Initial Studies in Forensic Entomology in Saskatchewan: Decomposition and Insect Succession on Pig Carrion in the Prairie Ecozone

A thesis submitted to the College of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Archaeology
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ABSTRACT

Research was conducted on decomposition and insect succession in the Prairie Ecozone of Saskatchewan. Twenty-four domestic pig carcasses were employed as human models for applications to future homicide investigations in this region. Several variables were considered including the effect of season, habitat (sun versus shade), and clothing. Research was conducted over three seasons: spring summer and fall. Ambient temperature, internal carcass temperature, faunistic succession over time, and the rate of decay were all compared for each experimental variable. Results indicated that habitat was only a factor in the decompositional rate of carrion in the spring season. The ambient temperature was the chief factor determining the seasonal variations in decay rate. Patterns of insect succession occurred in a predictable sequence that was unique in different habitats and different seasons. Clothing was shown to decelerate decay, but not to alter the arrival times of major taxa.

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DEDICATION

This thesis is dedicated to my best friend and husband, Terry Sharanowski. I am forever grateful for your endless support, encouragement, patience, and love. I love, adore, and thank-you with all of my heart.

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CHAPTER 1: INTRODUCTION

Forensic entomology, the study of insects associated with the decomposition of human remains, has been widely employed to determine the elapsed time since death in homicide cases (1, 2, 3, 4). Faunistic examinations of cadavers can also yield information pertaining to the manner of death (5) and post-mortem events such as secondary movement or burial of a corpse (4,6).

Time since death estimations can be based on the growth rates of necrophagous flies in the early stages of decomposition (7). These estimations require knowledge on the growth rates of the species recovered at the crime scene. Development rates of several species of flies of forensic significance have been well documented (8, 9, 10, 11). Establishing the age of the oldest insects on a corpse will lead to an estimation of the minimum time since death (4). However, this methodology can only be used until the first wave of adult flies begins to emerge, after which it is not possible to differentiate which generation is present (1).

Postmortem intervals can also be established by examining patterns of insect succession on carrion (12). Certain families of insects colonize carrion in a predictable sequence, either attracted to the products of decomposition or to prey on these necrophagous insects (4). Intervals based on succession patterns require knowledge of insect fauna in the geographic region in which the corpse is discovered; species vary widely with geographic region (13). Ambient temperature and microclimate of the postmortem habitat also play major roles in the determination of the invertebrate assemblage on carrion (5). Thus, it is crucial to examine seasonal insect activity on carrion in specific geographic regions and various habitats within these regions.

This investigation specifically examines the decompositional processes and the successional patterns of insects on carrion in the Prairie Ecozone of

Saskatchewan in a forensic context. Several variables are investigated including season, microclimate (sun and shade), and the effect of clothing on decomposition and faunistic patterns using pig carcasses as human models.

The main objective of the present study is to provide entomological data that can be employed in forensic cases in Saskatchewan and other similar biogeoclimatic regions. Locally generated data on arthropod succession and development increases the precision of postmortem interval estimations (14). In the United States, many researchers have completed excellent regional studies in the past few decades (12, 15, 16, 17). In Canada, extensive research in forensic entomology has been completed in British Columbia and new studies have initiated in Alberta, Manitoba, and Nova Scotia (Anderson, personal communication).

Anderson (18) has proposed the development of an entomological database for utilization in forensic cases in Canada. Data generated from this research are not only intended for this countrywide database, but also for the development of knowledge in forensic entomology in Saskatchewan.

CHAPTER 2: OBJECTIVES

The present study is divided into two major experiments, each with specific objectives. Each experiment is an investigation of the effect of specific variables on decomposition and arthropod succession on pig carrion. Microclimate and season are investigated in Experiment-1. The effect of clothing and nudity are examined in Experiment-2.

2.1 Experiment-1: Sun-exposed Versus Shaded Carrion in Three Seasons

Experiment-1 is designed to investigate the pattern and rate of decomposition and arthropod succession on clothed carrion placed above ground in two different habitats (sun exposed and shaded) and in three different seasons (spring, summer, and fall). Shean *et al* (19) investigated the differential decomposition on sun exposed versus shaded carrion in Washington State. They concluded that the variation in ambient temperature between the two habitats significantly affected blow fly (Diptera: Calliphoridae) activity. Carrion exposed in the sun demonstrated a faster rate of decomposition and thus, a shorter period of insect colonization.

The seasonal distribution of carrion-frequenting arthropods may alter their colonization of corpses (4). Activity cycles are mediated by a complex set of cues including the daily light/dark cycle and yearly variations in weather patterns (20). Kaufmann (21) investigated beetles associated with carrion in different seasons of the year and noted that insect succession on carrion was largely affected by seasonal abundance. Introna *et al* (22) collected the blow flies *Calliphora livida* (Hall), *C. vicina* (Robineau Desvoidy), and *Lucilia illustris* (Meigen) during spring surveys, but not in summer.

The temperature drift in Saskatchewan is extremely broad, ranging from +40°C to -40°C throughout the year (23). Furthermore, temperatures may

fluctuate over 30°C in a given day. Experiment-1 is based on the premise that the pattern of insect succession on carrion will not only be unique in Saskatchewan, but will also change significantly in different seasons of the year. Carrion placed in sunlight should experience higher ambient temperatures and therefore, decompose at a faster rate than shaded carrion. Faster decomposition should shorten the period of species-specific colonization.

2.2 Experiment-2: Clothed Versus Nude Carrion in Summer

Experiment-2 is designed to investigate the rates of decay and patterns of insect succession on clothed versus nude carcasses in summer. This experiment is performed in summer only to lower the overall research expenses. Human corpses in forensic investigations may be discovered nude or clothed, thus, it is important to understand how clothing impacts insect activity and estimations of postmortem intervals. Rodriguez and Bass (16) noted that clothing affected the rate of decomposition of human cadavers, but they did not examine how clothing influenced insect colonization in comparison to nude cadavers. Most publications on forensic entomology do not mention clothing as a factor in the pattern of arthropod succession (4, 12, 13, 15, 17, 19, 24). If clothing affects decomposition, it may also impact insect colonization. Clothing may provide shady conditions on a corpse that is otherwise in full sunlight. This may create a small, but very different microclimate.

Experiment-2 is based on the premise that clothed carrion will demonstrate a different pattern of insect succession than nude carcasses due to differential decomposition. Species not common to sunlit habitats may colonize a clothed carcass when the clothing creates shaded areas. Clothing may also provide protection for developing larvae from lethal temperatures experienced in summer.

CHAPTER 3: DECOMPOSITION

3.1 Processes of Decomposition

The cessation of normal body function immediately after death begins a process of destructive changes. The rate and pattern of these changes are largely dependent upon the environment in which the body is decaying and the ante-, peri- and postmortem circumstances. Cellular integrity becomes comprised in the absence of circulating oxygen and necrosis ensues (25). However, these changes are not morphologically evident in the initial phases of decomposition. Three observable processes dominate within the first 72 hours: algor mortis, livor mortis, and rigor mortis (26).

Algor mortis is the cooling of the body as it loses heat to the external environment. The rate of this heat loss can be used to establish the postmortem interval, typically within the first 24 hours of death (27). Livor mortis, or hypostasis, is the gravital pooling of blood within the body tissues. Starting one-hour postmortem, the aggregation of blood cells causes a mottled, burgundy discoloration of the external skin (26). Lividic discoloration reaches its maximum intensity within 8-12 hours postmortem and remains until haemolysis ensues. Stiffening of the muscles, or rigor mortis, initializes as oxygen ceases to be delivered to the muscle tissue. Cells continue with anaerobic respiration and lactic acid accumulates causing muscular rigidity (25). Rigor typically occurs within 2 to 4 hours of death and ends when muscle tissue begins to decompose from putrefactive liquids, usually around 36 hours after death (28).

Autolysis and putrefaction are processes of soft-tissue decomposition. Autolysis is an intrinsic process where cellular enzymes begin to self-digest cellular components (25). Putrefaction is a bacterial process initiated by the body's pre-existing micro fauna and later by extrinsic organisms (29). As autolysis ensues, an increasingly anaerobic environment is created that promotes

bacterial activity (30). Thus, putrefaction is enhanced by cellular self-digestion. Putrefaction is also enzymatic and accounts for the major decomposition of carbohydrates, proteins, and fats (25).

Putrefaction is characterized by five major events: distension, discoloration, odour, liquefaction, and skeletonization (25,30,31). The bacterial breakdown of tissues creates foul-smelling gaseous by-products, such as hydrogen sulphide, methane, cadaverine, and putrescine (25). The gases are released into the body cavity, causing distension of the abdomen and organs and cyan marbling of the outer skin (29). As pressure builds in the abdomen, gases and fluids are forced out of bodily orifices and eventually the body cavity erupts exposing the internal organs (32). Liquefaction ensues and breakdown occurs until only the skeleton remains, surrounded in the foul liquids of the putrefactive process (30). Comprehensive reviews on all of the biochemical processes of decomposition can be found in Janssen (25). The estimation of time since death using pathological methodologies can be found in Henssge *et al* (28) and others (26,27,30).

The postmortem environment may promote processes of preservation that can counteract decomposition. Enzymatic reactions of autolysis and putrefaction are inhibited at temperatures below 10°C and tissues can be preserved for extended periods at sub-zero temperatures (32). Temperatures above 40°C can also delay decomposition by deactivating autolytic and bacterial enzymes, and causing the heat fixation of tissues.

Typically, lipids are hydrolysed into fatty acids, which are then subject to oxidization (33). However, if the cadaver is in an anaerobic environment, oxidization of the fatty acids cannot occur. In the presence of water and bacteria, fatty acids may become deposited on the skeleton causing saponification, the formation of a soap-like substance called adipocere (34). Adipocere can remain on a skeleton for extended periods and is most common in females and other cadavers with a higher percentage of body fat (32,35).

Mummification is caused by the complete evaporation of moisture, causing tissues to shrivel and harden, resembling parchment paper (36). This preservation process is most common in hot, arid climates. In temperate, arid climates,

mummification can be common on naked peripheral areas such as the hands, arms, and scrotum (37).

The decompositional sequence is not a set of exclusive phases, rather a continuum of interrelated processes that continue until all organic matter is recycled. However, researchers of forensic entomology have commonly divided these processes into discrete stages for ease in associating decay with waves of insect infestation. Payne (15), following the work of Mégnin (38), Fuller (39), and Reed (24), outlined 6 stages in the decay process for domestic pigs in north-western South Carolina: fresh, bloated, active decay, advanced decay, dry, and remains. More recently, Canadian researchers Anderson and VanLaerhoven (13), followed by Komar and Beattie (40), employed 5 stages of decomposition of fresh pigs, combining the last 2 stages of Payne. Table 3.1 outlines the stages of Anderson and VanLaerhoven and the associated indicators of decomposition.

Table 3.1: Stages of decomposition in British Columbia (13)

Stage	Characteristics of Decompositional Stage
Fresh	Starts at time of death
	Algor mortis
	No odour
	Ends with first sign of bloating
Bloated	Starts with first signs of bloat
	Accumulation of gases in abdomen
	Skin discoloration and marbling
	Strong odour of decay
	Ends with deflation of body
Active Decay	Starts with deflation of body
	Bodily fluids seep out
	Very strong odour of decay
	Peak of internal carcass temperature
	Major decrease in body mass
Advanced Decay	Mass departure of fly larvae
	Decrease in internal body temperature
	Majority of flesh removed by larval feeding
	Slower rate of body mass loss
	Substantially less odour of decay
Dry/ Remains	Carcass mostly cartilage and bone
	Some mummified skin remnants
	No detectable odour

3.2 Effect of Variables on Decomposition

The variables of the postmortem environment will determine the pattern and the rate of decomposition. Understanding the nature, effect, intensity, and interrelation of all of the variables affecting decomposition would be an overwhelming course of study. Mann *et al* stated:

> The variables affecting the decay rate of the human body cannot be isolated or controlled in experimental field studies precisely because they are so interrelated – to isolate one variable would, in reality, only give us a tiny piece of a biased puzzle (41; p. 104).

Decomposition and necrophagous insect activity are highly interrelated, both being affected by variables such as season and climate, habitat, geographical location, clothing, scavengers, and the manner of death, just to name a few. Insects are key contributors to decomposition, a fact highlighted by Payne's (15) summer carrion study. He investigated the difference between pigs exposed in an open field versus pigs enclosed in an insect-proof cage. The stages of decomposition were not as discernable when the pigs were free from insect activity. The fresh stage was noticeably longer in insect-free carrion and Payne contributes this to the insects being a factor in the dissemination of bacteria. Pigs free from insects demonstrated a gradual rate of weight loss over several weeks, whereas pigs exposed to insects exhibited a rapid reduction in biomass within the first 5 days. Thus, insects are believed to be the largest contributor to animal decomposition.

The most influential variables affecting the decomposition of carrion are discussed in the remaining sections of this chapter. For ease of discussion, the effect of these variables on insect succession will be discussed separately in Chapter 5.

3.2.1 Geographical Location, Temperature, Climate, and Season

The geographical location determines the ecological characteristics of an area, including temperature, climate, seasonal change, terrain, soil type and pH, and the native flora and fauna. These ecological characteristics impact the pattern and rate of decomposition and the species of flora and fauna that invade the decomposing remains (36).

Ambient temperature has a profound impact on the pattern and rate of decomposition (41). Generally, decay is accelerated at higher temperatures and decelerated, or even halted, at lower temperatures. Two factors lead to an increased rate of decomposition at elevated temperatures: the acceleration of biochemical processes involved in decomposition (30), and the increased rate at which insects develop (see Chapter 4). Insects also generate a great deal of metabolic heat when aggregated in masses (42). This can have a compound effect on decomposition as the larval aggregations raise the internal carcass temperature, which in turn, increases the metabolic rate of bacteria.

Different rates of decomposition of pig carcasses in various regions of the world are outlined in Table 3.2. A striking comparison is that of Payne (15) and Anderson and VanLaerhoven (13) who both placed pig carcasses above-ground in summer in South Carolina and British Columbia, respectively. It took approximately 8 days for the pigs to reach the dry stage in South Carolina. Alternatively, it took 43 days to reach the dry/remains stage in British Columbia. The major factor in the difference of decay rates was ambient temperature, as summer in South Carolina is significantly warmer than in British Columbia. However, it should be noted that Payne (15) utilized frozen carcasses, which exhibit greater aerobic decay and skeletal disarticulation, potential factors affecting the faunistic succession on carrion (43).

Decay rate in Southern Queensland, Australia (44) was similar to O'ahu, Hawaii (45), both areas possessing similar maximum daily temperatures in summer (Table 3.2). More than a two-week difference was observed in the decompositional rates in Southern Queensland in different seasons (44). The vast

difference in decay rates, demonstrates the need to establish baseline data in several geographical regions and seasons.

Table 3.2: Summary of various decay studies on animal and human remains

Location (Reference)	Season	Animal	Number of Decay Stages	Number of Days To Final Stage of Decomposition
S.W. British Columbia (13)	Summer	Pig	5	43 (dry/remains)
Southern Queensland (44)	Summer	Pig, Sheep, Dog	5	14 (After advanced decay)
Southern Queensland (44)	Winter	Pig, Sheep, Dog	5	28+ (After advanced decay)
O'ahu (45)	Summer	Pig	5	14 (Remains)
South Carolina (15)	Summer	Pig	6	10-12 (Remains)
Tennessee (16)	Spring	Human	4	34 (Dry)
Tennessee (16)	Summer	Human	4	13 (Dry)

Cold environments with low humidity tend to minimize the process of putrefaction and enhance the desiccation of tissues (43). Desiccation is also common in hot, dry climates and may lead to the natural mummification of soft-tissues (36). The hot air evaporates tissue moisture causing partial preservation and a leather-like appearance on external tissues.

Sub-zero temperatures can preserve soft-tissue, sometimes in a near perfect state. Beattie *et al* (46) exhumed the body of John Torrington, a crewmember of Sir John Franklin's disastrous nineteenth century Arctic expedition. The permafrost grave preserved Torrington's tissues so well that one could recognize his facial features over one hundred years after his death.

Climatic factors such as humidity and precipitation also have a significant effect on decompositional rate. Arid environments diminish anaerobic bacterial processes and tend to desiccate the carcass, slowing species-specific insect activity and decomposition (29). Humid environments tend to saturate tissues, potentially slowing down decomposition, similar to aquatic burial environments (3).

3.2.2 Size and Species of Remains

Various animals have been used as human models in decomposition experiments, ranging from cats and mice to pigs and sheep (12, 13, 15, 19, 44, 47,

48). If the pattern of decomposition and arthropod succession in the animal model does not mimic that of a human corpse, then extrapolation of results from these studies to humans may not be relevant (14). For example, Mann *et al* (41) compared decomposition rates of dog carcasses and human corpses. They noted that dogs decayed more rapidly and insects left the carcass much sooner than in human corpses. Animals used in decomposition experiments should be freshly killed before use. Micozzi (43) examined differences in decomposition between fresh carcasses and frozen carcasses that had been thawed before use. Decomposition of the two carcasses varied significantly; aerobic decomposition dominated the frozen-thawed carcasses, whereas fresh carcasses primarily exhibited anaerobic decomposition.

It is best to use an animal model that is inexpensive, easy to obtain, and imitates the pattern and rate of decomposition of humans (14). Anderson and VanLaerhoven (13), along with others (4, 49) suggested the use of pigs as carrion because of the similarity in decomposition to humans. Unlike other mammals such as cats and dogs, pigs have similar skin and gastro-intestinal fauna to that of humans (13).

Smith (4) and Catts and Goff (49) have recommended that pigs approximately 23 kilograms in weight be used. This size was chosen as it approximates the size of a human torso, the area where decomposition is centralized. Other researchers have utilized pigs within this recommended size range (13, 15). Hewadikaram and Goff (45) investigated the effect of carcass size on the rate of decomposition. Although they noted a difference in decompositional rate in the decay and post-decay stages, they observed no difference in the pattern of arthropod succession and concluded that size was not a major factor in succession studies. However, they utilized 8.4 and 15.1-kilogram pigs, both below the previously recommended weight. Komar and Beattie (40) examined the differential rates of decomposition between small (19-26 kg), medium (36-80 kg) and large (156-162 kg) domestic pig carcasses to determine an appropriate human model size. When compared to previous homicide cases in Alberta, they noted that small sized carcasses reached skeletonization at a

significantly faster rate than humans. Komar and Beattie suggested that middle to large-sized carcasses are better models for human decomposition in their geographic location. Rodriguez and Bass (6) noted that size is not always the most important factor, but rather, body fat. For example, a 110-kilogram cadaver of high body fat decayed faster than a 65-kilogram body laid out on the same day in the same environment. Clearly, more research examining the effect of carcass size, species, and body fat on decomposition is needed.

3.2.3 Habitat

The postmortem habitat plays an important role in decomposition, affecting level of sun exposure, insect development, vegetation and soil, scavengers, and the microclimate in which the corpse will decompose. Areas of direct sunlight typically have higher ambient temperatures than shaded areas, and this temperature differential has a greater impact on the rate of decay than other variables, such as open wounds (41). Shean *et al* (19) noted that decomposition is significantly faster in sun-exposed versus shaded carrion in coastal Washington. Additionally, habitat influences insect composition. Many carrion insects are heliotropic and may develop faster due to the higher temperatures experienced at sun-exposed sites (19).

The vegetation surrounding outdoor remains may impact decomposition. Leaf litter can introduce additional bacteria to the carrion microenvironment and increase the rate of decomposition (36). In later stages of decay, the chemical and physical processes of plants and fungi can aid in the break down of bone. Botanical evidence provides one method of estimating the postmortem interval as plant roots grow through clothing, hair, tissue, and bone as the remains continue with decomposition (50).

3.2.4 Burial and Depth

Burial creates a unique microenvironment, susceptible to a variety of complex and unpredictable variables that can alter the pattern and rate of decay of

carrion. These variables include, but are not limited to, depth of burial, access to floral and faunal agents of decay, and characteristics of the burial substrate (51).

Rodriguez and Bass (6) completed a unique and noteworthy study on the decomposition of human cadavers buried at various depths in Tennessee. They noted that burial slows decomposition at a rate that increases with depth of interment. For example, a cadaver buried at a depth of 0.3 meters was almost completely skeletonized after three months, whereas a body interred at 0.6 meters retained a vast majority of soft-tissue six months postmortem. Rodriguez and Bass contribute the differential rate of decomposition to two main factors: cooler temperatures and reduced access to insects in the deeper underground environment.

Generally, soil temperature, and thereby decomposition, decreases with depth. At shallow depths, solar radiation causes daily fluctuations in soil temperature (6). In deeper soils, daily temperatures tend to fluctuate less, but are still susceptible to major seasonal changes in ambient temperature. Similar to above-ground carrion, interred decomposing remains generate heat from the putrefactive processes. Rodriguez and Bass (6) observed that the difference between soil temperature and internal cadaver temperature was directly proportional to the depth of burial. Increased depth resulted in a smaller difference in temperature. The temperature differential was greatest during the active decay stage of decomposition, which corresponded with the results from the above-ground study performed by the same authors (16).

Mant (52), after excavating over 150 interred bodies from the Second World War, described the effects of some soil characteristics on decomposition. Porous, acidic soils accelerated decomposition. Water rich soils held thermal input better, thus accelerating decomposition. Deep burials with moist anaerobic conditions promoted saponification. Soil particle size affects drainage and thermal input (53) and thus, rate of decomposition or preservation. Dense soils, such as heavy clay, not only hinder aerobic decomposition, but also can seriously limit the ability of insects and other scavengers from accessing the body (54).

The deeper the burial, the less likely insects and other scavengers will be able to access the body. Rodriguez and Bass (6) noted insect activity on shallow-buried cadavers (0.3 meters), but not on bodies interred at greater depths (0.6 and 1.2 meters). Likewise, the authors witnessed mammalian burrowing evidence only at the shallow graves. Turner and Wiltshire (54) reported that pigs buried (0.4 meters) in heavy clay soil did not experience insect activity until scavengers exposed the carcass. The majority of soft-tissue decomposition is the result of insect activity, and without appropriate access, the rate of decomposition will be significantly reduced (41, 55, 56). In shallow burials (0.3 meters) of pig carcasses in British Columbia (57), insects colonized the carrion, but demonstrated unique composition and delayed arrivals in comparison to above-ground carcasses performed in a previous study (13). Thus, insects will colonize shallowly buried remains, but the soil covering may delay their arrival.

3.2.5 Scavengers

Vertebrate scavengers are common visitors to decomposing remains and can accelerate and alter the pattern and rate of decomposition. Scavengers remove significant quantities of flesh and bone, thereby reducing available resources for invertebrates (6, 41, 58). Mann *et al* (41) reported that carnivores, such as dogs, prefer the hands, feet and skeletal components of the body, including vertebrae, the spongy ends of the femur and humerus, and the innominate bones. Scavengers will attack above-ground carcasses, burrow for buried remains, and may disarticulate components and move them away from the carrion proper (41). Studies conducted on pig carcasses in British Columbia revealed scavenger activity on carrion in above-ground, buried, and aquatic habitats (59). Scavengers were also noted to be more attracted to shaded carrion in both terrestrial and buried environments. Heavy vertebrate scavenging rendered some decompositional stages obsolete, thereby accelerating decay and altering the patterns of insect succession.

3.2.6 Clothing

There have been conflicting reports in the literature on the effect of clothing and other textiles on decomposition. Mann *et al* (41) stated that clothing accelerates decay, serving as a thermal protector for developing larvae. Mant (52) noted that clothing on buried remains inhibited decay by protecting regions of the body from the soil environment and burrowing scavengers. Mant (52) and Mellen *et al* (35) reported that clothing promoted saponification of tissues and kept body parts well preserved. Dillon and Anderson (59) stated that clothing provided more oviposition sites as the textiles become saturated with putrefactive liquids, thereby accelerating decomposition. However, nude carrion attracted an overwhelming number of blow fly larvae in comparison to clothed, resulting in the rapid depletion and mummification of soft-tissues (59). Clothing may also provide shade on a corpse in full sunlight and possibly attract negatively phototropic species. Clearly, more research is needed to determine the nature clothing and other textiles have on decomposition and insect succession.

