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Etoniru, Izuchukwu Stanley

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POST-MORTEM INTERVAL ESTIMATION AND INSECT SUCCESSION PATTERNS IN THE TROPICAL CLIMATE OF NIGERIA

Izuchukwu Stanley Etoniru

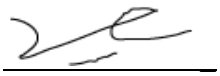
A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree:

Ph.D. (Anatomical Sciences)

Johannesburg, 2022

DECLARATION

I, Izuchukwu Stanley Etoniru, declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in Anatomical Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

20th day of July 2022 in Johannesburg

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

1. Poster presentation at the Humanitarian and Human Rights Poster Session, 73rd American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting, virtual event (15-19 February 2021). Etoniru IS, Myburgh J, Steyn M, Brits D.

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3. Academic presentation at the Anatomical Society of Southern Africa (ASSA) World Anatomy Day celebration, online symposium (13th October 2021). Etoniru IS, Myburgh J, Steyn M, Brits D.

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   Insect succession patterns during decomposition in southern Nigeria

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ABSTRACT

Post-mortem interval (PMI) estimation is the first step in the identification of badly decomposed remains. Apart from identifying the victim, obtaining the PMI is an important aspect in investigation into the cause and manner of death, and helps to narrow down the number of suspects. The ongoing armed conflict in Nigeria which has lingered over a decade has left a large burden of human remains. These remains are mostly left in the fields where the attacks occurred for fear of further attacks, especially in cases of terrorism. They are, therefore, badly decomposed at the time they are recovered, and identification becomes more difficult in a country that has very few forensic scientists. Law enforcement agencies usually resort to mass burials without identification. The aim of this study was to assess decomposition rates in southern Nigeria and to derive formulae for PMI estimation using the quantitative variables Accumulated Degree Days (ADD) and total body score (TBS), and to obtain the arthropod succession pattern during decomposition using a pig model. To achieve this aim, a longitudinal examination of quantitative variables, TBS and ADD, was conducted over a period of 14 months. This period included both the dry and wet seasons. Scatter plots between TBS and PMI, and TBS and ADD were used to show decomposition patterns. Arthropod succession patterns were also observed during the study for each carcass. Decomposition was found to progress rapidly, and desiccation was a frequent occurrence during decomposition. There were marked differences in decomposition patterns between the seasons, with the wet season exhibiting a more rapid decomposition. Linear regression formulae for ADD and PMI, and 95% confidence interval charts for TBS for ADD were derived. The arthropods arrived very early on the pig cadavers. There was more arthropod abundance and species richness in the wet season than in the dry season. There were also some arthropods that were observed only in the wet season. A combination of these formulae and insect activity will lead to a more precise PMI estimation in Nigeria and regions with similar climate. The data on insect succession developed from this study will serve as a reference for forensic researchers.

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LIST OF ABBREVIATIONS

ADD accumulated degree days

CDI cadaver decomposition island

CPI Corruption Perception Index

GTI Global Terrorism Index

PIA period of insect activity

PMI post-mortem interval

SDG Sustainable Development Goal

TBS total body score

WISPI World Internal Security and Police Index

Chapter 1: Introduction

One of the first questions that needs to be addressed when analysing decomposed human remains, is to determine how long the individual has been deceased. This is referred to as the post-mortem interval (PMI). The PMI is an important aspect of the investigation into the possible cause and manner of death. Accurate estimation of the PMI enables verification of witness testimony, thereby narrowing down the number of possible suspects (Li *et al.*, 2016). Inaccurate PMI estimation will confuse and complicate an investigation (Kaliszan *et al.*, 2009).

Nigeria, in the past decade, has witnessed an alarmingly high turnout of human remains from mostly armed conflicts. The root of these conflicts are perennial corruption, poverty, and insecurity (Ngwube and Okoli, 2013, p. 92), and reports of reputable monitoring organizations appear to confirm that these factors are very present in Nigeria.

The Corruption Perception Index (CPI), an index published yearly by Transparency International, ranks countries by their perceived level of corruption in the public sector. CPI presently ranks 180 countries on a scale of 0 to 100, that is, from highly corrupt to very honest, respectively. Nigeria has witnessed a steady decline on the index in the past five years moving from a score of 28 in the 136th position in 2016 to a score of 24 in the 154th position in 2021 (https://www.transparency.org/en/cpi/2021/index/nga).

The World Poverty Clock provides real-time estimates for almost every country, and monitors progress against Ending Extreme Poverty which is the UN's first Sustainable Development Goal (SDG1). An August 2021 projection by The World Poverty Clock compiled by the Brooklyn Institute revealed that Nigeria, the largest economy in Africa, had 86.8 million people living in extreme poverty (less than $1.90/day) (https://worldpoverty.io), second only to India (https://blogs.worldbank.org/opendata/half-world-s-poor-live-just-5-countries).

The combined effects of extreme poverty and high levels of corruption have severe impacts on the safety and security in the country. The World Internal Security and Police Index (WISPI) measures the ability of the police and other security agencies to address internal security problems based on assessing the following domains: capacity, process, legitimacy, and outcome, as well as measuring the public's confidence in such services. Whereas capacity assesses "whether the level of resources devoted to internal security in a country are sufficient to deal with existing internal security issues, and whether these resources are adequate to deal with any unexpected outbreak of civil unrest," (*World Internal Security and Police Index*, 2016, p. 7), process assesses issues like corruption, effectiveness of criminal justice, bribe payment and under reporting of crime. Legitimacy measures whether the police and security services act to the best benefit of the country and its people, and outcome assesses aspects such as rates of homicide, violent crime, terrorism, and public safety perceptions (www.wispindex.org). The WISPI report of 2016 revealed that Nigeria performed worst of the 127 countries on the index (www.wispindex.org). Although Nigeria scored poorly across all four domains assessed, the indices in which it scored the poorest were the process and outcome domains, which indicates a corrupt and ineffective law enforcement and criminal justice system on which criminals capitalize and commit violent crimes uncontrollably. This report also showed that over 12,000 people were killed in terrorist attacks between 2006 and 2016 with 7,512 of these deaths occurring in 2014 alone (www.wispindex.org).

This report is further supported by The Global Terrorism Index (GTI), an index published by the Institute for Economics and Peace which analyses the impact of terrorism in 163 countries. The GTI showed that Nigeria was the third most terrorized nation in the world after Afghanistan and Iraq, between 2014 and 2019. Although this happened in a nation that was

not at war or under military invasion like Afghanistan and Iraq at the time, Nigeria harboured two of the five deadliest terrorist groups in the world – Boko Haram and Fulani militants. In 2014 alone, Boko Haram, the deadliest terrorist group according to the GTI, was responsible for the death of 6,644 people out of a total of 7,512 deaths. These killings have continued through very frequent attacks on farming communities all over the country but especially in the Middle Belt, mostly by Fulani militants.

In addition to these problems with crime and terrorism and the resulting number of unidentified remains, ritualist dens with decomposing human remains are discovered from time to time (http://news.bbc.co.uk/2/hi/africa/3540306.stm; https://thenationonlineng.net/ibadan-forest-horror-police-uch-forensic-experts-begin-probe-2/). Unfortunately, none of the badly decomposed victims have been identified and no report has been made available. In Nigeria today, there is no association or academy of forensic scientists and so most cases are either sent abroad for identification or investigated arbitrarily (Olotu and Didia, 2015) or not investigated at all. With the lingering dearth of forensic experts and cheaper methods of investigation adapted to the Nigerian setting, law enforcement agents resort to mass burials without any victim identification (Obafunwa *et al.*, 2015). This has led to numerous cases of missing persons who may never be identified, and many families mourn without any confirmation that their loved ones have died. In a nation that is deeply immersed in culture, including burial rites, the lack of closure could be intensely traumatizing. Slowly, a few scientists and other concerned individuals are starting to come together to provide the scientific background and expertise that may become useful to start investigating some of these cases. This has led to a slowly growing number of studies in this field. Also, the acceptance that victim identification requires, apart from the pathologist, the

expertise of other forensic scientists (Obafunwa *et al.*, 2015), is hopefully a boost for this growing body.

Studies have generated many models for PMI estimation based on gross tissue decomposition, but these are mainly from North America (Megyesi *et al*., 2005; Moffatt *et al.*, 2016), Europe (Gelderman *et al.*, 2018), Australia (Fitzgerald and Oxenham, 2009) and South Africa (Myburgh *et al.*, 2013; Forbes *et al.,* 2019). Research in this important field has advanced considerably especially as evidenced by the increasing number of research articles and the proliferation of taphonomy research facilities since 1981. Such facilities where human remains are used for decomposition research are still only found in the United States, Canada, Australia, and the Netherlands. They are not yet universally available due to funding (Williams, 2018) and, to some extent, the general repulsion to the public. Most decomposition facilities favour pigs as a proxy for humans due to the comparable internal anatomy, fat to muscle ratio, gut flora, hair follicles, sweat glands, skin thickness, torso size and sparse skin hair (Hall, 1988; Hopewell, 1991; Goff, 1993; Byrd and Castner, 2001; Pakosh and Rogers, 2009; Reeves, 2009). They are also readily available with less ethical scrutiny than when human remains are used (Keough *et al*., 2017). Although some studies have pointed out differences between human and pig decomposition (Connor *et al*., 2018; Matuszewski *et al.*, 2019), the use of pigs continue for the reasons stated above and can still aid in understanding the decomposition process in specific regions.

The introduction of innovative methods such as accumulated degree days (ADD) by Vass *et al*. (1992) has introduced some standardization that allows studies from different regions to be compared (Simmons *et al.*, 2010). However, validation studies of popular models in various regions clearly point to the need to develop region-specific models for precise PMI estimation

since models developed in one specific region cannot be generally applied (Myburgh *et al.*, 2013; Marhoff *et al.*, 2016; Suckling *et al.*, 2016; Marhoff-Beard *et al*., 2018; Forbes *et al.*, 2019). These methods cannot be directly applied to Nigeria due to obvious environmental differences. There are no studies to address the development of a method for PMI estimation in Nigeria and therefore no locally derived methods. In addition to other factors such as funding, this unavailability of locally derived and easy to use methods discourages the few forensic experts saddled with the identification of the numerous human remains burden which reinforces the practice of mass burials by law enforcement agencies (Obafunwa *et al.*, 2015) which has become common in Nigeria following conflicts. The availability of cheap, easy-to-use and locally derived methods of PMI estimation in Nigeria has the potential to strengthen the effectiveness and reliability of the criminal justice system, especially with the agreement that victim identification requires the inclusion of other forensic experts such as forensic anthropologists (Obafunwa *et al.*, 2015).

Southern Nigeria is climatologically distinct from the north with a longer wet season and shorter dry season. Although Nigeria experiences persistently high temperatures, the proximity of southern Nigeria to water bodies, including the Atlantic Ocean, means more humid and lower daytime temperatures. Most of southern Nigeria, including the study area, therefore has a hot humid climate, with temperature and relative humidity ranges of 20 ^{o}C - 37 ^{o}C and 70 – 100%, respectively (Mobolade and Pourvahidi, 2020).

The aim of this study was to observe and assess decomposition patterns in southern Nigeria and using this to derive formulae for PMI estimation using the quantitative variables Accumulated Degree Days (ADD) and total body score (TBS). In addition to this, we assessed the arthropod succession pattern during decomposition. For this purpose, a pig model was

used, as using human remains in Nigeria is not possible due to the strong cultural affinity for burials as a sign of respect to the dead. Therefore, currently, there is no body donation programme or taphonomic research facility.

To address this aim, the following specific objectives were assessed:

1. To conduct longitudinal observations of quantitative variables of decomposition during the wet and dry seasons respectively.
2. To demonstrate patterns of decomposition with scatter plots of TBS and PMI, and TBS and ADD.
3. To develop new formulae to predict PMI for southern Nigeria using morphological quantitative variables and accumulated temperature.
4. To compare decomposition patterns observed in southern Nigeria with those on the Highveld region of South Africa as a representative of a completely different climate but still on the same continent. Both countries also have distinct seasonal differences.
5. To collect and observe insects associated with the decomposition process in order to develop a database for the insects of forensic importance, i.e., the insects that are commonly associated with remains, and their succession pattern in southern Nigeria.
6. To develop an integrated practical model using both insects and morphological changes to estimate PMI.

Chapter 2: Literature Review.

2.1 Introduction

The events following death are geared towards returning the constituents of the remains back to the surrounding environment, a type of energy recycling. After death, there is cellular deprivation of oxygen and build-up of carbon dioxide with a fall in pH. This signals autolysis, a process of self-digestion by which intracellular enzymes begin to digest the cells from within and release nutrient rich fluid (Vass, 2001). Under this anaerobic condition and rich supply of nutrients, micro-organisms in the body, especially from the breached intestinal tract, multiply and begin the phase of putrefaction with destruction of body tissues (Clark *et al.*, 1997; Vass, 2001). These changes follow a predictable pattern until the body disintegrates into the surrounding environment (Mathur and Agrawal, 2011).

Changes and processes associated with decomposition include those occurring within the remains and those occurring outside it, but which are due to the remains. Less obvious changes in the remains include a series of chemical reactions resulting in changes in post-mortem biochemical markers, microbial colonization and succession, whereas obvious physical changes include the mortis triad (a trio of algor mortis, livor mortis and rigor mortis), formation of skin blisters, skin slippage, bloating, the obvious destructive effect of arthropods in active decomposition, and skeletonization. Changes in the soil chemical and microbial community composition, succession of arthropods and vertebrates that visit the remains, and vegetation changes in the immediate vicinity of the remains are examples of changes that occur outside the remains, but due to, the remains. The observation and measurement of these changes with respect to time and the factors affecting them form the basis for the estimation of the PMI. Research in PMI estimation therefore involves researchers who have carried out studies in various fields such as thanatochemistry (Agrawal *et al.*, 1983; Cina, 1994;

Mathur and Agrawal, 2011; Tumram *et al.*, 2011; Salam *et al.*, 2012), forensic ecogenomics (Sidrim *et al.*, 2010; Metcalf *et al.*, 2013; Finley *et al*., 2015a; Fu *et al.*, 2015; Hyde *et al.*, 2015; Guo *et al.*, 2016; Adserias-Garriga *et al.*, 2017; Zhou and Bian, 2018; Liu *et al.*, 2020), forensic entomology (Payne and King, 1969; Louw *et al*., 1993; Carvalho *et al.*, 2000; Okiwelu *et al*., 2008; Shi *et al.*, 2009; Kelly *et al*., 2009; Azwandi *et al.*, 2013; Brown and Harvey, 2014; Abajue *et al*., 2015; Iancu *et al.*, 2015; Mądra *et al.*, 2015; Reid *et al*., 2020; Maisonhaute and Forbes, 2020; Tembe and Mukaratirwa, 2021; Matuszewski, 2021), and forensic anthropology (Forbes *et al.*, 2004, 2019; Sutherland *et al.*, 2013; Myburgh *et al.*, 2013; Keough *et al*., 2017; Marais-Werner *et al.*, 2018; Finaughty and Morris, 2019; Knobel *et al.*, 2019; Keyes *et al*., 2020, 2021; Spies *et al.*, 2020).

2.2. Decomposition of remains

Although environmental factors like temperature alter the rate of decomposition, the key characteristics are unchanged (Micozzi, 1996). The physical changes of decomposition are based on visual observation and have been divided into varying number of stages by different researchers. This includes the eight stages recognized by Megnin (1894), the three stages of Fuller (Fuller, 1934), the two stages of Howden (1950), and the more popular five stages *viz* fresh, bloat, active decay, advanced decay and dry (Reed, 1958; Payne, 1965; Galloway *et al.*, 1989; Megyesi *et al*., 2005; Myburgh *et al.*, 2013). These five stages will be briefly described below with characteristics of the stages summarized in Table 2.1. The duration of these stages depends on the season, the local faunal characteristics, behaviour and abundance of insects, and the type, size and location of the carcass (Greenberg, 1991). These stages are therefore not expected to be of the same duration in all places. The various stages of decomposition can coincide within one carcass. Although decomposition is typically divided into qualitatively

defined stages, the biological process is a continuous one and stage delineators are, essentially, arbitrary.

2.2.1. Fresh Stage

The fresh stage of decomposition encompasses all the events that occur from the time of death to the time the gas generated from putrefaction begins to distend the coelomic spaces. The collapse of the respiratory and circulatory systems that follows death leads to low cellular oxygen concentration and accumulation of metabolic waste. These changes lead to a fall in pH which initiates autolysis (Vass, 2001; Tsokos, 2005). Hydrolytic enzymes which are abundant in abdominal organs such as the liver, pancreas and stomach are released and digest cells and tissues, distorting cellular and intercellular adhesive structures. This also implies that such organs will experience autolytic changes more rapidly (Vass, 2001). Although the above changes are hardly recognised visually, clouding of the cornea and the mortis triad are more obvious in this stage of decomposition.

The fresh stage lasted for two days in both the monsoon season of Malaysia (Chin *et al.*, 2011) and the hot season in Thailand (Apichat *et al.*, 2007). It also lasted for the same duration (two days) in both seasons in Kwa-Zulu Natal, South Africa (Tembe and Mukaratirwa, 2021), urban Tasmania (Magni *et al.*, 2019) and outdoor setting in south-western Iran in autumn (Keshavarzi *et al.*, 2019). A shorter duration of one day was found at the end of the dry season in south-eastern Nigeria (Ekanem and Dike, 2010). Although the fresh stage lasted for a day in the summer of central Argentina, it continued for up to five days in the autumn, and 14 days in the winter (Battán Horenstein *et al.*, 2010). It is also at this stage of decomposition that the early necrophagous insects arrive at the cadaver to lay eggs.

2.2.2. Bloat Stage

As autolysis continues, the cell membrane of the intestinal cells, and therefore the intestinal integrity, is breached with release of intestinal bacteria into the anaerobic and nutrient rich milieu (Vass *et al.*, 2002; Bull *et al.*, 2006; Forbes, 2008). Autolysis therefore gradually gives way to putrefaction as the released bacteria rapidly multiply in the absence of a defence mechanism and act on the remains with obvious change in physical characteristics (Gill-King, 1997). Intestinal gas, gaseous products of putrefaction and other by-products are released into the coelomic spaces resulting in progressive bloating and the strong odour that characterize this stage. This is most obvious in the abdomen and is also seen in the most proximal aspects of the limbs and in the neck. The demarcation between autolysis and putrefaction in terms of sequence is not entirely clear-cut as both can occur concurrently, with putrefaction beginning when autolysis is advanced. The progressive gaseous distension that is most obvious in the abdominal region marks the end of the fresh stage of decomposition and continues until the gaseous pressure is released from the body orifices like the mouth (stomach purge), nostrils (lung purge), the anus (rectal purge) or a skin wound which may have been sustained ante-, peri-, or post-mortem, causing a deflation in the initially distended remains. The combined factors of gaseous pressure and the destruction of tissues leads to breakdown of adhesive forces between the dermis and epidermis causing skin slippage as the epidermis sloughs off (Henderson, 1987). The deflation of the initially distended remains ends the bloat stage. Various studies have found this stage to last between days 2 – 5 depending on the regional climate, open versus shaded areas, and the season (Apichat *et al.*, 2007; Ekanem and Dike, 2010; Chin *et al.*, 2011). In the presence of gas-forming bacterial septicaemia at death, this stage will appear earlier (Yamaguchi *et al.*, 2015).

2.2.3. Active Decay Stage

The deflation of the bloated remains allows opportunity for additional aerobic decay. The released purges, decomposition gases and by-products like methane, hydrogen sulphide, ammonia, putrescine and cadaverine both attract more insects with their strong odour and cause skin blisters (Vass *et al.*, 1992; Marshall and Bal'a, 2001). The newly attracted insects and the larvae that hatched from oviposition in the fresh stage discussed above gain access into the inner parts of the remains. They inoculate and spread bacteria more rapidly as they feed (Janaway *et al*., 2009). The larvae are responsible for the most significant removal of soft tissue (Simmons *et al*., 2010; Singh *et al*., 2016). As the mature larvae move away from the remains to pupate, they create space for adult insects to lay eggs that hatch into larvae that will continue feeding in a second wave of entomological activity (Rattenbury, 2018). These larvae could be aged to determine the PMI and will be discussed later in this chapter. As the state of the food source changes over time, there is a succession of insects in a largely predictable fashion on the basis of their ability to handle the state of the remains at the time, or the availability of other insects on which they scavenge. This stage lasts between three and six days (Apichat *et al.*, 2007; Chin *et al.*, 2011; Kyerematen *et al.*, 2013; Tembe and Mukaratirwa, 2021).

2.2.4 Advanced Decay Stage

The advanced decay stage is characterized by a considerable decline in decomposition fluid, entomological activity, and the odour of decomposition. Tissue loss has slowed down considerably and depending on the prevailing weather conditions, tissue preservation could occur. Decomposition rate becomes variable. By the amendment method of scoring physical changes of decomposition (Keough *et al*., 2017), bone exposure and mummification, if weather conditions allow, occur at this stage of decay. Validation studies of various models

for PMI estimation have reported a reduction in precision at this stage and beyond (Vass, 2011; Suckling *et al*., 2016; Keough *et al*., 2017; Marhoff-Beard *et al*., 2018; Forbes *et al.*, 2019). Studying the changes that occur in the states of preservation like mummification (Connor *et al*., 2019) over time may hold the key to improving the accuracy of PMI estimated in this stage and beyond. This is important in places like tropical West Africa with high temperatures all year round, and where desiccation rates are expected to be high. There are no such studies in the region, to the knowledge of the authors, aimed at improving decomposition scoring and PMI accuracy when remains are found in this stage and beyond.

2.2.5 Dry Stage

The dry or skeletonization stage occurs when bone exposure exceeds half of the body region being scored (Keough *et al.*, 2017). Carter *et al.* (2007) suggest that the regrowth of vegetation on the edges of the cadaver decomposition island (CDI) is an indication that decomposition has moved to the dry stage. Studies show that this stage is reached between seven and 16 days (Apichat *et al.*, 2007; Chin *et al.*, 2011; Kyerematen *et al.*, 2013; Tembe and Mukaratirwa, 2021). This stage may last for very long at which time the focus for PMI estimation moves to diagenesis which utilizes the bone modification scoring system by Behrensmeyer (1978). Qualitative assessment of taphonomic processes on skeletonized remains such as bone weathering, sun bleaching and cortical exfoliation is gradually being replaced by quantitative methods like identification or analysis of lipid moieties obtained from bone marrow which have yielded excellent results for prolonged PMI estimates running into years (Dudzik, 2017). Also the finding of a reduction in microbial taxa richness in skeletal remains over time holds potential for PMI estimation (Damann *et al*., 2015).

There have been calls to apply standard definitions of these stages to limit the subjectivity in distinguishing between decomposition stages (Michaud and Moreau, 2011).

Table 2.1. Characteristics of the five stages of decomposition (Keough *et al.*, 2017)

Decomposition stages	Fresh	Bloat	Active decay	Advanced decay	Dry
Characteristics	From the time of death to the appearance of early signs of bloating	Gaseous distension of the abdomen, neck and proximal parts of the limbs	Deflation of gaseous pressure. Wet decomposition with high entomological activity, especially maggots	Reduced entomological activity and evidence of bone exposure and desiccation	Dry bones and a few pieces of desiccated skin

2.3. Secondary changes in decomposition

2.3.1. Mummification

Aufderheide (2003) defines mummification as the survival of soft tissue beyond the postmortem interval normally expected for it to completely decay. The very dry darkened skin overlies dry bones. This is different from drying or desiccation which causes a shortlived pause in the process of decomposition. When the prevailing environmental variables favour putrefaction, decomposition continues to skeletonization without soft tissue preservation. However, when conditions change to favour rapid desiccation, the process of decomposition ceases and mummification takes over, preserving the remains in the state at which putrefaction was arrested. In other words, the forces in favour of dessication are always present in surface decomposition but may be overpowered by environmental variables in favour of putrefaction such as the right temperature, humidity, insects and the bacteria they spread, among other factors. Environments with dry and hot weather encourage rapid desiccation by deactivating biotic factors of decomposition leading to mummification. This is typical of the weather in southwestern Africa and the deserts of North Africa. This is also common in cold environments as long as there is low or absent moisture (Amy *et al.*, 1986; Mann *et al*., 1990; Clark *et al*., 1997; Vass, 2001; Hayman and Oxenham, 2016c; Finaughty and

Morris, 2019). Examples of temperate regions with dry weather conditions are southwestern North America, southwestern and southeastern Australia, generally high altitude areas, cold regions of north Asia and Antarctica (Micozzi, 1986; Peel *et al.*, 2007), and rapid mummification has been reported in some of these places (Kashimura *et al.*, 1984; Galloway *et al.*, 1989). In temperate regions without arid conditions, mummification is a long-term process occuring in at least three months postmortem (Piombino-Mascali *et al*., 2017). However, rare instances of precoccious mummification, defined as its occurrence within 4 weeks, have been reported in temperate regions such as Italy (Marella *et al.*, 2013), Bulgaria (Tsranchev *et al.*, 2017) and South Africa (Finaughty and Morris, 2019). These are attributed mostly to considerable perimortem trauma through blood loss and rapid fluid loss without loss of skin (Marella *et al.*, 2013), and the prevailing weather condition at the time (Kashimura *et al.*, 1984; Campobasso *et al.*, 2009).

Mummification leaves behind a desiccated odourless mass with brittle leathery skin stretched over bones with little or no shrunken muscle and internal organs. Finding of such remains could cause challenges with PMI estimation since they could remain in this preserved form for years. Nigeria falls within the regions where desiccation is expected to be common on account of weather conditions (Koppen-Geiger: Af, Am, Aw, BWh, BSh, Csb) (Peel *et al.,* 2007), and there are no studies on natural desiccation there. In addition to enabling forensic scientists to predict the state of decomposition or preservation when a suspected missing person is found, such research in Nigeria will incorporate this state in scoring remains to improve PMI estimation locally.

2.3.2. Adipocere formation

Remains decomposing under warm, humid, mildly alkaline pH and anaerobic conditions are often characterized by body fat that is converted to adipocere, a waxy substance consisting

of saturated fatty acids, through bacteria-induced hydrolysis and subsequent hydrogenation (Forbes *et al*., 2005a; Ubelaker and Zarenko, 2011). The finding of adipocere in a case study under dry concealment appears to indicate that external moisture is not an absolute requirement for this form of presevation as the body contains sufficient moisture for the process (Nushida *et al.*, 2008).

Research has examined the formation of adipocere and its possible use in PMI estimation (Gotouda *et al.*, 1988; Yan *et al.*, 2001; Forbes *et al.*, 2004; S. Forbes *et al*., 2004; O'Brien and Kuehner, 2007). The absence of a correlation between the degree of decomposition and the level of adipocere formation shows that its formation is not a function of time since death, but the prevailing factors in that environment (Forbes *et al.*, 2004). Hayman and Oxenham (2016d) suggest that accounting for the unique environmental factors causing this state may result in establishing the relationship necessary for the use of adipocere formation in PMI estimation.

2.4. PMI studies in Africa

The majority of PMI studies and the validation of the derived models were done outside Africa. South Africa (Koppen-Geiger: Af, Am, Aw, BWh, BWk, BSh, BSk, Csa, Csb, Cwa, Cwb, Cfa, Cfb) (Peel *et al.,* 2007) is clearly championing studies in forensic anthropology in Africa, but also has a temperate climate unlike the tropical climate in Nigeria.

There are presently no human decomposition facilities in South Africa but there are decomposition facilities using pig models both in the Northern and Southern parts of the country. The presence of these facilities has allowed research in taphonomy to flourish. Some of these studies examined decomposition progress and factors influencing them (Kelly *et al.*, 2011; Sutherland *et al.*, 2013; Marais-Werner *et al.*, 2018; Spies *et al.*, 2020) while others

examined preservation (Finaughty and Morris, 2019) and popular models for PMI estimation to see how they perform in South Africa for possible application locally (Myburgh *et al.*, 2013; Forbes *et al.*, 2019). Other studies have examined the role and behaviours that vertebrate scavengers (Spies *et al*., 2018; Keyes *et al*., 2020, 2021; Spies *et al.*, 2020) and insects (Braack, 1986; Louw *et al*., 1993; Williams, 2003; Kelly *et al*., 2009; Richards *et al.*, 2009b; Gilbert, 2014; Parry *et al*., 2016; Williams and Villet, 2019; Tembe and Mukaratirwa, 2021) have during decomposition in South Africa.

In tropical west Africa, there are much fewer studies in forensic anthropology, especially decomposition. To the knowledge of the authors, there are no studies on PMI estimation using the gross changes in decomposing remains in Nigeria. There are, however, one on estimation of PMI using the period of insect activity (PIA) (Ahmed and Joseph, 2016). The rest of the studies are in forensic entomology, especially pertaining to insect succession pattern on animals such as the domestic pig, the giant cane rat and monkeys (Okiwelu *et al.*, 2008; Ekanem and Dike, 2010; Ekrakene and Iloba, 2011; Ndueze *et al.*, 2013; Abajue *et al.*, 2015; Alafia *et al*., 2017). It appears that the same applies to Ghana (Kyerematen and Boateng, 2012; Kyerematen *et al.*, 2013) and Egypt (Hegazi *et al*., 1991; Tantawi *et al.*, 1996). Part of the reason for the preponderance of such entomological studies may be the seemingly low cost when compared to large scale studies utilizing a high number of pig cadavers for post-mortem interval estimation. Furthermore, research funding for studies on developmental biology of local insect communities may constitute an obstacle. Other methods of PMI estimation have been explored in Africa such as biochemical methods with encouraging results in Egypt (Salam *et al.*, 2012). These studies are expensive and are, therefore, few. Some of the research on PMI estimation are shown in Table 2.2.

Although substantial work has been done, decomposition research is still evolving. This is more so in tropical sub-Saharn Africa where there are much fewer studies to strengthen the practice of forensic science for PMI estimation and ultimate victim identification. More research in this region is needed to explore the factors influencing decomposition that are unique to the region, and adequately consider them in fashioning out a model adapted to the region.

Table 2.2. Examples of research on PMI estimation in Africa.

Country	Tissue/sample	Method	Authors
South Africa			
	Pigs	ADD/TBS	Myburgh *et al.,* 2013
	Pigs	ADD/TBS	Sutherland *et al.,* 2013
	Pigs	ADD/TBS	Forbes *et al.,* 2019
	Pigs	Effect of trauma and clothing	Kelly *et al.,* 2011
	Pigs	Surface vs buried decomposition using TBS	Marais-Werner *et al.*, 2018;
	Pigs	Influence of scavenging	Spies *et al.*, 2018
	Pigs	Effect of clothing and scavenging	Spies *et al.*, 2020
	Pigs	TBS scoring table	Keough *et al.,* 2017
	Pigs	Taphonomic bone trauma and scavengers	Keyes *et al.,* 2020
	Pigs	Influence of scavengers	Keyes *et al.,* 2021
Nigeria			
	Guinea pigs	Insects – PIA	Ahmed and Joseph., 2016
	Monkey, rat	Insect succession pattern	Ndueze *et al.*, 2013
Egypt			
	Human remains	Thanatochemistry and early physical changes	Salam *et al.,* 2012

2.5. Emerging methods of PMI estimation

Studies on PMI estimation in Western nations have advanced. Apart from those discussed below, a relatively new method of PMI estimation that utilizes the predictable microbial community dynamics in/on the remains or the surrounding soil is the field of forensic ecogenomics or forensic microbiology. This new field applies the knowledge of microbiology to investigate crime including PMI estimation (Metcalf *et al.*, 2013, 2016; Finley, Benbow and

Javan, 2015b). Using genomic sequencing of bacteria samples obtained from the rectal and oral cavities (Guo et al., 2016), or bones from human skeletonised remains (Na, 2020), bacterial community composition and succession have been demonstrated to show promise for PMI estimation. Liu *et al.* (2020) have also suggested the use of an integrated model that combines the characterization of microbial community, sequencing of microbiome from organs, and machine learning algorithms to assess microbial succession pattern for accurate PMI estimation in the first couple of weeks following a study on mice. These are emerging methods and large validation studies, especially utilizing human samples, will go a long way in translating these findings to real forensic caseworks. The Australian Facility for Taphonomic Experimental Research (AFTER) on the outskirts of Sydney has an ongoing work titled "Taphonomic investigation of human skeletal remains in an Australian context" which is examining, among other things, the changes in the gross and microscopic structure of bone due to the activities of bacteria, fungi and plant with the aim of finding better methods for PMI estimation for bodies deposited on land surface (https://www.uts.edu.au/about/faculty-science/after-facility/research-and-training).

2.6. Factors influencing decomposition

Decomposition rate and pattern are affected by factors which, by extension, determine when the stages of decomposition begin and how long they last. These factors also determine if secondary changes or preservation will occur. The point of reference in most of the validation studies of popular models for PMI estimation appear to be these influencing factors and how they affect decomposition in different environments and climates. These factors include climatic factors like temperature, humidity, season and rainfall, and other factors like body weight or size, insect access, clothing, scavenging, burial, health status, drugs, trauma and location. These factors, particularly those that are uniquely related to the remains, are

important as they may account for the seeming disparity between the observed state of decomposition of the remains and what is expected under the circumstance.

2.6.1. Temperature

Temperature, together with insects and burial depth, is considered to be the most important factor affecting decomposition (Mann *et al*., 1990; Vass *et al.*, 2002) due to its effect on enzyme activity (Henderson, 1987; Gill-King, 1997; Sheridan *et al.*, 2000; Pecsi *et al.*, 2020), insect viability and activity (Reed, 1958; Payne, 1965; Galloway *et al.*, 1989; Mann *et al*., 1990; Campobasso *et al*., 2001; Dadour *et al.*, 2001; Dadour *et al.*, 2001; Brown and Peckmann, 2013) and bacterial growth (Micozzi, 1991) which influence decomposition. Due to the wide effect of temperature and the relationship with other key factors that influence decomposition like rainfall and humidity, researchers recognize the difficulty in considering temperature as a standalone factor (Galloway *et al.*, 1989; Mann *et al*., 1990; Komar, 1998; Myburgh, 2010).

Decomposition is faster in warmer seasons or climates than it is in colder seasons or climates (Galloway *et al.*, 1989; Komar, 1998; Prieto, Magana and Ubelaker, 2004; Cockle and Bell, 2017). The faster development of fly larvae which cause the highest tissue removal during decomposition in higher temperatures (Rivers *et al*., 2011) supports this. This difference in decomposition rate is also seen when works from different climatic regions are compared (Battán Horenstein *et al.*, 2010; Ekanem and Dike, 2010; Myburgh *et al.*, 2013). Moreover, at subzero temperatures seen in some winters of temperate regions, decomposition ceases (Vass *et al.*, 1992) or is inhibited due to bacterial and enzyme inactivation and the absence of vertebrate scavengers at such temperatures (Di Maio and Di Maio, 2001). Decomposition in colder areas, therefore, generally takes a longer time to complete than in sub-Saharan tropical climates such as is found in Nigeria that have high temperatures throughout the year.

With the aim of improving standards in the field of forensic anthropology in general, including to meet the requirements of Daubert & Frye criteria for scientific evidence in court, studies have looked at how measurement of these influencing factors should be taken. For example, Megyesi *et al.*'s (2005) use of retrospective temperature data from a weather station for the calculation of ADD was faulted on the grounds that there was no consistent relationship between weather station data and that of the site (Fitzgerald, 2007; Fitzgerald and Oxenham, 2009). Using paired sample t-tests between the research site and each of the two weather stations used in a study in Arizona, Dabbs (2010) found a significant difference between the average daily temperature data at the research site and the weather stations and therefore discouraged the use of weather station temperature data when ADD is used to estimate PMI. The finding of a high correlation between the site readings and that of the weather station, on the other hand, justifies the use of both readings (Myburgh *et al.*, 2013). Furthermore, an earlier study in Victoria and New South Wales, Australia, found that retrospective temperature data correction between the weather station and the body discovery site was reliable. The accuracy of correlation was found not to be affected by season, the length of correlation period, and a distance between the weather station and the body discovery site of up to 15 km (Johnson *et al*., 2012). A multiple model that considers other weather parameters like wind volume and speed, humidity and rainfall which are found to correlate with temperature, was found to be more accurate than the single model variety than considers only temperature data at the weather station for ADD calculation (Jeong *et al.*, 2020).

2.6.2. Humidity

Humidity refers to the water vapour concentration in the atmosphere. Increased humidity ensures that the food resource does not dry out but remains in an easily digestible state while

also encouraging insect feeding and microbial growth on which maggots partly depend to ingest the decomposing remains since they have non-articulated jaws (Campobasso *et al.*, 2001; Byrd and Castner, 2010). Humidity is therefore a key determining factor in tissue dessication during decomposition. Giles *et al.* (2020) suggest that humidity may be the most crucial factor for the advancement of early decomposition over temperature. The influence of humidity in accelerating decomposition was documented by earlier studies (Prieto *et al*., 2004; Gunn, 2009; Shi *et al.*, 2009).

2.6.3. Season

The effect of season is especially difficult to assess since decomposition changes are attributed to temperature, humidity and rainfall which make up the season (Mann *et al*., 1990; Vass *et al.*, 1992; Bass, 1997; Kyerematen *et al.*, 2013). Earlier studies show that decomposition is faster in summer (Bass, 1997; Meyer *et al.*, 2013; Myburgh *et al.*, 2013; Gilbert, 2014; Knobel *et al.*, 2019). The observed difference between seasons accounts for the improved accuracy demonstrated when separate equations were derived for each season (Myburgh *et al.*, 2013). However, a recent study by Giles *et al.* (2020) which assessed the effect of season on decomposition rate showed that although decomposition was faster in the summer, this difference was not significant between seasons. Furthermore, the finding of both peak insect abundance and activity (Reed, 1958) and higher scavenger activity (Keyes *et al*., 2021) in the summer, could contribute to this higher decomposition rate in this season through tissue loss, especially if these factors were not controlled for. Mądra *et al.* (2015) also suggest that the season of death influences not only the insect species that will colonize the remains after death, but also those that will recolonize the remains in subsequent years.

2.6.4. Rainfall

The effect of rainfall on decomposition rate is not very clear, again because of its strong association with other seaonal variables like humidity and temperature. After rainfall, humidity increases but temperature falls. Some studies suggest an increase in decomposition rate in both large (Mann *et al.*, 1990; Lopes de Carvalho and Linhares, 2001) and small (Archer, 2004a) animals with increasing rainfall. Rainfall also influences insect activity during decomposition as desiccated remains in the preceding season are remoistened and attract insects as decomposition resumes. However, insects avoid cadavers in the early stages of decomposition that are drenched by rainfall therefore reducing decomposition rate (Myburgh *et al.*, 2013). This is also the case when the remains dry out in the absence of rainfall and the arthropods move away. Archer (2004a) pointed out that the difficulty with assessing the effect of rainfall on decomposition rate is because rainfall as a standalone factor has not been statistically tested.

2.6.5. Body weight

There appears not to be a concensus on the effect of weight / body size on decomposition. Contrary to the findings of researchers who believe that larger remains decomposed faster than smaller remains (Mann *et al.,* 1990; Campobasso *et al.,* 2001; Zhou & Byard, 2011), a study on the effect of body size in South Africa showed that smaller pigs decomposed 2.82 times faster than larger pigs, and that the decomposition pattern between these two groups differed significantly (Sutherland *et al.*, 2013). This is in keeping with the findings of Simmons *et al*. (2010) but only when insects could access the remains; in the absence of insects, carcass mass had no significance. Megyesi *et al.*'s (2005) suggestion that their equation and, therefore, the TBS system is for application in adult points to the importance of body mass. This is confirmed by another study which found only 35% of the progress of decomposition

was accounted for by ADD when juvenile carcasses were used instead of the 80% found by Megyesi *et al.* (2005) for adult remains (Ross and Hale, 2018).

2.6.6. Insect access

As the earliest visitors to carrion, insects attracted to remains by the smell of decomposition are the objects of study by entomologists for the estimation of PMI based on either the larval developmental biology or the arthropod community composition and succession pattern. The latter can be valuable when the time since death is suspected to run into months or years (Amendt *et al.*, 2011; Mądra, Frątczak, Grzywacz, Matuszewski, *et al.*, 2015). These insects have been found to vary from one region to the next due to factors like food abundance, habitat, other insect species and season (Williams, 2003; Matuszewski *et al.*, 2013; Parry *et al.*, 2016). The majority of these insects are of the Diptera and Coleoptera (Byrd and Castner, 2010). Their influence in decomposition have long been recognised (Payne, 1965; Jirón and Cartín, 1981; Mann *et al.*, 1990; Vass *et al.*, 2002; Bachmann and Simmons, 2010; Simmons *et al.*, 2010; Simmons, Adlam and Moffatt, 2010). Insects exert their influence in decomposition through tissue consumption, spread of bacteria, and heat generation by the immature insects, necessary for decomposition (Payne, 1965; Mann, Bass and Meadows, 1990; Simmons *et al.*, 2010). According to Simmons *et al.* (2010) in the northwest of England, insect access was the most important factor with respect to decomposition rate when decomposition from different environments were compared and temperature was standardized with ADD. Even in buried remains, those remains with prior insect exposure decomposed significantly faster than those without (Bachmann and Simmons, 2010). This supports the findings of an earlier work in South Carolina, that 90% of the carrion exposed to insects was disposed of in six days when it was compared with carrion protected from insects (Payne, 1965).

2.6.7. Clothing

There appears not to be a uniform finding on the effect of clothing on decomposition. Whereas some studies found that clothing has negligible or insignificant effects on decomposition of surface remains in Poland, North West England and Canada (Kelly *et al.*, 2011; Matuszewski *et al.*, 2014b; Card *et al.*, 2015; Cockle and Bell, 2017), other studies in western Australia, Italy and Tennessee, USA, found an increase in decomposition rate as the clothing provided a condusive environment for the larvae to multiply (Mann *et al*., 1990; Campobasso *et al*., 2001; Voss *et al*., 2011). However, when the effect of clothing was assessed in Cape Town, South Africa, it was found to appreciably decrease the rate of decomposition (Spies *et al.*, 2020) in agreement with an earlier finding in Tennessee, USA (Miller, 2002). This decrease in the rate of decomposition was as a result of limited access to scavengers. This led to a call for study designs on the effect of scavenging on decomposition to factor in clothing. For buried bodies clothing was found to delay decomposition by encouraging adipocere formation (Forbes *et al*., 2005b).

2.6.8. Scavengers

Vertebrate scavengers, just like insects, remove a large chunk of the decomposing remains (Junkins and Carter, 2017). Access to scavengers, therefore, encourages rapid decomposition (Spies *et al*., 2018). Besides facilitating rapid decomposition, the bite marks left by vertebrate scavengers could mimic signs of antemortem trauma both in soft tissue and bone (Mann *et al*., 1990; Rothschild and Schneider, 1997; Tsokos and Schulz, 1999; Byard *et al*., 2002; Janjua and Rogers, 2008). Studies have shown that scavenger activity is affected adversely by clothing (Spies *et al.*, 2020) and the winter (Keyes *et al*., 2021). Scavengers may disrupt insect activity such as succession pattern after the mutilation of remains such that insect succession in that instance may no longer be a reliable PMI estimation tool (Ellison, 1990).

2.6.9. Burial

Burial of decomposing remains limits access to insects, scavengers and the warmer aerial temperature which are factors that accelerate decomposition. Therefore, buried remains exhibit a slower decomposition rate than surface ones (Mann *et al*., 1990; Fiedler and Graw, 2003; Megyesi *et al.*, 2005; Marais-Werner *et al.*, 2018). The deeper the body is buried, the slower the decomposition (Rodriguez and Bass, 1985).

Soil type also influences the decomposition rate as carcasses buried in pasture soil decomposed faster than woodland and upland soil (Wilson *et al.*, 2007). This is based on soil characteristics such as moisture retention, nutrient availability and pH which determine the soil capacity to support microbial activity. Soil moisture is considered the most important factor influencing carcass decomposition in soil (Carter *et al.*, 2010). Soil with more moisture retention resulted in accelerated decomposition up to a certain limit as very wet soils caused a decrease in decomposition rate due the impairment of gas diffusion and therefore decreased aerobic metabolism.

