | 9 | 0 | 55 |
| Necrophagous Diptera| Calliphoridae | Chrysomya | putoria | 5 | 4 | 9 | 0 | 0 | 12 |
| Necrophagous Diptera| Calliphoridae | Chrysomya | albiceps | 14 | 13 | 7 | 5 | 0 | 34 |
| Necrophagous Diptera| Calliphoridae | Chrysomya | chloropyga | 11 | 15 | 5 | 0 | 0 | 19 |
| Necrophagous Diptera| Calliphoridae | Lucilia | cuprina | 1 | 4 | 1 | 2 | 7 | 9 |
| Necrophagous Diptera| Muscidae | Musca | domestica | 11 | 22 | 26 | 13 | 5 | 81 |
| Necrophagous Diptera| Muscidae | Atherigona | soccata | 0 | 0 | 0 | 0 | 0 | 2 |
| Necrophagous Diptera| Sarcophagidae | Sarcophaga | calcifera | 1 | 4 | 6 | 2 | 7 | 24 |
| Total Diptera | | | | 34 | 76 | 92 | 62 | 31 | 225 |
| χ²/Fisher exact P-value | | | | P = 0.082a | P = 0.150a | P = 0.116a | P = 0.102a | P = 0.001a* | P = 0.134a |
| Necrophagous Coleoptera | Dermestidae | Dermestes | maculatus | 0 | 0 | 0 | 22 | 26 | 66 |
| Predators Coleoptera | Cleridae | Necrobia | rufigipes | 0 | 0 | 0 | 0 | 0 | 48 |
| Predators Coleoptera | Silphidae | Thanatophilus | micans | 0 | 0 | 0 | 13 | 20 | 44 |
| Coprophagous Coleoptera | Scarabaeidae | Onthophagus | crassicolis | 0 | 0 | 0 | 12 | 5 | 42 |
| Incidental Coleoptera | Meloidae | Hyleus | lunatus | 0 | 0 | 0 | 2 | 0 | 4 |
| Total Coleoptera | | | | 0 | 0 | 0 | 4 | 0 | 264 |
| χ²/Fisher exact P-value | | | | N/A | N/A | 28 | 62 | 46 | 125 |
| | | | | P = 0.002a* | P = 0.08a | P = 0.002a* | P < 0.001a* |

CS, cold season; WS, warm season; Nd, not done; *Fisher’s exact; Significant at P < 0.05.
number of beetle individuals collected continued to increase on both warm \((n = 94)\) and cold \((n = 51)\) seasons, and the difference in the number of beetle individuals collected was statistically significant \((P = 0.002)\) (Table 3). \textit{T. micans} disappeared from the pig carcass during the cold season (Table 3).

**Discussion**

The decomposition pattern and time taken by the carcass exposed to warm season was the same as that of the carcass exposed to the cold season. This was not expected as most studies show that carcasses exposed to the summer season decompose faster than that one exposed to winter (Bass 1996, Gilbert 2014). The observed similar period of decomposition of carcasses between seasons in this study may have been due to rainfall experienced mostly when the warm season experiment was taking place. According to Lyu et al. (2016) rainfall wets the carcass and expel fly maggots from the carcass (Singh and Bala, 2019), and as a result affect the rate at which the carcass decomposes. Archer (2004) also reported that rainfall delay the rate of decomposition by reducing the carcass temperature through evaporative cooling, and hence as the carcass becomes waterlogged, the mass loss become slower.

