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Decomposition Dynamics and Arthropod Colonization Patterns in Shifting Environmental Conditions: Subaerial to Freshwater Aquatic Transitions and Vice Versa

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2025
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Lindsay, Alexandra Helen . (2025). Decomposition Dynamics and Arthropod Colonization Patterns in Shifting Environmental Conditions: Subaerial to Freshwater Aquatic Transitions and Vice Versa [Master's dissertation, University of the Witwatersrand, Johannesburg]. WIRerDSpace. https://hdl.handle.net/10539/49781

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Decomposition Dynamics and Arthropod Colonization Patterns in Shifting Environmental Conditions: Subaerial to Freshwater Aquatic Transitions and Vice Versa

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A Dissertation submitted to the Division of Forensic Medicine, Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of MSc (Med) in Forensic Medicine.

Johannesburg, 2025

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Dedication

This work is dedicated to the memory of all those whose silent stories shape our understanding of life and death, inspiring deeper compassion, and respect in forensic science. Thank you, dad (Bruce Lindsay 05/10/1968 – 14/05/2016).

To my family and partner, Jarred Wright, who have been my unwavering support system, providing encouragement, understanding, and love throughout this journey.

This research is a tribute to understanding the impermanence of life and honouring the lives that teach us. The dead teach the living.

Abstract

Decomposition studies are pivotal for forensic science, offering insights into postmortem events. While decomposition dynamics in static environments are well-documented, transitions between terrestrial and aquatic settings remain underexplored, particularly in diverse ecological regions like South Africa.

This study investigated decomposition rates and arthropod colonization patterns in juvenile piglets subjected to subaerial-to-freshwater aquatic and freshwater aquatic-to-subaerial transitions. Twelve piglets were used in total: five in each experimental group and two controls, each exposed exclusively to either subaerial or aquatic conditions. Decomposition progression was quantified using Adjusted Total Body Scores (ATBS) and Total Aquatic Decomposition Scores (TADS), which were standardized for cross-environment comparison. Accumulated Degree Days (ADD) were calculated to account for thermal input, and arthropods were sampled using sticky traps and direct collection.

Subaerial-to-aquatic transitions exhibited delayed decomposition initially, followed by rapid tissue breakdown post-submersion, often leading to near-complete skeletonization. In contrast, aquatic-to-subaerial transitions displayed rapid decomposition during submersion, followed by reduced activity and partial or complete mummification after environmental change. These shifts were reflected in scoring results but also revealed morphological changes not accounted for in current scoring systems. Notably, *Anthomyia* spp. were found exclusively on remains transitioning from aquatic to terrestrial environments, suggesting their potential as indicators of post-submersion exposure.

Transitions between environments significantly alter decomposition rates, patterns, and arthropod succession. Existing scoring systems proved limited in accounting for these shifts, particularly in cases of mummification. This study highlights the need for a novel scoring framework tailored to fluctuating environments and calls for further research into decomposition morphology and entomological markers specific to environmental transitions.

Acknowledgments

This research would not have been possible without the support, guidance, and encouragement of many individuals and institutions, to whom I am deeply grateful.

Thank you to…

- My supervisors, Dr Craig Keyes and Mr Lawerence Hill, for their unwavering support, expertise, and patience. Your guidance has been instrumental in shaping both this research and my growth as a forensic medicine student. Thank you for responding to the hundreds of emails sent and always being there to listen and provide guidance.
- Ms Phumzile Mdluli and Mr Johannes Mosikili (JHB FPS mortuary managers), and Dr Tinyiko Zelda Nkondo (Chief Forensic Pathologist) for granting me permission to use JHB FPS mortuary as my study site.
- Victor Chungwane and Neo Mphaphuli for their assistance in helping set up the study site.
- Marius from Rooiport Farm, GHB Farms for his efforts in collecting piglets and storing them for my collection.
- Allison Gibson for all your assistance and guidance in identifying arthropods.
- And finally, to the animals whose lives, even in death, have contributed to the advancement of science. Your silent contribution bridges the gap between theory and application, reminding us of the shared threads in the tapestry of life.

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1. Introduction

Human decomposition is a complex process that is influenced by a wide range of abiotic (Galloway, 1997; Megyesi *et al.,* 2005; Goff, 2009; Vass, 2011) and biotic factors (Pechal *et al.,* 2013; Cobaugh *et al.,* 2015; Metcalf *et al.,* 2015). In forensic science, understanding this complex process is crucial for determining postmortem intervals (PMI), postmortem submersion intervals (PMSI), cause of death, and if the body was moved postmortem. Arthropods play a significant role in the decomposition process. Their colonization patterns provide vital information for forensic investigations. However, studying human decomposition directly is often limited by ethical and logistical challenges, which is why animal models such as pigs are commonly used as proxies due to their similar body size, skin composition, fat-to-muscle ratio, and physiology (Matuszewski *et al.*, 2019).

Globally, studies on human decomposition have informed forensic practices in various climates and environmental conditions. However, decomposition rates and colonization patterns can vary significantly between different ecosystems (Knight, 1996), making region-specific studies essential. In South Africa, the diverse environmental conditions present unique challenges and opportunities for forensic research. For example, transitions between subaerial (terrestrial) and aquatic environments, which occur naturally or through flooding events, can dramatically affect decomposition dynamics and arthropod activity.

1.1. Research focus

Remains in natural settings can transition between subaerial (exposed to air) and aquatic (submerged in water) environments due to factors such as seasonal flooding (Fitchett *et al.* 2016; Dalu *et al*. 2018), drought, tides and drifting of remains or deliberate disposal (Salfati *et al.* 2014). These transitions create unique conditions where decomposition processes and arthropod colonization can differ significantly compared to static environments. In freshwater

environments, for example, oxygen depletion, and temperature may slow decomposition and alter the arthropod community, while subaerial environments may promote faster breakdown due to higher temperatures and access to oxygen as well as subaerial scavenger activity. For example, Olson *et al.* (2012) found that when key scavengers were removed from an ecosystem, it took longer for carcasses to be fully consumed suggesting that subaerial scavengers play an important role in efficiently breaking down carcasses. However, de Donno *et al.* (2014) conducted a study on sequestered and non-sequestered remains and found that among the 52 sequestered bodies examined, most showed only partial decomposition. Decomposition was mostly localized to areas exposed to water, such as the head, neck, and hands, where clothing did not provide protection. Signs of interaction with aquatic scavengers were observed in these areas (de Donno *et al.,* 2014).

Although decomposition dynamics in strictly subaerial environments have been extensively studied and strictly aquatic environments have also been explored (although less thoroughly than the former), there remains a striking lack of empirical research on the decomposition in a transitional context, where bodies move between land and water. In forensic contexts, remains often experience environmental transitions, such as being submerged by floods, washed from inland into water bodies, or intentionally relocated by offenders, disrupting the applicability of standard decomposition models. Traditional tools like the Total Body Score (Megyesi *et al.,* 2005), Total Aquatic Decomposition Score (Heaton *et al.,* 2010), and Accumulated Degree Days (ADD) were developed and studied under static conditions and may estimate incorrect post-mortem intervals (PMI) when remains transverse terrestrial-aquatic boundaries. Investigating how decomposition progresses across such transitions is therefore critical for calculating a correct PMI estimation, accurately interpreting taphonomic evidence, and guiding recovery procedures in complex forensic scenarios. Moreover, in regions such as South Africa, where remains are frequently recovered from diverse settings

(vegetated areas, resides, farms, and aquatic environments), there is a need for locally relevant taphonomic, entomological, and decomposition morphology data to improve forensic identification and timeline reconstruction. Furthermore, while existing work such as Heaton et al. (2010), van Daalen et al. (2017), Reijnen et al. (2018) and Palazzo et al. (2020), has focused on bodies directly from water, with limited consideration of those that decompose in one environment before being displaced in another. This gap is particularly concerning given the increasing frequency of the severe flooding events worldwide. In 2022, South Africa's Durban region endured the most catastrophic flood on record for the province, with hundreds killed, thousands displaced, and immense damage to infrastructure and housing (Naidoo, 2022). Over 459 people died and 88 were reported missing and some bodies were recovered weeks and even months later. For instance, the skeletal remains of a Durban man, Jayishwar Baijalall, who went missing in April 2022, were only discovered nearly two years later in late 2024 when rebuilding began on the site of his old home (Marriah-Maharaj, 2024). Additional global examples further emphasize the forensic complexity inherent in transitional environment cases. In Nigeria's 2025 Mokwa flood the death toll was 153 with bodies discovered under a bridge, long after the flooding subsided (Egba, 2025). In central Texas, 2025, at least 135 people died after the surge of the Guadalupe River over the 4th of July weekend. A missing camp counsellor's (Katherine Ferruzzo) remains were found seven days after the flooding incident (Brunner, 2025).

These real-world events reveal a disturbing pattern: many flood victims, especially those displaced across land-water boundaries, remain unrecovered for prolonged periods, complicating decomposition assessments and PMI estimations. This uncertainty reinforces the necessity for forensic models to account for transitional decomposition dynamics. This study aims to fill a critical gap by experimentally investigating how decomposition

progresses across aquatic and subaerial environments and how such insights could improve forensic practices in regions with dynamic ecological conditions.

2. Literature review

2.1. Decomposition Dynamics and Arthropod Colonization

Human decomposition is a complex biological process that begins immediately after death, involving the systematic breakdown of bodily tissues and organic matter, ultimately returning nutrients to the environment. Arthropods, particularly insects, are integral to this process as they are drawn to decomposing remains and utilize the resources for feeding and reproduction.

Decomposition stages can vary depending on environment (Knight, 1996), in a subaerial environment can be divided into five stages. The first stage, known as the fresh stage (autolysis), occurs immediately after death and is driven primarily by the body's own enzymes. Cells break down, releasing enzymes that cause tissue degradation (Michaud and Moreau, 2010). This stage is also characterised by abdominal discolouration, specifically a dark blue-green discolouration (Saukko and Knight, 2015; Shkrum and Ramsay, 2007). This discolouration is due to the anaerobic bacteria in the intestines that begins to colonize the venous system. Skin slippage is also a common feature of this stage; a result of the skin and tissue losing its anatomic integrity.

The second stage is known as the bloat stage (putrefaction) which typically occurs within the first few days' post-mortem. During this stage, gases (hydrogen sulphide, ammonia, methane, and carbon dioxide) produced by bacteria in the gastrointestinal tract cause the body to swell and bloat (Michaud and Moreau, 2010). The bloating is a result of the gases permeating into the soft tissue of the gastro-intestinal tract (Shkrum and Ramsay, 2007). At this stage, notable features include swelling of the abdomen and neck, protrusion of the anus, elevation of the

limbs, greenish discoloration of the abdominal area, and a brownish hue affecting the fingers, nose, and ears (Rodriguez and Bass, 1983; Galloway *et al.,* 1989; Shkrum and Ramsay, 2007; Michaud and Moreau, 2010). This discolouration is caused by sulfhaemoglobin formation in settled blood (Vass, 2011).

The active decay stage is characterized by significant tissue breakdown and fluid release, leading to the collapse of the body's structural integrity (Rodriguez and Bass, 1983; Galloway *et al.,* 1989; Michaud and Moreau, 2010). This decomposition is considered a moist decomposition with partial bone exposure, aided by proteolytic enzymes secreted by maggots (Saukko and Knight, 2015).

The advanced decay stage follows, marked by the near absence of soft tissues, leaving behind primarily bones, hair, and non-biodegradable materials. During this phase, maggots typically migrate away from the body to pupate (Michaud and Moreau, 2010) and there is an increase in beetle activity as they feed on the dried skin, hair, and tendons (Kolver, 2009; Parks, 2010). At this stage, the decomposition process may involve either wet decomposition or mummification, which can result in the preservation or autolysis-driven loss of internal organs, often exacerbated by insect activity. Additionally, adipocere may form, a substance that appears waxy, greasy, and clay-like with a chalky-white colour. It develops as a result of the enzymatic activity of anaerobic bacteria or endogenous lipase acting on subcutaneous fat (Shkrum and Ramsay, 2007).

Mummification is a variation of decomposition in which the typical liquefaction of tissues is replaced by the transformation of the skin into a brown-black, leathery tissue. This process results from a combination of the desiccation of tissue and the suppression of bacterial activity. (Ambach *et al.* 1992; Saukko and Knight, 2015). A dry environment is essential for mummification to occur, and this can result in localized, partial, or extensive mummification

over the entire body (Shkrum and Ramsay, 2007). However, it should be noted that it is not just a dry or low humidity environment that plays a role, as high solar radiation and wind have an effect on mummification as well (Finaughty and Morris, 2019). Furthermore, the exclusion of insect-driven decomposition may also play a role in decomposition as seen in subterranean environments (Dix and Graham, 2000; Janaway *et al*., 2009).

The final stage is known as skeletonization where only skeletal remains and dried, mummified tissues may persist (Rodriguez and Bass, 1983; Galloway *et al.,* 1989; Michaud and Moreau, 2010). Arthropods may continue to scavenge on any desiccated tissues and other organic matter in the surrounding area. This structured understanding of human decomposition not only highlights the biological processes involved but also underscores the critical role of arthropods in recycling nutrients back into the ecosystem.

Decomposition in aquatic environments follows a distinctive progression of stages that diverge in both pace and morphology from that of terrestrial decomposition. Based on Zimmerman and Wallace (2010), aquatic decomposition can be categorized into five sequential stages: submerged fresh, early floating, floating decay, advanced floating decay, and sunken remains. In the submerged fresh stage, the remains undergo early post-mortem changes underwater, with limited insect activity due to immersion. As gases from tissue breakdown accumulate, particularly in warmer or still water, the body enters the early floating stage where gas-induced buoyancy brings it to the surface. The floating stage sees accelerated soft tissue breakdown and surface scoring by aquatic scavengers, often accompanied by skin slippage and tissue loss. Advanced floating decay is marked by progressive loss of soft tissue and emergence of adipocere formation, particularly in anaerobic conditions that favour fat saponification (Shkrum and Ramsay, 2007). Finally, in the sunken remains stage, much of the soft tissue has degraded or detached, and the body may sink as skeletal remains become exposed (Caruso, 2016; Wang *et al*., 2022).

When compared to terrestrial decomposition, aquatic environments generally slow early putrefactive processes due to cooler temperatures and reduced oxygen availability, while enabling processes that are less common on land like adipocere formation (Caurso, 2016). Terrestrially, decomposition follows a more predictable progression driven largely by aerobic microbes and arthropod succession. In contrast aquatic decomposition is also influenced heavily by buoyancy dynamics, water currents, immersion duration, water chemistry (e.g., salinity and pH), and scavenging by aquatic animals (Caruso, 2016; Martlin *et al.,* 2022; Guareschi *et al.,* 2025).

Arthropods, particularly insects, play an integral role in decomposition by colonizing remains and accelerating tissue breakdown through feeding, reproduction, and microbial interactions (Voss *et al.,* 2009). These activities expedite the breakdown of decaying tissue, thereby accelerating the decomposition process. Additionally, arthropods contribute to the dispersion of remains through burrowing and engage in microbial interactions that further enhance decomposition. The study of insect succession patterns is a crucial tool for estimating the PMI, as the pattern of specific insect species colonization and their activity on a deceased body is closely linked to the stages of decomposition and VOC profiles (Voss *et al.,* 2009; Martin *et al.,* 2019). The presence and necrophagous activities of insects directly influence the speed and extent of soft tissue breakdown (Simmons *et al*., 2010). Decomposing remains provide a rich source of nutrients for various arthropods, thereby facilitating the decomposition process. Studying the patterns of arthropod colonization not only aids in determining the PMI but also underscores the essential role of arthropods in the decomposition ecosystem.

While decomposition has been extensively studied in static subaerial or aquatic environments, transitions between these settings introduce additional complexities. For instance, bodies moved from subaerial to aquatic environments, or vice versa, may

experience altered decomposition rates and arthropod colonization patterns, yet these transitional phases remain underexplored.

2.1.1. Variables that affect decomposition

Decomposition is influenced by both intrinsic and extrinsic factors. Intrinsic factors originate from the remains themselves and include elements such as body size, age, the integrity of the remains, and the presence of any diseases or injuries at the time of death (Campobasso *et al.,* 2001). These factors can affect the rate and specific process by which decomposition occurs. For example, the age of an individual influences the rate of decomposition. Putrefaction has been shown to be slower in younger individuals, such as foetuses and newborns, due to the absence of a developed gut microbiome (Griest, 2009). Studies conducted on both humans and pig models have found that body mass and size (body fat) does not play a notable role in decomposition, but it is rather muscle mass that has a greater effect (Mann *et al.,* 1990; Simmons *et al.,* 2010; Matuszewski *et al*., 2010; Mason *et al*., 2022). More studies into body constitution need be conducted to fully understand the effect it has on decomposition rate. The integrity of the remains refers to the state in which body is in. The open wounds allow for external bacteria and Diptera to enter the body, speeding up decomposition (Campobasso *et al*., 2001). Furthermore, decomposition occurs more rapidly in individuals who were emaciated, suffered from septic infections, or died from asphyxia, as these conditions promote bacterial development due to increased blood fluidity (Campobasso *et al.,* 2001). However, it should be noted that recent cases have shown that the presence of penetration injuries has a limiting effect on the rate of decomposition (Cross and Simmons, 2010; Smith, 2014; Bates and Wescott, 2016; Rivers *et al.,* 2023) but does have an impact on the pattern of decomposition.

On the other hand, extrinsic factors are derived from the external environment surrounding the body. These include temperature, humidity, exposure to sunlight, presence of scavengers, and the specific location where the body is found (Galloway *et al.,* 1989; Galloway, 1997; Megyesi *et al.,* 2005; Goff, 2009; Vass, 2011; Pechal *et al.,* 2013; Cobaugh *et al.,* 2015; Metacalf *et al.,* 2015). Temperature has the greatest influence on decomposition (Michaud and Moreau, 2010). Temperature directly influences the biological, biochemical, and enzymatic reactions responsible for the decomposition of soft tissue. Research indicates that decomposition rates are higher during the summer months when temperatures are warmer. Bodies discovered during this time tend to be in more advanced stages of decomposition (Rodriguez and Bass, 1983; Galloway *et al.* 1989; Mann *et al.,* 1990; Shean *et al.*, 1993; Komar, 1998; Sharanowski, *et al.*, 2008). The elevated temperatures contribute to increased putrefaction rates, more rapid colonization by arthropods, and dehydration of soft tissues. Studies done on decomposition rates in winter found that there was a decrease in the rates (Sharanowski, *et al.* 2008). These colder temperatures result in a reduction or complete halt of biochemical and bacterial activity. Furthermore, the colder temperatures influence arthropod activity as well as mortality of maggots (Galloway *et al.,* 1989).

Moisture significantly impacts the decomposition process, influencing both the rate and manner in which a body decomposes. The moisture content of the air changes from region to region (Jaworski and Hilszczański, 2013), necessitating the need for region specific studies.

In general, humid environments accelerate decomposition by promoting the growth of bacteria and fungi, essential for breaking down tissues (Galloway *et al.,* 1989). Higher moisture levels enhance microbial activity, leading to faster decomposition. Additionally, moisture attracts certain types of insects, like flies, that lay eggs on moist bodies, increasing maggot activity and accelerating tissue breakdown. Giles *et al.* (2020) suggested that humidity serves as the primary influencing factor in the early stages of decomposition across

both seasons. Moisture is also said to accelerate microbial activity and arthropod colonization in humid, warmer climates (Mann *et al.*, 1990).

A moist environment refers to conditions where high humidity, damp surfaces, or water-saturated surroundings are present, but the body is not fully submerged in water (Haglund and Sorg, 2002). In contrast, an aquatic environment or submerged environment involves complete or partial immersion in water (Haglund and Sorg, 2002). The key differences lie in the level of water exposure and its effects on decomposition.

The environment itself can affect the manner in which the remains will decompose. Different environments - subaerial, aquatic, and buried - will have their own set of characteristics and effects on the remains.

2.1.2. Subaerial decomposition

Subaerial decomposition occurs on land and is influenced by factors such as climate, insect activity, and scavengers. Key indicators used in this environment include the presence and developmental stages of insects, algor mortis in the early stages of decomposition, and physical changes such as skin discoloration, bloating, and tissue breakdown (Michaud and Moreau, 2011). Algor mortis is the process by which a deceased body equilibrates with the ambient temperature (Henssge and Madea, 2004). The body equilibrates with environmental temperature due to thermoregulation ceasing. After death, the body stops producing metabolic heat, and heat loss continues until the body reaches thermal equilibrium with its environment. The rate of cooling can be influenced by several factors, including ambient temperature, humidity, clothing, body size, and the surface on which the body rests (Henssge and Madea, 2004).

Anderson (2011) conducted a study on the decomposition rates of pig remains in outdoor conditions, finding that such environments accelerated decomposition. This acceleration was

attributed to exposure to direct sunlight, rapid insect colonization, and significant temperature fluctuations.

Sluis *et al.* (2023) investigated the decomposition of piglet remains in a subaerial environment in the Netherlands and found that decomposition rates were lower during the winter season. This slower progression of decomposition contrasted with the faster rates observed in the subsequent spring season. Additionally, the study noted delayed bloating of the small cadavers in both winter and spring. Similar findings were reported by Matuszewski *et al.* (2010) and Díaz-Aranda *et al.* (2018), who observed that the bloating stage began later and lasted longer during colder months. The delayed bloating in winter means that cadavers from spring, which have already passed this stage, could be misidentified as winter cadavers, leading to inaccuracies in determining the time since death. Galloway *et al.* (1989) investigated human decomposition using cadavers and discovered that outdoor exposure to rainfall slowed decomposition rates. This deceleration occurred because the tissues retained moisture, preventing dehydration. Rainfall also influences decomposition by altering insect colonization on the remains (Mann *et al*. 1990). Additionally, research has shown that moisture exposure leads to the formation of adipocere, which helps preserve the remains (Mant and Furbank, 1957; Galloway *et al.* 1989). Consequently, in aquatic environments, the formation of adipocere is commonly observed.

Despite advances in understanding Subaerial decomposition, key gaps remain. For instance, while insect succession patterns are well-documented, most studies focus on static Subaerial environments, leaving transitional or fluctuating conditions, such as seasonal flooding, underexplored. Addressing these gaps is essential to refining forensic techniques for PMI estimation in diverse and changing ecological settings.

2.1.3. Aquatic decomposition

Bodies found in water do not exhibit the same characteristics as those found on land. Medical parameters such as algor mortis, rigor mortis (muscle stiffening after death as a result of adenosine triphosphate (ATP) breakdown (Smith and Bendall, 1947; Bendall, 1973)), and livor mortis (a passive process that causes blood pooling in blood vessels, resulting in a dark purple discolouration (Olhovsky *et al.,* 2019) are altered by aquatic environments (Petrik *et al.* 2004). Moreover, the floatation of the bodies creates unique scenarios and poses difficulties in establishing PMI (Petrik *et al.* 2004).

Establishing PMI in an aquatic environment is challenging due to the unique variables affecting decomposition compared to Subaerial settings. Heaton *et al.* (2010) identified factors such as currents and tides, dissolved oxygen levels, water acidity, depth, pressure, temperature, and debris as potential influences on decomposition. Dolinak *et al.* (2005) found that the rate of decomposition decreases with increased depth of submergence. This was corroborated by similar findings in studies done by Heaton *et al.* (2010) and Caruso (2016). Thus, depth of submersion strongly influences the forensic relevance of aquatic decomposition studies. While deep-water research, such as that conducted by Hibischak and Anderson (1999) has provided important biological insights into taphonomy, these contexts are not representative of most forensic recoveries, which typically occur in shallow setting such as pools, river, dams, and near-shore waters (Anderson, 2011; Anderson and Bell, 2016). Shallow submersion environments are characterised by warmer and more variable temperatures, greater potential for insect access, and higher likelihood of mechanical disturbance (Pinherio, 2006; Heaton *et al.,* 2010). As such, studies of shallow-water decomposition are more directly applicable to forensic casework, where bodies are rarely recovered from deep-sea contexts.