CHAPTER 4: TEMPERATURE AND INSECT DEVELOPMENT

Temperature is one of the key factors affecting the growth rate of poikilothermic animals such as insects. Insect development occurs in a range of optimal temperatures that is specific to each species (60). If development time is plotted against constant temperature, an inverted J-shaped curve is formed (Figure 4.1). This simple graphical relationship describes longer development times at low temperatures and shorter development times at higher temperatures. Growth is impeded at very high temperatures and development time becomes lengthened (61), creating the hook in the backwards "J".

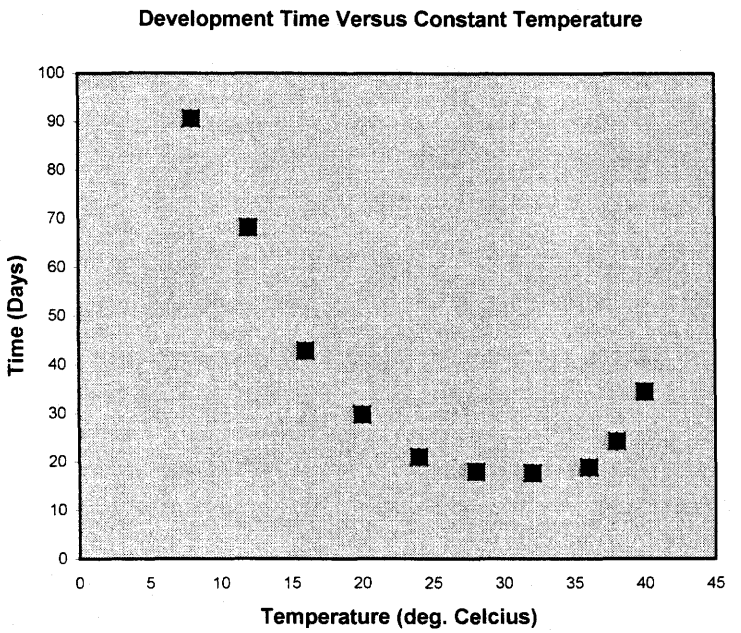

Figure 4.1: A hypothetical graph demonstrating the relationship between constant temperature and insect development time. Modified from Wagner *et al* (61).

Conversely, plotting the development rate (reciprocal of development time) against temperature results in a shallow S-shaped curve (Figure 4.2). This graphical relationship has three thermally distinct phases: lower temperature threshold, optimal temperature range, and upper temperature threshold (62). At low temperatures there is a threshold at which development ceases; insects possess the ability to survive at cold temperatures without growth (61). The second phase describes the rate of development above the lower threshold through a range of favourable temperatures. A positive linear slope characterizes this phase, as development rate is proportional to temperature. The third phase begins when a maximum temperature for growth is reached, above which a severe decline in the development rate occurs, along with an increase in the mortality rate (63). This curvilinear relationship between temperature and development rate is the primary feature of growth in poikilothermic animals (64).

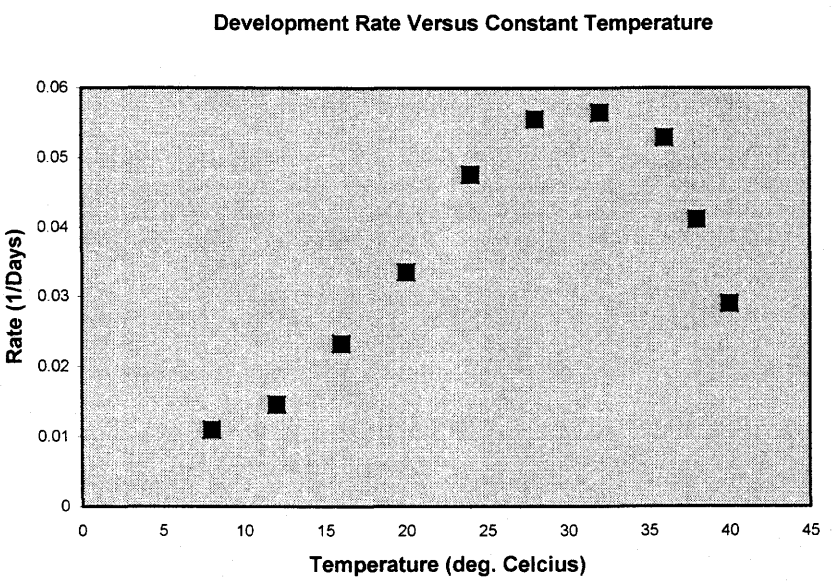

Figure 4.2: A hypothetical graph describing the relationship between constant temperature and insect development rate. Modified from Wagner *et al* (61).

Pradhan (65) describes two principles associated with the relationship between temperature and development in insects that corresponds with phase two

and three of this curvilinear relationship (Figure 4.2). First, he states that organic development occurs in accordance with the laws of compound interest. If one considers simple cellular division, whether the speed of division remains constant or increases arithmetically, the amount of growth increases in geometrical progression (phase two). The only difference between the two is the amount of time required to achieve this growth. An increase in temperature increases the speed of cellular division, thus decreasing the developmental time. From a more holistic viewpoint, the rate of an organism's cellular and enzymatic reactions are increased until the point where the temperature is so high that it hinders the rate of these reactions (60).

Pradhan's (65) second point is that a retardation rate occurs at high temperatures. The rate of cellular and enzymatic reactions increases within a unit of time corresponding with a rise in temperature. Simultaneously, there is a resistance against these reactions that also increases with temperature (65). This resistance retards the rate of development, which is demonstrated on the higher inflexion of the curve (Figure 4.2) (phase three). Thus, the relationship between temperature and development is based on the biochemical processes that are affected by temperature.

Sharpe and DeMichelle (66) describe the biochemical basis for the relationship between temperature and development rate. They maintain that the arrangement of metabolic pathways, usually convergent parallel, allows for a rate-controlling enzyme to regulate the velocity of biochemical processes in the pathway. The control enzyme can exist in one of three energy states, each associated with different temperature ranges (low, mid, and high). Only the energy state associated with the mid-temperature range (the linear portion of a development curve) is catalytically active. According to Sharpe and DeMichelle (66), the development rate in the mid-temperature range is proportional to the product of the rate constant of active enzymes (which are temperature dependent) and the concentration of those enzymes. At high and low temperature extremes, the control enzyme undergoes a conformational transition that alters the ability of

the enzyme to conform to a catalyst. Thus, development is inhibited at high and low temperature extremes, the two thresholds being specific for each species.

4.1 Thermal Summation

Throughout the nineteenth century, scientists in several disciplines were describing the relationship between temperature and development mathematically. One theory evolved, called thermal summation, which used the product of time and temperature to express a constant (65). Compared to other mathematical models, thermal summation provided the most accurate fit to the development data of insects, resulting in wide usage of this model for predicting insect development (61).

Thermal summation, or the degree-day approach, has been used for over a century due to its simplicity in calculation, application, and data formulation (61). The main premise of the degree-day model is that the rate of development is a linear function of temperature (67). However, this assumption is only true in the optimal temperature range for a given species, as demonstrated by the curvilinear relationship presented in Figure 4.2 (61). At low and high temperatures, a threshold exists where development can no longer occur (minimum and maximum threshold) (68).

Subtracting the base temperature (minimum threshold) from the average daily temperature, results in one degree-day (61). The base temperature is the minimum temperature at which development proceeds and is determined graphically using the x-intercept method (see Arnold (67) for more details). The following equation describes the calculation for degree-days for one day (69):

$$\text{Degree-days} = \frac{(\text{max. temp} + \text{min. temp}) - (\text{base temp}) \times 1 \text{ day}}{2} \quad (1)$$

The duration of development is determined by the sum of degree-days.

Despite its popularity, this model has been widely criticized for the lack of flexibility over a broad range of temperatures, inaccuracies at temperature extremes, and empirical calculations of the base temperature (61, 68, 70-72).

Allen (70) proposed a non-linear degree-day model to incorporate development over a wider range of temperatures and to allow for both an upper and lower threshold. In Allen's model, the temperature cycle insects experience is assumed to be a sine wave, thus creating six possible relationships between the temperature cycle and the two thresholds. There is a different equation associated with each relationship for the computation of degree-days (see Allen (70) for detailed explanation and all calculations). This sine wave simulation of the temperature cycle provided better accuracy in the prediction of insect development rates (72).

4.1.1 Minimum and Maximum Thresholds

Degree-day models rely on approximations in calculating development thresholds. The developmental minimum temperature is usually calculated by measuring development over a range of temperatures, graphing the growth rates, and then extrapolating the line until development reaches zero (67). Alternatively, the maximum temperature threshold can be very difficult to estimate; variability in the development rate and the mortality rate increase significantly at higher temperatures (61). The point at which the development rate plateaus or declines is usually chosen as the developmental maximum (63). These empirically calculated values introduce a certain degree of inaccuracy into development time predictions. This error is compounded if the temperature cycle falls far above or below the threshold for long periods.

In the degree-day model, the developmental minimum and maximum temperatures are calculated as single values for the entire life history of the species. Insects may have different threshold temperatures for each of the different life stages (68). Most degree-day models do not incorporate these variable thresholds to maintain simplicity in the model. Research describing

variation in threshold temperatures with life stage is scarce, thus compounding the problem.

Higley and Haskell (73) argued that the utilization of incorrect minimum thresholds is not a significant issue provided the daily temperatures are above the actual developmental minimum. However, if daily temperatures fall below the minimum threshold, significant errors can result from using the incorrect threshold. Since most forensic species have minimum thresholds between 6-10°C (73), the need for accurate data on developmental minima are of significant importance in Saskatchewan, as daily temperatures fall below 10°C throughout most of the year.

4.1.2 Thermoregulation

Inherent in the thermal summation model is the assumption that insects are ideal poikilotherms, possessing a body temperature that is equal to ambient temperature. However, several studies demonstrate that insects have a preferred temperature range for growth (3, 9, 74) and will actively try to achieve this temperature range through behavioural and physiological responses. Insects primarily employ three strategies to regulate body temperature when not in flight: postural control of sun exposure, selection of microhabitat, and alteration of diel activity patterns (75).

Insects can orient their bodies in a particular way to maximize or minimize solar heating. For example, butterflies direct their dorsal side toward the sun and stretch their wings apart to maximize solar input (76). To minimize exposure, the wings are held vertically over the back with the thin edge toward the sun. Desert tenebrionids possess long legs adapted for stilting (74). Stilting is the raising of their bodies away from the ground surface where high air temperatures persist. This postural orientation also allows the insects to reduce body temperature through greater ventilation from higher wind speeds.

Insects can regulate body temperature by selecting microhabitats of thermal preference. For example, developing blow fly larvae tend to aggregate in masses while feeding, possibly to optimize their growth rate during a vulnerable

time in their life history (3). Significant heat is generated within the larval mass, often producing temperatures that approach the lethal temperature for blow fly species. Byrd and Butler (9) observed individual maggots moving in and out of the centre of a larval mass. By rotating in and out of the mass, the maggots can avoid lethal temperatures and regulate themselves at an optimal temperature. Mosquito larvae may also aggregate when water temperatures drop below 15°C and disperse when water temperatures rise (77). Other aquatic insects move around the temperature gradient found in varying depths of water bodies, selecting shallow water in spring and deeper water on hot summer days (75).

The activity cycle of insects acts as a regulatory mechanism for temperature preference. Diurnal species have preferred periods of activity depending on temperature (75). Rove beetles (Staphylinidae) possess bimodal activity cycles, preying on dipteran larvae in early morning and early evening (49). This diel cycle allows the beetles to avoid the scorching mid-day temperatures of summer.

These thermoregulation strategies may allow insects to experience a temperature that varies from ambient. Two sources of error arise in development time predictions. First, development models assume that the temperature data used in the calculations are the same as the temperature the insect actually experiences. Thermoregulation renders this assumption false creating inaccuracies in predictions of development time. Second, field temperatures are often collected from a single site, such as a weather station, causing only an approximation of the ambient temperature an insect is actually exposed to.

4.1.3 Constant Versus Fluctuating Temperature

Development times of many species are known to differ between constant and fluctuating temperature regimes (78). Several studies on insect development under variable temperatures have been completed (7, 78-80). These studies report conflicting results on whether development is accelerated, unchanged, or retarded under fluctuating temperatures. Thus, the relationship between insect development and variable temperature conditions is not well understood.

Hagstrum and Milliken (81) suggest that the frequency and amplitude of temperature cycles has a greater significance on development rates than the mean of the temperature regime. Furthermore, fluctuating temperatures and the corresponding mean temperature may not have the same influence on the development rate. These statements create obvious problems with using insect development models; rates of development are usually based on constant temperature regimes from the laboratory and applied to field populations (80).

Additionally, Worner (82) warns of the problems associated with utilizing linear and non-linear functions derived from field data. In linear models, the lower threshold may shift depending on the characteristics of the fluctuating temperature regime, creating erroneous predictions. Eubank *et al* (78) propose that insects may not even possess a lower threshold in fluctuating cycles, but rather, development rates become asymptomatically smaller. Non-linear models may provide an excellent fit to development data at constant temperatures, but their mathematical characteristics may have a different reaction in fluctuating conditions, especially at temperature extremes. This varying behaviour is known as the Kaufmann or rate summation effect (82). For non-linear models, development rates are accelerated at low temperatures and retarded at high ones. Hagstrum and Milliken (81) and Manel and DeBouzie (80) developed models to overcome the Kaufmann effect by incorporating variable temperatures in non-linear models. A complete review of development models designed for variable temperatures can be found in Worner (82).

Although degree-day methods have been vastly improved over the years with the advent of computer programs, thermal summation remains underestimated at low temperatures, and overestimated at high temperatures (61). However, if the temperature cycles are rarely in the extremes, degree-day estimates can be very accurate and effective (83). Currently, it is still the most widely used model by forensic entomologists (73).

4.1.4 Application to Forensic Entomology

Forensic entomologists employ degree-days to establish the amount of time a corpse has been exposed to necrophagous insects. This time is considered to be the minimum amount of time a body has been deceased, as necrophagous insects typically do not feed on living human tissue (73). Necrophagous flies are attracted to human remains within hours of death provided there are appropriate climatic conditions for activity (49). Adult calliphorids and sarcophagids immediately oviposit or larviposit on the corpse in a natural orifice or wound, if present. The offspring develop on the corpse at a rate determined largely by ambient temperature. Establishing the age of the oldest insects on a corpse will lead to an estimation of the time of death. However, this methodology can only be used until the first wave of adult flies begins to emerge, after which it is not possible to differentiate which generation is present (1).

To determine the age of an insect, the development rate of the species found must be known. Several researchers have generated growth rate data on blow flies and flesh flies at constant (8-11, 84-86) and fluctuating temperatures (7, 85, 86). Most of these publications report the accumulated degree-days at each life stage for all species investigated. This information, coupled with the weather records from the weather station closest to the death scene can yield the postmortem interval. For example, if the mean temperature at the crime scene for the weeks prior to discovery was 25°C, the development rate of the species found at 25°C must be known, in addition to the developmental minimum for that species. If the minimum threshold were 10°C, then 15 degree-days would be accumulated each day. The calendar date of oviposition is determined by simple reverse summation of accumulated degree-days (1). If the species required a total of 150 degree-days to reach the developmental stage in which the insect was recovered from the body, then oviposition would have occurred 10 days prior to the date of recovery. This would be the minimum elapsed time since death as an unknown amount of time could have occurred between death and oviposition. Although this example is extremely simplified, the basis for using degree-days in forensic entomology is generally the same in all cases.

CHAPTER 5: INSECT SUCCESSION ON CARRION

Decomposing animal remains provide a temporary microhabitat for a multitude of organisms, including bacteria, fungi, plants, and animals. Arthropods, especially insects, are the primary colonizers of carrion (4). Insects use carrion as a food source, a reproductive medium for offspring, and/or as a source for hosts or prey (14). Others are attracted to molds and fungi that thrive in putrefactive liquids, while some insects are indigenous to the surrounding habitat (4). As remains are modified throughout the biochemical processes of decomposition, so too are the insects that exploit this microhabitat. This sequence of insect colonization, termed heterotrophic succession, occurs in a predictable pattern for a given set of circumstances (87, 88).

Arthropod succession has typically been associated with stages of decay for forensic applications (13, 15, 16, 24, 38, 39). In baseline studies, specific taxa are noted for their arrival time on a carcass and are associated with specific stages of decomposition (14, 88). The baseline data can be compared to collected species from bodies of unknown time of death to yield the postmortem interval, provided circumstances are similar (58).

Carrion-breeding flies and their offspring are the primary colonizers in the first few stages of decomposition and account for the majority of carcass degradation (15, 19, 41, 89). Specifically, blow flies (Calliphoridae) are usually the first to arrive at a corpse, often within one hour of death and ovipositing shortly thereafter (13, 15, 19, 57). As mentioned in the previous chapter, the growth rate of insects is dependent on temperature and can be predicted with great accuracy. However, estimating the elapsed time since death using development data is only accurate within the lifecycle of the first progeny, after which the time of oviposition moves further away from the time of death (58). Thus, after one

generation of larval development, successional patterns are the best source for determining postmortem interval estimations.

Although insects of several orders may inhabit a corpse, the taxa that colonize carrion in a predictable sequence are the most forensically significant. Taxa that reoccur on a carcass, with successive visitations and absences, are less reliable as indicator species for postmortem interval estimations (90). For terrestrial locations, members of the orders Diptera and Coleoptera are typically the best indicators for postmortem interval estimations based on succession (14). Furthermore, carrion-frequenting flies and beetles are the most active, abundant, and predictable insects as they are necrophagous, predaceous on necrophagous insects, or both (4).

5.1 Attraction and Oviposition of Blow flies

The attraction of necrophagous insects to decomposing remains is mediated by a complex set of cues and physiological responses that are species-specific. Blow flies are typically the first insects to arrive at outdoor carrion in temperate regions (4, 13, 15, 57). Calliphorids require protein for proper ovarian and oocyte development (91) and an appropriate substrate for oviposition and development of offspring (3).

Although visual cues play a role in locating resources, olfactory cues are the chief stimuli initially leading blow flies to carrion (92). Hall *et al* (92) compared ratios of attracted flies to bait with and without odour. They noted a 72:1 ratio for the blow fly *Lucilia sericata* (Meigen) between odorous and non-odorous baits. This phenomenon was not observed in the sarcophagid *Wohlfahrtia magnifica* (Schiner), and may account for blow flies typically colonizing carrion before other families of necrophagous flies. Calliphorids have been reported to arrive at remains within minutes of death with oviposition occurring within hours (13, 15, 19, 57), and sooner in the presence of blood or other bodily excretions (93, 94). The sulphur-rich compounds produced by bacterial decomposition stimulate host location by triggering upwind orientation and landing (95).

Furthermore, the olfactory receptors on the ovipositor respond to the ammonia-rich products of decay and stimulate oviposition in blow flies (95-97)

Oviposition is also related to moisture (98), pheromones (99, 100), and physiological changes in the adult female (101). Chemical stimuli from pheromones deposited by previously ovipositing females constitute a major factor in site selection for calliphorids and muscids (94, 95). These chemical cues elicit group oviposition causing the clustering of hundreds of eggs, sometimes of several different species (3). However, the exact origin of the pheromone is not entirely known. Greenberg (3) suggests that group oviposition behaviour is a strategy to lower the risk of egg predation in the core of the cluster. Perhaps clustering is also a thermal strategy to hasten the time spent in this vulnerable stage and to avoid low, lethal temperatures.

Barton Browne (98) revealed that blow flies are not only sensitive to water, but that tarsal contact with water increased the oviposition rate of *Lucilia cuprina* (Wiedemann). Perhaps this explains the earlier oviposition in the presence of blood, as whole blood contains approximately 80 percent water. Collatz and Hoeger (101) discovered lower lipid values in female populations of *Phormia terraenovae* (Meigen) (= *Protophormia*) during periods of high oviposition rates. Thus, physiological changes within the adult blow fly may also stimulate behavioural responses.

5.2 Factors Affecting Insect Succession on Carrion

In temperate climates, heterotrophic succession is typically initiated by blow flies and variously advanced by other prominent dipteran invaders, such as the Sarcophagidae and Muscidae (3, 4, 13, 14). Coleopterans ensue in the sequence and are characteristically represented by the families Staphylinidae, Silphidae, Histeridae, Nitidulidae, Dermestidae, Scarabaeidae, and Cleridae (4, 5, 14). Tables 5.1 and 5.2 demonstrate the generalized pattern of faunistic sequence in Tennessee for adults and larvae, respectively (16).

Table 5.1: The main families of adult insects observed on decaying human remains in spring/summer in Tennessee (modified from Rodriguez and Bass (16))

		Stages of Decomposition (days postmortem)			
Insect Family	**Common Name**	**Fresh** (0-4)	**Bloated** (5-7)	**Decay** (8-13)	**Dry** (14-26)
Calliphoridae	blowflies				
Muscidae	muscid flies				
Silphidae	carrion beetles				
Sarcophagidae	flesh flies				
Histeridae	clown beetles				
Staphylinidae	rove beetles				
Nitidulidae	sap beetles				
Cleridae	checkered beetles				
Dermestidae	dermestid beetles				
Scarabaeidae	scarab beetles				

Table 5.2: The main families of immature insects observed on decomposing human remains in spring/summer in Tennessee (modified from Rodriguez and Bass (16))

		Stages of Decomposition (days postmortem)			
Insect Family	**Common Name**	**Fresh** (0-4)	**Bloated** (5-7)	**Decay** (8-13)	**Dry** (14-26)
Calliphoridae	blowflies				
Muscidae	muscid flies				
Silphidae	carrion beetles				
Sarcophagidae	flesh flies				
Staphylinidae	rove beetles				
Dermestidae	dermestid beetles				
Scarabaeidae	scarab beetles				

For comparison, Tables 5.3 and 5.4 exhibit the generalized pattern of carrion insect succession in British Columbia (13). The abundance of each family was undeterminable from Anderson and VanLaerhoven (13), hence the difference in the style of the Tables. In terms of decompositional stage, the time of arrival of many families are similar in both regions. However, the rate of decomposition varies significantly. Furthermore, the particular species involved in the pattern of arthropod succession on carrion is distinct for each region.

Table 5.3: The main families of adult insects observed on pig carrion in summer in British Columbia (13)*

Order	Family	Stage of Decomposition				
		Fresh	Bloated	Active	Advanced	Dry/Remains
Diptera	Calliphoridae	■	■	■	■	■
	Muscidae	■	■	■	■	■
	Sarcophagidae	■	■			■
	Fanniidae		■			■
	Heleomyzidae		■			
	Piophilidae		■	■	■	■
Coleoptera	Staphylinidae		■		■	■
	Silphidae		■	■	■	■
	Cleridae		■	■	■	■
	Dermestidae				■	■
	Nitidulidae				■	■
	Histeridae					■
Days Postmortem		Days 0-1	Days 2-10	Days 11-16	Days 17-42	Days 43-271

* Shaded areas denote the presence of an insect family.

Table 5.4: The main families of immature insects observed on pig carrion in summer in British Columbia (13)*

Order	Family	Stage of Decomposition				
		Fresh	Bloated	Active	Advanced	Dry/Remains
Diptera	Calliphoridae	■	■	■	■	
	Muscidae					■
	Sarcophagidae					
	Fanniidae					
	Heleomyzidae					
	Piophilidae				■	■
Coleoptera	Staphylinidae					■
	Silphidae					
	Cleridae					■
	Dermestidae				■	■
	Nitidulidae					■
	Histeridae					
Days Postmortem		Days 0-1	Days 2-10	Days 11-16	Days 17-42	Days 43-271

* Shaded areas denote the presence of an insect family.