Cadavers buried in warmer seasons had a more rapid decomposition than those buried in colder seasons (Breitmeier *et al.*, 2005), and those which had prior access to insects before burial decomposed 30% faster than those that did not (Bachmann and Simmons, 2010).

2.6.10. Health status

Disease states that increase the body temperature in the perimortem period result in rapid decomposition (Davy, 1839) by accelerating the onset of autolysis through enhanced action of intracellular hydrolytic enzymes. The subsequent autolysis of tissues leads to accumulation of nitrogenous products of protein hydrolysis such as ammonia and amines which increases pH to alkaline levels and is conducive for putrefactive bacterial growth and multiplication

(Zhou and Byard, 2011). In addition to hastening autolysis and providing the requisite pH for their growth, antemortem fever also provides the necessary temperature for endogenous bacteria to grow and multiply easily, which is at an optimum of 21°C – 38°C (Jain, 2004; Vij, 2008), and easily invade the rest of the body from the intestine (Skopp, 2004). The disease states that result in antemortem fever include cerebrovascular accident, heat stroke, intracranial haemorrhage, and hyperthyroidism (Byard *et al*., 2000; Green *et al.*, 2001; Tsokos, 2005). Sepsis accelerates decomposition through increased perimortem temperature and increased bacterial load in the body (Tsokos, 2005).

A few case studies found accelerated decomposition rates with patients that had diabetes (Zhou and Byard, 2011; Hayman and Oxenham, 2016b). This is attributed to the abundance of organic carbon in glucose for which putrefactive bacteria have affinity and which is made available through fermentation (Riedel *et al.*, 2001). Also, diabetics are more at risk of infections that may result in septicaemia (Rayfield *et al.*, 1982), and those that are not treated with insulin easily retain fluid (Evans *et al.*, 1986; Nesto *et al.*, 2003).

2.6.11. Drugs

The use of prescription or illicit drugs in the period before death modifies the process of decomposition. The nature of modification depends on the type of drug. For example, prescription drugs like neuroleptics and benztropine, and illicit drugs like cocaine and ring-derivative amphetamines (“ectasy”) cause antemortem fever (Byard *et al*., 2000; Zhou and Byard, 2011) and facilitate decomposition as already discussed above. Hayman and Oxenham (2016b) found that treatment with cytotoxics and antibiotics before death delayed decomposition. Cytotoxics act by targeting and destroying rapidly multiplying cancer cells but may also reduce the rapidly dividing cells of the upper gastrointestinal tract that produce hydrolytic enzymes that initiate decomposition through autolysis (Gill-King, 1997). Very high

doses of antibiotics such as gentamycin and cephazolin have been reported to reduce the survival of blowfly larva (Sherman *et al.*, 1995). It is therefore important to note that the progress of decomposition may differ while working with human remains from hospitals on whom medical intervention in the form of drug therapy may have been performed to save life.

2.6.12. Trauma

Earlier works on the effect of trauma on decomposition rate suggested that trauma significantly altered decomposition rate (Micozzi, 1986, 1991; Mann *et al.,* 1990). Since the study by Micozzi (1986) was with an initially frozen carcass, it is not certain whether the witnessed more rapid decomposition was due to the effect of the freeze-thaw cycle, or the trauma (cervical dislocation) which actually had no skin disruption. The work by Mann *et al.* (1990) was performed with two human remains, one of which had a gunshot trauma to the chest and the other had no trauma. Although a more rapid decomposition was observed in the remains with trauma, the sample size may be insufficient to adequately examine the effect of trauma on decomposition rate. More recent findings by researchers from various regions including South Africa (Kelly *et al*., 2011), the UK (Cross and Simmons, 2010), the US (Smith, 2014; Munro *et al*., 2019) and Germany (Breitmeier *et al.*, 2005) suggest that trauma had no significant effect on the decomposition rate in both surface and buried remains. However, blood loss, which often follows trauma, was reported to delay decomposition (Cockle and Bell, 2019) probably due to the adverse effect of blood loss on the ability of bacteria to spread through the body (Bell *et al.*, 1996; Bell, 2011, 2012) and the likely contribution of blood loss to tissue dessication (Cockle and Bell, 2019). Since the regions where all the above were tested have temperate climate, it will be important, in the future,

to test the effect of trauma on decomposition rate in the tropical climate of sub-Saharan Africa.

2.6.13 Location

The location of remains affects the rate of decomposition with a more rapid decomposition reported in remains located outdoors than those indoors (Prieto *et al.*, 2004). This is related to a more rapid insect access and colonization of the outdoor carcass with blowflies and other insects both in absolute number and species richness (Campobasso *et al*., 2001; Pohjoismäki *et al.*, 2010; Anderson, 2011). Therefore, tissue destruction by insects during decomposition is more rapid outdoors than it is indoors (Payne, 1965; Anderson, 2011). Srnka (2003) also reported a more rapid decomposition in the early stages in bodies exposed to direct sunlight than those which were shaded.

2.7. Estimation of PMI

Researchers have used some of the physical changes of decomposition to attempt PMI estimation. The mortis triad, consisting of algor mortis, livor mortis, and rigor mortis, is one such example (Adelson, 1952; Henssge, 1992; Vanezis & Trujillo, 1996; Althaus & Henssge, 1999; Goff, 2009; Janaway et al., 2009; Usumoto et al., 2010).

2.7.1. Estimation of PMI in the early decomposition phases

Algor mortis, or body cooling, results from the failure of the body's thermoregulation after death. Body temperature subsequently equilibrates with ambient temperature over time by radiation, conduction, convection, and evaporation for a wet body (Pounder, 2000; Tracqui, 2000). The rate of this loss is dependent on factors such as the ambient temperature, the presence or otherwise of clothing, the presence or absence of fever in the perimortem period, body position and weight to body surface area ratio. Although a fall in body temperature of

1°C per hour is generally accepted, temperature gradients between the surface and core of the body could cause a variable time lag or plateau before cooling begins (Hayman and Oxenham, 2016a). A time lag of up to three hours before the resumption of cooling was reported by Al-Alousi *et al.*(2002). This initial lag, or even an increase in temperature in very warm climates, before commencement of cooling, and the slowing as the body temperature approaches the containing medium or ambient temperature (Rainy, 1868) show that heat loss does not occur at a uniform rate until equilibration with the containing medium or ambient temperature. The complex nature of body cooling is captured in the time-dependent Z equation (TDZE) method of Green and Wright (1985a, 1985b) to improve the accuracy of this method.

Noting the complex nature of body cooling and the need for mathematical definition with a formula in order to ensure accuracy using this method, Marshall and Hoare (Marshall, 1962; 1962) set the stage for development of Henssge's nomogram, one of the most accurate methods for PMI estimation using body cooling (Henssge, 1979; Nokes *et al*., 1985). The Henssge's nomogram provided a means for PMI estimation by reading off a single rectal temperature taken at least 8 cm within the rectum on a nomogram. Apart from situations like perimortem hyperthermia, hypothermia, and finding the body somewhere different from the place of death in which case the method cannot be used due to high error rates, there is ordinarily an error rate of 2 – 4 hours in the first 12 hours following death. There have been refinements to accommodate influencing factors such as body weight, covering, ambient temperature and other crime scene conditions to produce a more complex form of the Henssge's equation (Henssge *et al.*, 2002, 2004; Madea and Henssge, 2016).

The recent introduction of a non-contact and quick 3D thermal photogrammetry for PMI estimation of bodies in any posture has increased the accuracy of PMI determination to 0.26

h ± 1.38 h for actual PMI between 2 and 35 h (Wilk *et al.*, 2021). This novel approach goes beyond the first 24 hours suggested for the use of algor mortis for PMI estimation (Mathur and Agrawal, 2011).

Livor mortis occurs due to **t**he failure of the circulatory system leads to gravitational pooling of the red blood cells to blood vessels in the most dependent parts of the body. The relaxation of the capillary and venous bed also facilitates this gravitational pooling, producing a pink to reddish colouration in light skin individuals. Body parts in contact with hard surfaces will blanch and leave pale areas due to compression of vessels (Krompecher, 2002). Minor signs of lividity are noticed 30 minutes post-mortem, are obvious in three to four hours, and are set in eight to twelve hours (Adelson, 1952; Tracqui, 2000). Although Clark *et al.* (1997) reported a short onset of 15 minutes, a much longer time interval for the appearance of livor mortis was suggested by Polson (1985). This timing is used when livor mortis is used as a guide to PMI determination, but like rigor mortis and algor mortis, the time of onset and/or being set is highly variable. Factors that influence the colour of lividity include carbon monoxide poisoning in which case there is a cherry red lividity; poisoning with chlorate-containing herbicide which produces a blue/brown lividity due to production of methaemoglobin; and a very cold environment which produces a pinkish hue (Hamilton and Green, 2017). These are only clues to the possible cause of death and should not take the place of proper toxicological testing.

Due to its variable time of onset, duration and the fact that it may not be visible in infants, the senile and the anaemic (Saukko and Knight, 2004), this method is not reliable in estimating post-mortem interval. Also, the difficulty in standardizing the occurrence rate, colour intensity and redistribution of lividity (if the body was disturbed) limits its use as a standalone method of post-mortem interval estimation (Vanezis and Trujillo, 1996).

Generalised muscle flaccidity results after death as the muscles are unable to contract. Over a variable period of time, rigor mortis occurs as muscle fibres crosslink permanently as a result of cellular ATP depletion and the muscle cells' inability to actively pump out calcium (Swift, 2006; Goff, 2009; Janaway et al., 2009). This begins from the smaller muscles like the eyelids, lower jaw, and fingers and spread to larger muscle groups until the entire body stiffens (Green, 2000; Tracqui, 2000). As decomposition proceeds and the contractile proteins are degraded by proteolytic enzymes, rigor mortis disappears, and flaccidity returns after 24 – 48 hours in temperate climate (Goff, 2009; Janaway *et al.*, 2009). Rigor mortis begins to develop in 2 – 6 hours, is fully developed in 6 – 12 hours, and then gradually disappears until the muscle becomes flaccid again; a period lasting between 72 and 84 hours (Gill-King, 1996; Mathur and Agrawal, 2011). The onset and duration of rigor mortis is affected by temperature, muscle bulk, pre-existing ante-mortem illness, age, the degree of physical activity in the immediate ante-mortem period, death from strychnine poisoning, carbon monoxide poisoning or electrocution (Bate-Smith and Bendall, 1947; Bate-Smith and Bendall, 1949; Krompecher *et al.*, 1983; Krompecher and Bergerioux, 1988; Krompecher, 1994; Huff-Lonergan *et al.*, 1996; Knight, 2005). High temperatures lead to early onset but short-lived rigor whereas low temperature causes the opposite – delayed onset and a prolonged duration. Rapid onset rigor mortis is also seen in if there was considerable perimortem muscular exertion. It is rapid but short-lived in children and the elderly due to less muscle bulk (Myburgh, 2010). Whereas individuals with large muscle bulk exhibit very strong rigor, those with very low muscle mass like the elderly will demonstrate weak rigor in extreme cases (Green, 2000; Pounder, 2000; Tracqui, 2000; Hamilton and Green, 2017).

Other methods used in the early post-mortem period are electrical excitability of striated muscles, vitreous potassium concentration, and stomach content (Henssge *et al.*, 2002).

The major drawback of these methods is the generally subjective nature of changes, the variable time of onset and duration, considerable individual variation, and the effects of multiple factors which may be intrinsic or extrinsic. They can only be used for PMI estimation in the first two to three days post-mortem (Amendt *et al.*, 2004), and are not applicable for dismembered, decomposed, and burnt remains (Vanin *et al.*, 2013). The use of any of the early physical changes like the mortis triad as a standalone method to estimate PMI is not encouraged for the above reasons (Vanezis & Trujillo, 1996; Henssge and Madea, 2004; Kaliszan et al., 2009). Furthermore the required accuracy, robustness and reliability needed to withstand a court testimony is lacking (Tibbett, 2008; Pittner *et al.*, 2016). A recent review showed that post-mortem biochemical markers are not yet admissible in court as a credible method for PMI estimation in the United States where Frye and Daubert criteria are used as standards due to lack of standardization (Meurs *et al.*, 2019).

As the search for more reliable methods for PMI estimation continues, research has explored larval developmental biology and arthropod community composition and succession pattern of insects attracted to remains (to be discussed later in this section); community composition and succession pattern of the necrobiome (Metcalf *et al*., 2013; Pechal *et al*., 2013; Olakanye *et al*., 2015; Cobaugh *et al*., 2015; Metcalf *et al*., 2016); and changes in chemical composition of body fluid or tissue after death, the commonest of which is vitreous humour potassium (Adjutantis & Coutselinis, 1972; Stephens & Richards, 1987; Agrawal *et al*., 1983; Foster *et al*., 2016). Other biochemical methods include the use of protein, DNA and RNA degradation rates (Cina, 1994; Di Nunno *et al.*, 1998; Sampaio-Silva *et al.*, 2013; Lee *et al.*, 2016; Lv *et al.*, 2016; Pittner *et al.*, 2016, 2017)and lipidomic analysis of skeletal muscle tissue (Langley *et al.*, 2019) and bones (Dudzik, 2017).

Tremendous gains have been made from decades of research in PMI estimation, but many questions remain. Pittner *et al.* (2016) suggest that the major inadequacy lies with the aspect of decomposition that involves soft tissue decay as estimation error margin increases with time. This part of forensic taphonomy which involves quantification of gross morphological change of putrefaction with a quantitative consideration of influencing factors to estimate PMI, is central to anthropological estimation of PMI (Wescott, 2018), and has witnessed considerable modification over time.

2.7.2. The use of morphological changes for PMI estimation

Physical changes of the remains are generally used for PMI estimation in late decomposition. Earlier research on the use of the gross physical changes of decomposition like bloating and bone exposure to assess decomposition rate and, by extension, the PMI, described these changes and grouped them into more or less well defined categories with one or more of these categories representing a stage in the process of decomposition (Reed, 1958; Payne, 1965; Galloway *et al*., 1989).

Furthermore, decomposition rate was expressed in absolute time by matching the observed physical changes to the time in calendar days taken to reach that state (Reed, 1958; Payne, 1965). Decomposition was therefore not treated as a continuous process without discernible demarcations since the features blend with one another (Vass *et al*., 1992; Pinheiro, 2006; Vass, 2011). Even with experience, this could produce only a rough estimate of the PMI in a particular region (Mann *et al*., 1990; Bass, 1997). This approach to earlier research was of little use in forensic casework since they did not consider variables such as climatic conditions, seasonality and insect access, which could cause considerable variation in decomposition rate and pattern (Mann *et al*., 1990; Vass *et al*., 2002; Iancu *et al*., 2018). To rectify these problems

and further refine PMI estimates, there has been a gradual shift from a qualitative to a quantitative approach.

2.7.3. Quantitative research in PMI estimation

The quantitative approach to PMI estimation attempts to assess, in a measurable way, the influencing factors such as temperature, humidity, rainfall, and insect access which, according to Matuszewski *et al.* (2014b), are poorly understood. This is important for the precision, robustness and reliability required in a court of law. Quantitative methods used to estimate PMI are described below.

Accumulated Degree Days (ADD): A popular attempt at quantification of the influencing factors and, by extension, standardization was the introduction of ADD into decomposition research by Vass *et al.* (1992). ADD denotes the average daily temperature a decomposing body is exposed to. The aim of using ADD was to avoid problems arising from temperature dependent results which may vary from one region to another. This is significant given that temperature is considered the most influential factor in decomposition (Mann *et al.*, 1990; Vass *et al.*, 2002) due to its effect on enzyme activity (Sheridan *et al.*, 2000), bacterial growth (Micozzi, 1991), insect activity (Galloway *et al.*, 1989; Mann *et al*., 1990; Campobasso *et al.*, 2001; Simmons *et al.*, 2010) and even secondary changes like mummification. The use of measurable variables such as the ADD has revolutionized decomposition research. Simmons *et al.* (2010) found that the standardization of temperature using ADD, an index of heat accumulation over time, allows decomposition studies from different environments to be easily compared when decomposition scores were utilised. This also allowed the whole process to be expressed as linear equations.

Total Body Score (TBS): Megyesi *et al.*'s (2005) introduction of the TBS by applying quantitative descriptions or measurements of the physical changes of decomposition based on the staging method by Galloway *et al.* (1989) is a further step in developing a quantitative approach. This appears to be the meeting point between the qualitative and the quantitative techniques. It was done by dividing the body into head and neck, torso and limbs due to differences in the progress of decomposition in these body regions. Point-based scores based on the five stages of decomposition listed above were assigned to these body regions on the grounds of the extent of decomposition assessed visually. The total of these assigned scores from the body regions gives the TBS. For example, for remains in advanced decomposition with desiccation and bone exposure less than half of the head and neck region (9 points), moist decomposition (without desiccation) with bone exposure less than half of both the trunk and the limbs (7 and 6 points, respectively), the TBS is the total of these scores (9+7+6=21).

By plotting the TBS of each sample as dependent variable against the known PMI on one hand, and against the known ADD for the time since death (TSD) on the other hand, Megyesi *et al.* (2005) obtained a graph that showed a rapid rise in TBS before levelling off in a log linear fashion. Transformation of the variables with log linear regression led to the derivation of a formula for calculating the estimated ADD since death. For an adult body (not fully skeletonised, not burned, not submerged), the ADD required for the body to reach the observed stage of decomposition can be calculated by scoring and fitting the TBS in the equation. The PMI is obtained by adding the average daily temperature backwards from the day of discovery (actual ADD) until it equals the calculated ADD. Approximately 80% of the variation in decomposition among the samples was due to ADD and this led to the suggestion that decomposition should be modelled as being dependent on ADD and not just the elapsed

time since death. Megyesi *et al.* (2005) proposed that temperature or heat accumulation over time as expressed in ADD was considered as the major factor affecting the progress and rate of decomposition while other factors such as animal scavenging and rainfall were not considered as it may not be possible to quantify them in a retrospective study. This suggestion also leaves out the significant influence of insects (Payne, 1965; Galloway *et al.,* 1989; Bachmann & Simmons, 2010).

On validating the Megyesi *et al.*'s (2005) model, Myburgh *et al.* (2013) in the Highveld of South Africa found it to be unable to predict ADD from TBS in late decomposition (especially TBS>17) at which time the decomposition pattern was highly variable, as other researchers have also found (Suckling *et al*., 2016; Marhoff-Beard *et al.*, 2018). The fact that most of the predictions were underestimated are also in keeping with the finding of Marhoff *et al*. (2016) in Australia. Like the Megyesi *et al.* (2005) study where, following linear regression, 80% the observed changes in decomposition could be accounted for temperature as expressed in ADD, Marhoff *et al.* (2016) found an improvement to 94% in Australia. Marhoff *et al.* (2016) suggest that the remaining 6% may represent other factors influencing decomposition which were not accounted for in the study. These factors, one of which may be the use of the pig model instead of human remains used by Megyesi *et al.* (2005), may be reponsible for the witnessed inaccuracy in predictions. The inability of the Megyesi *et al.'*s (2005) model to predict ADD from TBS is supported by the finding of Forbes *et al.* (2019) in Cape Town, South Africa, but only in the winter when significant underestimations were found and which increased with increasing TBS. In summer, however, Megyesi *et al.'*s (2005) model was found to be less reliable in early decomposition and more reliable in the later stages of decomposition (Forbes *et al.*, 2019). This agrees with an earlier study in West Central Montana (Parsons, 2009). This difference observed between the seasons of the same study in Cape Town (Forbes *et al.,*

2019) is an indication of the importance of season and climatic factors generally in decomposition studies. Also, the difference in the accuracy of the Megyesi *et al.* (2005) model with respect to the season and the period of decomposition (early versus late) observed in Pretoria (Myburgh *et al.,* 2013) and Cape Town (Forbes *et al.,* 2019) highlights the effects of environmental factors on decomposition. South Africa exhibits climatologic diversity more than most countries in sub-Saharan Africa (Conradie and Kumirai, 2010). There are obvious climatic differences between Pretoria (Köppen Geiger: Cfa) in the northeast and Cape Town (Köppen Geiger: Csb) in the south. While Pretoria experiences summer rainfall and hardly any in winter, and generally lower temperatures, Cape Town typically has winter rainfall and a generally warmer temperature. There are therefore differences in temperature, humidity and rainfall between these locations which affect decomposition either directly or indirectly. Also, since insects are ectothermic, the effect of temperature differences might be obvious on the necrophagous insect population for each location. This is even more so as environmental temperature is one of the key factors that drive circadian rhythm which in turn influences oviposition time (Villet *et al*., 2010; Villet, 2011).

Using the Megyesi *et al.*'s (2005) model, Myburgh *et al.* (2013) developed a linear regression equation with a 95% prediction interval for PMI from ADD. An r^2 value of 0.623 obtained when TBS was regressed against log ADD implied that only 62% of the variation in decomposition, represented by TBS, was explained by ADD compared to the 80% found by Megyesi *et al.* (2005). The rest of the variation could be accounted for by humidity differences, seasonality and its far reaching effects, scavenger activity and the state of health at the time of death. On validating this method, only one of the 16 pigs used for the validation had a PMI that fell within the lower limit of the 95% prediction interval. The accuracy of PMI estimation in

northern South Africa will, therefore, be affected by the considerable variation observed in decomposition (Myburgh *et al.*, 2013).

Moffatt *et al.* (2016) attempted a validation of the Megyesi *et al.*'s (2005) method and introduced a new equation. Fifteen of the 68 human remains used by Megyesi *et al*. (2005) were utilized after eliminating those cases from which PMI was derived from entomological methods on the ground of inaccuracy; indoor remains on the ground of differences in the pattern and rate of decomposition as the outdoor remains; remains with ADD beyond 3000 on the ground of being fully skeletonized; and one other set of remains on the ground of being of very low weight. Using inverse regression models to derive ADD from TBS, Moffatt *et al.* (2016) derived a formula with a narrower confidence interval (CI). Forbes *et al.* (2019) found the Moffatt *et al.*'s (2016) model to be generally unreliable in winter, but close to accurate in early decomposition with increasing error rates as decomposition progressed.

Another validation study of the Megyesi *et al.*'s (2005) equation was performed by Suckling *et al*. (2016), using 10 donated human remains allowed to decompose outdoors in a longitudinal study in Texas. Statistical analysis showed a significant difference between the overestimated ADD derived from Megyesi *et al.*'s (2005) equation and the actual ADD. This led to the suggestion that ADD as a lone or major variable in estimating PMI may not be proper in different regions of the world. Scavenger activity which was not assessed by Megyesi *et al*. (2005) was found to significantly increase decomposition rate. Suckling *et al*. (2016) pointed out the wide confidence interval as an indication that the equation lacked precision in PMI estimation making it of little use in actual forensic anthropology casework. The Megyesi *et al*.'s (2005) equation was also found to be unable to predict ADD from TBS in advanced decomposition in keeping with an earlier validation study in South Africa (Myburgh *et al.*, 2013). Also, the finding of a significant difference between the overestimated ADD derived

from Megyesi *et al.'*s (2005) equation and the actual ADD led to the suggestion that ADD as a lone or major variable in estimating PMI may not the appropriate in other regions.

Following a validation study in Hawkesbury region of Australia, Marhoff-Beard *et al*. (2018) supported the Megyesi *et al.*'s (2005) equation but only if the remains are found during early decomposition. Although frequent ADD overestimations were found, the accuracy was put at within 2 weeks. However, this study had a small sample and was performed in summer alone.

While investigating the environmental factors involved in juvenile decomposition and whether the Megyesi *et al.'*s (2005) approach for scoring decomposition of adult remains also applied to juveniles, Ross and Hale (2018) made some important discoveries when juvenile and foetal pigs were deposited through the four seasons. Examination of the relationship between PMI and both TBS and ADD with linear regression models, showed that only 35% of the progress in decomposition was due to ADD unlike the 80% found by Megyesi *et al.* (2005). This led to the suggestion that either TBS was not appropriate for scoring juvenile remains or that differences in environmental conditions may render TBS inapplicable in other places as affirmed by Cockle and Bell (2017).

In their study in Canada, Cockle and Bell (2017) found that time and temperature alone, which are the components of ADD, were not substantial dependent variable for decomposition. Therefore, the significant levels of correlation between ADD and progress of decomposition found by Megyesi *et al.* (2005) were not confirmed. Clothing and amount of rainfall were also found to have only negligible effects on the progress of decomposition. This accentuates the unforeseeable nature of the onset of the visible changes of decomposition which constitute scoring systems, and probably the failure of a single equation to successfully predict PMI in all environments.

Vass (2011) proposed a two-formulae method for PMI estimation of surface and buried remains. The formulae were based on the number of ADD's required for a decomposing body to stop liberating volatile fatty acids (VFAs), 1285, which signifies the end of decomposition (Vass *et al.*, 1992). For surface remains, the formula incorporates the percentage progress of soft tissue decomposition as visually assessed on the remains as the numerator, and the environmental factors like temperature and humidity driving this change as the denominator. The conditions to be met for the formula to be used include being at least one day postmortem, having pliable soft tissue, being as intact as possible, being in the preskeletonised phase of decomposition with little or no adipocere formation, and at a temperature over 0 ^{o}C. Although Vass (2011) attested to the success of these formulae, the need for an expert to assess the stage of decomposition, and adipocere formation for buried remains, and the use of percentage ratio instead of quantitative scale may affect both its use and error rates (Hayman and Oxenham, 2016c).

The Vass' (2011) equation, like that of Megyesi *et al.* (2005), meets most of the criteria proposed by Henssge & Madea (2007) for a PMI estimation method to gain wide practical application, but does not provide an error rate (Wescott, 2018). These criteria include quantitative measurement, quantitative consideration of influencing factors, numerical description, and statement and validation of precision. On testing the Vass' (2011) formula's ability to correctly predict the PMI of surface remains in Australia using linear regression, Marhoff-Beard *et al.* (2018) found a low accuracy of about 31% when pig carcasses were used. Validation with photographs of human remains as in the original work showed the formula to be accurate for PMI estimation in advanced decomposition but not in skeletonized remains. For PMI estimation, Vass' (2011) formula performed better than Megyesi *et al.*'s (2005) method but the error margin of 1 – 2 weeks was said to probably not find practical use in

actual forensic casework in Greater Western Sydney region of Australia (Marhoff-Beard *et al*., 2018).

In order to address questions raised about the TBS method of Megyesi *et al*. (2005) to quantify decomposition accurately and in all conditions, the degree of decomposition index (DDI) was proposed by Fitzgerald and Oxenham (2009) as a predictive model for PMI estimation in recent deaths. Ranging from 0 – 5, the DDI was obtained by adding the scores of individual body segments (i.e. just like the total body score of Megyesi *et al*. (2005), but there were 8 body segments) and dividing this value by the number of segments. When DDI was plotted against the known PMI, regression modelling showed that PMI, which they believe represents both elapsed time and unquantified environmental factors affecting the remains within that time, accounts for most of the variations in decomposition. This, they suggest, is preferred to ADD which factors only temperature over time. They also believe that the lack of significant differences in the DDI of the carcasses despite being placed in different environmental conditions, and undergoing decomposition expected of these different environmental conditions, shows that the DDI method is a better method for quantifying decomposition and can solve concerns that may arise from differential decomposition processes on account of different environmental conditions. Until these unquantified environmental factors incorporated in PMI are known and quantified, it is difficult to say what their contribution to decomposition is. On testing the findings of Fitzgerald & Oxenham (2009) in the Hawkesbury region of Australia, Marhoff *et al.* (2016) found that the DDI method underestimated the post-mortem interval of the remains. However, regression analysis to demonstrate the relationship between PMI and DDI showed that 92% of the variation observed in the decomposition process, represented by DDI, could be accounted for by time (PMI) alone. This is similar to the 95% found by Fitzgerald & Oxenham (2009). Using data obtained from the

study, two alternative predictive equations were proposed for the Hawkesbury region (Marhoff *et al.*, 2016).

2.7.4. Examination of the TBS system and introduction of other scoring parameters

TBS is generally considered a good indicator of the progress of decomposition. It was found to be not only a good quantitative descriptor of the decompositional stages, but also for the rate of change between the TBS values in the Highveld of South Africa (Myburgh *et al.*, 2013). Also, in a study in the UK that examined the effect of insect pre-exposure on decomposition, Bachmann and Simmons (2010) found TBS to be a justifiable parameter since it best mirrored the progress of decomposition when used with ADD. In Poland, Nawrocka *et al*. (2016) found the inter-rater reliability when TBS is used to score the extent of decomposition to be high (a Krippendorff's alfa of 0.818 for all volunteers) irrespective of the user's experience. A validation study of the TBS method for scoring human remains in the United States found no statistically significant difference between scorers for any of the body regions (Wescott *et al.,* 2018). Using the TBS method to score human remains in different stages of decomposition, a two-way random model interclass correlation showed a very high correlation between participants (average absolute correlation coefficient of 0.991) (Dabbs *et al*., 2016). Nevertheless, the subjective nature of the TBS method and its lack of precision in describing the decomposing remains have been pointed out as possible sources of interobserver error (Nawrocka *et al.,* 2016; Forbes *et al.*, 2019; Michaud & Moreau, 2011). In order to avoid this, refinements such as specifying the bones being examined instead of using nonspecific phrases such as "bone exposure more than half or 50% of the area being scored" have been suggested (Nawrocka *et al*., 2016). Another problem created by the above non-specified phrase is the fact that it does not accommodate typical situations like hot and dry weathers such as the dry seasons in Nigeria where desiccation occurs with little or no bone exposure, or even the

unexpected finding of precocious spontaneous desiccation in a temperate region such as South Africa (Finaughty and Morris, 2019). Forbes *et al.* (2019) suggest that a broader criterion of skeletonization be utilized to accommodate such situations. Suckling *et al.* (2016) believe that the various patterns of decomposition would have been accommodated by the Megyesi *et al.* (2005) TBS method if the TBS was treated as a categorical variable instead of a continuous variable like ADD, in which case a mixed model statistical analysis would be applied.

With the aim of addressing the rigidity associated with previous staging methods, and producing an ADD model that can be applied to various decomposition processes, Michaud & Moreau (2011) developed a staging scale by representing the duration of each stage with a realistic interval (the degree-day index). Although a scale that comprised the five stages of decomposition viz fresh, bloat, active, advanced and dry decay was used, a new scale, the degree day index, was developed that reflected realistic time frames for the different decomposition stages instead of strictly assigning values as though they were of the same duration. Following multiple regression analysis using ADD and the developed decomposition scale to determine the onset of each decomposition stage (the decomposition index) from the degree-day index, decomposition stages were found to be a reliable depiction of the process of decomposition, and 97% of the variation in decomposition was accounted for by temperature in this model.

Another scoring method for PMI estimation that went a step beyond the TBS as used by Megyesi *et al*. (2005) is that developed by Hayman and Oxenham (2017). This scoring system involved scoring the degree of decomposition externally according to the standard format of autopsy report by pathologists using the early physical changes like the mortis triad and marbling, and internally by assessing four internal organs (brain, heart, liver and spleen), the

total of which gives the TBS, a deviation from the TBS of Megyesi *et al*. (2005) which scored only the external changes of decomposition. A good correlation was found between the PMI and TBS, with TBS accounting for 76.4% of PMI variability. The external body score alone was also found to have a strong positive relationship with the TBS which could be used to obtain a quick tentative PMI while awaiting the outcome of autopsy for a more accurate PMI. Due to the unforeseeable manner by which decomposition of internal organs progresses, the authors believe that this model may not give accurate results for remains with PMI beyond 14 days. This may also not be practical for low resource settings, and for the recently dead but badly scavenged remains with missing organs since the required thorough autopsy may not be possible in this situation.

Further refinement of the scoring method by Megyesi *et al*. (2005) and development of PMI estimation formula was performed in the Netherlands by Gelderman *et al*. (2018) using 91 adult human remains with known PMI and selected from closed forensic cases. For the scoring method, events in decomposition were merged into fewer stages to eliminate the rigidity in scoring these phenomena as though they always occurred in the same sequence. Also, easily recognizable events like rigor mortis were used instead of colours which is only practical in light skinned remains. Percentages or ratios in previous scoring systems were replaced with ''partial'' and ''gross'' with a description of what they mean to eliminate subjectivity. On validating the scoring method, a high inter-rater reliability was found, and the developed formulae produced a narrower error margin compared to that by Megyesi *et al.* (2005). The formulae, however, did not give accurate results for actual PMI beyond 10 days, so caution should be exercised when these formulae are used for remains with longer PMI. The authors cautioned that this formula can only be used in regions with climates similar to that in the Netherlands.

2.8. The use of pigs as proxies for human decomposition study

The original description of stages of decomposition by Galloway *et al.*(1989) and later adapted by Megyesi *et al.* (2005) were based on human remains. The majority of research on decomposition, however, make use of other animals but the domestic pig is by far the most popular (Payne *et al.*, 1968; Terneny, 1997; Shalaby *et al*., 2000; Schiel, 2008; Bunch, 2009; Callahan, 2009; Fitzgerald and Oxenham, 2009; Reeves, 2009; Brown and Peckmann, 2013; Kyerematen *et al.*, 2013; Myburgh *et al.*, 2013; Marhoff *et al.*, 2016). This is due to the comparable internal anatomy, fat to muscle ratio, gut flora, sparse skin hair, hair follicles, sweat glands, skin thickness and torso size (Hall, 1988; Hopewell, 1991; Goff, 1993; Byrd and Castner, 2001; Pakosh and Rogers, 2009; Reeves, 2009). They are also readily available with less ethical scrutiny than when human remains are used (Keough *et al*., 2017). Also, since they are easily available, sample size could be easily altered in a controlled longitudinal study to examine the influence of one or a suite of factors on decomposition. Although most of the studies that utilized human remains were retrospective, longitudinal studies with human remains continue in a few human taphonomy research facilities like the Forensic Anthropology Facility (ARF) in Knoxville, Tennessee. Decomposition studies with human remains allow a direct interpretation and application of research findings to real forensic caseworks as influencing factors are varied either artificially or by the siting in different climatic areas. A number of studies have pointed out differences between human and pig decomposition (Turner and Wiltshire, 1999; Notter *et al.*, 2009; Connor *et al*., 2018; Knobel *et al.*, 2019; Matuszewski *et al.*, 2019) and the need to be cautious when findings from such studies are transferred for use in actual forensic cases. The use of human remains for

decomposition research is not without challenges. Since it is dependent on the availability of human remains from human donation programs, oftentimes the required sample size, treatment and control group for a scientifically valid result may not be available (Simmons, 2017). Technical difficulties such as delay in transportation and storage are not uncommon and during which decomposition continues and the accumulated temperature cannot be fully accounted for. Furthermore, refrigeration to halt decomposition will interfere with decomposition subsequently (Roberts and Dabbs, 2015). There are other factors that could either delay or hasten decomposition in each of the donated bodies as already discussed above.

Neither of these approaches is without difficulty, but in many regions of the world researchers have no choice but to continue using pigs as proxies. The limitations should, however, be kept in mind when pigs are used as proxies.

A refinement to improve the accuracy in scoring the TBS when pigs are used was introduced by Keough *et al.* (2017). This was an amendment of the Megyesi *et al.*'s (2005) scoring method based on that developed by Galloway *et al*. (1989). This became necessary due to the important differences in anatomy, and the decomposition rate and pattern between pigs and humans (Keough *et al.*, 2017; Connor *et al.*, 2018; Knobel *et al.*, 2019), and therefore allow for a better interpretation when pigs are used. These differences in decomposition between humans and pigs were marked in the early decomposition stages; there were no diffeences in the gross appearance and sequence in advanced decomposition and skeletonization (Keough *et al.*, 2017). Researchers are able to score the near commensurate changes in the decomposition of human remains when pigs are used since the differences in the sequence and rate were evaluated. This is significant because the domestic pig is the most popular proxy

for decomposition research. Therefore, works can be easily compared when this scoring method is used.

2.9. Entomology in decomposition research

The use of the knowledge of insects in legal investigation dates back to 13 th century China when a murderer was identified by Sung Tz'u due to the presence of flies attracted by blood stains on the murderers sickle (McKnight, 1981). Forensic entomology is therefore a discipline which has evolved over a long time.

The basis for the use of forensic entomology in legal investigation is the fact that insects are the earliest visitors to remains, attracted by the smell of decomposition. Some of the insects lay their eggs in moist body orifices which hatch to larvae. The larvae feed on the remains and subsequently move away to form pupae from which the adult insects emerge to mate and continue another life cycle. These are the necrophagous insects and are of great forensic importance. Diptera (Calliphoridae and Sarcophagidae) and Coleoptera (Silphidae and Dermestidae) are the dominant species in this group of insects. Other insects are the predators and parasites of the former group which include some species of Calliphoridae, Coleoptera and Hymenoptera; omnivores like ants and wasps that feed on both the remains and the necrophagous insects; and adventitious species that use the remains as shelter like spiders and centipedes (Hayman and Oxenham, 2016d).

Insects exert their effect by spread of bacteria and feeding by larvae which account for the greatest amount of soft tissue loss during decomposition (Payne, 1965; Mann *et al*., 1990; Bachmann and Simmons, 2010). Studies have shown that the family Calliphoridae, to which the blowfly belongs, is the first to visit a decomposing body (Wall and Warnes, 1994; Anderson, 2000; Kyerematen *et al.*, 2013; Ndueze *et al.*, 2013; Keshavarzi *et al.*, 2019). These

blowflies are commonly found on decomposing bodies, garbage and faeces (Apichat *et al.*, 2007; Chin *et al.*, 2011; Ekrakene and Iloba, 2011; Aigbodion *et al.*, 2013).

Insect activity and the clues they leave behind in the form of dead adult insects, larvae and pupal casings which are resistant to decay (Vanin and Huchet, 2017) are important to the forensic entomologist for forensic investigation, particularly the PMI estimation. The association of different insects with the various stages of decomposition makes PMI estimation the most important use of forensic entomology (Stefanuto *et al.*, 2017). Some of the common methods for PMI estimation using these clues will be discussed below.

One of the methods for PMI estimation using insects is based on larval developmental biology of the necrophagous insects. Several methods for this have been developed by forensic entomologist (Reiter, 1984; Wells and LaMotte, 1995, 2010; Marchenko, 2001; Reibe *et al*., 2010). Necrophagous insects are the most useful in this regard because the time of their appearance on the remains is predictable, mostly appearing within the first hour (Tantawi *et al.*, 1996). By asessing the age of the oldest egg, larva or pupa, the minimum PMI (PMI_{min}) is obtained. Minimum PMI because some time may have elapsed between death and colonization (Villet *et al.*, 2010; Villet and Amendt, 2011). From the report that they commonly arrive within the first hour (Tantawi *et al.*, 1996), it is clear that places which favour shorter colonization times produce more accurate results with this method. This is expected in tropical Africa with consistently high ambient temperatures and insect abundance (Ekanem and Dike, 2010).

Places like South Africa with more literature in forensic entomology are bound to have developed more data on developmental biology of the prevalent population of carrion insects. There are far fewer such studies in Nigeria in comparison (Ahmed and Joseph, 2016).

Since these data on developmental biology may be different for various regions due to differences in species composition, reference data from the closest population is suggested for use when age estimation of the immature insect is performed (Greenberg and Kunich, 2002; Amendt *et al.*, 2007; Richards *et al*., 2008; Owings *et al.*, 2014). The use of age estimation of the immature forms of necrophagous flies for PMI estimation is restricted to two to four weeks depending on the species and ambient temperature but the use of adult flies for this purpose could extend this to several weeks if the body was found indoors (Amendt *et al*., 2021). The use of several methods simultaneously for adult fly age estimation has been shown to give more accurate PMI estimation (Moon and Krafsur, 1995; Perez-Mendoza *et al.*, 2002; Butler *et al.*, 2009).

Another method for PMI estimation is arthropod community composition and succession patterns. This is based on the definite pattern of appearance and disappearance of the various insects that visit the carcass (Greenberg, 1991) as they interact with the food source and with one another. As the food source changes, for example from moist to dry remains, adaptation determines which insects stay or leave. This pattern coincides with the stages of decomposition (Catts and Goff, 1992; Carvalho *et al.*, 2000; Wolff *et al.*, 2001). This method is valuable when the time since death is suspected to run into months or years (Amendt *et al.*, 2011; Mądra, Frątczak, Grzywacz, Matuszewski, *et al.*, 2015). This is important as a complementary method for PMI estimation in forensic anthropology where validation of popular models have shown that their precision generally diminished in later stages of decomposition. Again, because insect community composition varies from place to place, studies on succession pattern from different regions are common in the literature (Early and Goff, 1986; Louw *et al.*, 1993; Tantawi *et al.*, 1996; Bharti and Singh, 2003; Battán Horenstein *et al.*, 2010; Matuszewski *et al.*, 2011; Kyerematen and Boateng, 2012; Ndueze *et al.*, 2013;

Bygarski and Leblanc, 2013; Kyerematen *et al.*, 2013; Alafia *et al.*, 2017; Magni *et al.*, 2019; Martín-Vega *et al.*, 2019). Comparisons have also been made between seasons (Lopes de Carvalho and Linhares, 2001; Shi *et al.*, 2009; Tembe and Mukaratirwa, 2021), human and non-human models (Jiang *et al.*, 2017), burnt and unburnt pig model (McIntosh *et al.*, 2017), trauma (Kelly *et al.*, 2011), clothing (Kelly *et al.*, 2009) and carcass size (Hewadikaram and Goff, 1991) to determine how these variables affect succession pattern, and ultimately the PMI. For example, it was shown that trauma had no effect on decomposition rate, and that the early colonizing flies prefered natural body orifices, instead of trauma sites, for oviposition (Cross and Simmons, 2010; Kelly *et al*., 2011). Clothing also provided protection for fly larvae causing them to multiply with a consequent more rapid decomposition, especially in the summer (Kelly *et al.*, 2009; Kelly *et al*., 2011). The authors also suggested that this could also be as a result of moisture preservation which allowed the remains to be in active decomposition for longer (Kelly *et al.*, 2011). Besides their importance for PMI estimation, insect succession pattern provides an inventory of the local carrion insects which is a starting point for further research in forensic entomology in that locality. The rising amount of literature on insect succession patterns from tropical Africa like Nigeria (Okiwelu *et al*., 2008; Ekanem and Dike, 2010; Ekrakene and Iloba, 2011; Ndueze *et al.*, 2013; Abajue *et al.*, 2015; Alafia *et al.*, 2017) and Ghana (Kyerematen and Boateng, 2012; Kyerematen *et al.*, 2013) is encouraging in this regard, and provides information for further study in forensic entomology in the particular location. Studies in Africa on these entomological methods are shown in Table 2.3.

Examination of pupal casing for weathering and chemical degradation with molecular methods like gas chromatography and spectrometry is another method of PMI estimation which is at the developmental stages. This requires expensive equipments which are not

feasible in resource-restricted places. Since the larvae mostly seek shelter away from the remains to pupate, it is important to know where to look for and collect empty puparia for analysis.

Entomological methods have thus found usefulness, but their dependence on regional environmental variables and fauna limits its widespread use (Pittner *et al.*, 2016).

Table 2.3. Some forensic entomology studies in Africa.