Heaton *et al.* (2010) study on postmortem submersion intervals showcased that decomposition in aquatic environments follows three stages, namely fresh, bloat and then decay. These results were first seen by Hobischak and Anderson (1999) in their investigation into freshwater related deaths in British Columbia between 1995 and 1996. However, their study revealed the challenges of accurately determining PMI in aquatic settings, particularly when prolonged immersion occurs. Most PMIs were estimated based on the last time the deceased was seen alive, a method that may be unreliable, especially in cases of homicide. The study highlighted the limited use of entomological evidence, which can provide valuable information about the time since death in Subaerial and aquatic cases. In their review, faunal succession and insect evidence were rarely documented, underscoring the need for improved training in the collection of aquatic invertebrates. The authors advocate for more detailed observations of decomposition changes in freshwater bodies, including the use of faunal evidence, to enhance the accuracy of PMI estimations in water-related deaths (Hobischak and Anderson, 1999). However, Barrios and Wolff (2011) provided a detailed categorization of decomposition in aquatic environments, identifying six distinct decomposition stages in pig carcasses placed in freshwater ecosystems. Their expanded classification emphasizes the complexity of aquatic decomposition and suggests that a more nuanced approach is necessary for refining PMI estimations in submerged cases.

Caruso (2016) examined decomposition changes in bodies retrieved from aquatic environments, confirming that decomposition slows with submergence but may accelerate once the body is removed from the water. This finding underscores the importance of conducting postmortem examinations promptly after the body is extracted from water, which was recommended by Dolinak *et al.* (2005) and further supported by Caruso's (2016) research.

Tomberlin and Adler (1998) studied the decomposition and insect colonization of rat (*Rattus rattus L.*) carrion in both subaerial and aquatic environments and focused on differences between summer and winter conditions. They found that during summer, fewer than 30 insect species colonized the carcasses in both environments. However, in winter, only three species of blowflies were observed on the subaerial carcasses, and no insect colonization occurred on the submerged carcasses. This study highlighted significant seasonal variations in insect colonization of carrion, illustrating distinct patterns and dynamics across different times of the year (Tomberlin and Adler, 1998).

Anderson and Bell (2016) looked at decomposition in a marine environment. The study focused the impact of marine submergence and seasons on the faunal colonization and decomposition of pigs. Lyssianassid amphipods (Family Lyssianassidae) rapidly colonized the carcasses, leading to complete skeletonization within three to four days. The activity of these amphipods significantly depleted local oxygen levels, creating conditions that excluded other marine fauna. Interestingly, the amphipods consumed muscle tissue before skin, feeding from the inside out. Seasonal differences were also observed: spring carcasses became covered in silt, while fall carcasses remained exposed, attracting more scavengers such as shrimp. Although sharks fed on the exposed carcass during the spring deployment, amphipods remained the dominant force driving decomposition in both seasons. These findings contribute valuable insights into deep-sea taphonomy, highlighting the impact of marine environments on the decomposition process and informing forensic recovery strategies in aquatic contexts.

Alley (2007) investigated how decomposition rates in chlorinated water compare to freshwater environments using pig carcasses as proxies for human bodies. The research focused on answering key questions about how chlorine influences PMI, how trauma impacts decomposition, and what additional factors may contribute. The study found that chlorine

delayed the onset of decomposition, with significant effects observed in bodies submerged in chlorinated water. Additionally, trauma altered decomposition rates, particularly in chlorinated environments.

Siri *et al*. (2019) investigated the potential use of chironomid larvae in forensic investigations involving bodies found in aquatic environments. The study presented two forensic cases from Buenos Aires, Argentina, where the presence of chironomid larvae, specifically *Cricotopus* and *Goeldichironomus holoprasinus*, was documented on submerged human remains. The goal of the study was to explore whether these larvae could assist in estimating the postmortem submersion interval (PMSI) or provide insight into other forensic questions such as whether the bodies had been moved postmortem. Chironomid larvae, especially those from the *Cricotopus* and *Goeldichironomus* genera, are not true necrophages; instead, they feed on decaying organic matter or biofilm on the remains rather than the body itself. This makes them less reliable as indicators of specific stages of decomposition but useful in corroborating environmental conditions at the scene of death, such as water temperature, pH, and the presence of silt or biofilm. This is because chironomid have specific requirements in order to survive and because of these specific requirements, chironomid assemblages can differ between aquatic environments (Saether, 1979; Olafsson, 1992). The study suggests that chironomids could be useful in determining whether a body has been moved postmortem. Barrios and Wolff (2011) also mention chironomids in their study, specifically as arthropods associated with the submerged phase of aquatic decomposition.

In a study by Guareschi and Magni (2021), the discovery of human remains in a floodplain in Northern Italy was investigated, providing key insights into the challenges of postmortem examination in aquatic and semi-aquatic environments. The body, found after a flooding event, was uncovered in mud and colonized by *Calliphora vomitoria* larvae and raft spiders (*Dolomedes fimbriatus*), marking the first recorded instance of raft spider colonization on

remains. This case highlighted the complexities of estimating the postmortem interval (PMI) and postmortem submersion interval (PMSI) in such environments. Cold and wet conditions contributed to the formation of early stage adipocere, while blowfly larvae colonization, which occurred four to five days before the body was recovered, offered a partial estimate of PMI. The raft spiders were likely attracted to the presence of the blowfly larvae, adding an unusual aspect to the forensic entomology findings. The study emphasized the importance of a multidisciplinary approach, integrating taphonomy, anthropology, and toxicology, which revealed the probable cause of death as suicide by drowning. Additionally, forensic evidence indicated that the body had been transported by floodwaters, showcasing the unique challenges posed by such dynamic environments in forensic investigations (Guareschi and Magni, 2021).

Studies have highlighted the challenges of determining PMI in aquatic environments due to the variability in faunal colonization and environmental conditions (Barrios and Wolff, 2011). Despite these differences, aquatic decomposition research is crucial for forensic investigations, particularly in drowning cases or bodies discarded in water.

Contradictions also emerge when comparing decomposition in freshwater and marine environments. For example, Alley (2007) demonstrated that chlorinated water delays decomposition compared to freshwater, with trauma further altering decomposition rates in aquatic settings. Additionally, Siri *et al.* (2019) introduced chironomid larvae as potential indicators of postmortem submersion interval (PMSI) and environmental conditions, though their necrophagous limitations reduce their reliability compared to traditional insect evidence. These findings suggest that while aquatic invertebrates may assist in PMI and PMSI estimations, their applicability is context-dependent and varies significantly with environmental conditions.

Overall, while aquatic decomposition research is crucial for forensic investigations, especially in drowning cases or water-related homicides, significant variability in faunal colonization, seasonal effects, and environmental conditions limits the accuracy of PMI estimations. Addressing these gaps through region-specific studies and enhanced methodologies is essential for improving forensic applications in aquatic contexts.

2.1.4. Transitional environments

Transitional environments in decomposition studies remain an underexplored area of forensic taphonomy. These environments can be characterized by fluctuating conditions, such as exposure to intermittent wetting and drying cycles, which can influence the decomposition process in complex and unpredictable ways. Given that human remains are often discovered in such settings, understanding the taphonomic processes that occur in these environments is crucial for improving PMI estimations and forensic reconstructions.

Du Toit *et al.* (2024) conducted a study on the influence of rehydration on decomposing pigs in Highveld, South Africa. Their study demonstrated that exposure to moisture led to an increase in Total Body Score (TBS) values, even after decomposition had plateaued. Additionally, rehydration was associated with heightened arthropod activity, further accelerating the breakdown of soft tissue. As described by du Toit *et al.* (2024), these findings suggest that episodic moisture exposure can “artificially inflate” TBS values, producing values that suggest a more advanced stage of decomposition than is truly present. In this context, “artificially inflates” refers to an increase in TBS caused by altered tissue appearance due to rehydration and mechanical alteration by the impact of rainfall and insect activity following rehydration, rather than the natural progression of decomposition. The re-initiation of decomposition after rehydration can mask a preceding stagnant period and potentially lead to an underestimation of the post-mortem interval.

To the author's knowledge, this is the only study that directly examines decomposition within a transitional environment, highlighting a critical gap in forensic taphonomy. Given the forensic relevance of such environments, further research is necessary to refine PMI estimation models and account for the influence of fluctuating environmental conditions.

2.2. Methodologies for Studying Decomposition

The Total Body Score (TBS) method was developed by Megyesi *et al.* (2005) using human remains. The TBS is calculated by evaluating distinct regions of the body—namely the head and neck region, trunk region, limbs region—and assigning a score to each region based on observed decomposition changes. The scores are then combined to produce an overall TBS. The scoring system accounts for a variety of qualitative decomposition indicators, including colour changes of the skin, presence of bloating or swelling, degree of skin slippage, development of purge fluids, and the extent of skeletonization. Keough *et al*. (2017) advanced this methodology by developing the Adjusted Total Body Score (ATBS) for pig decomposition studies, adapting the Megyesi *et al*. (2005) approach to better suit non-human subjects.

Another method derived from Megyesi *et al*. (2005) is the Total Aquatic Decomposition Score (TADS), developed by Heaton *et al*. (2010), for assessing decomposition in aquatic environments. The TADS scoring system specifically considers factors influenced by water, such as skin slippage and discoloration, presence of bloating, skeletonization, the phenomenon known as 'washer woman' immersion, and adipocere formation. This methodology was developed using crime scene photos and cold cases from the United Kingdom (Heaton *et al.* 2010). Other aquatic environment scoring methods exists such as Aquatic Decomposition Score (ADS) was developed by van Daalen *et al.* (2017), derived from Megyesi *et al*. (2005), to potentially predict the postmortem submersion interval of bodies that had been recovered from the North Sea.

These scoring systems are often used in conjunction with Accumulated Degree Days (ADD). ADD is a tool borrowed from agricultural entomology and employed by forensic entomologists to estimate a minimum PMI by quantifying the accumulated thermal energy experienced by the body since death. This method integrates time and temperature data to account for the thermal conditions influencing decomposition (Simmons *et al.* 2010). It is calculated using the sum of the daily averages of body temperature (Megyesi *et al*., 2005; Myburgh *et al.,* 2013). The utility of ADD is predicated on the assumption that temperature is the primary environmental driver of the biological processes underlying decomposition. Time alone does not directly cause decomposition, rather, it is the cumulative exposure of decomposition agents (such as bacteria and invertebrates) to specific thermal conditions that drives the rate and pattern of decay. Consequently, accumulated thermal energy over time provides a more accurate explanatory variable than elapsed time alone. By incorporating both decomposition scoring systems and ADD, forensic scientists can achieve a more comprehensive and precise determination of the PMI or PMSI.

These methodologies have been used for both human and animal remains, offering forensic scientists standardized tools for decomposition analysis. However, their application in dynamic environments, such as those with environmental transitions, remains limited. This is because these methods were developed and validated under relatively stable environmental conditions. In dynamic settings, such as transitions between subaerial and aquatic environments (and vice versa), decomposition may proceed in a non-linear manner with abrupt changes in tissue appearance and decomposition rates that are not fully captured by existing scoring systems and ADD models. Such variables could disrupt the assumed relationship between accumulated thermal energy and decomposition stage, reducing the accuracy of PMI estimations derived from these methods.

Furthermore, while stage-based assignment provides a useful framework for describing decomposition, it has been criticised as typological and reductionist, potentially obscuring and overlapping the continuous process of decomposition. As different anatomical regions may simultaneously exhibit characteristics of multiple stages, such as moist decomposition and partial skeletonization occurring at once (Ceciliason *et al.,* 2023). Nonetheless, decomposition staging remains a pragmatic and necessary tool for forensic taphonomy. These standardized frameworks (e.g., TBS, ATBS, TADS) facilitate consistent observation and comparative analysis across studies. By systematically documenting morphological changes, stage-based scoring enables clearer communication of taphonomy progress and helps structure complex data into interpretable patterns. In this current study, stages are used not to impose rigid categories but to provide a structured approach for comparing decomposition trajectories across experimental conditions and environments.

2.3. Arthropod Colonization Across Environments

Forensic entomology is application of arthropod study to criminal and death investigations (Hall and Huntington, 2010). Although primarily used to assist in determining a minimum PMI, arthropods can also be used to provide insight into the circumstances surrounding death, including whether a body was moved postmortem (as many insect species have distinct habitat preferences or are endemic to specific regions (Goff, 1991)), the seasonality of death, and potential signs of trauma or drug use that could affect insect succession (Goff, 1991; Anderson, 1997; Amendt *et al.,* 2006; Marchetti *et al.,* 2013).

The study of insect succession and colonization of decomposing remains is how forensic entomologists aid in a minimum PMI estimation (Hall and Huntington, 2010). For example, forensic entomologists can estimate the time elapsed since a Calliphoridae larvae hatched from its egg. Backtracking development time is how they deduce this which can be done through phenological information (Staerkeby, 2001) or by subtracting the ADD that would be

required to complete development from total number of days required for larval development to complete (Higley and Haskell, 2001).

There are four ecological groups linked to decomposing remains (Smith 1986). Smith (1986) grouped them based on biological roles. Group one consists of necrophagous species, that directly consume carrion and remains. Group two includes the predators and parasites that feed and target group 1 (necrophages) species. The third group is the omnivorous species that feed on the remains as well as the other colonizing insects. Finally, group four is the group of species that use the remains as a habitat rather than for nutrition. Insects, particularly necrophagous species, arrive at a body in a predictable sequence, influenced by factors such as environmental conditions, volatile organic compounds (VOCs) released by the remains (Martin *et al.,* 2019), and geographic location (Gennard, 2007). The *Calliphoridae, Sarcophagidae, Phoridae*, and *Muscidae* families for the Dipteran order, exhibit a strong correlation with specific stages of decomposition in both human and animal studies and can thus be used to estimate minimum PMI (Byrd and Castner, 2010; Villet, 2011).

The estimation of PMI relies on the sequential colonization of a body by different assemblages of Diptera and Coleoptera (Rodriguez & Bass, 1983; Goff, 1993; Amendt *et al.,* 2006; Gennard, 2007). Additionally, it takes into account insect development and life cycle progression to determine the time elapsed since death (Goff, 1993; Gennard, 2007).

2.3.1. Subaerial arthropod decomposition

During the fresh and bloat stages of decomposition, blowflies (*Calliphoridae*) are among the first to colonize remains, laying eggs that hatch into larvae (Michaud and Moreau, 2010; Saukko and Knight, 2015). Beetles (*Coleoptera*) and other scavengers appear as decomposition progresses (Michaud and Moreau, 2010; Saukko and Knight, 2015). Thus, in the active stage of decomposition various species of beetles (*Coleoptera*), ants, and other arthropods, including maggots from blowflies are present, further accelerating decomposition

through the secretion of proteolytic enzymes (Saukko and Knight, 2015). Advanced decomposition sees maggots typically vacating the body to pupate (Michaud and Moreau, 2010) and an increase in beetle activity as they feed on the dried skin, hair, and tendons (Kolver, 2009; Parks, 2011). Insect succession on decomposing remains is used in conjunction with other methods and characteristics of stages to estimate the post-mortem interval of the remains.

However, this predictability can vary depending on environmental conditions. For instance, factors such as temperature, humidity, and access to remains may alter the timing and composition of arthropod colonization. In cold climates or highly arid environments, insect activity can be delayed or suppressed, potentially skewing PMI estimations (Sharanowski *et al.,* 2008). These contradictions highlight the need for regional studies to refine our understanding of insect succession under diverse environmental conditions.

Additionally, while the literature emphasizes the utility of blowflies and beetles as primary decomposers, gaps remain in understanding the role of other arthropods, such as ants and scavenger beetles, particularly in later stages of decomposition (Al-Qurashi *et al.,* 2023). The ecological interactions between these species—whether competitive, complementary, or disruptive—are underexplored and could provide deeper insights into decomposition dynamics (Matuszewski and Madra-Bielewicz, 2022).

Furthermore, studies often focus on static environments, leaving a significant gap in understanding how insect succession may differ in transitional settings, such as remains exposed to both subaerial and aquatic conditions. Addressing this gap would improve forensic entomology's applicability in complex cases where remains are subjected to fluctuating environments.

2.3.2. Aquatic arthropod decomposition

Forensic entomology in subaerial and aquatic environments presents distinct challenges due to environmental variability, which influences insect colonization patterns, decomposition rates, and the accuracy of postmortem interval estimations (Campobasso *et al.,* 2001). While insect succession in subaerial settings is often predictable, aquatic environments introduce complexities influenced by factors such as water temperature, oxygen levels, and depth. Barrios and Wolff (2011) offer valuable insights into the succession of aquatic arthropods, with key species classified as collectors (e.g., *Chironomus*, *Simulium*), shredders (e.g., *Tropisternus*, *Hyallela*), and predators (e.g., dragonflies and damselflies) dominating the submerged fresh stage.

A notable pattern in their study is the gradual shift from aquatic-dominant arthropod communities in early stages to a mix of aquatic and subaerial species during later stages. For instance, subaerial species such as blue bottle flies (*Calliphoridae*) and carrion beetles (*Silphidae*) begin colonizing the body during the later floating stage. This phase represents an intermediate stage of decomposition, where increased accessibility to terrestrial scavengers coincides with ongoing aquatic activity. This transition reflects the interplay between decomposition processes and environmental conditions, with aquatic environments slowing decomposition while still attracting necrophagous and sarcosaprophagous species as the body surfaces.

However, their research also highlights a gap in understanding how these patterns may differ across ecological regions. The specific species identified—such as *Chironomus* and *Simulium*—are characteristic of the Colombian Andes and may not generalize to other ecosystems. This limitation raises questions about the universality of arthropod succession patterns in aquatic environments and underscores the need for region-specific studies.

A key contradiction in the literature lies in the predictability of arthropod succession in aquatic environments. While Barrios and Wolff (2011) identified consistent stages, other studies have noted variability in arthropod colonization depending on local water conditions, such as salinity or water movement (Heaton *et al.*, 2010). This discrepancy indicates a need for further research to determine how environmental factors mediate arthropod activity in aquatic decomposition.

Finally, the present proof-of-concept study provides limited insights into transitional environments, where remains shift between aquatic and subaerial settings. Understanding how arthropod colonization patterns adapt in such dynamic contexts remains a critical gap, particularly for forensic investigations in regions with fluctuating water levels or seasonal flooding. Addressing this gap could refine PMI estimations and enhance the applicability of forensic entomology in diverse ecosystems.

2.4. Animal proxies in Forensic research

Many decomposition studies in subaerial and aquatic environments use pigs (*Sus scrofa*) as human proxies. Pigs are widely recognized as effective proxies for human cadavers in forensic science research due to their physiological, anatomical, and genetic similarities to humans (Matuszewski *et al.*, 2019). These shared traits include comparable body fat distribution, skin composition, and internal organ structures, making pigs highly suitable for studying decomposition processes where the establishment of baseline data is concerned. Their use is particularly advantageous because they are readily available, cost-effective, and ethically permissible, addressing logistical challenges associated with human cadaver research. This is especially relevant in countries like South Africa, where cultural and ethical concerns surrounding the use of human remains often limit research opportunities (Matuszewski *et al.,* 2019). Thus, many decomposition and forensic taphonomy studies in

South Africa have used a porcine model (Sutherland *et al*., 2013; Marais-Werner *et al.,* 2017; Spies *et al.*, 2020; Keyes *et al.,* 2020; Tembe and Mukaratirwa, 2021; Adams *et al.,* 2024)

Comparative studies have consistently demonstrated significant overlap in the arthropod fauna associated with pig and human cadavers. For example, Schoenly *et al.* (2007) and Wang *et al*. (2017) observed that the succession patterns of insect colonization and the species attracted to decomposing remains were largely consistent between the two species. This similarity strengthens the validity of using pigs as proxies in forensic entomological research, enabling realistic simulations of human decomposition dynamics under various environmental conditions.

Despite these advantages, the application of pig models has its limitations. While pigs provide reliable insights into general decomposition processes, differences in skin structure, fat content, and other factors mean that results must be cautiously interpreted when applied to human decomposition. This is particularly true in scenarios involving environmental transitions, such as shifts between subaerial and aquatic environments, which remain unexplored for both species in forensic research.

Addressing these limitations is critical for improving the applicability of pig-based findings to human forensic cases. Future studies should aim to quantify and account for these discrepancies, particularly in dynamic environmental settings. By refining the use of animal proxies, forensic researchers can better bridge the gap between experimental results and real-world forensic applications.

3. Research Gap and Significance

While decomposition dynamics and arthropod colonization are well-documented in static environments, the effects of environmental transitions remain underexplored. This gap is particularly relevant in South Africa, where diverse ecosystems and climatic variability

create unique forensic challenges. Such is the case of 2022, KwaZulu-Natal which suffered one of the most catastrophic flood disasters in Sout African History. Over 400 people died, more than 7 000 were displaced and many victims were unaccounted, with some bodies being recovered weeks or even months late (Naidoo *et al.,* 2022). Investigating these transitional phases is crucial for advancing forensic science, particularly in regions prone to flooding or other environmental shifts.

4. Aim and objectives

The aim of the study was to describe the rate and pattern of decomposition, as well as the colonization dynamics of arthropods, in response to alterations in the surrounding environment of the remains from subaerial to freshwater aquatic conditions and vice versa.

The objectives of the study were:

1) To describe the rate and pattern of decomposition of the remains that were initially subjected to subaerial conditions and then moved to freshwater aquatic conditions.
2) To describe the rate and pattern of decomposition of the remains that were initially subjected to freshwater aquatic conditions and then moved to subaerial conditions.
3) To evaluate the effectiveness of the methods (Total Body Score and Total Aquatic Decomposition Score) in remains that decomposed in alternating environments.
4) To conduct a comprehensive survey of arthropod species inhabiting carrion across the two different decomposition environments
    a. Analyse variations in the arthropod population density on the remains in response to alterations in decomposition environments (subaerial vs aquatic).
    b. Evaluate alterations in the species composition of arthropods inhabiting the remains across the two decomposition environments.

5. Materials and methods

5.1. Study Design

A longitudinal, comparative, observational study with an experimental component. This study is classified as a longitudinal study as decomposition was monitored at multiple predefined intervals over the course of the research period, enabling temporal patterns to be identified and analysed. It is comparative as it systematically evaluates the differences in decomposition progression between experimental groups exposed to alternating environments and control groups remaining in a single environment. The research also incorporates an experimental component, wherein the environmental transition was deliberately introduced under controlled conditions to isolate and assess its specific effects on decomposition rate, morphological change, and associated arthropod activity.

5.2. Sample

The study involved a total of 12 juvenile piglets (*Sus scrofa domesticus*) that died from natural causes. The piglets were donated by GHB Farms, a registered pig farm that breeds pigs for commercial consumption. Natural causes of death should not significantly impact decomposition rates or patterns for reasons such as a lack of physical trauma or no release of inhibitory substances (Munro *et al.,* 2019). The piglets that were used in this study had a mass of 1.5 to 6.0 kgs. The piglets had been refrigerated for 2 days prior to the onset of the study. Piglets were used in this study as opposed to adult pigs due to the limited size of the study site as well as the fact that this was a proof-of-concept study.

Pigs are frequently employed as models of human cadavers in forensic science studies, owing to numerous physiological and anatomical similarities with humans (Matuszewski *et al.* 2019). Their availability and cost-effectiveness further enhance their suitability for research endeavours. Notably, comparative studies involving pig and human cadavers have demonstrated substantial overlap in insect faunas (Schoenly *et al.,* 2007; Wang *et al.,* 2017).