The specific species involved in the succession are affected by the postmortem circumstances, such as habitat, season and climate, and manner of death (58). It is essential to understand the impact of variables on carrion insect succession patterns for accurate postmortem interval estimations based on these

patterns (14). Discussed below are many of the factors that affect insect succession on carrion.

5.2.1 Geographical Location

The geographical location has a major impact on insect succession patterns. The biogeoclimatic zone determines the ecological characteristics of an area, including temperature and climate, altitude, seasonal change, terrain, and the flora and fauna that reside in the region. Although many carrion insects are ubiquitous, several species are adapted for particular climates. For example, there are over 1000 species of blow flies worldwide, but more than eighty percent are restricted to the Old World (102).

Early and Goff (12) examined insect succession on cat carcasses at two ecologically different regions in O'ahu. Separated by only four kilometres, the carrion at each site demonstrated localized variation in the sequence of insect colonization. These findings are summarized in Table 5.5 for the fresh and bloated stages of decay at both sites. Unfortunately, the studies were not performed simultaneously and do not account for seasonal effects on succession.

Table 5.5: Summary of species collected in the early stages of decay on domestic cat carrion in two ecologically different regions of O'ahu, Hawaii (12)

Diamond Head		**Manoa**	
Semi-arid tropical		Tropical	
2.06 centimeters precipitation (during study)		31.4 centimeters precipitation (during study)	
Vegetation: Xerophytic (primarily grasses)		Vegetation: Mesophytic and Xerophytic	
Fresh Stage (Days 1-2)		**Fresh Stage (Days 1-2)**	
Order	Genus and Species	Order	Genus and Species
Sarcophagidae	*Bercaea haemorrhoidalis*	Sarcophagidae	*Helicobia morionella*
	Parasarcophagula ruficornis		*Sarcophagula occidua*
	Sarcophagula occidua		
		Calliphoridae	*Phaenicia cuprina*
Bloated Stage (Days 2-6)		**Bloated Stage (Days 2-6)**	
Order	Genus and Species	Order	Genus and Species
Sarcophagidae	*Bercaea haemorrhoidalis*		
	Parasarcophagula ruficornis		
	Sarcophagula occidua		
Calliphoridae	*Chrysomya megacephala*	Calliphoridae	*Chrysomya megacephala*
	Chrysomya rufifacies		*Chrysomya rufifacies*
Muscidae	*Ophyra chalcogaster*	Muscidae	*Ophyra sp.*
	Musca domestica		

In British Columbia, *Lucilia illustris* (Meigen), *Phormia regina* (Meigen), and *Calliphora vomitoria* (Linnaeus) were the first to arrive at pig carcasses in summer (13). The same species were observed in the early stages of decay in nearby Washington State (19). However, the species and arrival times of carrion (Silphidae) and rove (Staphylinidae) beetles varied significantly between the two regions. Additionally, only one species of Histeridae was reported in British Columbia, and it did not appear until the dry/remains stage (43+ days postmortem) (13). Alternatively, four species of clown beetles colonized remains in Washington, beginning as early as the bloated stage (10 days postmortem). These differences demonstrate the need to conduct baseline studies in several regions for accurate and valid estimations on elapsed time since death.

5.2.2 Season

Different insects have different activity cycles throughout the year. In temperate regions, the winter season is characterized by a decrease in insect activity. Most blow flies have a number of generations per year and are active from early spring to late fall in temperate regions (4). Thus, a carcass exposed in summer will have a richer fauna and invariably a different pattern of insect succession than carrion exposed in winter. Introna *et al* (22) collected insects from decomposing liver in Maryland in both spring and summer. The blow flies *Calliphora livida* (Hall), *C. vicina* (Robineau Desvoidy), and *L. illustris* were collected from the spring survey, but not in summer. The summer collections included *Phaenicia sericata* (= *Lucilia sericata*) and the flesh fly, *Sarcophaga sarracenioides* (Aldrich), which were absent in spring.

Lowered insect activity in cold seasons also decreases the rate of decay. However, insects can continue to develop in lethal temperatures provided shelter can be found under the skin of the body or under clothing (59). Maggots can also generate their own metabolic heat in larval aggregations, thereby protecting themselves from lethal dips in ambient temperature (42). Insects can also over-winter, provided they are in the appropriate species-specific stage, and the inductors for diapause are triggered by environmental cues (103). Some species of

blow flies are resistant to cold weather and have longer periods of activity. In Iowa, *P. regina* was collected throughout winter, but peaked in abundance in spring and fall (104). Another cold-resistant fly, *Cynomya cadaverina* (Robineau-Desvoidy), was not collected in Iowa in winter, but was the most abundant blow fly in early spring. The presence of *P. regina* in winter is likely due to the ability of this insect to over-winter in the adult stage (105).

In summer, especially in hot dry climates, a corpse can rapidly dehydrate and potentially eliminate the arrival of some insect taxa (58). Dried skin is not attractive to blow flies, which prefer humid environments and require moisture for oviposition (98). Dermestid beetles, which prefer dried carrion, typically dominate a dehydrated corpse and accelerate skeletonization (106).

Climatic factors such as humidity and precipitation also have a significant effect on insect activity. Insects are sensitive to humidity and are least abundant in extreme temperatures of hot and dry climates (4). Precipitation can also decrease fly activity and egg deposition (41). However, larvae will continue to develop in rain as they seek shelter under skin, clothing, and vegetation during periods of heavy rainfall (19).

5.2.3 Diel Activity Cycles

Blow flies and other carrion-frequenting insects have daily rhythms of activity and are considered to be the most active when temperatures are likely to be optimal for development (107). Inter-species variation in periodicity is common and can change in different habitats (108) and seasons (109). Parker *et al* (109) noted that mid-day temperatures above 31°C significantly reduced blow fly activity. Thus, warmer seasons may lead to a bimodal activity pattern as insects take advantage of cooler temperatures in mid-morning and early evening. Alternatively, cooler seasons may result in a unimodal activity pattern, where insects become most active during the mid-day when temperatures are highest.

Although blow flies are considered a diurnal family, the literature presents conflicting information on whether or not blow flies oviposit at night. Green (110) observed that flies in the genus *Calliphora* were active and oviposited at

night, but that flies in the genus *Lucilia* did not. Greenberg (111) and Singh and Bharti (112) both reported nocturnal oviposition in blow flies. Greenberg (111) noted that the numbers of eggs were reduced in nocturnal egg batches, but that oviposition still occurred in 33 percent of trials. Singh and Bharti (112) determined that Greenberg's experiment did not prove nocturnal flight or attraction to remains at great distances due to the placement of the bait on the ground; blow flies could simply crawl on the ground to the bait. After altering the bait placement, Singh and Bharti (112) substantiated Greenberg's (111) results, observing oviposition in 33 percent of trials. Tessmer *et al* (107) and Spencer (113) refute the occurrence of nocturnal oviposition even in the presence of artificial light. There may be differences between species, but clearly the issue needs to be resolved as nocturnal oviposition can underestimate postmortem interval estimations by up to twelve hours.

5.2.4 Habitat

Characteristics of the habitat, such as level of sun exposure, indoor or outdoor setting, and rural or urban locality affect decay rates and the sequential pattern of arthropods on carrion. Very few indoor locations are impervious to insects. Goff (114) examined 35 cases (14 indoor and 21 outdoor) to determine potential differences in arthropod succession. A total of 22 different species were collected from all 35 cases, however, only five species were common to both habitats. In the initial stages of decomposition in outdoor locales, the blow flies *Chrysomya megacephala* (Fabricius) and *C. rufifacies* (Macquart) dominated the insect composition. In contrast, early stages in indoor locales were characterized by a richer fauna of Dipteran larvae and were often dominated by sarcophagids. In later stages of decay (3-4 weeks postmortem), outdoor remains attracted a greater diversity of insects, primarily coleopteran species. Indoor remains attracted only one coleopteran in later stages, the leather beetle *Dermestes maculates* (DeGeer).

Examining over 40 homicide cases involving insects in British Columbia, Anderson (1) observed that certain blow fly species were more commonly found

indoors. Of the indoor cases, *P. regina* and *P. sericata* were present in over 30 and 40 percent of cases, respectively. Alternatively, *C. vomitoria*, *Eucalliphora latifrons* (Hough) and *P. terraenovae* were not recovered from any of the indoor locations, but were readily collected from outdoor remains. These studies highlight the need for more research examining the impact of indoor habitats on insect succession, not just from case studies, but also in experiments allowing for replication.

Anderson (1) also reported that *P. terraenovae* was exclusive to rural habitats and *P. sericata* to urban settings in British Columbia. Other authors have also reported a rural or urban preference in certain blow flies and the use of this information in determining secondary movement of a corpse (4, 14). However, rural and urban classifications may be specious as blow flies may migrate into different habitats in response to temperature gradients, synanthropic behaviour, inter-specific competition, and other biotic factors (115). Furthermore, some rural areas may be close in distance to urban regions, thereby creating considerable species overlap (116).

A higher level of sun exposure stimulates higher temperatures and evaporation rates, which leads to corpse dehydration, resource reduction and intensified insect competition (115). Increased temperature results in accelerated decomposition, potentially eliminating decay stages and waves of insects associated with those stages (58). Dillon and Anderson (59) noted that rapid dehydration can occur in open sunlit habitats in British Columbia. This rendered the carcass unattractive to blow flies, causing a mass exodus of underdeveloped larvae in search of another food source.

Shean *et al* (19) examined the differential decomposition and insect succession on a pig carcass in shade versus one in full sunlight. Temperatures at the sun-exposed site were consistently higher in the daytime, but were often lower during evening hours. Oviposition began at the same time at both sites, but was extended on shaded carrion. As decay was decelerated at the shaded site, there was a prolonged availability of carrion suitable for ovipositioning. Generally, the

temporal sequence of insects occurred at a faster rate on the sun-exposed carrion due to the higher temperature and accelerated decomposition.

Insects may be positively or negatively heliotropic and may not colonize remains in habitats that are not preferential. Smith (4) states that members of European strains of the genus *Lucilia* (greenbottles) are positively heliotropic, preferring direct sunlight, whereas species of *Calliphora* (bluebottles) are negatively heliotropic, preferring shady conditions. Shean *et al* (19) noted the same habitat preferences in blow flies in Washington State, but noted that the species involved are not necessarily exclusive to their preferential habitat.

Habitat preferences are also common among coleopterans. For example, the burying beetle *Nicrophorus investigator* (Zetterstedt) (Coleoptera: Silphidae) prefers to bury small carrion in open sunlit habitats versus shaded areas (117). Smith and Heese (117) purport that adults gain a reproductive advantage by having their progeny develop in higher temperatures. Shean *et al* (19) noted that 11 species of insects (of a total of 49) were found exclusively at the shaded site and 16 exclusively at the sunlit site. Many of the insects with a clear habitat preference were forensically significant beetles, including species of Dermestidae, Histeridae, Scarabaeidae, Cleridae, and Nitidulidae. The rove beetles (Staphylinidae) demonstrated no clear preference as all species appeared at both sites, albeit with sooner arrival at the shaded site (19). Thus, habitat plays a strong role in determining the type and sequence of insects that frequent carrion.

Aquatic habitats significantly alter the succession patterns of insects and the process of decomposition. The habitat is so different from terrestrial habitats that an entire chapter could be devoted to forensically significant aquatic insects, the types of marine habitats, and the different stages of decay associated with these habitats. Submersion deaths are beyond the scope of this paper, however, an extensive review can be found in Merritt and Wallace (118).

5.2.5 Burial

Buried remains, although hidden from the human eye, are typically not hidden from the olfactory senses of insects (4). Illicit burials are usually shallow

compared to ritual burial, as perpetrators rush to dispose of remains to avoid detection (58). However, burial impacts the species, sequence, and arrival times of insects. Like decomposition rate, insect succession on buried remains is also affected by depth of interment, soil type, climate, seasonal change, and perturbation by vertebrate scavengers.

To date, VanLaerhoven and Anderson (57) have completed the best research on insect succession on buried carrion. Although previous research on pigs in coffins (55) and on humans at varying depths (6) revealed excellent insights into decomposition and arthropod succession patterns, the design of each study had inherent flaws that either lacked application to forensic scenarios or did not allow for replication or control of variables. VanLaerhoven and Anderson (57) buried 27 pigs in two biogeoclimatic zones and compared the decomposition, various temperature readings, and insect succession patterns with above-ground pigs and with previous research (13). Three pigs in each zone were exhumed at intervals of two weeks, six weeks, and three months after death. Another three pigs were exhumed after sixteen months postmortem.

Similar to other studies (6, 55), decomposition was decelerated in the burial environment in British Columbia, which in turn impacted the timing of insect colonization (57). Insect species varied considerably between the zones and several species were exclusive to one zone or the other. The lesser housefly, *Fannia canicularis* (Linnaeus) (Diptera: Fanniidae) arrived earlier on carcasses in both zones than in previous above-ground studies (13). The presence of *F. canicularis* was attributed to soil moisture and may not be typical in drier climates (57). Additionally, various species of muscid flies and their offspring commonly colonized the buried carcasses in early decay (1, 57), unlike above-ground carrion (13). Muscid flies were also better suited as an indicator species for postmortem interval estimations in burials, compared to above-ground carrion in British Columbia (57). Burials at different times of the year will also impact succession, especially in Canadian climates where the ground is subject to freezing. Thus, baseline studies on buried carrion in different regions and seasons,

in addition to above-ground studies, are essential to forensic applications on buried corpses.

Heavy soils and deep burials may be resistant to insects. Turner and Wiltshire (54) noted that insects were unable to colonize a pig carcass buried 40 centimetres in heavy clay in Britain. Decay of the carrion proceeded slowly and it was not until three months interment that vertebrate scavengers were able to detect the putrefactive odours. Eventually, the scavengers burrowed to the carcass and exposed the flesh, allowing insects to colonize the remains. The burial environment is cooler at greater depths, thereby slowing decomposition and the timing of insect colonization (6). Currently, there is on-going research on the effect of depth on *Calliphora* colonization (reported in Anderson (58)).

5.2.6 Other Factors Affecting Insect succession on Carrion

The manner and cause of death can alter faunistic succession patterns and the rate of decomposition. Bodily trauma causing blood seepage will hasten the arrival of fauna, whether vertebrate or invertebrate (41). Blood can also provide more oviposition sites and may stimulate egg-laying by providing moisture to gravid females (98). On cadavers without traumatic injury, oviposition tends to be centralized in natural orifices, such as the ears, nose, eyes, mouth, anus, and genitals (5).

Death by drug overdose can impact the development rate of flies feeding on the body. Goff *et al* (119, 120) discovered that lethal doses of cocaine or heroin in rabbits stimulated accelerated development in feeding larvae of the flesh fly *Boettcherisca peregrina* (R.-D.) (Sarcophagidae). The larvae feeding on tissues containing heroin were significantly larger and remained in the pupal stage longer than larvae fed on control tissue without heroin. In addition to the overestimation of the postmortem interval by development methods, decomposition and insect arrival times would be accelerated. Other circumstances such as hanging, burning (whether peri- or postmortem), or corpse wrapping for concealment may delay insect invasion or render a corpse unsuitable for insect activity (58). However, extensive research in this area is still yet to be completed.

Vertebrate scavengers can quickly deplete a carcass of flesh, thereby accelerating decay. Dillon (121) and Dillon and Anderson (59) reported that scavenging was more common on shaded carrion in British Columbia. The vertebrate activity equalized the effect of lower temperatures in shaded regions on the rate of decomposition. However, if scavenging is extensive, some stages of decay may be eliminated, thus reducing the number and types of insects colonizing remains.

Opportunistic invertebrate scavengers, such as ants (Hymenoptera: Formicidae) may deplete the number of immature blow flies developing on carrion. Early and Goff (12) observed the aggressive ant *Solenopsis geminata* (F.) preying on dipteran larvae throughout decomposition. The removal of eggs and larvae by the ants slowed the rate of biomass loss. However, most insect scavengers are reported as normal in the succession pattern and few are so aggressive as to significantly alter the sequence of arrival (58, 59, 121).

Clothing and other textiles can alter faunal succession by altering the characteristics of the microhabitat in which the corpse decomposes. Clothing can soak up decompositional fluids, thereby extending moisture retention (59). This may delay species that prefer dried remains, such as dermestid beetles. Dillon and Anderson (59) reported accelerated decomposition on clothed carrion, as wet clothing can provide additional oviposition sites, similar to blood seepage from bodily trauma. Clothing may also shelter insects from lethal temperatures both hot and cold, thereby allowing insect activity in otherwise inhospitable conditions (41).

While this chapter discusses many factors affecting insect succession, it certainly is not an exhaustive list of all variables potentially impacting faunistic patterns. However, the factors presented here are the most influential from a forensic perspective and are the most relevant to the research discussed in subsequent chapters.

CHAPTER 6: MATERIALS AND METHODS

6.1 Geographical Location

Saskatchewan is divided into four broad ecological zones, which are subdivided into eleven ecoregions (23) (Table 6.1). The research land, located in Saskatoon, Saskatchewan, lies in the Moist Mixed Grassland Ecoregion in the Prairie Ecozone. The Mixed Moist Grassland Ecoregion encompasses 6,789,000 hectares of land, and 11 percent of Saskatchewan's total land area (122). This semi-arid area receives an average of 380 millimetres of precipitation and 1100 millimetres of snowfall annually. The mean temperature is 18.8 °C in July and – 18.9°C in January. Soils tend to be dark brown and well-drained, characteristic of the level to rolling lacustrine and morainic plains. There are also numerous sloughs associated with un-drained depressions and surrounded by aspen groves (23).

Saskatoon also lies extremely close to the southern border of the Aspen Parkland Ecoregion (Figure 6.1). This region has similar characteristics, although slightly colder and less arid (122). There is also a larger concentration of aspen groves and sloughs supporting a greater diversity of wildlife. In contrast, the Mixed Grassland Ecoregion to the south of Saskatoon is drier and warmer with less vegetation.

Table 6.1: Subdivision of Saskatchewan ecozones into ecoregions

Ecozone	Prairie	Boreal Plain	Boreal Shield	Taiga Shield
Ecoregion	Cypress upland	Boreal Transition	Churchill River Upland	Tazin Lake Upland
	Mixed Grassland	Mid Boreal Lowland	Athabasca Plain	Selwyn Lake Upland
	Moist Mixed Grassland	Mid Boreal Upland		
	Aspen Parkland			

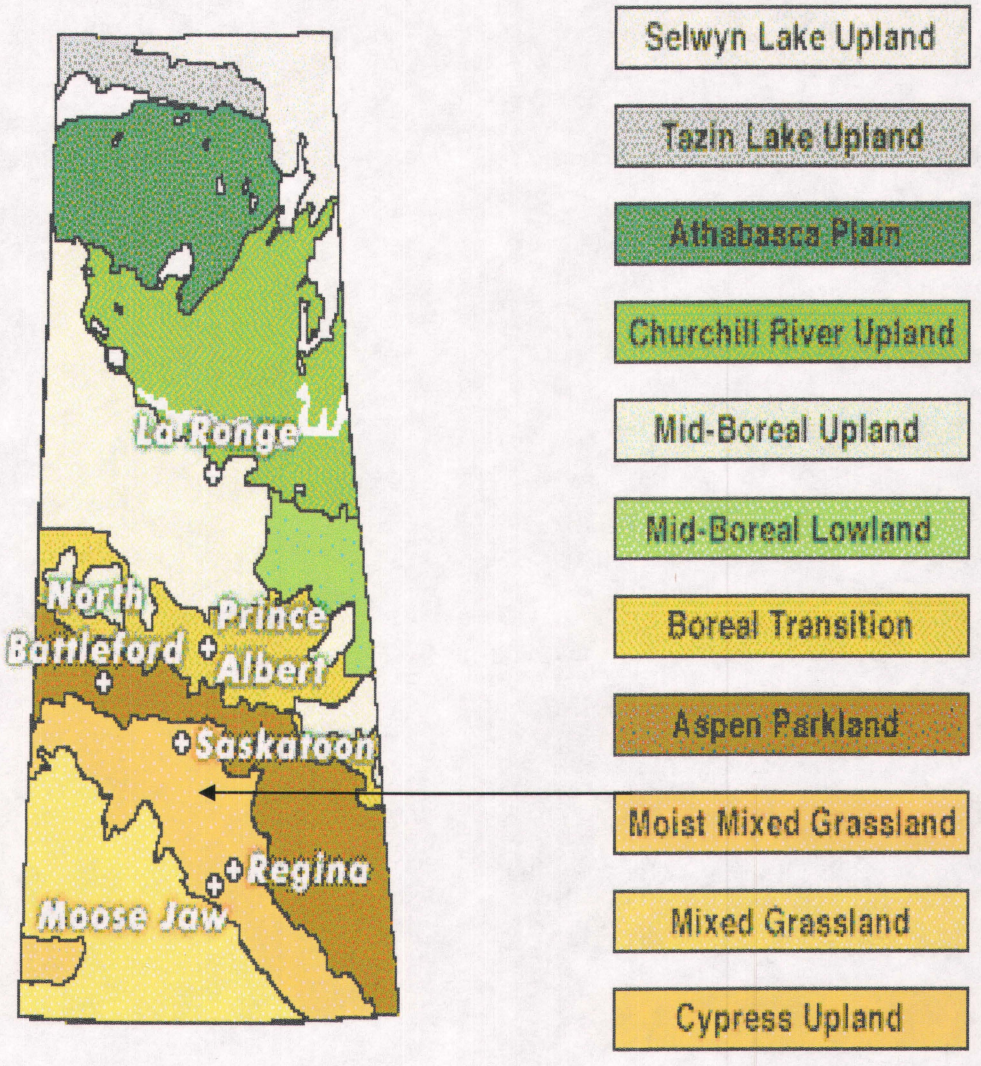

Figure 6.1: The Ecoregions of Saskatchewan. Reproduced and modified from Saskatchewan Interactive (122).

The Prairie Ecozone occupies all of southern Saskatchewan with the Grassland regions dominating the landscape. Broad-leaved herbs and mixed grass (a mosaic of spear and wheat grasses) are characteristic of uncultivated land in the area (123). A variety of deciduous shrubs dot the landscape, including buckbrush wolf willow, chokecherry and saskatoon berry (23). However, approximately

80% of all native lands have been converted for the cultivation of cereal grains (123). The majority of terrestrial wildlife in the region includes prairie dog, sage grouse, sharp-tailed grouse, pronghorn antelope, and mule deer, along with various other birds, mammals, and reptiles (23). The Prairie Ecozone has also been titled the Grassland Ecozone (123).

This region was chosen for several reasons. First, the Prairie Ecozone possesses the highest population density, having the two largest Saskatchewan cities (Saskatoon and Regina) within its boundaries. Thus, the Prairie Ecozone has the most forensic significance in Saskatchewan, as homicides tend to be concentrate in more densely populated areas. Furthermore, the Boreal Plain Ecozone is being researched in Edmonton (124).

The University of Saskatchewan research land, located approximately 2 kilometres northeast of the campus proper, was chosen in part for convenience and the mixed habitats of open sunlit fields and long stretches of trees and shrubs that characterize the ecoregion. Approximately half of the research land had been cultivated for agriculture research, although sites were chosen on fringe areas, within the bushes away from the crops, or on native, uncultivated lands.

6.2. Animal Model

Twenty-four domestic swine, *Sus scrofa* (Linnaeus), were purchased from the Prairie Swine Centre in Saskatoon. Several researchers have recommended the use of pigs as human models (4, 13, 49). Not only are human cadavers difficult to obtain, but also there are obvious moral and ethical issues surrounding their use. Pigs have similar skin and gastro-intestinal fauna as humans, and therefore, have a very similar pattern of decomposition (13).

Smith (4) recommended the use of 23-kilogram pigs as it approximates the size of a human torso. However, Komar and Beattie (40) concluded that swine weighing less than 26 kilograms were too small to mimic the decomposition of a human adult. Pigs weighing between 42 and 79 kilograms were used in this study to simulate the average size of an adult female (Table 6.2). This range falls into the mid weight category established in Komar and Beattie's (40) research on

carcass size, shown to be a more appropriate size model for human decomposition. However, the inconsistency in average carcass weights in different seasons is a potential source of error in the decay rates of carcasses.