This study also showed that the duration of the decomposition stages was similar to that described by Wolff et al. (2001) in a pig carcass with the difference in the dry stage in our study, which was shorter. During the first stage (fresh) of decomposition, the carcasses had soft torsos (abdomen and thorax) and flexible limbs and there was no odor during both warm and cold seasons. However, there was an observed decrease in the body temperature of carcasses in both warm and cold seasons. Similar observations were made by Kelly et al. (2008, 2011), where the fresh stage was associated with soft torso and flexible limbs with no odor during the winter and summer experiments. Furthermore, Grisales et al. (2010) made similar observations where body temperature of pig [\textit{Sus scrofa} (L.)] carcass decreased from 38°C to 22°C during the fresh stage of decomposition. Species from the families Calliphoridae (\textit{Ch. marginalis}, \textit{Ch. putoria}, \textit{Ch. albiceps}, \textit{Ch. chloropyga}, \textit{L. cuprina}), Muscidae (\textit{M. domestica}), and Sarcophagidae (\textit{S. calcifera}) were the first colonizers in both carcasses mainly for feeding and breeding purposes. Similarly, Shi et al. (2009), confirmed Sarcophagidae species as primary colonizers of a rabbit carcass in warmer temperatures and tropical areas. However, Mabika et al. (2014) and Martinez et al. (2007) recorded Sarcophagidae family as secondary colonizers and Calliphoridae and Muscidae as primary colonizers in rabbit and pig carcasses respectively. According to Mabika et al. (2014), species from these families play an important role during the early stages of decomposition, and due to the predictable sequence of arrival on the carcass they are potential indicators of PMI and determining clues in cases of criminal investigations especially if the body tissue is still fresh (Padonou et al. 2017, Tembe and Mukaratirwa 2020).

The bloated stage of both carcasses during the two seasons was associated with a change in body color from white to dark, with a foul odor. Similar observations were made by Kelly et al. (2011) where during the bloated stage the pig carcass body color darkened. Verheggen et al. (2017), reported that the bloated stage of vertebrate carcasses is characterized by the presence of a perceived odor as also confirmed by Mabika et al. (2014) in a rabbit carcass. Furthermore, during the bloated stage we observed an increase in the body temperature of the carcasses of which according to Martinez et al. (2007), this may be due to high insect activity taking place during this stage. It was further observed that during both seasons the carcass body temperature was slightly higher than that of the soil temperature. Similar observations were made by Payne (1965), whereby the temperature of the pig carcass was slightly higher than the soil temperature during the bloated stage. Furthermore, as the carcasses released foul odor, the numbers of \textit{Ch. marginalis}, \textit{Ch. putoria}, \textit{Ch. albiceps}, \textit{Ch. chloropyga}, \textit{L. cuprina}, \textit{M. domestica}, and \textit{S. calcifera} visiting the carcasses also increased during both seasons and according to Verheggen et al. (2017), odor plays a crucial role in attracting necrophagous insect species.

Our results further showed that Coleoptera species, \textit{D. maculatus}, \textit{T. micans}, \textit{O. crassicollis}, and \textit{H. lunatus} were the first beetle species to visit the pig carcass during the bloated stage and persisted through to the last stage of decomposition (dry stage) during the warm season with exception of \textit{H. lunatus}, which was only found during the bloated stage. The presence of these beetles on the carcass as early as bloated stage supports the findings of other several studies that reported the early arrival of Coleoptera species on carcasses (Early and Goff 1986, Braack 1987, Mayer and Vasconcelos 2013, Singh and Bala 2019). According to VanLaerhoven and Anderson (1999), the presence of these beetles during the bloated stage may be due to seasonal peaks appearance rather than the decomposition stages (Mabika et al. 2014). This could also explain the absence of these species on the carcass during the bloated stage of decomposition during the cold season. The occurrence of \textit{H. lunatus} in lower numbers only in the bloated stage of the warm season may indicate that the beetles could have accidentally landed on the carcass, as they are normally associated with crops (Gorthy et al. 2017) than dead bodies and their role in decomposing carcasses is still not clear.

Arthropod taxa (Dipteran and Coleopteran) richness increased during the bloated stage in the warm and cold season, and similarly Shi et al. (2009) observed and reported the highest taxa richness during the bloated stage of a rabbit carcass. Furthermore, the number of dipteran species collected during the bloated stage were higher compared to other stages during both cold and warm season and the dominant species were \textit{Ch. albiceps}, \textit{M. domestica}, and \textit{Ch. marginalis}. However, the highest number of Dipteran species were collected during the warm season. Similar results were reported by Keshavarzi et al. (2019), where the highest number of arthropods were observed during the bloated stage of albino rats and the dominant species were \textit{Ch. albiceps}, \textit{Calliphora vicina}, and \textit{S. africana} during the autumn season.