Country	Method	Authors
South Africa	Succession pattern	Braack, 1986; Louw, Linde and Van der Linde, 1993; Kelly *et al.,* 2009; Richards *et al.,* 2009b; Parry *et al.,* 2016; Tembe and Mukaratirwa, 2021
Nigeria	Developmental biology Succession pattern	Ahmed and Joseph, 2016 Okiwelu, Ikpamii and Umeozor, 2008; Ekrakene and Iloba, 2011; Ndueze *et al.*, 2013; Abajue, Ewuim and Akunne, 2015
Ghana	Succession pattern	Kyerematen, Boateng and Twumasi, 2010; Kyerematen *et al.*, 2013
Egypt	Succession pattern	Tantawi *et al.*, 1996

2.9.1. Standardization in forensic entomology

Standardization in forensic entomology which may improve both uniformity and accuracy is still evolving. There has been a call for further standardization especially in the areas of collection and analysis of insect specimen at a death investigation scene (Matuszewski, 2021). For example, it is important to ensure that the needed insects, that is "the most

developmentally advanced life stage of the most successionally advanced species" (Matuszewski, 2021, p. 2) are not missed in a bid to ensure that representative samples are collected according to existing guidelines (Lord and Burger, 1983; Amendt *et al.*, 2007; Sanford *et al.*, 2019). It is further suggested that guidance be provided when non-entomologists are saddled with the responsibility of insect specimen collection at the investigation scene.

Since the development and succession of insects on remains depends heavily on temperature (Higley and Haskell, 2001; Michaud and Moreau, 2009; Matuszewski *et al*., 2014a), it is important to reconstruct temperature conditions when PMI is estimated from developmental biology or succession pattern. Correction of frequently used local weather station temperature data to represent that at the research site can be done (Archer, 2004b; Johnson *et al*., 2012; Hofer *et al.*, 2017, 2020; Lutz and Amendt, 2020) through regression of recordings from both locations (Charabidze and Hedouin, 2019). Although there are conflicting views about the benefit of this correction (Archer, 2004b; Johnson *et al.*, 2012; Hofer *et al.*, 2020; Lutz and Amendt, 2020), Matuszewski (2021) recommends its frequent use especially in outdoor investigation scenes. These steps have been suggested to improve accuracy with entomological PMI estimation.

Chapter 3: Materials and Methods

3.1. Materials

3.1.1. Location of the study

The study was performed on an unfenced, uncultivated private property in Nibo (6°10'0"N, 7°4'0"E), a suburban town in Anambra state, Nigeria (Figure 3.1). It is about 7 km from Awka, the state capital, at an altitude of 123 m (403.54 ft) above sea level.

Following application for ethical clearance from the Research and Ethics Committee (REC) of the Veterinary Services Department of the Ministry of Agriculture and Rural Development in Nigeria, this location was assessed for suitability for this research with respect to public health safety, among other indices, and approved (MOA/ANV/441/Vol. 1/40, see Appendix A). Ethical clearance for this research was also granted by the Animal Research Ethics Committee, University of the Witwatersrand, South Africa (2019/08/46/A, see Appendix B).

Nigeria has a tropical climate (BWh, BSh, Aw, Am) (Peel *et al.,* 2007) with wet and dry seasons. The difference in these seasons in the arid northern Nigeria and the humid southern Nigeria is the duration, with a shorter wet season and longer dry season in the north compared to the south. The extreme northern states are closer to the Sahara Desert (BSh, BWh) (Peel *et al.,* 2007) whereas the southern states are closer to large water bodies (Aw, Am) (Peel *et al.,* 2007). Nibo, where this study was conducted, is located in southern Nigeria (shown in Figures 3.1 to 3.4). The Koppen-Geiger climate classification for this region is tropical Savanah (Aw) (Peel *et al.,* 2007). It has an average temperature of 26.9°C, average wind speed of 7.5 km/h, and minimum and maximum humidity of 35% and 90% respectively. The average rainfall is 1862 mm per year with September accounting for the highest average precipitation while December has the lowest (Figure 3.5). The difference in precipitation between the driest and wettest months is approximately 300 mm. The variation in daily mean temperatures

throughout the year is about 3.6 °C. The average temperature in the wet season is 26.3°C while it is 27.5°C in the dry season. (https://www.worldweatheronline.com/awka-weather-averages/anambra/ng.aspx; https://en.climate-data.org/africa/nigeria/anambra/nibo-1022872/#climate-graph). Temperature differences between the wet and dry seasons are thus low. Temperatures in Nigeria never go below 0°C.



Figure 3.1. The location of Anambra state where the research site is located (Map of Nigeria, 15 January 2021 from https://en.wikipedia.org/wiki/File:Nigeria_Anambra_State_map.png).



Figure 3.2. The research site during the wet season (taken 30th September 2020)



Figure 3.3. Research site during the dry season (taken 6th February 2020).

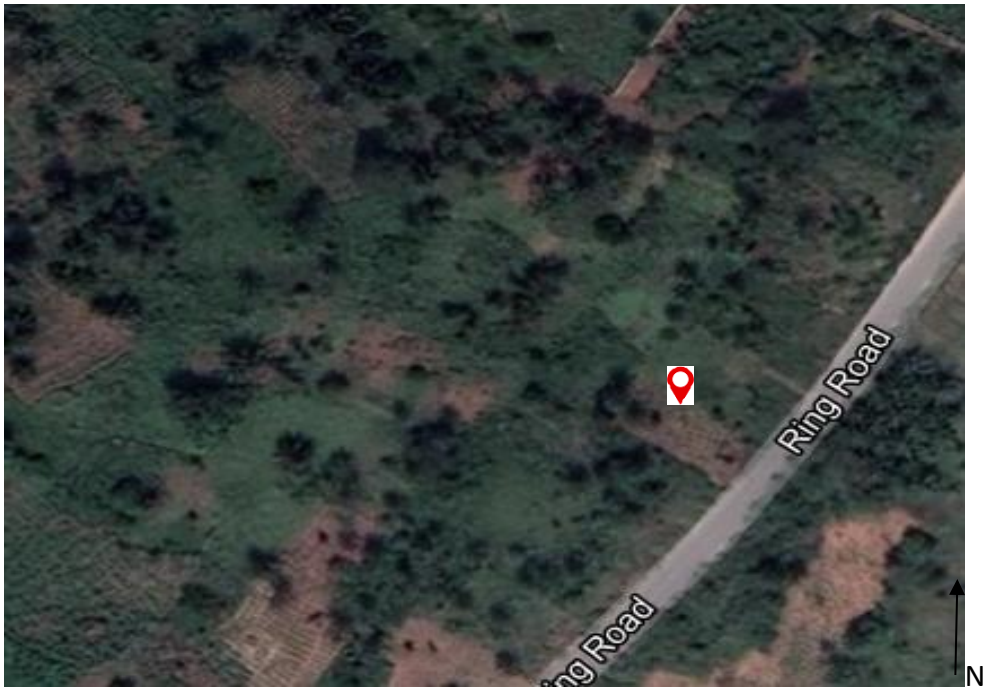

Figure 3.4. Satellite image of the research site (15 January, 2021 from https://www.google.com/maps/@6.1897804,7.0847684,428m/data=!3m1!1e3).

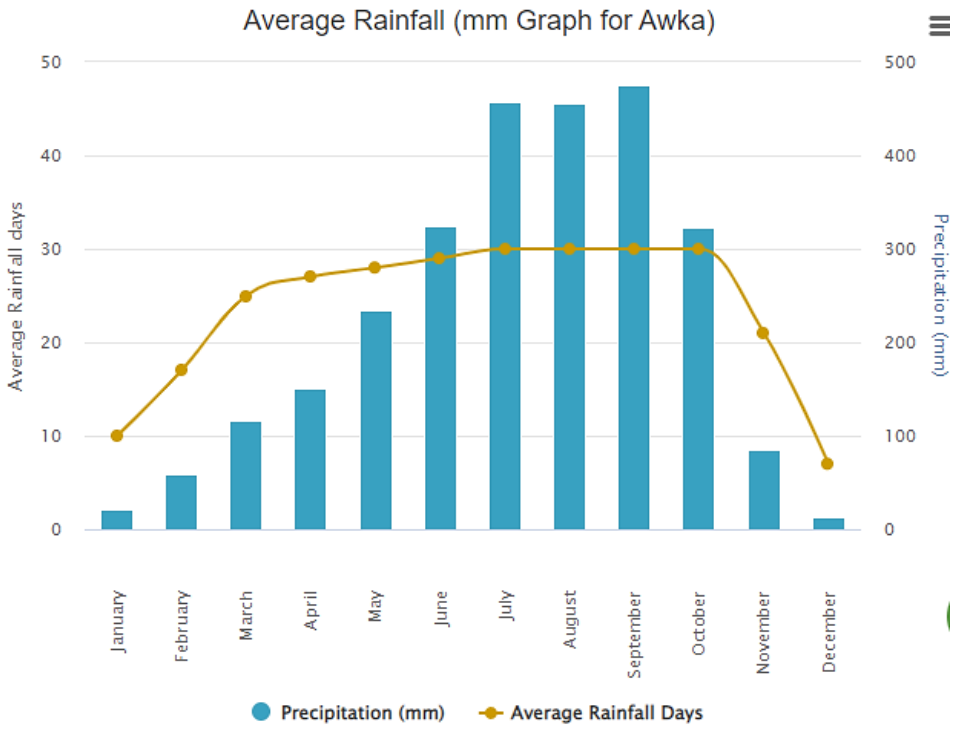

Figure 3.5. Average monthly rainfall around the research site (20 May 2021 from https://www.worldweatheronline.com/awka-weather-averages/anambra/ng.aspx;).

3.1.2. Study sample

The study sample consisted of 20 domestic pigs (*Sus scrofa*) of the large white breed. The pig

carcasses were bought, freshly killed, from a butcher after inspection for any external wounds apart from the slaughter wound. Personnel from the State Veterinary Services are often present at the butchers to ensure that the animals are healthy before they are slaughtered. Pigs were killed by piercing the heart and/or great vessels with a sharp knife through the chest area close to the left forelimb. This created a knife puncture wound. In order not to leave an open wound that leaked body fluids and thus attract insects and other animals, the lesion was closed. The skin and subcutaneous tissue of the slaughter laceration were repaired with non-absorbable nylon suture size 2 (Agary Pharmaceuticals), washed with water, dried and covered with plaster. This was an attempt to minimise the effect of insect colonization and activity on the wound site and the consequent overall increase in the rate of decomposition which has been demonstrated (Galloway *et al*., 1989; Mann *et al*., 1990; Rodriguez, 1997; Campobasso *et al*., 2001). The pigs were weighed at the butcher's shop and the length, height, and thoracic and abdominal girths were measured following suggestions by Myburgh *et al.* (2013) with a slight modification such as the girth measurements (Table 3.1, Figure 3.6). This gives an idea on the body mass and dimensions of the pigs, but the detailed measurements were not used in subsequent analyses. The pigs used for this study ranged between 30 kg and 70 kg, a proposed value to minimise the effect of body size on decomposition rate (Sutherland *et al.,* 2013; Matuszewski *et al.*, 2014b) and reflecting the body/torso size of adult humans. They were then bagged and transported to the study site for deposition within three hours of death.

Table 3.1. Descriptions for the measurements of the pigs (adapted from Myburgh *et al*., 2013).

Measurement	Description
Length (L)	The distance measured from the snout to the root of the tail along the spine.
Height (H)	The distance measured from the furthest portion of the back to the furthest point of the hind hoof.
Thoracic girth (TG)	The circumference of the thoracic region just below the forelimb.
Abdominal girth (AG)	The maximum circumference of the abdominal region.



Figure 3.6. Measurements taken from each pig (length = black; height = yellow; thoracic girth = blue; abdominal girth = red).

At the site, each pig was numbered and the sex, date of death and date of placement recorded. The pigs were placed in chicken mesh metal cages (120cm x 80cm x 60cm) in direct sun and the cages were anchored to the ground to protect them from scavengers and disturbance (Figure 3.7). Each cage had an open base that allowed the carcass to be in direct contact with the ground, and a hinged lockable door at the top. A distance of at least 10 m was maintained between cages to avoid arthropod cross colonization (Shahid *et al.*, 2003). A

total of 10 pigs were deposited at different times in the dry season (December to March 2020, and November 2021) and the other ten were placed in the wet season (May to August 2020). Once skeletonization was reached, the skeletonized remains were removed from the cage, the cage was moved to a new site, and a new pig carcass was deposited. Five pig skeletons were donated to the Anatomy Department of Chukwuemeka Odumegwu Ojukwu University while the rest were buried.



Figure 3.7. Metal cage used to cover pig carcasses.

3.2. Methods

3.2.1. Scoring of decomposition

In order to get an overall idea of decomposition rates in Nigeria, one pig was used in a pilot study. It was observed that decomposition progressed very rapidly such that early advanced decomposition was reached in 4 days. Following on this, it was decided for data collection to proceed as follows: daily during the first week after a pig was placed out, once every 3 days during the second week, weekly for the following 3 weeks, then every second week until the late stages of advanced decomposition (almost complete skeletonization) or skeletonization for both dry and wet seasons were reached. These observations were not done at exactly the

same time on the days of observation. The stage of decomposition was assessed and scored using the amendment to Megyesi *et al.*'s (2005) method by Keough *et al.* (2017) as shown in Tables 3.2, 3.3 and 3.4, in which different body regions (head and neck, trunk and limbs) were scored separately due to differences in decomposition rates for the various body regions. The scores obtained from each body region were added to give the total body score (TBS) which represents the overall stage of decomposition for that pig carcass. For example, Figure 3.8 shows a TBS value of 17. A score of 8 was assigned to the head and neck region; moist decomposition with bone exposure less than one half of the area. The trunk was scored 5; the trunk is deflated from the initial gas-filled abdomen and there is skin slippage. The limbs were scored 4 as there are brownish shades at the edges and skin drying that is more pronounced proximally. Photographs were also taken of the complete pig and the different body regions scored on each visit to allow for scoring by an independent observer.

Table 3.2 Modified categories and stages of decomposition for the head and neck (Keough *et al*., 2017).

A: Fresh	
(1pt)	Fresh, no discoloration – slight lividity (pink/red)
B: Early decomposition	
2pts	Insect activity; pronounced lividity (dark pink/red)
3pts	Dark – red discoloration with some flesh still relatively fresh; oedema of ears; maggot colonization (mouth); initial bloating of neck and skin slippage
4pts	Discoloration and/or brownish shades particularly at the edges, drying of nose, ears, and lips; prominent bloating of neck; maggot colonization (mouth and eyes); purging of decompositional fluids (mouth)
5pts	Purging of decompositional fluids (mouth, eyes, nose); brown discoloration; hair loss and skin slippage; drying of lips, nose and ears.
6pts	Black discoloration of flesh; extensive maggot colonization and migration
C: Advanced decomposition	

7pts	Caving in of the flesh and tissues of eyes and throat
8pts	Moist decomposition with bone exposure less than one half that of the area being scored
9pts	Mummification with bone exposure less than one half that of the area being scored
D: Skeletonization	
10pts	Bone exposure of more than half of the area being scored
11pts	Bone exposure of more than half of the area being scored with desiccation or mummified tissue
12pts	Bones largely dry, but retaining some grease
13pts	Dry bone

Table 3.3: Modified categories and stages of decomposition for the trunk (Keough *et al.,* 2017).

A: Fresh	
1pt	Fresh, no discoloration – slight lividity (pink)
B: Early decomposition	
2pts	Skin appears shiny/glossy with early bloating and may show purple-black discoloration over abdominal area
3pts	Gray-purple to green discoloration: some flesh still relatively fresh; marbling of abdomen with maximum bloat
4pts	Purple black discoloration and purging of decompositional fluid; skin slippage with maggot-filled blisters present; hair loss
5pts	Post-bloating following release of the abdominal gases, with extensive skin slippage and drying out of blisters
C: Advanced decomposition	
6pts	Decomposition of tissues producing sagging of flesh; caving in of the abdominal cavity
7pts	Moist decomposition with bone exposure less than one half that of the area being scored
8pts	Mummification with bone exposure less than one half that of the area being scored

D: Skeletonization	
9pts	Bones with decomposed tissue, sometimes with body fluids and grease still present
10pts	Bones with desiccated or mummified tissue covering less than one half of the area being scored
11pts	Bones largely dry, but retaining some grease
12pts	Dry bone

Table 3.4: Modified categories and stages of decomposition for the limbs (Keough *et al.,* 2017).

A: Fresh	
1pt	Fresh, no discoloration – slight lividity (pink) with rigor present
B: Early decomposition	
2pts	Pink-white appearance with bloating of proximal parts of limbs
3pts	Gray to green discoloration: marbling and shiny appearance of skin; some flesh still relatively fresh; skin slippage and hair loss
4pts	Discoloration and or brownish shades particularly at edges, drying of skin (starting distal to proximal)
5pts	Brown to black discoloration, skin having a leathery appearance
C: Advanced decomposition	
6pts	Moist decomposition with bone exposure less than one half of the area being scored
7pts	Mummification with bone exposure less than one half of the area being scored
D: Skeletonization	
8pts	Bone exposure over one half of the area being scored, some decomposed tissue and body fluids remaining
9pts	Bones largely dry, but retaining some grease
10pts	Dry bone

3.2.2. Ambient temperature recording and calculation of Accumulated Degree-Days

With the failure of two onsite temperature and humidity data loggers (Tzone TempU03) due to extreme humidity, data pertaining to temperature and humidity were obtained from the Nigerian Meteorological Agency (NIMET) weather station on Enugwu Agidi Road, about 7.4 kilometres from the research site. Temperatures in Nigeria do not reach zero or sub-zero; the temperature at which decomposition is either severely inhibited or stops (Micozzi, 1986; Catts and Haskell, 1990). The average of the maximum and minimum temperatures for each day was obtained and the accumulated degree days (ADD) were calculated by adding the daily averages starting from the date of death to the end of data collection for each individual pig.

3.2.3. Collection and identification of arthropods

Following deposition, the pig carcass was observed in order to note the arthropod visitors. Adult flies were collected by aerial sweeps using a hand net with gloved hands. As decomposition progressed, larvae and beetles were also collected using forceps. Insect collection was done once daily in the first one week after deposition, every three days in the second week, then every second week until the bones were exposed. Caution was exercised in collecting these specimens so that only the minimum number required for identification was sampled in order not to alter the course or duration of the decomposition process. The collected adult insects and beetles were killed in boiling water and preserved in 70% ethyl alcohol for identification (Amendt *et al.*, 2007). The larvae were divided into two groups: one group was reared to adult stage on ground chicken liver, in an improvised insect cage for identification while the other group was immersed in hot water for five minutes and fixed in 70% ethyl alcohol for preservation and subsequent identification. Photographs of the collected arthropods and larvae were sent to, and identified by, an entomologist at the Department of Forensic Medicine, University of the Witwatersrand (Holloway, 1991; Lutz *et*

al., 2018; Picker *et al.,* 2004; Prins, 1982; Prins, 1983; Smith, 1986; Zumpt, 1965) as no such specialist could be found in Nigeria. The references used for the arthropod identification

The insect succession patterns obtained from this identification were compared between the wet and the dry seasons, and between Nigeria and South Africa (Kelly et al., 2009; Kelly et al., 2011; Tembe and Mukaratirwa, 2021).

3.3. Statistical analysis

The weight, length, height, thoracic and abdominal girths were taken and were beyond the scope of the current study; they were used to ensure that the sample corresponds to the samples in other similar studies.

3.3.1. Patterns of decomposition

Scatter plots were drawn up for all the pigs in order to assess the decomposition pattern for the PMI in relation to the TBS, and subsequently the ADD in relation to the TBS. These patterns were visually compared with those in South Africa using the work by Myburgh *et al.* (2013).

Differences in decomposition patterns due to seasonal variations were assessed by grouping the observations according to season. All observations between November and March were regarded as the dry season group while those that occurred between April and October were the wet season group. Scatter plots were then drawn up to demonstrate the relationship between the PMI and TBS, and the ADD and TBS for the seasonal data. Decomposition patterns were then visually compared between the seasons.

3.3.2. Random effects maximum likelihood regression

Random-effects Maximum Likelihood regression was used to model ADD and PMI due to the longitudinal nature of the data. Two models were derived. In the first one, ADD and PMI were

each modelled against TBS alone. Then, ADD and PMI were each modelled against TBS, together with season and with the interaction between TBS and season.

PMI and ADD were log transformed in order to be linearly related with TBS because the values of PMI and ADD resulted in a skewed distribution on the original scale (Myburgh *et al.*, 2013). Their coefficients of determination (r^2) were used to compare these relationships. When r^2 was multiplied by 100, it showed the percentage of the variation in logPMI and logADD that can be explained by the variation in TBS.

Models were reported on the original scale and for all possible combinations of the TBS predicted values of ADD and PMI were tabulated along the 95% confidence intervals. The formulated equations were, therefore, used to produce a forecast of ADD and PMI for each TBS value including the standard error with the upper and lower limit forecast at a 95% confidence interval.

3.3.3 Inter-observer repeatability

Inter-observer repeatability was assessed by having a different individual score the decomposition of the various body regions, using the photographs of two randomly selected pig carcasses (34 in all) taken at the time of physical scoring. This different individual was another student working on a similar project.

The scores by the original observer were compared to that of the additional individual using Pearson's correlation. A perfect correlation exists if the coefficient of coefficient of correlation is 1; values between 0.75 and 0.99 indicate a high degree of correlation; 0.50 to 0.74 indicate a moderate degree of correlation whereas values below 0.5 indicate a low degree of correlation (Allan, 1982).

3.3.4. Intra-observer repeatability

Intra-observer repeatability was assessed by the primary observer scoring the TBS of two randomly selected pig samples using photographs. These scores were then compared with the scoring done at the research site by the primary observer using Pearson's correlation. A perfect correlation exists if the coefficient of coefficient of correlation is 1; values between 0.75 and 0.99 indicate a high degree of correlation; 0.50 to 0.74 indicate a moderate degree of correlation whereas values below 0.5 indicate a low degree of correlation (Allan, 1982).

3.3.5. Entomology data

No statistical analysis was done for the insect data since the aim in this section is to produce data on succession patterns/species assemblage and insects of forensic importance in southern Nigeria and comparing same with the findings in other regions.

Chapter 4: Results

The results are set out in two major parts. The first part will deal with the scoring of the rate and pattern of decomposition in Nigeria which has not been assessed before and comparing decomposition patterns between wet and dry seasons. This section will include the details of the repeatability of the scoring. The rate and pattern of decomposition in Nigeria will be compared with other regions but focusing mostly on South Africa (Pretoria) since the particular study by Myburgh *et al.* (2013) had a similar aim and also looked at seasonality like the present study. For this part, the modified staging method by Keough *et al*. (2017) was used. This method considers TBS 1 – 3 as fresh, 4 – 16 as early decomposition, 17 – 24 as advanced decomposition, and 25 – 35 as skeletonized stage.

The second part of the results will deal with a description of the insect succession patterns during decomposition in Nigeria. This will be compared between the wet and the dry season, and with other studies in other regions. The expected rapid decomposition in Nigeria on account of high temperatures may also indicate higher insect activity during decomposition.

4.1 Pig sample

A total of 20 pigs were scored for decomposition. The pigs were numbered according to the sequence of deposition and were placed in cages separated at a minimum of 10 m from each other to minimize the effect of arthropod cross colonization (Early & Goff, 1986; Anderson & VanLaerhoven, 1996; Anderson, 2000). Table 1 shows the dates of placement, indicating that 10 pigs were put out in the dry season (December 21, 2019 – March 16, 2020, and November 13, 2020), and 10 pigs in the wet season (May 19, 2020 – August 18, 2020). The weights of the pigs ranged between 30 kg and 66 kg (Tables 4.1 and 4.2). These measures of body size are used only to demonstrate that the pigs represent adult-sized human individuals since

Sutherland *et al*. (2013) have shown that values smaller than this experience faster decomposition.

Table 4.1. Summary of the date of death/deposition, weight, and dimensions of the sample.

Pigs in order of placement	Date of death/ placement	Sex	Weight (kg)	Length (cm)	Height (cm)	Thoracic girth (cm)	Abdominal girth (cm)
01	21/12/2019	M	38	114	57	92	108
02	18/01/2020	M	50	124	71	93	117
03	17/02/2020	M	31	114	56	87	93
04	04/03/2020	M	55	129	72	99	105
05	04/03/2020	M	30	104	61	66	81
06	16/03/2020	M	66	126	74	102	129
07	19/05/2020	M	35	117	61	81	84
08	22/05/2020	M	49	125	67	93	111
09	30/05/2020	M	40	116	61	93	105
10	28/07/2020	M	42	117	62	75	87
11	28/07/2020	M	33	112	60	78	84
12	04/08/2020	M	34	116	61	78	85
13	04/08/2020	F	41	118	61	96	105
14	15/08/2020	M	31	110	59	78	83
15	15/08/2020	M	32	112	60	75	84
16	18/08/2020	F	32	112	60	72	81
17	13/11/2020	M	37	115	63	77	93
18	13/11/2020	M	45	115	67	78	90
19	13/11/2020	M	50	131	66	87	101
20	13/11/2020	M	41	116	62	71	90
Minimum			30	104	56	66	81
Maximum			66	131	74	102	129
Mean			40.60	117.15	63.05	83.55	95.80
Standard deviation			9.51	6.71	4.90	10.30	13.59

4.2 Decomposition patterns of individual pigs

4.2.1. Complete/Combined pig data set

To demonstrate the progression of decomposition, the total body score (TBS) of each individual pig was plotted against the post-mortem interval (PMI) (Figure 4.1) (Appendix C), and accumulated degree days (ADD) (Figure 4.3) to see if there was a relationship between decomposition and time, and between decomposition rate and temperature, respectively. Here the complete sample including all 20 pigs is shown.

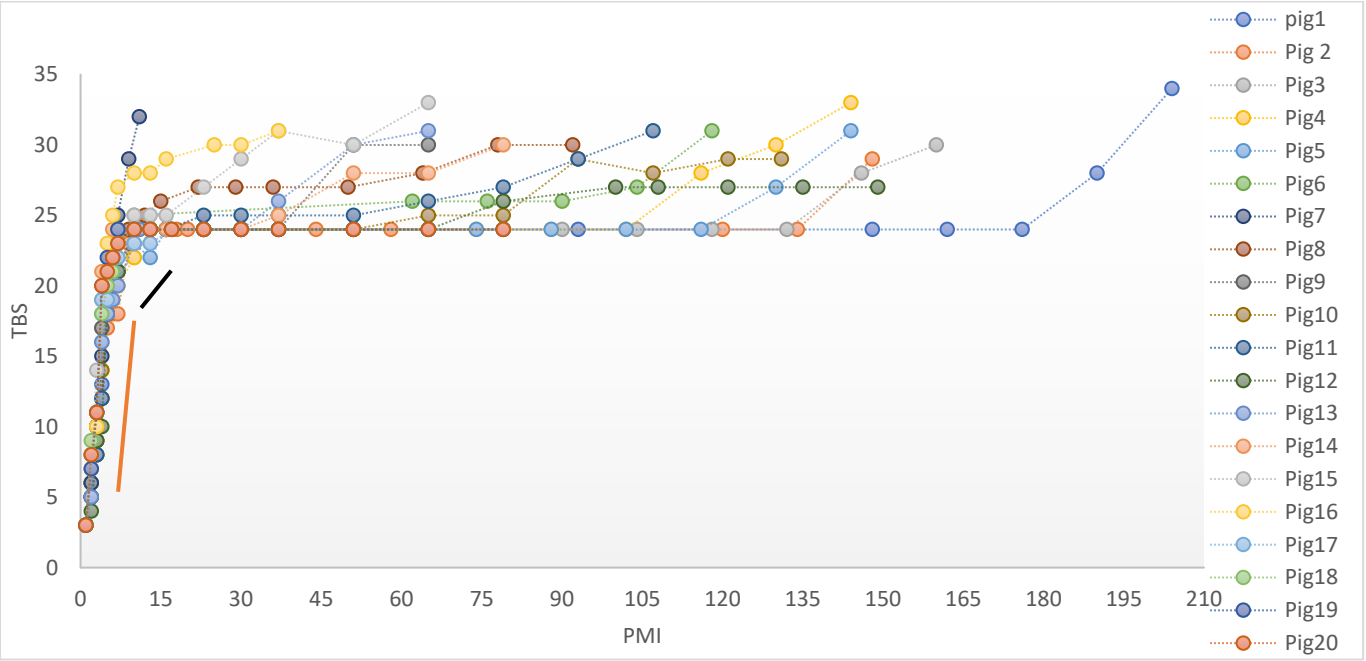

Figure 4.1. Scatter plot of TBS vs. PMI (in days) for each pig in the sample (n = 20). The orange bar represents the period of rapid decomposition, and the black bar shows the period of slowing in decomposition rate before desiccation.

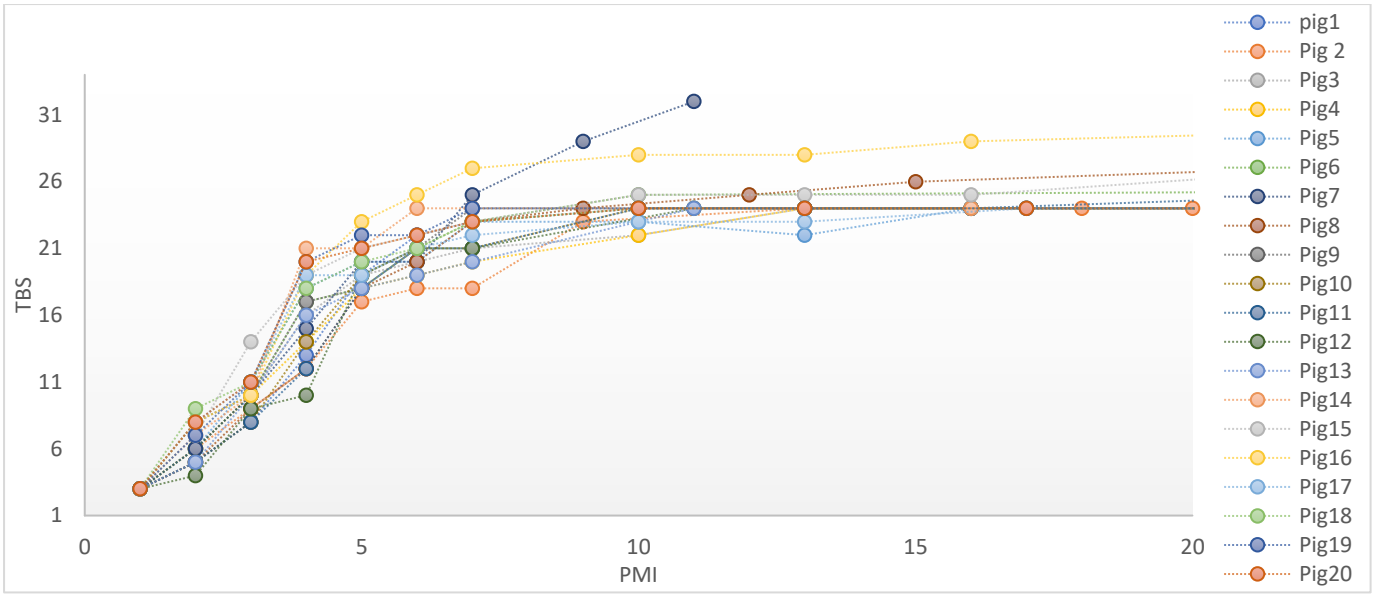

Figure 4.2. Scatter plot of TBS vs. PMI (in days) for each pig in the sample (n = 20) for the first 20 days post-mortem when decomposition was rapid.

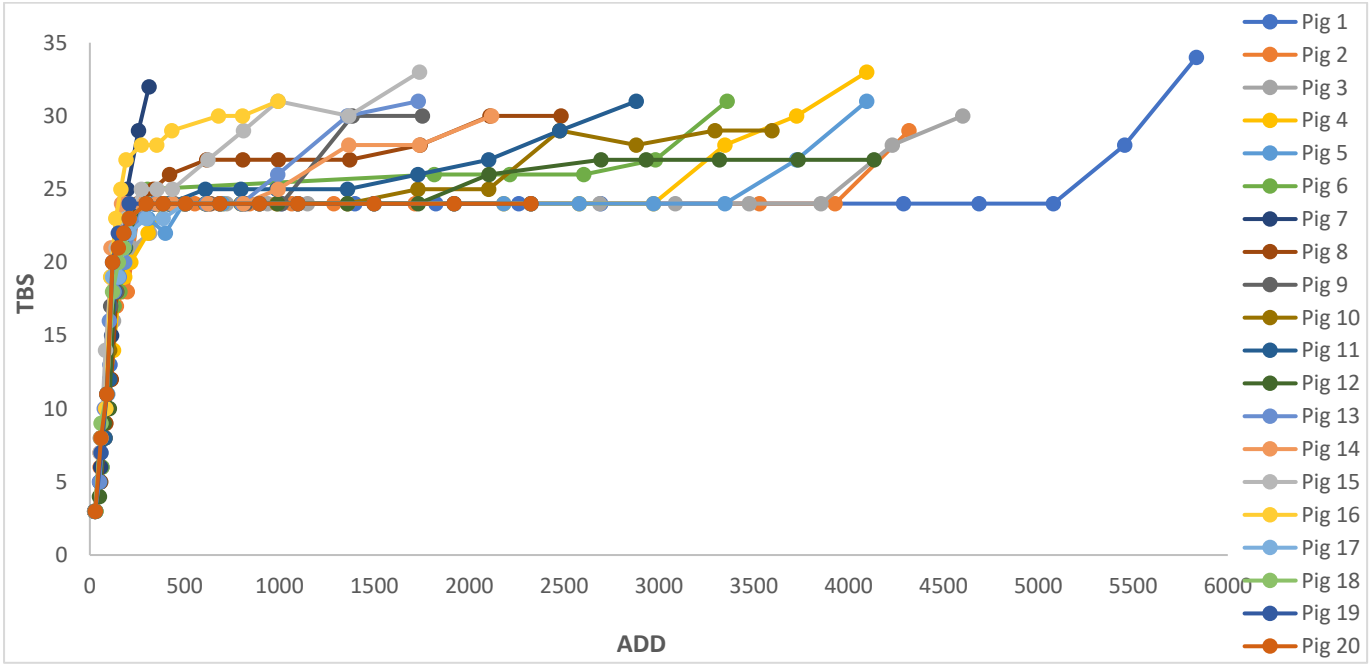

Figure 4.3. Scatter plot of TBS vs. ADD for each pig in the sample (n = 20).

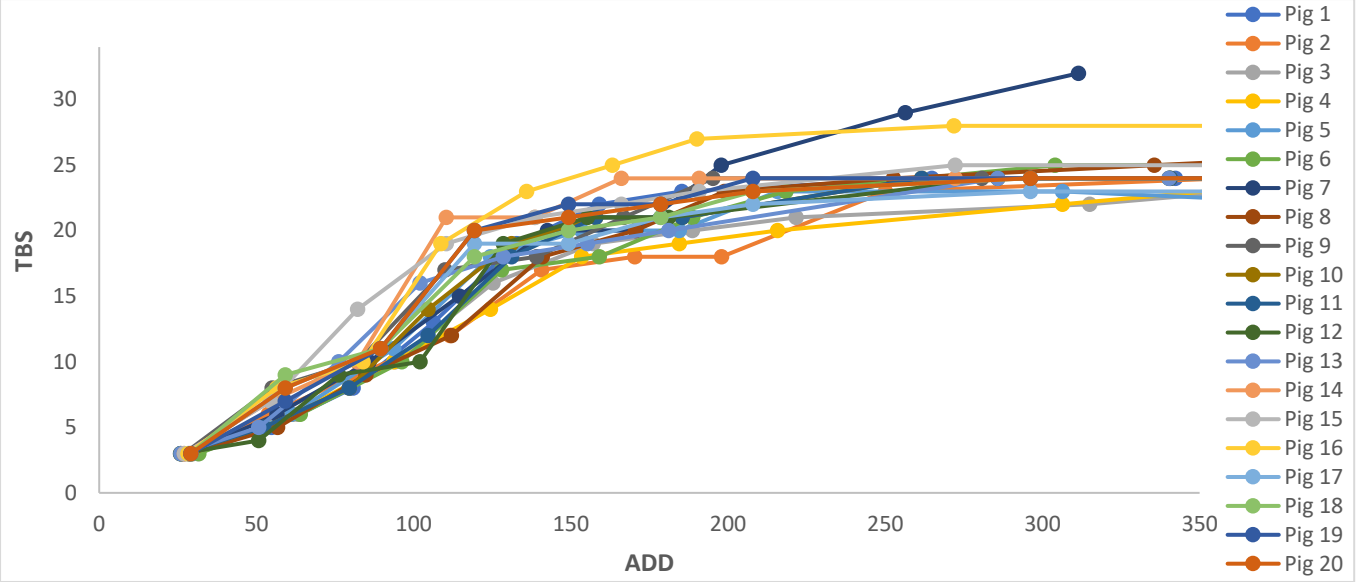

Figure 4.4. Scatter plot of TBS vs. ADD for each pig in the sample (n = 20) up to 350 ADD to show the rapid decomposition phase in detail.

The average temperature in the period of the study was 28.2°C, with an average of 27.1°C in the wet season, and 29.3°C in the dry season. The average daily temperatures between the two seasons were thus not much different, but slightly higher in the dry season. For example, the average ADD on the fifth day of decomposition for all carcasses, irrespective of season, is 142; it was lower (135.5) in the wet season, and higher (149.25) in the dry season on the same day (day 5). The decomposition pattern for each individual pig can be seen in Appendix C. The decomposition rate produced a sigmoid pattern with a very rapid rate of decomposition during the fresh, early, and advanced decomposition stages followed by a distinct plateau phase where decomposition rate slowed down. This plateau was then followed by an increase in the rate of decomposition until skeletonization was reached.

Decomposition occurred rapidly during the initial decomposition stages (orange bar in Figure 4.1, Figure 4.2), reaching a TBS of 22 – 27 in the first 7 days after placement in the majority of the carcasses (15 out of 20 pigs). There was a gradual and short period of slowing in decomposition rate as the carcasses began to dry out before a distinct plateau was reached for most of the carcasses (75%). Most of this slowing occurred between a TBS of 18 and 24

(black bar in Figure 4.1, Figure 4.2). There were, however, a few of the carcasses that went through very rapid decomposition and progressed linearly until they reached advanced decomposition. Examples are Pigs 7 and 16 which reached skeletonization in this linear phase, i.e., a TBS of 32 (11 days PMI, ADD of 311.45) and 27 (seven days PMI, ADD of 190.10), respectively. When decomposition is examined for these two pig cadavers on day 7 PMI, Pig 7 had a TBS of 25 at ADD of 197.85, while Pig 16 had a TBS of 27 at ADD of 190.1.

A prominent feature of advanced decomposition was desiccation which starts at a TBS of 22 when only one body region was desiccated, and completed at a TBS of 24 when it involves all the three body regions (Megyesi *et al*., 2005; Keough *et al.,* 2017) at the end of advanced decomposition.

Around a TBS of 22, decomposition also became more variable, with the earliest time to begin desiccation occurring after 6 days while the longest was 17 days with an average of 11 days after placement. Also, the duration of the plateau phase lasted anywhere between 6 to 166 days after placement.

Overall, the head reached advanced decomposition, especially desiccation (TBS of 9, average of 7 days), faster than the rest of the body, followed by the limbs (TBS of 7, average of 9.1 days) and then the trunk (TBS of 8, average of 9.7 days). It was common for the three body regions to reach desiccation on the same day in the wet season, but the head always advanced faster in the dry season. Skeletonization also progressed in a similar order, with the head skeletonized first, followed by the neck, limbs, and trunk.

In Figures 4.1 and 4.3 the curvilinear pattern of decomposition can be observed in detail. The patterns of TBS versus ADD/PMI are similar. The greater amount of variation noticed in the later stages of decomposition is clear from these figures and may be the effect of depositing the pigs in different seasons with temperature and rainfall fluctuations. To assess this, the

data were further analysed by the season of deposition, namely dry (deposited from December 2019; January to March 2020 and observed until July 2020; November 2020 and observed until January 2021) and wet (deposited from April to August and observed until October 2020) groups.

4.2.2. Dry season sample

Ten out of the 20 pigs were deposited in the dry season. The dry seasons of 2019 and 2020 were a typical dry season in Nigeria with no rainfall until late March/early April. The average temperature in the period that the dry season samples were under observation (which included some periods of the wet season as the mostly desiccated dry season samples had to be observed beyond the dry season until they skeletonized) was 28.8^{o}C. The average relative humidity was 65.2%. Figures 4.5 and 4.6 show the average daily temperatures and relative humidity data for the months of observation of the dry season sample. The months shown in the figure represent the months during which the pig cadavers were observed, and for the dry season (Figure 4.5) this includes the dry seasons of 2019 and 2020. There was no dry season sample under observation for the period between August and October 2020. A spike in humidity (85%) in late March 2020 is indicative of the first rainfall as the wet season approached (Figure 4.7). The scatter plots of TBS vs PMI are shown in Figure 4.8 for all the dry season pigs.

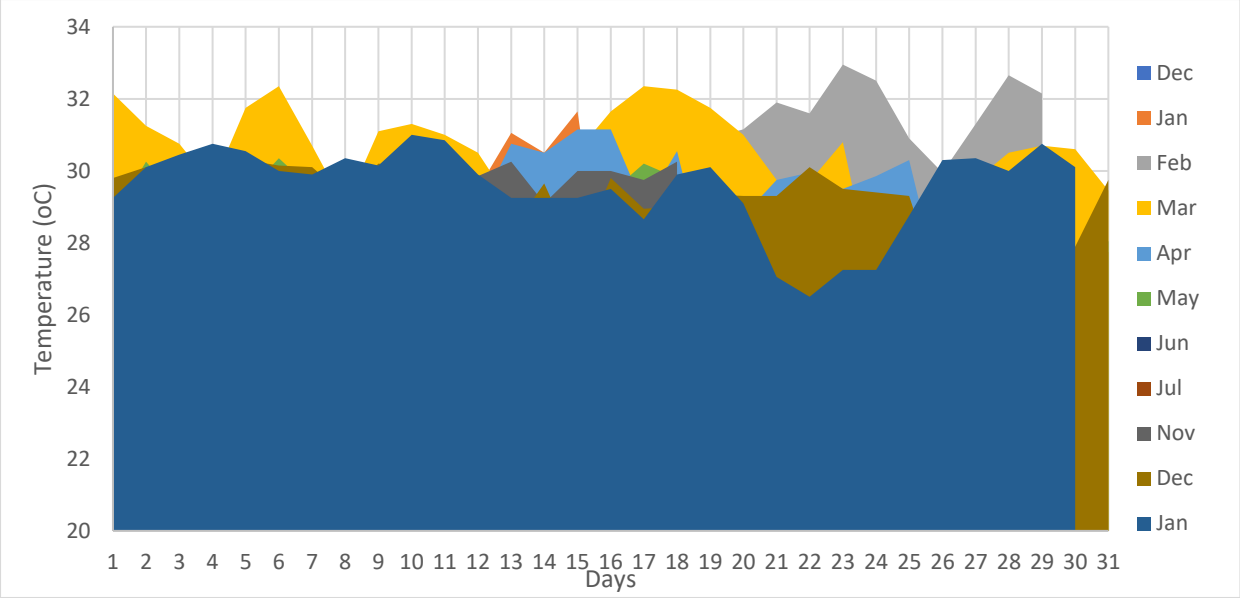

Figure 4.5. Average daily temperatures for the months of observation of the dry season sample.