Table 6.2: Weight and girth of pig carcasses. Abbreviations: EXP, sun-exposed; SH, shaded; NU, nude; CL, clothed.

Season	Pig	Weight (kg)	Girth (cm)
Spring	1EXP-1	78.8	99.2
	1EXP-2	73.8	115.0
	1EXP-3	76.2	106.0
	1SH-1	73.0	101.9
	1SH-2	70.8	108.0
	1SH-3	74.6	106.3
	Average	**74.5**	**106.1**
Summer	2EXP-1	51.5	103.5
	2EXP-2	60.0	103.0
	2EXP-3	57.7	101.5
	2SH-1	72.7	108.0
	2SH-2	58.2	113.5
	2SH-3	67.3	109.0
	Average	**61.2**	**106.4**
Clothed vs. Nude	NU-1	66.6	109.5
	NU-2	70.0	114.5
	NU-3	71.0	115.5
	CL-1	64.1	103.0
	CL-2	65.7	109.5
	CL-3	71.0	113.0
	Average	**68.1**	**110.8**
Fall	3EXP-1	41.9	80.0
	3EXP-2	43.9	80.5
	3EXP-3	45.8	82.0
	3SH-1	44.4	84.0
	3SH-2	45.0	79.5
	3SH-3	45.6	81.5
	Average	**44.4**	**81.3**
	Total Average	**62.1**	**101.1**

6.3 Season

The experiment for carcasses placed in different microclimates (sun vs. shade) was performed in three separate seasons: spring, summer, and fall (Table 6.3). To reduce research costs, the study investigating the effects of clothing was

performed in the summer season only. All experiments were completed in the year 2000.

Table 6.3: Schedule of fieldwork for all experiments. (2000)

		Data collection	
Season	**Experiment**	**Start Date**	**End Date**
Spring	Experiment-1: sun vs. shade	17-May	18-Jul
Summer	Experiment-1: sun vs. shade	19-Jul	31-Aug
Summer	Experiment-2: clothed vs. nude	19-Jul	31-Aug
Fall	Experiment-1: sun vs. shade	01-Sep	26-Oct

The spring delivery occurred on May 17th, 2000. May was an ideal month to begin, as the temperature was warm enough for overwintering flies to complete development and emerge as adults. Minimum temperatures in May can decline to 3°C, which may impede blow fly activity. However, low temperatures are usually experienced at night when blow fly activity is diminished. Certain species of blow flies, notably species in the tribe Calliphorini and Phormini, will oviposit at temperatures as low as 5°C (86). All experiments were completed by October 26th. The fieldwork in its totality occurred over 25 weeks, spanning the three seasons in which insects are most active in Saskatchewan.

6.4 Habitat

Two different microhabitats were investigated in Experiment-1: sun-exposed and shaded. Shaded sites were chosen for maximum shade throughout most of the day. Depending on the position of the sun, the carcasses were exposed to streams of sunlight through gaps in the overhanging vegetation. Sun-exposure through vegetation usually covered less than twenty percent of the carcass and

lasted four hours or less. The pigs exposed in full sunlight were placed in open areas devoid of overhanging vegetation to prevent late afternoon shadows.

6.5 Experiment Design

All experiments are based on the protocols outlined by Anderson (125). The experiments have been modified from the protocols for cost-effectiveness, without sacrificing validity. For example, Anderson calls for the use of five pigs per habitat and season, 2 of which act as control carcasses without insect collection. However, subsequent studies demonstrated that representative insect collection from carrion does not impede the natural succession of the insects (13). Thus, the two control pigs were omitted from this study and efforts were made to keep carcass disturbance to a minimum during sampling.

6.5.1 Experiment-1: Sun-exposed Versus Shaded Carrion in Three Seasons

On May 16th, 6 pigs were weighed and ear-tagged at the Prairie Swine Centre, with the weight recorded on the tag. On May 17th, the weighed pigs were killed with a high-powered bolt to the frontal portion of the skull. The bolt-technique is the standard method for the slaughter of swine at the Prairie Swine Centre and was approved by the University Committee on Animal Care and Supply. The time of death of all swine occurred between 9:00 and 9:40am. The pigs were delivered to the research site in a covered 4x4 trailer at 10:00am and were immediately prepared for placement.

Six sites above ground were chosen in a fenced area of the research land owned by the University of Saskatchewan. Three of the sites were in open, sunlit areas and the other three sites were largely shaded by vegetation. Each site was approximately 40-50 meters apart to minimize crossover of insects. Individual pigs were measured for girth and dressed with one piece of clothing, typically a t-shirt or button up collared shirt. Next, the pigs were wounded with a non-serrated knife in the thoracic region. Wire-mesh (2.5 centimetre grid), approximately 0.5 metres longer than the length of the pigs, was placed on the ground. The pigs were then placed atop the wire mesh. All pigs were placed on their side. One

pitfall trap was placed at each site to collect insects throughout the day. Traps were fashioned from jars filled half full with soapy water and buried so that the lip of the jar was level to the ground.

One pig in each of the two habitats had a data-logger (Hobo®, Hoskins Scientific, Vancouver, British Columbia) inserted approximately 5 centimetres into the thoracic wound to record internal carcass temperature every thirty minutes throughout the experiment. Cages, made from PVC piping and wire mesh, were placed atop the pigs and secured with wire. The cages and underlying wire mesh served to minimize potential scavenging by vertebrates. Another data logger was attached to the cage of the same two pigs that had the internal data loggers. This logger recorded ambient temperature every thirty minutes throughout the experiment. After carcass placement, a sketch of the area was completed noting site location, direction of the carcass, position of the data loggers and pitfall traps. All pigs were placed within 3 ½ hours of the time of death.

In the first week after placement, observations, photographs, temperature readings and collections were made daily at varying times of the day (between 7:00am and 7:00pm). For the remainder of the experiment, collections were made every two to three days. Observations were made of the decompositional state and of the insects collected and seen. Temperature readings included carcass skin temperature and core maggot mass temperature when large aggregations were witnessed. Major insect collections occurred from May 17th to July 18th. The summer and fall experiments followed the same protocols as the spring experiment. The summer experiment transpired from July 19th to August 31st, and the fall experiment lasted from September 1st to October 26th.

6.5.2 Experiment-2: Clothed Versus Nude Carrion in Summer

This experiment closely imitates the protocols followed in Experiment-1. The Prairie Swine Centre delivered an additional six freshly killed pigs on July 19th, the delivery date for all pigs investigated in the summer season. All pigs were measured for girth and wounded in the thoracic region. However, of the six, three were heavily clothed, two with a sweater and pants and one with a long

dress. The remaining three pigs were left nude. A data logger was placed in the wound of one clothed pig and one nude pig to measure internal carcass temperature. Ambient temperatures were taken from the data logger placed on the cage of the Experiment-1 sun-exposed pig of the same season. All six of the pigs were placed in open, sun-exposed areas 40-50 meters apart. All other materials and methods were identical to those described for Experiment-1, including pitfall traps, insect collection, photographs, and protective cages.

6.6 Collections, Rearing, and Identification of Arthropods

During collection days, representative samples of immature and adult insects were collected on and in the carcass, as well as the surrounding soil. While all insects observed were sampled, there was a definite focus on flies and beetles. Adult flies were collected with an aerial sweep net and then transferred to 70% alcohol. Adult beetles, immature insects and other hard-bodied crawling insects were collected by hand or with forceps and immersed in 70% alcohol.

Soil samples were taken on occasion, especially after the observation of pupae. Soil was sifted through a mesh strainer to collect any burrowing insects or immature flies that had pupated in the soil. While beetles were preserved, pupae were placed in jars with wet tissue paper and sugar and covered with paper towel secured with rubber bands. These jars were then transferred to the growth chamber at the phytotron facility within 3 hours of collection to be reared to adulthood.

For each carcass, approximately 20 immature fly specimens in various stages of development were collected from every region of the body where present. Approximately half of the immature fly specimens collected were preserved while the other half were kept alive for rearing. The live specimens were placed in jars atop a layering of beef liver and wet tissue. The jars were covered with paper towel, secured with rubber bands and transferred to the phytotron facility. All samples were labelled with the date and time of collection, the carcass number, the area of the carcass the samples came from, and the stage of development at the time of collection.

All insects reared were taken to the Department of Agriculture phytotron facility at the University of Saskatchewan. The growth chamber conditions were kept constant at 22°C ± 0.2°C with a 16:8 light:dark cycle and 65% relative humidity. Insects in the chamber were checked every two days. Upon adult emergence, beyond the teneral stage, the adults were immersed in 70% alcohol and relabelled.

All insects were identified to the minimum of the family level with several entomological keys (105,126 – 135). All efforts were made to identify Dipteran and Coleopteran members to the species level.

6.7 Data Analysis

The average daily ambient temperatures for the entire study period were obtained from the Saskatoon Research Council weather station, located within three kilometres of all carcass deposition sites. The Saskatoon Research Council average temperatures were correlated (Pearson correlation coefficient) against the average daily temperatures from the shaded and sun-exposed sites to determine how well the weather station data fit that of the study sites. The average temperatures for this correlation were derived from the average of the maximum and minimum daily readings from the data-loggers, similar to the average temperature reported for the weather station.

The Student's t-test was employed to compare the mean temperatures between the sun-exposed and shaded sites for each season separately. For the t-test, the mean temperatures were derived from the average of every temperature reading (every thirty minutes) in each habitat. For test validity, all treatments were tested for equal variance at a 99% confidence interval. Maximum, minimum, and average temperatures (averaged from all data points within the defined duration of time) are also reported in for each stage of decomposition for each experiment.

CHAPTER 7: RESULTS

For ease in presenting the results, decomposition and insect succession are discussed in terms of decompositional stages, similar to other researchers (12, 13, 15, 16, 24, 38, 39). The stages of Anderson and VanLaerhoven (13) are used as a schematic model, as this study is Canadian-based. The morphological characteristics of these stages are presented in Chapter 3 (Table 3.1). Although each decompositional stage is associated with identifiable morphological changes and waves of insect infestation, decay is a continuous process. The day of death (and delivery) is marked as day 0 for all seasons and experiments.

Data loggers recorded several temperature readings including: ambient temperature in both the sun and shaded habitats, and internal carcass temperatures for pigs in all habitats and for clothed and nude carcasses. During collections, maggot mass and skin temperature readings were taken by hand with a standard thermometer. The data loggers had a recording range from –5°C to 37°C (± 0.1°C accuracy/resolution). Temperatures rarely fell below -5°C, with the exception of a few cold days in October. However, temperatures were often recorded above 37°C and are marked on the graph as a straight line at the highest point of the trend line on temperature graphs. Temperatures recorded below -5°C are marked as a line at the lowest point of a trend line. Based on temperatures from the weather station and readings from a standard thermometer, ambient, internal carcass, and core maggot mass temperatures were never above 43°C. The daily average temperature from the weather station, when correlated with the daily average temperature in each of the habitats for the entire study period, was 0.967 and 0.965 for the sun-exposed and shaded sites, respectively.

7.1 Experiment-1: Spring

7.1.1 Temperature

Ambient temperature readings for both the sun-exposed and shaded sites are presented in Figures 7.1 and 7.2, respectively. There was a significant difference between the means of these treatments ($p = 0.01$). Temperatures at the

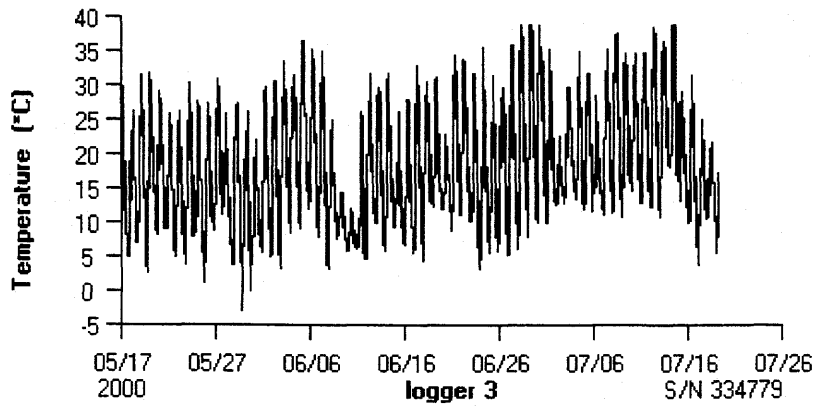

Figure 7.1: Ambient temperature at sun-exposed site in spring. Average temperature from May 17th to July 19th, 2000 was 17.83°C (± 0.15 (SE); n = 3026).

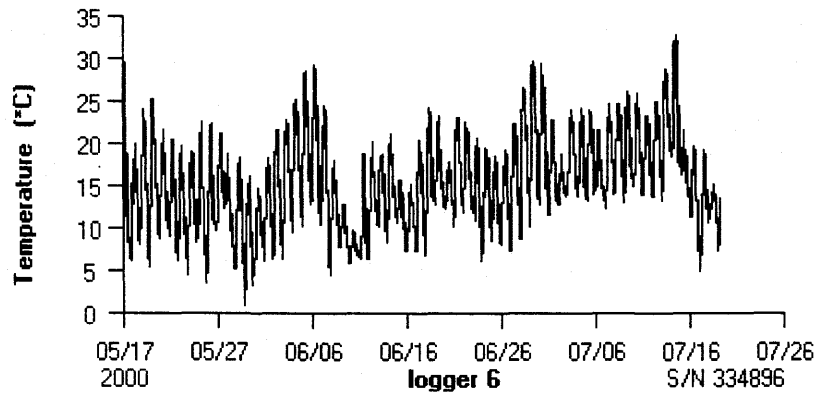

Figure 7.2: Ambient temperature at shaded site in spring. Average temperature from May 17th to July 19th, 2000 was 15.44°C (± 0.10 (SE); n = 3026).

sun-exposed site tended to be higher during the day, but often fell below that of the shaded site at night. For the duration of the spring experiment (May 17-

July19), the average temperature at the sun-exposed site was 17.8°C with maximum temperatures reaching above 38°C and minimum temperatures reaching as low as -2.8°C. Alternatively, the average temperature at the shaded site during this same time period was 15.4°C. The maximum temperature reached at the shaded site was 32.6°C and the minimum temperature was 1.0°C. Generally, the shaded site demonstrated less fluctuation in temperature than the exposed site.

There was a striking difference in the internal carcass temperatures between the sun-exposed and shaded sites, with a significant difference between the means of the two treatments ($p = 0.01$). The average internal temperature over the duration of the experiment was 26.0°C and 18.2°C at the sun-exposed and shaded sites, respectively (Figures 7.4 and 7.5). Thus, larval development and decomposition at the sunlit site occurred at a 7.8°C higher average temperature. During late evening hours, when ambient temperatures were at their lowest, the shaded carrion experienced slightly higher internal temperatures than the sun-exposed carrion. Generally, there was far less fluctuation in internal carcass temperatures at the shaded site.

Internal carcass temperatures at both sites were very similar during the first and second days. However, on the third day (May 19th), the average temperature difference was approximately 3.0°C, with the sunlit site reaching temperatures as high as 10.7°C above internal temperatures at the shaded site. This trend continued until day 23 (June 9th), reaching maximum disparity on day 12 (May 29th), when internal carcass temperature was over 27°C higher at the sun-exposed site than the shaded site. Internal carcass temperatures were always higher at the exposed site, with the exception of two days: day 24 (June 10th) and day 34 (June 20th). On day 24, internal temperatures at the sun-exposed site rapidly declined from the elevated temperatures experienced in the previous 10 days. By day 25, internal temperature was once again higher at the sunlit site. On day 34, internal carcass temperature at the shaded site increased and surpassed temperatures at the sun-exposed site. Again, this lasted only one day, and internal temperatures were once again higher at the exposed site on day 35.

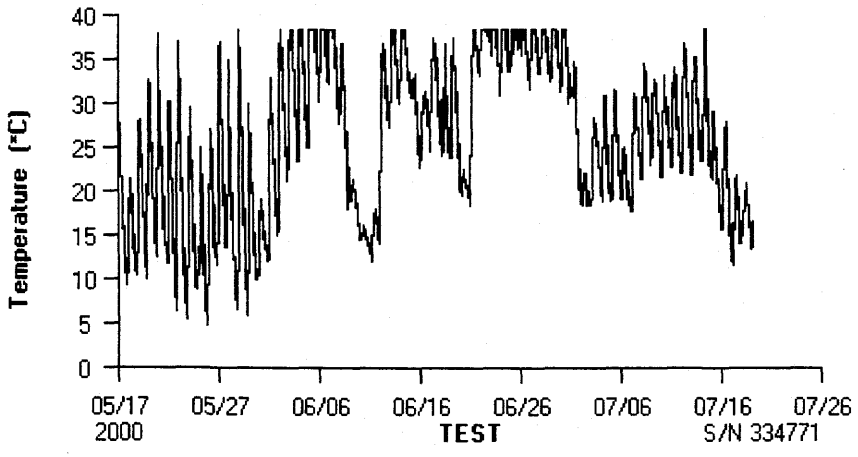

Figure 7.4: Internal carcass temperature at sun-exposed site in spring. The average internal carcass temperature from May 17th to July 19th was 25.99°C (± 0.16 (SE); $n = 3026$).

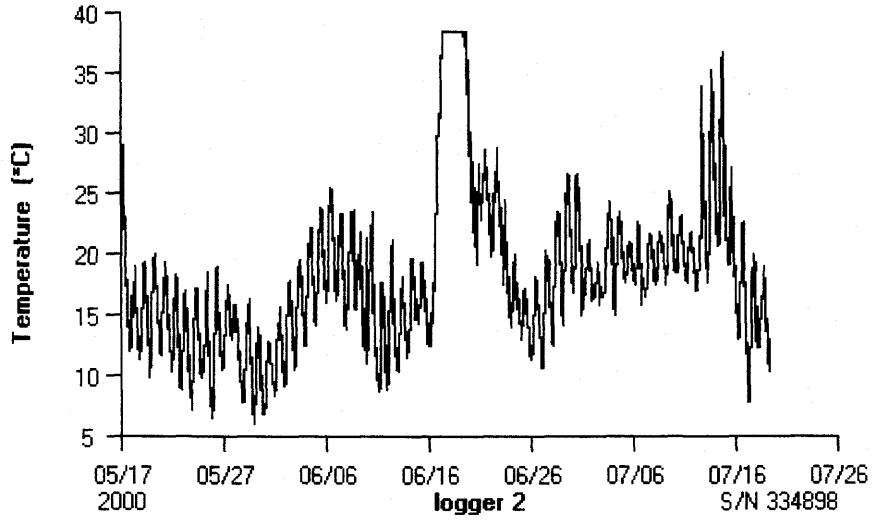

Figure 7.5: Internal carcass temperature at shaded site in spring. The average internal carcass temperature from May 17th to July 19th was 18.20°C (± 0.12 (SE); $n = 3026$).

The average internal carcass temperature at the sun-exposed site was close to, or above, maximum ambient temperature from day 14 (May 31st) to day 47 (July 3rd). Dips in internal temperature after periods of maximum recordings in sunlit carrion during this period correspond with major waves of post-feeding larval migration. Before day 14 and after day 47, internal carcass temperatures were closer to the average ambient temperature. In contrast, internal carcass temperatures at the shaded site did not approach maximum ambient temperature until day 21 (June 7th). On days 31 to 33 (June 17 –19) at the shaded site, internal temperatures soared above the maximum ambient temperature. This temperature differential reached almost 15°C above maximum ambient temperature on day 32.

7.1.2 Decomposition

The fresh stage begins with death and ends when bloating is initiated. In spring the fresh stage lasted from day 0 to day 1 for sun-exposed carcasses. The fresh stage was extended slightly in shaded carrion from 0 to 2 days postmortem. Carcasses in both habitats exhibited signs of rigor and algor mortis on days 0 to 1. Rigidity ended on day 2 for sun-exposed carcasses and on day 3 for shaded carcasses. There was no odour of decay associated with the carcasses in either habitat during the fresh stage.

Ambient temperature readings on day 0 were nearly identical in both habitats, averaging approximately 15°C. Internal carcass temperatures also demonstrated similar rates of cooling in each habitat on days 0-1. However, on day 1, sun-exposed carcasses experienced higher ambient temperatures of up to 8°C, leading to the earlier initiation of bloat in this habitat.

The beginning of the bloated stage was marked by the slow accumulation of gases, first exhibited as a slight bloat in the abdomen on day 1 for sun-exposed carrion and day 2 for shaded carrion. By day 2, abdominal bloating increased in sunlit carcasses and extended to the posterior regions of the body. On day 3, bloating was obvious and had spread to the limbs, anus, and head. For shaded carrion, bloat initiated on day 2 in the abdominal area, but did not spread to the remainder of the body until day 6, and did not peak until day 9.

During the bloated stage, lividic patches became evident on the lower portion of the carcass and remained throughout the duration of this stage. Skin discoloration occurred from the accumulation of gases and was exhibited as a blue-green marbling, mainly in the abdominal region. A moderate odour emanated from open wounds and natural orifices. This odour increased in strength throughout this stage.

Both ambient and internal carcass temperature readings for each decompositional stage are summarized in Table 7.1. Ambient temperatures were typically 3°C higher at the sun-exposed site versus the shaded site. At the shaded site, internal carcass temperatures approximated ambient temperatures throughout the bloated stage, although never reaching the same degree of daily fluctuation as ambient readings. At the sun-exposed site, internal carcass temperatures surpassed ambient temperatures, reaching as high as 16°C above ambient on day 12. Additionally, internal carcass temperatures were typically 6°C higher in the sun-exposed carrion versus the shaded carrion. The temperature differential in both ambient and internal readings between the two habitats accounts for the slower rate of decomposition at the shaded sites.

Table 7.1: Summary of ambient and internal carcass temperatures in each decay stage in spring

		Temperatures (degrees Celsius)				
Habitat	**Decay Stage (Days Postmortem)**	**Ambient Temp. Range**	**Ambient Temp. Avg.**	**Internal Temp Range**	**Internal Temp Avg.**	**Precipitation (mm)**
Sun	Fresh (0-1)	5.0 - 30.4	15.2	9.3 - 27.5	17.3	0.4
Shade	Fresh (0-2)	6.2 - 31.7	14.8	11.3 - 28.9	16.8	
Sun	Bloated (2-12)	1.3 - 31.4	15.5	4.9 - 38.1	18.5	11.0
Shade	Bloated (3-15)	1.0 - 25.1	12.8	6.1 - 19.9	12.7	
Sun	Active Decay (13-30)	-2.8 - 36.2	16.4	6.0 - 38.1	27.4	38.0
Shade	Active Decay (16-35)	4.7 - 29.0	14.5	8.7 - 38.3	20.5	
Sun	Advanced Decay (31-42)	3.2 - 38.4	18.6	18.4 - 38.1	33.2	8.8
Shade	Advanced Decay (36-45)	6.2 - 29.6	16.7	10.7 - 28.7	18.5	
Sun	Dry (42-63+)	4.0 - 38.4	20.4	11.6 - 38.1	25.5	72.0
Shade	Dry (46-63+)	5.1 - 32.6	17.6	7.9 - 36.5	19.7	

The end of the bloated stage is typically marked by the deflation of the carcass due to insects piercing the skin (13, 15). For carcasses in both habitats, deflation occurred over a period of several days, typically starting in the head region, then occurring in the extremities and posterior, and lastly in the abdomen. Deflation was always most noticeable where maggot activity was the greatest. These regions included those areas with natural orifices, such as the eyes, ears, nose, mouth, and anus, and the thoracic region where the artificial wound was created.

Anterior deflation initiated on day 11 and abdominal deflation initiated on day 15 for sun-exposed carcasses. As a group, the shaded carcasses exhibited a greater variety in decomposition rate. For one shaded carrion, deflation initiated on day 12 at the anterior and posterior ends of the carcass. Abdominal deflation of this carcass also began on day 15. The other two shaded carcasses deflated at a slower rate. Anterior deflation initiated on day 15 and abdominal deflation on day 21. Maggots were packed into natural body orifices so tightly that they resembled a package of tightly packed pipe cleaners open at one end. Thus gaseous diffusion to the air was sluggish, being partially blocked by the infestation of larvae.