A small sample size was used due to logistical constraints, the need for controlled experimental conditions, resource limitations, intensive data collection requirements, and the study's exploratory nature, ensuring detailed and manageable data collection while maintaining research integrity. Furthermore, as this is a proof-of-concept study, the piglets were unclothed in order to limit the number of variables within the study. Future studies could explore more variables to add to forensic realism.

5.3. Site of study

The location for this study was a secured open lot connected to the Johannesburg Forensic Pathology Service Medico-legal Mortuary (JHB FPS) and adjacent to the abandoned hospital that sits on the same site as the JHB FPS (Figure 1). Permission to use the site was obtained from the JHB FPS mortuary managers (Ms Phumzile Mdluli and Mr Johannes Mosikili), Chief Forensic Pathologist (Dr Tinyiko Zelda Nkondo), the Chief State Veterinarian (Dr D Mofu), and Director of Animal Health: Department of Agriculture, Land Reform, and Rural Development (Dr Mpho Maja) (Appendix C). Entomological analysis occurred in the Forensic Entomology Laboratory at the JHB FPS.

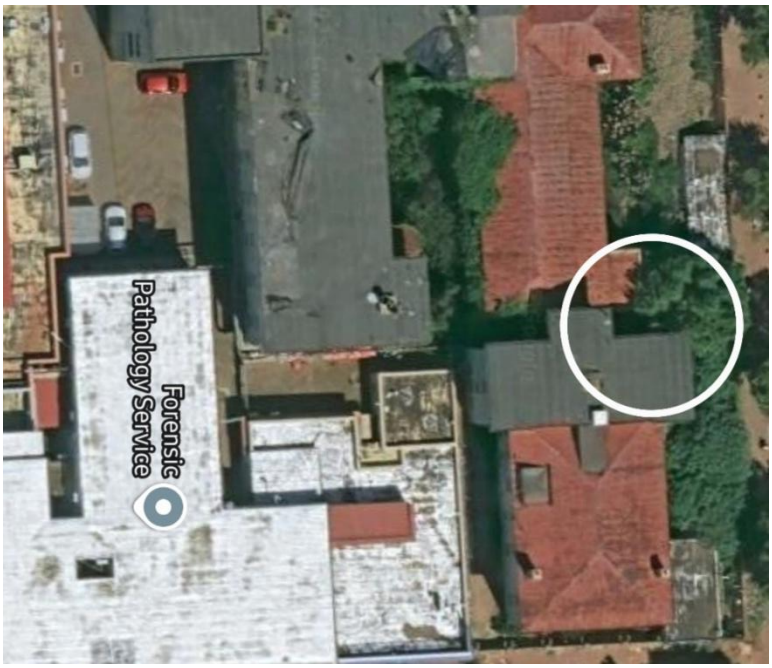


Figure 1: Shows the study site location (circled in white) at the Forensic Pathology Services in Hillbrow.

5.4. Data collection

The sample (N=12) was divided into two experimental groups (Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group) of five piglets each, with an additional two controls. Within the groups piglets were labelled LW1 to LW5 (Subaerial-to-Aquatic Group where “L stands for Land and “W” stands for Water, with LW indicating a “Land” to “Water” environmental transition) and WL1 to WL5 (Aquatic-to-Subaerial Group where “W” stands for Water and “L” stands for Land, with WL indicating a “Water” to “Land” environmental transition). This naming convention was done in order to compare the different piglets within and across groups. The Subaerial-to-Aquatic Group was initially exposed to subaerial conditions (placed on concrete with vegetation such as fallen leaves occurring naturally) for seven days followed by a transition to freshwater aquatic conditions for the same amount of time. The Aquatic-to-Subaerial Group went through the reverse sequence, commencing with immersion in freshwater aquatic conditions before the remains were transitioned to subaerial conditions (placed on concrete with vegetation such as fallen leaves occurring naturally). The time frame of seven days for decomposition was to avoid having pigs in the advanced stage of decomposition before changing environments. This time frame was determined by the decomposition rates observed in a study done by Coertze (2022) in the same location (JHB FPS). One control pig remained in a subaerial environment for the full two-week duration and one control pig remained in an aquatic environment for the full two-week duration of the study.

The study commenced on 3 December 2024 with all twelve piglets being placed at the study site. The piglets were placed in a subaerial environment with partial shade. These conditions were kept the same for both groups. The piglets were placed one meter apart, due to the limited space in the study site. Cross contamination of insects likely occurred; however, the effect was not quantified as this falls outside the scope of the objectives of this study

(Schoenly *et al.,* 2015). Subaerial piglets were placed on one side of the study site and the aquatic piglet group were placed on the opposing side of the study site. See Figure 2 for schematic. Piglets were uncovered, as no cages were required due to the lack of scavengers at the study site (Keyes *et al.,* 2022). Minimal disturbance to the remains was maintained throughout the study to preserve the integrity of the decomposition process. In the subaerial environment, piglets were not moved once placed, except for minor adjustments necessary during scoring. In the aquatic environment, piglets were only lifted while still submerged. These precautions were taken to minimize the introduction of additional variables that could influence the rate or pattern of decomposition, thereby ensuring consistency and reliability in the observational data.

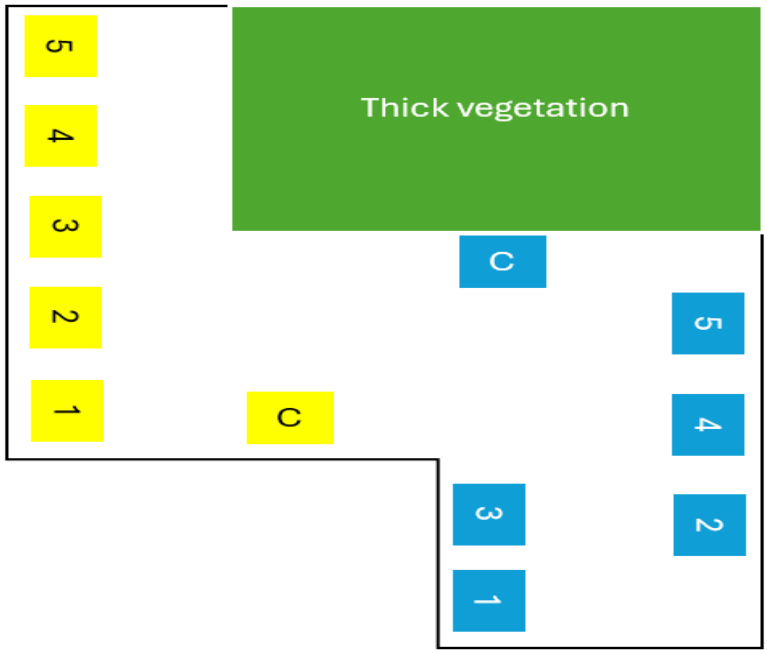


Figure 2: A schematic of the layout of the study. Piglets were placed 1 meter apart. Yellow represents the Subaerial-to-Aquatic piglets and the Subaerial control (C). Blue represents the Aquatic-to-Subaerial piglets and the Aquatic control (C).

The subaerial environment was a controlled concreted outdoor environment with ambient air exposure, replicating natural subaerial conditions. The controlled freshwater aquatic environment consisted of placing the pig carcasses in a 30-liter plastic container filled with municipal tap water. This choice was deliberate to control the study conditions by mitigating

numerous variables, including water flow, acidity, salinity, water current, and tides as this is a proof-of-concept study. As part of the study design, the water in the container remained stagnant. Replenishment of the water was not needed as the containers were not exposed to direct sunlight and thus minimal evaporation occurred. Each piglet carcass had its own container to avoid possible influence from other carcasses. Finally, each piglet was equally exposed to sunlight, shade, and rainfall.

5.5. Decomposition Rate

Photographs of each carcass were taken every day of the week to visually document the decomposition process. These photos were taken at midday to avoid shadow casts on the carcass. Photographs were taken using the camera of a Samsung A73 phone at a consistent height (30 cm) above the carcasses.

The carcasses' decomposition was scored daily on-site using an Adjusted Total Body Scores (ATBS) by Keough *et al.* (2017) (developed for pig TBS scoring based on the human TBS method by Megyesi *et al.* (2005)) (Appendix A) for the subaerial environment and Total Aquatic Decomposition Score (TADS) for the aquatic environment from Heaton *et al*. (2010) (Appendix B). Scoring backwards was allowed and accounted for, allowing the scorer to assess decomposition based on observations from that specific day, regardless of whether a higher score had been recorded on a previous day. It should be noted that TADS is a scoring system developed using human remains. Since this study uses piglets as a proxy, minor wording adjustments were made to account for anatomical differences. Although the scoring criteria themselves were not altered, TADS has not yet been validated for porcine remains and will require more validation. Nonetheless, given the proof-of-concept nature of the present study and the absence of a standardised aquatic decomposition scoring system for non-human remains, TADS was applied in its original form with an adjustment (the addition of "trotter" in place of hands and feet).

The supervisors of this study also scored the remains from photographs to assess inter-observer error. Supervisors have extensive experience with several TBS methods and provided training to the principal examiner. Although inter-observer error analysis was conducted, only the scores provided by the primary researcher were utilized for the final analysis, as the inter-observer error was low. This approach aimed to maintain consistency and reliability in the data interpretation, while also acknowledging the importance of corroborating observations from multiple perspectives when necessary.

All scores (ATBS and TADS) were converted into a standardised TBS (STBS) (out of a total of 35) to allow for direct comparisons between the groups (Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group) throughout the decomposition period and environmental changes. ATBS is already out of a total score of 35, so those were not changed, but the scores for TADS are out of a total score of 24, so they converted into a STBS out of 35 using the following equation: $STBS = \left(\frac{x}{24}\right) \times 35$.

The ambient temperature of the air and water in each container were recorded daily at the time of each documentation session. Temperature was recorded using iButton data loggers (Maxim iButton DS1922L) in latex gloves for protection, these were secured on site and set to take temperature measurements hourly. iButtons were placed either directly next to the piglets (when in a subaerial environment) or in the water container with the piglets (when in the aquatic environment). This data were used to calculate accumulated degree days (ADD) (calculated as the sum of daily average temperatures) which was used in correlation with STBS to plot decomposition rates. ADD was calculated using the following formula: $ADD = \Sigma(\frac{\max \text{temperature} + \min temperature}{2})$. Many of the iButtons for the experimental piglets stopped recording data due to technical issues, thus the data from the iButtons with two control piglets were used for all piglets.

STBS was plotted against logADD for each pig to show the change in decomposition rate during the experimental period. The logarithmically transformed ADD was used to in order to have a linear association between STBS and ADD for comparability to other studies.

5.6. Decomposition Pattern

To describe the pattern of decomposition in the two experimental groups, gross morphological changes of the remains were described and categorized into established decomposition stages and compared between the two experimental groups. These changes were recorded and classified according to widely accepted decomposition stages, such as fresh, bloat, active decay, advanced decay, and skeletal remains. Each stage was identified based on key visual indicators, including tissue discoloration, bloating, maggot activity, soft tissue breakdown, and skeletal exposure. These stages typically include i) early decomposition, ii) bloated, iii) active decay, iv) advanced decay, and v) dry remains as guided by published literature (Myburgh *et al.,* 2013; van Daalen *et al.,* 2017; Reijnen *et al.,* 2018). This approach ensures repeatability by relying on standardized classification criteria, allowing for objective comparisons and potential replication in future studies.

5.7. Arthropod Colonization Dynamics

Sticky traps were placed around the carcasses in order to capture arthropods. Cruise *et al.* (2018) showed that sticky traps were effective in sampling arriving insects on neonate pigs. The sticky traps used by Cruise *et al.* (2018) were mounted to an open wooden frame above the carcasses. However, the method is ineffective in sampling beetle species (Cruise *et al.,* 2018) and thus active sampling from the carcasses was also implemented in the present study to account for this limitation of sticky traps. Traps were checked, and contents collected at each daily documentation session, with some (not a set amount each day) arthropods also directly collected from decomposing carcasses for identification purposed. This was done in order to document alterations in arthropod species. Arthropods were stored in small plastic

jars. These were then euthanised by placing them in the freezer. Maggots were killed using hot water from a kettle and then placed in 70% ethanol for preservation. Arthropods were then identified using a stereo microscope, a digital microscope, and established keys (Zumpt, 1965; Smith, 1986; Holloway 1991; Brink, 2009; Szpila and Villet, 2011).

Insects were not specifically removed for the changing of decomposition environments in order to limit the variables changed. This was done to mimic real scenarios where remains would suddenly be submerged into an aquatic environment or emerged into a subaerial environment and arthropods inhabiting the remains would thus be subjected to environment changes as well. However, due to active sampling some of the insects that were not easily identifiable were removed for analysis.

5.8. Data Analysis

Software used to run statistical tests and generate graphs and maps included Microsoft Excel, R studios, and IMB SPSS Statistics V28.

Table 1: Statistical analysis run on the data to achieve the outlined objectives of the study.

Objectives	*Variables*	*Data analysis*
To describe the rate and pattern of decomposition of the remains that were initially subjected to subaerial conditions and then moved to aquatic conditions.	Gross morphological changes of the remains Ambient temperature of the air and water Adjusted Total Body Scores (ATBS) Total Aquatic Decomposition Score (TADS).	Generated graphs to illustrate the progression of decomposition over time, separately for subaerial and aquatic conditions as well as together. Graphing the ATBS and TADS against ADD for each pig.
To describe the rate and pattern of decomposition of the remains that were initially subjected to aquatic conditions and then moved to subaerial conditions.		
To evaluate the effectiveness of the methods (Total Body Score and Total Aquatic Decomposition Score) in remains that decompose in alternating environments.	Standardised TBS scores. Multiple observers (inter observer)	Non-parametric test run was Friedman test (5% level of significance) with a post-hoc Wilcoxen Signed Rank test (Bonferroni correction applied by IMB SPSS). To compare the decomposition patterns between the two groups, the change in decomposition rates over time will be described using the TBS vs ADD graphs. Intraclass correlation coefficient (ICC) for inter observer error. Specifically model ICC (C, k) (Two-way random effects, absolute agreement, average measures. ICC for intra observer error. Specifically model ICC(A, k) (Two-way mixed effects, absolute agreement, average measures).
To conduct a comprehensive survey of arthropod species inhabiting carrion across two different decomposition environments to assess alterations in species composition.	Types of arthropods collected	Jaccard similarity heatmap to provide graphical representation of dissimilarities in arthropod communities between the two decomposition environments.

5.9. Ethics

An ethics waiver was obtained from the Animal Research Ethics Committee (AREC) at the University of the Witwatersrand. **Waiver 08-02-2024-O (Appendix D)**

5.10. Funding

Funding came from RINC funds in the Division of Forensic Medicine and Pathology.

6. Results

During the period of the study, a heatwave was ongoing (Figure 35 Appendix H). Rainfall was recorded on day five, six, eight, and nine of the study.

6.1. Morphological decomposition patterns

Observations were recorded daily, noting visible changes such as bloating, discolouration, blistering, skin slippage, maggot and insect activity, and skeletal exposure.

6.1.1. Subaerial-to-Aquatic Group morphological decomposition description

All piglets in the Subaerial-to-Aquatic Group (Piglets that were first placed in a subaerial environment and then transitioned into an aquatic environment) had a STBS of 3 on day one, except for the LW2 and LW3 piglets (STBS values of 6 and 4, respectively). During the subaerial decomposition phase (See Appendix D - Table 12 for panel images of all Subaerial-to-Aquatic specimens), the piglets exhibited rapid discolouration, bloating, and blistering, with progressive exposure of internal organs by day four. Bloating was first observed on day two and became pronounced by day five, with all piglets exhibiting distinct bloating. Some piglets rolled onto their dorsal trunk during this time. By day five blackening of the lips, eyes, abdomen, neck, and chest was observed, along with a grey-green discolouration in areas connecting the trotters and abdomen (see Figure 3 of LW1 specimen).



Figure 3: Day 5 - LW1 specimen of Subaerial-to-Aquatic group.

On day six, the piglets were lifted for the first time to examine the body areas in contact with the ground. This revealed large maggot colonies and exposed tissue, particularly in the abdomen and face. Maggot activity was also visible within the cavities of the neck, abdomen, and parts of the face, where decomposition had removed tissue and skin.

On the final day of subaerial decomposition (day seven), bloating decreased, resulting in the caving-in of abdomens, except for LW3, which remained bloated. Bone exposure was observed in the face and neck regions of LW1 and LW2 (see Figure 4 of LW1 specimen), while the other piglets exhibited blackened and collapsed face and neck areas.



Figure 4: Day 7 - LW1 specimen for Subaerial-to-Aquatic Group

The piglets were transitioned to an aquatic decomposition environment on day eight(See Appendix D - Table 13 for panel images of Subaerial-to-Aquatic specimens and Figure 5 for LW1 specimen). Upon placement in water, all piglets floated. On the same day, increased bone exposure was observed in LW1 and LW2, particularly in the upper trotter bones of the front limbs and the ribs. The other piglets displayed greater tissue exposure compared to the previous day.



Figure 5: Day 8 - specimen LW1 of Subaerial-to-Aquatic group.

By days nine and ten, trotters showed advanced signs of decomposition, including soggy tissue and discolouration. Facial bones were exposed in four out of five piglets, with LW4 being the exception. Day eleven marked the disarticulation of the heads of LW1 and LW2 (see figure 6).



Figure 6: Day 11 - specimen LW1 of Subaerial-to-Aquatic group.

By day twelve, skeletonization of the front limbs was evident in LW1 and LW2. LW3 and LW5 displayed disarticulation of the mandible and bone exposure in the neck and chest regions.

On day thirteen, the trotters began degloving or turning black. All piglets exhibited varying degrees of bone exposure in the head region, with LW5 showing head disarticulation from the neck.

On the final observation day (day fourteen) (Figure 7), LW1 exhibited extensive skeletonization, with more than 90% of its tissue lost, and disarticulation of the front limbs from the trunk. LW2 showed significant skeletonization of the abdomen. LW3 exhibited no abdominal skeletonization; however, the head and neck region showed bone exposure, and the front limbs began to disarticulate from the trunk. LW4 displayed bone exposure in the head, neck, and the rear trunk near the tail. The trotters of LW4 were predominantly black and soggy. LW5 exhibited bone exposure in the chest and neck regions, while the trotters remained attached to the trunk but were soggy to the touch.



Figure 7: Day 14 - specimen LW1 of Subaerial-to-Aquatic group.

6.1.2. Aquatic-to-Subaerial Group morphological decomposition description

During the first seven days of the study, the Aquatic-to-Subaerial piglets exhibited distinct stages of decomposition in the aquatic environment (See Appendix D - Table 14 for panel images). Two of the piglets began with an initial TADS of 3, except for WL1 (TADS = 5), WL3 (TADS = 4) and WL5 (TADS = 4). TADS was converted to STBS resulting in higher STBS in comparison to the Subaerial-to-Aquatic group. This conversion resulted in initial STBS values of 7.29, 4.38, 5.83, 4.38, and 5.83 for piglets named WL1, WL2, WL3, WL4 and WL5 respectively. Early discoloration, primarily in the head, neck, and abdomen, was observed, progressing from dark pink to purple tones. Day two saw WL1, WL2, WL3, and WL4 floating. By day three, bloating and blistering were evident, accompanied by green discoloration of the abdomen and neck (Figure 8). A green-grey tinge was noted around the eyelids in WL1 and WL5. Gas bubbles were observed escaping through orifices, evidenced by bubbles in the water around the mouth and anus, as well as purging of blood from the mouth.



Figure 8: Day 3 - specimen WL4 of Aquatic-to-Subaerial group.

On day four, the piglets displayed further decomposition, with bloated trotters and dark purple-red abdomens. WL5 was the last piglet to float (seen on Day four) and showed no

colour changes up to this point. By day five, WL1 and WL2 had exposed internal organs and blackened abdomens. WL1 and WL3 exhibited green discoloration on their limbs. WL4 was the first piglet to exhibit tissue exposure with maggot activity. By day six, black discoloration had become prominent on the abdomen, chest, neck, and head of many piglets. Signs of skin slippage were noted on the trotters, which were soggy to the touch. WL1 displayed exposed internal organs, while other piglets had tense abdomens indicative of pooled blood.

On the final day (day seven) of the aquatic environment, green discoloration was observed on the neck, dorsal trunk, and face of several piglets (Figure 9). WL2 and WL4 showed additional brown discoloration on the trotters and dorsal trunk, respectively.



Figure 9: Day 7 - specimen WL4 of Aquatic-to-Subaerial group.

On day eight, the piglets of the Aquatic-to-Subaerial group were transferred to the subaerial environment, where fly activity increased significantly (See Appendix D - Table 15 for panel images). The trotters remained relatively fresh, as per the scoring system, although WL2's front trotters had degloved, exposing the underlying soft tissue. WL2 and WL4 developed a brown, leathery appearance on their dorsal trunks (Figure 10). All piglets appeared bloated.



Figure 10: Day 8 - specimen WL4 of Aquatic-to-Subaerial group.

By day nine, abdominal caving was evident in WL1, WL2, and WL3. WL5 was classified as being in the fresh stages of decomposition, with green-red coloration and black discoloration on parts of the face and abdomen. WL2 and WL4 exhibited extensive tissue exposure and were characterized by deep red brown to black coloration. On day ten, WL2 and WL4 were considered mummified, although internal maggot activity persisted. The abdomens of all piglets showed deflation, and trotters appeared bloated. WL1 and WL3 displayed black and grey discoloration of the head and neck, while WL5 remained bloated but showed progressing decomposition, including a marbled appearance on the neck, greening limbs, and exposed tissue around the eye.

On day eleven, WL3 was partially mummified in the head region. Internal maggot activity was noted in WL1, WL2, WL3, and WL4. WL5 remained bloated, with light red trotters and pronounced black and green discoloration across the body. By day twelve, WL3 exhibited hind limb bone exposure, marking the first instance of skeletonization in the Aquatic-to-Subaerial group. WL1 and WL5 continued to show discoloration, and WL4's limb tissue was exposed (Figure 11).



Figure 11: Day 12 - specimen WL4 of Aquatic-to-Subaerial group.

On day thirteen, WL1 displayed rib bone exposure, with black discoloration on the limbs, face, and chest. Tissue exposure was also noted in these regions. WL5 showed blackening of the abdomen, face, and neck, with red discoloration on the trotters. WL4 demonstrated signs of wet decomposition in the abdomen while maintaining a mummified appearance in the skin and external tissue.

By the final day (day fourteen), WL2 and WL4 remained mummified with no bone exposure, while internal maggot activity continued (Figure 12). WL1, WL3, and WL5 exhibited rib and trotter bone exposure. WL5's body had deflated and was predominantly black in the abdomen, neck, and head. WL3 displayed a mummified head and blackened abdomen.



Figure 12: Day 14 - specimen WL4 of Aquatic-to-Subaerial group.

6.1.3. Subaerial Control morphological decomposition description

The Subaerial Control piglet remained in the subaerial environment for the entire duration of the study (See Appendix D - Table 16 for panel images). On day one, the Subaerial Control piglet had a Total Body Score (TBS) of 3 (translated to and STBS of 3), consistent with fresh remains in a subaerial decomposition environment. By day two, the abdomen had become firm to the touch, with a slight pink discolouration developing. On day three, active decomposition was evident, characterized by blistering and abdominal bloating (Figure 13). By day four, the abdomen exhibited a greying appearance. Day five saw an increase in discolouration, with browning around the nose, mouth, and eyes, along with black spots on the abdomen that extended to the upper portion of the limbs. On day six, neck tissue was no longer present, exposing internal maggots ranging from 1st to 3rd instar stages. Although the limbs appeared relatively fresh compared to the rest of the body, the piglet's abdomen remained slightly bloated but had begun to deflate. On day seven, extensive soft tissue loss was observed in the neck region, and the face appeared grey black (Figure 14).