The active stage was also characterized by the presence of foul odor of decay and skin peeling. However, as the insect maggots fed on the carcass the foul odor became more pronounced. Our results correspond with those reported by Kelly et al. (2008, 2011), where the active stage of a pig carcass was characterized by a strong foul odor which became prominent as maggots fed on the carcass and persisted until the carcass started deflating. Furthermore, the observed skin peeling of carcasses might have occurred due to the skin drying out and erupting and tearing (Kelly et al. 2008). The early days of this stage were also characterized by a decrease in carcasses body temperature, which subsequently increased from day two until the last day of this stage in the warm season. However, in the cold season it decreased on the second day and subsequently increased until the last day of decomposition. Martinez et al. (2007), also observed low body temperature of pig carcass that increased in the last days of this stage. The observed increase in carcass body temperature may be due to insect larval activity as the body burst (Wolff et al. 2001) or peel which allows insect maggots to feed underneath it (Kelly et al. 2011). Additionally, according to Kelly et al. (2011), the
observed slightly decrease in carcass body temperature may be due to the emission of gases by the body as the skin erupted.

*Chrysomya chlorophaaga* numbers were slightly higher during the warm season as compared to the cold season and it persisted on the carcass up to the advanced stage in the warm season, whereas it did not pass through this stage during the cold season. Similarly, *Williams and Villet* (2019), found *Ch. chlorophaaga* in high numbers during November (warm season), and stated that this species seems to be restricted by the maximum and minimum temperature excesses. This might explain the observed low numbers in both seasons. The number of flies collected during the active stage, decreased in both seasons, coinciding with the introduction of *N. rufipes* and *D. maculatus* and increase of *D. maculatus* and *O. crassicollis* in cold and warm seasons respectively. *Campobasso et al.* (2001) and *Kelly et al.* (2008) also noted that Dipteran species usually increase in numbers during the early stages of decomposition, whereas Coleoptera species only increase in numbers as the decomposition progresses. This may be caused by the presence and the increase in the number of predator beetles, which feeds on the fly maggots and consequently reducing the number of the dipteran flies produced. Although Goff (1993) listed only three predator beetle families, which included Silphidae, Staphylinidae, and Histidae as the main fly maggot’s feeders, only the species from the family Silphidae was collected along with other predator beetles from the family Cleridae in this study.

The advanced stage of decomposition was characterized by a reduction in foul odor and the carcasses showed extensive peeling and drying out of the skin and there was significant loss of soft tissue. The carcass body temperature was consistent with that of the surrounding soil temperature. Our finding corresponds with that of *Wolff et al.* (2001), where the recorded pig body temperature was almost similar to that of the environment surrounding the carcass during both advanced and dry stages of decomposition. The number of *Chrysomya* flies (*Ch. marginalis, Ch. albiceps*, and *Ch. Chloropyga*) continued to decrease and were last observed during this stage in both seasons. However, only *M. domestica* and *S. calcifera* remained on the carcass up to the dry stage. Similar observations were made by *Rosa et al.* (2011), where the numbers of fly species from the families Calliphoridae, Sarcophagidae, and Muscidae decreased from the advanced decay stage as compared to the early stages of a decomposing pig. This might be explained by the association and attraction of most Dipteran species to the carcass when the tissues are still soft (Goff 1993) and the presence of strong foul odor (Verheggen et al. 2017). The number of *N. rufipes* and *D. maculatus* continued to increase during both seasons and *T. micans* and *O. crassicollis* during the warm season of this study. *Kelly et al.* (2011) also observed *N. rufipes* and *D. maculatus* to be associated with the advanced stage of decomposition in all seasons and *T. micans* in warmer seasons. Furthermore, in the study of *Kelly et al.* (2008), *D. maculatus* were observed in high numbers during this stage, however, *N. rufipes* remained in the same numbers as in the active stage of decomposition. The presence of these species in high numbers during this stage could be due to their preference towards the dry skin (Mashaly et al. 2018).

During the dry stage of decomposition, the carcass showed little moisture and dry skin and the foul odor was reduced. Subsequently, most of the time the body temperature of the carcass was consistent with that of the surrounding soil temperature. This stage was mainly dominated by the presence of *D. maculatus* and *N. rufipes* followed by *T. micans* and *O. crassicollis* in the warm season. However, in the cold season this stage was dominated by *D. maculatus* and *N. rufipes*. These observations were supported by *Mayer and Vasconcelos* (2013) and *Mashaly et al.* (2018), where *D. maculatus* and *N. rufipes* were reported to be strongly associated with the dry stages of a decomposing pig (Arnaldos et al. 2004). Due to their feeding preference, these species can be used as potential indicators of PMI in forensic investigation.