A better marker for the end of the bloated stage and beginning of the active decay stage was evidence of liquefaction. The ground surrounding the carcass became wet as the gaseous pressure finally forced fluids out of natural orifices. The appearance of the liquid was frothy, resembling muddy dishwater. This event was associated with a burst of maggots being forced out along with the fluid. Evidence of liquefaction first occurred on day 12 for sun-exposed carcasses and day 15 for shaded carcasses.

During the active decay stage, the carcass became entirely infested with maggots in all stages of development. Insects devoured extremities such as the head, anus, and limbs. The remaining skin in this area had several perforations and rips from maggot activity. These areas became deflated, while the abdomen remained intact and bloated. The odour of decay increased dramatically and became putrid and offensive. Clothing became soaked with putrefactive liquids and eventually became a growth medium for mold. The clothing also trapped

escaping gas as deflation ensued. Outer edges of skin and the underlying grass took on the characteristics of black putrefaction: a blackened, cooked or burned appearance (Figure 7.5). Skin began to separate from bone, most commonly in the shoulder region and limbs.

During the active decay stage, ambient temperatures reached an average of 16.4°C at the sun-exposed site and 14.5°C at the shaded site (Table 7.1). Internal carcass temperatures averaged 27.4°C at the sun-exposed site, often reaching over 25°C over ambient. Alternatively, internal carcass temperatures reached an average of 20.5°C in the shaded carrion. This was the highest average for internal carcass temperature of shaded carrion. Precipitation was still meagre, as only 38 millimetres of rainfall fell during this stage.

Full deflation of all carcasses was highly variable and occurred between days 22 and 30. Near the end of this stage the carrion odours changed to the characteristic smell of organ fluids. Day 30 marked the end of the active decay stage and the beginning of the advanced decay stage for sun-exposed carcasses. Two of the shaded carcasses reached advanced decay by day 35. Skunk kittens (*Mephitis mephitis*) were constantly attacking the other shaded carcass. Initially, the skunks preyed on the insects, thereby slowing decomposition. By day 38, the skunks had reduced the majority of visible insects and began to attack the flesh of the carcass, which hastened the rate of decomposition. Thus, by day 39, this slower shaded carcass had reached the advanced decay stage and the same level of decomposition as the other shaded carrion.

Characteristics of advanced decay have typically included the removal of most flesh, a decrease in odour, and migration of prepupal larvae from the carrion (13, 15). All of these characteristics marked the advent of advanced decay in both habitats in spring, with the exception of larval migration. Prepupal larvae were more common in active decay, although waves of migration were observed over several weeks, starting from day 18 in sun-exposed carcasses and day 20 in shaded carcasses. The loss of skin on the head, limbs, and posterior was common to all carcasses. However, carrion retained most skin in the abdominal area (Figure 7.6).

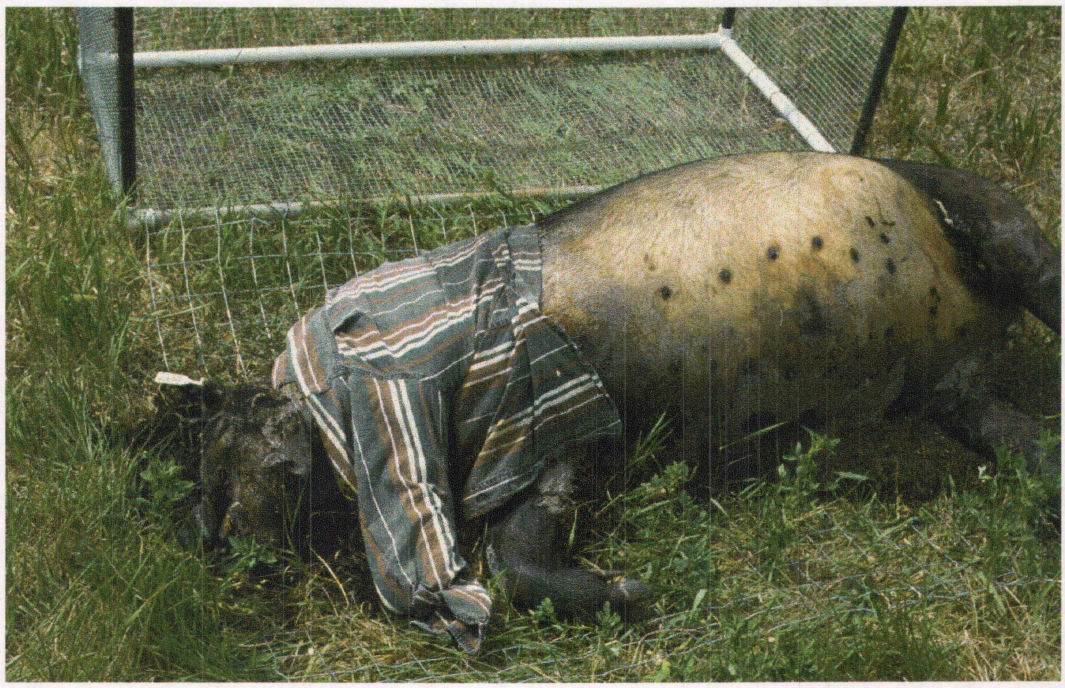

Figure 7.5: Sun-exposed carcass in active decay in spring (day 20). There is extensive anterior decomposition and blackened grass from putrefactive liquids.

Figure 7.6: Shade carcass in advanced decay in spring (day 43). Note the retention of skin over the majority of the carcass.

Remaining hair and skin rapidly dehydrated in the advanced decay stage. Clothing was covered in pupae by the start of this stage and became hard from the drying of decompositional fluids. Cranial and limb bones were exposed on all carcasses and one carcass in each habitat also had the ribs partially exposed. Odour decreased dramatically and smelled like dried organ tissue. The rapid desiccation of the carcass shortened the length of this stage. During periods of rainfall, the carcass retained small puddles of water in depressions on the remaining skin.

Ambient temperatures in both habitats increased during the advanced decay stage, as the summer season approached. At the sun-exposed site ambient temperatures averaged 18.6°C, nearly 2°C higher than the shaded average of 16.7°C (Table 7.1). Sun-exposed internal carcass temperatures peaked during this stage and ranged from 38.1°C to 18.4°C, with an average of 33.2°C. Shaded internal carcass temperatures decreased from the peak experienced in active decay and ranged from 28.7°C to 10.7°C, with an average of 18.5°C. A total of 8.8 millimetres of rain fell during this stage.

The warmer temperatures and lack of precipitation in the advanced decay stage hastened the advent of the dry stage. The characteristics of this stage include little to no odour and only dry skin, cartilage and bone remaining (13, 15). The final stages of decomposition in spring in Saskatoon best follow the stages of Payne (15), separating final decay into two stages: dry and remains. This experiment however, did not last long enough to observe the remains stage. The defining characteristics for each stage of decomposition for both sun-exposed and shaded carcasses are summarized in Table 7.2.

The carcasses became so dry as to preserve the remaining outer skin and tissue. Under the hardened skin a dark brown sludge was all that remained of the tissues. Heavy rainfall in the beginning of July prevented the sludge from drying out and provided a remnant breeding ground for insects. The outer skin and clothing did not moisten from the rain, remaining crusty and rigid.

Table 7.2: Summary of stages of decomposition in spring and their associated characteristics.

Decay stage	Days Postmortem	Defining Characteristics
Fresh	Sun (0-1)	No odour
	Shade (0-2)	Rigor mortis
		Fresh appearance
Bloated	Sun (2-12)	Bloated appearance, initiating in abdomen
	Shade (3-15)	Livor mortis
		Discoloration (Cyan marbling)
		Moderate odour, stronger near body openings
		Maggots developing inside body openings
Active Decay	Sun (13-30)	Maggot infestation outside of cavities, all over body
	Shade (16-35)	Liquefaction of tissues, frothy liquid outside of body
		Very strong odour of decay
		Evidence of black putrefaction
		Major migration of prepupal larvae
		Deflation occurring throughout stage, lastly in abdomen
		Skin beginning to separate from bone
		First pupae near end of stage
Advanced Decay	Sun (31-42)	Removal of flesh at extremities (head, limbs, anus)
	Shade (36-45)	Odour moderate, resembles smell of dried organ tissue
		Dehydration of remaining tissues
		Bone exposure evident at extremities
Dry	Sun (43-63+)	Little to no odour
	Shade (46-63+)	Hardened, dried, and wrinkled skin
		Exposed bone and tissue remnants whitish-grey

By day 41, one exposed carcass was completely dehydrated. All remaining skin had a cheesecloth-like appearance. By day 43, all sun-exposed carcasses were dried. The remaining skin on the abdomen resembled parchment paper and took on a wrinkled, mummified appearance (Figure 7.7). The carcass remnants began to turn a whitish-grey colour. By day 45, all shaded carcasses had reached the dry stage. Generally, shaded carcasses retained more abdominal skin than sun-exposed carcasses. Collections continued until day 63. Between day 45 and 63 there were few changes in the overall appearance of the carcasses.

Approximately 72 millimetres of rain fell during this stage, the majority of which resulted from late afternoon thunderstorms after hot days. Four thunderstorms contributed to over 85% of the total rainfall. Ambient temperatures during the dry stage were the highest of all decompositional stages, as the majority of this stage fell in the month of July (Table 7.1). Internal carcass temperatures remained higher than ambient in both habitats, although there was an overall decrease in the average internal temperatures from the previous stage.

Figure 7.7: Sun-exposed carcass in dry stage in spring (day 56). The remaining tissues have dehydrated and have a mummified appearance.

7.1.3 Insect Succession

Most carcasses experienced fly activity immediately after placement and all carcasses experienced activity within four hours of death. Oviposition was not observed immediately, but eggs were detected on carcasses the following day and were likely deposited on day 0, as light rain and overcast skies limited fly activity on day 1. The blow fly *Cynomya cadaverina* (R.-D.) was the most abundant fly attracted to carcasses in both habitats on day 0. Both *Phormia regina* (Meig.) and *Protophormia terraenovae* (R.-D.) were also collected from shaded carrion during the fresh stage. Eggs were observed but not collected during the fresh stage to minimize intrusion to this fragile life stage.

Day 2, the start of the bloated stage for sun-exposed carrion, was sunny and warm, causing a dramatic increase in fly activity. Shaded carcasses had 10 to 35 blow flies feeding and ovipositing in several regions, including the ears, nose, mouth, thoracic wound, and anus. Several egg masses were found in these areas. Sun-exposed carrion had anywhere from 30 to 70 blow flies on each carcass. In both habitats, the bloated stage attracted a greater diversity and number of insects (Table 7.3). However, the Calliphorids remained the most numerous flies in both habitats. The blow flies *P. regina* and *P. terraenovae* were by far the most common flies feeding and ovipositing on the sun-exposed carrion. At shaded carrion, adult *C. cadaverina* and *P. terraenovae* were the most abundant. The difficulty in identifying immature stages of blow flies forced the majority of larval identification to come from reared adults. In both habitats, several larvae were collected from various areas. However, the only two species that emerged from laboratory rearing were *P. regina* and *P. terraenovae*. It is possible that the rearing technique could have favoured the development of Calliphorid flies over that of other Dipteran species.

Table 7.3: Forensically important insects collected from pig carcasses in spring in the fresh and bloated stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Fresh (0-1)	Diptera	Calliphoridae	*Cynomya cadaverina*	ad
			Scathophagidae	*not identified beyond family*	ad
			Muscidae	*Phaonia sp.*	ad
			Anthomyidae	*Hydrophoria sp.*	ad
Shade	Fresh (0-2)	Diptera	Calliphoridae	*Cynomya cadaverina*	ad
				Phormia regina	ad
				Protophormia terraenovae	ad
			Muscidae	*Phaonia sp.*	ad
Sun	Bloated (2-12)	Diptera	Calliphoridae	*Protophormia terraenovae*	ad, imm
				Cynomya cadaverina	ad
				Phormia regina	ad, imm
				Phaenicia sericata	ad
				Calliphora vicina	ad
				Lucilia illustris	ad
			Sarcophagidae	*Sarcotachinella sinuata*	ad
			Anthomyidae	*Hydrophoria sp.*	ad, imm
			Muscidae	*Fannia sp.*	ad
				Musca sp.	ad
				Phaonia sp.	ad
				Hydrotaea sp.	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Phoridae	*Megaselia sp.*	ad
			Sphaeroceridae	*Leptocera wirthi*	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad
			Scarabaeidae	*Phanaeus sp.*	ad
				Aphodius sp.	ad
			Histeridae	*Saprinus distinguendus*	ad
Shade	Bloated (3-15)	Diptera	Calliphoridae	*Cynomya cadaverina*	ad
				Protophormia terraenovae	ad, imm
				Phormia regina	imm
				Lucilia illustris	ad
				Bufolucilia silvarium	ad
			Sarcophagidae	*Liopygia sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Phoridae	*Phora sp.*	ad
				Anevrina sp.	ad
				Megaselia sp.	ad
			Sphaeroceridae	*Leptocera wirthi*	ad
			Chloropidae	*not identified beyond family*	ad
		Coleoptera	Staphylinidae	*Lobrathium sp.*	ad
			Silphidae	*Thanatophilus lapponicus*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

The blow flies *Lucilia illustris* (Meig.), *Phaenicia sericata* (Meig.) and *Bufolucilia silvarum* (Meig.) made their first appearances during the bloated stage. *P. sericata* was collected only at the sun-exposed sites, whereas *B. silvarum* was collected only at the shaded sites. Similar to the muscid flies, *P. sericata* and *B. silvarum* were not common in the collections. Other dipterans appearing in the bloated stage included members of Sarcophagidae, Piophilidae, Phoridae, Heleomyzidae, and Chloropidae. Flesh flies (Sarcophagidae) were almost exclusively captured near the carcass, but not on it. The cheese skipper *Piophila casei* (L.) was collected late in the bloat stage (day 9) and only at one sun-exposed carcass. The humpbacked flies (Phoridae) were common visitors in the bloated stage in both habitats. At sun-exposed carrion, only species of *Megaselia* (Rondani) were collected. Alternatively, three species of Phoridae were collected from shaded carrion, although members of the genus *Anevrina* (Lioy) were the most common. Members of the genus *Tephrochlamys* (Loew) (Heleomyzidae) were common visitors to carrion in both habitats, starting from day 5 and increasing in number throughout the bloated stage. Members of Chloropidae were rare visitors, and were only collected from one of the shaded carrion.

The elusive carrion beetle, *Thanatophilus lapponicus* (Herbst) (Silphidae), was first collected on day 2 from sun-exposed carrion and day 5 from shaded carrion. Collections of this beetle came almost exclusively from pitfall traps, as attempts at hand collecting were unsuccessful. During the bloated stage, *T. lapponicus* was the most abundant coleopteran in both habitats. The only other coleopteran collected from shaded sites was one rove beetle (Staphylinidae). Only one beetle of each of the families Scarabaeidae and Histeridae were collected from sunlit carrion, on day 5 and day 9, respectively. Thus, the only beetle that has a predictable arrival time during the bloated stage is the carrion beetle, *T. lapponicus*. This beetle arrived early in bloat and increased in abundance throughout the bloated stage.

In the active decay stage, ants (Hymenoptera: Formicidae) started to increase in numbers, preying on dipteran eggs and larvae. The number of ants never rivalled the numbers of blow fly eggs or larvae. However, the effect of

predation by ants could not be quantified. Maggots were teeming in several areas of the carcass, although many of the masses were still contained inside the cavities of the orifices and wounds, especially in the shaded carrion. Maggots were not developing underneath clothing at this point, but could be seen underneath the skin where the clothing covered the carcasses. The heads of all carcasses were entirely infested with larvae in varying stages of development.

Although *P. casei* was first collected in the bloated stage, there numbers increased dramatically in the active decay stage in both habitats. The number of adult blow flies visiting the carcass was also greatest during this stage. The weather was becoming increasingly favourable for blow fly activity and development. Furthermore, oviposition increased and every carcass had thousands of freshly laid eggs. In this stage, egg-laying tended to be centralized around the crevices in the outer skin, where the carcass appeared to be sweating. The relative abundance of adult *P. regina* increased at the sun-exposed carrion. By day 20, huge maggot masses were evident in several areas of the sun-exposed carcasses. Maggot mass temperatures ranged between 35-38°C. Day 20 was also the first day that pupae were observed at the sun-exposed carrion. These trends occurred on day 21 in shaded carrion.

The number of coleopterans increased considerably during the active decay stage. The first larval carrion beetles (Silphidae) were collected on day 20 and day 28 at sun-exposed and shaded carrion, respectively (Table 7.4). The larval silphids equalled the number of adult carrion beetles during this stage. The silphid *Heterosilpha ramosa* (Say) was restricted to the sun-exposed carrion, while *T. lapponicus* was found in both habitats.

Adult rove beetles (Staphylinidae) made their first appearance at the sun-exposed carrion on day 18 and began to rival the numbers of Silphidae. The shaded carrion also experienced a major increase in the number of adult Staphylinidae during the active decay stage. The most abundant adult Staphylinidae are in the subfamily Aleocharinae. Unfortunately, identification to species level was not possible. The second most abundant species of Staphylinidae was *Creophilus maxillosus* (Gravenhorst). Immature Staphylinidae

were first collected on day 35 on shaded carrion, the last day of active decay. However, the presence of larval Staphylinidae was more characteristic of advanced decay for both habitats.

Table 7.4: Forensically important insects collected from pig carcasses in spring in the active decay stage of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Active Decay (13-30)	Diptera	Calliphoridae	*Protophormia terraenovae*	ad, imm
				Phormia regina	ad, imm
			Muscidae	*Hydrotaea sp.*	ad
			Anthomyidae	*Hydrophoria sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Phoridae	*Anevrina sp.*	ad
				Megaselia sp.	ad
			Piophilidae	*Piophila casei*	ad
				not identified beyond family	ad
		Coleoptera	Staphylinidae	*Creophilus maxillosus*	ad
				Lobrathium sp.	ad
			Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	ad, imm
			Histeridae	*Saprinus distinguendus*	ad
			Scarabaeidae	*Phanaeus sp.*	ad
				Aphodius sp.	
			Nitidulidae	*Carpophilus sp.*	ad
Shade	Active Decay (16-35)	Diptera	Calliphoridae	*Phormia regina*	ad, imm
				Protophormia terraenovae	ad, imm
				Cynomya cadaverina	ad
				Bufolucilia silvarium	ad
			Anthomyidae	*Hydrophoria sp.*	ad
			Muscidae	*Morellia sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Phoridae	*Anevrina sp.*	ad
			Chloropidae	*not identified beyond family*	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
			Staphylinidae	*Lobrathium sp.*	ad, imm
				Creophilus maxillosus	imm
			Scarabaeidae	*Aphodius sp.*	ad
			Nitidulidae	*Carpophilus sp.*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

There was more diversity in species of coleopterans at the sun-exposed sites. The number of scarab (Scarabaeidae) and clown beetles (Histeridae) was also greater at the sun-exposed sites. The histerid beetles were not collected from shaded carrion during this stage and the Scarabaeidae were infrequent visitors. The sap beetles (Nitidulidae) made their first appearance during this stage on day 27 and 30 at the shaded and sun-exposed sites, respectively.

From day 31 to 41 of the advanced decay stage, fly activity decreased in both habitats. At the sun-exposed sites, there were no adult blow flies collected until day 41, when the first major adult emergence occurred. Laboratory rearing of immature specimens collected from sun-exposed carrion were identified as *P. regina* and *P. terraenovae*. On day 41, there was hundreds of newly emerged *P. regina* near every sun-exposed carcass (Figure 7.8).

Figure 7.8: Newly emerged flies on a rock near a sun-exposed carcass in spring (day 41).

Another wave of fly emergence occurred on day 43 at the sun-exposed carrion. Flies from this wave were identified as *P. terraenovae*. At shaded carrion, the first major emergence of adult flies occurred between days 43 and 45. Flies collected included *C. cadaverina* and *P. terraenovae*. Thus, immature *C. cadaverina* must have been present on shaded carrion, even though they were never identified from larval specimens or adults emerging from laboratory rearing. During the advanced decay stage, *P. casei* disappeared from sun-exposed remains, but increased dramatically at shaded remains (Table 7.5).

Table 7.5: Forensically important insects collected from pig carcasses in spring in the advanced decay stage of decomposition

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Advanced Decay (31-42)	Diptera	Calliphoridae	*Protophormia terraenovae*	ad, imm
				Phormia regina	ad, imm
			Anthomyidae	*Hydrophoria sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
		Coleoptera	Staphylinidae	*Creophilus maxillosus*	imm
				Lobrathium sp.	ad, imm
			Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	ad, imm
			Histeridae	*Saprinus distinguendus*	ad
			Scarabaeidae	*Aphodius sp.*	ad
Shade	Advanced Decay (36-45)	Diptera	Calliphoridae	*Protophormia terraenovae*	ad, imm
				Cynomya cadaverina	ad
			Anthomyidae	*Hydrophoria sp.*	ad
			Muscidae	*Morelia sp.*	ad
			Phoridae	*Anevrina sp.*	ad
			Piophilidae	*Piophila casei*	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Oiceoptoma noveboracense	imm
				Heterosilpha ramosa	imm
			Staphylinidae	*Lobrathium sp.*	ad, imm
				Creophilus maxillosus	imm
			Histeridae	*Hister furtivus*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

Immature Silphidae and Staphylinidae were very common in both habitats during the advanced decay stage. The carrion beetle, *Oiceoptoma noveboracense* (Forster), appeared for the first time on shaded carrion. The number of scarabs remained abundant at the sun-exposed sites but disappeared from shaded carrion. The histerid beetle *Saprinus distinguendus* (Marseul) remained relatively abundant at sun-exposed carrion. Only one histerid beetle, *Hister furtivus* (LeConte) was collected from a shaded carcass on day 45. The Histeridae were more common in the dry stage at shaded carrion. Muscid flies disappeared from sun-exposed carrion and were rarely collected from shaded carrion. Although humpbacked flies (Phoridae) and black scavenger flies (Sepsidae) were collected during this stage, they were also rare in the collections.

Insect diversity increased during the dry stage, especially at the sun-exposed carcasses (Table 7.6). The blow fly *Cochliomyia macellaria* was collected for the first time at sun-exposed carrion. This species is known to have a more southern distribution and only flies north during summer months. New fly emergence on day 50 released a new wave of *P. terraenovae* on sun-exposed carcasses.

Coleopterans were the dominant insects during the dry/remains stage. Larval staphylinids and silphids remained abundant in both habitats. The histerid beetle *S. distinguendus* finally made its appearance on shaded carrion on day 56. At sun-exposed sites, *S. distinguendus* remained abundant throughout the dry stage. The histerid *H. furtivus* was unique to the shaded carrion and increased in abundance during the dry stage. The red-legged ham beetle, *Necrobia rufipes* (DeGeer), made its first appearance on day 50 and 52 in the shaded and sun-exposed habitats, respectively. The only dermestid beetle collected was one larva of *Dermestes maculatus* from a sun-exposed carcass on day 56. Final collections were made on day 62.