Figure 13: Day 3 - Subaerial Control piglet.



Figure 14: Day 7 - Subaerial Control piglet.

By day eight, complete deflation and caving in of the head and neck region had occurred, and the limbs began to display a green-grey discolouration consistent with the rest of the body. Day nine marked the onset of skeletonization, with partial visibility of the mandible bone. The head and neck region exhibited a blue-black discolouration. By day ten, skeletonization progressed to include the ribs, front limb bones, and chest bones, while the abdomen was completely black. By day eleven, less than 50% of the trunk bones were exposed, and the mandible was fully visible (Figure 15). The trotters retained some pink colouration towards the distal ends, though greying was spreading. By day twelve, the remains were dry with minimal skin and tissue, and the front limbs had disarticulated from the trunk. Skeletal exposure of the abdomen and face had advanced. Results on day thirteen were consistent with those observed on day twelve.



Figure 15: Day 11 - Subaerial Control piglet.

On the final day of the study, day fourteen, the remains were classified as dry, with extensive skeletonization (Figure 16). The remaining tissue was grey-black and retained some hair. The decomposition concluded with a TBS score of 26 (STBS = 36), consistent with advanced decomposition in a subaerial environment.



Figure 16: Day 14 - Subaerial Control piglet.

6.1.3.1. Subaerial Control vs Subaerial Decomposition Phases of the Subaerial-to-Aquatic Group

The Subaerial Control and Subaerial-to-Aquatic Group both began with abdominal bloating, blistering, and early discolouration within the first three days. However, the

Subaerial-to-Aquatic piglets generally progressed more rapidly, with internal organ exposure and advanced maggot colonization evident by Day 4, compared to Day 6 in the Subaerial Control.

By Day 7, the Subaerial Control had extensive tissue loss but retained less facial bone exposure compared to several Subaerial-to-Aquatic piglets, particularly LW1 and LW2. In contrast, the Subaerial-to-Aquatic Group showed more pronounced blackening of facial and abdominal regions, and in some individuals, partial skeletonization had begun in the head and neck.

6.1.3.2. Subaerial Control vs Subaerial Decomposition Phases of the Aquatic-to-Subaerial Group

During the second week, the Aquatic-to-Subaerial Group transitioned to the subaerial environment. Similarly seen in the Subaerial Control, this group exhibited abdominal caving, blackening of soft tissues, and maggot activity. However, decomposition in the Aquatic-to-Subaerial Group was more variable. For example, while the Subaerial Control displayed consistent skeletonization of limbs and face by Day 14, several Aquatic-to-Subaerial piglets retained mummified soft tissue with no bone exposure by the same point.

The Subaerial Control exhibited a more predictable and steady progression into advanced decomposition. In contrast, the Aquatic-to-Subaerial Group showed instances of delayed tissue breakdown and mummification, particularly in the limbs and abdomen, despite active internal decomposition.

6.1.4. Aquatic Control morphological decomposition description

This Aquatic Control piglet remained in the aquatic environment for the entire duration of the study (See Appendix D - Table 17 for panel images).

On day one, the Aquatic Control piglet had a Total Aquatic Decomposition Score (TADS) of 3 (translates to an STBS value of 4.38), indicative of the fresh stage of decomposition. By

day two, bloating became apparent, accompanied by a bruised appearance around the eyes. By day three, the head and neck displayed green discolouration, the abdomen was blistered, and the trotters appeared soft and bloated (Figure 17). Day four revealed visible internal organs, and the face had developed a green-grey appearance with initial signs of skin slippage. On day five, black discolouration appeared on the face and abdomen, which progressed to include the neck region by day six. By day seven, tissue exposure on the trunk was observed, and skin slippage continued (Figure 18). The trotters were soggy to the touch.



Figure 17: Day 3 - Aquatic Control piglet.



Figure 18: Day 7 - Aquatic Control piglet.

By day eight, bloating decreased, leading to deflation and making the outline of ribs visible. Extensive maggot activity on the trunk obscured the exposed tissue, while the head region was grey black. On day nine, the trunk displayed a yellow-green colour beneath the maggots, and the body felt fragile during handling for scoring. Degloving of the trotters was first noted on day ten. The majority of the tissue on the body was exposed, and the neck and face showed dark green discolouration. Extensive maggot activity persisted on day eleven, during which the trotters continued degloving. Brown discolouration was noted on the abdomen, face, and trotters. By day twelve, the abdominal tissue had turned black, and the face continued to darken in colour (Figure 19). On day thirteen, the trotters were fully degloved and black, with Culicidae larvae observed in the water feeding on exposed tissue.



Figure 19: Day 12 - Aquatic Control piglet.

On the final day (day fourteen), the piglet exhibited signs of sinking, and ribs became visible beneath the maggots (Figure 20). The decomposition concluded with a TADS of 16 (STBS = 23.33), consistent with advanced decomposition in an aquatic environment.



Figure 20: Day 14 - Aquatic Control piglet.

6.1.4.1. Aquatic Control vs Aquatic Decomposition Phases of the Aquatic-to-Subaerial Group

The Aquatic Control piglet and the Aquatic-to-Subaerial Group both exhibited early signs of aquatic decomposition, including bloating, abdominal discolouration, skin slippage, and purging from orifices. By day three, blistering and green discoloration of the neck and abdomen were observed in both, along with softening of the trotters. By day five, internal organ exposure was evident in both, although the Aquatic Control presented more consistent green-grey facial discoloration.

The Aquatic Control showed continued tissue exposure and persistent sogginess of limbs, with blackening of facial features and advanced skin slippage. This mirrored the Aquatic-to-Subaerial Group, although some variability was noted for maggot activity and when bloating reduction occurred. The Aquatic Control displayed a more homogenous decomposition pattern, while the Aquatic-to-Subaerial Group showed more variation in discoloration and organ exposure timing across individuals.

6.1.4.2. Aquatic Control vs Aquatic Decomposition Phases of the Subaerial-to-Aquatic Group

Following their environmental transition, the Subaerial-to-Aquatic Group exhibited many of the same aquatic-stage decomposition changes noted in the Aquatic Control, including floating, tissue maceration, bone exposure in the head and limbs, and disarticulation events. Both showed pronounced sogginess of the trotters and facial skeletonization.

However, the Aquatic Control displayed slightly earlier degloving of the trotters (noted by Day 10), while the Subaerial-to-Aquatic Group demonstrated varying rates of degloving and disarticulation across individuals. Bone exposure in the facial and chest areas was observed in both, although the Aquatic Control showed more uniform tissue darkening and earlier onset of sinking signs. Overall, both groups concluded with advanced aquatic decomposition stages by Day 14, characterized by soft tissue loss, discoloration, and skeletonization.

6.2. Decomposition Body Score Results

6.2.1. Inter-observer and Intra-observer error

Inter-observer error was assessed using ICC (Two-way random effects, absolute agreement, average measures) between the main scorer and two other scorers. P value < 0.001 suggests that reliably consistent scoring between three different scorers is statistically significant. Intra-observer error was assessed using ICC (Two-way mixed effects, absolute agreement, average measures). P value < 0.001 suggests that reliably consistent scoring between the same scorer is statistically significant.

6.2.2. Standardized Total Body Scores for Subaerial-to-Aquatic pigs

The trend of the standardized total body scores (STBS) values for –Subaerial-to-Aquatic environment piglets - steadily increased over the 14-day period (Figure 21). During the initial subaerial phase (days 1 – 7), all piglets showed a gradual rise in STBS, with some variability in the rate of increase. Following the environmental transition on Day 8, there was no

immediate sharp change; however, from approximately Day 9 onward, most experimental piglets exhibited a more rapid increase in STBS compared to the initial subaerial phase.

The Subaerial Control piglet displayed a more gradual increase in STBS compared to the Subaerial-to-Aquatic Group specimens and had a lower final STBS than four of the five specimens, suggesting that the aquatic environment accelerated decomposition for the group. The rapid increase in STBS from Day 9 could be due to the biological acceleration of decomposition in water and/or the switch from Adjusted Total Body Score (ATBS) to Total Aquatic Decomposition Score (TADS) that highlights aquatic decomposition features.

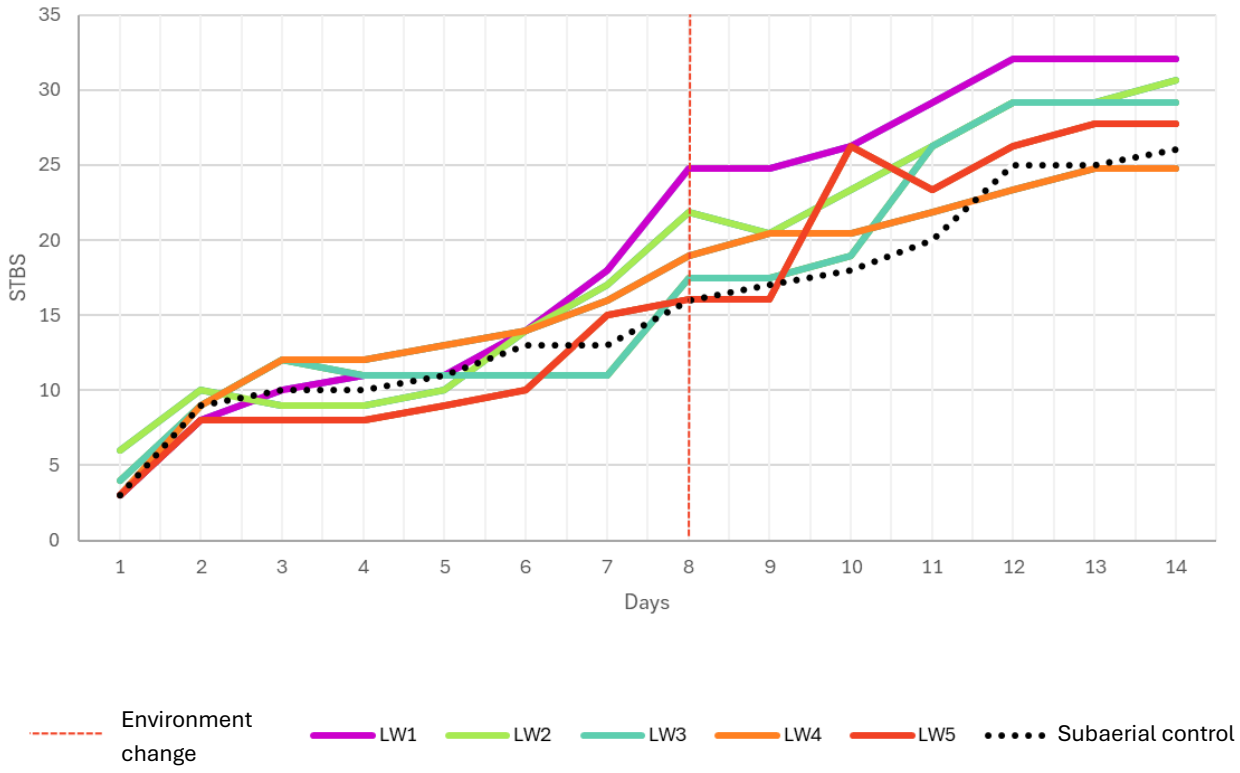


Figure 21: STBS vs Days for Subaerial-to-Aquatic Group piglets and Subaerial Control piglet.

increases in STBS, reaching values of 24.79–29.17 within this range, whereas LW5 showed a delayed increase, with an STBS of 16.04 at logADD 2.37 before accelerating later. These variations suggest differences in decomposition rates potentially influenced by microclimatic factors, moisture levels, or insect activity.

By the late stages (logADD ~2.4–2.5), most subjects' STBS values plateaued, reflecting the stabilization of soft tissue breakdown. LW1 reached a consistently high STBS of 32.08, while LW5, despite its slower initial progression, accelerated and reached 27.71. The eventual convergence of STBS values suggests that early-stage differences in decomposition rate may be overridden by cumulative environmental effects over time.

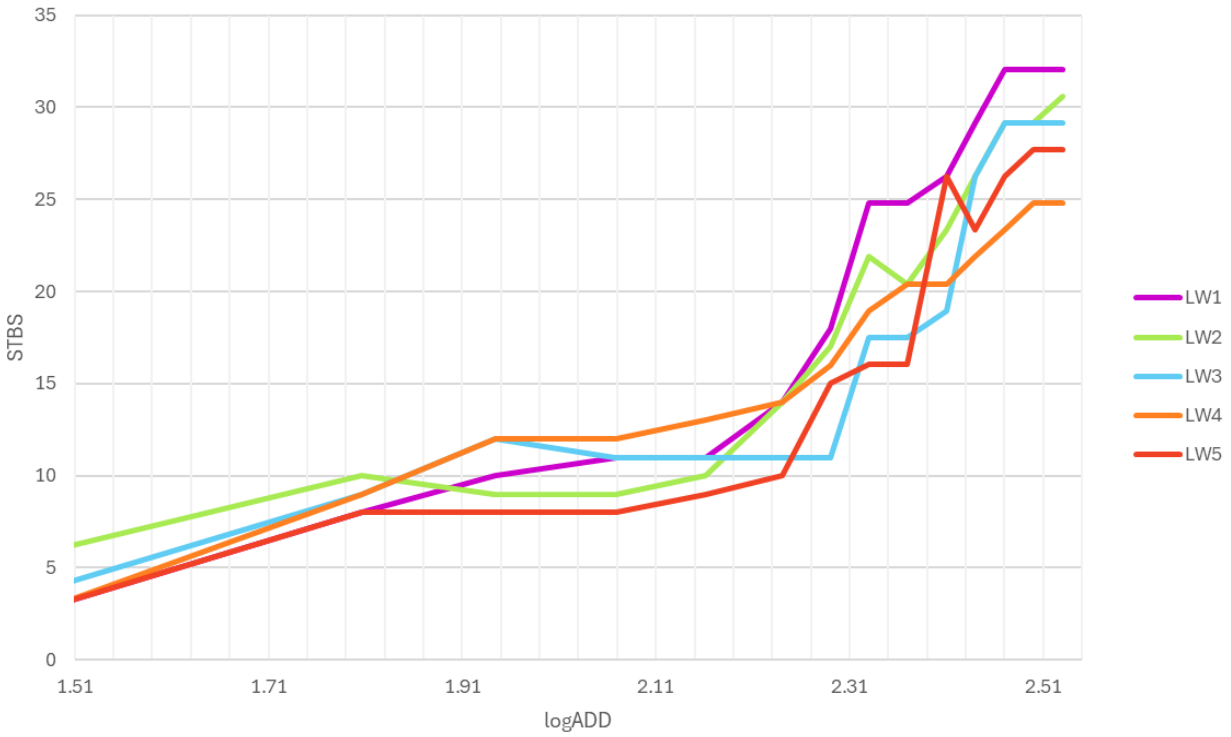


Figure 22: STBS vs logADD for Subaerial-to-Aquatic Group piglets.

The R^2 values for the STBS vs logADD trend lines (Table 2) indicate a generally strong relationship between STBS and logADD (average 0.8009) , supporting the use of accumulated thermal energy as a predictor of decomposition progression. Although differences in fit highlight variability in decomposition rates across subjects—likely influenced by environmental factors, microclimatic differences (e.g., being in more shade or direct sunlight), or insect activity. This sample showed overall consistency, with decomposition trends remaining comparable, among subjects. LW4 had the highest R^2 value (0.9313) suggesting a highly predictable and steady decomposition pattern.

Table 2: R squared values of the STBS vs logADD graphs of the Subaerial-to-Aquatic Group. This measures how well the trend line (regression model) fits the data points, an indication of the behaviour of STBS and logADD in correlation with each other.

Piglets ID	R^2 value	Analysis
LW1	0.8442	This is a relatively strong fit, indicating the trend line accurately represents the data.
LW2	0.7737	While this is weaker than LW1, it is still a good fit, showing the model captures most of the trend.
LW3	0.6998	This is a moderate fit, suggesting that while the trend line captures some of the general pattern, there is more variability in the data that the model does not account for.
LW4	0.9313	This is the strongest fit among all the samples, showing the model very closely aligns with the data.
LW5	0.7557	Similar to LW2, this is a good fit, with the model capturing most of the data's trends.

6.2.3. Standardized Total Body Scores for Aquatic-to-Subaerial Group

STBS for Aquatic-to-Subaerial Group (transitioning from an aquatic to a subaerial environment) showed variable trends across replicates, with most specimens experiencing a steep increase in decomposition scores over time (Figure 23). Notably, WL4 exhibited a sharp rise in STBS by Day 6, followed by a steep decline on Day 8, coinciding with the transition from water to land.

This common decline in STBS observed across Aquatic-to-Subaerial Group is attributable to the scoring system change rather than an actual reduction or reversal in decomposition, highlighting a limitation of scoring systems being limited to single environments and not accounting for environment changes. Upon transitioning to land, ATBS was applied instead of TADS, which does not account for certain water-related decomposition characteristics (e.g., skin slippage, floating, and submerged bloating). As a result, piglets that had already undergone substantial aquatic decomposition scored lower when reassessed under ATBS, which focuses more on terrestrial features of decay.

Despite transitioning to a land environment, the piglets exhibited morphological characteristics typical of early terrestrial decomposition, aligning with fresh subaerial decay patterns. Meanwhile, the Aquatic Control group, which remained in the aquatic environment throughout the study, displayed a consistent increase in STBS without any sharp declines, reinforcing the conclusion that environmental transitions—particularly from water to land—significantly impact decomposition scoring when different systems are applied.

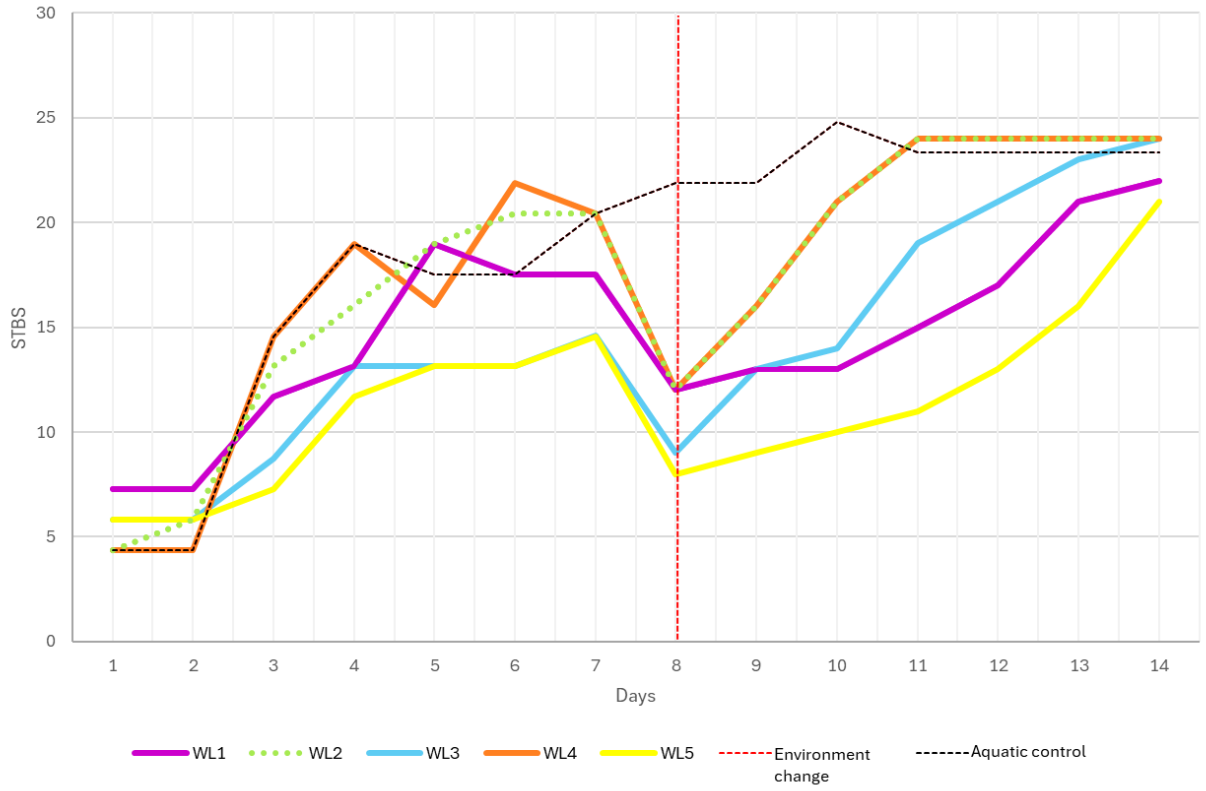


Figure 23: STBS vs Days for the Aquatic-to-Subaerial Group piglets and the Aquatic control piglet.

During the early stages of decomposition (logADD ~1.38 to ~1.82), STBS values remained relatively low across all Aquatic-to-Subaerial Group subjects (Figure 24). This slow increase in decomposition is likely due to environmental constraints in the aquatic environment, such as temperature regulation, which may have delayed microbial and insect activity.

Between logADD ~1.94 and ~2.17, a period of accelerated decomposition was observed, suggesting that anaerobic microbial activity may have become dominant in the aquatic

environment. This period of increased breakdown aligns with the expected shift from early to active decay in submerged remains.

Following the environmental transition (logADD 2.17 to 2.30), a sudden drop in STBS was observed. This decline does not represent a reversal in decomposition but rather reflects the change in scoring systems. Before the transition, decomposition was assessed using TADS (for aquatic remains), but after the transition to a subaerial environment, ATBS (for terrestrial remains) was applied. As a result, the piglets, now exposed to air, displayed morphological characteristics of early terrestrial decomposition rather than advanced aquatic decomposition, which lowered their STBS scores within the new scoring criteria. However, decomposition itself did not slow; instead, the framework for assessing it changed.

During the final days of the study (logADD ~2.36 to ~2.53) Aquatic-to-Subaerial Group showed a sharp increase in STBS, followed by stabilization at higher values. This suggests rapid soft tissue dissection post-transition, with scores plateauing as decomposition neared its endpoint. The scoring system shift is likely contributed to this trend, as ATBS is structured to capture terrestrial decomposition patterns, which tend to progress rapidly once remains are exposed to air. However, four of the five Aquatic-to-Subaerial Group specimens exhibited signs of mummification after transitioning into the aquatic environment. When Aquatic-to-Subaerial Group specimens were moved onto land, the sudden exposure to air may have caused rapid desiccation rather than immediate breakdown, altering the typical decomposition trajectory. Furthermore, since mummification slows soft tissue decay, ATBS may not have fully captured the decomposition process in these specimens, resulting in greater variability in STBS trends.