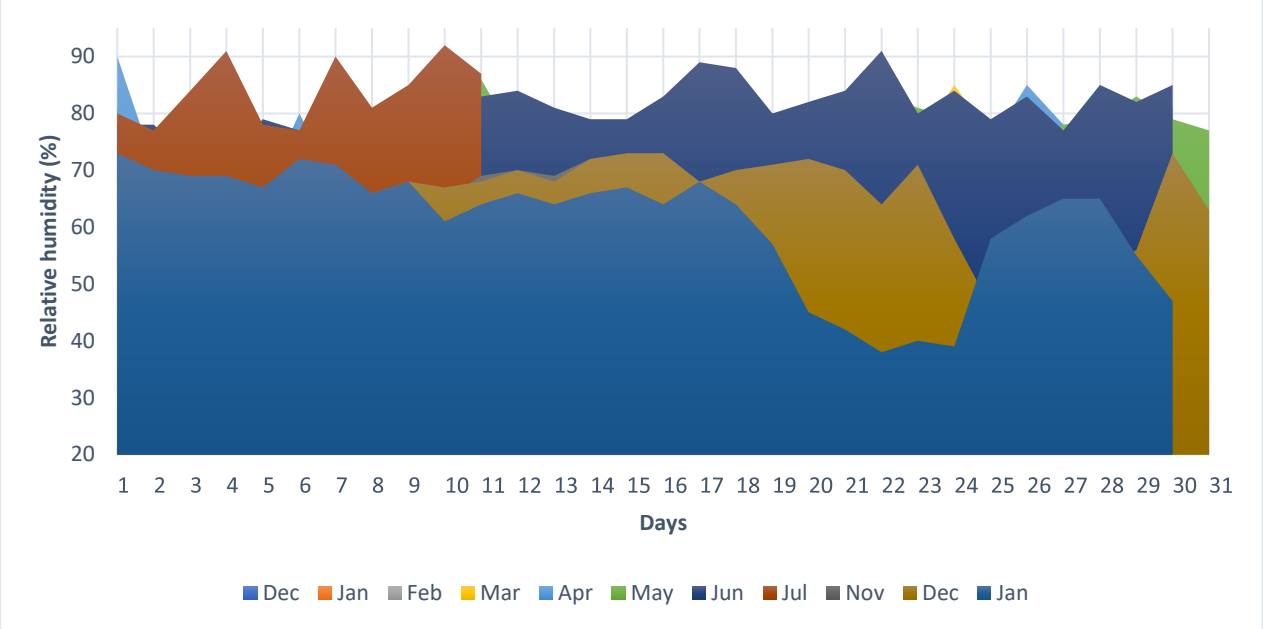

Figure 4.6. Daily relative humidity for the months of observation of the dry season sample.

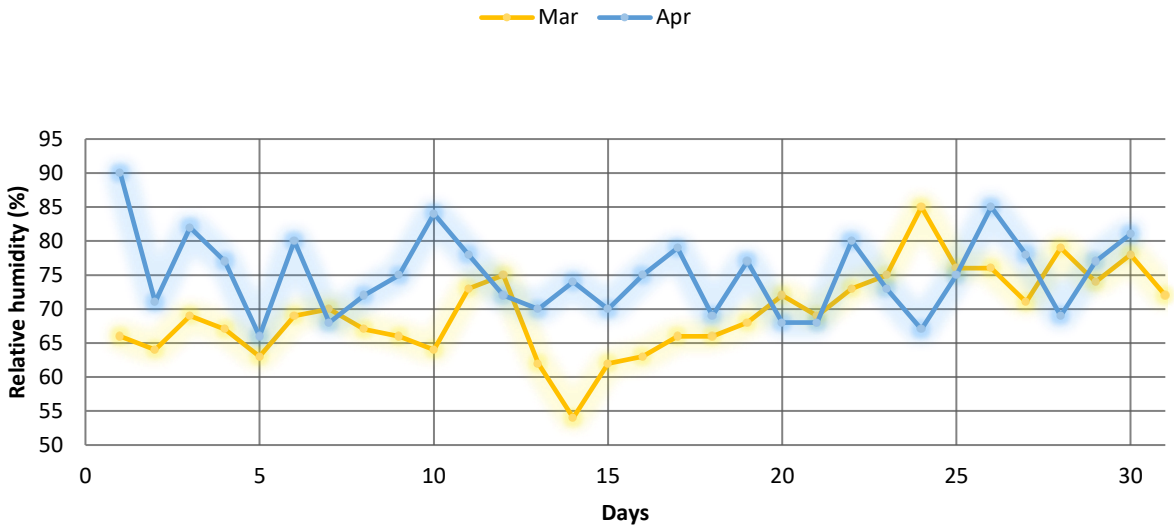

Figure 4.7. Daily relative humidity for the months of March and April 2020 showing a spike in humidity (85%) in late March after the first episode of rainfall for the wet season.

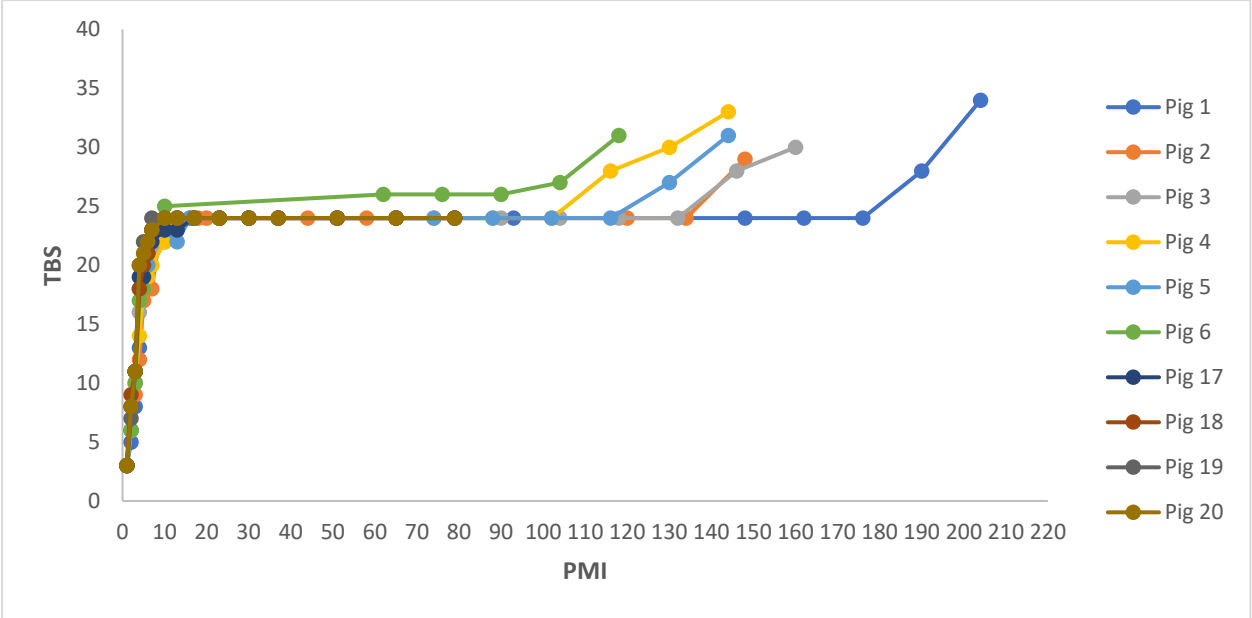

Figure 4.8. Scatter plot of TBS vs PMI (in calendar days) for the dry season pigs (n = 10).

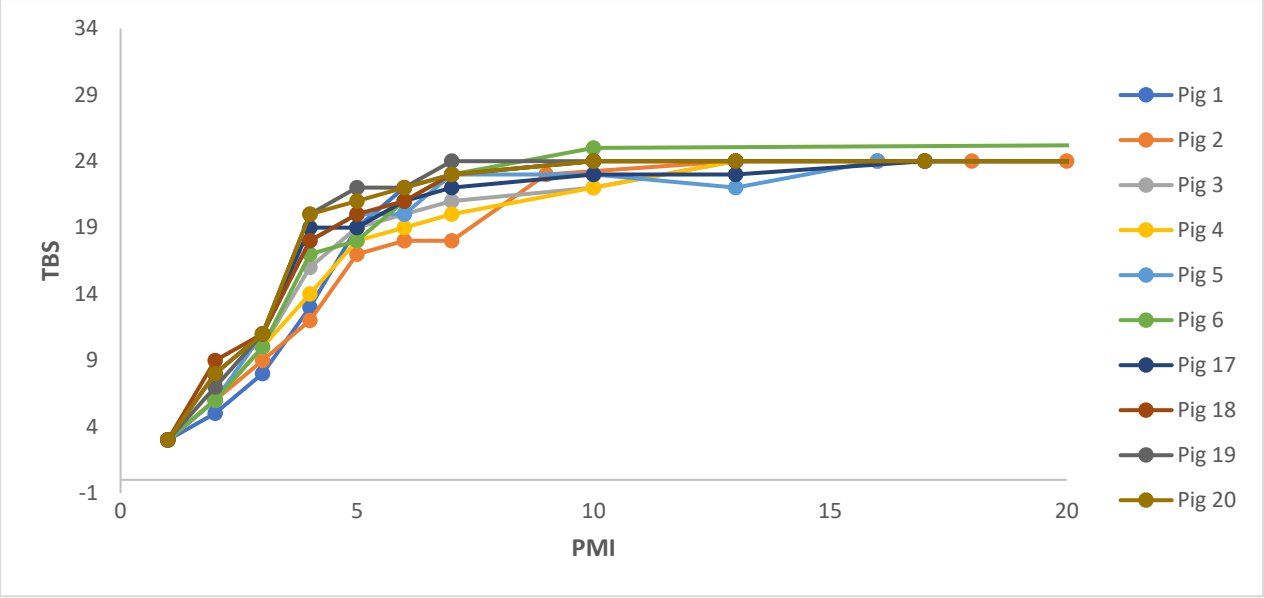

Figure 4.9. Scatter plot of TBS vs PMI (in calendar days) for the dry season pigs (n = 10) for the first 20 days post-mortem when decomposition was rapid.

The pattern of decomposition followed a sigmoid curve in all the samples, as was the case for the combined sample. The fresh, early and most of the advanced decomposition stages (TBS scores less than 24) was completed in the first 13 days after placement. Following complete desiccation (TBS score of 24 and above, Figure 4.10a) there was a plateau at which point there was an almost complete cessation of the progression of decomposition. Ninety percent of the dry season sample plateaued at a TBS of 24. This period of plateau lasted between 89 and 166 days. During this period maggot activity was absent.

Six carcasses deposited before the wet season were rehydrated at the start of the wet season, however, the resumption of the decomposition progress in the desiccated remains remained slow, especially in those specimens that have spent longer times in the plateau stage. For example, in Pig 1, with the remoistening of the desiccated remains, the insects returned, and decomposition continued gradually into skeletonization (Figure 4.10 b, c and d). However, one of the pigs (Pig 6, placed on the 16th of March 2020 and the last pig to be placed in the dry season of that year) followed a slightly different decomposition pattern (Figure 4.11). It experienced a sigmoid curve but levelled off at the skeletonization stage (TBS score of 26)

where it remained for the following four weeks, with no observable change. Thereafter, decomposition progressed to the later stages of skeletonization. This pig was thus at the plateau phase for a shorter period than the others. This may be due to the combined factors of a comparably shorter time allowed for desiccation and the increasing humidity usually witnessed prior to the change of seasons from dry to wet and onset of the rains at the end of the same month or early in the following month (April).



Figure 4.10. Stages of advanced decomposition of a dry season pig (Pig 1) a) before rainfall at a PMI of 37 days, TBS 24; b) about eight weeks from the first episode of rainfall in the wet season, at a PMI of 162 days, TBS 24; c) four weeks later at a PMI of 190 days, TBS 28 and d) one week later, close to the peak of rainfall and at a PMI of 204 days, TBS 34.



Figure 4.11. Stages of advanced decomposition of a wet season pig (Pig 6) a) before rainfall at a PMI of 7 days, TBS of 23 with early skeletonization of the head; b) three days later after the first episode of rainfall, at a PMI of 10 days, TBS of 25. This is the only dry season pig that skipped uniform desiccation of all body region (TBS of 24) before skeletonization; c) PMI of 62 days, TBS of 26, and the start of a plateau that lasted for four weeks before proceeding to higher TBS; d) PMI of 118 days, TBS of 31.

When ADD was plotted against TBS for all the dry season pigs, the pattern of decomposition resembled that found when PMI was plotted against TBS (Figure 4.12). It showed that the rate of decomposition was dependent on the ambient temperature or heat accumulation over a specified period (number of days).

The variability in the later phases (as described under section 4.2.1) persisted, showing that variation is not only influenced by dry versus wet seasons but that there are also other factors at play that introduce variation.

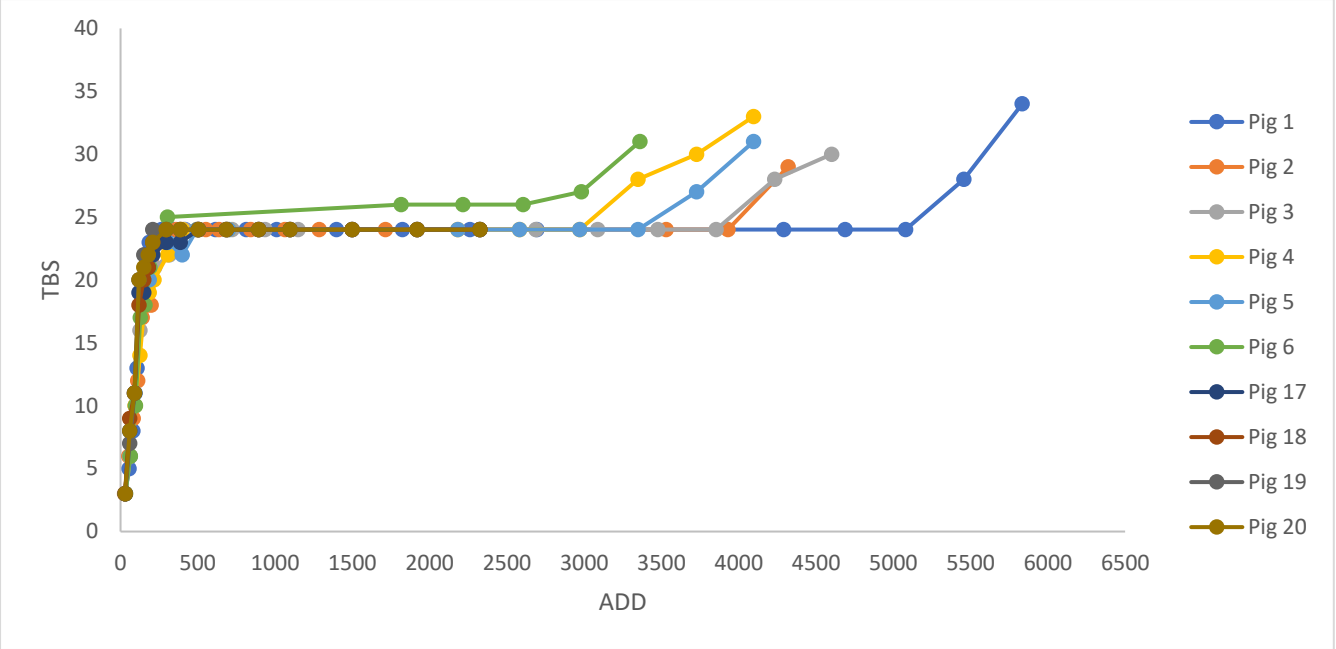

Figure 4.12. Scatter plots of TBS vs ADD for the dry season pigs (n = 10).

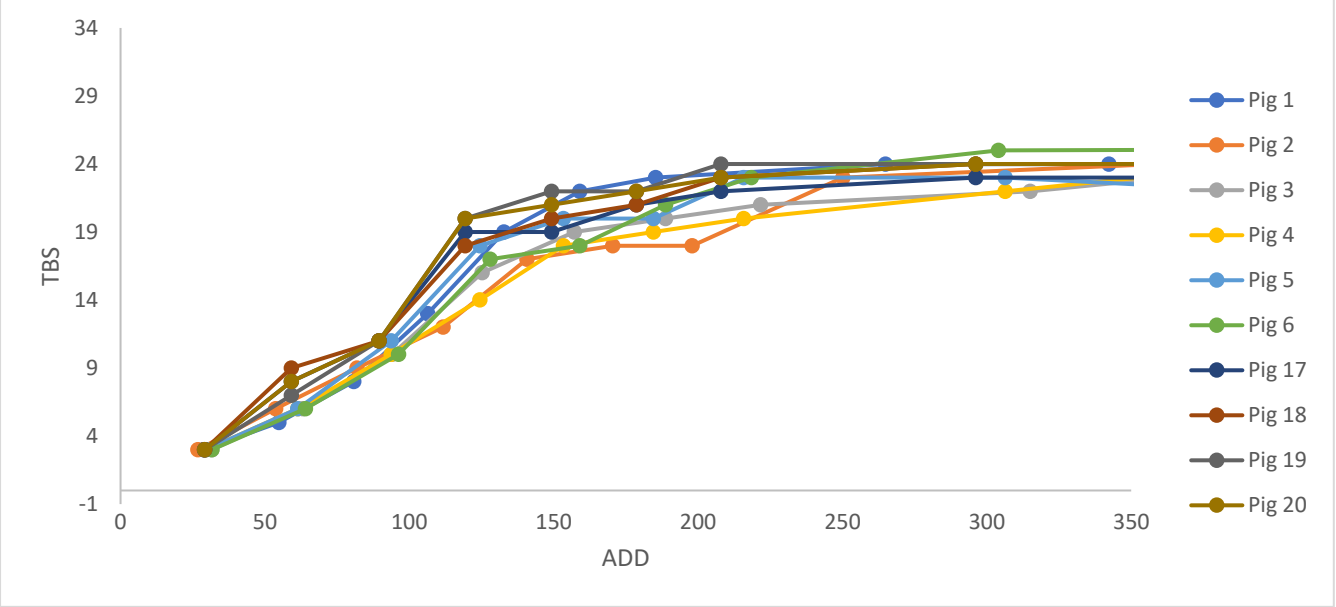

Figure 4.13. Scatter plots of TBS vs ADD for the dry season pigs (n = 10) up to 350 ADD to show the rapid decomposition phase in detail.

4.2.3. Wet season sample

Ten pigs were deposited in the wet season, and all were placed in the same wet season. It was a typical wet season. The average temperature and relative humidity in the period when the wet season samples were under observation, which included a part of the dry season,

were 27ºC and 81%, respectively. The average daily temperature and relative humidity for the period are shown in Figures 4.14 and 4.15.

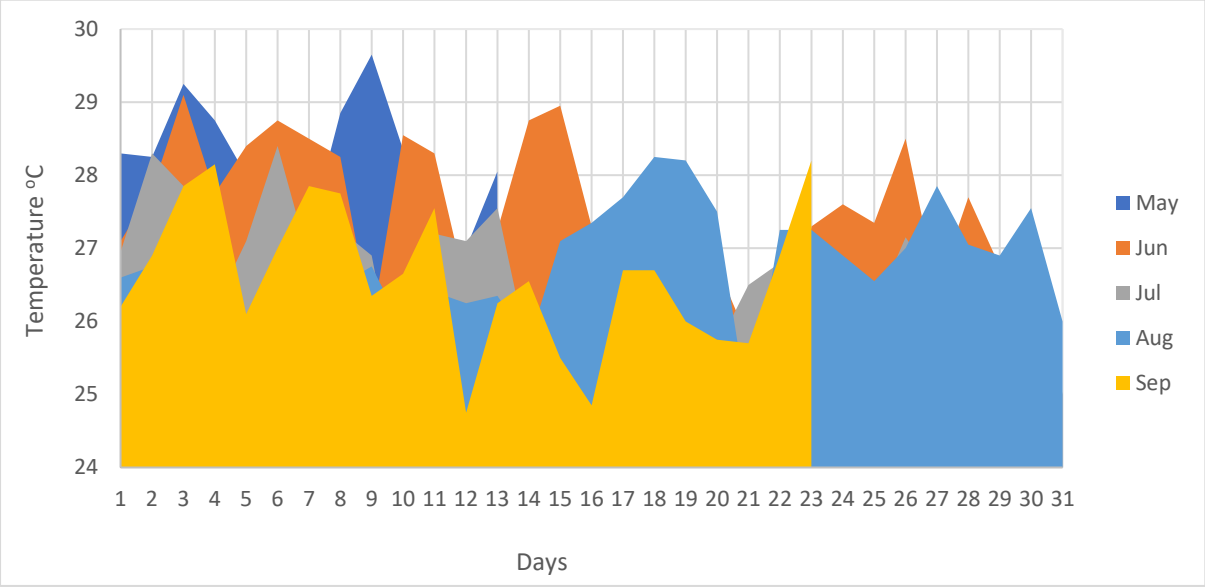

Figure 4.14. Daily temperature averages for the period of observation of the wet season sample.

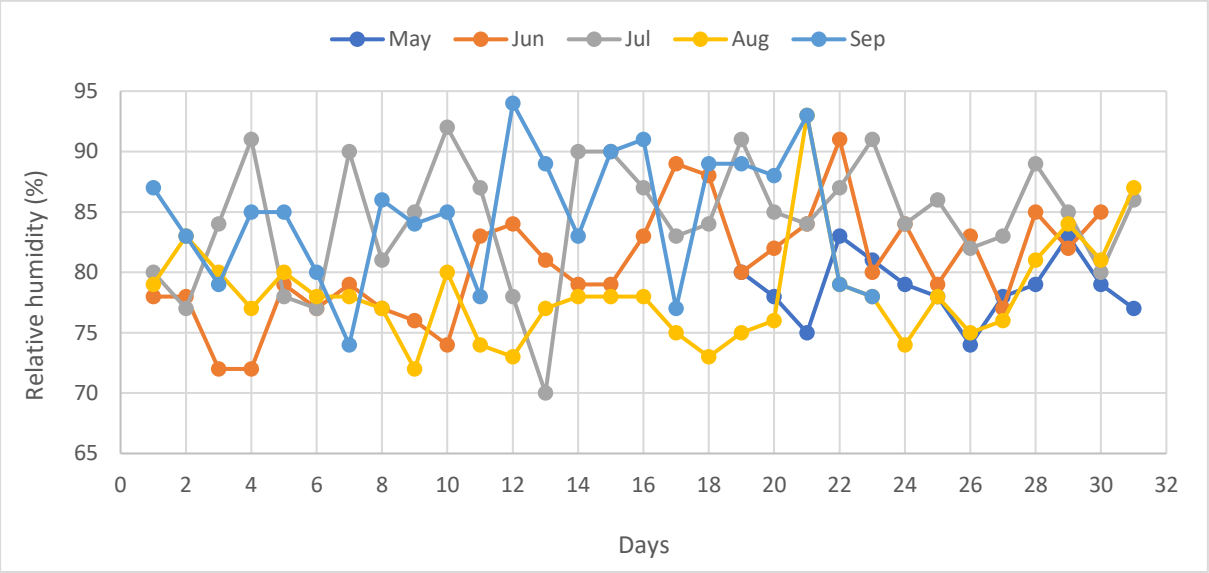

Figure 4.15. Daily relative humidity for the period of observation of the wet season sample.

Decomposition was rapid (Figure 4.16) with 40% of the sample reaching skeletonization (TBS score above 24) at an average of only 8.75 days after placement: the earliest occurred 6 days and the latest 12 days after placement.

Six of the sample attained complete desiccation (TBS score of 24) at which time there was a plateau with no observable progress of decomposition. This plateau lasted for a variable period between 6 and 41 days before decomposition progressed to the skeletonization stage. At the skeletonization stage, four of these carcasses (Pigs 9, 11, 12 and 14) experienced one more plateau (at TBS of 30, 25, 27 and 28, respectively) which lasted between 2 and 7 weeks before further progress. One of the remaining two (Pig 10) had two plateaus in the skeletonization phase (at TBS of 25 and 29) while the other (Pig 13) moved to higher stages of skeletonization without a further plateau (See Appendix C for decomposition pattern of individual pigs). After a TBS score of 24 was reached (or 6 – 17 days after placement), the decomposition pattern became highly variable more than the dry season pigs. This could be due to temperature fluctuations between the earlier and later parts of the season (and transition between seasons) and their effects on insect activity.

Of the remaining four pigs, one (Pig 7) progressed to skeletonization without a plateau while the other three attained mostly brief plateau (except Pig 8 which lasted for 4 weeks) in the skeletonized stage of decomposition (TBS of 25 and above when at least a body region is more than 50% skeletonized).

Five of the pigs whose placements were evenly distributed over this wet season were plotted separately to show the differences in decomposition (Figure 4.18: Pig 7 placed on May 19, 2020; Pig 9 placed on May 30, 2020; Pig 10 placed on July 28, 2020; Pig 15 placed on August 15, 2020, and Pig 16 placed on 18 August 2020).

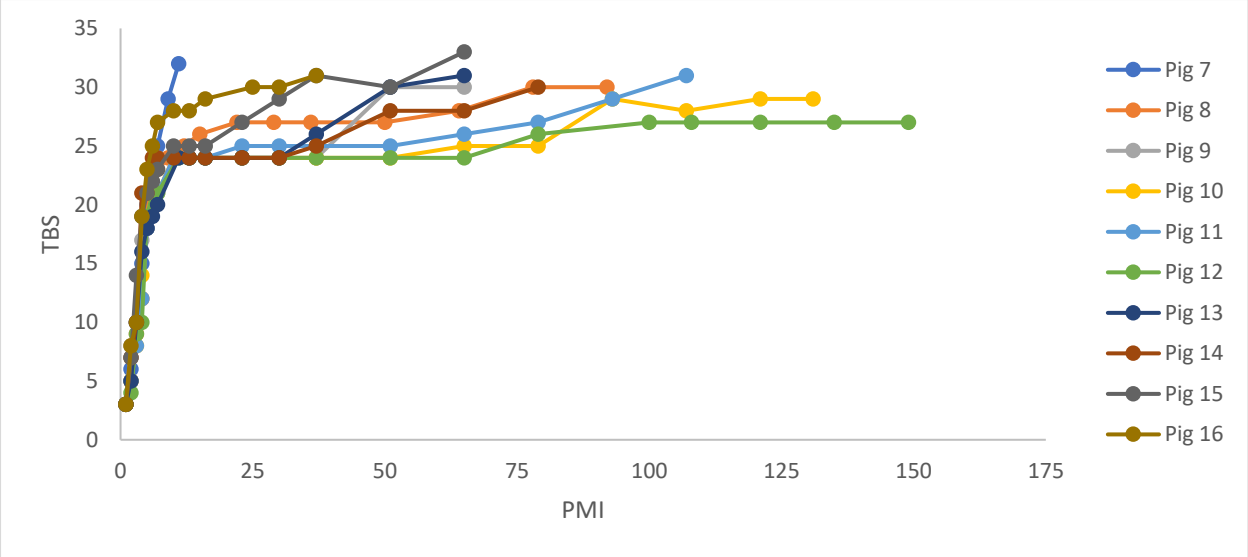

Figure 4.16. Scatter plot of TBS vs. PMI (in calendar days) for the wet season pigs.

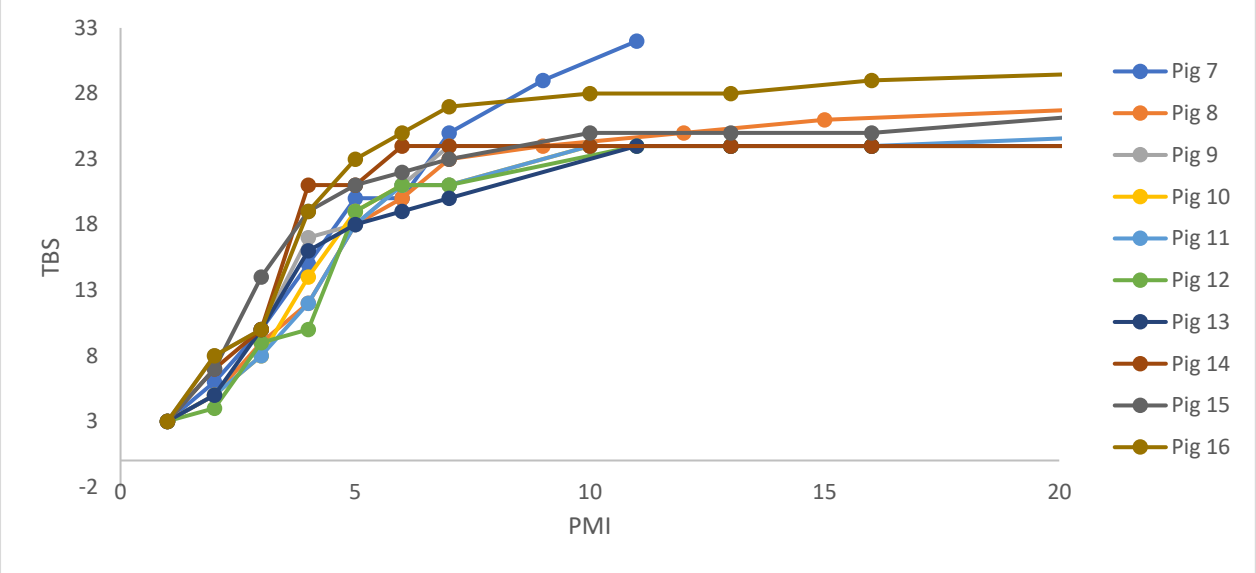

Figure 4.17. Scatter plot of TBS vs. PMI (in calendar days) for the wet season pigs (n=10) for the first 20 days post-mortem showing this rapid decomposition phase in detail.

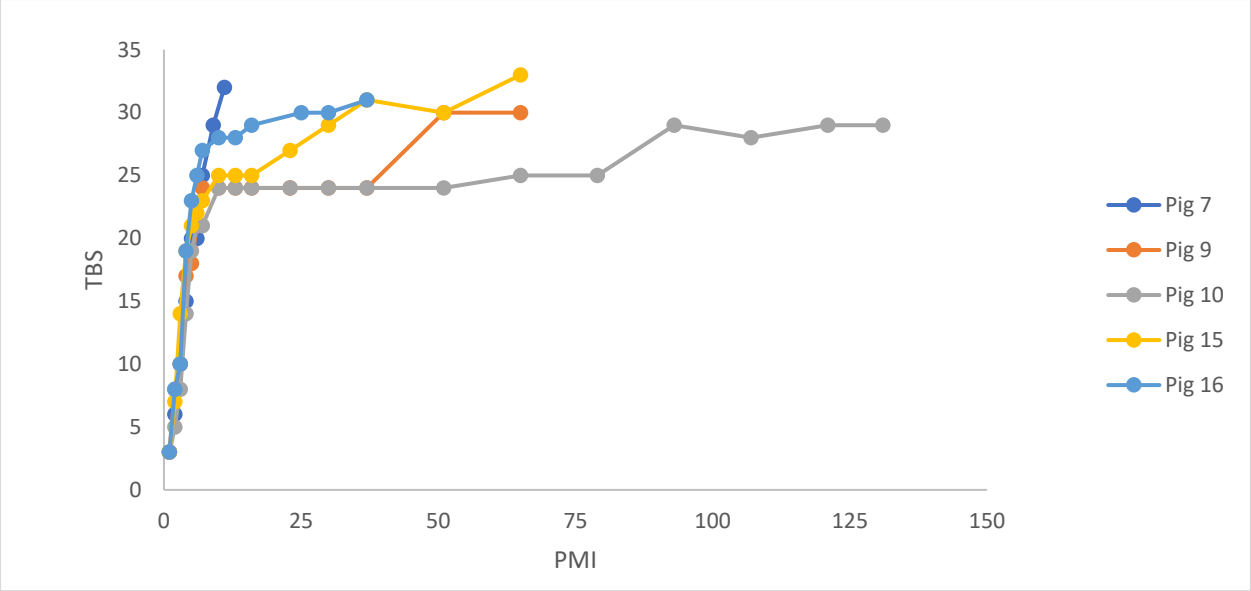

Figure 4.18. Scatter plot of TBS vs PMI (in calendar days) for five wet season pigs, demonstrating the variability.

In Pig 7 (Figure 4.18), all the stages of decomposition progressed rapidly and advanced skeletonization (TBS score of 32) was reached in just 11 days. The period of placement corresponds with change of seasons from dry to wet and the early rains. One noticeable characteristic of pigs placed around this period (Pig 6 placed in the last month of the dry season, and Pig 8 placed about one month into the wet season) is that they did not plateau at a TBS score of 24 but continued directly to reach skeletonization (TBS scores above 24) quickly: day 10 and day 12 after placement, respectively. Pig 7 reached the skeletonization phase (TBS score above 24) 7 days after placement.

The decomposition pattern of Pig 9 showed a deviation from that seen in Pig 7 (deposited only 11 days earlier), attaining complete desiccation (TBS score of 24) 7 days after placement and remaining in this state for 30 days. The same pattern of early and long desiccation is seen for Pig 10 which was deposited at the peak of the wet season. It reached complete desiccation on day 10 and remained in this state for a longer period (41 days).

Figure 4.19 shows the decomposition of Pigs 10 (a1 – d1; left column) and 16 (a2 – d2; right column). Figure 4.19 a1 shows Pig 10 about an hour after rainfall on the second day after placement. There is reduced insect activity and maggot activity in the mouth when compared to Pig 16 also on the second day after placement (Figure 4.19 a2). This could most probably be attributed to the rains. By disturbing adult insect activity (oviposition) and washing away larva, rainfall in early decomposition delays decomposition and allows enough time for desiccation to occur. Decomposition has also progressed further in Pig 16 (TBS score of 8) than in Pig 10 (TBS score of 5). The same trend continues until the fourth day after placement (Figure 4.19 c1 and c2). For Pig 10, there is the presence of high larval feeding, but desiccation has begun to set in at the same time (at the ear and around the eye, with a brownish shade) (Figure 4.19 c1). This is not the case in Pig 16 where decomposition is continuing with the head almost completely covered with feeding larvae with bone exposure of both the maxilla and mandible (Figure 4.19 c2). By the fifth day after placement, Pig 10 has shown very clear signs of desiccation with a TBS score of 19, and a drastic reduction in the number of larvae present (Figure 4.19 d1). Pig 16, on the other hand, did not desiccate but continued in moist decomposition at a TBS of 23 (Figure 4.19 d2). Complete desiccation followed for Pig 10 on day 10 after placement and continued for 41 days. Therefore, apart from the persistently high temperatures, significant insect activity and desiccation are two strong opposing factors operating on remains in Nigeria; a delay in insect colonization or activity, for whatever reason (in this case rainfall in early decomposition), appears to play a significant part in when the remains will experience desiccation.

The decomposition patterns of Pigs 15 and 16 seemingly showed a reversal of the trend seen in Pigs 9 and 7, respectively (Figure 4.18). In other words, carcasses deposited at the beginning and end of the wet season showed rapid decomposition while those deposited around the

middle or peak of the wet season showed slower decomposition. Pigs 7 and 16 reached the skeletonization phase 7 and 6 days after placement, respectively. Pigs 15 and 9 reached the skeletonization phase later at 16 days and after 37 days after placement, respectively.

Like the last pig that was put out in the dry season (Pig 6) which showed a faster decomposition, Pigs 7 and 16 were deposited at a time which could be regarded as a transition period between the seasons (wet to dry).

1. 

a2 

b1. 

b2 

c1. 

c2 

d1.   d2

Figure 4.19. Decomposition of Pig 10 (a1 – d1; left column) and Pig 16 (a2 – d2; right column) on day 2 (row 1), day 3 (row 2), day 4 (row 3), day 5 (row 4) after placement.

Figures 4.20 and 4.22 show the decomposition pattern when ADD is plotted against TBS for all 10 wet season pigs and for the five wet season pigs used for illustration of variability, respectively. Again, ADD appeared to produce similar plots to that of PMI (Figures 4.16 and 4.18). This was also the case with the scatter plots for the dry season pig data.

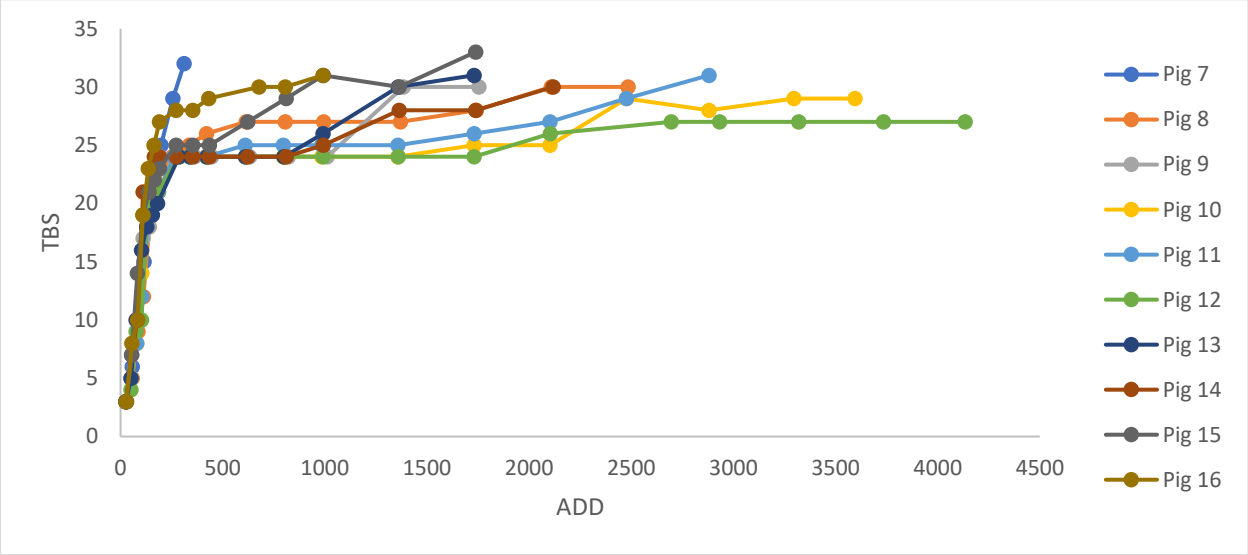

Figure 4.20. Scatter plot of TBS vs. ADD for all wet season pigs (n=10).

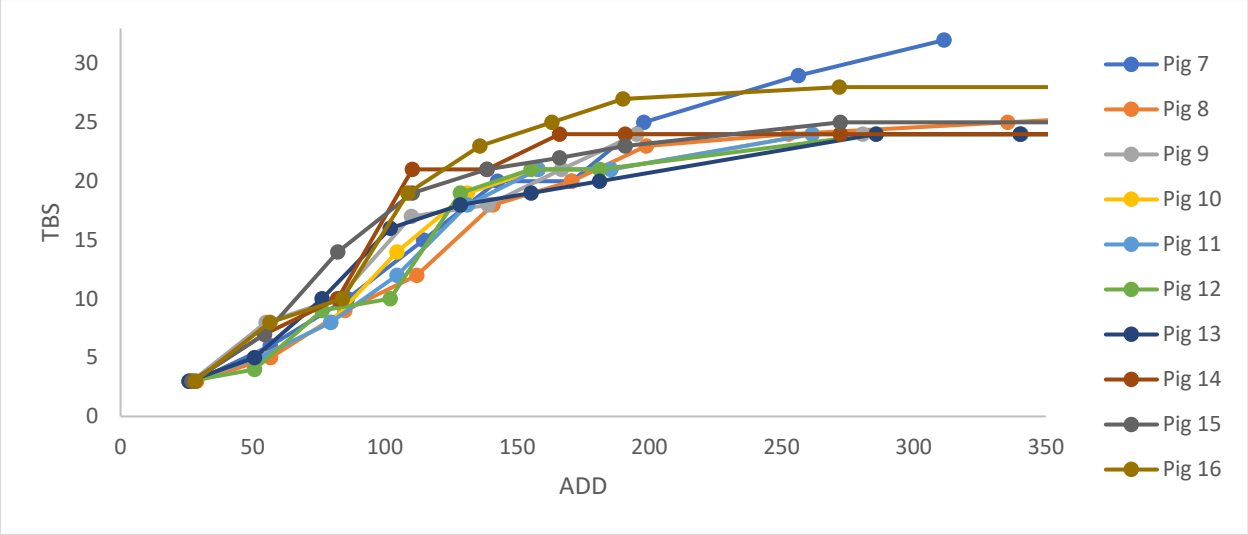

Figure 4.21. Scatter plot of TBS vs. ADD for all wet season pigs (n=10) up to 350 ADD to show the rapid decomposition phase in detail.

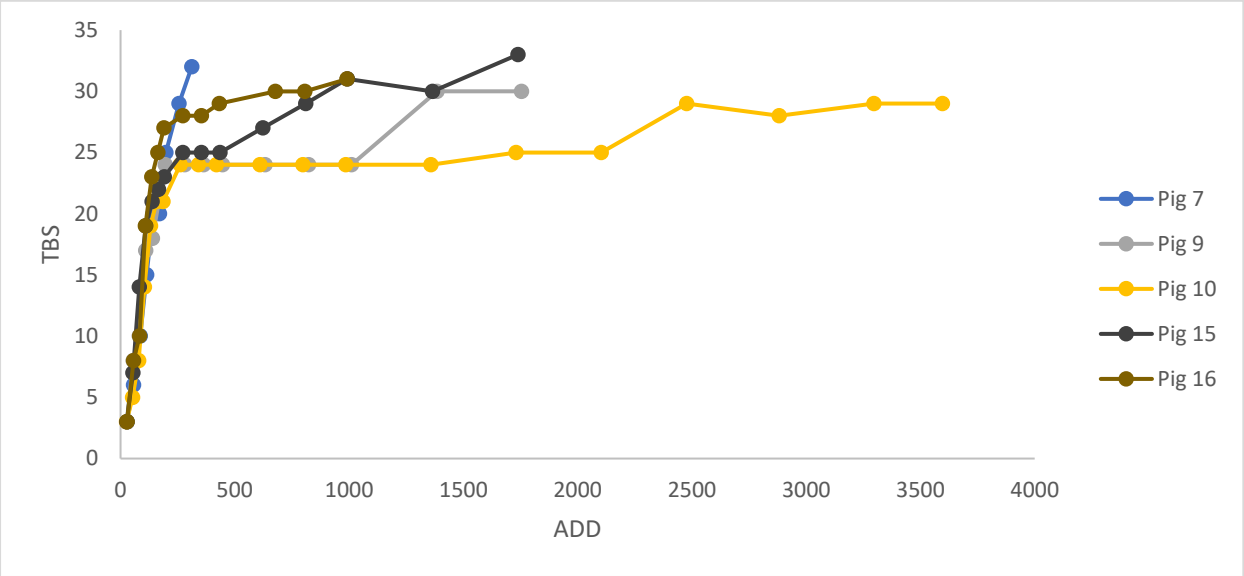

Figure 4.22. Scatter plot of TBS vs. ADD for 5 wet season pigs to illustrate the variability.

4.3. Log-transformed regression analysis of pig data

The resulting data for the above scatter plots between TBS vs PMI, and TBS vs ADD was skewed, and to produce a linear graph for clearer interpretation, PMI and ADD were log transformed. This gives a clearer illustration of the relationship between PMI and TBS, and ADD and TBS and allows the use of standard least-squares linear regression to predict ADD. Something similar was done for PMI. The predicted ADD could be used to predict the PMI by

counting backwards from the day of discovery of the remains until the actual ADD equals the predicted ADD. Following transformation of PMI and ADD, the equation took the form below:

$$Log_{10}(y) = B(x) + constant$$

Where B indicates the slope, and the constant represents the y-intercept. For this study, the dependent (y) was PMI or ADD and the independent (x) variable was the TBS. This led to the equations below:

For ADD:

$$Log_{10}ADD = B*TBS + constant, \text{ or}$$

$$ADD = 10^{(B*TBS + constant)}$$

For PMI

$$Log_{10}PMI = B*TBS + constant, \text{ or}$$

$$PMI = 10^{(B*TBS + constant)}$$

4.3.1. Complete data set

The r-squared value which indicates the proportion of the change in the dependent variables (PMI and ADD) that is accounted for by the independent variable (TBS) were improved by log transformations. Figures 4.24 to 4.21 show the regression relationship between untransformed PMI and ADD, and TBS, and the same relationship when PMI and ADD are log transformed. For that of PMI the r-squared value increased from 29% to 70% (Figures 4.23 and 4.24); for ADD the r-squared value increased from 28% to 70% (Figures 4.25 and 4.26).

A similar percentage of variability in decomposition was accounted for by both ADD (70%) and PMI (70%). Therefore, both PMI and ADD are equally good indicators of the decomposition process.