Table 7.6: Forensically important insects collected from pig carcasses in spring in the dry stage of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Dry (42 to > 63)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
				Protophormia terraenovae	ad
				Calliphora vicina	ad
			Anthomyidae	*Hydrophoria sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
		Coleoptera	Staphylinidae	*Creophilus maxillosus*	ad, imm
				Lobrathium sp.	ad, imm
			Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	imm
			Histeridae	*Saprinus distinguendus*	ad
				Hister furtivus	ad
			Scarabaeidae	*Aphodius sp.*	ad
			Nitidulidae	*Carpophilus sp.*	ad
			Cleridae	*Necrobia rufipes*	ad
			Dermestidae	*Dermestes maculatus*	imm
Shade	Dry (46 to > 63)	Diptera	Calliphoridae	*Protophormia terraenovae*	ad, imm
				Cynomya cadaverina	ne-ad
				Lucilia illustris	ad
				Phormia regina	ne-ad
			Anthomyidae	*Hydrophoria sp.*	ad
			Heleomyzidae	*Tephrochlamys sp.*	ad
			Phoridae	*Anevrina sp.*	ad
			Piophilidae	*Piophila casei*	ad, imm
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Oiceoptoma noveboracense	ad
			Staphylinidae	*Lobrathium sp.*	ad, imm
				Creophilus maxillosus	imm
			Histeridae	*Saprinus distinguendus*	ad
			Scarabaeidae	*Aphodius sp.*	ad
			Cleridae	*Necrobia rufipes*	ad
			Nitidulidae	*Carpophilus sp.*	ad

ad=adult, imm=immature, ne-ad=newly emerged adult; * Elapsed Time Since Death (days)

7.2 Experiment-1: Summer

7.2.1 Temperature

Ambient temperature readings for both the sun-exposed and shaded sites are presented in Figure 7.9 and 7.10, respectively. There was no significant difference between the means of the two treatments ($p = 0.01$). The sun-exposed

site had an average temperature of 19.5°C throughout the experiment, with a range of 0°C to over 38°C. The shaded site experienced an average temperature of 19.1°C with a range of 5°C to over 38°C. Daily maximums were similar in both habitats, whereas daily minimums were lower at the sun-exposed site.

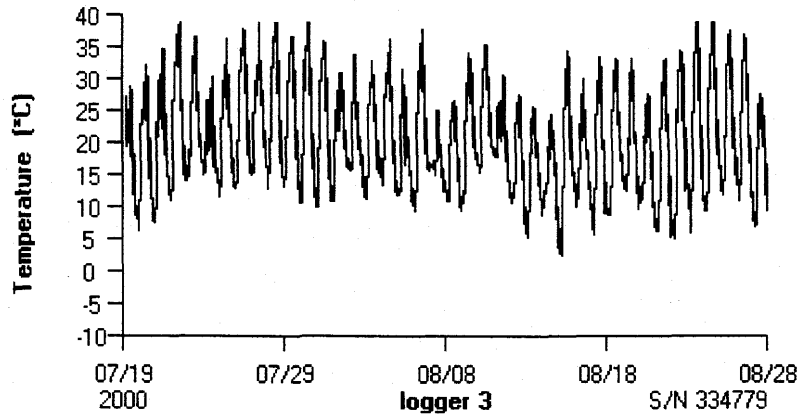

Figure 7.9: Ambient temperature at sun-exposed site in summer. The average temperature from July 19th to August 31st, 2000 was 19.47°C (± 0.19 (SE); n = 2093).

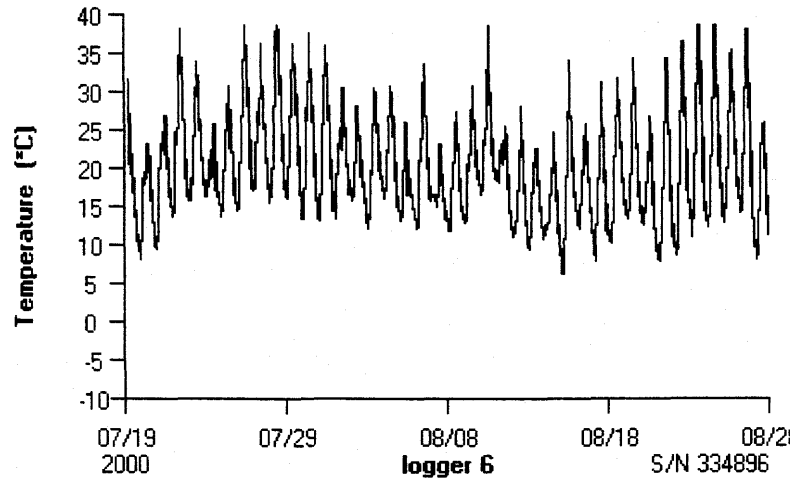

Figure 7.10: Ambient temperature at the shaded site in summer. The average temperature from July 19th to August 31st, 2000 was 19.08°C (± 0.15 (SE); n = 2093).

Internal carcass temperatures are graphically represented in Figures 7.11 and 7.12, for sun-exposed and shaded carrion, respectively. There was a

significant difference between the means of these two treatments ($p = 0.01$). Interestingly, carrion at the shaded sites experienced a 3.9°C higher average

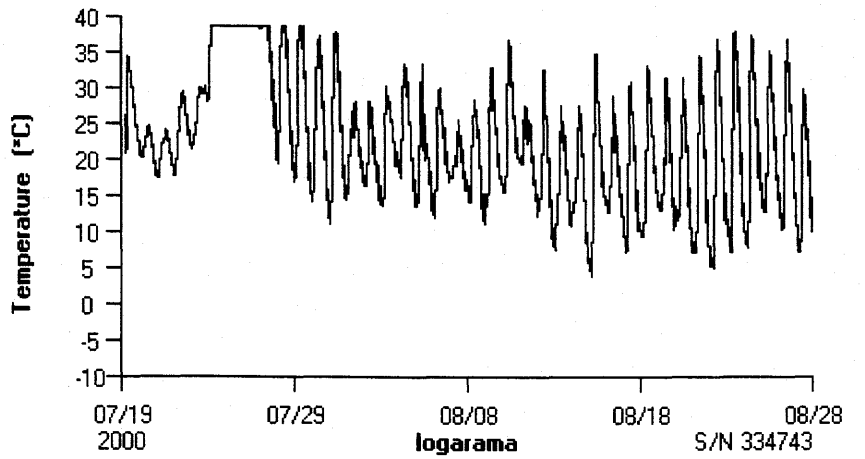

Figure 7.11: Internal carcass temperature at sun-exposed site in summer. The average temperature from July 19th to August 31st, 2000 was 21.56°C (± 0.20 (SE); $n = 2093$).

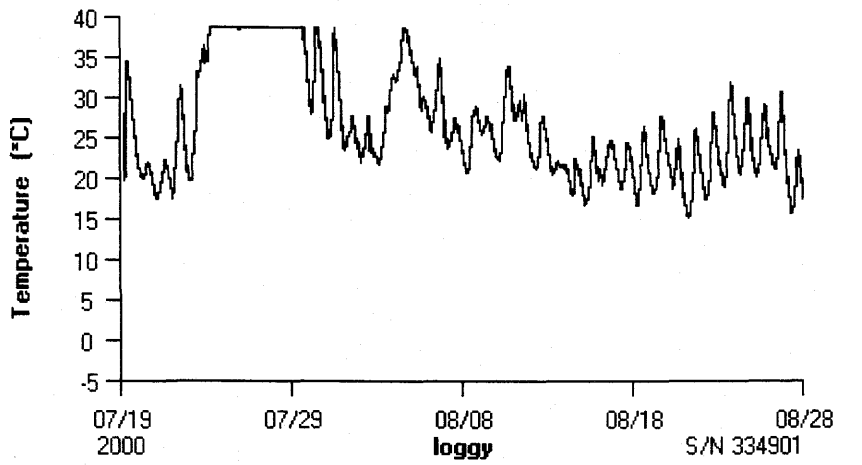

Figure 7.12: Internal carcass temperature at shaded site in summer. The average temperature from July 19th to August 31st, 2000 was 25.50°C (± 0.16 (SE); $n = 2093$).

internal temperature than sun-exposed carrion. From day 0 to day 8, internal temperatures were nearly identical in both habitats. Internal temperatures increased on day 5 and remained consistently over 38°C until day 8. From day 8

onward, sun-exposed carrion experienced a decline in internal temperatures with large daily fluctuations. Shaded carrion retained maximum temperatures until day 10 before daily fluctuations re-occurred. At the shaded site, internal temperatures exhibited less daily fluctuation than ambient temperatures throughout the remainder of the experiment.

7.2.2 Decomposition

Decomposition occurred at a significantly faster rate in summer due to the higher ambient temperatures. Carcasses in both habitats exhibited bloating on day 1. Algor mortis lasted less than 24 hours after placement, after which the internal temperature of the pigs began to mimic ambient. Both ambient and internal carcass temperature readings for each decompositional stage are summarized in Table 7.7.

Table 7.7: Summary of ambient and internal carcass temperatures in each decay stage in summer

		Temperatures (°C)				
Habitat	**Decay Stage (Days Postmortem)**	**Ambient Temp. Range**	**Ambient Temp. Average**	**Internal Temp. Range**	**Internal Temp. Average**	**Precipitation (mm)**
Sun	Fresh (0)	8.6 - 28.4	19.8	20.7 - 34.1	28.4	0.0
Shade	Fresh (0)	10.9 - 31.3	18.8	19.7 - 34.3	27.8	
Sun	Bloated (1-4)	6.1 - 38.4	21.3	17.3 - 29.7	23.0	0.0
Shade	Bloated (1-4)	8.1 - 37.8	20.0	17.3 - 34.9	22.7	
Sun	Active Decay (5-11)	10.6 - 38.4	23.4	14.1 - 38.1	32.9	10.8
Shade	Active Decay (5-11)	13.4 - 38.2	23.0	27.8 - 38.3	37.4	
Sun	Advanced Decay (12-25)	5.4 - 37.5	19.9	7.4 - 36.4	20.6	49.6
Shade	Advanced Decay (12-25)	9.5 - 38.2	19.3	20.36 - 38.3	26.8	
Sun	Dry/Remains (26+)	0.1 - 38.4	17.2	2.1 - 37.5	17.1	3.0
Shade	Dry/Remains (26+)	4.8 - 38.2	17.2	11.5 - 31.7	20.2	

There were very few differences in decomposition between the sun-exposed and shaded carcasses during the first two stages. By day 2, all carcasses were significantly bloated in all body regions. Rigor mortis initiated on day 1 and evidence of hypostasis was clearly evident by day 2. By day 4 the bloat was so severe that the organs burst through the abdominal wall. The carcasses expanded

to twice their original size and filled the entire cage. Skin started to exhibit the characteristic blue-green marbling from gaseous build-up in the abdomen. Abdominal distension pushed clothing against the shoulders and neck of the pigs.

Ambient temperatures in the bloated stage were slightly higher at the sun-exposed site, averaging a 1.3°C higher temperature than the shaded site (Table 7.7). Internal temperatures were nearly identical in both habitats during the bloated stage. Furthermore, internal temperatures were higher than ambient, exhibiting a 1.7°C and 2.7°C increase over ambient at the sun-exposed and shaded sites, respectively. The first four days of the summer experiment were sunny with clear skies and no precipitation.

On day 5, the abdominal wall, thoracic region, anus, genitals, and orifices of the head of all carcasses ruptured, spilling developing larvae and decompositional fluid. The carcasses did not deflate upon rupture. Day 5, however, is considered to be the first day of active decay due to the rupturing of the skin, the release of decompositional fluids, gas and subsequent froth, and the associated putrid odour.

During active decay, the edges of broken skin blackened from putrefaction and started to flake from maggot activity. The larvae created several holes in the carcass as they moved from one area to another. By day 6, deflation initiated in the head, neck, and exposed organs of carcasses in both habitats. At this point, maggots had infested every region of the carcasses. By day 7, deflation had extended to the abdomen and buttocks (Figure 7.13). Full deflation occurred by day 9. The sun-exposed pig carrion started to attract numerous scavengers, which were not deterred by the cages and mesh underlay. By day 7, every sun-exposed carcass displayed some evidence of scavenging. However, the animal responsible was likely quite small and concentrated on removing flesh from the distal ends of the forelimbs. Shaded carrion displayed almost no evidence of scavenging throughout the experiment.

By day 7, the skin of the sun-exposed carcasses started to dehydrate from the intense heat and exhibited an orange-brown discoloration. Similar events took place on shaded carrion by day 8, although the moisture level remained higher

compared to sun-exposed carrion. Between the maggot activity and the high ambient temperatures, the sun-exposed carrion were rapidly diminishing as a resource for insects. Maggots began to retreat to the underside of the carcasses, under clothing, and into the internal cavities where moisture was still available. As shaded carcasses were protected from direct sunlight, the larvae were able to continue feeding all over the body for an extended period of time. The skin of shaded carcasses also remained pliable and damp longer. Cranial exposure was evident on most carcasses by day 7.

Figure 7.13: Sun-exposed carrion in active decay in summer (day 7). Exposed organs are completely deflated and shrivelled from dehydration.

The active decay stage occurred during the hottest days of summer. The average ambient temperature was approximately 23°C in both habitats, with daily highs over 38°C. Internal carcass temperatures also peaked during the active decay stage. At the shaded site, internal temperatures never dropped below 27.8°C, as maggot activity continued feverishly throughout this stage. The sun-

exposed carrion experienced a 4.5°C lower internal temperature than shaded carrion, averaging 32.9°C. The rapid desiccation of the sun-exposed carrion led to the early departure of underdeveloped larvae. Thus, maggot mass heat generation declined by day 8 and internal temperatures began to approximate ambient.

At the shaded sites, drops in internal carcass temperature were consistent with the mass migration of post-feeding larvae. Mass migration first occurred on day 10 at shaded sites, but was not observed until day 12 at sun-exposed sites. Core maggot mass temperatures at the sun-exposed site ranged from 40-43°C. At shaded sites, core temperatures ranged from 34-37°C. The high temperatures at the sun-exposed site likely exceeded the maximum threshold for most species, causing a slower rate of development. This was confirmed by the presence of heat stressed and dead larvae at the sun-exposed sites.

Advanced decay started on day 12 in both habitats. The stench of decay had decreased and changed to a dried, charred odour. Waves of migration continued on day 12. At sun-exposed carcasses, many larvae were leaving the carcass in search of lower temperatures. Several non-post-feeding, 3rd instar larvae were found underneath the grass away from the carcass. The grass provided protection from the intense heat, as the temperature was 4-5°C cooler than the skin temperature of the carrion. The extent of carcass desiccation was greater at the sun-exposed sites and left maggots scrambling for an alternative food source. By day 14, more flies and larvae were found dead near the sun-exposed carrion.

Shaded carrion in the advanced decay stage experienced a dramatic decline in insect activity. Developing larvae were not readily visible, although they may have withdrawn to areas underneath or deep within the carcasses. The surrounding soil was sampled for pupae and larvae, but none were found. Post-feeding larvae may have migrated a great distance to areas that were not sampled. Fly activity was extremely low and beetles were rarely sighted. This low activity occurred until day 14, when the first emergence of blow flies occurred. At the sun-exposed sites, the first emergence of adult flies occurred on day 16. The increased fly activity coincided with an increase in beetle activity, especially at the

sun-exposed sites. The defining characteristics for each stage of decomposition for both sun-exposed and shaded carcasses are summarized in Table 7.8.

By day 16, a vast majority of the abdominal skin remained intact, and appeared wrinkled, brittle, mummified, and roasted. The complete desiccation of the skin resulted in few changes in the overall appearance of the carrion for the remainder of the experiment. However, vertebrate scavenging continued to occur at sun-exposed carrion, removing more skin and bones as the advanced decay stage progressed.

Table 7.8: Summary of stages of decomposition in summer and their associated characteristics.

Decay stage	Days Postmortem	Defining Characteristics
Fresh	Sun (0)	No odour
	Shade (0)	Fresh appearance
		Algor mortis
Bloated	Sun (1-4)	Bloated appearance, extreme bloating at end of stage
	Shade (1-4)	Rigor Mortis
		Discoloration (Cyan marbling)
		Moderate odour, stronger near body openings
		Maggots teeming inside body cavities
		Livor mortis
Active Decay	Sun (5-11)	Rupture of abdominal wall and body cavities
	Shade (5-11)	Infestation of larvae all over body
		Liquefaction of tissues
		Strong, putrid odour
		Evidence of black putrefaction
		Deflation initiating at extremities
		Full deflation half way through stage
		Skin discoloration from heat (orange-brown)
		Dehydration of skin and extremities beginning
		First major migration of post-feeding larvae
Advanced Decay	Sun (12-25)	Skin completely dehydrated, signs of mummification
	Shade (12-25)	Odour moderate, resembling smell of charred skin
		Removal of flesh at extremities (head, limbs, anus)
		Bone exposure evident at extremities
		Larval migration continuing
		Decrease in insect activity
Dry/Remains	Sun (26+)	Odour negligible, resembling smell of charred skin
	Shade (26+)	Remaining skin brittle and rigid
		Skin easily separated from bone

The beginning of the dry/remains stage in summer was difficult to distinguish from the end of the advanced decay stage. There were no discernible events or gross morphological changes that marked the separation. However, by day 26 the odour of decay was slight at sun-exposed sites, resembling the smell of burnt skin. Approximately 30 % of the skeleton was exposed on sunlit carrion, mostly due to scavenging. Remaining skin was extremely brittle and could be easily separated from the skeleton (Figure 7.14). At the shaded sites, the outer skin remained intact and preserved, although rigid (Figure 7.15). The odour of decay was negligible. A saw-dust-like residue was observed in the abdominal region of all carcasses by day 34 (Figure 7.16). Final collections were made on day 43. However, a return to the carcasses 99 days after death yielded no forensically significant insects and few changes in the overall appearance of the carrion (Figure 7.17 and Figure 7.14).

Figure 7.14: Sun-exposed carcass in dry/remains in summer (day 26). Evidence of scavenging is easily visible on left forelimb and skull.

Figure 7.15: Shaded carcass in dry/remains in summer (day 26). There is extensive retention of outer tissues and no evidence of scavenging.

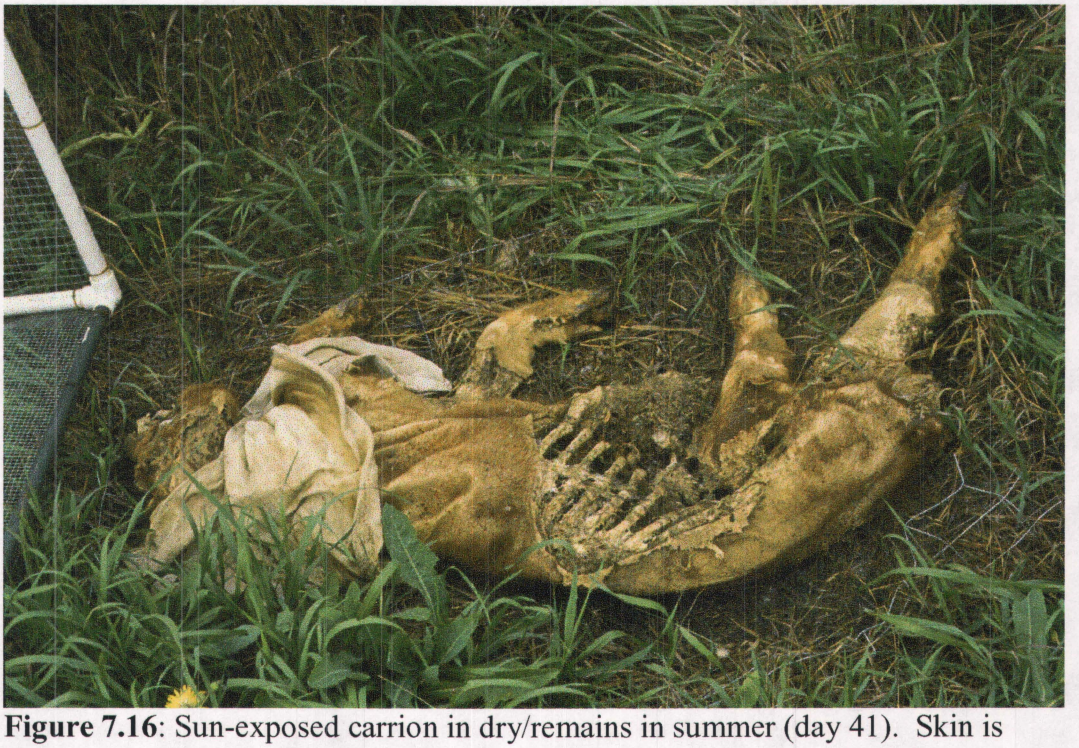

Figure 7.16: Sun-exposed carrion in dry/remains in summer (day 41). Skin is brittle and flaky. A sawdust-like residue from organ degradation is visible.

Figure 7.17: Sun-exposed carcass in dry/remains in summer (day 99). With the exception of additional skin degradation and further desiccation, there are few gross morphological changes from day 26 to 99 (compare with Figure 7.14).

7.2.3 Insect Succession

The first day of the summer experiment was warm and sunny. Fly activity occurred immediately upon the arrival of the carcasses in the covered flatbed. Oviposition occurred on some carrion before placement on the mesh underlay. Only blow flies were collected on day 0 (Table 7.9). Fly activity was higher at the sun-exposed sites and three species were collected: *C. macellaria*, *P. regina*, and *P. sericata*. At the shaded site, *C. macellaria* was the only insect collected.

By day 1 there were dozens of blow flies on every carcass. In the bloated stage, *C. macellaria* remained the dominant fly at shaded carrion, followed by *Musca domestica* (Muscidae). There was a greater diversity of flies at the sun-exposed carrion, which additionally attracted *P. regina*, *P. sericata*, and *Agria housei* (Shewell) (Sarcophagidae). At least one other species of flesh fly was observed at the sunlit carrion, but unfortunately never collected. At both habitats,

P. casei arrived at the end of the bloated stage on day 4. Although all of these flies were collected in the near vicinity of the carrion, only the blow flies were actually observed on the carrion proper. By day 3 small maggot masses had developed on various locations of the carcasses. By day 2, the carrion beetles *H. ramosa* and *T. lapponicus* were collected from sun-exposed and shaded carrion, respectively.

Table 7.9: Forensically important insects collected from pig carcasses in summer in the fresh, bloated, and active decay stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Fresh (0)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
				Phaenicia sericata	ad
Shade	Fresh (0)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
Sun	Bloated (1-4)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad, imm
				Phaenicia sericata	ad
			Sarcophagidae	*Agria housei*	ad
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Heterosilpha ramosa*	ad
Shade	Bloated (1-4)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad
Sun	Active Decay (5-11)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad, imm
				Phaenicia sericata	imm
			Sarcophagidae	*Agria housei*	ad
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
				Parapiophila sp.	ad
			Phoridae	*Megaselia sp.*	ad
			Sepsidae	*Meroplius stercorarius*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
Shade	Active Decay (5-11)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Calliphora vicina	ad
			Muscidae	*Morellia sp.*	ad
				Musca domestica	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm

ad=adult, imm=immature; * Elapsed Time Since Death (days)

The active decay stage was characterized by an increased diversity of Dipteran colonizers, especially at the exposed sites. On day 6, new arrivals at the sun-exposed sites included *Megaselia* (Phoridae), *Parapiophila* (McAlpine) (Piophilidae), and *Meroplius stercorarius* (R.-D.) (Sepsidae). At the shaded sites, *Morellia* (R.-D.) (Muscidae) and *C. vicina* arrived on day 6 and day 8, respectively. Larval identifications demonstrated the presence of immature *C. macellaria* in both habitats, and immature *P. sericata* and *P. regina* at the sun-exposed sites. However, *P. sericata* was not identified from laboratory rearing of larvae. The number of adult *P. casei* and *M. domestica* also increased in the active decay stage in both habitats. Immature and adult *T. lapponicus* remained the only coleopterans to colonize the remains during this stage. Immature silphids first appeared on day 8 in both habitats.

In the advanced decay stage the diversity of insects decreased dramatically (Table 7.10). Daily maximum temperatures were often above 30°C, approaching as high as 37°C. Many of the developing larvae were showing signs of heat stress. The rapid depletion of the carrion would have made competition for resources intense. Immature *P. casei* were abundant at the shaded sites, and larvae were observed jumping in the characteristic spring-like manner for this species. Interestingly, the dominant blow fly larvae differed between shaded and sunlit carrion during this stage. At the shaded carcasses *C. macellaria* dominated, while *P. regina* was in the majority at the sun-exposed carrion.