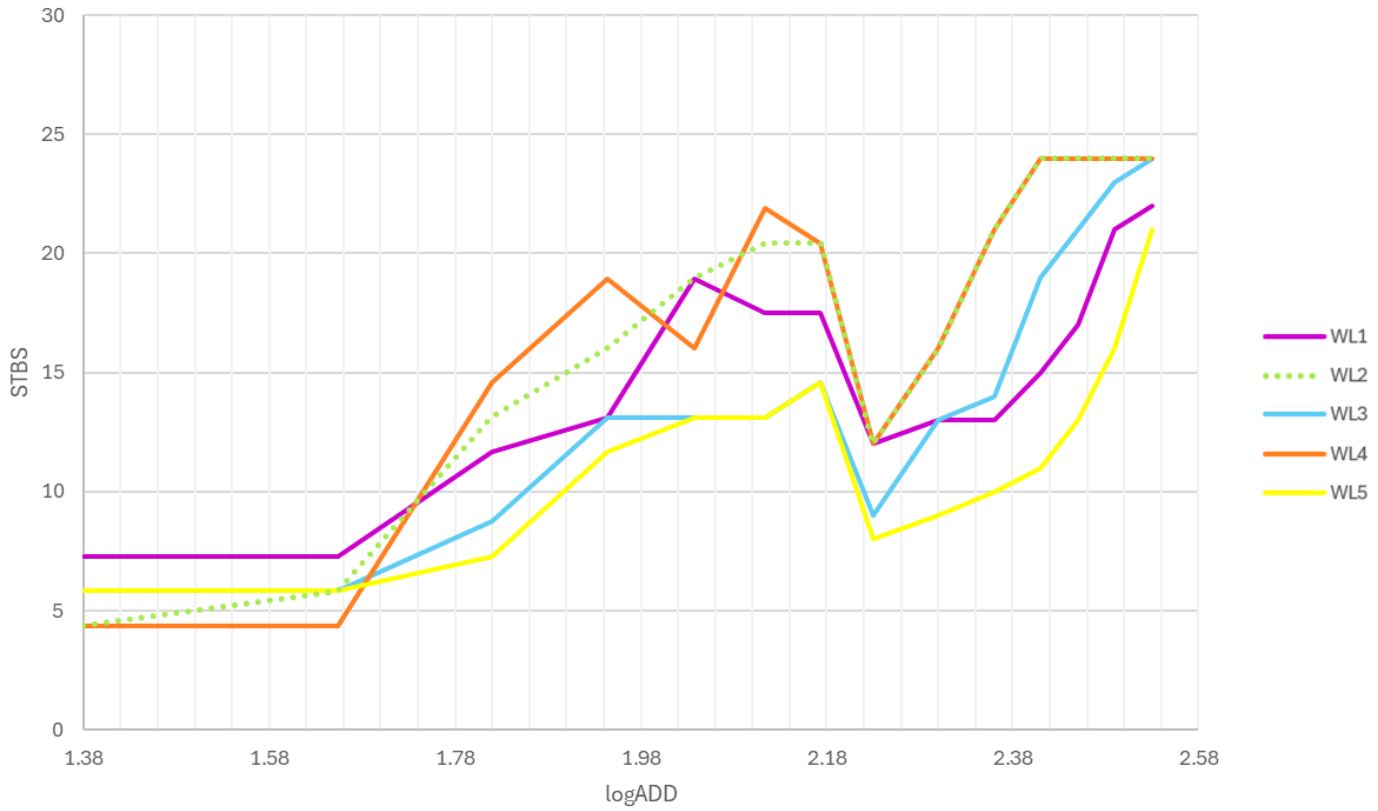


Figure 24: STBS vs logADD for Aquatic-to-Subaerial Group piglets.

The R^2 values for the trend line for Aquatic-to-Subaerial Group (Table 3) STBS vs logADD, indicate variability in how well STBS progression aligns with logADD (average value = 0.6646). While WL2 (0.7938) showed the strongest fit, suggesting a predictable decomposition pattern, other piglets exhibited more variability. WL3 (0.7383) and WL4 (0.7425) demonstrated moderate-to-strong fits, indicating that while decomposition followed a general trend, some fluctuations were present. WL1 (0.5694) showed a moderate fit, suggesting additional influencing factors, while WL5 (0.4788) had the weakest fit, indicating substantial variability in decomposition progression that may be due to external microenvironmental influences or internal influences such as gut biome, as well as the ATBS scoring system not accounting for prior aquatic-associated morphological changes.

Table 3: R squared values of the STBS vs logADD graphs of the Aquatic-to-Subaerial Group. This measures how well the trend line (regression model) fits the data points, an indication of the behaviour of STBS and logADD in correlation with each other.

Piglets ID	R^2 value	Analysis
WL1	0.5694	This indicates a moderate fit, meaning about half of the variability in STBS is captured by the trend line.
WL2	0.7938	This is a strong fit, suggesting the trend line closely represents the data. Decomposition for this piglet follows a predictable pattern aligned with the model.
WL3	0.7383	This is a moderate-to-strong fit. The data is fairly well-aligned with the trend line, though there is some variability unaccounted for by the model.
WL4	0.7425	Similar to WL3, this is a moderate-to-strong fit, indicating that the model is fairly reliable in describing the decomposition dynamics for this piglet
WL5	0.4788	This is a weak fit, suggesting the trend line does not capture the data well. Decomposition dynamics for this piglet may be influenced by additional factors not captured by the model.

6.2.4. Comparison of Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Standardized Total Body Scores

On Day 7, the day before the environment switch, the mean STBS for Subaerial-to-Aquatic Group (originally on land) was 17.4, while the mean STBS for Aquatic-to-Subaerial Group (originally in water) was 17.5. This suggests that, despite being in different environments, both groups had undergone similar levels of decomposition up to this point.

On Day 8, after switching environments, the mean STBS for Subaerial-to-Aquatic Group (now in water) increased to 19.83, while the mean for Aquatic-to-Subaerial Group (now on land) dropped to 10.6. Subaerial-to-Aquatic Group's increase reflects the accelerated soft tissue breakdown in water, as immersion speeds up skin slippage, bloating, and microbial activity, all of which contribute to higher scores under TADS, which is used for aquatic decomposition. Conversely, the drop Aquatic-to-Subaerial Group's score can be attributed to the change in scoring system, as ATBS does not emphasize water-related changes like skin

slippage and floating, leading to an apparent reduction in STBS despite continuous decomposition.

By Day 14, the final day of observation, the mean STBS for Subaerial-to-Aquatic Group had further increased to 28.88, while Aquatic-to-Subaerial Group had risen to 23. Subaerial-to-Aquatic Group's higher STBS by the end of the study reinforces that remains transitioning from land into water have an accelerated decomposition due to increased moisture exposure, bacterial activity, and tissue maceration. The slower increase in Aquatic-to-Subaerial Group could be a result of the tissue desiccation or that decomposition when switched from water onto land occurs more gradually compared to the rapid progression observed when switching from land into water.

As stated previously, during the first seven days of the study, both experimental groups (Subaerial-to-Aquatic and Aquatic-to-Subaerial) exhibited closely aligned decomposition patterns as measured by the Standard Total Body Score (STBS). This similarity was evident despite the differences in environmental exposure, with Subaerial-to-Aquatic piglets initially in a subaerial setting and Aquatic-to-Subaerial piglets submerged in water. A comparison of the scoring systems (ATBS and TADS) revealed overlap in the morphological indicators used to describe early decomposition stages (Table 18 in Appendix C). Both systems assess early postmortem changes based on skin discoloration (ranging from pink to green), marbling, initial bloating, and the onset of skin slippage or purging. These shared criteria likely contributed to the consistent scores observed between the groups in the early postmortem period. Table 18 in Appendix C summarizes the decomposition descriptors for each stage (fresh, early, active, advanced, and final) as defined by ATBS and TADS, and highlights the considerable similarities in early-stage descriptors, followed by increasing divergence in the advanced and final stages.

A Friedman Test yielded a p-value < 0.001 for all days, indicating a statistically significant difference in STBS values over the 14-day period. This indicates that decomposition progressed in a non-uniform and measurable manner over time, with significant changes in STBS scores between multiple timepoints. To determine which specific days showed significant differences in STBS values across the piglets, a Wilcoxon Signed-Rank Test was performed as a post-hoc analysis, which also returned a p-value < 0.001 the Bonferroni correction corrected p-value was also significant. The significance differences confirmed by the statistical tests can be attributable to notable shifts in decomposition scoring associated with the environmental transitions. In the Subaerial-to-Aquatic Group, a sharp increase in STBS was observed from Day 8 onwards, coinciding with immersion in water and reflecting accelerated tissue breakdown. Conversely, the Aquatic-to-Subaerial Group exhibited a drop in STBS on Day 8 due to the change from the aquatic scoring system (TADS) to the subaerial system (ATBS), which does not account for prior aquatic features. Subsequent increases in STBS were more variable, particularly in piglets displaying signs of mummification. These contrasting trends (rapid score increases, abrupt drops, and scoring plateaus) resulted in statistically significant day-to-day differences, which could confirm that decomposition was influenced by biological progression, the effects of environment and, scoring system transitions.

While both groups experienced a scoring system change, the scoring system switch may have been more disruptive for Aquatic-to-Subaerial Group. The ATBS system (used after Day 8 for Aquatic-to-Subaerial Group) is designed for terrestrial decomposition and may not have accounted well for prior aquatic-associated changes. Additionally, four of the five Aquatic-to-Subaerial Group specimens exhibited signs of mummification after transitioning from the aquatic to the terrestrial environment. This likely contributed to the higher R^2 values observed in Subaerial-to-Aquatic Group compared to Aquatic-to-Subaerial Group, as mummification

slows soft tissue decomposition, leading to a less predictable and more variable scoring pattern in Aquatic-to-Subaerial Group.

6.2.5. Subaerial Control vs Subaerial Decomposition Phases of the Experimental Groups

During the early stages of decomposition, logADD ~1.49 to 1.81 (ADD 31 to ADD 64), the STBS values were relatively low, ranging from 3 to 9. This corresponds with the fresh stages of decomposition. LogADD ~ 1.94 to ~ 2.29 (ADD 88 to ADD 195) shows a gradual increase (Figure 25), likely a representation of the transitioning phase into active decomposition. A more notable increase in STBS occurs from logADD ~ 2.34 (ADD 217) onward. This rapid increase could indicate a peak in active decomposition. The final days (logADD ~2.52 to ~2,58, ADD 331 to 381) show STBS plateau and stabilize around 25 and 26. Subaerial Control R^2 value = 0.8551. This is a very strong fit, suggesting the decomposition pattern in the Subaerial Control group is highly predictable and well-aligned with the trend line.

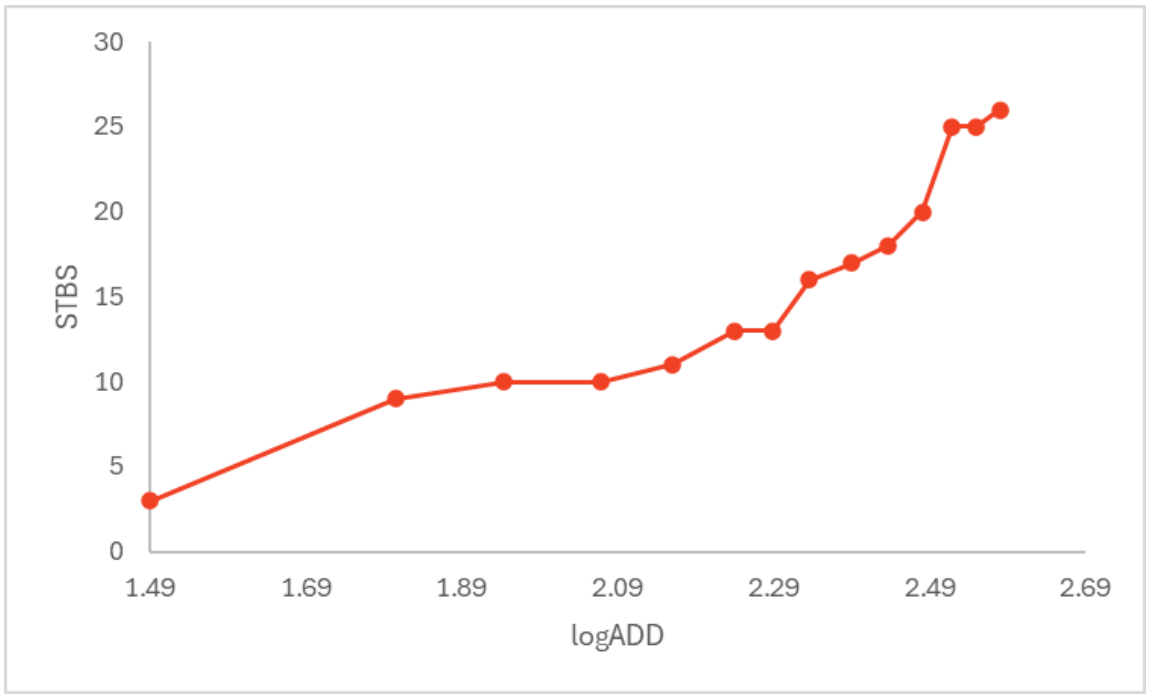


Figure 25: STBS vs logADD for the Subaerial Control piglet.

Both the Subaerial Control and the Subaerial-to-Aquatic Group were scored using ATBS in the seven days. The Subaerial-to-Aquatic Group showed a higher mean STBS (17.4 on Day 7) compared to the Subaerial Control (lower than four of the five LW piglets by Day 7). This indicates a more rapid decomposition trajectory in the experimental piglets prior to immersion.

After transitioning into the subaerial environment, Aquatic-to-Subaerial Group piglets were scored using ATBS. While some individuals (WL1, WL3, WL5) exhibited final STBS values approaching those of the Subaerial Control due to bone exposure which yields a higher score with ATBS, others (WL2, WL4) remained lower due to mummification, which can reduce scoring potential despite ongoing decomposition.

6.2.6. Aquatic Control vs Aquatic Decomposition Phases of the Experimental Groups

Early decomposition (logADD ~ 1.4 to ~ 1.8, ADD 24 to ADD 66) of the Aquatic Control (Figure 6) showed low, uniformed STBS values indicating minimal observational indications of decomposition (Figure 26). The onset of decomposition seen by the sharp increase in STBS between logADD ~1.8 and ~2.0 (ADD 66 and ADD 109). This corresponds with the initial acceleration of decomposition. STBS is then seen to fluctuate during the period of logADD ~2.1 to ~2.3 (ADD 130 and ADD 210). This can be attributed to some stabilization in the rate of decomposition. The final days of the study (logADD ~2.3 to ~2.5, ADD 210 to ADD 292) shows STBS reaching a plateau, indicating a levelling off of decomposition rate.

Aquatic Control R^2 value = 0.8983. This is a very strong fit, suggesting the decomposition pattern in the Aquatic Control group is highly predictable and well-aligned with the trend line.

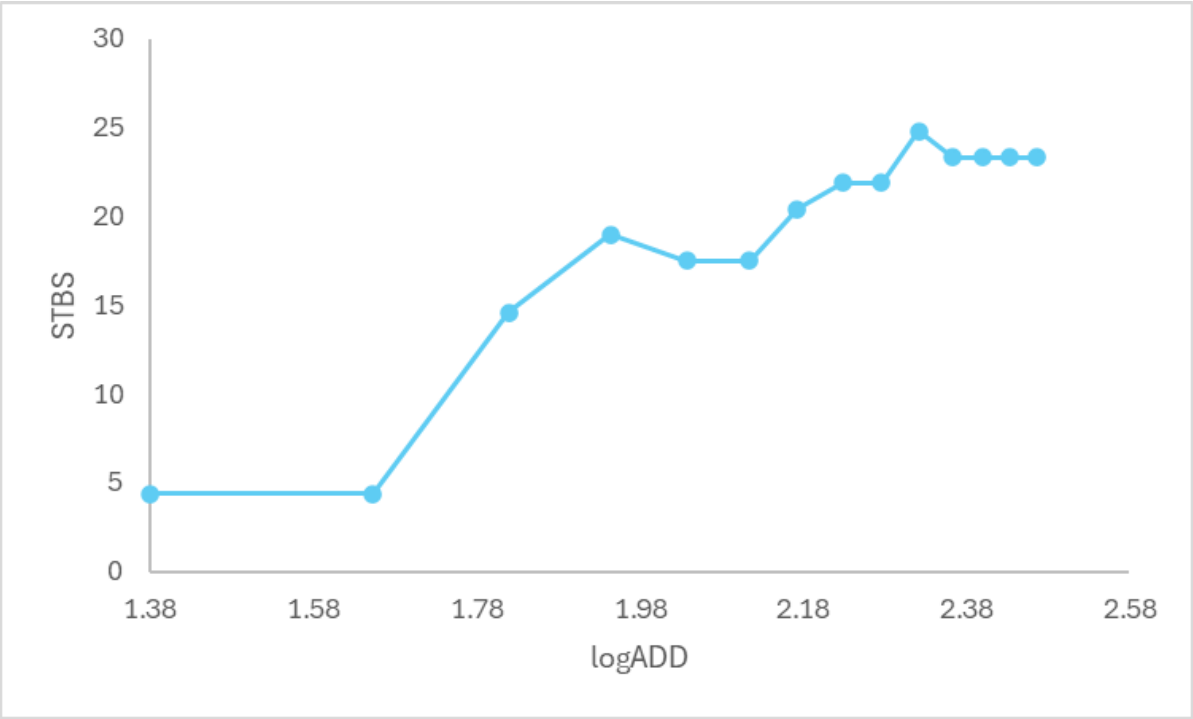


Figure 26: STBS vs logADD for the Aquatic Control piglet.

The Aquatic Control piglet and the Aquatic-to-Subaerial Group piglets were scored using TADS during aquatic decomposition. STBS trends across Days 1 to 7 for the Aquatic-to-Subaerial Group showed a gradual rise with variability in individual piglet scores. Final STBS values prior to transition ranged from approximately 16 to 19.5, closely aligning with the Aquatic Control's Day 7 STBS of 17.

Upon transition to the aquatic environment, Subaerial-to-Aquatic piglets were also assessed using TADS. These piglets showed a rapid increase in STBS post-immersion, with end-of-study values (Day 14) ranging from 27.71 to 32.08. Compared to the Aquatic Control's final STBS of 26, the Subaerial-to-Aquatic Group displayed a higher magnitude of decomposition acceleration.

6.3. Arthropod resultsI

The presence and type of arthropod species (Table 4) over the 14-day period varied between the Subaerial-to-Aquatic Group (yellow bars in Table 4) and Aquatic-to-Subaerial Group (blue bars in Table 4) groups, with some species persisting across both environments (represented by dotted lines in Table 4). Persistence into the next environment (environments split by solid black vertical line in Table 4) refers to species that were observed in the original setting on a given day and remained present after the environmental shift. This suggests that some insects were able to withstand or adapt to the change rather than abandoning the remains entirely.

Despite the transition between terrestrial and aquatic environments, the majority of species (12 out of 15, namely, *Dermestus maculatus,* Histeridae*, Fannia canicularis, Lucilia sericata*, *Chrysomya marginalis, Chrysomya megacephala, Chrysomya chloropyga, Chrysomya putoria, Chrysomya albiceps, Musca domestica*, and Sarcophagidae) were documented on both groups, indicating that the environment did not strongly influence which insects colonized the remains. Instead, the stage of decomposition (and thus VOC's) seemed to be the primary factor driving insect activity, with species arriving in response to tissue changes rather than specific environmental conditions.

Musca domestica were early arriving species on the remains, being seen as early as day two on the Aquatic-to-Subaerial Group. *Chrysomya marginalis, Chrysomya megacephala,* and *Chrysomya albiceps* were among the other species first to colonise both remains. *Chrysomya chloropyga* were among the earliest species observed Aquatic-to-Subaerial Group and was only seen on day nine for Subaerial-to-Aquatic Group after the environment was shifted to aquatic, suggesting a possible preference to the aquatic environment remains.

Anthomyia spp. and Scathophagidae were unique to Aquatic-to-Subaerial Group, with the former present from Day 3 to Day 11 and the latter appearing only on Day 4. This

discrepancy could be a result of collection methods and identification limitations, as these arthropods are relatively small and may have been missed during sampling or not consistently documented. The absence of these species in Subaerial-to-Aquatic Group does not necessarily indicate a true difference in colonization but may instead reflect variability in detection.

Histeridae were seen in both groups, for Subaerial-to-Aquatic Group this was from day 6 to 9, persisting into the environment change to aquatic. This is most likely a result of the species trying to avoid drowning. Aquatic-to-Subaerial Group saw the presence of Histeridae from day 11 to 14, when they were in the subaerial environment. *Dermestus maculatus* was another species of Coleoptera that was seen in both Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group, these were seen on day 11 and day 13 respectively.

Lepidoptera were seen on day 3 of the study only and specifically in the Aquatic-to-Subaerial Group piglets. This could suggest that it was incidental.

Table 4 also shows the presence of arthropod species seen and collected from the two control piglets (aquatic (red) and subaerial (green) control piglets). The results show that *Musca domestica, Sarcophagidae, Chrysomya albiceps, Chrysomya megacephla, and Chrysomya chloropyga* were the first species to colonize. *Lucilia sericata* were seen on both controls, however they were seen earlier in the Aquatic Control (day 4) than the Subaerial Control (day 6). *Anthomyia spp*. were also seen as an early arriving arthropods for the Aquatic Control only, similarly, seen in the Aquatic-to-Subaerial Group. Possibly suggesting a preference for the aquatic decomposition environment.

Calliphora vicina were only seen or collected from the Aquatic Control piglet. Other species seen and collected only from the Aquatic Control were *Culcidae* larvae and Arachnida which

are incidental species in decomposition and most likely using the remains and subsequent maggots as a food source.

Chrysomya putoria were seen in both control groups. However, they were seen relatively early (day 5) for the Aquatic Control and towards the end of the study (day 10) for the Subaerial Control. Both control groups also saw species of Coleoptera, namely *Dermestus maculatus* and Histeridae. However, Histeridae were see on the Subaerial Control only and were found towards the end of the study (day 9 to 11) when remains were dry. *Dermestus maculatus* were seen on both remains towards the end of the study as well. This further supports the importance of decomposition stage in insect colonization, as some species may have remained on remains despite environmental shifts due to their ecological roles.

6.3.1. Controls vs Subaerial-to-Aquatic Group: Arthropod Succession

The Subaerial Control piglet and Subaerial-to-Aquatic Group showed overlapping colonization by *Musca domestica*, *Chrysomya megacephala*, *Chrysomya albiceps*, and *Sarcophagidae*. *Lucilia sericata* appeared slightly later on the Subaerial Control (Day 6) but was present in both. Later-stage decomposers such as Histeridae and *Dermestus maculatus* were seen in both groups toward the end of the subaerial period. These beetles typically appeared after substantial tissue breakdown.

After transition, Subaerial-to-Aquatic piglets exhibited several of the same insect species seen on the Aquatic Control, including *Chrysomya albiceps*, *Chrysomya megacephala*, *Musca domestica*, and *Sarcophagidae*. *Chrysomya chloropyga* was observed on Day 9, post-immersion, potentially indicating a preference for aquatic-stage remains.

The Subaerial Control piglet and Subaerial-to-Aquatic Group showed overlapping prescence by *Musca domestica*, *Chrysomya megacephala*, *Chrysomya albiceps*, and *Sarcophagidae*. *Lucilia sericata* appeared slightly later on the Subaerial Control (Day 6) but was present in

both. Later-stage decomposers such as Histeridae and *Dermestus maculatus* were seen in both groups toward the end of the subaerial period. These beetles typically appeared after substantial tissue breakdown.

6.3.2. Controls vs Aquatic-to-Subaerial Group: Arthropod Succession

The Aquatic Control piglet and the Aquatic-to-Subaerial Group piglets shared similar activitty patterns during aquatic decomposition. Both were initially associated with common Dipteran species such as *Musca domestica*, *Chrysomya marginalis*, *Chrysomya megacephala*, and *Chrysomya albiceps*. *Lucilia sericata* appeared slightly earlier on the Aquatic Control (Day 4) than in the Aquatic-to-Subaerial Group.

Importantly, *Anthomyia spp.* was observed on both the Aquatic Control and the Aquatic-to-Subaerial Group piglets. Its presence in both suggests a potential association with aquatic decomposition environments. This finding was consistent across both groups despite their independent environmental exposures. Other unique aquatic taxa, such as *Culicidae* larvae and *Arachnida*, were observed only on the Aquatic Control. These are likely incidental aquatic colonizers using the decomposing remains and associated maggot masses as a resource. The absence of these taxa on the experimental group may be due to the shorter duration in the aquatic environment or differences in microhabitat exposure.