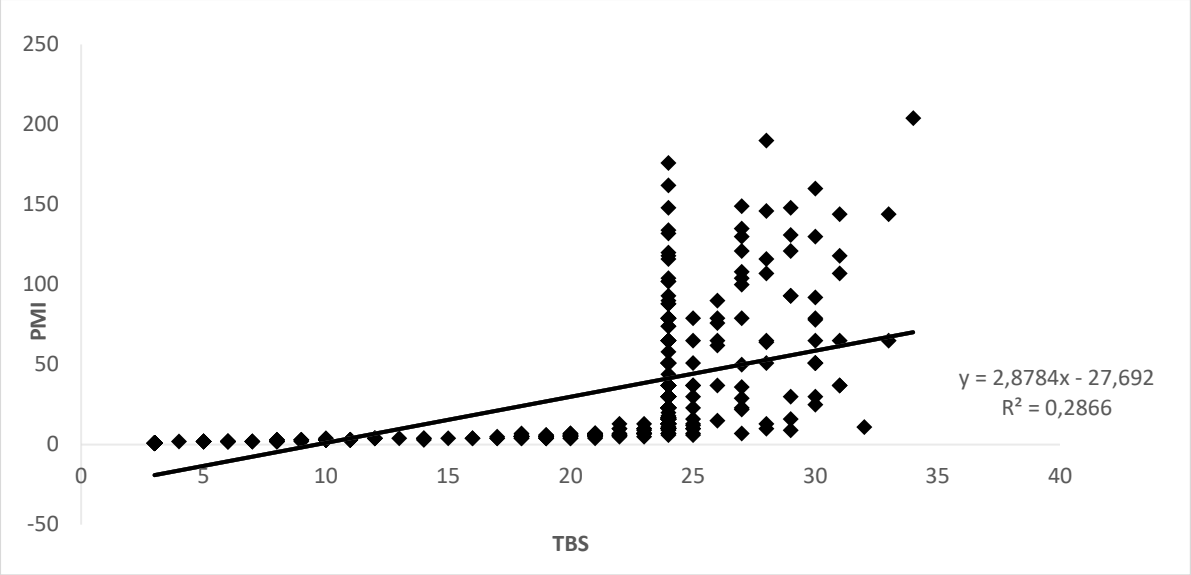

Figure 4.23. PMI vs TBS for all pigs.

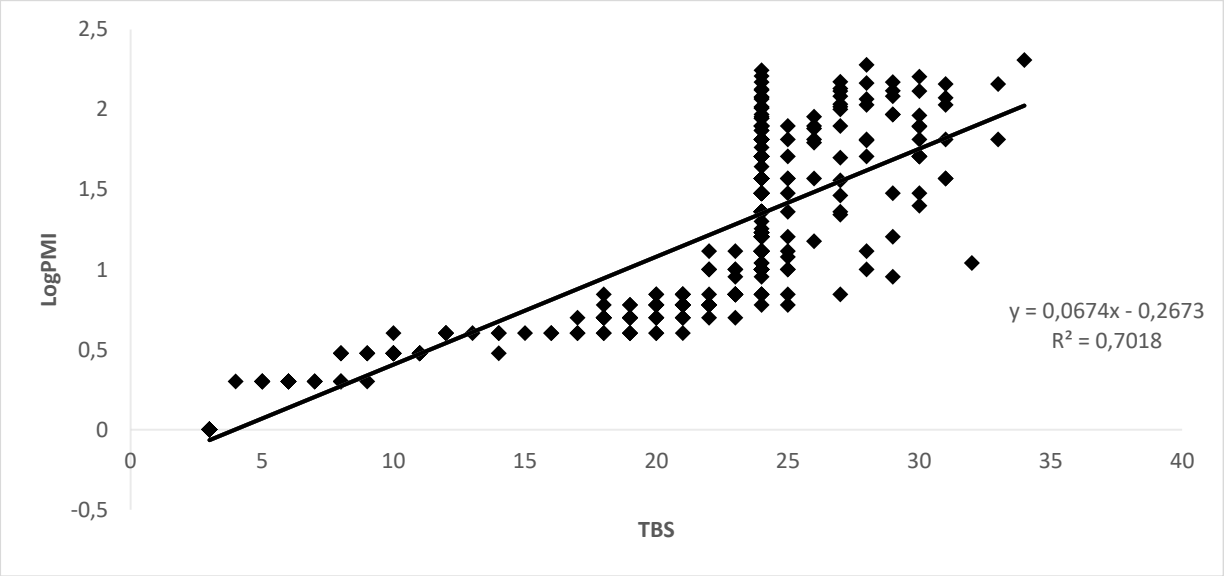

Figure 4.24. LogPMI vs TBS for all pigs.

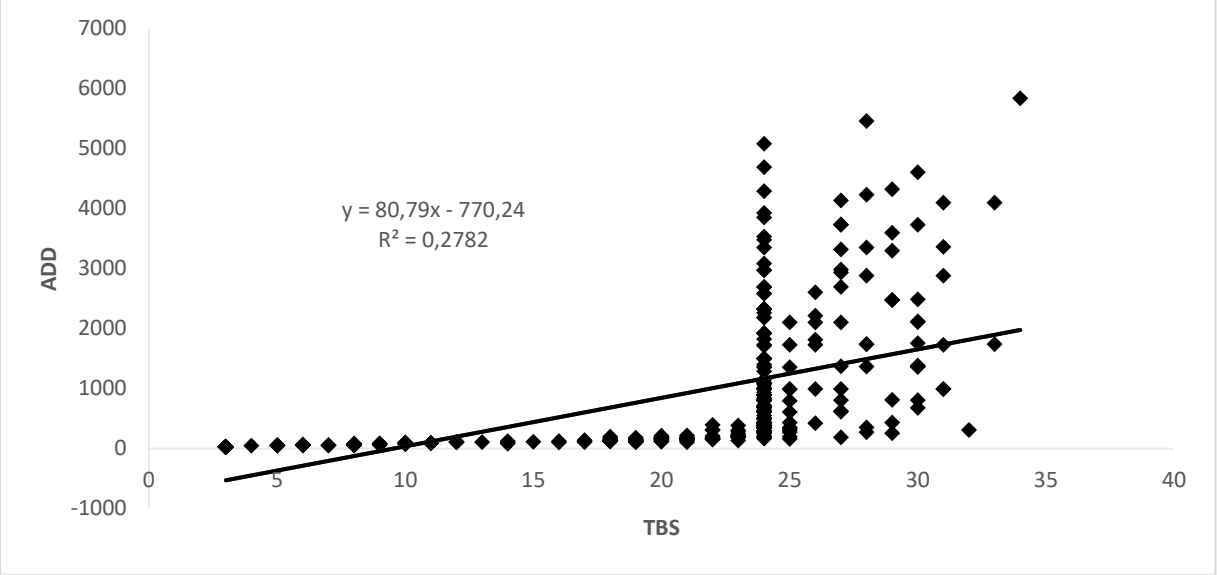

Figure 4.25. ADD vs TBS for all pigs.

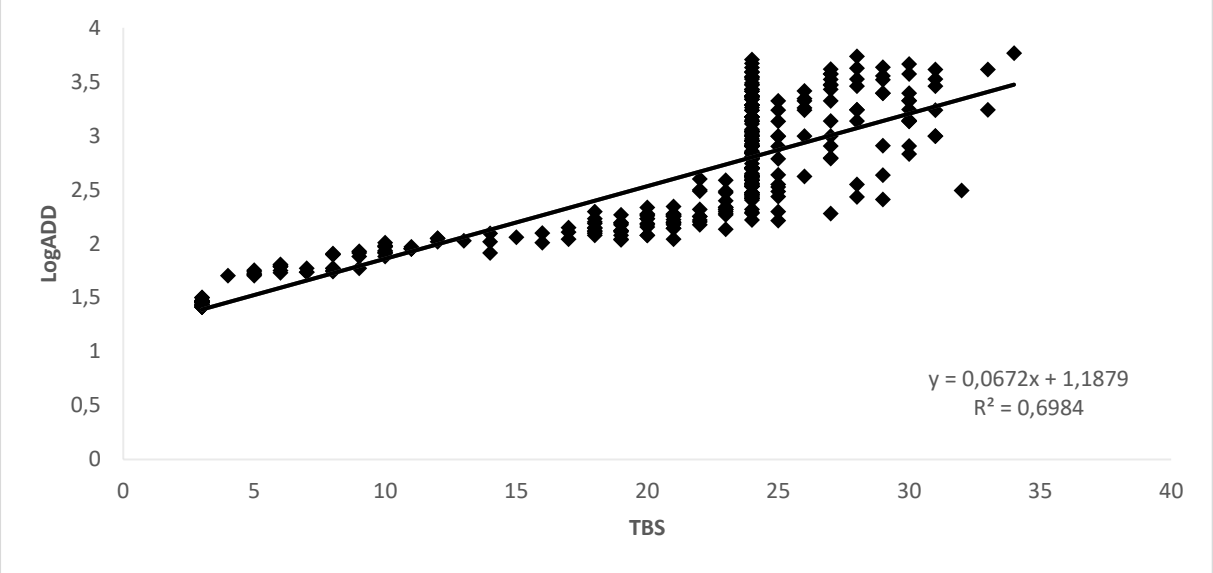

Figure 4.26. LogADD vs TBS for all pigs.

4.3.2. Dry season pig data

For the untransformed dry season pig data, random-effects maximum likelihood regression lines of PMI vs TBS yielded an r-squared value of 0.3183, or 32% (Figure 4.27). This implies that that 32% of the observed variation in the decomposition progress (represented by TBS) can be accounted for by PMI. Following log transformation, the r-squared value appreciated to 0.7042, or 70% (Figure 4.28).

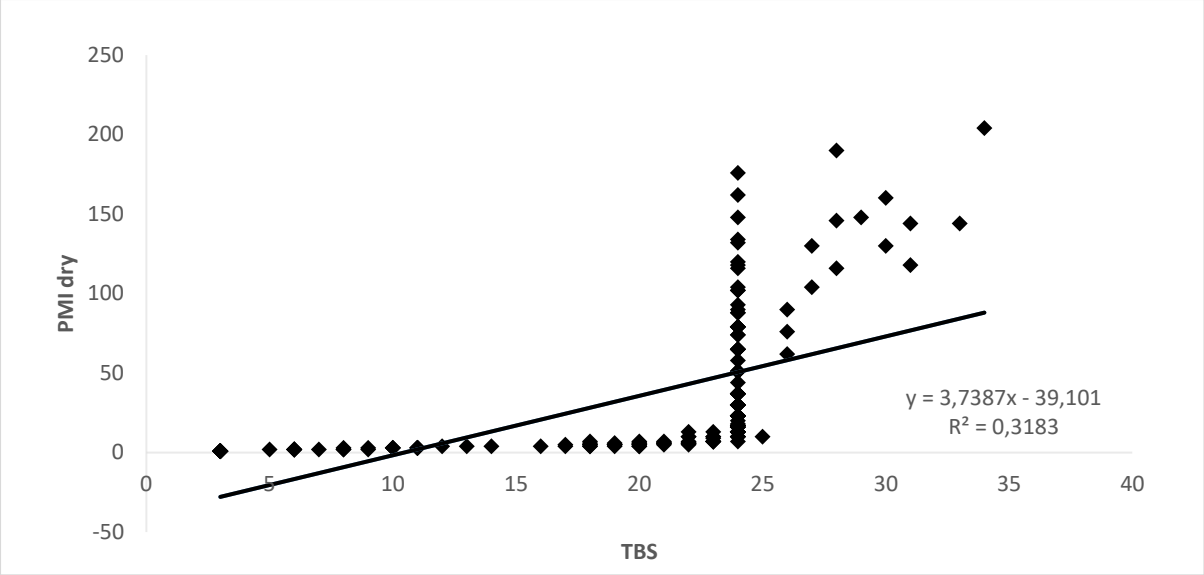

Figure 4.27. PMI vs TBS for the dry season.

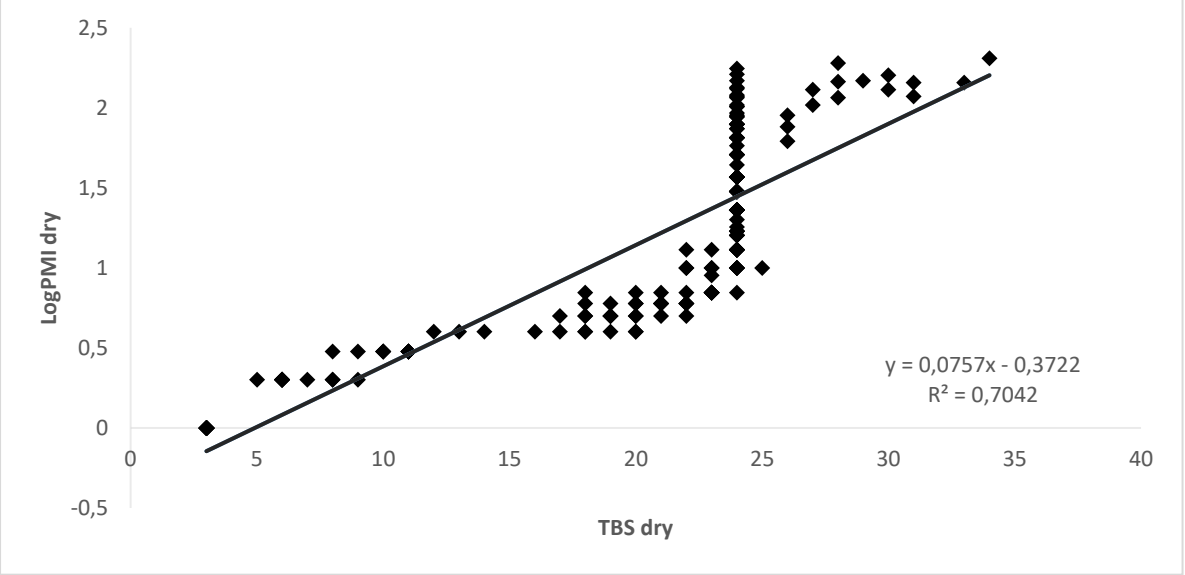

Figure 4.28. LogPMI vs TBS for the dry season.

The same trend is witnessed when log transformation is applied to the plot of ADD and TBS, with an increase in the r-squared value from 0.3192, or 32%, to 0.7058, or 71% (Figures 4.29 and 4.30). Like the finding in the complete data set, PMI and ADD are both good descriptors of decomposition progress in the dry season.

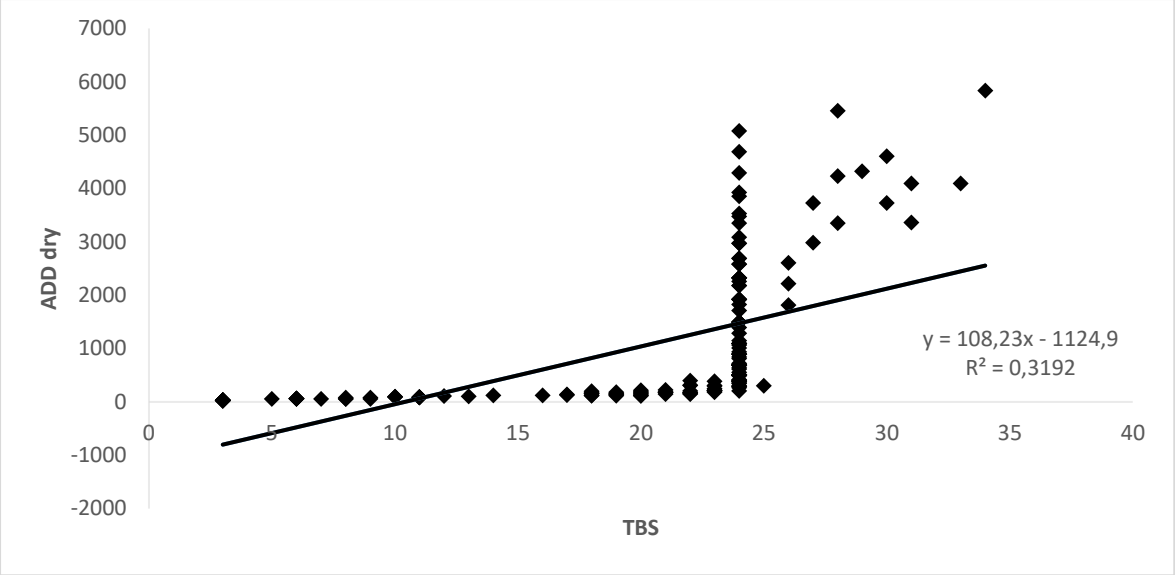

Figure 4.29. ADD vs TBS for the dry season.

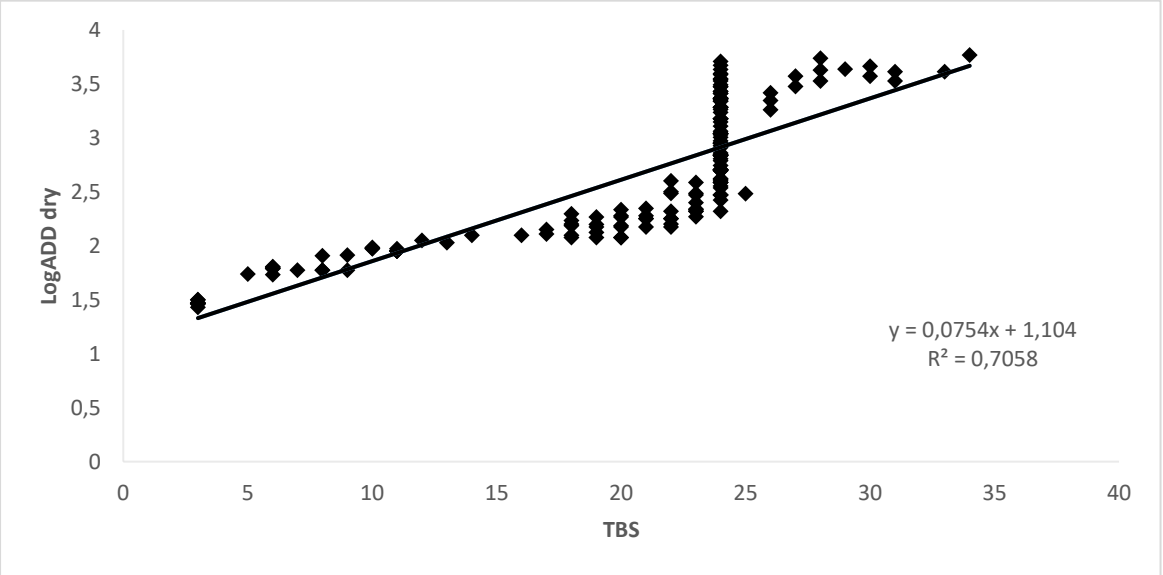

Figure 4.30. LogADD vs TBS for the dry season.

4.3.3. Wet season pig data

When the logarithmic curve is used on the regression of PMI and ADD against TBS, the r-squared values appreciated from 0.3159 to 0.7412 (Figures 4.31 and 4.32) and from 0.3133 to 0.7444 (Figures 4.33 and 4.34), respectively. The correlation between TBS and both PMI and ADD were similar to that in the dry season data.

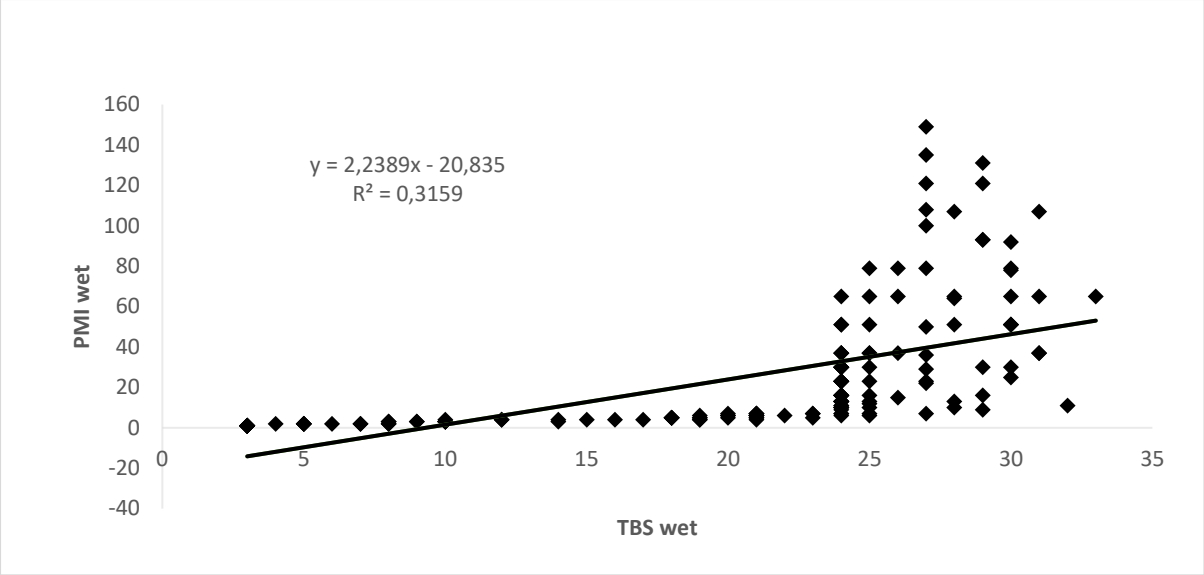

Figure 4.31. PMI vs TBS for the wet season.

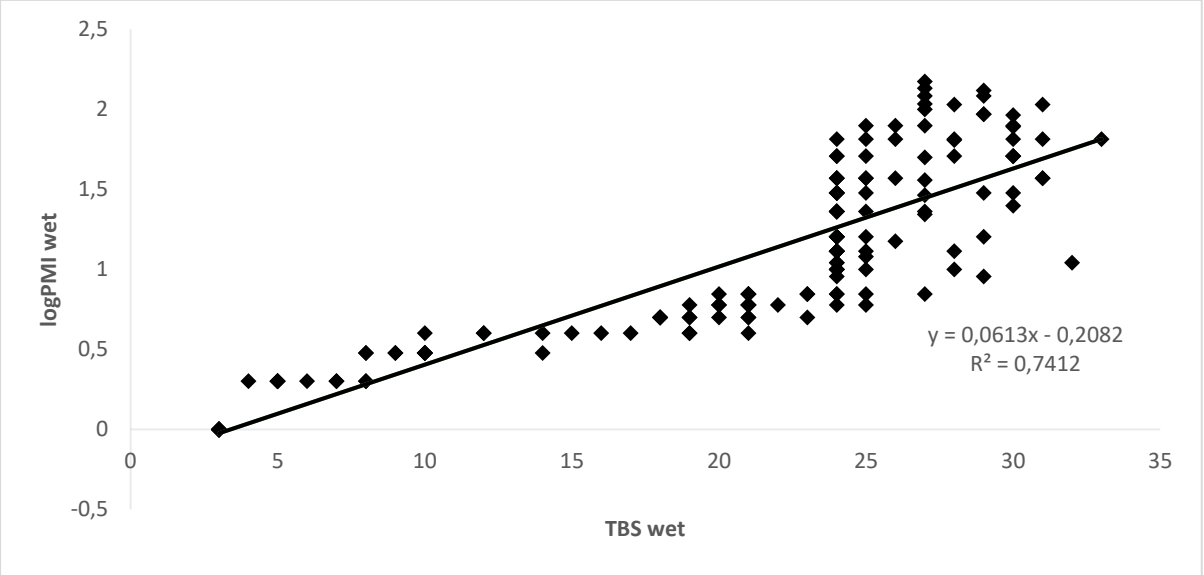

Figure 4.32. LogPMI vs TBS for the wet season.

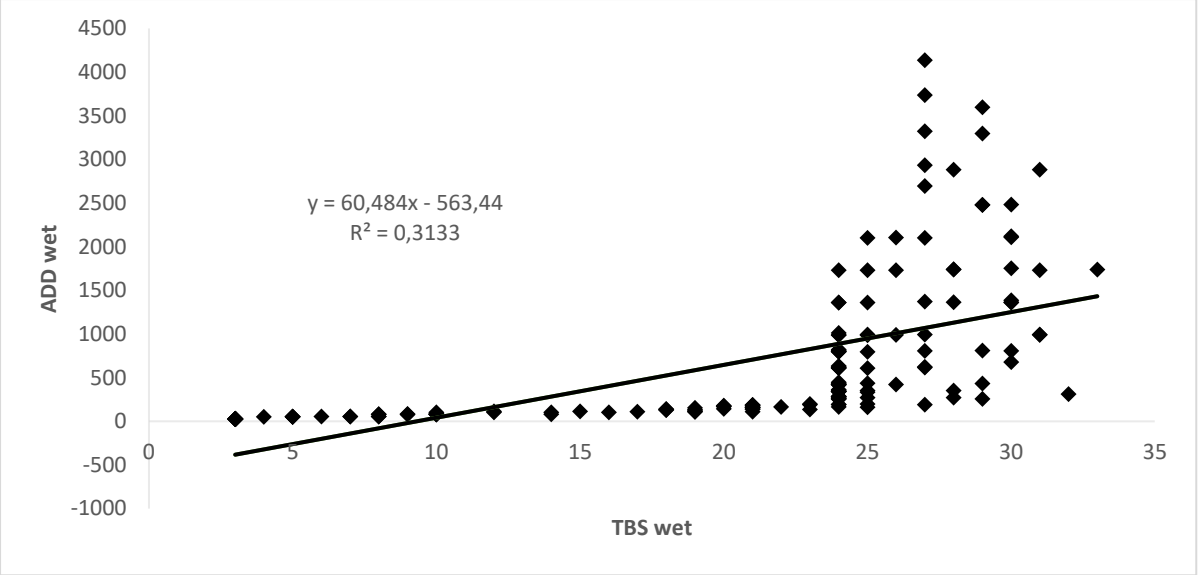

Figure 4.33. ADD vs TBS for the wet season.

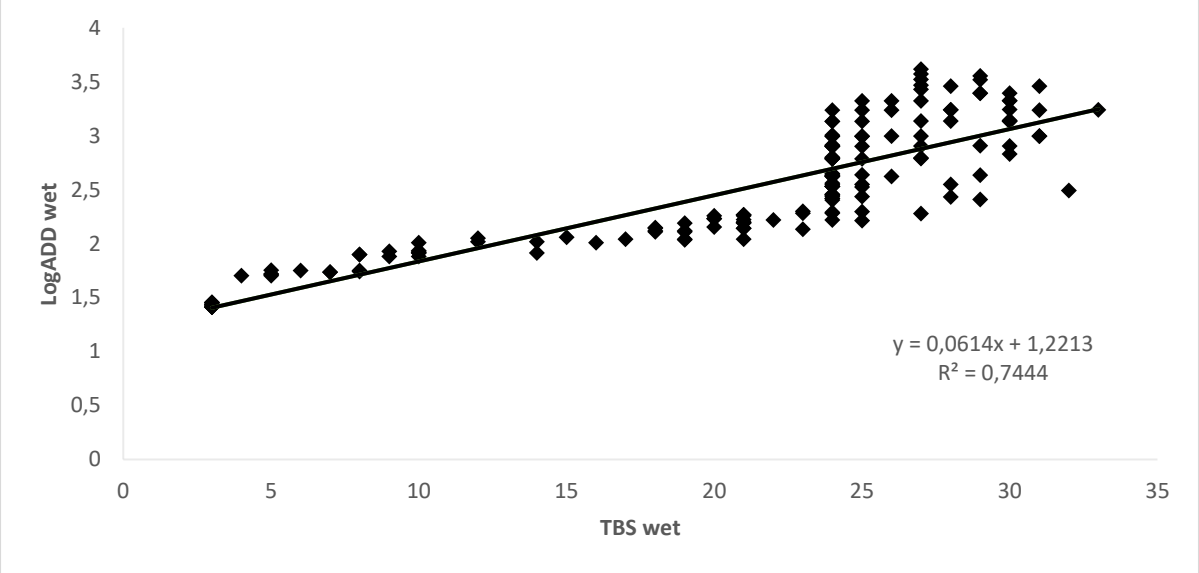

Figure 4.34. LogADD vs TBS for the wet season.

4.3.4. Comparison between wet and dry season decomposition

Figure 4.35 shows the seasonal distribution of decomposition when ADD is plotted against TBS for all the pigs. Although decomposition progressed rapidly in both seasons until a TBS of about 24 and above, it was more rapid in the wet season. For example, at an ADD of 1000, the TBS range for the wet season (green data) was 24 – 31 while it was only 24 - 26 for the dry season (blue data) (Figure 4.35). Furthermore, it took an average ADD of 121.6 to reach advanced decomposition (TBS between 17 and 24) in the wet season compared to an average

of 136.9 for the dry season. The average ADD to both reach and exit complete desiccation (TBS of 24) was 132.3 and 1,021.8, respectively, for the wet season and 367.9 and 3,836.6, respectively, for the dry season. Therefore, it took a lower heat accumulation to reach the same level of decomposition (complete desiccation) in the wet season than it did in the dry season. This implies that although heat contributes, seasonality and other factors which it influences such as humidity and insect activity, as will be discussed, also contributed. There was also more variability in the wet season than in the dry season.

Compared to the 90% (9 pigs) of the dry season sample that underwent complete desiccation, only 60% (6 pigs) did so in the wet season. This was attained earlier in the wet season sample (6 – 11 days; average of 9.2 days) (Figure 4.16) than in the dry season sample (10 – 17 days; average of 13 days) (Figure 4.8). The average duration of this state was also shorter (29 days) in the wet season than in the dry season (119 days).

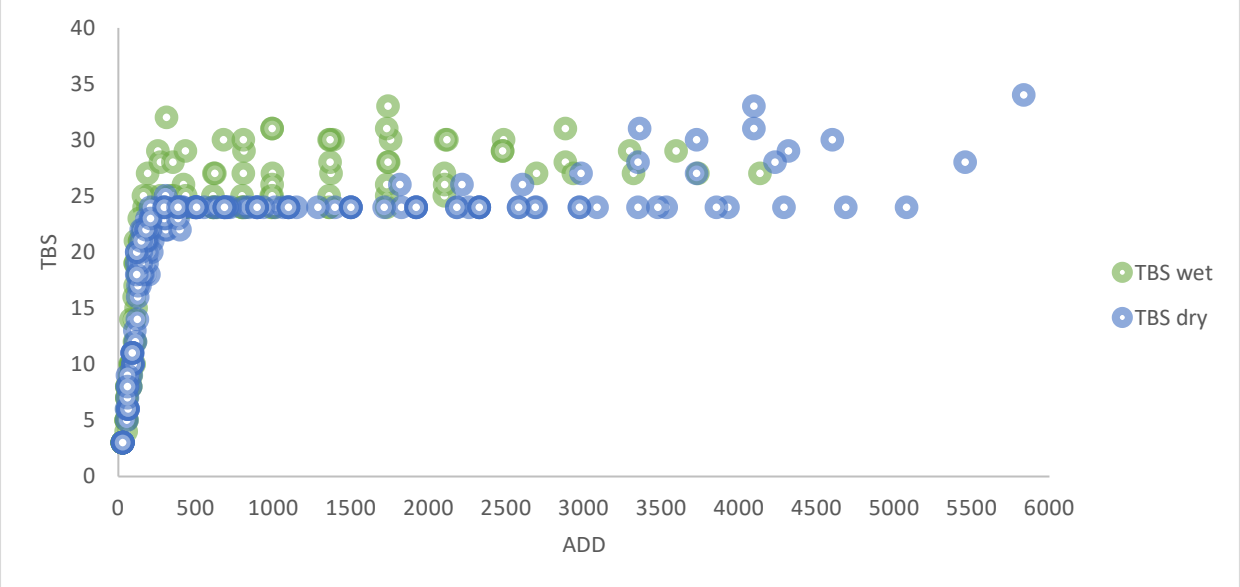

Figure 4.35. Scatter plot of TBS vs ADD for all the pigs indicating differences in decomposition between wet (green) and dry (blue) season groups.

Using the regression mentioned above (LogADD regressed against TBS), predictive equations for calculating the estimated ADD for discovered remains were developed.

For the dry season:

$$ADD = 10^{(0.075*TBS + 1.104)}$$

$$PMI = 10^{(0.076*TBS - 0.372)}$$

For the wet season:

$$ADD = 10^{(0.061*TBS + 1.221)}$$

$$PMI = 10^{(0.061*TBS - 0.208)}$$

When season is not known, the log transformed regression for the all the pigs is used:

$$ADD = 10^{(0.067*TBS + 1.188)}$$

$$PMI = 10^{(0.067*TBS - 0.267)}$$

Because decomposition is mostly driven by the accumulated heat over time (ADD), the formula for estimated ADD can be applied irrespective of the climatic region. When using this formula, the number of days it took for this estimated ADD (from this formula) to be equal to the actual ADD (computed from daily weather station temperature data) is the PMI. The PMI formula, which is less reliable because temperature is not taken into account, can only be used when the daily ADD cannot be obtained (from a weather station).

The ADD predictions for all the TBS values from 3 – 35 were obtained from the equations for the complete dataset without the influence of season (Table 4.2). Also, the upper and lower limits within the 95% confidence interval were obtained and can be read off from Table 4.2. These ADD values were calculated by fitting the corresponding TBS into the regression equation: $ADD= 10^{(0.067xTBS+1.188)}$. For example, if a body is found with an unknown season of death, and the TBS is scored 7, the estimated ADD is 45.39 or between 42.86 and 47.92. As can be expected, the 95% CI increases at higher TBS scores since decomposition becomes more variable in the later stages as seen from the scatter plots.

Similarly, the ADD predictions and the 95% confidence interval were obtained from the respective equations with the influence of season (Tables 4.3 and 4.4, for the dry and wet seasons respectively). The ADD forecast used is dependent on the known season of death. The gaps (-) are seen where there exists only one sample for a particular TBS value, therefore, the standard deviation or 95% CI cannot be calculated.

Table 4.2. Forecast of ADD and the associated upper and lower limits within the 95% confidence interval without the effect of seasons.

TBS	ADD	ADD (95% Confidence Interval)	
		Lower Limit	Upper Limit
3	24.49	23.75	25.23
4	28.58	-	-
5	33.34	31.48	35.21
6	38.90	35.97	41.83
7	45.39	42.86	47.92
8	52.97	44.65	61.28
9	61.80	52.07	71.53
10	72.11	66.96	77.26
11	84.14	82.68	85.60
12	98.17	94.28	102.07
13	114.55	-	-
14	133.66	114.13	153.19
15	155.96	-	-
16	181.97	165.89	198.05
17	212.32	198.05	226.60
18	247.74	233.49	261.99
19	289.07	275.09	303.04
20	337.29	320.51	354.07
21	393.55	380.46	406.64
22	459.20	405.60	513.80
23	535.80	499.42	572.17

24	625.17	431.98	818.38
25	729.46	422.75	1036.17
26	851.14	334.93	1367.35
27	993.12	304.66	1681.58
28	1158.78	81.15	2236.41
29	1352.07	353.03	2351.12
30	1577.61	900.85	2254.37
31	1840.77	892.71	2788.83
32	2147.83	-	-
33	2506.11	872.34	4139.88
34	2924.15	-	-
35	3411.93	-	-

Table 4.3. Forecast of ADD and the associated upper and lower limits within the 95% confidence interval in the dry season.

TBS	ADD	ADD (95% Confidence Interval)	
		Lower Limit	Upper Limit
3	21.33	20.52	22.14
5	30.13	-	-
6	35.81	32.62	39.00
7	42.56	-	-
8	50.58	39.06	62.10
9	60.12	44.39	75.85
10	71.45	70.17	72.73
11	84.92	83.46	86.38
12	100.93	-	-
13	119.95	-	-
14	142.56	-	-
16	201.37	-	-
17	239.33	230.53	248.13
18	284.45	263.03	305.87
19	338.06	318.60	357.53

20	401.79	376.77	426.81
21	477.53	457.15	497.91
22	567.54	508.32	626.76
23	674.53	634.53	714.53
24	801.68	544.94	1058.42
25	952.80	-	-
26	1132.40	768.06	1496.74
27	1345.86	829.96	1861.76
28	1599.56	621.51	2577.61
29	1901.08	-	-
30	2259.44	1652.89	2865.98
31	2685.34	2174.71	3195.98
33	3793.15	-	-
34	4508.17	-	-
35	5357.97	-	-

Table 4.4. Forecast of ADD and the associated upper and lower limits within the 95% confidence interval in the wet season.

TBS	**ADD**	**ADD (95% Confidence Interval)**	
		Lower Limit	**Upper Limit**
3	25.35	24.70	26.00
4	29.17	-	-
5	33.57	31.37	35.77
6	38.64	-	-
7	44.46	-	-
8	51.17	39.50	62.84
9	58.88	52.89	64.87
10	67.76	61.37	74.16
12	89.74	84.55	94.94
14	118.85	103.33	134.37
15	136.77	-	-
16	157.40	-	-

17	181.13	-	-
18	208.45	203.35	213.55
19	239.88	225.07	254.69
20	276.06	262.01	290.10
21	317.69	303.64	331.74
22	365.59	-	-
23	420.73	389.08	452.38
24	603.95	489.51	718.38
25	557.19	234.67	879.70
26	641.21	2.35	1280.07
27	737.90	-2.93	1478.73
28	849.18	133.87	1564.49
29	977.24	26.28	1928.20
30	1124.60	749.82	1499.38
31	1294.20	536.95	2051.45
32	1489.36	-	-
33	1713.96	-	-
34	1972.42	-	-
35	2269.86	-	-

4.4. Inter-observer repeatability

Inter-observer repeatability was tested by an external individual scoring decomposition of the three body regions (head and neck, trunk, and limbs) for two of the pig cadavers using photographs taken at the time when the primary observer was scoring. Pearson's correlation coefficient was used to assess the relationship between the scores. A perfect correlation exists if the coefficient of correlation is 1; if it is between 0.75 and 0.99, a high degree of correlation exists; values between 0.50 and 0.74 indicate moderate degree of correlation, and values less than 0.50 show a low degree of correlation (Allan, 1982).

The data for the scores of the various body regions (head and neck, trunk, and limbs) and the TBS for two of the pigs scored by the primary and the external observer are shown in Appendices D and E, respectively.

The degree of decomposition as represented by TBS can be consistently repeated if a high degree of correlation (0.75 and above) exists. Pearson's correlation test scores for the three body regions separately and the TBS are shown in Table 4.5. The TBS had the highest r-value of all the description scores (0.988) and so was correctly repeated 98.8% of the time. For the head and neck, trunk, and limbs, they were 0.962, 0.974 and 0.963 respectively, indicating that this method has a very high degree of repeatability.

Table 4.5 Correlation coefficients for the inter-observer results

Body part	Correlation coefficient
Head and neck	0.962
Trunk	0.974
Limbs	0.962
Total Body Score	0.988

4.5. Intra-observer repeatability

Intra-observer repeatability was assessed by the primary observer scoring the TBS of two randomly selected pig samples using photographs. These scores were then compared with the scoring done at the research site by the primary observer using Pearson's correlation.

The data for the scores of the various body regions (head and neck, trunk, and limbs) and the TBS for two of the pigs scored by the primary observer at the research site and with photographs are shown in Appendices D and F, respectively. Pearson's correlation test scores for the three body regions separately and the TBS are shown in Table 4.6 The TBS had the highest r-value of all the description scores (0.994) and so was correctly repeated 99.4% of

the time. For the head and neck, trunk, and limbs, they were 0.992, 0.980 and 0.981 respectively, indicating that this method has a very high degree of repeatability.

Table 4.6 Correlation coefficients for the intra-observer results

Body part	Correlation coefficient
Head and neck	0.992
Trunk	0.980
Limbs	0.981
Total Body Score	0.994

4.6. Comparison of decomposition patterns between Nigeria and South Africa

The decomposition patterns in Nigeria were observed and compared with those observed in a temperate, Highveld region of South Africa (Myburgh *et al.*, 2013). This was done because the data for this study are available and were collected in a comparable manner to the data in the current study. The Myburgh *et al.* (2013) study, conducted around Pretoria, also assessed decomposition in two seasons – in this case summer (wet) versus winter (dry). This comparison would bring to the fore the similarities and differences between these climatically different regions and specifically help to better understand the influence of two key factors in decomposition – temperature and rainfall. In the South African study there were vast differences in temperature between the two seasons, as opposed to the current study where mean temperature differences between the seasons were small. Also, in South Africa, the wet season was also the warmest, whereas in Nigeria the wet season is slightly cooler than the dry season.

The curvilinear pattern of decomposition in both the complete dataset plot and those observed in the two different seasons in Nigeria is common to both studies like other studies around the world (Rodriguez & Bass, 1983; Galloway *et al.,* 1989; Megyesi *et al.*, 2005; Schiel, 2008; Brown & Peckmann, 2013; Knobel *et al*., 2019; Marhoff *et al*., 2016; Suckling *et al*.,

2016). There are, however, a few differences, bearing in mind that environmental differences exist between Nigeria which has a tropical climate, and South Africa, a temperate region. Comparison of different segments of this curvilinear shape of decomposition which includes an early rapid phase with a steep slope, a plateau, and a resumption phase, reveals some peculiarities. Whereas the rapid decomposition phase (TBS score < 16) in the South African Highveld winter occurred within 20 days of placement, this rapid decomposition phase stopped short of the final score in advance decomposition (TBS < 24) and occurred within a shorter time (13 days) in the Nigerian dry season. This shows a more rapid decomposition in the Nigerian dry season than in the South African winter (Figure 4.36). In the Pretoria summer, this rapid phase was completed within 15 days of placement at a TBS less than 10, while it occurred earlier in the wet season of Nigeria in 9.2 days on average, at a higher TBS range (24 – 32) (Figure 4.37).

Completely shielding the remains of this study from all kinds of scavengers may have contributed to some changes in decomposition rate and pattern, as opposed to that witnessed in the study by Myburgh *et al.* (2013) where, although scavengers were generally excluded, aerial scavengers like cattle egrets had access.

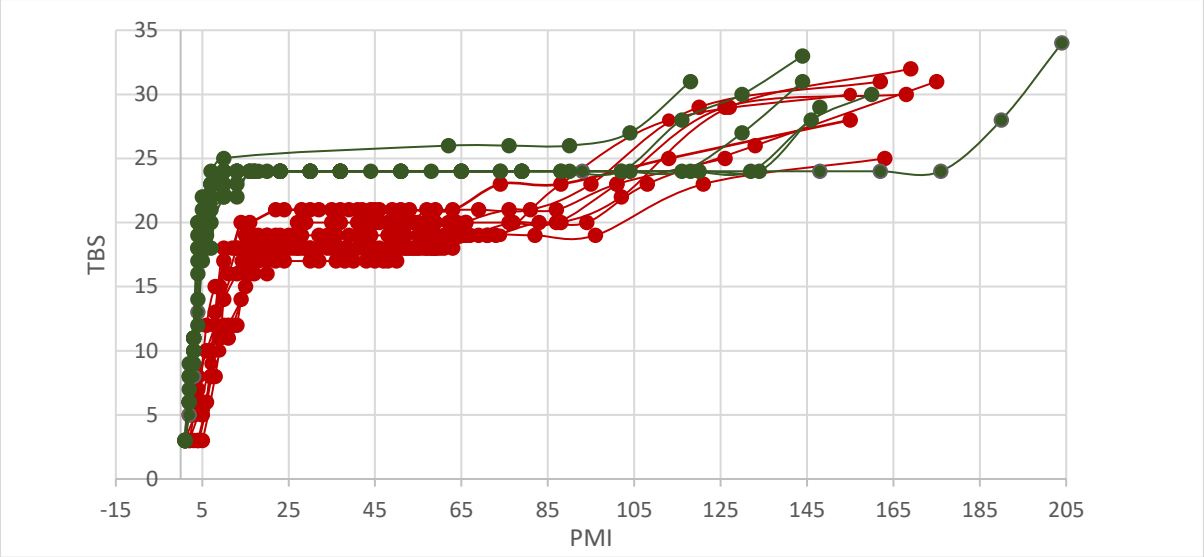

Figure 4.36. Scatter plot of TBS vs PMI (in calendar days) for the dry season sample of this study (n = 10) in green, and the winter season pigs in Pretoria, South Africa (n = 8) in red (Myburgh e*t al.*, 2013).