Coleopterans were restricted to carrion beetles on the sun-exposed carcasses during the advance decay stage. Both adult and larval *H. ramosa* and *T. lapponicus* were collected from sun-exposed carrion. The return of *H. ramosa* occurred on day 12 and larvae of this species were collected on day 26. On day 14, adult Staphylinidae (subfamily Aleocharinae) were first collected on shaded carrion. Immature Staphylinidae were collected on day 16 and included larvae of *C. maxillosus*. Although adult *C. maxillosus* were never collected in summer, their offspring confirm their presence. Due to the high daily temperatures in summer, it is likely that adults were hidden underneath or deep within the carcasses. Compared to spring, the advanced decay stage in summer had a very

low diversity of coleopteran species. Many underdeveloped fly larvae were either dying or leaving the carcass for a new resource, and may account for the lack of predaceous beetles. Additionally, many of the sun-exposed carrion had been partially scavenged during this stage and may also account for the lowered diversity.

Table 7.10: Forensically important insects collected from pig carcasses in summer in the advanced decay and dry/remains stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Advanced Decay (12-25)	Diptera	Calliphoridae	*Phormia regina*	ad, imm
			Muscidae	*Morellia sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	ad
Shade	Advanced Decay (12-25)	Diptera	Calliphoridae	*Cochliomyia macellaria*	imm
			Piophilidae	*Piophila casei*	ad, imm
			Phoridae	*Megaselia sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
			Staphylinidae	*Creophilus maxillosus*	imm
				Lobrathium sp.	ad, imm
Sun	Dry/ Remains (26+)	Diptera	Calliphoridae	*Phormia regina*	ad
			Muscidae	*Hydrotaea sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	imm
			Nitidulidae	*Carpophilus sp.*	ad
Shade	Dry/ Remains (26+)	Diptera	Calliphoridae	*Calliphora vicina*	ad
			Calliphoridae	*Cochliomyia macellaria*	ad
			Muscidae	*Hydrotaea sp.*	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
			Phoridae	*Megaselia sp.*	ad
				Phora sp.	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
			Staphylinidae	*Creophilus maxillosus*	imm
				Lobrathium sp.	imm
			Cleridae	*Necrobia rufipes*	ad
			Nitidulidae	*Carpophilus sp.*	ad
			Histeridae	*Hister furtivus*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

In the dry/remains stage, beetles increased in number and diversity. Clerids were the first newcomers at the shaded site, arriving on day 26. Histerids appeared on day 32 at the shaded sites, but were never abundant. Sap beetles

(Nitidulidae) first arrived on day 26 and day 34 at sun-exposed and shaded carrion, respectively. Interestingly, scarab beetles were never collected from carrion in either habitat. The number of larval Silphidae and Staphylinidae were extremely abundant on carrion in both habitats, feeding on remnant dipteran larvae. These two families remained the dominant coleopterans for the remainder of the experiment.

7.3 Experiment-1: Fall

7.3.1 Temperature

Ambient temperatures in fall exhibited the most daily fluctuation and the coolest average compared to spring and summer. Ambient temperature readings for the sun-exposed and shaded sites are presented in Figures 7.18 and 7.19, respectively. The average ambient temperature in both habitats did not differ significantly ($p = 0.01$). Generally, the shaded site demonstrated less fluctuation in temperature than the exposed site, especially in October. In both habitats, the average temperature in September was just above 12°C, whereas the average temperature in October was just above 5°C.

The data-logger measuring internal carcass temperature at the shaded site was never recovered at the end of the experiment. The surrounding soil and leaf litter and the carcass were searched extensively in an effort to find the data logger. Extensive scavenging of this carcass may have led to the disappearance of the data logger. The average internal carcass temperature for the duration of the experiment at the sun-exposed site was 10.0°C (Figure 7.20). In September, the average internal temperature was 14.6°C. Maximum temperatures were recorded on September 17th (day 16), after which the internal temperature mimicked ambient. The average internal temperature in October was considerably lower, averaging 4.5°C.

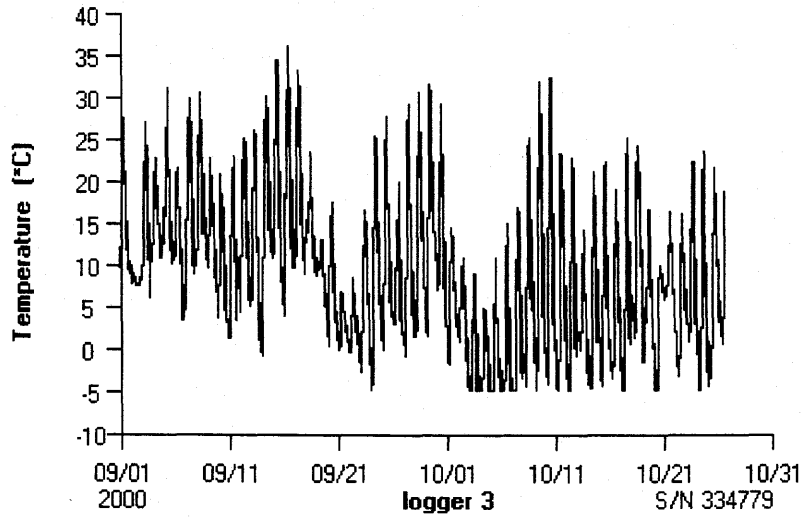

Figure 7.18: Ambient temperatures at sun-exposed site in fall. Average temperature from September 1st to October 26th was 9.00°C (± 0.17 (SE); n = 2669).

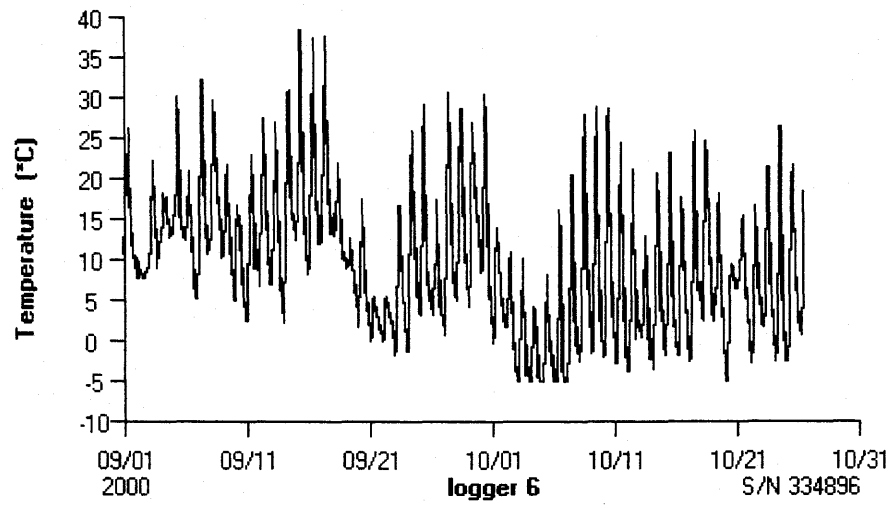

Figure 7.19: Ambient temperature at shaded site in fall. Average temperature from September 1st to October 26th was 9.36°C (± 0.16 (SE); n = 2669).

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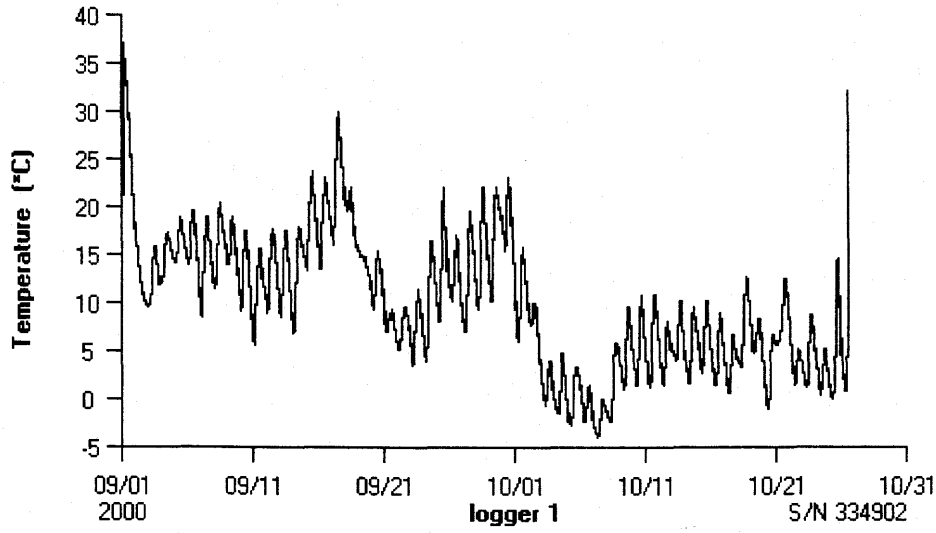

Figure 7.20: Internal carcass temperatures at sun-exposed site in fall. Average temperature from September 1st to October 26th was 10.0°C.

7.3.2 Decomposition

Although the pigs used in the fall experiment were smaller than in previous experiments and some had hernia deformities, they remained within the mid-weight range established by Komar and Beattie (40) as the most appropriate size model for human decomposition. However, the smaller pig size may have had an impact on the rate of decay, an effect that was not quantified in this experiment. On day 0, fly activity was abundant and diverse. Flies were seen feeding at the thoracic wound of most carcasses even before placement on the mesh underlay. The rainy, overcast, and cool weather of days 1 to 4 limited fly activity and bloating. By day 3, a very slight distension was visible in the necks and hernias of sun-exposed carrion. Similar bloating was observed on shaded carrion on day 4. Both ambient and internal carcass temperature readings for each decompositional stage are summarized in Table 7.11. The average temperature in the fresh stage was 12.6°C and 12. 8°C for the sun-exposed and shaded sites, respectively. After death, the carcasses cooled at a slow rate, resulting in a higher average internal temperature during the fresh stage.

During the bloated stage, the wounds of all carcasses became gaping holes filled with developing larvae. Bloating had extended to the remainder of the body by day 4 in sunlit carrion and day 6 in shaded carrion. Carcasses in either habitat never reached the same degree of bloating exhibited in carrion in the previous seasons. There was little disparity between the decomposition of shaded and sun-exposed carcasses from the bloated stage onward. Lividity was apparent on all carcasses by day 4. By day 5, the odour of decay remained negligible. Bloating peaked on day 8 and skin discoloration became evident. By day 8, large maggot masses infested all orifices of the body and the artificial thoracic wound. Temperatures were slightly higher in the bloated stage, averaging 14°C in both habitats (Table 7.11). Internal carcass temperature mimicked ambient during the bloated stage, but exhibited less daily fluctuation.

Table 7.11: Summary of ambient and internal carcass temperatures in each decay stage in fall

		Temperatures (°C)				
Habitat	Decay Stage (Days Postmortem)	Ambient Temp. Range	Ambient Temp. Avg.	Internal Temp Range	Internal Temp Avg.	Precipitation (mm)
Sun	Fresh (0-2)	6.3 - 27.5	12.6	9.6 - 36.8	17.7	13.8
Shade	Fresh (0-3)	7.6 - 26.0	12.8	lost data	lost data	
Sun	Bloated (3-10)	1.4 - 30.8	14.0	5.6 - 20.4	14.5	0.4
Shade	Bloated (4-10)	2.4 - 32.1	14.3	lost data	lost data	
Sun	Active Decay (11+)	-4.6 - 35.8	9.0	-3.8 - 36.8	8.5	7.9
Shade	Active Decay (11+)	-4.8 - 38.2	9.3	lost data	lost data	

The first evidence of liquefaction occurred on day 11 and coincided with the initiation of deflation (Figure 7.21). Maggots had infested the entire head region of the carcasses. In other areas, larvae remained inside the body cavities. A putrid stench was associated with the remains by day 11. One-sun-exposed carrion was slightly scavenged by a magpie on day 11. This carcass became increasingly scavenged by a variety of animals throughout the experiment. A badger had built a den near the shaded carrion and continually attacked two of the carcasses. The extensive scavenging resulted in variability in the decay rates of these carrion.

Figure 7.21: Shaded carcass on first day of active decay in fall (day 11). Accumulated gases have forced developing larvae and putrefactive liquids out of the thoracic wound and natural orifices.

Deflation occurred slowly and varied widely among all of the carcasses. However, all of the pigs were 50% deflated by day 15, and 95% deflated by day 21. By day 17, the skin and surrounding soil blackened and the head and neck regions were overcome with decompositional fluids (Figure 7.21). Maggot activity could be seen underneath the skin on day 19. All developing larvae had returned to the inner cavities of the carcass and underneath clothing at sun-exposed carcasses. These areas provided protection from the cold September nights, dropping below –5.0°C at the sun-exposed sites. On sunny, warm days, maggots would reappear outside of the cavities (Figure 7.23). In comparison, the shaded site experienced higher evening temperatures and larvae could still be exposed to air without lethal effects. Thus, larvae at shaded carrion were visible outside of the carcass for a longer period of time.

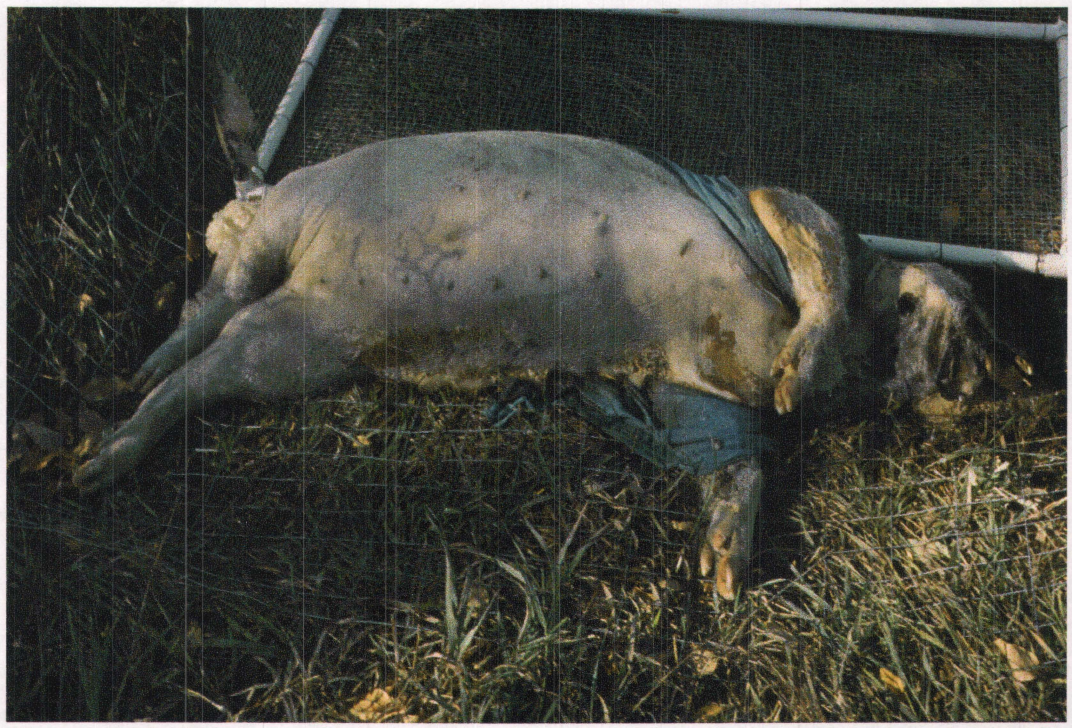

Figure 7.22: Sun-exposed carcass in active decay in fall (day 19). Liquefaction is evident in several areas and the carcass remains bloated in the abdominal region.

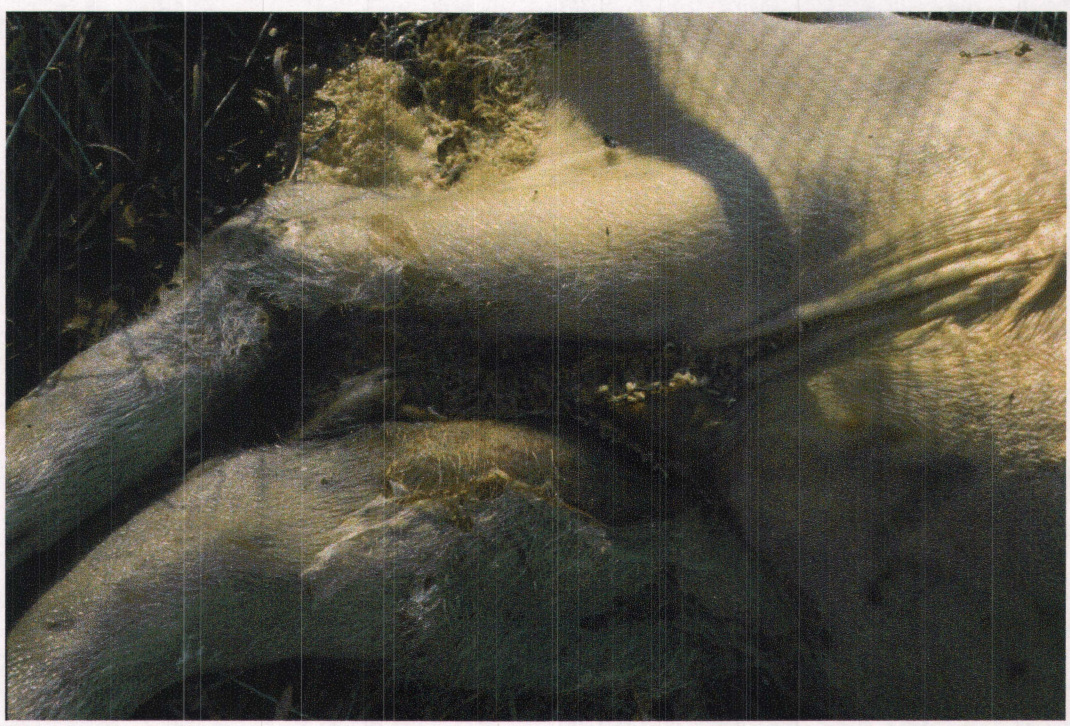

Figure 7.23: Sun-exposed carcass in active decay in fall (day 26). Larvae have reappeared on outer tissues of the carcass on a warm, sunny day.

Beyond day 22, decomposition varied from the typical patterns. The head of the carcasses dehydrated, exposing the cranium. By day 26, the anterior portion of the carcass appeared to be in the advanced decay stage. Meanwhile, putrefaction continued in the abdominal region, producing frothy liquid and further blackening the soil and outer edges of the skin. A mid-ventral seam was created by internal maggot activity where the abdomen met the ground (Figure 7.24). The remainder of the abdominal skin was intact. By day 31, the outer skin on all of the carcasses had become leathery and hard. Once the skin was dry and rigid, the carcass could virtually be lifted in half at the mid-ventral seam.

Figure 7.24: Sun-exposed carcass in active decay in fall (day 34). Internal larval activity has created a seam where the abdomen meets the ground.

On the inside of the carcass, active decay continued at a slow rate, and various larvae were continuing development in the wet tissues (Figure 7.25). In October (day 30+), the cold ambient temperatures slowed decay to a near halt. The average temperature in October was approximately 5°C in both habitats. Evening frost was also a common occurrence in October. Between day 31 and day 54 there were few overall changes in the appearance of the carrion (Figure 7.26). Final collections were made on day 54.

Figure 7.25: Close-up of inner tissues of sun-exposed carrion in fall (day 34). Calliphorid larvae develop in the moist, protective environment under the skin.

Figure 7.26: Sun-exposed carcass on final day of experiment in fall (day 54). There are very few observable changes from day 34 (compare with Figure 7.24).

Due to the continual decrease in ambient temperatures in the fall experiment, decomposition followed a unique pattern. During the observational period of the experiment, the carcasses progressed through only three stages of decay. The characteristics of these stages are summarized in Table 7.12. Carcasses continued in active decay stage from day 11 onward, although decomposing at an extremely slow rate. Although this experiment did not last long enough to observe complete preservation of the carrion, sub-zero temperatures typically experienced in November and the remainder of the winter would have prevented the progression of decay. The following spring, decomposition and insect activity would be resumed when temperatures rose to appropriate levels for such activity.

Table 7.12: Summary of stages of decomposition in fall and their associated characteristics.

Decay stage	Days Postmortem	Defining Characteristics
Fresh	Sun (0-2)	No odour
	Shade (0-3)	Fresh appearance
		Algor mortis
Bloated	Sun (3-10)	Bloated appearance
	Shade (4-10)	Discoloration (Cyan marbling)
		Rigor Mortis
		Livor mortis
		Moderate odour, stronger near body openings
		Maggots teeming inside body cavities
Active Decay	Sun (11-54+)	Rupture of body cavities of head
	Shade (11-54+)	Strong, putrid odour
		Deflation of body, initiating in anterior regions
		Evidence of black putrefaction
		Larvae return to body cavities at mid-point in stage
		Increasing dehydration of abdominal regions
		Remaining skin mummified half way through stage
		Anterior regions of body with significant flesh removal
		Bone exposure of skull
		Eventual preservation of body in sub-zero weather

7.3.3 Insect Succession

The delivery day of the fall experiment was sunny and temperate. Fly activity was observed immediately after placement. Blow flies were feeding at all of the orifices and the thoracic wounds. Days 1 and 3 were cold and rainy and severely limited fly activity. Day 2 was also cool and overcast, but precipitation was minimal. Oviposition was first observed on day 2. There was a greater diversity of flies attracted to the carrion in the fresh stage compared to other seasons. At the sun-exposed site, *C. macellaria*, *C. cadaverina*, *P. regina* and *Phaenicia caeruleividiris* (Macquart) were collected (Table 7.13). At the shaded site, *C. macellaria*, *L. illustris*, and *P. caeruleividiris* were collected.

Table 7.13: Forensically important insects collected from pig carcasses in fall in the fresh and bloated stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Fresh (0-2)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Cynomya cadaverina	ad
				Phaenicia caeruleividiris	ad
				Phormia regina	ad
Shade	Fresh (0-3)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Lucilia illustris	ad
				Phaenicia caeruleividiris	ad
Sun	Bloated (3-10)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad, imm
				Phaenicia caeruleividiris	ad
				Cynomya cadaverina	ad
				Lucilia illustris	ad
			Muscidae	*Morellia sp.*	ad
			Piophilidae	*Piophila casei*	ad
			Phoridae	*Parapiophila sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad
			Staphylinidae	*Lobrathium sp.*	ad
Shade	Bloated (4-10)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Lucilia illustris	ad, imm
				Phaenicia caeruleividiris	ad
		Coleoptera	Silphidae	*Heterosilpha ramosa*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

The bloated stage coincided with the arrival of several new species. The first to arrive were the carrion beetles. On day 4, *T. lapponicus* was collected from the sunlit carrion and on day 5, *H. ramosa* was collected at the shaded carrion. Adult Staphylinidae, in the subfamily Aleocharinae, were also collected on day 4 at the sunlit site. Members of the families Phoridae and Muscidae arrived at sunlit carrion on day 4 and day 8, respectively. The blow fly *L. illustris* and the cheese skipper *P. casei* arrived on day 11 at sunlit carrion. There was far less diversity in insects at the shaded site; silphid beetles were the only new insect to arrive during the bloated stage.

At the beginning of the active decay stage, core maggot mass temperatures ranged between 35-36°C in both habitats. These high temperatures were not reflected in the internal temperature readings from the sun-exposed carcasses. From day 19 to 23, core temperatures decreased, ranging between 19-23°C. Pupae were first observed on the one shaded carcass without extensive scavenging on day 19. Attempts at rearing the pupae were unsuccessful. However, larval rearing from collections on day 19 resulted in the emergence of two species of blow flies: *L. illustris* and *C. macellaria*. It is possible that pupae had entered diapause due to the shortened day length and low temperatures.