After transitioning to the subaerial environment, the Aquatic-to-Subaerial Group were associated with similar taxa to the Subaerial Control, including *Chrysomya albiceps*, *Musca domestica*, *Sarcophagidae*, and *Dermestus maculatus*. Histeridae also appeared during this stage, overlapping with the advanced stages of decomposition. Despite prior immersion, arthropod activity on the Aquatic-to-Subaerial Group largely mirrored that of the Subaerial Control. However, partial mummification observed in some piglets may have limited arthropod access, which could explain subtle differences in the frequency or distribution of certain taxa.

Table 4: Arthropod presence over the period of the study for the two experimental groups and the two controls. Blue = Aquatic-to-Subaerial, Yellow = Subaerial-to-Aquatic, Red = Aquatic Control, and Green = Subaerial Control, --- represents arthropods seen across the environment transition.

Arthropod	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Chrysomya megacephala			---	---	---	---	---	---	---					
			---	---	---	---	---	---	---	---	---	---	---	---
Chrysomya albiceps			---	---	---	---	---	---	---	---	---	---	---	
			---	---	---	---	---	---	---	---	---	---	---	
Anthomyia spp.			---	---	---	---	---	---	---	---	---			
Musca domestica		---	---	---	---	---	---	---	---	---				
				---	---	---	---	---	---	---	---	---	---	
Scathophagidae														
Sarcophagidae														
Culicidae larvae														
Arachnoid														


Arthropod
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Lepidoptera
Dermestus maculatus
Histeridae
Fannia cancularis
Calliphora vicina
Lucilia sericata
Chrysomya chloropyga
Chrysomya marginalis
Chrysomya putoria

6.3.3. Jaccard Similarity Heatmap

The Jaccard similarity heatmap (Figure 27) illustrates the arthropod community composition between Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group piglets over time. Overall, clustering patterns reveal that similarity is primarily influenced by decomposition stage rather than the initial environment. While some grouping by environment is observed, there are notable overlaps between Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group samples at specific time points.

Early-stage arthropod communities (Days 1–7) differ between environments. Subaerial-to-Aquatic Group piglets show relatively stable and predictable succession patterns, with high similarity between consecutive days (e.g., LW_Day3 to LW_Day6). In contrast, Aquatic-to-Subaerial Group piglets exhibit greater variation in similarity, particularly in later stages (e.g., WL_Day7 to WL_Day9), indicating more dynamic insect colonization in the aquatic environment as well as variations in the level of aerial tissue exposure.

Following the environmental switch on Day 8, similar patterns suggest stage-driven community shifts rather than environment-driven differences. Moderate similarity between certain days for Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group (e.g., LW_Day9 and WL_Day8) suggests overlapping arthropod species present in both environments, albeit at different times. This implies that arthropod succession aligns more with decomposition progression rather than environmental conditions alone.

The hierarchical clustering dendrogram further supports these findings. While some separation between Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group is present, cross-environment similarities indicate that arthropod colonization is not strictly environment-dependent. Instead, the observed clustering patterns reinforce that

decomposition stage is a stronger determinant of arthropod succession than initial environmental exposure in this study.

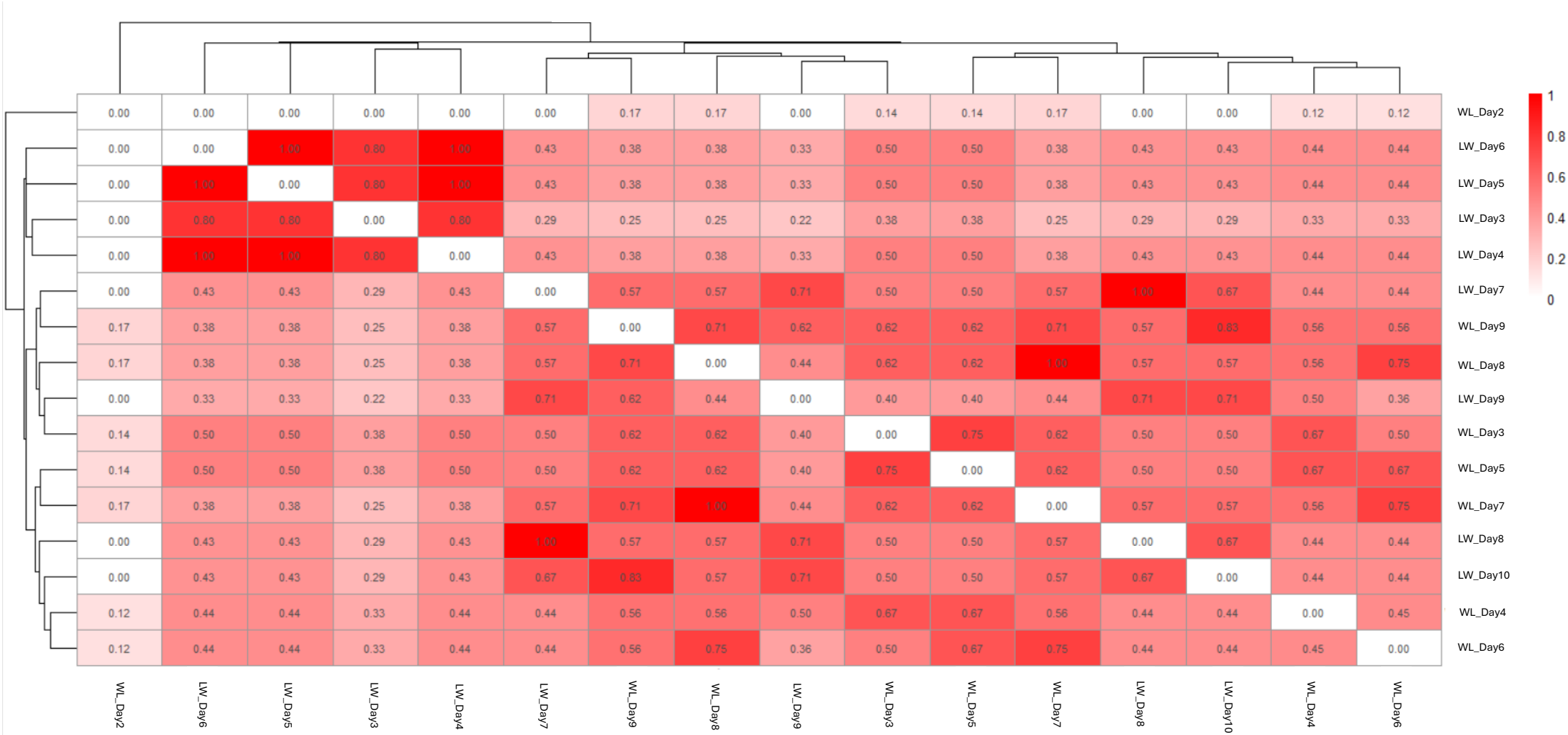


Figure 27: Jaccard Similarity Heatmap for the Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group. LW refers to Subaerial-to-Aquatic Group and WL refers to Aquatic-to-Subaerial Group.

7. Discussion

While decomposition dynamics and arthropod colonization are well-documented in static environments, the effects of environmental transitions remain underexplored. Thus, the aim of this study was to describe the rate and pattern of decomposition, as well as the colonization dynamics of arthropods, in response to alterations in the surrounding environment of the remains from subaerial to freshwater aquatic conditions and vice versa.

7.1. Morphological Decomposition Progression

7.1.1. Skeletonization and Rehydration of Subaerial-to-Aquatic Remains

By the conclusion of the study, most Subaerial-to-Aquatic specimens had reached partial to near complete skeletonization with rapid decomposition. This is an intriguing finding, as prior studies suggest that remains that decomposed primarily in aquatic environments typically do not undergo complete skeletonization (Alfsdotter and Petaros, 2021). For instance, in a study done by Alfsdotter and Petaros (2021) in Sweden on outdoor human decomposition, of the 51 aquatic corpses included in the study, only one reached complete skeletonization.

The near complete skeletonization of Subaerial-to-Aquatic Group piglet remains could be attributed to the remains with initially desiccated tissue due to subaerial exposure being rehydrated once placed into an aquatic environment. This phenomenon was also observed by de Toit *et al.* (2024) in the Highveld of South Africa using a porcine model. Their study qualitatively explored subaerial body tissue rehydration and observed an increased decomposition rate following rehydration. These findings were then further corroborated by Adams *et al.* (2024) in the Western Cape of South Africa also using a porcine model, where previously desiccated remains were rehydrated by rainfall and saw increases in decomposition during this hydrated period.

It should be noted that the repeated mechanical disturbances in the current study, namely lifting the piglets for decomposition scoring, may have influenced decomposition trajectories. Previous research has shown that frequent handling of the remains in a subaerial environment can alter insect colonisation patterns and reduce measurable weight loss and temperature progression, thereby slightly suppressing or delaying observable decomposition changes (Schoenly *et al.,* 2007; Sharanowski *et al.,* 2008). In an aquatic environment, this disturbance could disrupt buoyancy, cause tissue to slough off, detach aquatic insect larvae, or algal growth, thereby masking certain morphological features of aquatic decomposition (Anderson and Bell, 2016). While necessary for observational purposes, such disturbances should be recognised as a methodological limitation when interpreting the decomposition patterns.

7.1.2. Mummification in Aquatic-to-Subaerial Remains

The Aquatic-to-Subaerial specimens, which were initially submerged in water for seven days, underwent mummification after transitioning to a subaerial environment. Mummification was seen across all areas of remains, however, in some piglets it was only seen in the head, neck, and abdominal regions. This is particularly significant as Adams *et al.* (2024) saw that the porcine remains that were exposed to rainfall did not later desiccate. It is worth noting that rainfall occurs predominantly in the winter months in the Western Cape, whereas this study was conducted in summer. Temperature, a key driver of decomposition, may explain this discrepancy. Furthermore, mummification requires a lack of moisture to occur (Mant, 1987; Janaway, 1997; Saukko and Knight, 2004; Shkrum and Ramsay, 2007) which preserves soft tissues by preventing microbial activity and decay. The findings of previously wet remains mummifying are unusual as mummification is generally not expected to occur in tissues that have been saturated with moisture. Instead, one would anticipate continued decomposition which is common in moist environments (Caruso, 2016). This

contradiction suggests that environmental transitions, such as moving from an aquatic to a subaerial setting, may have played a crucial role in facilitating unexpected tissue desiccation.

Further research could explore whether rapid drying due to variations in the combination of temperature and humidity changes influenced this outcome resulting in the quick mummification seen on Aquatic-to-Subaerial Group specimens. Forbes *et al.* (2019) reported that the warm and dry conditions of Cape Town's coastal region facilitated mummification in their study. Similarly, the present study was conducted in December, when subaerial temperatures were relatively high (Appendix F), and rainfall was minimal. These conditions likely contributed to the rapid desiccation of Aquatic-to-Subaerial Group specimens following their removal from water, leading to the skin drying tightly around the skeletal frame. These findings align with observations from both Forbes *et al.* (2019) and Coertze (2022). Furthermore, the concrete surface on which the Aquatic-to-Subaerial remains were placed following the environmental transition may have influenced the observed decomposition patterns. Mann *et al.* (1990) reported that bodies positioned on concrete decompose at a slower rate and exhibit earlier onset of mummification compared to those placed on natural substrates such as grass. These findings were supported by subsequent studies conducted by Shattuck (2009) and Cogswell and Cross (2020), both of which documented reduced decomposition rates and accelerated mummification on concrete surfaces. The delayed decomposition observed on concrete may be attributed to its antimicrobial properties, which stem from the material's high alkalinity due to its composition (Wei *et al.,* 2013). Although external decomposition appeared to halt due to mummification, internal decomposition persisted, as evidenced by maggot activity and the presence of decomposition fluids leaking from orifices. The internal maggot activity would have reduced the water content and thus, played a role in preserving the remains in a mummified state (Bereuter *et al.,* 1996). Future studies could investigate whether submerged

remains, when exposed to high temperatures and low humidity following retrieval from water, undergo a unique decomposition trajectory in which external mummification occurs while internal microbial and insect activity continues. One avenue for such research could involve investigating whether chemical changes to lipids in the dermis (potentially induced by prolonged submersion) promote desiccation when the skin is later exposed to dry and warm conditions, thereby contributing to this pattern of external preservation alongside internal decay (Bereuter *et al.,* 1996). Additionally, further investigation is warranted into the influence of the post-submersion surface substrate, to determine whether the type of surface onto which previously submerged remains are placed significantly affects the rate and pattern of decomposition.

The present study highlights the significant impact of environmental transitions on decomposition outcomes, particularly in terms of tissue desiccation and mummification. However, before transitioning to a terrestrial setting, the Aquatic-to-Subaerial Group specimens exhibited decomposition patterns consistent with aquatic exposure.

7.1.3. Floating and Sinking Behaviour in Water

Notably, at the beginning of the study, all Aquatic-to-Subaerial Group piglets and the water control remains sank when placed into the water. By day three, all but one of the Aquatic-to-Subaerial Group piglets (WL5) had resurfaced and exhibited signs of bloating. These remains continued to float and showed bloating throughout their time in water. Similarly, the Aquatic Control piglet floated with only the head and limbs submerging as bloating softened. This aligns with findings by Anderson and Hobischak (2004) and Heo *et al*. (2008) which both showed that remains will sink/submerge initially, re-emerge during the bloated stage and then sink again after bloating subsides. In comparison, all Subaerial-to-Aquatic Group floated upon entering the water and continued to do so, even after appearing deflated. The exception was LW1, which sank after reaching near complete skeletonization. The initial floating of

Subaerial-to-Aquatic Group remains is likely due to active decomposition, which facilitated sufficient gas accumulation within the soft tissues before submersion. The continued floating seen in other Subaerial-to-Aquatic Group piglets may be a result of the entrapment of gases in the abdomen and soft tissues, a phenomenon observed by Anderson and Hobischak (2004) where some remains continued to float long past the bloating stage. Dalal *et al*. (2020) reported similar findings, noting that remains eventually sunk, suggesting that the Subaerial-to-Aquatic Group remains in this study may have eventually sunk had the study been extended.

The position of remains in water can influence patterns of disarticulation (Franceschetti *et al.*, 2021). Franceschetti *et al.* (2021) observed that disarticulation followed a progressive and region-specific pattern, typically beginning in the distal extremities. The hands, feet, and mandible were the first to disarticulate, sometimes as early as three months post-submersion, while complete separation of larger elements such as the skull, limbs, and torso occurred in bodies with longer postmortem submersion intervals (five months and beyond). When transitioned into the aquatic environment, the Subaerial-to-Aquatic Group remains showed disarticulation of the forelimbs but not of the hindlimbs as well as disarticulation of the head and neck from the trunk. However, it began first in the head and neck region and was then later seen in the limbs. Franceschetti *et al.* (2021) found that prolonged submersion resulted in full disarticulation, suggesting that the partial disarticulation observed in this study may be due to the remains floating on the water's surface rather than being fully submerged. The study defined full disarticulation as the complete separation of skeletal elements from one another, including the detachment of limbs and the skull from the vertebral column. Franceschetti *et al*. (2021) associated this with the highest decomposition scores (e.g., Limb Aquatic Decomposition Score (LADS) of 9 and a TADS of 25), even in cases where

disarticulated parts remained physically present within clothing but were no longer anatomically connected.

7.2. Decomposition Body Score

7.2.1. Decomposition Body Score Trends

During the first seven days (before the environment change), Aquatic-to-Subaerial Group exhibited similar Standardized Total Body Score (STBS) values to Subaerial-to-Aquatic Group. However, after transitioning environments, Subaerial-to-Aquatic Group displayed greater STBS scores. The similarity in decomposition scores between the Subaerial-to-Aquatic piglets and Aquatic-to-Subaerial piglets during the initial seven days is likely attributable to the overlap in early-stage descriptors within the ATBS and TADS scoring systems, as detailed in Table 18 (Appendix C). Both systems rely heavily on internal autolysis-driven changes (such as discoloration, marbling, and bloating) which are common to all remains regardless of environmental context during the early stage of decomposition (Megyesi *et al.,* 2005; Heaton *et al.,* 2010; Keough *et al.*, 2017). This reflects the biological reality that, in the early postmortem period, autolysis (an internal cellular process) dominates decomposition (Vass, 2011). At this stage, the body remains largely intact and fleshed, which provides a level of protection from the external environment and slows the influence of external variables (Putman, 1977; Carter *et al.,* 2007). As noted by Carter *et al.* (2007), the transition from early to active decomposition is marked by ruptures in the skin caused by putrefactive bloating and maggot activity, which allow oxygen and environmental agents to interact with internal tissues. This indicates that the intact, fleshed state of the body during early decomposition offers a degree of protection from external environmental influences. However, as decomposition progresses and putrefaction becomes the primary driver, the influence of the environment becomes more pronounced due to the exposure of soft tissue (Forbes *et al.,* 2005). Thus, this shift coincides with arthropod colonization, fluid purging,

and tissue breakdown, which expose internal structures to external conditions (Rodriguez and Bass, 1983; Anderson, 2011). These environment-sensitive changes are distinctly emphasized in the scoring systems: ATBS highlights features like mummification and desiccation typical of terrestrial remains (Megyesi *et al.,* 2005; Keough *et al.*, 2017) , while TADS captures aquatic-specific phenomena such as adipocere formation and disarticulation (Heaton *et al.,* 2010). These differences explain the divergence in decomposition scores observed between the groups in the later stages, as external environmental factors increasingly shaped the rate and pattern of decomposition. Thus the change in scoring systems could account for the perceived increase in decomposition seen for the Subaerial-to-Aquatic group and perceived decrease in decomposition for the Aquatic-to-Subaerial group.

Previous studies have found that decomposition generally progresses more slowly in water compared to land (Mann *et al.,* 1990; Rodriguez 1997; Haglund and Sorg, 1997; Heo *et al.,* 2008; Dalal *et al.,* 2020). However, while submerged, Aquatic-to-Subaerial Group remains exhibited higher STBS values than Subaerial-to-Aquatic Group remains on land during the initial environment period. After the transition, Subaerial-to-Aquatic Group remains in water decomposed more rapidly than Aquatic-to-Subaerial Group remains on land, further reiterating that in the present study's decomposition progresses faster in water than on land. Du Toit *et al*. (2024) saw similar results in their study on the influence decomposition of rehydrated remains. They concluded that rehydration influenced the progression of decomposition and subsequent rate, with rehydration increasing decomposition score values (du Toit *et al.,* 2024).

The control piglets initially deviated from this general trend observed in previous studies by land (Mann *et al.,* 1990; Rodriguez *et al.,* 1997; Haglund and Sorg, 1997; Heo *et al.,* 2008; Dalal *et al.,* 2020). The Aquatic Control decomposed more rapidly at first before plateauing, whereas the Subaerial Control followed a gradual decomposition pattern, ultimately reaching

a higher STBS value. Ayers (2010) reported similar results to this study, with pig carcasses in freshwater decomposing more rapidly than those on land. Similarly, Coertze (2022) noted accelerated decomposition in the piglet carcasses placed in water. Notably, their study found that water temperatures remained cooler than ambient air temperatures—a trend also observed in the present study. This suggests that the unexpected rapid decomposition in the aquatic environment is unlikely to be driven by water temperatures exceeding those of the subaerial environment. A possible factor contributing to this accelerated decomposition is insect activity. Diptera species, particularly blowflies, are known to favour moist environments for oviposition (Cruise *et al.,* 2018). The prolonged presence of Diptera on piglets in aquatic environments may have resulted in extensive oviposition on floating remains, leading to significant tissue degradation. This aligns with findings by Cruise *et al.* (2018), which highlight the role of moisture in facilitating sustained insect activity and accelerating soft tissue breakdown.

Subaerial-to-Aquatic Group showed a strong correlation between STBS and logADD, suggesting that remains initially decomposing on land and later submerged can still be effectively scored using Adjusted Total Body Score (ATBS) (Megyesi *et al.,* 2005; Keough *et al.,* 2017) and Total Aquatic Decomposition Score (TADS) (Heaton *et al.,* 2010). This implies that PMI estimation may still be feasible in such cases. However, as this study serves as a proof of concept, further research is needed to confirm these findings. In contrast, Aquatic-to-Subaerial Group demonstrated poor reliability between STBS and logADD, indicating that remains initially decomposing in water and later found on land may not be accurately scored with existing systems. This suggests PMI estimation in such cases would likely be unreliable. Future research should explore how environmental transitions, particularly aquatic-to-terrestrial shifts, influence decomposition dynamics and PMI accuracy.

7.2.2. Effectiveness of the Decomposition Body Scores

The effectiveness of decomposition scoring systems in alternating environments is a critical consideration when evaluating postmortem changes. This study demonstrated that while the Standardised Total Body Score (STBS) provided a means of comparing decomposition progression across different environments, the underlying scoring systems (ATBS and TADS) varied in their effectiveness.

Subaerial-to-Aquatic Group exhibited decomposition trends suggesting that both ATBS and TADS were effective in describing the decomposition changes in remains that initially decomposed in a subaerial environment before transitioning into an aquatic setting. However, Aquatic-to-Subaerial Group, which underwent the reverse transition (aquatic to subaerial), demonstrated that these scoring systems' descriptions were not as applicable or effective, even when standardized.

The initial similarity in STBS between Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group on Day 7 suggests that both groups experienced comparable levels of decomposition prior to the environmental switch. However, following the transition, a clear divergence in decomposition trajectories was observed. Subaerial-to-Aquatic Group, moving from land into water, exhibited a rapid increase in STBS, whereas Aquatic-to-Subaerial Group, transitioning from water to land, displayed a substantial decline in STBS. This decline is most likely attributed to the change in scoring system. The Aquatic-to-Subaerial remains were initially scored using TABS while in the aquatic environment and then ATBS after transitioning into the subaerial environment. ATBS does not account for some of the active decomposition characteristics resulting from an aquatic environment.

Forbes *et al.* (2019) highlighted the limitations of defining skeletonization based solely on bone exposure, noting that mummification can present with varying patterns of tissue desiccation that may not be adequately captured by existing scoring criteria. This suggests

that the drying of soft tissues may have influenced the scoring results, leading to the observed decline in STBS for Aquatic-to-Subaerial Group as with no bone exposure, one cannot score higher with ATBS. Similar to the present study, Forbes *et al.* (2019) further highlighted that Total Body Scores (TBS) (Megyesi *et al.,* 2005) became less reliable during the later stages of decomposition of the remains in their study.

Another potential reason for these findings is that terrestrial-based scoring systems, such as ATBS (Keough *et al.*, 2017), do not account for the unique morphological decomposition patterns associated with aquatic environments. Aquatic decomposition is influenced by factors which can result in distinct soft tissue preservation and loss patterns that differ significantly from terrestrial decomposition. Consequently, when remains transition from an aquatic to a terrestrial setting, pre-existing decomposition features may not align with the criteria used in traditional terrestrial scoring systems, leading to inaccurate assessments of decomposition progression.

Future research should explore the development of an integrated scoring system that accounts for the complexities of decomposition in alternating environments. Such a system would need to incorporate both terrestrial and aquatic decomposition processes, ensuring a more accurate representation of postmortem changes when remains experience environmental transitions. This could involve refining existing scoring criteria or introducing new morphological markers that capture the intermediate and transitional stages of decomposition more effectively. Additionally, experimental studies that systematically track decomposition progression across different environmental sequences could provide further insights into how postmortem changes manifest under varying conditions, ultimately enhancing forensic estimations of the postmortem interval.