The plateau phases of the decomposition in the South African winter occurred over a wider range of TBS (16 – 21) and lasted between 20 and 90 days after placement. In contrast, the sample in the Nigerian dry season levelled off over a narrower TBS range (24 – 26) with 90% occurring at a TBS of 24 and lasted longer (Figure 4.36) and were attained earlier. The same applies to the Nigerian wet season and the Highveld summer seasons where plateaus were attained at TBS ranges of between 24 and 30, and 18 and 35, respectively (Figure 4.37). However, like in the Nigerian dry season, there were more pigs with plateaus in the South African winter than summer.

A remarkable feature of the decomposition in Pretoria (Myburgh *et al.*, 2013) was the higher frequency of multiple inactive phases (multiple plateaus) per sample. An example is seen in the 8th winter pig on the uppermost part of the scatter plot of the Pretoria winter sample (Figures 4.36 and 4.38) where it achieved a very brief plateau between days 14 and 16 at a TBS of 20, a longer plateau between days 22 and 63 at a TBS of 21, and a third at a TBS of 23 that lasted for 14 days. All the Highveld winter pigs (n=8) and 73% of the summer samples had multiple plateaus. In the present study, a higher frequency of double plateaus is seen in

the wet season (73%) than in the dry season (40%) (scatter plots for each are shown in Appendix C).

Similar to the lower heat energy required for complete desiccation (TBS of 24) in the wet season of Nigeria relative to that in the dry season, a considerably lower heat energy was required to reach this state of decomposition (TBS of 24) in the summer (ADD of at least 397.84) than in the winter (ADD of at least 1035.18) in Cape Town, South Africa (Finaughty and Morris, 2019). The authors found that desiccation occurred earlier in the summer than the winter; the earliest desiccation took only 17 days in the summer, and at least 77 days in the winter. Considering that Cape Town has a Mediterranean climate with winter rainfall and therefore a dry summer, the early desiccation in summer is at variance with the finding in Nigeria which occurred earlier in the wet season.

With the onset of rainfall in the South African summer (Myburgh *et al.,* 2013) as in the Nigerian wet season, and corresponding with remoistening of the samples, resumption of decomposition occurred in both countries. The high variability witnessed in the South African summer was also found in the Nigerian wet season. However, whereas this was noticed after a TBS score of 20 in the South African study, it was seen at a more advanced state of decomposition (after a TBS score of 24) in the current study. A significant factor common to these seasons in both countries is rainfall, and its effect on other factors like humidity, temperature, and insect activity.

There was a generally rapid decomposition in the late winter/dry season and early summer/dry season in both countries. Also, remains deposited at or nearest to the peak of rainfall decomposed slowest as judged by the TBS score attained over a particular time or

ADD (Pig 15 in the South African study by Myburgh *et al*. (2013) and Pig 10 in this study; Figure 4.22). After this peak in rainfall, decomposition rate increased in both locations.

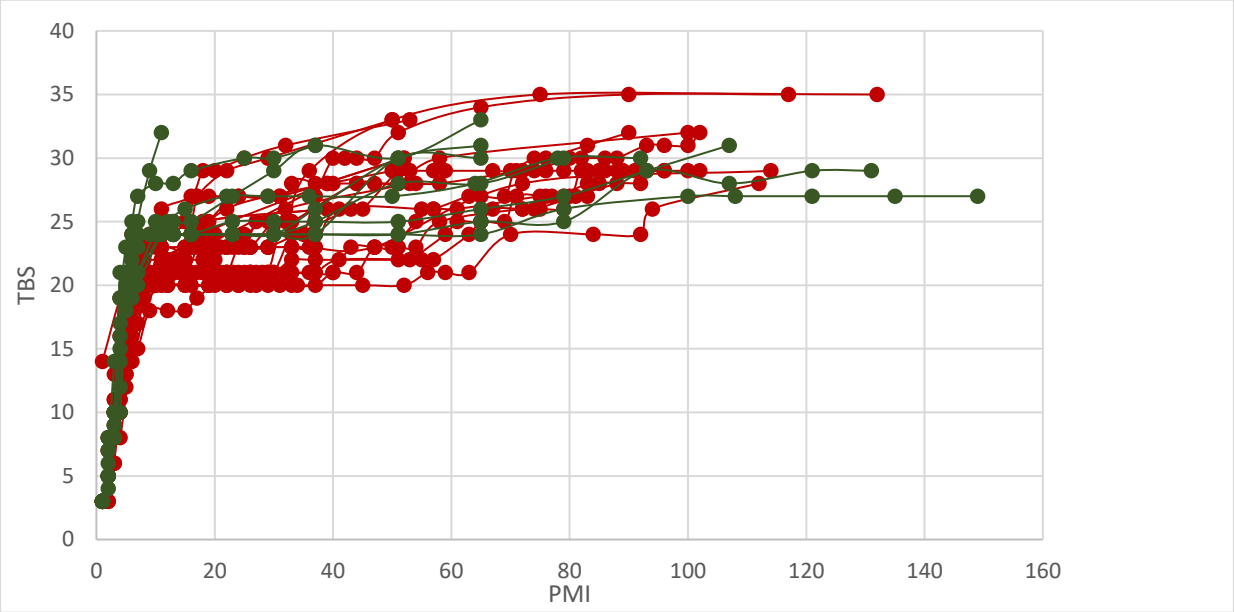

Figure 4.37. Scatter plot of TBS vs PMI (in calendar days) for the wet season sample of this study (n = 10) in green, and the summer season pigs in Pretoria, South Africa (n = 22) in red (Myburgh e*t al.*, 2013).

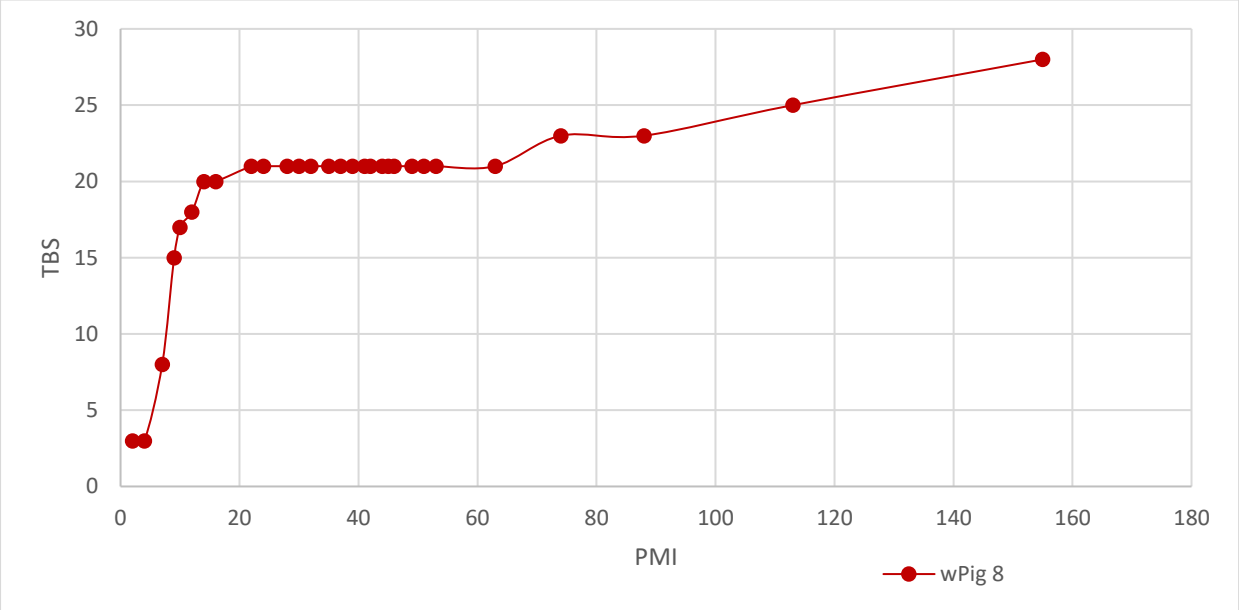

Figure 4.38. Scatter plot of the TBS vs PMI for Pig 8 (winter) in South Africa showing multiple plateaus

4.7. Insect succession patterns in southern Nigeria

The stages of decomposition, although practically difficult to separate due to blending of one stage with the next is, for the sake of convenience, divided into 5 namely fresh, bloat, active, advanced, and dry decay/skeletonization stages. In the present study, the fresh stage lasted for only one day, i.e., from the time of placement to the next day (day 0 – 1). The housefly, *Musca domestica,* was mostly the first to arrive in this stage followed closely by the blowfly *Chrysomya marginalis*, visiting within 5 minutes and 10 minutes after placement, respectively (Figure 4.39).

a.  b. 

Figure 4.39. The various flies present in the fresh stage. *Musca domestica* (a), Family Muscidae. *Chrysomya marginalis* (b), Family Calliphoridae.

The bloat stage lasted between days two and three after placement (days 2 and 3, with TBS ranging from five to 14). The insects associated with this stage were the very occasional *Chrysomya chloropyga* (a blowfly) observed on the second and third days, and the usually few flesh flies of the Family Sarcophagidae appearing from day three (Figure 4.40a and b). Other insects that visited the pig cadavers in the bloat stage were the ants (Family Formicidae) on day two, and beetles (Family Gyrinidae), the black soldier fly (*Hermetia illucens*) and a group of tiny flies (Family Phoridae) which all appeared on day three (Figure 4.40c to f, respectively). Whereas the beetles of the Gyrinidae family and the black soldier flies were exclusively present in the wet season and lasted until about the 11th day, the flies of the Phoridae family were found in both seasons and lasted for about 22 days. Overall, Calliphoridae was the most

dominant family, reached a peak appearance by the third to fourth day, or the time of maximum bloat, and gradually thinned out and eventually disappeared between the fourth and seventh day.



Figure 4.40. The various insects present in the bloat stage. *Chrysomya chloropyga* (a), Family Calliphoridae. Flesh fly (b), Family Sarcophagidae. Ant (c), Family Formicidae. Beetle (d), Family Gyrinidae. Black soldier fly/*Hermetia illucens* (e), Family Stratiomyidae. Fly (f), Family Phoridae.

The beginning of active decay stage is marked by the initially tiny, newly hatched maggots from the eggs deposited in body orifices in the fresh stage gaining access to the internal remains on which they feed voraciously and grow. As they feed and decomposition proceeds, body fluid and products of tissue destruction flow out into the surrounding area. As desiccation sets in and progresses, the grown larvae begin to move away from the carcass to

pupate due to the changing food resource. This migration coincided with the end of the active decay stage. This study found the active decay stage, which witnessed peak maggot activity, to last between days four and six after placement (days 4 – 6, with TBS from 20 to 24). There was, however, an observed difference in the movement of the maggots to pupate between the wet and the dry season. Whereas the maggots favoured the area immediately surrounding the carcass to burrow into the soil and pupate in the dry season, they moved further away in the wet season. Consequently, far more new/juvenile flies and pupal casings were observed within the cage, or around the carcass, in the dry season than in the wet season where pupal casings were mostly found away from the carcass.

The insects found in this decomposition stage were mainly beetles. Dermestid beetles (*Dermestes maculatus*) appeared between the fourth and the sixth day and lasted for a variable time depending on the season; up to the 16th day in the dry season and 30th day in the wet season (Figure 4.41a). The scarab beetles (Family Scarabaeidae) and bees (Family Apidae) appeared only briefly between the fourth and fifth days (Figures 4.41b and 4.41c, respectively). However, whereas the former was exclusively observed in the wet season, the latter were common to both seasons. Another beetle *Platycorynus dejeani* (Family Chrysomelidae) appeared on day four while the rove beetle (Family Staphylinidae) was observed in only one wet season pig between days six and 16 (Figure 4.41e).

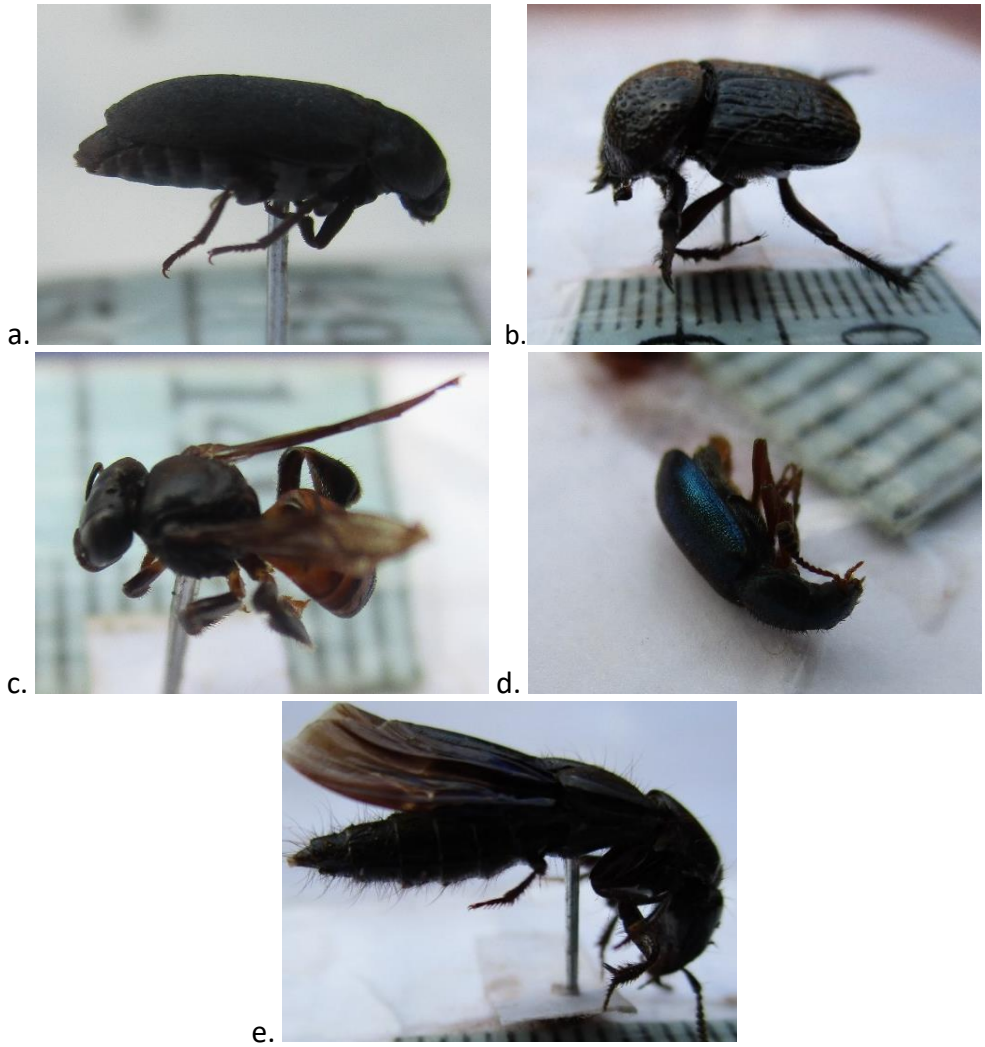

Figure 4.41. The various insects present in the active decay stage. *Dermestes maculatus* beetle (a), Family Dermestidae. Scarab beetle (b), Family Scarabaeidae. Bee (c), Family Apidae. *Platycorynus dejeani* beetle (d), Family Chrysomelidae. Rove beetle (e), Family Staphylinidae

All the preceding stages of decomposition were found not to differ in duration between the seasons. Although starting around day seven after placement, the advanced decay stage was found to be considerably shorter in the wet season than the dry season. It lasted between day seven and anytime between day 51 and 107 in the wet season (Table 4.4) with a TBS range of 20 to 35, while it was from day seven to between day 118 to 190 after placement in the dry season (Table 4.3) with a TBS range of 18 to 33. Arthropods that appeared late were mainly incidental, like the jumping spider (Family Salticidae), centipedes, crickets, and grasshoppers (Figures 4.42) appearing later than two weeks after placement. These

arthropods use the carcass as shelter or an extension of their habitat. A few of the arthropods in the earlier stages were also found in these late stages of decomposition.

a.  b. 

c.  d. 

Figure 4.42. The various insects present in advanced decay and dry stages of decomposition. Jumping spider (a), Family Salticidae. Centipede (b), Class Chilopoda. Cricket (*Gryllus sp*) (c), Family Grylloidea. Variegated grasshopper (*Zonocerus variegatus) (d),* Family Pyrgomorphidae.

For the group of pig remains placed in the first dry season (December 21, 2019 – March 16, 2020), there was an obvious interlude in insect activity which coincided with late advanced decomposition at which time the remains were completely desiccated. With the remoistening of the remains in the wet season, there was a return of some insects to continue decomposition which eventually led to skeletonization. These insects were much fewer in number and included the housefly, the flesh fly (Family Sarcophagidae), and very scanty or no blowflies. There were also very few maggots, and few or absent larvae predators like the black soldier ants. These were shortly followed by few beetles (*Platycorynus dejeani* and *Dermestes maculatus*).

Chrysomya marginalis was the most dominant blow fly, and *Platycorynus dejeani* was the most dominant beetle followed by *Dermestes maculatus*.

Ants (Family Formicidae) and beetles, especially *Platycorynus dejeani* (Family Chrysomelidae), were found to scavenge on the maggots (Figure 4.43) and the flightless juvenile flies which emerged around day 13. The time of appearance of these arthropods and their duration of stay on the carcass coincided with the presence of these insect food sources. Both families (Formicidae and Chrysomelidae) typically stayed on or visited the carcass until either complete desiccation in the dry season, or rapid skeletonization in the wet season and made the food resource unsuitable for the maggots and they leave to pupate. The holes/nests dug by these ants are often located within 50 cm of the decomposing pig cadavers around the time the maggots are present (Figure 4.44).



Figure 4.43. Maggot scavenging by an ant, Family Formicidae.



Figure 4.44. The black ant nest.

It is important to point out the role of arthropods in facilitating skeletonization by biting off chunks of the initially desiccated tissues remoistened in the wet season (Figure 4.45).



Figure 4.45. Beetle and beetle larva damage to remoistened desiccated tissue for skeletonization to occur.

The arthropods collected from the decomposing pig cadavers included three classes (Table 4.7): Insecta, Arachnida and Chilopoda; six orders: Dipthera, Hymenoptera, Hemiptera,

Coleoptera, Araneae and Orthoptera; and 16 families. The families consisted of Muscidae, Calliphoridae, Sarcophagidae, Phoridae, Apidae, Stratiomyidae, Staphylinidae, Formicidae, Chrysomelidae, Gyrinidae, Dermestidae, Scarabaeidae, Salticidae, Grylloidea, Pyrgomorphidae, and Acrididae. Some of the arthropods could not be identified beyond the class, order, or family levels.

Table 4.7. Arthropods according to classes, orders, and families.

Class	Order	Family
Insecta	Diptera	Muscidae Calliphoridae Stratiomyidae Phoridae Sarcophagidae
	Coleoptera	Chrysomelidae Gyrinidae Dermestidae Scarabaeidae Staphylinidae
	Hymenoptera	Formicidae Apidae
	Hemiptera	
	Orthoptera	Grylloidea Acrididae Pyrgomorphidae
Arachnida	Araneae	Salticidae
Chilopoda		

4.8. Differences in insect succession patterns between the dry and wet seasons

The dry season witnessed fewer families of arthropods than the wet season. There were seven families in the dry season and twelve in the wet season (Tables 4.8 and 4.9). Apart from this obvious difference in the number of arthropod families present for each season, there

was a considerable increase in the absolute number of insects in the wet season when compared to those observed in the dry season.

Table 4.8. Summary of the mature arthropods associated with the stages of decomposition in the dry season ("+" denotes present; "-" denotes absent).

Family	Stages of decomposition				
	Fresh (Day 0-1)	Bloat (Day 2-3)	Active (Day 4-6)	Advanced (Day 7-190)	Dry (>Day 118)
Muscidae	+	+	+	+	+
Calliphoridae	+	+	+	+	-
Formicidae	-	+	+	+	-
Chrysomelidae	-	-	+	+	-
Phoridae	-	+	+	+	-
Dermestidae	-	-	+	+	-
Apidae	-	+	+	-	-

Table 4.9. Summary of the mature arthropods associated with the stages of decomposition in the wet season ("+" denotes present; "-" denotes absent).

Family	Stages of decomposition				
	Fresh (Day 0-1)	Bloat (Day 2-3)	Active (Day 4-6)	Advanced (Day 7-107)	Dry (>Day 51)
Muscidae	+	+	+	+	-
Calliphoridae	+	+	+	-	-
Formicidae	-	+	+	+	+
Chrysomelidae	-	-	+	+	+
Gyrinidae	-	+	+	+	-
Stratiomyidae	-	+	+	+	-
Phoridae	-	+	+	+	-
Dermestidae	-	-	+	+	-
Scarabaeidae	-	+	+	-	-
Staphylinidae	-	-	+	+	-
Apidae	-	+	+	-	-
Salticidae	-	-	-	+	-

The arthropods also acted on the carcass for longer in the wet season with little or no interlude caused by complete desiccation of the remains at which time the arthropods leave the carcass only to return with the remoistening of the remains. The tissues therefore remained supple for longer for them to feed without drying out. The families of insects that returned after remoistening of the remains are common to both seasons and included Muscidae, Chrysomelidae (*Platycorynus dejeani),* Dermestidae (*Dermestes maculatus*) and

Formicidae. Also, the emergence of new flies occurred mostly in or around the decomposing carcass in the dry season, and only occasionally in the wet season. This may be due to the post-feeding larvae moving away from the wet carcass to pupate and emerge from the pupa in a dry environment, as opposed to the burrowing around the carcass to pupate within the dry soil in the dry season.

At the time of peak maggot activity, the blowflies that colonized the pig cadavers in early decomposition were mostly found in the surrounding bushes (Figure 4.46) and not on the carcass, although a few visited occasionally.



Figure 4.46. A group of the green bottle fly (*Chrysomya marginalis*) in the bushes surrounding the carcasses after they have left the carcass around the time of peak maggot activity.

Chapter 5: Discussion

5.1. Introduction

The milestone research by Megyesi *et al*. (2005) popularized the quantitative approach to PMI estimation. These authors attempted to account for the factors that influence decomposition, the most important of which was temperature. Using the concept of accumulated degree days (ADD) introduced by Vass *et al*. (1992) to standardize temperature, and modifying the staging method by Galloway *et al*. (1989) to produce quantitative descriptions of the physical changes of decomposition or the total body score (TBS), some of these factors, especially temperature, were accounted for. Subsequent studies in various parts of the world have continued to either examine this method or investigate factors that are important in their particular environments, attempting to quantify such factors and their influence (e.g., Schiel, 2008; Parsons, 2009; Fitzgerald & Oxenham, 2009; Simmons *et al*., 2010; Myburgh *et al*., 2013; Moffatt *et al*., 2016; Suckling *et al*., 2016; Marhoff *et al*., 2016; Marhoff-Beard *et al*., 2018; Forbes *et al*., 2019).

The aim of this study was to assess the patterns of decomposition and to estimate the post-mortem interval (PMI) in southern Nigeria by using the quantitative variables, TBS and ADD, and to assess the arthropod community composition and succession patterns on decaying remains. For this purpose, a pig model was used. To the knowledge of the authors, this is the first study on PMI estimation in Nigeria aimed at demonstrating decomposition patterns, insect succession dynamics, and deriving a locally adapted formula for PMI estimation. It is hoped, therefore, that the findings of this research will stimulate interest in this field of forensics and arm forensic anthropologists and entomologists working in Nigeria with the necessary, affordable, and easy tool for PMI estimation.

Nigeria has a tropical climate with wet and dry seasons. Southern Nigeria, where this study was done, is a warm, humid environment with a narrow temperature fluctuation throughout the year. For the town where the research was performed, this fluctuation in temperature is, on average, 3.6 °C. The average temperature in the wet season is 26.3°C while it is 27.5°C in the dry season, while the minimum and maximum humidity are 35% and 90% respectively. The average rainfall per year is 1862 mm, with a difference in precipitation of 300 mm between the driest and wettest months (https://www.worldweatheronline.com/awka-weather-averages/anambra/ng.aspx; https://en.climate-data.org/africa/nigeria/anambra/nibo-1022872/#climate-graph). The results of this study may apply to other African countries with similar weather conditions, but this will need to be investigated further.

To achieve the aim of this study, TBS and ADD data were collected over 14 months (December 2019 to January 2021), using a pig model. The pigs weighed between 30 and 60kg in order to be similar to the adult human weight, and to minimize the possible effect of weight on decomposition rate. The effect of absolute time in days, as well as temperature accumulation over time on decomposition was assessed by plotting TBS against PMI and ADD, respectively. Scatter plots were produced to demonstrate the general decomposition pattern (Figures 4.1 and 4.3). Log linear formulae in which ADD and PMI were estimated with TBS were developed. The repeatability in scoring the TBS was also assessed and found to be highly repeatable with the TBS having the highest r-value (0.988) when compared with those of the three different body regions. Arthropods were collected through the stages of decomposition and were identified to produce a comprehensive data set of insects of forensic importance and their succession pattern in southern Nigeria. Comparisons were made between the succession patterns in the wet and dry seasons of Nigeria, and between Nigeria and other countries.

5.2. Decomposition

For all the pigs, decomposition advanced rapidly and linearly initially, reaching a TBS of 22 – 27 (advanced decomposition) in only 7 days. This rapid decomposition phase implies a very steep plateau when compared with the finding of another study in Nova Scotia, Canada, where advanced decomposition was first reached in about 28 days after placement (Brown and Peckmann, 2013). However, another study in the summer of Western Sydney, Australia, showed that the earliest advanced decomposition was reached was 10 days, i.e., 10 - 35 days (Knobel *et al.*, 2019). The comparable earliest onset of advanced decomposition between the latter and the present study could be due to the high summer temperatures in Western Sydney (average daily temperature of 23.5°C, 14.7–40.6°C.). Another similarity with this Australian study due to the high temperatures common to both locations was the early onset of desiccation in the active phase in both studies.

A plateau was reached in the late stages of advanced decomposition (TBS score of 24) for 75% (n=15) of the total sample after which decomposition became more variable. Of the remaining 25% (n=5), 20% (n=4) achieved plateau(s) at higher TBS than 24, and 10% proceeded to skeletonization without a plateau. This, therefore, produced a curvilinear decomposition pattern as found in previous studies (Brown & Peckmann, 2013; Galloway *et al*., 1989; Knobel *et al.*, 2019; Marhoff *et al*., 2016; Megyesi *et al*., 2005; Rodriguez & Bass, 1983; Schiel, 2008; Suckling *et al*., 2016).

Studies on decomposition are difficult and there are many unforeseen factors that can play a role. For example, on one occasion, two crows were found in the cage of Pig 11. They had dug up the corner of the cage in contact with the ground to gain entrance and feed on the maggots

and, possibly, some decomposing tissues. Although this happened at the time when desiccation had set in and maggots had started migrating, reduction of the maggot mass generally reduces decomposition rate. Crows have also been observed feeing on the remains at the Anthropology Research Facility (ARF) (Bass, 1997; Rodriguez, 1997). The crows in this study were released from the cage as soon as they were discovered. It is not always possible to account for all these unforeseen events.

5.3. Comparison between the wet and dry seasons in Nigeria

The initial linear phase of decomposition which progressed rapidly included the fresh, bloat, active and advanced decay stages of decomposition up to a TBS of 24 at which time this phase levelled into complete desiccation/plateau for the 75% (n=15) of the whole sample that attained this state. Five percent (n=1) of the remaining 25% progressed to full skeletonization in 11 days without a plateau, while another 20% (n=4) attained a plateau at higher TBS values (skeletonization). When the same phase was compared between the seasons, it was faster in the wet season. For example, at an ADD of 1000, the TBS range for the wet season (green data in Figure 4.35) was 24 – 31, while it was only 24 – 26 for the dry season (blue data in Figure 4.35). This implies that at the same heat accumulation, decomposition progressed further in the wet season than it did in the dry season. This initial linear phase of decomposition was completed between 166 and 286 ADD in the wet season, and 208 and 505 ADD in the dry season (Appendix C for individual pig scatter plots). Therefore, the dry season pigs needed more ADD to reach the same phase of decomposition as the wet season sample.

The more rapid decomposition in the wet season in this early phase was likely due to rainfall and the associated increased humidity as these variables have been reported to accelerate decomposition rate. (Prieto *et al*., 2004; Gunn, 2009; Shi *et al*., 2009; Giles *et al.*, 2020). For rainfall, increased rates were noticed at the beginning and end of the season when episodes

of rainfall are infrequent. There was a reduction towards the peak of the season when there are more frequent episodes of rainfall. In a country like Nigeria with generally high temperatures all year round, the considerable changes in humidity due to season is expected to contribute significantly to decomposition rate. The similar daily temperature averages in both seasons, 27.1^{o}C in the wet season and 29.3^{o}C in the dry season, and comparable ADD values (e.g., an average of 142 on day 5 for all carcasses) irrespective of season may indicate that temperature, although an important driving force in decomposition, may not explain the differences seen in decomposition rates between the seasons. Differences in humidity and rainfall are what stands out between the seasons. The finding of a more rapid decomposition rate in the dry season in Ghana which also has both wet and dry seasons is at variance with the findings of the present study but there is agreement in humidity accounting for more rapid decomposition as there was higher humidity in the dry season of that study (Kyerematen *et al.*, 2013). Increased humidity ensures that the food resource remains in an easily digestible state while also encouraging maggot and microbial growth which these maggots partly depend on to ingest the decomposing remains since they have non-articulated jaws (Campobasso *et al*., 2001; Byrd & Castner, 2010). The effect of humidity in accelerating decomposition through insect activity, which was also observed in this study, is supported by other researchers (Shi *et al.*, 2009; Kyerematen *et al.*, 2013). According to Giles *et al*. (2020) humidity may be the most crucial factor for the advancement of early decomposition, more so than temperature. This is supported by the fact that there were more arthropods observed, and decomposition progressed faster, in the wet season of the present study. Beetles have chewing or articulated mouth parts which can dispose of more tissues and there were three more beetle families in the wet season than in the dry season. These are Gyrinidae, Scarabaeidae and Staphylinidae (Tables 4.7 and 4.8).

A visually noticeable feature of advanced decay in this study was desiccation which starts at a TBS of 22 when only one body region was desiccated, and completed at a TBS of 24 when it involves all the three body regions (Megyesi *et al*., 2005) at the end of advanced decomposition. There is usually no bone exposure on more than 50% of the body regions, no maggot activity or noticeable progress in decomposition in this state. This may be due to a combination of factors such as high temperature, the amount of insect activity in early decomposition, and other factors which may promote or hinder these two factors. More dry season samples (90%) attained stalled desiccation than the wet season samples (60%) (Figures 4.5 and 4.6). This was attained later in the dry season (average of 13 days) (Figure 4.8) than in the wet season (average of 9.2 days) (Figure 4.16) and lasted for longer in the dry season - an average of 119 days compared to an average of only 29 days in the wet season (see Appendix C for individual pig scatter plots). The earlier occurrence and short duration of desiccation (state of apparently arrested decomposition) in the wet season may indicate that this change is only superficial in the wet season; tissues beneath the skin may still be undergoing wet decay leading to earlier skeletonization (shorter duration) in the wet season than in the dry season. This is one of the drawbacks of the Megyesi *et al.*'s (2005) TBS method pointed out by Suckling (2011). Suckling (2011) pointed out that assigning a higher TBS value to desiccation than moist decomposition while it is possible that the external part of the remains may desiccate with the underlying soft tissue undergoing moist decomposition at the same time as a source of reduced precision of the Megyesi *et al.*'s (2005) model. Since photographs were used in the study by Megyesi *et al.* (2005), this could not have been taken into account. Even with the onset of the wet season, it took some time for the tissues of the dry season pigs to hydrate for decomposition to resume (Figure 4.10). This indicates that prolonged desiccation in the dry season resulted in true tissue preservation but not in the wet

season. This is considered mummification by the descriptions of Galloway *et al.* (1989) in southern Arizona, and Suckling *et al.* (2016) in central Texas. Since the more important requirement for mummification to occur is low humidity and not temperature (Mann *et al.*, 1990; Clark *et al.*, 1997; Vass, 2001), desiccation in the wet season with a high humidity is not likely to lead to true preservation. Mummification is considered to be a modification of the decomposition process whereby the normal decomposition pattern is altered. Since stalled or prolonged desiccation has been observed in numerous studies (Megyesi *et al.*, 2005; Myburgh *et al.*, 2013; Suckling *et al.*, 2016; Connor *et al.*, 2019; Finaughty and Morris, 2019) and the decomposition continued after the plateau phase, it would appear the stalled or prolonged desiccation is still a normal decomposition stage and not a true modification, such as mummification. Therefore, for clarity, the term stalled or prolonged desiccation is suggested to differentiate it from true mummification.

There appears to be different causes for prolonged desiccation in the two seasons. In the dry season, the dry weather, and high temperatures favour desiccation over insect activity, making the remains unsuitable for the arthropods and they move away. In the wet season, the slowest rates of decomposition were found to be in those pigs deposited at the peak of the wet season when there were more frequent episodes of rainfall which limited insect activity by washing off the feeding larvae and preventing activity of the adult insects too in early decomposition. When insects are not available to feed in early decomposition, early short-lived desiccation occurs due to the persistently high temperature with narrow fluctuation in the wet season (Figure 4.14). Desiccation, or its absence, and insect activity are obvious antagonistic factors in decomposition in the tropical climate of southern Nigeria. Considering that in aerial decomposition insects are considered the second most important factor after temperature (Mann *et al*., 1990; Vass *et al*., 2002; Simmons *et al*., 2010), this

agrees with the assertion that the changes seen in decomposition is a form of balance between putrefaction, at which time insect activity is the highest, and desiccation (Micozzi, 1986; Hayman & Oxenham, 2016).

It is important to take desiccation into account as most developed PMI estimation models based on TBS perform poorly in later decomposition (Vass, 2011; Myburgh *et al.*, 2013; Marhoff *et al.*, 2016; Nawrocka *et al*., 2016; Suckling *et al.*, 2016; Marhoff-Beard *et al.*, 2018). This poor performance has been attributed to the treatment of decomposition as a linear process, the early phase of which may be linear, but not the advanced stages (Suckling *et al.*, 2016; Connor *et al.*, 2019). Another factor to give consideration to in this seeming inactive phase is that, unlike in the temperate regions where desiccation did not have a specific order as desiccation occurred at different TBS (Myburgh *et al.*, 2013; Suckling *et al*., 2016), it did have a uniform order in the dry season of this study where 90% of the pigs had a uniform desiccation of all the body regions at a TBS of 24, otherwise referred to as stalled or prolonged desiccation.

The finding of long durations of prolonged desiccation especially in the dry season where this lasted between 89 and 166 days may be problematic for PMI estimation if remains are found in this state, in which it may have been for any period between a week and six months, or a short while after decomposition has resumed following remoistening by rainfall and return of the insects in the wet season. A possible way to resolve this would be to look for dead insects and pupal casings to attempt identification of insects which may have been involved in the active decomposition phase. If a dead insect is identified to be one of those that appear exclusively in the wet season, death may have occurred in the wet season. This study observed that the soil around the carcass (and possibly underneath) is a good place to gently excavate for the casings if the active/early advanced phase occurred in the dry season, and

away from the carcass in the wet season. In the dry season, most of the mature larvae burrow into the dry soil around the pig cadaver to pupate but in the wet season they move away from the moist soil around the carcass in search of a dry place to pupate. In order not to miss the insects in an outdoor scene like in this study, Amendt *et al.* (2007) suggests collection of soil samples and overlying leaf litter up to two metres away from the remains, 10 cm depth or more, and from more than one compass point. The place of desiccation in decomposition and PMI estimation is gaining attention as the desiccated tissue has been reported to undergo progressive visible change in this period of seeming inactivity due to the continued influence of the environment (Connor *et al.*, 2019).

With the onset of rainfall and remoistening of the desiccated carcasses in this study, decomposition resumed with the return of the insects (Tantawi *et al.*, 1996; Ayers, 2010; Godde, 2011; Myburgh *et al.*, 2013). This insect activity is of a much smaller scale than the insect activity in the active decay stage before desiccation and lasted for only a couple of days. Skeletonization followed the return of the insects as the ants and, especially, the beetles and their larvae, made holes in the remoistened desiccated outer shell of the remains to expose more bones (Figure 4.45) to above 50% of the body regions since desiccation occurred at a TBS of 24 (the last score in advanced decomposition phase – TBS range of 17 – 24) and the next score from this is in the phase of skeletonization (TBS of 25 – 35). There were more of the Muscidae family, including the housefly, and very scanty or no blowflies of the Calliphoridae family. There were also very few maggots. These were shortly followed by beetles (*Platycorynus dejeani* and *Dermestes maculatus*).

The amount of rainfall did not bear a direct relationship with decomposition rate. This is demonstrated by the longer periods of desiccation at the peak of the wet season as seen in Pigs 10 and 12 (Figures 4.16 and 4.18) when compared to the other samples at the beginning

and the end of the season when rainfall was less. This lower decomposition rate may be due to the fewer insects noticed immediately after rainfall, and the usually lower temperature recorded during the peak of the wet season unlike the higher temperatures at the transition periods between the seasons. Rainfall can reduce decomposition by saturation of the remains and reducing the number of insects feeding on the remains, and delayed recolonization (Myburgh *et al*., 2013; Kyerematen *et al*., 2013). Another explanation put forward for this delay in decomposition is through cooling by evaporation after the rains (Archer, 2004a) although the author reported that the influence of rainfall has not been separated from that of temperature in order to measure its influence separately. This is because there have been other reports of rainfall increasing decomposition rate (Mann *et al.*, 1990). There was also more variability in the decomposition pattern in the wet season of this study than there was in the dry season after a TBS score of 24 (green data above a TBS of 24 in Figure 4.35).

Changes in decomposition rate were also noted at the time of transition between the seasons. For example, the last pig placed in the dry season of 2019 (pig 6) experienced the fastest decomposition rate for that season, reaching the shortest plateau at the stage of skeletonization at a TBS score of 26 on the 62nd day of placement. Also, pig 7, the first wet season sample experienced the fastest decomposition for the season, reaching a TBS score of 32 in only 11 days. The infrequent rains at the start of the wet season ensured that the carcass remained hydrated enough for insect activity to continue but not too much as to interrupt feeding or colonization considerably. This was seen in pig 10 which was deposited at the peak of the wet season, and it experienced early and long desiccation. This appears to confirm that increased rainfall on its own does not increase decomposition rate (Archer, 2004a; Kyerematen *et al.*, 2013; Myburgh *et al.*, 2013), although rainfall in moderate amounts increased decomposition rate by keeping the carcass moist and therefore a usable food

resource for both bacteria and insects. Also, humidity, apart from promoting faster decomposition, appears to be a strong factor in determining how far decomposition proceeds before it is arrested (stalled or prolonged desiccation) since temperature was high throughout the year, only exhibiting more fluctuations in the dry season due to cooler nights. This is demonstrated in Figure 4.35 where a higher proportion of the wet season sample experienced desiccation of at least one body region at TBS greater than 24 (skeletonization phase).

When the stages of decomposition were compared with respect to the season (dry vs wet) viz fresh, bloat, active, advanced and skeletonization/dry remains (Swann *et al.*, 2010; Statheropoulos *et al.*, 2011), they were similar until active decay as follows: fresh: day 0-1; bloat: days 2-3; active decay: days 4-6. Much more variability was found in advanced decomposition and skeletonization stages where advanced decay was observed from days 7 – 190, with skeletonization occurring after more than 118 days in the dry season. For the wet season, on the other hand, advanced decay occurred from days 7 to 107, with skeletonization occurring as early as after day 50 (Tables 4.7 and 4.8). The duration of these stages differs with the finding of other studies that also used a pig model (Apichat *et al.*, 2007; Ekanem and Dike, 2010; Chin *et al.*, 2011). A study performed at the transition from the cold to the hot season in northern Thailand showed that the fresh and bloat stages had similar duration with the present study but had a much shorter active decay, advanced decay and dry stages (Apichat *et al.*, 2007). Chin *et al.* (2011) reported durations in the fresh, bloat, and active decay stages similar to the present study, and a much shorter advanced decomposition stage. Ekanem and Dike (2010) in southern Nigeria reported a duration of only the fresh stage that is similar to the present study. Temperature influences the duration of decomposition stages by its effect on enzyme catalysts, as well as intrinsic and extrinsic bacterial action (Amy *et al.*, 1986; Gill-King, 1997; Hayman and Oxenham, 2016d). The differences noted between the

seasons in the advanced stages of decomposition mirror the variability noted in these stages (TBS scores over 24) (Figure 4.35).

Higher decomposition rates were found both in the Pretoria summer and the Nigerian wet season. For example, the earliest desiccation occurred in only 17 days in the summer and took at least 77 days in the winter. Temperature explains this in Pretoria but not quite in Nigeria. Warmer summer temperature facilitates other positive influencers of decomposition such as bacterial growth, insect and enzyme activity, and Pretoria has a summer rainfall which increases humidity. Nigeria, on the other hand, has high average daily temperatures all year round with only a slight difference between the seasons (Figures 4.5 and 4.14). There is, however, a marked difference in humidity and rainfall between the wet and dry seasons (Figures 4.6 and 4.15).

5.4. Comparison between decomposition in Nigeria and other studies

Comparison between decomposition studies in places with climatic or environmental similarities or differences are important to highlight the effect of these environmental factors on decomposition, and possibly highlight the effect of other less known factors unique to the particular environment. The decomposition patterns in Nigeria were observed and compared with those observed in other African countries like South Africa and Ghana. The comparison between the present study and that in a temperate, Highveld region of South Africa by Myburgh *et al*. (2013) was more in-depth because comparable seasonal data for this study is available.

Comparing the present study with the study by Myburgh *et al*. (2013) in Pretoria, South Africa, the curvilinear pattern of decomposition is similar in both studies just like other decomposition studies around the world (Rodriguez & Bass, 1983; Galloway *et al.*, 1989;

Megyesi *et al*., 2005; Schiel, 2008; Brown & Peckmann, 2013; Knobel *et al.*, 2019; Marhoff *et al*., 2016; Suckling *et al.*, 2016). This appears to be an inherent characteristic of decomposition irrespective of the prevailing climatic factors. However, a comparison of different segments of this curvilinear shape of decomposition which includes an early rapid phase with a steep slope, a plateau and a resumption phase, reveals other peculiarities.

The early rapid phase was found to end at a lower TBS score in Pretoria, South Africa (Myburgh *et al.*, 2013) than in Nigeria. For example, during the South African winter in the Highveld, the rapid decomposition phase ended at a TBS score below 16 which is early decomposition, whereas it ended at a TBS score of just below 24 in the Nigerian dry season (Figure 4.36) which is advanced decomposition (Keough *et al*., 2017). Since this was reached over a shorter time in the Nigerian dry season, it indicates a more rapid decomposition which is expected in warmer tropical climates than a temperate climate. The same applies in the Pretoria summer (Myburgh *et al.*, 2013).

The occurrence of more multiple plateaus (more than one inactive phase) per sample exhibited by all the winter samples and 73% of the summer samples in Pretoria is common in temperate regions like the United States and Australia especially in winter (Suckling *et al*., 2016; Marhoff *et al*., 2016). This could be due to the wide differences between the possible maximum and minimum average daily temperature (12.7 ^{o}C – 26.25 ^{o}C) and therefore the wider daily fluctuations, up to 5 ^{o}C or 6 ^{o}C every few days which do not occur in Southern Nigeria. It could also be due to the complete cessation of decomposition on the rare occasions when temperature drops below freezing point in South Africa. Conversely, multiple plateaus occurred more in the wet than dry season (70% vs 40%) in Nigeria. This could be due to the militating effect of moisture on desiccation. Rainfall may have remoistened the drying carcass and arrested complete desiccation so that true preservation was not achieved; the

incompletely dried carcass resumed decomposition. This could also be due to the generally lower peak temperatures and the wider intervals between peak temperatures (Figure 4.14). This is unlike the dry season with higher peak temperatures and shorter intervals between peaks (Figure 4.5). This maintenance of the high temperature required for desiccation to occur is probably needed to keep the carcass in the desiccated state. Deactivation of enzyme and bacterial action, which is temperature dependent, is a contributory factor to sustaining desiccation (Amy *et al*., 1986; Myburgh *et al.*, 2013; Hayman & Oxenham, 2016). The generally narrower average daily temperature fluctuation (typically about 2 ^{o}C, Figures 4.5 and 4.14) in this study may also explain the generally less variable decomposition pattern in this study when compared with the study in Pretoria (Myburgh *et al*., 2013).