As in other previously reported studies, *Ch. marginalis, Ch. albiceps*, and *M. domestica* were most dominant and abundant in this study throughout both seasons, although there were more in numbers in the warm season as compared to the cold season. *Kelly et al.* (2011) showed that *Ch. marginalis, Ch. albiceps*, and *M. domestica* were the dominant species in the summer and autumn experiments and *Calliphora vicina* and *Ch. chlorophaaga* during the winter season in Bloemfontein. However, the absence of *Calliphora vicina* in our study may be due to differences in geographic regions and climate (Shin et al. 2015), *Williams and Villet* (2019) also noted that *Ch. albiceps* occurred most of the year, although reduced in numbers from July to September in Eastern Cape province of South Africa. *Chrysomya albiceps* was also found in high numbers on the pig carcass during the spring and summer in Argentina (Battán Horenstein et al. 2007). Furthermore, *Braack* (1986) also reported that *Ch. marginalis* was more abundant in summer in the Kruger National Park of South Africa. However, *Gilbert* (2014), showed that *Ch. albiceps* appeared only during summer whereas in our study this species was one of the dominant species during both cold and warm seasons. Coleoptera species, *N. rufipes* and *D. maculatus* were the most dominant species in both warm and cold seasons, and *T. micans* was the third dominant species. However, *T. micans* and *O. crassicollis* were present in less numbers during the cold season. Similarly, *Kelly et al.* (2008), reported that species of *N. rufipes* and *D. maculatus* were the most dominant Coleoptera species present and breeding on the carcasses through all seasons and only recorded *T. micans* during the warm season. *Villet* (2011) reported that *D. maculatus* is more common in summer, and *Braack* (1986) reported their highest occurrence in late autumn. *Braack* (1987) reported that *D. maculatus* beetles are usually present and uses the carcass-habitat throughout the year and this could explain their observed high numbers in both seasons in this study. Although high number of flies were collected in warm season, these findings point out that there is still a huge debate on the correlation concerning temperature and arthropods taxa richness (Shi et al. 2009).

According to the classification of ecological relationship between the insects and carcasses by *Smith* (1986), Goff (1993), and *Martinez et al.* (2007), the following ecological categories were also observed in this study: necrophagous species which mainly fed on the soft tissues of the carcass and those species were *Chrysomya* species, *L. cuprina, M. domestica*, and *S. calcifera*. These species have been reported to be of forensic value because of their predictable time of appearance and hence may be used to estimate PMI in forensic investigation; predator species which prefer feeding on the larvae and pupae of necrophagous species and these were *N. rufipes* and *T. micans*. This group is considered as the second most significant group of species associated with the carcass decomposition and they are also significant in forensic investigations and; coprophagous species which feed mainly on the fecal material or excrement from the carcass and only *O. crassicollis* species were recorded in this category and lastly incidental species which according to *Villet* (2011), constitutes any organism (airborne or mobile terrestrial insect) that may land on the carcass unintentionally and according to *Braack* (1986) this group occurs in low numbers and in our study these species were recorded as *H. lunatus* and *Atherigona soccata* which
are normally associated with crops (Nikbakhtzadeh 2004, Gorthy et al. 2017).

Overall, the length of the stages of decomposition during the warm season was similar to that of the cold season. Furthermore, even though carcass temperature pattern changed with decomposition stage, the overall change in carcass temperature was observed to have also been influenced by the change in soil/environmental temperature. In conclusion, the pig carcass was ideal as a model in determining insect succession and decomposition pattern during warm and cold seasons in KwaZulu-Natal Province of South Africa. Consequently, the succession and decomposition pattern observed could be potentially useful in forensic investigation related to the estimation of PMI. We recommend that similar studies be conducted at other geographical locations of South Africa with a different ecological system in order to build a database of Dipteran species of forensic value which are endemic in these areas.

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