7.3. Arthropod succession

Calliphoridae, Muscidae, and Sarcophagidae are some of the first families to arrive at remains and are thus of forensic importance (Cruise *et al*., 2018; Salimi *et al*., 2018; Temba and Mukaratirwa, 2021). The findings of the present study support this, as Muscidae and Calliphoridae were the earliest colonizers observed across all remains. Shi *et al*. (2009) state that Sarcophagidae are primary colonizers in warmer climates; however, in this study, they were documented as secondary colonizers, appearing only once (on Day 12) in Subaerial-to-Aquatic Group and twice (on Days 5 and 6) in Aquatic-to-Subaerial Group. A similar pattern was observed by Mabika *et al*. (2014) in their study on rabbit carcasses in Harare, Zimbabwe, although no explanation was provided for this deviation from expected patterns. One possible reason for the delayed appearance of Sarcophagidae in the present study could be possible limitations of the insect collection method employed. It is possible that the collection approach was not optimally suited for capturing this particular family, or that the sampling intervals were insufficient to detect their earliest presence. Additionally, environmental microvariations at the site, such as shade, wind exposure, or proximity to vegetation, may have influenced Sarcophagidae colonization behaviour (Campobasso *et al.,* 2001).

Later-stage colonizers, such as Coleopterans, typically arrive once remains have dried, preferring desiccated tissue over fresh decomposition stages (Amendt *et al*., 2011; Cruise *et al*., 2018; Temba and Mukaratirwa, 2021). This study aligns with these findings, as *Dermestus maculatus* and Histeridae were documented in the later stages of decomposition across Subaerial-to-Aquatic Group, Aquatic-to-Subaerial Group, and the control specimens. Notably, Histeridae were only observed on the Subaerial Control, day 7 to 9 of Subaerial-to-Aquatic Group, and Days 11 to 14 of Aquatic-to-Subaerial Group. This pattern suggests that certain Coleopteran taxa, such as Histeridae, may favour terrestrial environments and are unlikely to colonize submerged remains. This is likely due to their biology as terrestrial

beetles, which are not adapted to survive or forage under aquatic conditions. Histeridae are typically predatory and rely on the presence of other arthropods and dry tissue, both of which are less accessible or absent in aquatic environments. Furthermore, Histeridae are burrowers and would be deprived of refugia in aquatic environments (Kovarik and Caterino, 2000).

Despite the environmental transitions experienced by Subaerial-to-Aquatic Group and Aquatic-to-Subaerial Group, the majority of documented species (12 out of 15) were found on remains across both groups throughout the study. These species included *Dermestus maculatus*, Histeridae, *Fannia canicularis*, *Lucilia sericata*, *Chrysomya marginalis*, *Chrysomya megacephala*, *Chrysomya chloropyga*, *Chrysomya putoria*, *Chrysomya albiceps*, *Musca domestica*, and Sarcophagidae. Of these, six species (*Chrysomya megacephala*, *Chrysomya chloropyga*, *Chrysomya putoria*, *Chrysomya albiceps*, *Musca domestica*, and Histeridae) persisted despite the environmental transition.

The species composition observed in this study is consistent with previous research on forensic arthropods in subaerial decomposition environments in South Africa. Kelly *et al*. (2009) studied decomposition in Bloemfontein and identified species from the genera *Chrysomya*, Muscidae, Sarcophagidae, and Dermestidae. Similarly, Tembe and Mukaratirwa (2021), in their study conducted in KwaZulu-Natal, documented species from *Chrysomya*, Muscidae, Sarcophagidae, and Dermestidae.

Studies examining forensic entomology in aquatic environments have also reported similar species compositions to those in the present study. Heo *et al*. (2008) found that species from the genera *Chrysomya* and Muscidae were dominant in aquatic remains. Barrios and Wolff (2011) similarly documented *Chrysomya*, Muscidae, and Sarcophagidae in their aquatic decomposition study. Haddadi *et al*. (2019) further expanded these findings by recording

Chrysomya, Muscidae, Sarcophagidae, Cleridae, Dermestidae, and Lepidoptera species in aquatic forensic settings.

These findings collectively suggest that environmental conditions do not strongly dictate which insect species colonize remains. Instead, decomposition stage and access to remains appear (most Aquatic-to-Subaerial piglets floated by day two, allowing for arthropod access) to be the primary factor influencing insect succession, with species arriving in response to tissue changes rather than specific environmental transitions.

A notable exception to this trend, however, was the presence of *Anthomyia* spp., which were exclusively found on Aquatic-to-Subaerial Group remains. The presence of *Anthomyia* spp. exclusively on Aquatic-to-Subaerial Group specimens, first appearing in the aquatic phase and persisting after the transition to a terrestrial environment, indicates that the presence of adults suggests that the remains might have experienced aquatic immersion. Coertze (2022) similarly documented the presence of Anthomyiidae on aquatic piglet carcasses, reinforcing the idea that this taxon may be adapted to, or preferentially attracted to, remains that have been submerged. The forensic significance of Anthomyiidae has been noted by Sharanowski *et al*. (2008) and Mabika *et al.* (2014), highlighting their potential use in forensic casework. Their exclusive presence on Aquatic-to-Subaerial Group remains in this study raises important questions regarding their ecological role in decomposition and whether they could serve as indicators of prior aquatic exposure if found on subaerial remains.

Further research could explore whether Anthomyiidae exhibit specific oviposition or feeding behaviours on submerged remains, as well as their potential role in distinguishing cases where bodies have been moved between environments postmortems. Additionally, investigating their life cycle in relation to varying aquatic conditions—such as water salinity, temperature, and depth—could provide valuable insights into their forensic applications.

Finally, as the Aquatic Control began sinking again (after the bloat stage subsided), subaerial arthropod larvae exhibited behavioural responses by either migrating away from the remains onto the container or relocating to body regions that remained above the water surface. This aligns with the observations of Payne and King (1972) and Ayers (2010), who documented maggots actively leaving partially submerged remains.

A similar pattern emerged on Day 8 when Subaerial-to-Aquatic Group specimens were transitioned into the aquatic environment. Existing larvae on these remains quickly migrated to regions that remained unsubmerged, prioritizing air-exposed areas to continue feeding and developing. This behavioural response suggests that while terrestrial colonization patterns establish early decomposition dynamics, a sudden shift to an aquatic environment alters insect activity and may impact overall decomposition rates. Future research could explore whether such larval relocation influences postmortem interval estimation by affecting larval development time, particularly in cases where remains experience multiple environmental transitions.

7.4. Limitations of the study

There are several limitations from the study that need to be addressed. The Total Aquatic Decomposition Score (TADS), developed by Heaton *et al.* (2010), was designed specifically for adult human remains. This study utilized piglets as a proxy for human decomposition, therefore, some modifications to the wording of the scoring criteria were necessary to account for anatomical and morphological differences. While the scores and descriptions themselves remained unchanged, these adjustments might influence the system's applicability or interpretation. Furthermore, the accuracy of using TADS on porcine remains was tested in the present study, but such use is not yet fully validated, which introduces an element of uncertainty.

As this was a proof-of-concept study with an objective to evaluate the accuracy of scoring systems in varying environmental conditions, the research represents novel work. These methodologies have not been extensively studied, either in South Africa or internationally, limiting the availability of comparative data and potentially affecting the generalizability of findings.

A notable limitation of this study, and all studies that use TBS methods, is that the limbs were scored as a single anatomical unit, without further subdivision. While Franceschetti *et al.* (2022) proposed and applied a modified aquatic decomposition scoring system for human remains that separates the limbs into upper and lower segments (e.g., arms, forearms, hands, thighs, legs, feet), this anatomical breakdown does not directly translate to pigs, which lack equivalent limb segmentation. Additionally, to the best of this author's knowledge, this modified system has not yet been validated across different species or formally adopted into standard decomposition protocols. As such, the original scoring structure, TADS (Heaton *et al.,* 2010) was used in this study. However, this presented a challenge, as in several cases one portion of a limb decomposed more rapidly than the other, complicating the scoring process and potentially reducing accuracy.

While piglets are commonly employed in forensic research due to similarities in soft tissue composition and decomposition processes, there are differences anatomically, morphologically, and physiologically from humans. These differences can influence decomposition rates and patterns, particularly in terms of body mass, fat distribution, and skin structure. Consequently, findings based on porcine models may not fully reflect the minutia of decomposition dynamics of human remains; however, it does present general trends on decomposition that are likely reflected in human remains. Using pigs in decomposition research presents a limitation, particularly when creating decomposition rate equations, as such equations cannot be directly and reliably applied to human remains for PMI estimations

(Gruenthal *et al.,* 2012). However, this study did not aim to establish decomposition rate equations, therefore, the results are relevant and applicable within the broader field of forensic science.

In the present study, the piglets were placed one meter apart due to space constraints within the research site. This limited spacing could have allowed for the occurrence of cross contamination of decomposition fluids, odours, and invertebrates between remains. In the current study, there was no observation of cross contamination of decomposition fluid. Experimental research underscores the importance of sufficient spacing to ensure independent decomposition. One such study ensured at least a 50-meter separation between the pigs to avoid interaction and maintain autonomous cadaver decomposition island (Indra *et al.,* 2024). Whereas some studies have maintained a minimum of 10-meter distance to precent arthropod cross-contamination (Etoniru *et al.,* 2025). These practices highlight how close placement, such as the 1-meter distance used in the current study, could affect decomposition dynamics by permitting transfer of fluids, altering arthropod colonisation patterns, and blending decompaction products between adjacent remains.

A further limitation in the current study is that all piglets were refrigerated for approximately two days prior to the placement in the study environments. Refrigeration slows, but does not arrest, decomposition by suppressing microbial activity and enzymatic breakdown, which can bias early-stage decomposition trajectories. Ashbridge *et al*. (2022) noted that refrigeration delays decomposition through moisture and heat retention effects. Roberts and Dabbs (2015) found that previously frozen pigs decompose significantly slower compared to fresh specimens. These factors should be considered when interpreting finding and extrapolating to forensic scenarios where remains have not been subject to cold storage.

7.5. Future Study Recommendations

To advance the understanding of decomposition in transitioning environments, future studies should incorporate several methodological refinements and broader investigative scopes. The findings of this study highlight several avenues for further investigation to enhance understanding of decomposition in transitional environments. Future research should examine the potential role of rapid drying, prompted by interactions between temperature and humidity, in facilitating the mummification observed in remains initially submerged in water and then placed in a subaerial environment. Particular attention could be given to whether lipid alterations in the dermis, possibly resulting from submersion, promote desiccation when remains are subsequently exposed to dry, warm conditions. This line of inquiry may shed light on the apparent preservation of external tissues despite ongoing internal decomposition. In addition, the influence of post-submersion surface substrates on decomposition dynamics warrants further exploration. Determining whether the type of surface onto which submerged remains are placed impacts decomposition rate or pattern could inform recovery and analysis protocols in forensic contexts.

Methodologically, there is a need to refine decomposition scoring systems to accommodate the complexities of alternating environments. Research could evaluate the proposed limb-specific scoring modifications suggested by Franceschetti *et al.* (2021), as well as explore the development of an integrated scoring system that captures transitional and hybrid decomposition features more accurately. Such a system would aid in better estimating the postmortem interval (PMI) for remains subjected to multiple environmental exposures.

Future research should characterise the VOC profiles of remains that alternate between terrestrial and aquatic environments, testing whether rehydration or immersion events produce distinct odour signatures relative to continuous single-environment decomposition. Prior studies demonstrate both the feasibility of VOC analysis and environment specific scent

profiles for terrestrial versus submerged remains (Irish *et al.,* 2019; Malevic *et al.,* 2023; Bone *et al.,* 2024), but comparative longitudinal datasets across transitional or non-stable environments is lacking. Research into this would address the gap and directly inform HRD dog training and electronic-nose calibration for remain recoveries in non-stable environments.

Finally, further investigation into arthropod activity, particularly the behaviour and developmental dynamics of Anthomyiidae on submerged remains, could contribute valuable forensic markers. Understanding how environmental variables such as salinity, depth, and temperature influence their colonisation, and larval development may improve PMI estimations and provide insight into postmortem body relocation between environments.

8. Conclusion

This study investigated the effects of environmental transitions on decomposition and arthropod succession, specifically examining how remains initially decomposing in a subaerial environment responded to subsequent aquatic immersion and vice versa. The findings provide valuable insights into how decomposition progresses under alternating environmental conditions and the limitations of existing forensic scoring systems in such scenarios.

The study demonstrated that remains initially exposed to a subaerial environment before submersion decomposed at an accelerated rate upon entering the water, leading to near-complete skeletonization. This contrasts with previous research that suggests that aquatic environments generally slow decomposition, and it highlights the role of tissue rehydration in accelerating soft tissue breakdown.

Remains initially submerged in freshwater before being placed on land underwent unexpected mummification, rather than the anticipated continued decomposition. This finding, challenges

traditional expectations of post-submersion decomposition and suggests that environmental transitions could play a significant role in soft tissue preservation along with pH changes, time in water, and chemical changes as a result of time in water.

The study assessed the reliability of decomposition scoring methods (Adjusted Total Body Score and Total Aquatic Decomposition Score) in remains that experienced environmental shifts. The results indicated that while the scoring systems effectively tracked decomposition in remains transitioning from a subaerial to an aquatic environment, they were less reliable for remains from an aquatic to subaerial setting, particularly due to mummification, which seemed to alter the scoring accuracy. These findings suggest the need for refining existing scoring models or developing new methodologies that account for decomposition in alternating environments.

A comprehensive survey of arthropod species across the two decomposition environments was provided. The study found that arthropod succession patterns were largely consistent across conditions, with Calliphoridae and Muscidae as primary colonizers and Coleopterans appearing later in the decomposition process. However, *Anthomyia* spp. were found exclusively on remains transition from aquatic to terrestrial environments, suggesting a potential marker for post-submersion exposure. Additionally, variations in larval migration behaviour were documented when remains transitioned between environments, providing more insight into arthropod activity in fluctuating decomposition settings.

Overall, the study contributes to forensics by enhancing the understanding of decomposition in alternating environments, refining the applicability of decomposition scoring systems, and identify potential forensic entomology markers. The findings have important implications for forensic investigations, particularly in cases where bodies are discovered in settings that could potentially differ from their original deposition environment, such as cases involving

areas of flooding. While this study serves as a proof-of-concept, further research is needed to explore the mechanisms driving these observed decomposition patterns, validate scoring systems in variable conditions, and refine forensic estimation methods for the postmortem interval of these particular scenarios.

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10. Appendix A

Table 5: Total Body Score (TBS) Categories for trunk from Megyesi et al., (2005). and Keough et al., (2017).

Anatomical region	Score	Description
Trunk		**Fresh Stage:**
	1	No discoloration or insect activity.
		Early Decomposition:
	2	Pink-white appearance with skin slippage and some hair loss.
	3	Gray to green discoloration; some flesh relatively fresh.
		Active Decomposition:
	4	Bloating with green discoloration and purging of decompositional fluids.
	5	Post bloating after release of abdominal gases, with discolouration changing from green to black.
	6	Decomposition of tissue leading to sagging of flesh; caving in of abdominal cavity.
		Advanced Decomposition:
	7	Moist decomposition with bone exposure, less than one-half of the area being scored.
	8	Mummification with bone exposure of less than one-half of the area being scored.
	9	Bones with decomposed tissue, sometimes with body fluids and grease still present.
	10	Bones with desiccated or mummified tissue covering less than one-half of the area being scored.
	11	Bones largely dry but still retaining some grease.
		Final Stage:
	12	Dry bone.

Table 6: Total Body Score (TBS) Categories for head and neck Megyesi et al., (2005). and Keough et al., (2017).

Anatomical region	Score	Description
Head and Neck		**Fresh Stage:**
	1	No discoloration or insect activity.
		Early Decomposition:
	2	Pink-white appearance with skin slippage and some hair loss.
	3	Gray to green discoloration; some flesh relatively fresh.
		Active Decomposition:
	4	Discoloration to brownish shades, particularly at edges, snout, and ears; some flesh still relatively fresh.
	5	Purging of decompositional fluids out of eyes, ears, snout, mouth; some bloating of the face may be present.
	6	Brown to black discoloration of flesh.
	7	Caving in of the flesh and tissues of eyes and throat.
		Advanced Decomposition:
	8	Moist decomposition with bone exposure, less than one-half of the area being scored.
	9	Mummification with bone exposure of less than one-half of the area being scored.
	10	Bones with decomposed tissue, sometimes with body fluids and grease still present.
	11	Bones with desiccated or mummified tissue covering less than one-half of the area being scored.
	12	Bones largely dry but still retaining some grease.
		Final Stage:
	13	Dry bone.

Table 7: Total Body Score (TBS) Categories for limbs Megyesi et al., (2005). and Keough et al., (2017).

Anatomical region	Score	Description
Limbs (each)		**Fresh Stage:**
	1	No discoloration.
		Early Decomposition:
	2	Pink-white appearance.
	3	Gray to green discoloration; marbling; some flesh still relatively fresh.
	4	Discoloration and/or brownish shades, particularly at edges of projecting extremities.
	5	Brown to black discoloration, skin having a leathery appearance.
		Advanced Decomposition:
	6	Moist decomposition with bone exposure, less than one-half of the area being scored.
	7	Mummification with bone exposure of less than one-half of the area being scored.
	8	Bone exposure over one-half of the area being scored, with some decomposed tissue and body fluids remaining.
		Final Stage:
	9	Bones are largely dry but still retaining some grease.
	10	Dry bone.

11. Appendix B

Table 8: Total Aquatic Decomposition Score (TADS) Categories for Trunk (Heaton et al. 2010).

Anatomical region	Score	Description
Trunk		**Fresh Stage:**
	1	No apparent changes are visible.
		Early Decomposition:
	2	There is a slight pink colour to skin.
	3	The abdomen and upper chest show yellow/green discoloration, marbling, and the internal organs are starting to decompose.
		Advanced Decomposition:
	4	The abdomen has dark green discoloration, mild bloating, and initial skin slippage.
	5	There is green/purple discoloration, extensive abdominal bloating (feels tense to touch), and exposure of underlying fat and tissues.
	6	Black discoloration is present, bloating is becoming softer, and there is initial exposure of internal organs and bones.
	7	More tissues and organs are lost, more bone is exposed, and initial formation of adipocere is happening.
		Final Stage:
	8	Complete skeletonization and disarticulation occur.

Table 9: Total Aquatic Decomposition Score (TADS) Categories for head and neck (Heaton et al. 2010).

Anatomical region	Score	Description
Head and Neck		**Fresh stage:**
	1	No noticeable changes are observed.
		Early Decomposition:
	2	There is a slight pink colour and darkened lips.
	3	The face and neck show reddening, with visible marbling on the face.
		Advanced Decomposition:
	4	Bloating of the face occurs, followed by green discoloration, and the skin starts to slough off.
	5	Tissues become exposed on the face and neck, displaying green/black discoloration.
	6	Bone is exposed, concentrated mainly over the orbital, frontal, and parietal regions, with some on the mandible and maxilla. Early adipocere formation is evident.
	7	More extensive skeletonization occurs on the cranium, leading to the disarticulation of the mandible.
		Final Stage:
	8	Complete disarticulation of the skull from the torso takes place, accompanied by extensive adipocere formation.

Table 10: Total Aquatic Decomposition Score (TADS) Categories for limbs (Heaton et al. 2010).

Anatomical region	Score	Description
Limbs (each)		**Fresh stage:**
	1	No noticeable changes are seen.
		Early Decomposition:
	2	There is mild wrinkling of the skin above the trotters, possibly with goosebumps.
	3	The skin above the trotters becomes white, wrinkled, and thickened, with a slight pink discoloration of the limbs.
		Advanced Decomposition:
	4	The skin above the trotters becomes soggy and loose, and there is marbling on the limbs, mainly on the upper portions.
	5	Skin above the trotters begins to slough off, revealing yellow green to green-black discoloration on the limbs, along with initial skin slippage.
	6	Lower appendages undergo degloving, exposing large areas of underlying muscles and tendons. There is patchy sloughing of skin on the upper portion of the limbs, with muscles, tendons, and small areas of bone exposed in the lower portion.
		Final Stage:
	7	Bones of the upper portion of the limbs become exposed.
	8	Complete skeletonization and disarticulation of the limbs occur.

12. Appendix C

Table 11: Comparison of Decomposition Stages in ATBS (Terrestrial) and TADS (Aquatic) Scoring Systems

Stage of Decomposition	ATBS (Subaerial)	TADS (Aquatic)	Similarities	Key Differences
Fresh	No discoloration or insect activity	No noticeable changes or discoloration	No visible changes; skin appears normal	No differences
Early	Pink-white appearance, skin slippage, some hair loss, grey-green discoloration, initial bloating (torso), purging (head)	Slight pink colour, darkened lips, marbling, yellow-green discoloration, mild bloating	Discoloration, marbling, bloating, early skin changes	TADS includes early internal decomposition (which cannot be seen when doing superficial scoring) and aquatic-specific signs like soggy skin and "goosebumps"
Active	Green to black discoloration, purging, sagging tissue, caving of abdomen (torso), brown to black leathery skin (limbs), soft tissue collapse (head)	Green/purple to black discoloration, exposure of internal organs and bone, significant bloating, early adipocere, degloving	Increased discoloration, bloating, skin/tissue breakdown, exposure of internal structures	TADS includes adipocere and limb degloving; ATBS emphasizes mummification and tissue sagging
Advanced	Moist decomposition with bone exposure (<50%), mummification, greasy or desiccated tissue, leathery appearance (limbs)	Extensive bone exposure, tissue loss, early disarticulation, widespread adipocere	Bone/tissue exposure, advanced decay indicators	TADS includes more severe disarticulation and adipocere; ATBS focuses on drying/mummification
Final	Dry bone, possibly retaining grease	Complete skeletonization and disarticulation, skull separation, extensive adipocere	Presence of dry bone and near-complete soft tissue loss	TADS emphasizes aquatic disarticulation and adipocere; ATBS ends with dry, intact skeletons

13. Appendix D – Panel images of decomposition progression

Table 12: Day 1 to 7 of the Subaerial-to-Aquatic Group piglets. Subaerial decomposition environment. The blue represents days where rainfall was recorded.

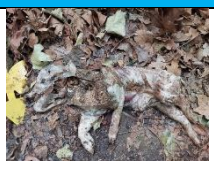
Day	LW1	LW2	LW3	LW4	LW5
1					
2					
3					
4					
5					
6					
7					

Table 13: Day 8 to 14 of the Subaerial-to-Aquatic Group piglets. Aquatic decomposition environment. The blue represents days where rainfall was recorded

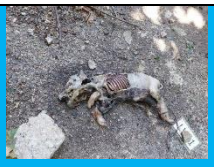
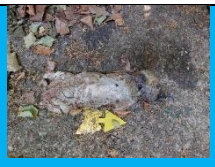
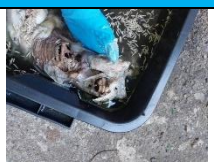
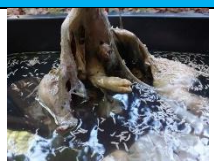
Day	LW1	LW2	LW3	LW4	LW5
8 Before transition					
8 After transition					
9					
10					
11					
12					
13					
14					

Table 14: Day 1 to 7 for the Aquatic-to-Subaerial Group piglets. Aquatic decomposition environment. The blue represents days where rainfall was recorded.

Day	WL1	WL2	WL3	WL4	WL5
1					
2					
3					
4					
5					
6					
7					

Table 15: Day 8 to 14 for the Aquatic-to-Subaerial Group piglets. Subaerial decomposition environment. The blue represents days where rainfall was recorded.