Another study in the hot Mediterranean-type climate of Cape Town, South Africa, reported a comparable desiccation rate to the present study due to high temperatures and low humidity especially in the summer (Finaughty and Morris, 2019). Precocious desiccation, occurring in less than 30 days in a temperate region, was found in about 31% of the carcasses with the earliest occurring in 17 days. This points out the diverse nature of the South African climate. Another similar finding between the present study and the Cape Town study (Finaughty and Morris, 2019) was the pattern of desiccation in the body regions, starting mostly from the head. Skeletonization also progressed in the same order, head and neck, limbs, and finally the trunk which was also the finding of Brown & Peckmann (2013) in the summer of Nova Scotia, Canada.

The narrower TBS range (TBS scores of 24 and 26) over which the plateau phases of the dry season samples in Nigeria occurred as compared to the winter sample in the South African Highveld (TBS scores between 17 and 23) (Figure 4.36) may point to the effect of complete desiccation (or preservation) on decomposition. The higher temperatures and lower humidity

in the Nigerian dry season encouraged desiccation (Figures 4.5 and 4.6). The scatter plot for the wet season/summer is not as distinct as that for the dry season/winter (Figure 4.37). This may be the effect of higher humidity in both seasons in Nigeria as Pretoria experiences summer rainfall like the Nigerian wet season. The plateau phases for the Nigerian wet season, however, occurred at a narrower TBS range than the Pretoria summer; TBS range between 24 and 30, and 18 and 35, respectively (Figure 4.37). This is likely due to the higher temperatures in Nigeria even in the wet season which had less daily fluctuation (Figure 4.14).

The samples that decomposed rapidly without a plateau were found mostly at the end of summer with diminishing rainfall. These samples were at the transition between seasons in the present study, and most of them had very brief plateaus. This rapid decomposition may be associated with the nature of the wet season which builds up gradually from April, peaks in July/August and gradually thins out until the beginning of the dry season. This same pattern was seen in decomposition rates as the rate was high at the transition between the dry and wet seasons and early wet season but diminished towards the peak and picks up thereafter as the rains diminished (Figure 4.18).

The most rapid decomposition was observed in Pig 7 which was the first wet season sample. It reached a TBS of 32 in only 11 days (ADD of 311.45) without a plateau. This implies that Pig 7 reached skeletonization in the linear phase of decomposition. Another pig which reached skeletonization in the linear phase was Pig 16 (TBS of 27, PMI of seven days and ADD of 190.1). When decomposition is examined between these pigs on day 7 PMI, Pig 7 had a TBS of 25 at ADD of 197.85, while Pig 16 had a TBS of 27 at ADD of 190.1. Although increasing ADD results in increasing decomposition rate, temperature does not fully account for all the changes of decomposition since Pig 7, with a higher ADD, had a lower TBS. Also, during the plateau phase,

ADD increases with little or no observable physical changes. This shows that other factors, both intrinsic and extrinsic, influence decomposition.

A striking feature of Pig 7 decomposition was the presence of numerous insects until skeletonization. The numerous post feeding larva seen in the skeletonization phase moved the bones around and partially buried some as they moved and burrowed. This was in the second month of the usual seven-month wet season, and the episodes of rainfall were still picking up. Also, the increased insect abundance and activity associated with the wet season (Tables 4.7 and 4.8), especially in early decomposition, aided its rapid decomposition. The delay in desiccation also allowed the insects to feed without inhibition.

Compared to another study in Ghana (Kyerematen *et al.*, 2013) which has a similar climate with Nigeria, differences were found in the duration of the stages of decomposition between the two studies. Differences were also found between the wet and dry seasons in Ghana in all the stages of decomposition. For example, while the fresh and bloat stages lasted one and two days, respectively, for both seasons in this study they lasted two and one day, respectively, in the wet season in Ghana, and one day each in the dry season. While the active decomposition lasted for three days in each season of this study, it lasted for longer in Ghana (six days for each season) (Kyerematen *et al.*, 2013). Also, whereas decomposition was more rapid in the wet season of this study, it was the reverse in Ghana which witnessed faster decomposition in the dry season. These differences between these two countries in the same region (tropical sub-Saharan Africa) and comparable weather conditions may be due to the differences in humidity when the study was performed; Ghana had higher humidity in the dry season of that year which resulted in more rapid decomposition in the dry season. This is in line with the report of Sharanowski *et al*. (2008) in Saskatchewan, Canada, that the prevailing weather condition will determine both the duration of overall decomposition and its stages.

In Saskatchewan, Canada, Sharanowski *et al*. (2008) found a fresh decomposition stage for sun-exposed remains of the same duration with the present study. The bloat and active stages of decomposition lasted from days 2 to 12, and days 13 to 30, respectively. Although these were much longer than was observed in the present study, the advanced decomposition which lasted from days 31 to 42 was shorter than in the present study. Carcasses deposited in fall and spring attracted more diverse species of insects than those deposited in summer.

The birds that were observed at the decomposition site of this study were mainly vultures and crows which typically had no access to the remains except on one occasion when two crows did. In South Africa, (Myburgh *et al.*, 2013) cattle egrets and smaller birds were the most common since the enclosure was demarcated with a wire mesh and, therefore, allowed only aerial scavengers except for a single case of meerkat. When scavengers have access to the remains, the black-backed jackals, mongooses, Cape porcupines and honey badgers were the observed scavengers in the Highveld of South Africa (Keyes *et al*., 2021).

These differences in decomposition from one region to the other reinforces the need for region-specific decomposition studies, and derivation of PMI models or formulae for the particular locality (Marhoff-Beard *et al.,* 2018).

5.5. Entomology

Apart from climatic conditions and other micro-environmental conditions, insects could cause considerable variation in the rate of decomposition (Mann *et al*., 1990; Vass *et al*., 2002; Bates & Wescott, 2016; Iancu *et al*., 2018). They are the first animals to arrive at the body, and exert their effect by spread of bacteria and larval feeding which accounts for the greatest amount of soft tissue loss during decomposition (Payne, 1965; Mann *et al.*, 1990; Bachmann & Simmons, 2010). Due to differences in species and behaviours of these arthropods among

regions and between seasons, and paucity of data on a generally accepted human proxy in Nigeria, it became important to observe, collect and identify these arthropods to produce a comprehensive data set of insects of forensic importance in southern Nigeria. Also, their succession patterns as they interact with their food source and other arthropods over the two seasons of the year were observed, despite studies carried out in other places, including studies of shorter duration in Nigeria (Bygarski & Leblanc, 2013; Carvalho *et al*., 2000; Ekanem & Dike, 2010; Grassberger & Frank, 2004; Kyerematen *et al.*, 2013; Mądra *et al*., 2015; Magni *et al*., 2019; Maisonhaute & Forbes, 2020; Martín-Vega *et al.*, 2019; Matuszewski *et al.*, 2011; Matuszewski *et al.*, 2013; Richards *et al*., 2009b; Wang *et al*., 2017). In this study, the succession patterns were then compared between the wet and dry seasons, and between this study and other studies.

The arthropods collected throughout the stages of decomposition included three classes, six orders and 16 families (Tables 4.7 and 4.8). Muscidae *(Musca domestica)* was mostly the first to arrive the scene followed closely by *Chrysomya marginalis*, visiting within 5 minutes and 10 minutes after placement, respectively. This is at variance with report of other studies in which Calliphorids arrived before Muscids (Byrd and Castner, 2010; Ekanem and Dike, 2010; Kyerematen *et al.*, 2013). There were very few instances in this study when the latter occurred. Ekanem and Dike (2010), following an earlier study on insect succession pattern at the end of the dry season in south eastern Nigeria, reported late arrival of Muscidae in the bloat stage. This difference could be due to slight microenvironmental or habitat differences (Villet, 2011); the location of the earlier study was near a ravine in a forest reserve which saw a different fauna that included crustaceans. It also captured succession for a short period which may not reflect the entire succession dynamics. The arrival of the first flies within minutes of deposition in this study could be exploited for more precise PMI estimates. This

implies that the minimum PMI or time of colonization derived from developmental biology of insect samples retrieved from the remains may be only a few minutes different from the actual PMI, giving a more precise estimate of the time since death.

A study in Jos, the central region of Nigeria, which utilised rabbit carcasses found other species of the Calliphoridae family like *Lucilia cuprina, Chrysomya albiceps, Chrysomya megacephala* and *Phormia regina* to be the first colonizers, closely followed by the common housefly *Musca domestica* in the fresh stage (Oladejo *et al.*, 2021). Carcass size and species difference, and differences in environment and climate may play a part in the observed differences in insect activity with the present study.

For the group of pig remains placed in the first dry season (December 21, 2019 – March 16, 2020), there was an obvious interlude in insect activity which coincided with late advanced decomposition at which time the tissues of the remains were completely desiccated and difficult to ingest. With the remoistening of the remains in the wet season, there was return of some insects, including those of forensic importance, to resume feeding and another round of their life cycle and decomposition that eventually led to skeletonization. This insect activity is of a much smaller scale with more of the Muscidae family, including the housefly, the flesh fly (Family Sarcophagidae) and very scanty or no blowflies. There were also very few maggots, and therefore the key larva predators like the black soldier ants were either very few or absent. These were shortly followed by beetles (*Platycorynus dejeani* and *Dermestes maculatus*). This resumption of decomposition following rehydration of the initially desiccated remains was also found in Pretoria, South Africa (Myburgh *et al.*, 2013).

The newly emerged insects from reared larva collected in the active decomposition stage were identified as *Lucilia sericata*, the common blowfly. Interestingly, the adult stage of this

insect was not observed at the research site throughout the period of study unlike other members of Calliphoridae like *Chrysomya marginalis* and the rare *Chrysomya chloropyga*. This may be due to nocturnal activity and oviposition of *Lucilia sericata* which was reported by an earlier study in the United States (Greenberg, 1990) and supported by another in north-western India (Singh and Bharti, 2001). A study in Nigeria which utilized the developmental biology of *Lucilia sericata* also reared the larvae (Ahmed and Joseph, 2016), so it is difficult to say if the adult fly was observed. This needs further study as earlier studies indicate that *Lucilia sericata* is widely distributed in Nigeria (Ekrakene & Iloba, 2011; Aigbodion *et al.*, 2013; Ndueze *et al*., 2013).

On account of carrying out part or their complete life cycle on or around the remains, habitually visiting the carrion and leaving clues to forensic experts, the insects of forensic importance in this study were of the families Calliphoridae (*Chrysomya marginalis, Chrysomya chloropyga and Lucilia sericata*), Muscidae, Chrysomelidae (*Platycorynus dejeani*), Dermestidae (*Dermestes maculatus*), Stratiomyidae (*Hermetia illucens*), Gyrinidae, Scarabaeidae and Staphylinidae.

For the Calliphoridae, *Chrysomya marginalis* was the most abundant and therefore of the greatest forensic importance. Irrespective of season, these insects appeared within 10 minutes of deposition of the remains and stayed on the carcass in various stages of their life cycle until the time the post feeding larvae moved away to pupate, or when desiccation occurred.

The beetles that were collected on the carrion were exclusively of the Coleoptera order. Chrysomelidae (*Platycorynus dejeani*) were the most dominant beetles, appearing in active decomposition and staying throughout until skeletonization in the wet season, or advanced

decomposition in the dry season. This is followed by Dermestidae (*Dermestes maculatus*) which left the carcass earlier and the larvae of which were observed. These two beetles appeared in both seasons. Other beetles of forensic importance were Gyrinidae which were observed from the bloat to the advanced stages, and Scarabaeidae which appearance were rather short-lived and both of which appeared only in the wet season. Their seasonal predilection could indicate the season of death (Matuszewski *et al*., 2013).

The arthropod succession patterns of the wet and dry seasons were similar. However, there were obvious differences in the absolute number and species composition which were both increased in the wet season. Those exclusively present in the wet season included the families Gyrinidae, Stratiomyidae (*Hermetia illucens*) and Scarabaeidae (Table 4.9). This difference in arthropod population composition and absolute number according to season or time of the year is documented in the literature (Richards *et al*., 2009a; Richards *et al.*, 2009b; Villet, 2011; Parry *et al.*, 2016; Tembe and Mukaratirwa, 2021). This is attributed to climatic factors such as temperature and humidity (Michaud and Moreau, 2009; Richards *et al.*, 2009a; Matuszewski *et al.* , 2013; Parry *et al.*, 2016; Tembe and Mukaratirwa, 2021). This seasonal difference is important as the insects found on the remains may indicate the season of death which also affects decomposition rate (Matuszewski *et al.*, 2011; Mądra, Frątczak, Grzywacz and Matuszewski, 2015; Díaz-Aranda *et al.*, 2018). Considering that three of the four families exclusive to the wet season were beetles which have articulated mouthparts for chewing hard or desiccated remains (Byrd and Castner, 2010), this is a contributor to faster decomposition.

Arthropod larvae also acted for longer in the wet season as the remains remained moist for longer than in the dry season. This ensured that insects like the Formicids which scavenged on larva also acted longer in the wet season. This confirms the generally acknowledged role of insects as a major force in decomposition (Payne, 1965; Jirón & Cartín, 1981; Mann *et al.*,

1990; Simmons *et al.*, 2010). For example, during desiccation which was more common and occurred for longer periods in the dry season, the dispersion of the insects and the generally lower number of beetle species in this season, worsened the low decomposition rate on account of desiccation alone. It appears obvious again that temperature, the key factor in desiccation and most other processes associated with decomposition including insect viability and activity (Reed, 1958; Payne, 1965; Mann *et al.*, 1990; Campobasso *et al*., 2001; Dadour *et al.*, 2001; Dadour *et al*., 2001; Simmons *et al.*, 2010) are the most important factors in sub-aerial decomposition (Simmons *et al.*, 2010).

The insect succession patterns between Nigeria and South Africa will likely vary due to expected differences in the composition of carrion insect population from region to region, especially when this comparison is between a tropical and a temperate region (Shin *et al.*, 2015; Parry *et al.*, 2016). Like the present study, Kelly *et al.* (2009) found that the first arthropod to visit the remains in central South Africa were the Muscids (*Musca domestica*) followed by Calliphorids. Unlike the present study, however, which recorded both flies in the fresh stage, Kelly *et al*. (2009) found the Calliphorids appearing for the first time in the bloated stage. This could be because the carcasses in their study were wrapped. Another study in KwaZulu-Natal found that Calliphorids such as *Chrysomya marginalis*, *Chrysomya chloropyga,* and *Chrysomya putoria* were the first visitors and that they appeared before the Muscids (*Musca domestica*). However, they all appeared within a few hours in the fresh stage. The bloat stage witnessed the highest number of Dipterans like in earlier studies (Shi *et al.*, 2009; Keshavarzi *et al.*, 2019; Tembe and Mukaratirwa, 2021). This has been reported to be due to the strong odour and their preference for soft tissues made even softer by both autolysis and putrefaction (Goff, 1993; Verheggen *et al.*, 2017). The period of activity of the *Chrysomya* species up to the advanced stage in the dry season of the present study is similar to the finding

in KwaZulu-Natal (Tembe and Mukaratirwa, 2021); the early termination of their activity in the wet season could be due to the abundance of both larva and their predators such as the ants (Formicidae) in the wet season. These ants also fed on adult flies.

With respect to the effect on rainfall on insect activity, both the present study and the study in Pretoria (Myburgh *et al.*, 2013) found that rainfall episodes reduced insect numbers but the insects return to continue their activity when the water content of the remains reduces. Overall, there was more insect activity in the wet season of Nigeria and the summer in the Highveld of South Africa. The summer season of the Highveld region is, therefore, similar to the wet season in Nigeria in that there was rainfall, increased humidity, and increased insect activity. These insects may attract other animals like the birds to the site of the study especially as they migrate to pupate.

The beetles that visited the remains in this study were exclusively from the order Coleoptera just like what was found in studies in South Africa (Kelly *et al*., 2009; Kelly *et al.*, 2011; Tembe and Mukaratirwa, 2021). The most dominant beetle was *Platycorynus dejeani* followed by *Dermestes maculatus,* but the former did not appear in the carrion-feeding insects in South Africa. The appearance of *Dermestes maculatus* in the active stage agrees with the findings of Kelly *et al*. (2009) in Central South Africa but not in KwaZulu-Natal (Tembe and Mukaratirwa, 2021) where they appeared earlier in the bloat stage. Unlike the present study in which the activity of *Dermestes maculatus* ended in advanced decomposition, it persisted to the dry remains stage in both KwaZulu-Natal and central South Africa (Kelly *et al*., 2009; Tembe and Mukaratirwa, 2021). This could be due to the prolonged advanced decomposition with desiccation during which time the carcasses usually consist of bones covered with desiccated hide which may be too tough for the beetles unless they are rehydrated by rain. This may be unsuitable for the beetles which may quit the site at this stage**.** Like our study,

studies in South Africa (Kelly *et al.*, 2009; Kelly *et al.*, 2011; Tembe and Mukaratirwa, 2021) agree that *Dermestes maculatus* is a dominant beetle species in carrion decomposition. Scarabaeidae appeared in the bloat stage both in the present study and in KwaZulu-Natal (Tembe and Mukaratirwa, 2021). The finding of *Hermetia illuscens* in the wet season confirms the report of its forensic significance in Africa by Villet (2011).

The species of insects that are common to Nigeria and South Africa appear to be those that are found in the warmer summers in South Africa (Braack, 1986; Villet, 2011; Williams and Villet, 2019). This is probably because Nigeria has no winter-like season which reinforces the effect of climatic factors in the species composition and abundance of carrion arthropods. However, among those insects which do not exactly match between any two regions may be representatives in the respective region that allow for fair comparisons to be made between regions with different climates (Villet, 2011).

In the present study, it was difficult to distinguish between the first and subsequent waves of colonization, but especially between the first and second waves. What was obvious was the increasing number of flies on the carcass in the first four to six days after placement. This could be due to the rapid progress of decomposition in this region. However, it appears that the usual late appearance of *Chrysomya chloropyga* on the second or third day after carcass placement, and more flies especially from the Muscidae family represents this second wave of colonization. The flesh flies, more blowflies, house flies are known to be the common flies in the second wave and this coincides with the time when the carcass has developed an odour unlike the first wave that occurred a few minutes after placement (Gullan and Cranston, 2010).

The blowfly was the most dominant fly in both the present study and another in Saskatchewan, Canada. However, whereas it was *Chrysomya marginalis* in the present study, it was *Phormia regina* and *Protophrmia terraenovae* in Saskatchewan. The blowflies appeared in the fresh stage in both studies, *Chrysomya marginalis* in the present study and *Cynomya cadaverina* in Saskatchewan (Sharanowski *et al*., 2008). These differences in the duration of decomposition stages and arthropod species and succession patterns are expected when comparing one study in tropical Africa with another with a continental climate.

There was a slight difference in both the available blowfly species and duration of decomposition stages between the urban and rural setting in the summer of urban Tasmania (Magni *et al.*, 2019). Whereas the urban site recorded the presence of *Calliphora stygia* and *Lucilia sericata* in the fresh stage, it was only *Calliphora stygia* at the urban site.

The blowfly was the most significant forensic fly in both the present study and the study in Ghana (Kyerematen *et al.*, 2013); while it was *Chrysomya marginalis* in this study, and *Chrysomya rufificacies* in Ghana. The difference could be due to factors such as competition, food quality, the prevailing insect species, and predation which are important factors that affect carrion insect availability and succession (Matuszewski *et al*., 2013; Richards *et al*., 2009b; Villet, 2011; Williams, 2003). The finding of more insect families and species in the dry season of Ghana (Kyerematen *et al.*, 2013), underlines the positive influence of humidity on insect abundance and decomposition rate.

Another study in the Kirimiro region of Burundi (Dushimirimana *et al.*, 2021) which utilized mice carcasses found *Monomoriun pharaonis* and *Leptothorax acervorum* , both of the Family Formicidae, to be both the first insects to visit the carcasses and by far the most dominant.

This could be explained by the difference in carcass species and size (Kneidel, 1984; Hewadikaram & Goff, 1991; Simmons, Adlam, *et al.*, 2010).

Being the first long term study on decomposition and carrion insect succession patterns in Nigeria, forensic science researchers can use the generated information and locally derived formula as a basis for further research and practical application. Also, relating entomological data to the TBS and ADD in future studies will provide valuable information about the role of insects in decomposition and how they can be better utilised for PMI estimation.

5.6. Estimating PMI when decomposed remains are found

A combination of the progress of decomposition represented by the TBS and insect activity from this study could be used to arrive at the PMI estimate of discovered remains.

When only adult blowflies (*Chrysomya marginalis*) and the common housefly (*Musca domestica*) are found on the remains, and the remains are in the fresh stage of decomposition with a TBS between 1 and 3, the PMI is within 24 hours.

If the flies are numerous (including *Chrysomya marginalis*, *Chrysomya chloropyga*, *Musca domestica* and the flesh fly) with the remains at about the maximum bloat and have a TBS between 8 and 20, the PMI is between 2 and 4 days. The finding of few mature fly larvae (brownish) with others burrowing into the surrounding soil (in the dry season) or around dry areas around the corpse (in the wet season), pupae or pupal casings, juvenile flies, and a TBS between 20 and 23, indicates a PMI of about 13 days.

As advanced decomposition continues, especially with the occurrence of stalled or prolonged desiccation, it becomes increasingly difficult to predict the PMI by only gross visual observation. This is because of the prolonged time the remains stay in this state of desiccation, especially in the dry season. However, the finding of an adult insect or its larva

that is exclusively found in the wet season, like the black soldier fly (*Hermetia illucens*) (Figure 4.40e) or the beetles of the Gyrinidae family (Figure 4.40d), in this state of desiccation indicates that death occurred in the wet season.

Burrowing by post-feeding larvae produce a characteristic breaking up of the mainly clay soil into smaller clods around the remains (Figures 4.19 d2 and 4.43). If remains which have reached the late active and advanced decomposition is found on soil without this finding of broken up soil, it may be assumed that the body was moved from its primary place of death.

The ADD formula should be used when weather station temperature data are available. The number of days it takes, counting backwards, for the actual accumulated temperature to equal the calculated ADD is the post-mortem interval in days. This is expected to be more accurate than the PMI method since temperature, not PMI, is the driving force for decomposition. But when weather station temperature data are not available, the PMI method should be used.

The variable nature of decomposition reflected in the widening limit between the upper and lower limits of the forecasted ADD as the TBS increases, however, means that the reliability of this method is reduced in later decomposition.

5.7. Difficulties, limitations and future directions

The limitations of this study included frequent bush burning pre-cultivation or for hunting in the dry season which affected one of the pig remains. This pig cadaver was replaced due to differences in taphonomy between burnt and unburnt carcass (Ubelaker, 2009). Access to the research site was limited due to complete restriction of movement to curtail the spread of COVID-19 and so data collection was halted for a couple of weeks. Data collection commenced immediately after restrictions were relaxed.

With the failure of numerous data loggers, we had to rely on data from the nearest weather station for temperature and relative humidity data which may not be exactly representative of the weather conditions at the research site but does reflect what will happen in reality when a case is discovered.

We were unable to identify all the insects to the species level. This was due to unavailability of local entomologists willing to identify the insects. The insects were then identified by an entomologist at the Department of Forensic Medicine, University of the Witwatersrand by the use of photographs of the insects.

An inter-carcass distance of 50m has been shown to reduce cross-contamination by migrating larvae (Matuszewski *et al.,* 2019; Tesmer and Meek, 1996; Lewis and Benbow, 2011). The shorter distance of 10m used in this study may present a confounder.

A future study will look at the effect of carcass size on decomposition in Nigeria. Data collected on weight and body dimensions obtained in this study could be useful in this regard. Studies on the influence of size on decomposition rate and the arthropod community attracted to the carcasses are, therefore, encouraged. Also, it is important to derive an ideal weight range of pig cadavers for studies representative of the various age weights in the general population in Nigeria so that these studies can bear some relationship to the populace and find practical use.

Since humidity, rather than temperature, was suggested to be largely responsible for the differences in decomposition between the wet and the dry seasons, further studies on the influence of humidity on decomposition should be carried out.

Desiccation and how it affects PMI estimation is an important area to explore, especially in regions where this secondary change occurs early. This will allow for models that incorporate

this change and assigns appropriate TBS with respect to the time of its appearance. This will ensure that less obvious changes in this seemingly inactive phase are accounted for in order to minimise errors in PMI estimation.

Future studies on arthropod succession patterns of a longer duration, say two years and above, will determine if arthropod succession patterns are the same for similar seasons for the duration of the study.

There is also a need to study the effect of previous decomposition sites, which may harbour arthropods in different stages of development, on decomposition rate through cross-colonization even when the skeletonized remains have been removed. This will enable researchers to eliminate this confounding factor in decomposition research if found to have an effect.

Forensic scientists are encouraged to utilise the formulae generated from this research. Testing of the PMI estimation formulae derived from this study on a pig model are encouraged both in southern Nigeria and in other regions to see if application is restricted to this region or can be used in other regions. Although differences are expected since pigs were used as proxies, the formulae could be tried for PMI estimation of human remains undergoing aerial decomposition to know what these differences are and how best to use them when human remains are discovered.

The weather observed during this study may not be absolutely representative of the climate in all the regions of Nigeria. This is because the more arid northern Nigeria, which is closer to the Sahara Desert, has a longer dry season and a shorter wet season. Studies are, therefore, encouraged in northern Nigeria to see what differences, if any, exist in the decomposition rate, pattern and insect community composition and succession patterns.

It is obvious that the findings of this study do not represent bodies that are exposed to carnivorous scavengers where decomposition progresses faster due to feeding. Post-mortem interval estimation studies to assess the effect of scavenger activity will give interesting and useful insights for the typical bodies which are not protected from scavengers. In the same vein, indoor studies will give an idea of the possible differences in the pattern and rate of decomposition in such conditions in Nigeria.

Testing of other formulae derived outside Nigeria will help determine whether those formulae are restricted to the regions where they were generated.

Chapter 6: Conclusions

This study provides the first data on long term decomposition and insect succession pattern on 20 pig carcasses studied over 14 months in an outdoor setting in southern Nigeria. It also produced the first locally derived formulae for the estimation of PMI in Nigeria.

It was found that:

1. Decomposition followed a characteristic curvilinear pattern.
2. Decomposition progressed rapidly with marked seasonal differences. In the dry season, late advanced decomposition took 6 – 11 days (average of 9.2 days) while it was 10 – 17 days (average of 13 days) in the wet season.
3. Decomposition was faster at the transition between the seasons.
4. Decomposition rate slowed at the peak of the wet season when there were more frequent episodes of rainfall. This is likely due to the usual avoidance of drenched remains, and physical disturbance of the adult fly by rainfall which prevents it from feeding. Larval feeding is also interrupted by rainfall as they are washed away from the pig cadaver.
5. Stalled or prolonged desiccation was a common experience. This was more common in the dry season with 90% (n=9) of the pig sample undergoing this change than in the wet season with 60% (n=6) of the wet season sample. This was reached earlier in the wet season (6 – 11 days; average of 9.2 days) than in the dry season (10 – 17 days; average of 13 days). Also, desiccation lasted longer in the dry season (average of 119 days) than in the wet season (average of 29 days).
6. Formulae were derived that can be used to estimate post-mortem interval for both when the season is known and unknown. The derived formulae provide a working tool

for forensic scientists when estimating PMI in southern Nigeria and other regions with similar climatic conditions. Although caution should be applied in the direct application of these formula to human remains due to obvious differences between pig and human decomposition, a combination of the use of these formulae and further studies are encouraged to find out the implication of these differences in Nigeria.

7. Humidity may mostly explain the obvious seasonal differences in decomposition with a relative humidity average of 65.2% in the dry season and 81% in the wet season. More insect species abundance was observed in the wet season which also acted for longer. Since the average daily temperature between seasons is not considerable (27.1^{o}C in the wet season, and 29.3^{o}C in the dry season), humidity is a stronger factor in this difference than temperature.
8. The first insects to visit the pig cadavers were the common housefly *Musca domestica* and *Chrysomya marginalis*, a blowfly. The former visited within 5 minutes after depositing the pig cadaver, and the latter followed about 5 minutes later. This short period of colonization could be exploited for more precise PMI estimates when these flies are found on remains.
9. Some arthropods visited the pig cadavers exclusively in the wet season. Examples are beetles of the Family Gyrinidae and the black soldier fly (*Hermetia illucens*). When these insects or their pupal casings are recovered from remains, it could indicate that early decomposition occurred in the wet season.
10. A list of the arthropods of forensic importance and the arthropod succession patterns in southern Nigeria was created. This would serve as a reference, a guide, or a starting point for forensic scientists in Nigeria.

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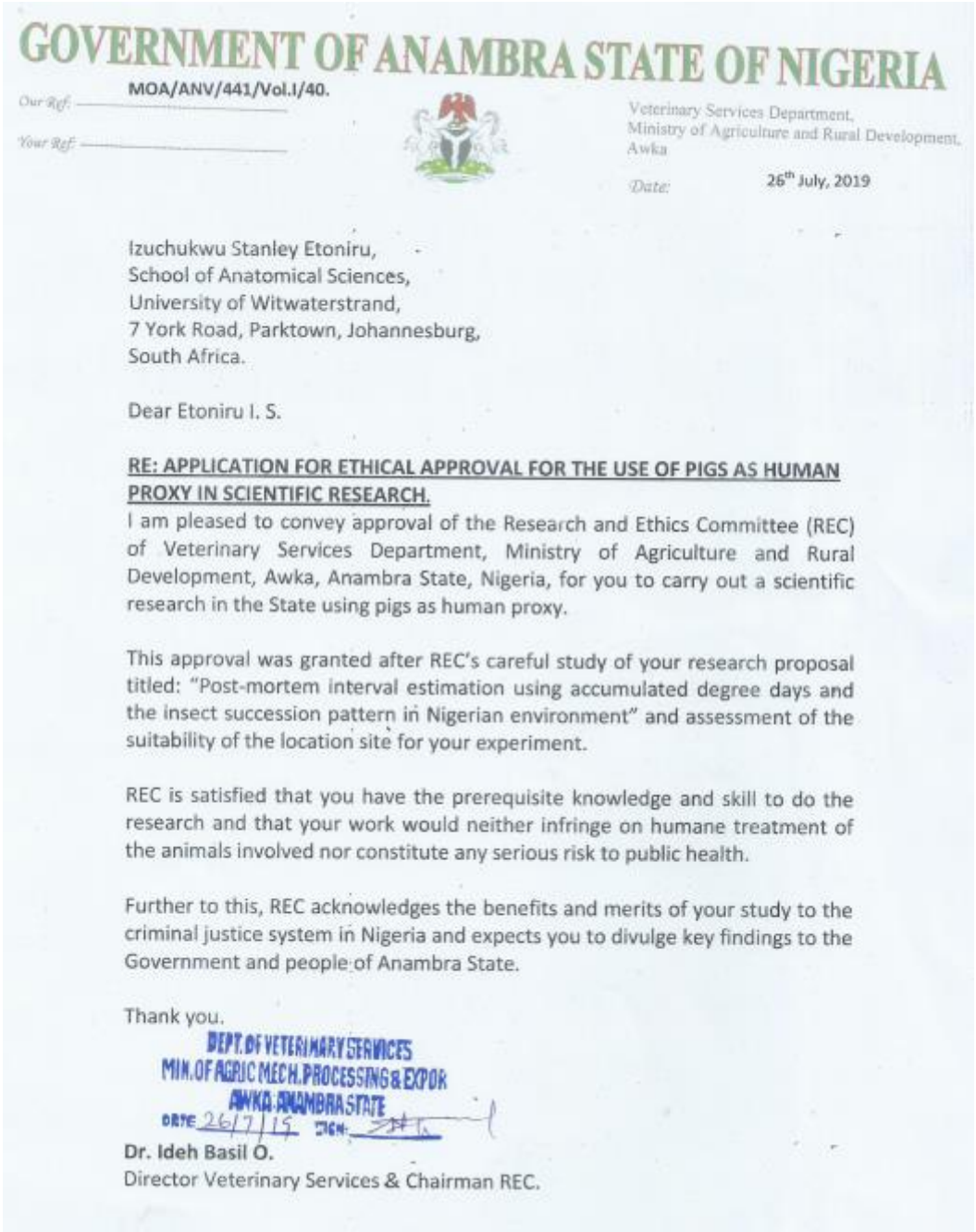
APPENDICES

Appendix A: ethical clearance from the Research and Ethics Committee (REC) of the Veterinary Services Department of the Ministry of Agriculture and Rural Development in Nigeria



GOVERNMENT OF ANAMBRA STATE OF NIGERIA

Our Ref: MOA/ANV/441/Vol.I/40.

Your Ref: _____

Veterinary Services Department,
Ministry of Agriculture and Rural Development,
Awka

Date: 26th July, 2019

Izuchukwu Stanley Etoniru,
School of Anatomical Sciences,
University of Witwaterstrand,
7 York Road, Parktown, Johannesburg,
South Africa.

Dear Etoniru I. S.

RE: APPLICATION FOR ETHICAL APPROVAL FOR THE USE OF PIGS AS HUMAN PROXY IN SCIENTIFIC RESEARCH.

I am pleased to convey approval of the Research and Ethics Committee (REC) of Veterinary Services Department, Ministry of Agriculture and Rural Development, Awka, Anambra State, Nigeria, for you to carry out a scientific research in the State using pigs as human proxy.

This approval was granted after REC's careful study of your research proposal titled: "Post-mortem interval estimation using accumulated degree days and the insect succession pattern in Nigerian environment" and assessment of the suitability of the location site for your experiment.

REC is satisfied that you have the prerequisite knowledge and skill to do the research and that your work would neither infringe on humane treatment of the animals involved nor constitute any serious risk to public health.

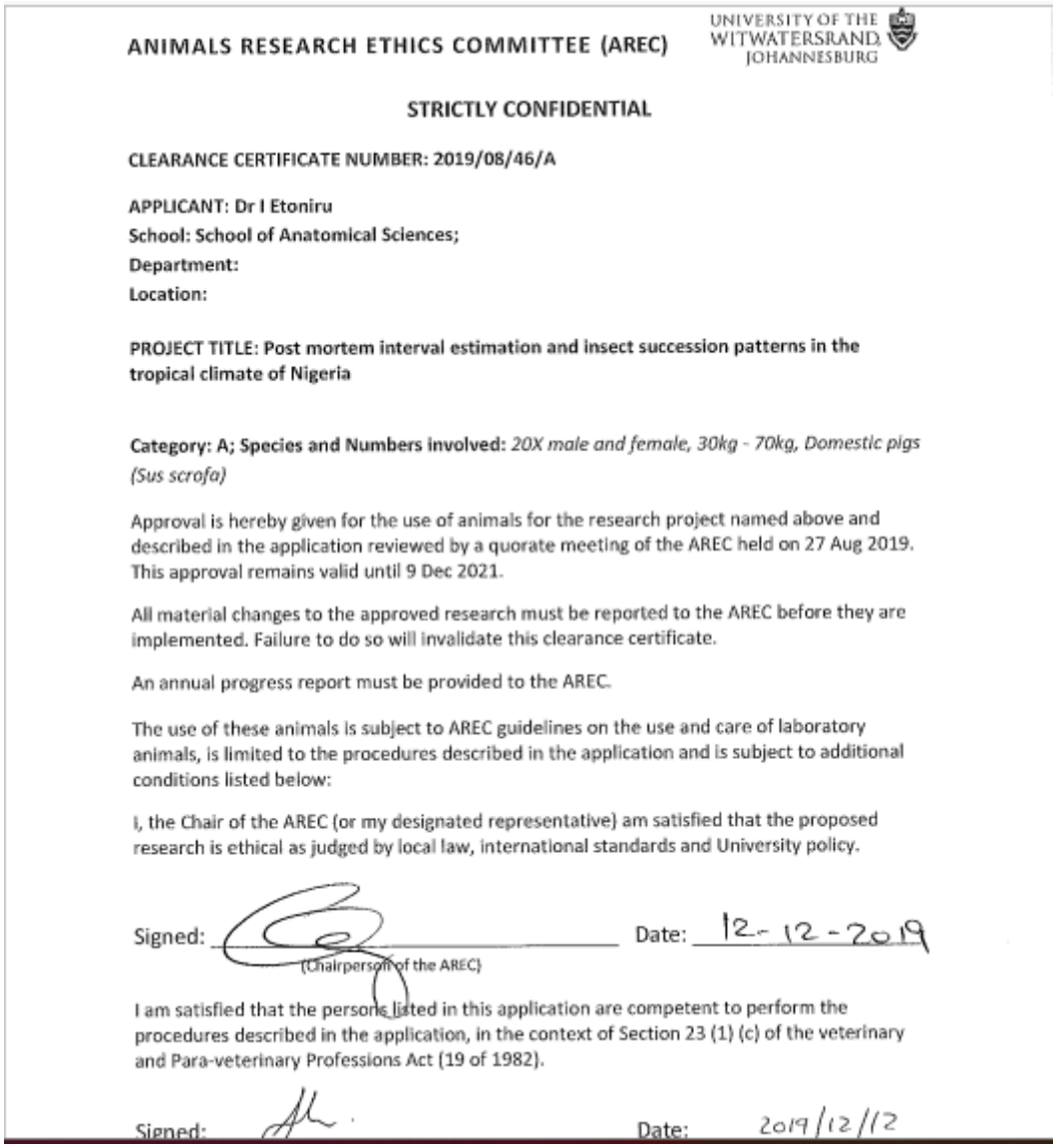
Further to this, REC acknowledges the benefits and merits of your study to the criminal justice system in Nigeria and expects you to divulge key findings to the Government and people of Anambra State.

Thank you.

DEPT. OF VETERINARY SERVICES
MIN. OF AGRIC MECH. PROCESSING & EXPOR
AWKA ANAMBRA STATE
DATE 26/7/19 SIGN:

Dr. Ideh Basil O.
Director Veterinary Services & Chairman REC.

Appendix B: Ethical clearance from the Animal Research Ethics Committee, University of the Witwatersrand, South Africa



ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2019/08/46/A

APPLICANT: Dr I Etoniru
School: School of Anatomical Sciences;
Department:
Location:

PROJECT TITLE: Post mortem interval estimation and insect succession patterns in the tropical climate of Nigeria

Category: A; Species and Numbers involved: *20X male and female, 30kg - 70kg, Domestic pigs (Sus scrofa)*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 27 Aug 2019. This approval remains valid until 9 Dec 2021.