Day	WL1	WL2	WL3	WL4	WL5
8					
9					
10					
11					
12					
13					
14					

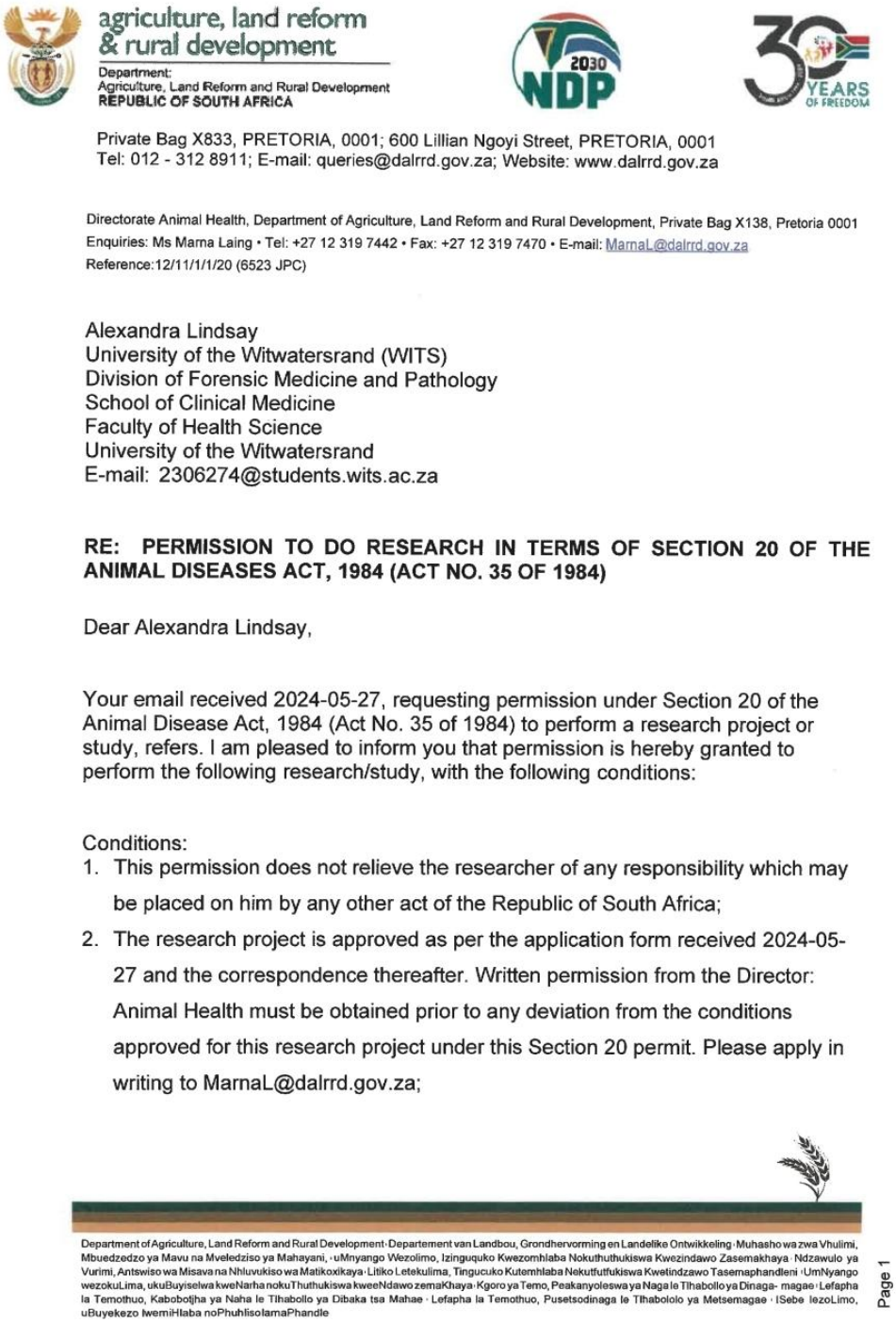
Table 16: Day 1 to 14 pictures of the subaerial decomposition Subaerial Control piglet. The blue represents days where rainfall was recorded.

Day	Picture of Subaerial Control	Day	Picture of Subaerial Control
1		8	
2		9	
3		10	
4		11	
5		12	
6		13	
7		14	

Table 17: Day 1 to 14 pictures of the aquatic decomposition Aquatic Control piglet The blue represents days where rainfall was recorded.

Day	Picture of Aquatic Control	Day	Picture of Aquatic Control
1		8	
2		9	
3		10	
4		11	
5		12	
6		13	
7		14	

14. Appendix E – Letters of permission to conduct study



agriculture, land reform & rural development
Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Private Bag X833, PRETORIA, 0001; 600 Lillian Ngoyi Street, PRETORIA, 0001
Tel: 012 - 312 8911; E-mail: queries@dalrrd.gov.za; Website: www.dalrrd.gov.za

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development, Private Bag X138, Pretoria 0001
Enquiries: Ms Marna Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MarnaL@dalrrd.gov.za
Reference:12/11/1/1/20 (6523 JPC)

Alexandra Lindsay
University of the Witwatersrand (WITS)
Division of Forensic Medicine and Pathology
School of Clinical Medicine
Faculty of Health Science
University of the Witwatersrand
E-mail: 2306274@students.wits.ac.za

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Alexandra Lindsay,

Your email received 2024-05-27, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:
1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The research project is approved as per the application form received 2024-05-27 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this research project under this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za;

Department of Agriculture, Land Reform and Rural Development·Departement van Landbou, Grondhervorming en Landelike Ontwikkeling·Muhasho wa zwa Vhulimi, Mbuedzedzo ya Mavu na Mveledziso ya Mahayani, ·uMnyango Wezolimo, Izinguquko Kwezomhlaba Nokuthuthukiswa Kwezindawo Zasemakhaya·Ndzawulo ya Vurimi, Antswiso wa Misava na Nhluvukiso wa Matikoxikaya·Litiko Letekulima, Tinguculo Kutemhlaba Nekutfutfukiswa Kwetindzawo Tasemaphandleni ·UmNyango wezokuLima, ukuBuyiselwa kweNarha nokuThuthukiswa kweeNdawo zemaKhaya·Kgoro ya Temo, Peakanyoleswa ya Naga le Tlhabollo ya Dinaga- magae·Lefapha la Temothuo, Kabobotjha ya Naha le Tlhabollo ya Dibaka tsa Mahae · Lefapha la Temothuo, Pusetsodinaga le Tlhabololo ya Metsemagae · ISebe lezoLimo, uBuyekezo lwemiHlaba noPhuhlisolamaPhandle

Page 1

Figure 29: Section 20 permit for permission to do research by the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa.

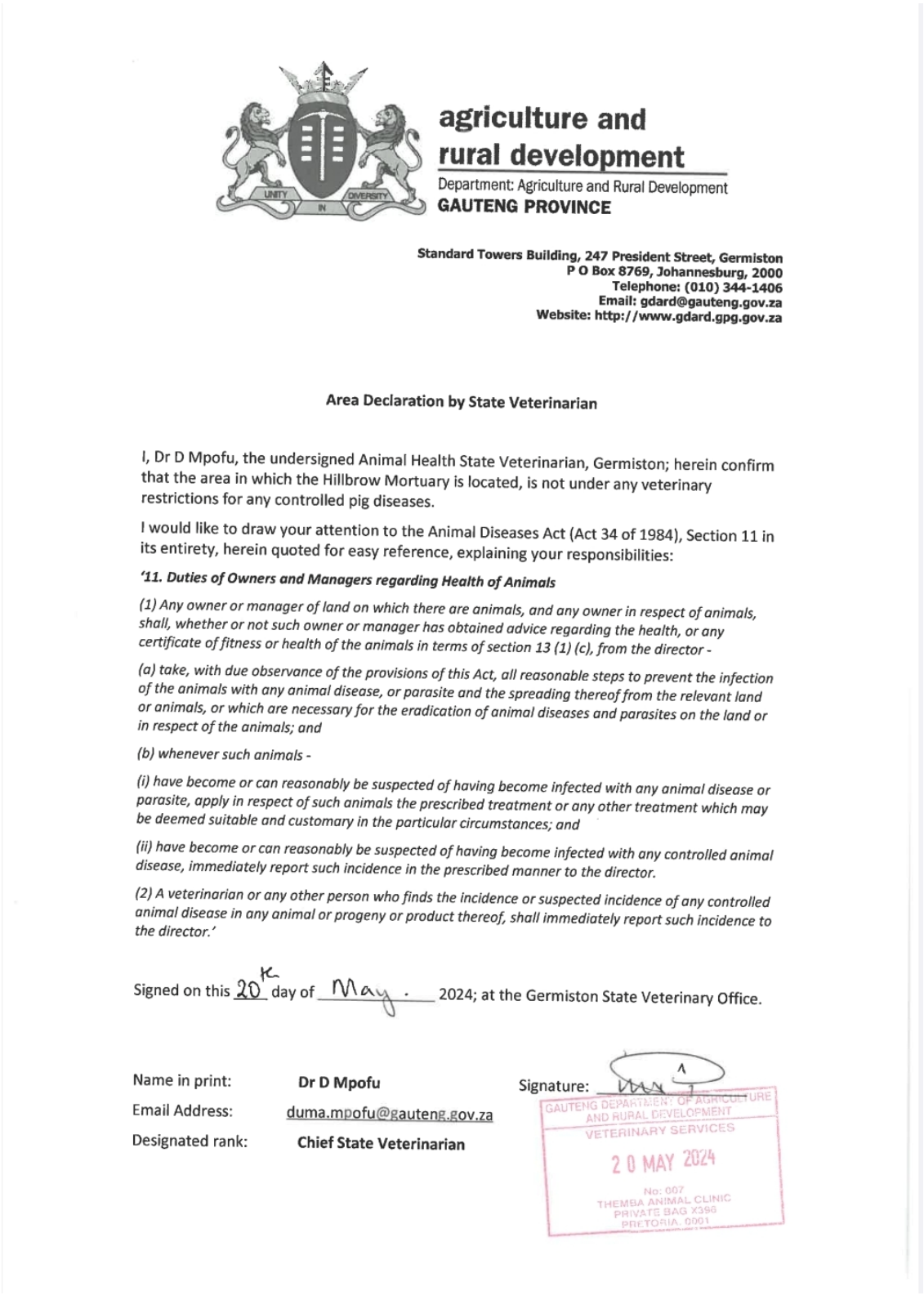
3. All potentially infectious material utilised, collected or generated during the study is to be destroyed at the completion of the study.
4. Only a waste disposal company registered for the disposal of biohazardous waste may be used for the removal of all potentially infectious waste from the research project;
5. No animal to be used in the study may be purchased or originate from an area under any State Veterinary restriction;
6. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act 1984 (Act no 35 of 84), must be reported immediately to the State Veterinarian of the area.
7. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za.
8. **This Section 20 approval is valid until 31 July 2025.**

Title of research/ study: "Decomposition Dynamics and Arthopod Colonization Patterns in Response to Shifting Environmental Conditions: Subaerial to Aquatic Transitions and Vice Versa"
Researcher: Alexandra Lindsay
Institution: University of the Witwatersrand (WITS)
Our ref Number: 12/11/1/1/20 (6523 JPC)

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date:
2024 -08- 26

Figure 30: Cont. Section 20 permit for permission to do research by the Department of Agriculture, Land Reform and Rural Development of the Republic of South Africa.



agriculture and rural development
Department: Agriculture and Rural Development
GAUTENG PROVINCE

Standard Towers Building, 247 President Street, Germiston
P O Box 8769, Johannesburg, 2000
Telephone: (010) 344-1406
Email: gdard@gauteng.gov.za
Website: http://www.gdard.gpg.gov.za

Area Declaration by State Veterinarian

I, Dr D Mpofu, the undersigned Animal Health State Veterinarian, Germiston; herein confirm that the area in which the Hillbrow Mortuary is located, is not under any veterinary restrictions for any controlled pig diseases.

I would like to draw your attention to the Animal Diseases Act (Act 34 of 1984), Section 11 in its entirety, herein quoted for easy reference, explaining your responsibilities:

'11. Duties of Owners and Managers regarding Health of Animals

(1) Any owner or manager of land on which there are animals, and any owner in respect of animals, shall, whether or not such owner or manager has obtained advice regarding the health, or any certificate of fitness or health of the animals in terms of section 13 (1) (c), from the director -

(a) take, with due observance of the provisions of this Act, all reasonable steps to prevent the infection of the animals with any animal disease, or parasite and the spreading thereof from the relevant land or animals, or which are necessary for the eradication of animal diseases and parasites on the land or in respect of the animals; and

(b) whenever such animals -

(i) have become or can reasonably be suspected of having become infected with any animal disease or parasite, apply in respect of such animals the prescribed treatment or any other treatment which may be deemed suitable and customary in the particular circumstances; and

(ii) have become or can reasonably be suspected of having become infected with any controlled animal disease, immediately report such incidence in the prescribed manner to the director.

(2) A veterinarian or any other person who finds the incidence or suspected incidence of any controlled animal disease in any animal or progeny or product thereof, shall immediately report such incidence to the director.'

Signed on this 20th day of May 2024; at the Germiston State Veterinary Office.

Name in print: **Dr D Mpofu**
Email Address: duma.mpofu@gauteng.gov.za
Designated rank: **Chief State Veterinarian**

Signature:

GAUTENG DEPARTMENT OF AGRICULTURE AND RURAL DEVELOPMENT
VETERINARY SERVICES
20 MAY 2024
No: 007
THEMBA ANIMAL CLINIC
PRIVATE BAG X396
PRETORIA, 0001

Figure 31 : Area Declaration by State Veterinarian for study site at JHB FPS mortuary.





University of the Witwatersrand
Faculty of Health Sciences
Department of Forensic Medicine & Pathology
Forensic Pathology Service: Southern Gauteng

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21 August 20245 April 2025

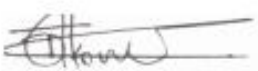
TO WHOM IT MAY CONCERN

PERMISSION TO CONDUCT MASTERS RESEARCH AT THE JOHANNESBURG FORENSIC PATHOLOGY SERVICES BY ALEXANDRA LINDSAY

Permission is hereby granted for the student to conduct her Masters research at the Johannesburg Forensic Pathology Services (FPS). The study has been approved by the Wits Faculty of Health Sciences and Animal Research Ethics Committee.

A secure and isolated site on the grounds of the FPS has been approved for use as her site of study. The site is very secure in an isolated, walled-off quad that is distant from the mortuary building and the mortuary staff. The site is secured with high brick walls, topped with barbed wire fences. The site is monitored by several security cameras. There is only one access gate that is accessed from within the mortuary grounds (i.e. no access from outside the secured mortuary grounds) and the gate remains locked 24/7. Only the security guards have a key to the gate, and they are on site 24/7.

Yours sincerely,





.....
Dr Tinyiko Zelda Nkondo
Head of Clinical Department (GDoH)/Chief Specialist
Southern Gauteng Forensic Pathology Service
Department of Forensic Medicine and Pathology

.....
Dr Shakeera Holland
Head of Academic Department
Department of Forensic Medicine & Pathology
Faculty of Health Sciences
University of the Witwatersrand

Figure 32: Letters from HOD's for Permission to Conduct Masters Research at the Johannesburg Forensic Pathology Services

15. Appendix F



ANIMAL RESEARCH ETHICS COMMITTEE
Registration number: AREC-101210-002

Date:08/02/2024

Certificate reference: Waiver 08-02-2024-O
Category: O
Applicant: Alexandra Lindsay
Department: Division of Forensic Medicine & Pathology, School of Clinical Medicine (WITS)
Email: 2306274@students.wits.ac.za

Re: Waiver from the Animal Ethics Research Committee of the University of the Witwatersrand

This letter is to confirm that Ms. Alexandra Lindsay (230627), MSc Candidate in the Division of Forensic Medicine & Pathology, School of Clinical Medicine, WITS, does not require full Animal Ethics Research Committee clearance to undertake the work titled **"Decomposition Dynamics and Arthropod Colonization Patterns in Response to Shifting Environmental Conditions: Subaerial to Aquatic Transitions an Vice Versa."**

Reason for waiver
The project will use cadavers of juvenile piglet (*Sus scrofa domesticus*, n=12) donated by an accredited abattoir (GHB Farms) to assess arthropod colonisation and decompensation of the cadavers under different environmental conditions. All animals died of natural causes.

Details of the study
The aim of the study is to describe the rate and pattern of decomposition, as well as the colonization dynamic of arthropods, in response to alteration in the surrounding environment of the remains from subaerial to aquatic conditions and vice versa.

A total of 12 juvenile piglets, divide into two experimental groups (n=5 in each group) and two controls (n=2) will be initially exposed to subaerial conditions for 1-2 weeks (depending of season (summer vs winter) followed by transition into aquatic conditions for the same amount of time (Group A). Group B will undergo the reverse sequence, commencing with immersion in aquatic followed by subaerial conditions with matched exposure times. Control pig cadavers will exclusively receive aquatic exposure (n=1) or subaerial exposure (n=1) for the experimental duration. Analyses in the variation and species of arthropod population density and colonisation between different decomposition environments will be assessed. All pig remains will be disposed of in biomedical waste containers and incinerated upon conclusion of the study.

The study location is a secured open plot connected to the Johannesburg Forensic Pathology Service's Medico-legal Mortuary (JHB FPS) or the secured Wits Frankenwald Research Site should it be required. Necessary permission to access these sites will be obtained prior to conducting the study. Entomological analysis will be conducted at the Forensic Entomology Laboratory at JHB FPS. These research sites and facilities are part of the Gauteng Sothern

Figure 33: Ethics Waiver obtained from the Animals Research Ethics Committee at the University of the Witwatersrand/

Cluster Forensic Pathology Services and are associated with the Division of Forensic Medicine and Pathology, University of the Witwatersrand.

The individuals covered by the waiver include Ms. Alexandra Lindsay, and supervisors Dr Craig Keyes and Mr Lawrence Hill - Division of Forensic Medicine & Pathology, School of Clinical Medicine, WITS.

Please contact me should you require further information.

Yours sincerely,

Dr Mark Killick
Deputy Chair: Animal Research Ethics Committee
University of the Witwatersrand

Figure 34: Cont. Ethics Waiver obtained from the Animals Research Ethics Committee at the University of the Witwatersrand.

16. Appendix H

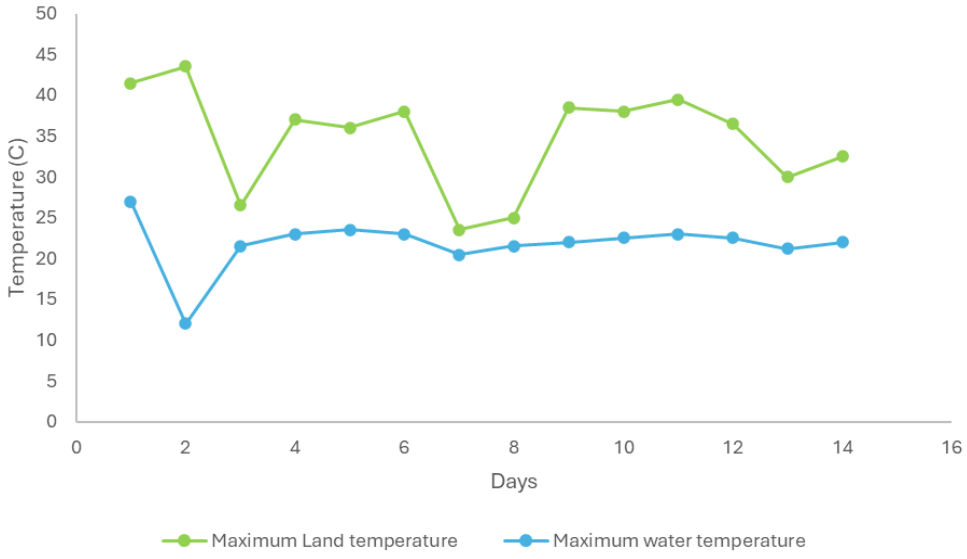
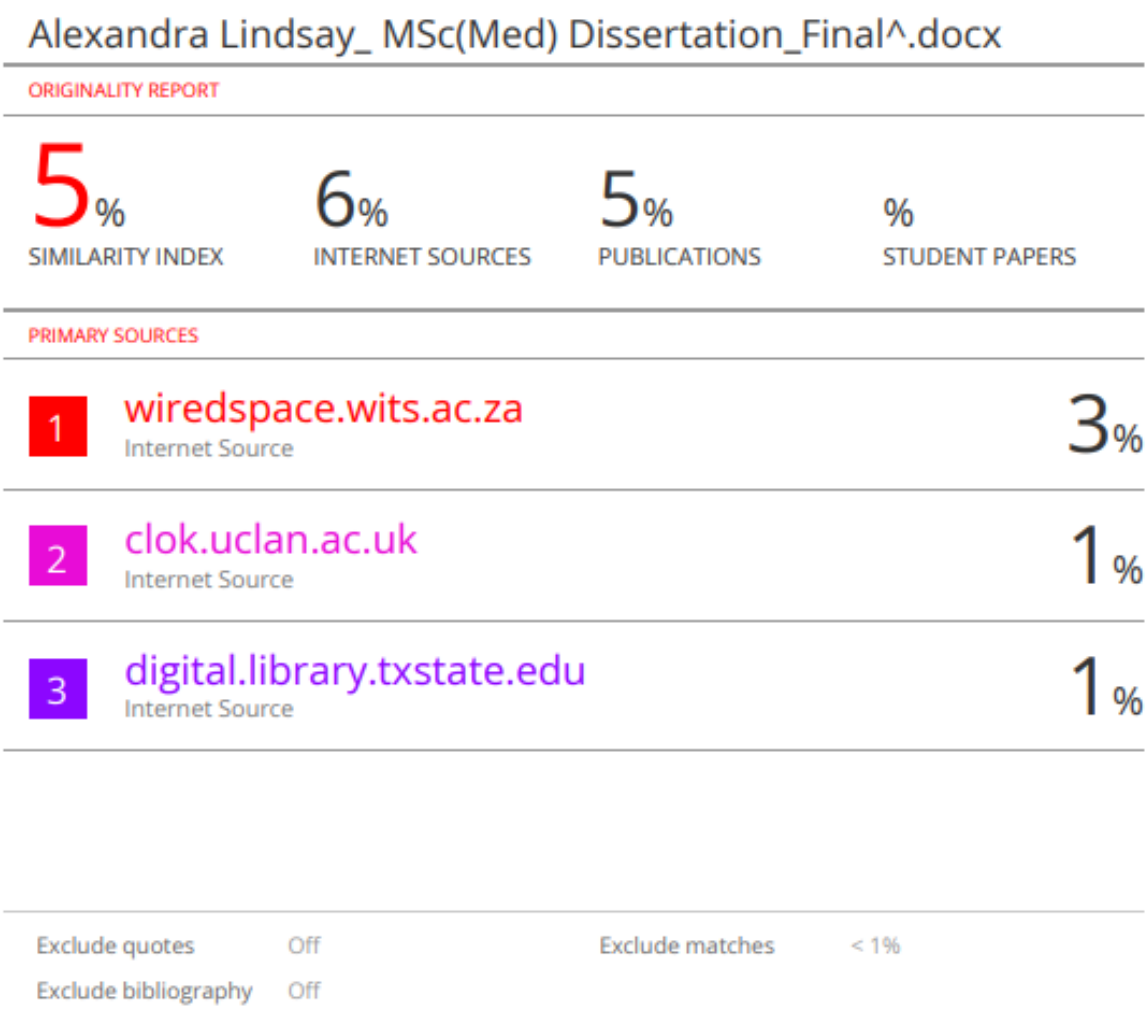


Figure 35: Maximum temperature experienced on each day of the experiment

Table 18: Accumulated Degree Days for Subaerial remains, and Water Remains.

Day	Land Accumulated Degree Days	Water Accumulated Degree Days
1	32	24
2	64	45
3	88	66
4	117	88
5	145	109
6	174	130
7	195	149
8	217	169
9	246	189
10	274	210
11	303	231
12	331	251
13	355	271
14	381	292

17. Appendix I – Turnitin report



Alexandra Lindsay_ MSc(Med) Dissertation_Final^.docx

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