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

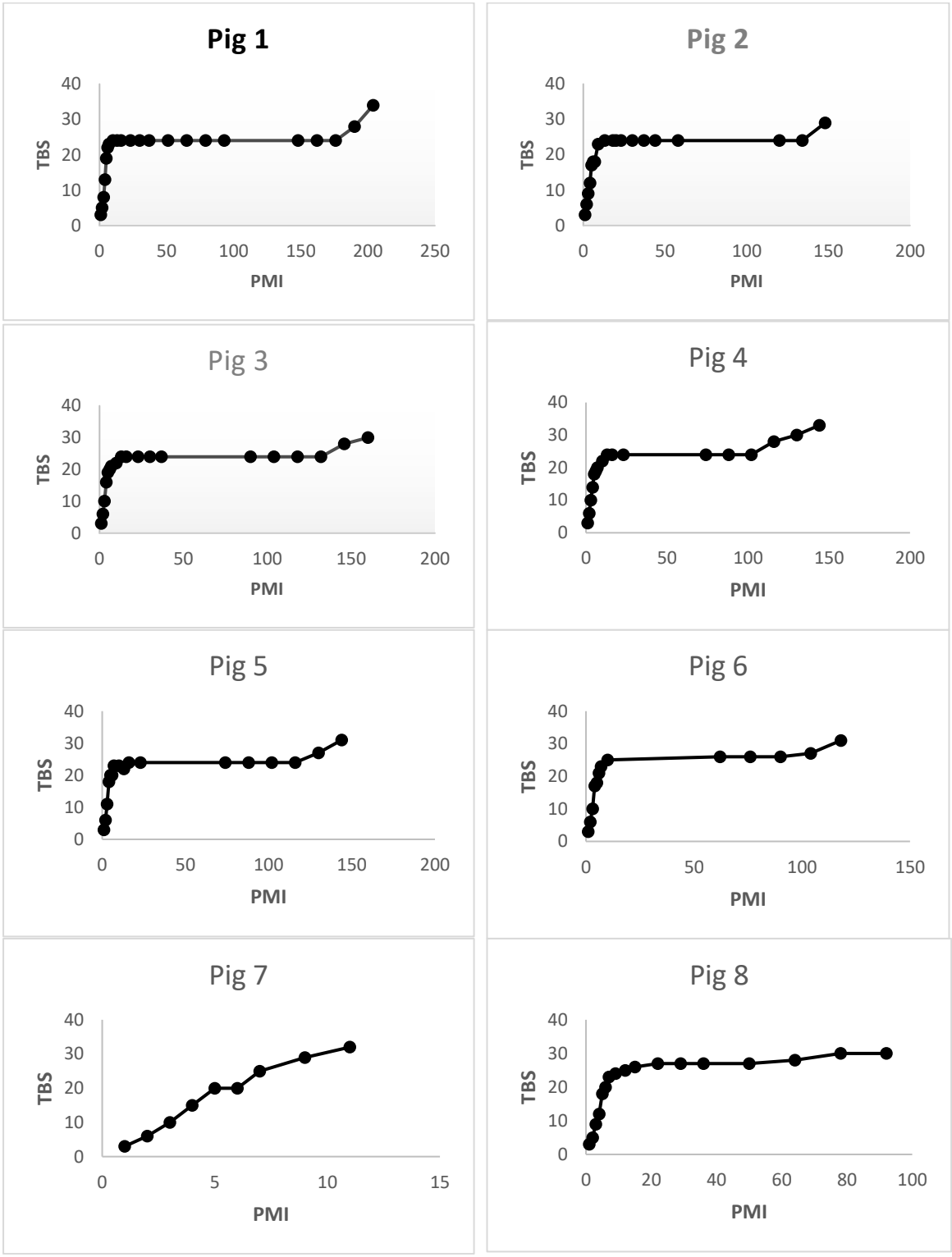
I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

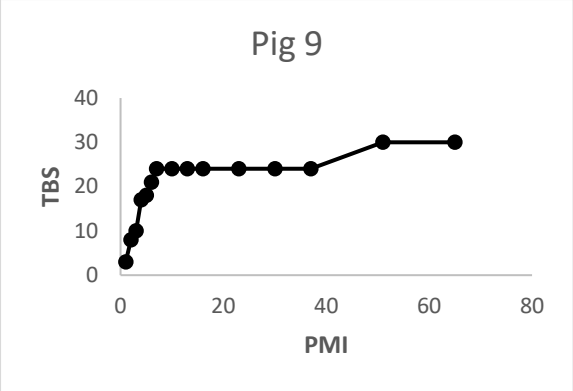
Signed: _____ Date: 12-12-2019
(Chairperson of the AREC)

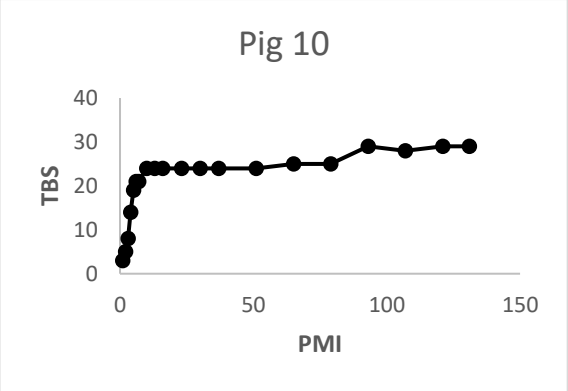
I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

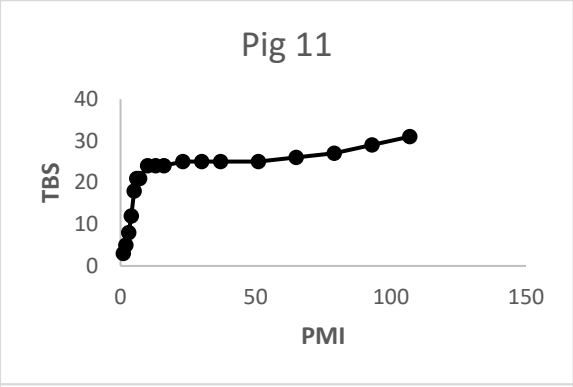
Signed: _____ Date: 2019/12/12

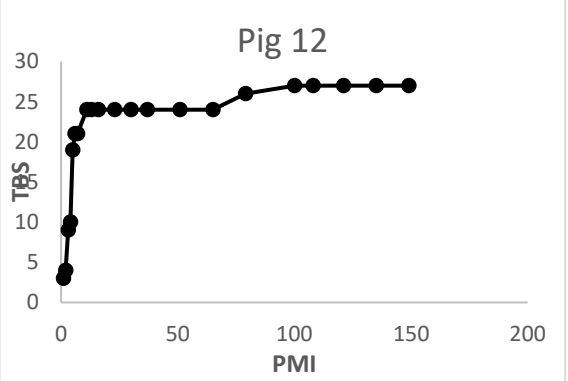
Appendix C: Scatter plots of TBS vs PMI for individual pigs

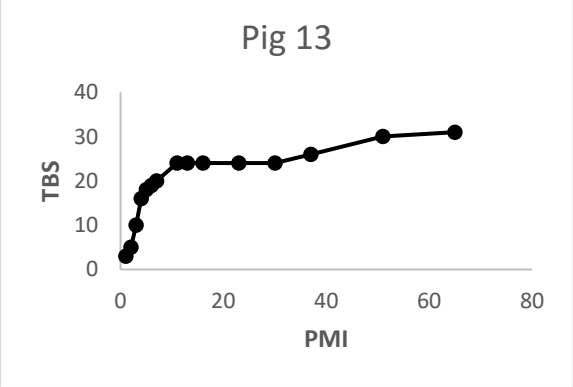


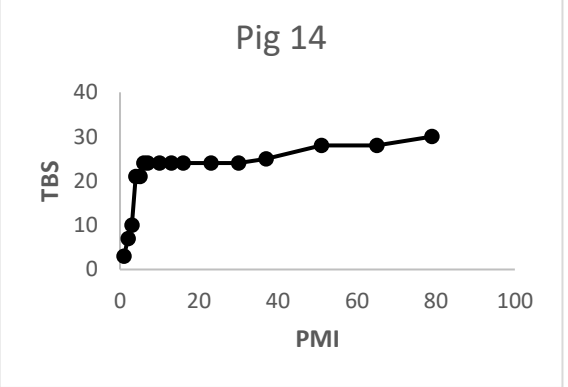

Pig 9
TBS
PMI
0
10
20
30
40
0
20
40
60
80

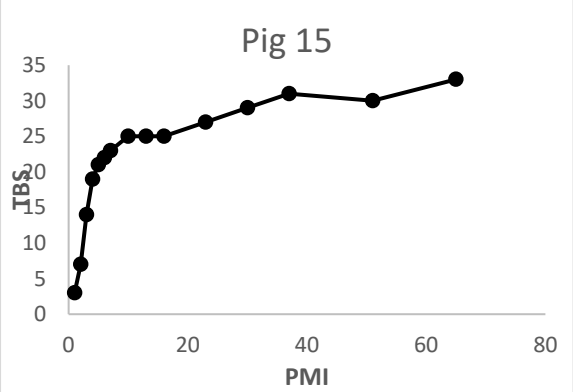

Pig 10
TBS
PMI
0
10
20
30
40
0
50
100
150

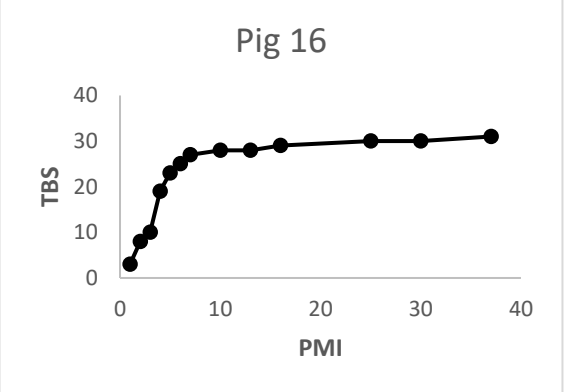

Pig 11
TBS
PMI
0
10
20
30
40
0
50
100
150

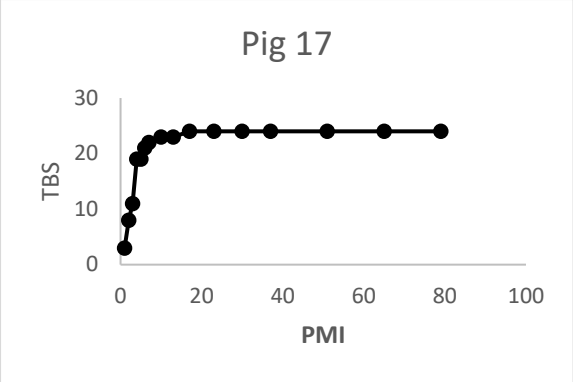

Pig 12
TBS
PMI
0
5
10
15
20
25
30
0
50
100
150
200

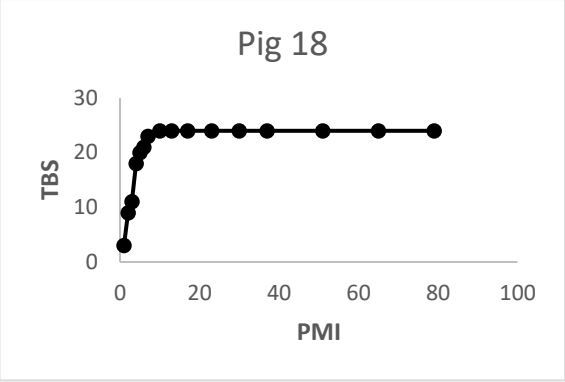

Pig 13
TBS
PMI
0
10
20
30
40
0
20
40
60
80

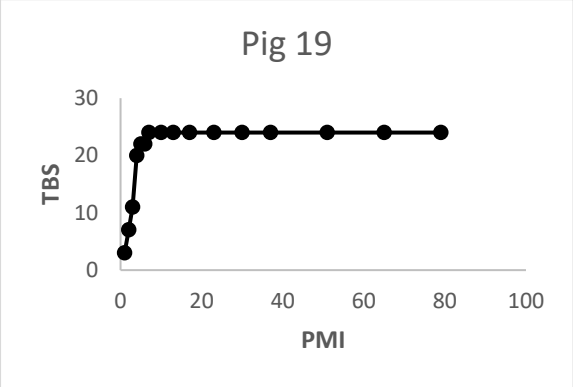

Pig 14
TBS
PMI
0
10
20
30
40
0
20
40
60
80
100

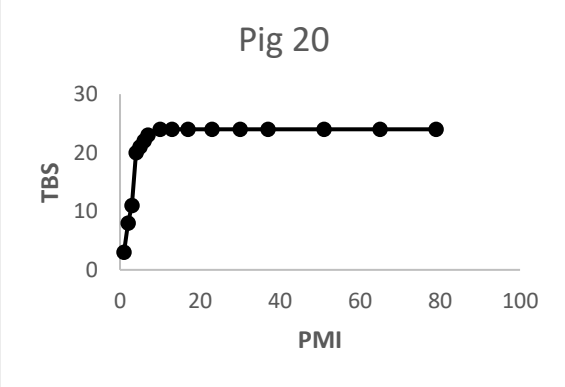

Pig 15
TBS
PMI
0
5
10
15
20
25
30
35
0
20
40
60
80


Pig 16
TBS
PMI
0
10
20
30
40
0
10
20
30
40


Pig 17
TBS
PMI
0
10
20
30
0
20
40
60
80
100


Pig 18
TBS
PMI
0
10
20
30
0
20
40
60
80
100


Pig 19
TBS
PMI
0
10
20
30
0
20
40
60
80
100


Pig 20
TBS
PMI
0
10
20
30
0
20
40
60
80
100

Appendix D: TBS of the 2 pigs scored by the primary observer for repeatability testing

Day		1	2	3	4	5	6	7	10	13	16	23	30	37	51	65	79	93	107
	Head	1	2	3	4	8	8	8	9	9	9	9	9	9	9	9	9	9	11
	Trunk	1	2	3	4	5	7	7	8	8	8	8	8	8	8	8	9	10	10
PIG 11	Limb	1	1	2	4	5	6	6	7	7	7	8	8	8	8	9	9	10	10
Total TBS		3	5	8	12	18	21	21	24	24	24	25	25	25	25	26	27	29	31
Day		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**13**	**16**	**23**	**30**	**37**	**51**	**65**	**79**		
	Head	1	3	4	8	8	9	9	9	9	9	9	9	9	9	9	11		
	Trunk	1	2	3	7	7	8	8	8	8	8	8	8	8	10	10	10		
PIG 14	Limb	1	2	3	6	6	7	7	7	7	7	7	7	8	9	9	9		
Total TBS		3	7	10	21	21	24	24	24	24	24	24	24	25	28	28	30		

Appendix E: TBS of the 2 pigs scored by the external individual, using photographs, for repeatability testing

Day		1	2	3	4	5	6	7	10	13	16	23	30	37	51	65	79	93	107
	Head	1	1	2	5	8	8	8	8	10	10	10	11	11	11	11	11	11	11
	Trunk	1	1	2	3	4	5	6	7	7	7	7	8	8	8	8	10	10	10
PIG 11	Limb	1	1	1	4	4	6	8	8	8	8	8	8	8	8	9	9	9	9
Total TBS		3	3	5	12	16	19	22	23	25	25	25	27	27	27	28	30	30	30
Day		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**13**	**16**	**23**	**30**	**37**	**51**	**65**	**79**		
	Head	1	1	3	8	8	8	8	8	8	8	8	8	9	9	10	11		
	Trunk	1	2	3	4	4	7	7	7	7	7	8	8	10	10	10	11		
PIG 14	Limb	1	2	2	5	5	5	5	6	6	6	7	7	8	9	9	9		
Total TBS		3	5	8	17	17	20	20	21	21	21	23	23	27	28	29	31		

Appendix F: TBS of the 2 pigs scored by the primary observer for intra-observer repeatability testing

Day		1	2	3	4	5	6	7	10	13	16	23	30	37	51	65	79	93	107
	Head	1	2	4	5	8	8	8	9	9	9	9	9	9	9	9	9	9	11
	Trunk	1	2	3	3	5	7	7	7	8	8	8	8	8	8	9	10	10	10
PIG 11	Limb	1	2	2	4	5	6	6	7	7	7	7	7	7	8	8	9	9	10
Total TBS		3	6	9	12	18	21	21	23	24	24	24	24	24	25	26	28	28	31
Day		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**	**13**	**16**	**23**	**30**	**37**	**51**	**65**	**79**		
	Head	1	3	3	8	8	9	9	9	9	9	9	9	9	9	10	11		
	Trunk	1	2	3	7	7	7	7	8	8	8	8	8	10	10	10	10		
PIG 14	Limb	1	2	3	6	6	6	6	7	7	7	7	7	8	8	9	10		
Total TBS		3	7	9	21	21	24	24	24	24	24	24	24	27	27	29	31		

Appendix G: Data for the Post-mortem Interval value, head and neck score, trunk score, limbs score, Total Body Score value, average temperature, Accumulated Degree-Days value, logarithmic Post-mortem Interval value and logarithmic Accumulated Degree-Days value for individual pigs.

pig_num	season	pig_obs	pmi_sco	han_sco	tru_sco	lim_sco	tbs_sco	tem_avg	add_avg	log_pmi	log_add
1	Dry	101	1	1	1	1	3	28.75	28.75	0	1.458638
1	Dry	102	2	2	2	1	5	26.05	54.8	0.30103	1.738781
1	Dry	103	3	3	3	2	8	26	80.8	0.477121	1.907411
1	Dry	104	4	4	5	4	13	25.55	106.35	0.60206	2.026737
1	Dry	105	5	8	6	5	19	26.45	132.8	0.69897	2.123198
1	Dry	106	6	9	7	6	22	26.25	159.05	0.778151	2.201534
1	Dry	107	7	9	7	7	23	26.25	185.3	0.845098	2.267875
1	Dry	108	10	9	8	7	24	26.6	264.85	1	2.423
1	Dry	109	13	9	8	7	24	24.8	342.3	1.113943	2.534407
1	Dry	110	16	9	8	7	24	26.95	418.15	1.20412	2.621332
1	Dry	111	23	9	8	7	24	29.5	614.9	1.361728	2.788804
1	Dry	112	30	9	8	7	24	26.95	813.65	1.477121	2.910438
1	Dry	113	37	9	8	7	24	25.7	1010	1.568202	3.004321
1	Dry	114	51	9	8	7	24	28.75	1396.85	1.70757	3.14515
1	Dry	115	65	9	8	7	24	32.95	1824.3	1.812913	3.261096
1	Dry	116	79	9	8	7	24	29	2261.3	1.897627	3.354358
1	Dry	117	93	9	8	7	24	29.7	2693.2	1.968483	3.430269
1	Dry	118	148	9	8	7	24	29.4	4290.55	2.170262	3.632513
1	Dry	119	162	9	8	7	24	27	4689	2.209515	3.67108
1	Dry	120	176	9	8	7	24	27.25	5079.2	2.245513	3.705795
1	Dry	121	190	9	10	9	28	26.45	5456.85	2.278754	3.736942
1	Dry	122	204	13	11	10	34	27.2	5834.5	2.30963	3.766004
2	Dry	201	1	1	1	1	3	26.8	26.8	0	1.428135
2	Dry	202	2	2	2	2	6	26.95	53.75	0.30103	1.730378
2	Dry	203	3	3	3	3	9	28.15	81.9	0.477121	1.913284
2	Dry	204	4	4	4	4	12	29.75	111.65	0.60206	2.047859
2	Dry	205	5	6	6	5	17	29.05	140.7	0.69897	2.148294
2	Dry	206	6	7	6	5	18	29.7	170.4	0.778151	2.23147
2	Dry	207	7	7	6	5	18	27.5	197.9	0.845098	2.296446
2	Dry	208	9	9	7	7	23	25.7	250.1	0.954243	2.398114
2	Dry	209	13	9	8	7	24	27	358.45	1.113943	2.554429
2	Dry	210	18	9	8	7	24	28	496.15	1.255273	2.695613
2	Dry	211	20	9	8	7	24	28.15	552.65	1.30103	2.74245
2	Dry	212	23	9	8	7	24	28.75	636.95	1.361728	2.804105
2	Dry	213	30	9	8	7	24	30.2	842.75	1.477121	2.925699
2	Dry	214	37	9	8	7	24	32.95	1064.4	1.568202	3.027105
2	Dry	215	44	9	8	7	24	32.15	1286	1.643453	3.109241
2	Dry	216	58	9	8	7	24	30.55	1714.85	1.763428	3.234226
2	Dry	217	120	9	8	7	24	29.4	3530.65	2.079181	3.547855
2	Dry	218	134	9	8	7	24	27	3929.1	2.127105	3.594293
2	Dry	219	148	9	12	8	29	27.25	4319.3	2.170262	3.635413

3	Dry	301	1	1	1	1	3	31.5	31.5	0	1.498311
3	Dry	302	2	2	2	2	6	31.65	63.15	0.30103	1.800373
3	Dry	303	3	4	3	3	10	30.9	94.05	0.477121	1.973359
3	Dry	304	4	5	6	5	16	31.15	125.2	0.60206	2.097604
3	Dry	305	5	8	6	5	19	31.9	157.1	0.69897	2.196176
3	Dry	306	6	8	7	5	20	31.6	188.7	0.778151	2.275772
3	Dry	307	7	9	7	5	21	32.95	221.65	0.845098	2.345668
3	Dry	308	10	9	8	5	22	29.95	315	1	2.498311
3	Dry	309	13	9	8	7	24	32.15	411.1	1.113943	2.613947
3	Dry	310	16	9	8	7	24	30.75	505.25	1.20412	2.703506
3	Dry	311	23	9	8	7	24	31.3	721.05	1.361728	2.857965
3	Dry	312	30	9	8	7	24	32.35	936.1	1.477121	2.971322
3	Dry	313	37	9	8	7	24	26.5	1147.85	1.568202	3.059885
3	Dry	314	90	9	8	7	24	29.4	2687.9	1.954243	3.429413
3	Dry	315	104	9	8	7	24	27	3086.35	2.017033	3.489445
3	Dry	316	118	9	8	7	24	27.25	3476.55	2.071882	3.541148
3	Dry	317	132	9	8	7	24	26.45	3854.2	2.120574	3.585934
3	Dry	318	146	9	10	9	28	27.2	4231.85	2.164353	3.62653
3	Dry	319	160	11	10	9	30	26.15	4601.9	2.20412	3.662937
4	Dry	401	1	1	1	1	3	29.6	29.6	0	1.471292
4	Dry	402	2	2	2	2	6	31.75	61.35	0.30103	1.787815
4	Dry	403	3	4	4	3	10	32.35	93.7	0.477121	1.97174
4	Dry	404	4	5	5	4	14	30.7	124.4	0.60206	2.09482
4	Dry	405	5	7	6	6	18	29	153.4	0.69897	2.185825
4	Dry	406	6	7	6	6	19	31.1	184.5	0.778151	2.265996
4	Dry	407	7	7	7	6	20	31.3	215.8	0.845098	2.334051
4	Dry	408	10	9	7	6	22	29.1	306.4	1	2.486289
4	Dry	409	13	9	8	7	24	31.65	398.5	1.113943	2.600428
4	Dry	410	16	9	8	7	24	31.75	494.85	1.20412	2.694474
4	Dry	411	23	9	8	7	24	29.15	700.05	1.361728	2.845129
4	Dry	412	74	9	8	7	24	29.4	2182.65	1.869232	3.338984
4	Dry	413	88	9	8	7	24	27	2581.1	1.944483	3.411805
4	Dry	414	102	9	8	7	24	27.25	2971.3	2.0086	3.472947
4	Dry	415	116	11	9	8	28	26.45	3348.95	2.064458	3.524909
4	Dry	416	130	11	10	9	30	27.2	3726.6	2.113943	3.571313
4	Dry	417	144	12	11	10	33	26.15	4096.65	2.158362	3.612429
5	Dry	501	1	1	1	1	3	29.6	29.6	0	1.471292
5	Dry	502	2	2	2	2	6	31.75	61.35	0.30103	1.787815
5	Dry	503	3	4	4	3	11	32.35	93.7	0.477121	1.97174
5	Dry	504	4	8	6	4	18	30.7	124.4	0.60206	2.09482
5	Dry	505	5	8	6	6	20	29	153.4	0.69897	2.185825
5	Dry	506	6	8	6	6	20	31.1	184.5	0.778151	2.265996
5	Dry	507	7	9	7	7	23	31.3	215.8	0.845098	2.334051
5	Dry	508	10	9	7	7	23	29.1	306.4	1	2.486289
5	Dry	509	13	9	7	6	22	31.65	398.5	1.113943	2.600428

5	Dry	510	16	9	8	7	24	31.75	494.85	1.20412	2.694474
5	Dry	511	23	9	8	7	24	29.15	700.05	1.361728	2.845129
5	Dry	512	74	9	8	7	24	29.4	2182.65	1.869232	3.338984
5	Dry	513	88	9	8	7	24	27	2581.1	1.944483	3.411805
5	Dry	514	102	9	8	7	24	27.25	2971.3	2.0086	3.472947
5	Dry	515	116	9	8	7	24	26.45	3348.95	2.064458	3.524909
5	Dry	516	130	11	8	8	27	27.2	3726.6	2.113943	3.571313
5	Dry	517	144	12	10	9	31	26.15	4096.65	2.158362	3.612429
6	Dry	601	1	1	1	1	3	31.65	31.65	0	1.500374
6	Dry	602	2	2	2	2	6	32.35	64	0.30103	1.80618
6	Dry	603	3	4	3	3	10	32.25	96.25	0.477121	1.983401
6	Dry	604	4	8	5	4	17	31.75	128	0.60206	2.10721
6	Dry	605	5	8	6	4	18	31	159	0.69897	2.201397
6	Dry	606	6	8	7	6	21	29.75	188.75	0.778151	2.275887
6	Dry	607	7	10	7	6	23	29.7	218.45	0.845098	2.339352
6	Dry	608	10	10	8	7	25	28.3	304.05	1	2.482945
6	Dry	609	62	11	8	7	26	29.4	1815.8	1.792392	3.259068
6	Dry	610	76	11	8	7	26	27	2214.25	1.880814	3.345227
6	Dry	611	90	11	8	7	26	27.25	2604.45	1.954243	3.415716
6	Dry	612	104	11	8	8	27	26.45	2982.1	2.017033	3.474522
6	Dry	613	118	12	10	9	31	27.2	3359.75	2.071882	3.526307
7	Wet	701	1	1	1	1	3	28.3	28.3	0	1.451786
7	Wet	702	2	2	2	2	6	28.25	56.55	0.30103	1.752433
7	Wet	703	3	4	3	3	10	29.25	85.8	0.477121	1.933487
7	Wet	704	4	6	5	4	15	28.75	114.55	0.60206	2.058995
7	Wet	705	5	8	6	6	20	28	142.55	0.69897	2.153967
7	Wet	706	6	8	6	6	20	28.1	170.65	0.778151	2.232106
7	Wet	707	7	10	7	8	25	27.2	197.85	0.845098	2.296336
7	Wet	708	9	12	9	8	29	29.65	256.35	0.954243	2.408833
7	Wet	709	11	12	11	9	32	26.75	311.45	1.041393	2.493388
8	Wet	801	1	1	1	1	3	28.75	28.75	0	1.458638
8	Wet	802	2	2	2	1	5	28	56.75	0.30103	1.753966
8	Wet	803	3	4	3	2	9	28.1	84.85	0.477121	1.928652
8	Wet	804	4	5	4	3	12	27.2	112.05	0.60206	2.049412
8	Wet	805	5	8	5	5	18	28.85	140.9	0.69897	2.148911
8	Wet	806	6	8	6	6	20	29.65	170.55	0.778151	2.231852
8	Wet	807	7	10	7	6	23	28.35	198.9	0.845098	2.298635
8	Wet	808	9	10	8	6	24	27	252.65	0.954243	2.402519
8	Wet	809	12	10	8	7	25	27.8	335.6	1.079181	2.525822
8	Wet	810	15	11	8	7	26	28.4	420.85	1.176091	2.624127
8	Wet	811	22	11	8	8	27	26.45	615.6	1.342423	2.789299
8	Wet	812	29	11	8	8	27	27	806.05	1.462398	2.906362
8	Wet	813	36	11	8	8	27	28.5	994.05	1.556303	2.997408
8	Wet	814	50	11	8	8	27	24.7	1370.95	1.69897	3.137022
8	Wet	815	64	11	8	9	28	26.55	1742.05	1.80618	3.241061

8	Wet	816	78	11	10	9	30	25.8	2109.25	1.892095	3.324128
8	Wet	817	92	11	10	9	30	24.75	2483.95	1.963788	3.395143
9	Wet	901	1	1	1	1	3	27	27	0	1.431364
9	Wet	902	2	3	3	2	8	28.05	55.05	0.30103	1.740757
9	Wet	903	3	3	4	3	10	27.1	82.15	0.477121	1.914608
9	Wet	904	4	6	5	6	17	27.8	109.95	0.60206	2.041195
9	Wet	905	5	7	5	6	18	29.1	139.05	0.69897	2.143171
9	Wet	906	6	8	7	6	21	27.75	166.8	0.778151	2.222196
9	Wet	907	7	9	8	7	24	28.4	195.2	0.845098	2.29048
9	Wet	908	10	9	8	7	24	28.25	280.7	1	2.448242
9	Wet	909	13	9	8	7	24	28.3	363.5	1.113943	2.560504
9	Wet	910	16	9	8	7	24	28.75	445.95	1.20412	2.649286
9	Wet	911	23	9	8	7	24	25.75	632.9	1.361728	2.801335
9	Wet	912	30	9	8	7	24	27.7	822.55	1.477121	2.915162
9	Wet	913	37	9	8	7	24	27.1	1011.2	1.568202	3.004837
9	Wet	914	51	11	10	9	30	26.3	1384.35	1.70757	3.141246
9	Wet	915	65	11	10	9	30	26.75	1754.1	1.812913	3.244054
10	Wet	1001	1	1	1	1	3	25.9	25.9	0	1.4133
10	Wet	1002	2	2	2	1	5	26.45	52.35	0.30103	1.718917
10	Wet	1003	3	3	3	2	8	27.2	79.55	0.477121	1.90064
10	Wet	1004	4	5	5	4	14	25	104.55	0.60206	2.019324
10	Wet	1005	5	8	6	5	19	26.6	131.15	0.69897	2.117768
10	Wet	1006	6	8	7	6	21	26.75	157.9	0.778151	2.198382
10	Wet	1007	7	8	7	6	21	27.5	185.4	0.845098	2.26811
10	Wet	1008	10	9	8	7	24	25.5	261.6	1	2.417638
10	Wet	1009	13	9	8	7	24	26.75	340.65	1.113943	2.532308
10	Wet	1010	16	9	8	7	24	26.25	419.2	1.20412	2.622421
10	Wet	1011	23	9	8	7	24	28.2	609.85	1.361728	2.785223
10	Wet	1012	30	9	8	7	24	27	797.05	1.477121	2.901486
10	Wet	1013	37	9	8	7	24	26.9	985.5	1.568202	2.993657
10	Wet	1014	51	9	8	7	24	24.85	1358.65	1.70757	3.133108
10	Wet	1015	65	9	8	8	25	26.65	1730.35	1.812913	3.238134
10	Wet	1016	79	9	8	8	25	25.2	2102.45	1.897627	3.322726
10	Wet	1017	93	9	10	10	29	27.25	2477.3	1.968483	3.393979
10	Wet	1018	107	9	10	9	28	29	2881.25	2.029384	3.459581
10	Wet	1019	121	9	10	10	29	30.25	3296.7	2.082785	3.518079
10	Wet	1020	131	9	10	10	29	30.3	3595.85	2.117271	3.555802
11	Wet	1101	1	1	1	1	3	25.9	25.9	0	1.4133
11	Wet	1102	2	2	2	1	5	26.45	52.35	0.30103	1.718917
11	Wet	1103	3	3	3	2	8	27.2	79.55	0.477121	1.90064
11	Wet	1104	4	4	4	4	12	25	104.55	0.60206	2.019324
11	Wet	1105	5	8	5	5	18	26.6	131.15	0.69897	2.117768
11	Wet	1106	6	8	7	6	21	26.75	157.9	0.778151	2.198382
11	Wet	1107	7	8	7	6	21	27.5	185.4	0.845098	2.26811
11	Wet	1108	10	9	8	7	24	25.5	261.6	1	2.417638

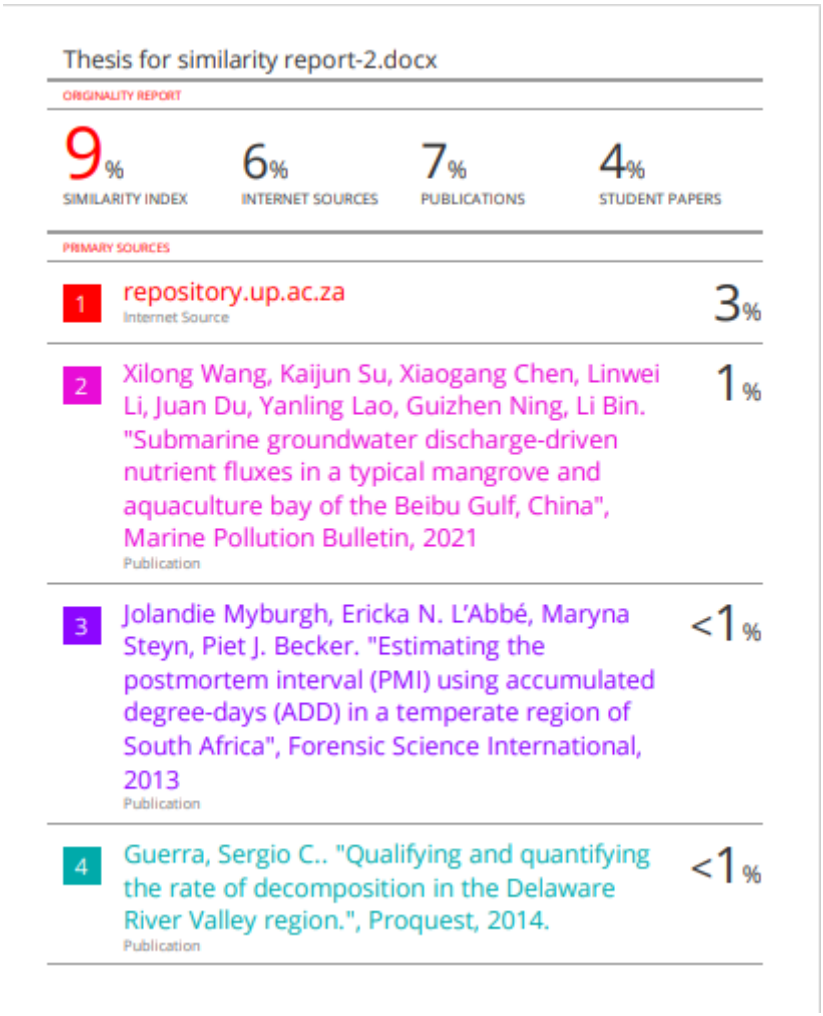
11	Wet	1109	13	9	8	7	24	26.75	340.65	1.113943	2.532308
11	Wet	1110	16	9	8	7	24	26.25	419.2	1.20412	2.622421
11	Wet	1111	23	9	8	8	25	28.2	609.85	1.361728	2.785223
11	Wet	1112	30	9	8	8	25	27	797.05	1.477121	2.901486
11	Wet	1113	37	9	8	8	25	26.9	985.5	1.568202	2.993657
11	Wet	1114	51	9	8	8	25	24.85	1358.65	1.70757	3.133108
11	Wet	1115	65	9	8	9	26	26.65	1730.35	1.812913	3.238134
11	Wet	1116	79	9	9	9	27	25.2	2102.45	1.897627	3.322726
11	Wet	1117	93	9	10	10	29	27.25	2477.3	1.968483	3.393979
11	Wet	1118	107	11	10	10	31	29	2881.25	2.029384	3.459581
12	Wet	1201	1	1	1	1	3	25.95	25.95	0	1.414137
12	Wet	1202	2	2	1	1	4	24.75	50.7	0.30103	1.705008
12	Wet	1203	3	3	3	3	9	25.5	76.2	0.477121	1.881955
12	Wet	1204	4	4	3	3	10	25.8	102	0.60206	2.0086
12	Wet	1205	5	8	5	6	19	26.5	128.5	0.69897	2.108903
12	Wet	1206	6	9	6	6	21	26.75	155.25	0.778151	2.191032
12	Wet	1207	7	9	6	6	21	25.9	181.15	0.845098	2.258038
12	Wet	1208	11	9	8	7	24	25.7	285.85	1.041393	2.456138
12	Wet	1209	13	9	8	7	24	27.35	340.3	1.113943	2.531862
12	Wet	1210	16	9	8	7	24	28.2	424.45	1.20412	2.627827
12	Wet	1211	23	9	8	7	24	27	611.65	1.361728	2.786503
12	Wet	1212	30	9	8	7	24	26.9	800.1	1.477121	2.903144
12	Wet	1213	37	9	8	7	24	26.35	991.15	1.568202	2.996139
12	Wet	1214	51	9	8	7	24	28.2	1359.2	1.70757	3.133283
12	Wet	1215	65	9	8	7	24	26.5	1731.2	1.812913	3.238347
12	Wet	1216	79	9	8	9	26	27.05	2104.35	1.897627	3.323118
12	Wet	1217	100	9	8	10	27	29	2695.85	2	3.430696
12	Wet	1218	108	9	8	10	27	29.35	2932.85	2.033424	3.46729
12	Wet	1219	121	9	8	10	27	30.1	3320.3	2.082785	3.521177
12	Wet	1220	135	9	8	10	27	29.8	3734.35	2.130334	3.572215
12	Wet	1221	149	9	8	10	27	27.9	4134.45	2.173186	3.616418
13	Wet	1301	1	1	1	1	3	25.95	25.95	0	1.414137
13	Wet	1302	2	2	2	1	5	24.75	50.7	0.30103	1.705008
13	Wet	1303	3	4	3	3	10	25.5	76.2	0.477121	1.881955
13	Wet	1304	4	8	4	4	16	25.8	102	0.60206	2.0086
13	Wet	1305	5	8	5	5	18	26.5	128.5	0.69897	2.108903
13	Wet	1306	6	8	6	5	19	26.75	155.25	0.778151	2.191032
13	Wet	1307	7	9	6	5	20	25.9	181.15	0.845098	2.258038
13	Wet	1308	11	9	8	7	24	25.7	285.85	1.041393	2.456138
13	Wet	1309	13	9	8	7	24	27.35	340.3	1.113943	2.531862
13	Wet	1310	16	9	8	7	24	28.2	424.45	1.20412	2.627827
13	Wet	1311	23	9	8	7	24	27	611.65	1.361728	2.786503
13	Wet	1312	30	9	8	7	24	26.9	800.1	1.477121	2.903144
13	Wet	1313	37	11	8	7	26	26.35	991.15	1.568202	2.996139
13	Wet	1314	51	11	11	8	30	28.2	1359.2	1.70757	3.133283

13	Wet	1315	65	12	11	8	31	26.5	1731.2	1.812913	3.238347
14	Wet	1401	1	1	1	1	3	27.1	27.1	0	1.432969
14	Wet	1402	2	3	2	2	7	27.35	54.45	0.30103	1.735998
14	Wet	1403	3	4	3	3	10	27.7	82.15	0.477121	1.914608
14	Wet	1404	4	8	7	6	21	28.25	110.4	0.60206	2.042969
14	Wet	1405	5	8	7	6	21	28.2	138.6	0.69897	2.141763
14	Wet	1406	6	9	8	7	24	27.5	166.1	0.778151	2.22037
14	Wet	1407	7	9	8	7	24	24.75	190.85	0.845098	2.280692
14	Wet	1408	10	9	8	7	24	26.9	272.25	1	2.434968
14	Wet	1409	13	9	8	7	24	27.85	353.65	1.113943	2.548574
14	Wet	1410	16	9	8	7	24	27.55	435.15	1.20412	2.638639
14	Wet	1411	23	9	8	7	24	27	623.35	1.361728	2.794732
14	Wet	1412	30	9	8	7	24	26.25	810.5	1.477121	2.908753
14	Wet	1413	37	9	8	8	25	25.75	992.55	1.568202	2.996752
14	Wet	1414	51	9	10	9	28	25.05	1364.85	1.70757	3.135085
14	Wet	1415	65	9	10	9	28	27.1	1738.95	1.812913	3.240287
14	Wet	1416	79	11	10	9	30	29.25	2118.5	1.897627	3.326028
15	Wet	1501	1	1	1	1	3	27.1	27.1	0	1.432969
15	Wet	1502	2	3	2	2	7	27.35	54.45	0.30103	1.735998
15	Wet	1503	3	8	3	3	14	27.7	82.15	0.477121	1.914608
15	Wet	1504	4	8	5	6	19	28.25	110.4	0.60206	2.042969
15	Wet	1505	5	9	6	6	21	28.2	138.6	0.69897	2.141763
15	Wet	1506	6	9	7	6	22	27.5	166.1	0.778151	2.22037
15	Wet	1507	7	9	8	6	23	24.75	190.85	0.845098	2.280692
15	Wet	1508	10	9	8	8	25	26.9	272.25	1	2.434968
15	Wet	1509	13	9	8	8	25	27.85	353.65	1.113943	2.548574
15	Wet	1510	16	9	8	8	25	27.55	435.15	1.20412	2.638639
15	Wet	1511	23	9	10	8	27	27	623.35	1.361728	2.794732
15	Wet	1512	30	11	10	8	29	26.25	810.5	1.477121	2.908753
15	Wet	1513	37	12	10	9	31	25.75	992.55	1.568202	2.996752
15	Wet	1514	51	12	9	9	30	25.05	1364.85	1.70757	3.135085
15	Wet	1515	65	13	11	9	33	27.1	1738.95	1.812913	3.240287
16	Wet	1601	1	1	1	1	3	28.25	28.25	0	1.451018
16	Wet	1602	2	3	3	2	8	28.2	56.45	0.30103	1.751664
16	Wet	1603	3	4	3	3	10	27.5	83.95	0.477121	1.924021
16	Wet	1604	4	8	6	5	19	24.75	108.7	0.60206	2.03623
16	Wet	1605	5	8	7	8	23	27.25	135.95	0.69897	2.133379
16	Wet	1606	6	10	7	8	25	27.25	163.2	0.778151	2.21272
16	Wet	1607	7	10	9	8	27	26.9	190.1	0.845098	2.278982
16	Wet	1608	10	10	10	8	28	27.85	271.85	1	2.434329
16	Wet	1609	13	10	10	8	28	27.55	353.35	1.113943	2.548205
16	Wet	1610	16	11	10	8	29	26.9	432.45	1.20412	2.635936
16	Wet	1611	25	11	10	9	30	27.55	677.7	1.39794	2.831037
16	Wet	1612	30	11	10	9	30	24.85	805.6	1.477121	2.906119
16	Wet	1613	37	11	11	9	31	28.2	991.55	1.568202	2.996315

17	Dry	1701	1	1	1	1	3	29.15	29.15	0	1.464639
17	Dry	1702	2	3	3	2	8	30.05	59.2	0.30103	1.772322
17	Dry	1703	3	5	3	3	11	30.35	89.55	0.477121	1.952066
17	Dry	1704	4	8	7	4	19	29.8	119.35	0.60206	2.076822
17	Dry	1705	5	8	7	4	19	29.9	149.25	0.69897	2.173914
17	Dry	1706	6	8	7	6	21	29.35	178.6	0.778151	2.251881
17	Dry	1707	7	9	7	6	22	29.35	207.95	0.845098	2.317959
17	Dry	1708	10	9	8	6	23	29.55	296.15	1	2.471512
17	Dry	1709	13	9	8	6	23	30.25	386.4	1.113943	2.587037
17	Dry	1710	17	9	8	7	24	29.75	505.25	1.230449	2.703506
17	Dry	1711	23	9	8	7	24	30.3	685.55	1.361728	2.836039
17	Dry	1712	30	9	8	7	24	28.75	894.05	1.477121	2.951362
17	Dry	1713	37	9	8	7	24	29.35	1096.75	1.568202	3.040108
17	Dry	1714	51	9	8	7	24	30.1	1498.65	1.70757	3.1757
17	Dry	1715	65	9	8	7	24	29.5	1919.8	1.812913	3.283256
17	Dry	1716	79	9	8	7	24	30.1	2325.85	1.897627	3.366582
18	Dry	1801	1	1	1	1	3	29.15	29.15	0	1.464639
18	Dry	1802	2	3	3	3	9	30.05	59.2	0.30103	1.772322
18	Dry	1803	3	5	3	3	11	30.35	89.55	0.477121	1.952066
18	Dry	1804	4	7	6	5	18	29.8	119.35	0.60206	2.076822
18	Dry	1805	5	8	7	5	20	29.9	149.25	0.69897	2.173914
18	Dry	1806	6	8	7	6	21	29.35	178.6	0.778151	2.251881
18	Dry	1807	7	9	7	7	23	29.35	207.95	0.845098	2.317959
18	Dry	1808	10	9	8	7	24	29.55	296.15	1	2.471512
18	Dry	1809	13	9	8	7	24	30.25	386.4	1.113943	2.587037
18	Dry	1810	17	9	8	7	24	29.75	505.25	1.230449	2.703506
18	Dry	1811	23	9	8	7	24	30.3	685.55	1.361728	2.836039
18	Dry	1812	30	9	8	7	24	28.75	894.05	1.477121	2.951362
18	Dry	1813	37	9	8	7	24	29.35	1096.75	1.568202	3.040108
18	Dry	1814	51	9	8	7	24	30.1	1498.65	1.70757	3.1757
18	Dry	1815	65	9	8	7	24	29.5	1919.8	1.812913	3.283256
18	Dry	1816	79	9	8	7	24	30.1	2325.85	1.897627	3.366582
19	Dry	1901	1	1	1	1	3	29.15	29.15	0	1.464639
19	Dry	1902	2	3	2	2	7	30.05	59.2	0.30103	1.772322
19	Dry	1903	3	5	3	3	11	30.35	89.55	0.477121	1.952066
19	Dry	1904	4	8	6	6	20	29.8	119.35	0.60206	2.076822
19	Dry	1905	5	9	7	6	22	29.9	149.25	0.69897	2.173914
19	Dry	1906	6	9	7	6	22	29.35	178.6	0.778151	2.251881
19	Dry	1907	7	9	8	7	24	29.35	207.95	0.845098	2.317959
19	Dry	1908	10	9	8	7	24	29.55	296.15	1	2.471512
19	Dry	1909	13	9	8	7	24	30.25	386.4	1.113943	2.587037
19	Dry	1910	17	9	8	7	24	29.75	505.25	1.230449	2.703506
19	Dry	1911	23	9	8	7	24	30.3	685.55	1.361728	2.836039
19	Dry	1912	30	9	8	7	24	28.75	894.05	1.477121	2.951362
19	Dry	1913	37	9	8	7	24	29.35	1096.75	1.568202	3.040108

19	Dry	1914	51	9	8	7	24	30.1	1498.65	1.70757	3.1757
19	Dry	1915	65	9	8	7	24	29.5	1919.8	1.812913	3.283256
19	Dry	1916	79	9	8	7	24	30.1	2325.85	1.897627	3.366582
20	Dry	2001	1	1	1	1	3	29.15	29.15	0	1.464639
20	Dry	2002	2	3	3	2	8	30.05	59.2	0.30103	1.772322
20	Dry	2003	3	5	3	3	11	30.35	89.55	0.477121	1.952066
20	Dry	2004	4	8	6	6	20	29.8	119.35	0.60206	2.076822
20	Dry	2005	5	8	7	6	21	29.9	149.25	0.69897	2.173914
20	Dry	2006	6	8	7	7	22	29.35	178.6	0.778151	2.251881
20	Dry	2007	7	9	7	7	23	29.35	207.95	0.845098	2.317959
20	Dry	2008	10	9	8	7	24	29.55	296.15	1	2.471512
20	Dry	2009	13	9	8	7	24	30.25	386.4	1.113943	2.587037
20	Dry	2010	17	9	8	7	24	29.75	505.25	1.230449	2.703506
20	Dry	2011	23	9	8	7	24	30.3	685.55	1.361728	2.836039
20	Dry	2012	30	9	8	7	24	28.75	894.05	1.477121	2.951362
20	Dry	2013	37	9	8	7	24	29.35	1096.75	1.568202	3.040108
20	Dry	2014	51	9	8	7	24	30.1	1498.65	1.70757	3.1757
20	Dry	2015	65	9	8	7	24	29.5	1919.8	1.812913	3.283256
20	Dry	2016	79	9	8	7	24	30.1	2325.85	1.897627	3.366582

Appendix H: **Turnitin similarity report**



Thesis for similarity report-2.docx

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