At the sun-exposed sites, pupae were first collected on day 21. Rearing attempts were also unsuccessful. Larval rearing from collections resulted in the emergence of *P. regina* and *C. macellaria*. After day 19, insect activity decreased dramatically. Adult blow flies were rarely seen after day 21. Several muscid flies were collected from the sun-exposed site during active decay, including *Hydrotaea* and *Morellia* (Table 7.14). Adult *P. casei* were collected on day 11 and day 13 at the sun-exposed and shaded sites, respectively. Although immature specimens were never collected, they may have been developing deep inside the carcass. The cheese skipper remained a dominant insect in both habitats until day 37 and was not collected past day 39.

The silphid *O. noveboracense* was observed at the shaded carrion on day 19, but unfortunately was too fast to collect. This silphid was first collected at sunlit carrion on day 23. Immature Silphidae were occasionally collected from

sun-exposed sites starting from day 26, but were never recovered from shaded sites. The lack of larval representation of Silphidae at the shaded sites may be a result of the extensive perturbation by badgers. The Scarabaeidae, represented by a single genus in both habitats, were first collected on day 26. The scarab beetles were typically hovering over the carcass or feeding in groups inside the carrion tissues. Throughout October, they were the most common beetles at sun-exposed carrion. They were also common at shaded carrion but were observed in much lower numbers. By the end of the experiment, the only insects at shaded carrion were developing *C. macellaria* larvae. The sun-exposed carrion had the addition of *Onthophagus*, which remained abundant even on the last day of collection.

Table 7.14: Forensically important insects collected from pig carcasses in fall in the final stages of decomposition

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Sun	Active Decay (11-54+)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad, imm
				Calliphora vomitoria	ad
				Calliphora vicina	ad
				Phaenicia sericata	ad
			Muscidae	*Hydrotaea sp.*	ad
				Morellia spp.	ad
				not identified beyond family	ad
			Piophilidae	*Piophila casei*	ad
				Parapiophila sp.	ad
		Coleoptera	Silphidae	*Oiceoptoma noveboracense*	ad
				Thanatophilus lapponicus	imm
			Staphylinidae	*Creophilus maxillosus*	ad
			Scarabaeidae	*Onthophagus sp.*	ad
Shade	Active Decay (11-54+)	Diptera	Calliphoridae	*Cochliomyia macellaria*	imm
				Lucilia illustris	imm
				Protophormia terraenovae	imm
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Oiceoptoma noveboracense*	ad-o
			Staphylinidae	*Creophilus maxillosus*	ad
			Scarabaeidae	*Onthophagus sp.*	ad

ad=adult, ad-o=observed adult (not collected) imm=immature; * Elapsed Time Since Death (days)

7.4 Experiment-2: Clothed Versus Nude Carrion

7.4.1 Temperature

Ambient temperatures were taken from the sun-exposed carrion in Experiement-1 and are depicted in Figure 7.9. The average ambient temperature for the duration of Experiment-2 (July 19th to August 31st) was 19.5°C. Internal carcass temperatures were taken for each type of pig, both clothed and nude (Figure 7.27 and 7.28).

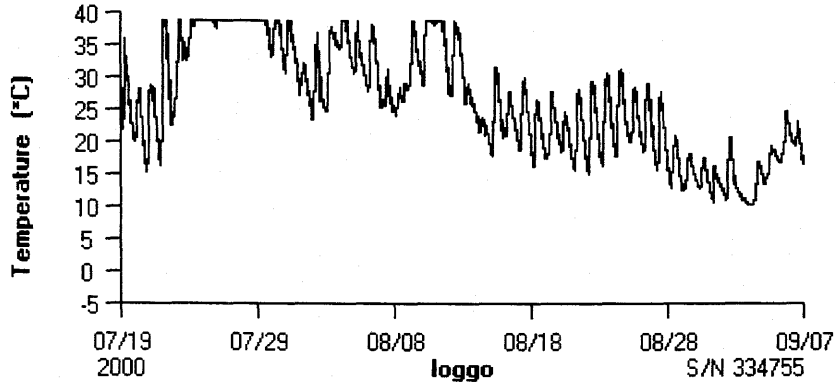

Figure 7.27: Internal carcass temperature of heavily clothed carrion in summer. Average temperature from July 19th to August 31st was 27.69°C (± 0.17 (SE); n = 2093).

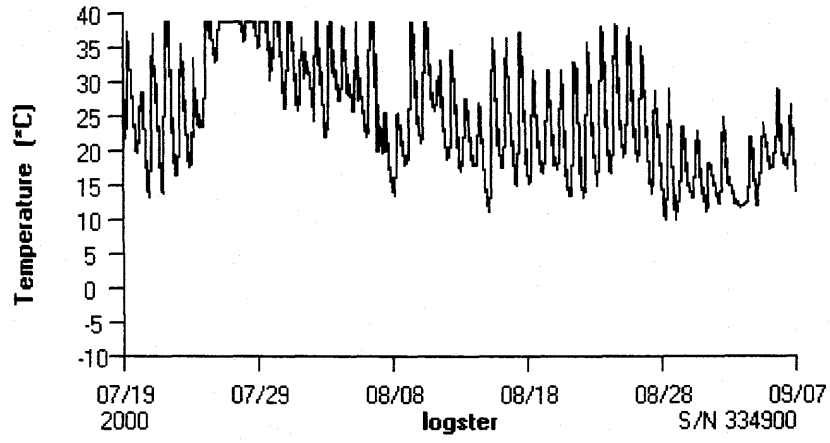

Figure 7.28: Internal carcass temperature of nude carrion in summer. Average temperature from July 19th to August 31st was 25.91°C (± 0.17 (SE); n = 2093).

A comparison of the data sets revealed that the average internal temperatures for clothed and nude pigs were significantly different ($p = 0.01$). Generally, the clothed carrion experienced less fluctuation and longer periods of maximum internal temperatures than nude carrion. Furthermore, clothed carrion retained maximum temperatures at night more often than nude carrion. Interestingly, the internal temperature for nude carrion was 4.3°C higher than sun-exposed carrion in summer, but only 0.4°C higher than shaded carrion. The internal carcass temperatures of clothed carrion were 6.7°C and 2.2°C higher than summer sun-exposed and shaded carrion, respectively. However, internal carcass temperature trends of nude carrion more closely approximated summer sun-exposed carrion, whereas the clothed carrion approximated the summer shaded carrion.

7.4.2 Decomposition

Decomposition of the heavily clothed and nude carrion were very similar to the decomposition of summer sun-exposed and shaded carrion described in section 7.2.2. Bloat occurred on day 1. By day 2, all carcasses were significantly bloated in all body regions. By day 5 the bloat was so severe that the organs burst through the abdominal wall. However, on the clothed carrion, the organs were not exposed to the air. The carcasses expanded to twice their original size and filled the entire cage.

Some clothing was partially displaced during bloat. Two of the pigs were clothed in pants and a sweatshirt. However, on only one carcass did the pants become displaced, exposing the rear end. The displacement of the one pair of pants was largely due to the elasticity of the fabric. These pants were tight fitting and could not sustain their original placement upon distension of the abdomen. Alternatively, the other pair of pants was loose fitting and could accommodate the expansion of the abdomen. Sweaters were also partially pushed up exposing the abdomen. One of the clothed carrion had a dress on instead of a sweater and pants, which managed to remain in place for the longest period of time.

During the bloated stage, ambient temperature ranged from 6.1°C to over 38°C (Table 7.15). The average temperature was only 21.3°C, a reflection of the cool evening temperatures. Internal temperatures did not decline as severely at night in the clothed carrion, resulting in a 1.7°C higher average temperature. There was no precipitation in either the fresh or bloated stages of decay.

Table 7.15: Summary of ambient and internal carcass temperatures in each decay stage for clothed versus nude carrion in summer

		Temperatures (°C)				
Habitat	Decay Stage (Days Postmortem)	Ambient Temp. Range	Ambient Temp. Avg.	Internal Temp. Range	Internal Temp. Avg.	Precipitation (mm)
Nude	Fresh (0)	8.6 - 28.4	19.8	21.6 - 37.0	29.8	0.0
Clothed	Fresh (0)	8.6 - 28.4	19.8	21.5 - 35.5	28.9	
Nude	Bloated (1-4)	6.1 - 38.4	21.3	13.1 - 38.3	24.3	0.0
Clothed	Bloated (1-4)	6.1 - 38.4	21.3	15.2 - 38.3	26.0	
Nude	Active Decay (5-11)	10.6 - 38.4	23.4	17.3 - 38.3	34.6	10.8
Clothed	Active Decay (5-13)	9.9 - 38.4	22.9	26.5 - 38.3	36.5	
Nude	Advanced Decay (12-25)	5.4 - 37.5	19.9	13.5 - 38.3	27.6	49.6
Clothed	Advanced Decay (14-32)	2.5 – 33.9	17.8	16.0 - 38.3	28.4	
Nude	Dry/Remains (26+)	0.1 - 38.4	17.2	9.9 - 38.1	21.5	3.0
Clothed	Dry/Remains (33+)	0.1 - 38.4	16.9	10.7 - 30.9	19.8	

On day 5, various areas of the carcass ruptured from maggot activity and gaseous pressure, signalling the start of active decay (Figure 7.29). Similar to the experiment-1 summer pigs, the carcasses did not deflate upon rupture. The edges of broken skin blackened from putrefaction and started to flake. The clothed carrion retained a great deal of moisture as the fabric absorbed the decompositional fluids. On day 6 deflation initiated in the head and neck of both clothed and nude carrion. On nude carrion, there was almost no part of the body not covered by huge maggot masses. The same was true for clothed carrion, although many of the developing larvae were underneath the clothing (Figure 7.30). By day 7, deflation had extended to the abdomen and buttocks on all carcasses except the one clothed in the dress. Abdominal deflation for this carcass occurred on day 8. Unlike the experiment-1 pigs, there was very little scavenging of the clothed or nude carcasses throughout the experiment.

Figure 7.29: Nude carcass in active decay in summer (day 5). The extensive bloating caused the rupture of the organs out of the abdominal wall.

Figure 7.30: Clothed carcass in active decay in summer (day 6). The clothing absorbs the putrefactive liquids and benefits the larvae developing underneath.

On day 7, the skin of the nude carcasses and exposed areas of clothed carcasses started to dehydrate from the intense heat. The skin turned orange-brown in colour and resembled the appearance of a roasted turkey. On nude carrion, larvae began to retreat to the underside of the carcasses and deep under the skin. The skin covered by clothing remained damp and provided protective areas for developing larvae. The added moisture also decelerated the desiccation of the carcasses. Exposure of the skulls of both clothed and nude carrion began on day 7.

During active decay, the highest ambient and internal carcass temperatures were recorded. The average ambient temperature throughout active decay was approximately 23°C (Table 7.15). Internal temperatures of clothed carrion remained almost 2°C higher than nude carrion. However, both clothed and nude carcasses experienced maximum internal temperatures, averaging more than 10°C over ambient. Clothed carrion experienced more frequent periods of maximum temperatures that lasted for longer periods of time. Furthermore, internal temperatures of clothed carrion never dropped below 26°C throughout the active decay stage.

Advanced decay started on day 12 for nude carrion and day 14 for clothed carrion. Exposed tissues were almost completely dehydrated (Figure 7.31). Tissues under clothing remained damp and frothy, thus delaying the onset of advanced decay (Figure 7.32). The stench of decomposition had decreased and changed to a dried, burnt odour. However, the smell remained stronger at clothed carrion in this stage.

For both types of carrion, post-feeding larvae began to leave in search of a suitable pupation substrate on day 12. Larvae travelled great distances to pupate, often over 3 metres away from the carrion. On clothed carrion, several dozen pupae were found attached to the underside of the fabrics on day 14. Pupae were never found in close association with the nude carrion. Thus, clothing provides a suitable substrate for pupation.

Figure 7.31: Nude carcass in advanced decay in summer (day 12). Exposed tissues are dehydrated and larvae have retreated to the underside of the carcass.

Figure 7.32: Clothed carcass in active decay in summer (day 12). Blow fly larvae continue development in the moist environment underneath the clothing.

The first wave of adult blow fly emergence occurred on day 15 at clothed carrion and day 16 at nude carrion. The later emergence at the nude carrion is likely a reflection of the near lethal temperatures experienced in core mass temperatures of developing larvae on these pigs. Similar to sun-exposed pigs in summer, core mass temperatures at nude carrion ranged from 40-44°C. The skin temperature of the nude carrion, without maggot mass heat generation, averaged 41°C in the active and advanced stages of decay. Alternatively, core temperatures of masses underneath clothing ranged from 35-38°C, similar to the findings of shaded carrion in summer. Thus, clothing provided a more optimal environment for developing larvae in summer.

Heat stressed maggots were found on top of clothing and at the nude carcasses. The extent of carcass desiccation was greater at the nude sites and left underdeveloped larvae scrambling for an alternative food source. Similar to sun-exposed carrion in summer, underdeveloped larvae were found underneath the grass in areas beside the carcasses. Protected by the vegetation, these sites were often 4-5°C cooler than the skin temperature of the carcass.

By day 22, the exposed skin of clothed carrion had completely dehydrated. The skin underneath clothed regions of the carcass was beginning to dehydrate, but still remained softer from the retention of decompositional fluids (Figure 7.33). Hair was still attached to remaining areas of skin underneath clothing. The pig clothed in the dress was decomposing at the slowest rate compared to other pigs. Generally, clothing acted like a layer of skin, preventing dehydration and allowing the completion of tissue liquefaction. More larvae were able to develop in the moist areas and this attracted more predaceous beetles. Thus, the active and advanced decay stages were extended in clothed carrion.

There were very few overall changes in the appearance of the nude carrion from day 14 to day 26, as the arid environment preserved the outer layer of remaining skin (Figure 7.34). By day 26 the odour of decay resembled the smell of burnt skin at nude carrion. At clothed carrion, the odour was stronger and more putrid-like. Nude carrion began to attract small scavengers that attempted to remove skeletal components.

Figure 7.33: Clothed carcass in advanced decay in summer (day 26). Exposed skin is dehydrated, although moisture remains underneath clothed areas.

Figure 7.34: Nude carcass in dry/remains stage in summer (day 26). There is extensive skeletal exposure and remaining skin has dehydrated.

During advanced decay, ambient and internal carcass temperatures declined from the previous stage (Table 7.15). Increased precipitation did not affect the nude carrion, but may have delayed the onset of the dry stage in clothed carrion. After thunderstorms, absorbent fabrics retained moisture until the following day. Daily maximum ambient temperatures were still high enough to evaporate the majority of moisture present in the clothing. However, the rainfall allowed the underlying skin and tissues to benefit from the added moisture, allowing continued larval development and putrefaction. At nude carrion, the outer skin acted as a preservative shield keeping inner tissues relatively dry. Internal carcass temperatures at nude carrion were similar to ambient, except the outer skin provided insulation during cool summer nights and prevented major fluctuation. The water retained by clothing probably acted as a thermal insulator, resulting in higher minimum internal temperatures in clothed carrion. Larval heat generation also contributed to high internal temperatures during early advanced decay, after which masses were rarely observed.

Similar to sun-exposed and shaded carrion in summer, the dry/remains stage did not possess definitive markers to distinguish the beginning of the stage. However, the start of the dry/remains stage was based on the following characteristics: major decrease in odour, increased bone exposure, desiccation of the majority of tissues, and skin separation from bone. Nude carrion entered the dry/remains stage on day 26, the same day as summer carrion in Experiment-1. Clothed carrion exhibited the same characteristics, although not until day 33. With the exception of further desiccation of the tissues, there were few, if any, gross morphological changes in the carrion between day 33 and the end of the experiment. Final collections were made on day 43.

7.4.3 Insect Succession

Fly activity occurred immediately upon arrival of the carcasses. Oviposition was observed even before placement on the mesh underlay. Two of the same species were collected from both nude and clothed carrion in the fresh stage (day 0): *C. macellaria*, and *P. regina* (Table 7.16). Additionally, *P. sericata* was also collected from nude carrion on day 0. All three of these blow fly species were present in the fresh stage on sun-exposed carrion in summer. At the start of the bloated stage on day 1, *M. domestica* arrived at both clothed and nude carrion. Similar to the blow flies, this muscid remained extremely abundant in the bloated stage and the early part of active decay. The cheese skipper *P. casei* arrived on day 2 at nude carrion and day 4 at clothed carrion. The arrival of *P. casei* at nude carrion predates its appearance on Experiment-1 summer carcasses by two days. The phorid *Megaselia* sp. arrived on day 4 at clothed carrion only. The carrion beetles first arrived on day 2 on both clothed and nude carrion. However, *T. lapponicus* was exclusive to clothed carrion in the bloated stage, similar to shaded carrion in summer.

A greater diversity of insects colonized the carrion in the active decay stage. On day 6, *Morellia* (Muscidae), *A. housei* (Sarcophagidae), and *Parapiophila* (Piophilidae) started colonizing nude remains. These insects remained exclusive to nude carrion. However, *Morellia* was the only common visitor. The blow fly *P. terraenovae* arrived on day 6 at clothed carrion. However, this blow fly was extremely rare in summer. At both clothed and nude carrion, *P. regina* and *C. macellaria* remained the dominant blow flies. Both *Meroplius stercorarius* and *Sepsidimorpha* sp. (Sepsidae) were initially collected on day 6 from both types of carrion. These flies peaked in abundance during the active decay stage.

Table 7.16: Forensically important insects collected from clothed and nude pig carcasses in summer in the fresh, bloated, and active decay stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Nude	Fresh (0)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
				Phaenicia sericata	ad
Clothed	Fresh (0)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
Nude	Bloated (1-4)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
				Phaenicia sericata	ad
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
		Coleoptera	Silphidae	*Heterosilpha ramosa*	ad
Clothed	Bloated (1-4)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Phormia regina	ad
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
			Phoridae	*Megaselia sp.*	ad
		Coleoptera	Silphidae	*Heterosilpha ramosa*	ad
				Thanatophilus lapponicus	ad
Nude	Active Decay (5-11)	Diptera	Calliphoridae	*Phormia regina*	ad
				Cochliomyia macellaria	ad, imm
			Muscidae	*Musca domestica*	ad
				Morellia sp.	ad
			Sarcophagidae	*Agria housei*	ad
			Piophilidae	*Piophila casei*	ad
				Parapiophila sp.	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
				Meroplius stercorarius	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
			Staphylinidae	*Creophilus maxillosus*	ad
			Scarabaeidae	*Aphodius sp.*	ad
Clothed	Active Decay (5-13)	Diptera	Calliphoridae	*Phormia regina*	ad, imm
				Protophormia terraenovae	ad
				Cochliomyia macellaria	ad, imm
			Muscidae	*Musca domestica*	ad
			Piophilidae	*Piophila casei*	ad
			Phoridae	*Megaselia sp.*	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
				Meroplius stercorarius	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
			Staphylinidae	*Creophilus maxillosus*	ad
			Scarabaeidae	*Aphodius sp.*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

The species and arrival times of coleopterans were nearly identical for clothed and nude carrion. Adult *T. lapponicus* were collected from nude carrion on day 5. The remainder of the new arrivals in the active decay stage were first collected on day 8 in both habitats, including immature *T. lapponicus* (Silphidae), *C. maxillosus* (Staphylinidae) and *Aphodius* sp. (Scarabaeidae) (Table 7.16). By far, the most abundant coleopterans in the active decay stage were both adult and immature *T. lapponicus*. This trend continued in the advanced decay stage in both habitats.

Massive maggot masses infested all carrion in active decay. The core temperature of masses under clothed areas was a minimum of 3°C cooler than masses at exposed areas or on nude carcasses. Thus clothing provided more favourable development conditions for blow flies and other dipterans. This environment allowed certain species to colonize the clothed carrion for longer periods of time. In particular, the dipteran larvae experienced lower mortality at clothed carrion. The rapid desiccation and lack of protective areas on nude carrion resulted in the death of several adult flies, dipteran larvae, and silphid larvae on nude carrion.

Fly activity decreased dramatically in the advanced decay stage, with the exception of emergence days. However, the number and diversity of beetles began to increase. This trend continued until the dry stage. In the advanced decay stage, *P. casei* was the most abundant dipteran at carrion except on blow fly emergence days. Immature *P. casei* were collected at shaded carrion on day 34, but never collected from nude carrion (Table 7.17). However, a gravid female was captured on day 41 from a nude carcass. It is possible that *P. casei* larvae were overlooked in the nude carrion collections. Blow fly emergence occurred in several waves, the first occurring between days 15-16. At nude carrion, only the blow fly *C. macellaria* emerged in massive waves. At clothed carrion, both *C. macellaria* and *P. regina* survived to emerge as adults. Adult *P. terraenovae* were never collected from nude carrion. However, pupae of this blow fly were collected on day 41, confirming the presence of this fly in advanced decay (Table

7.17). The sepsid flies disappeared from clothed carrion and were only rarely collected at nude carrion during this stage.

Table 7.17: Forensically important insects collected from pig carcasses (Experiment-2) in summer in the advanced decay and dry/remains stages of decomposition.

Habitat	Decay Stage (ETSD*)	Order	Family	Genus and Species	Insect Stage
Nude	Advanced Decay (12-25)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
			Piophilidae	*Piophila casei*	ad
			Sepsidae	*Sepsidimorpha sp.*	ad
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	ad
			Cleridae	*Necrobia rufipes*	ad
Clothed	Advanced Decay (14-32)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad, imm
			Piophilidae	*Piophila casei*	ad, imm
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
Nude	Dry/Remains (26+)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad
				Protophormia terraenovae	imm
			Piophilidae	*Piophila casei*	ad, imm
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	imm
				Heterosilpha ramosa	imm
			Cleridae	*Necrobia rufipes*	ad
				not identified beyond family	ad
			Staphylinidae	*Creophilus maxillosus*	ad
Clothed	Dry/Remains (33+)	Diptera	Calliphoridae	*Cochliomyia macellaria*	ad, imm
				Phormia regina	ad
			Muscidae	*Fannia sp.*	ad
			Piophilidae	*Piophila casei*	ad, imm
		Coleoptera	Silphidae	*Thanatophilus lapponicus*	ad, imm
				Heterosilpha ramosa	ad, imm
			Cleridae	*Necrobia rufipes*	ad
			Staphylinidae	*Creophilus maxillosus*	ad

ad=adult, imm=immature; * Elapsed Time Since Death (days)

The faster desiccation of the nude carrion hastened the arrival of the clerid *N. rufipes*. On nude carrion, *N. rufipes* arrived on day 12, but did not arrive on clothed carrion until day 41 (Table 7.17). Immature *T. lapponicus* were extremely abundant during advanced decay, especially on clothed carcasses. Groups of 15-20 larvae were often observed feeding in several areas underneath clothing.

Although a wide diversity of insects was collected in the dry/remains stage, immature silphids remained the most abundant on both clothed and nude carrion. Immature *H. ramosa* were collected from both nude and clothed carrion on day 32 and day 34, respectively. Other than remnant developing larvae and emergence of new adult blow flies and cheese-skippers, most other insects collected were rare and not observed on all carrion. The only exception was *N. rufipes*, which was a common visitor to nude carrion throughout the dry/remains stage.

CHAPTER 8: DISCUSSION

This study examined decomposition and insect succession on pig carrion in three different seasons (spring, summer, and fall) and two different habitats (sun and shade). The effect of clothing on decay and faunistic patterns was also investigated. It was hypothesized that the variables of season, habitat, and clothing would impact the rate of decomposition and the pattern of insect succession. Specifically, it was theorized that shaded carrion would decompose at a slower rate than sun-exposed carrion and exhibit a unique carrion fauna. It was assumed that carrion in summer would decompose at a faster rate than carrion in fall or spring and insect colonization times would be reduced. It was also hypothesized that clothing would create a unique microclimate, attracting a distinctive succession of insects.

8.1 Experiment-1

8.1.1 Habitat Differences

Previous research on the effect of habitat has been sparse. DeSouza and Linhares (136) examined two small pig carcasses in both shade and sunlight over four seasons. However, they did not make observations on decomposition and were mainly concerned with the comparative analysis of the seasonal abundance of blow flies. Furthermore, they did not report the differential insect patterns associated with sunlit and shaded habitats. Shean *et al* (19) specifically examined the effect of direct sunlight versus shade on carrion decomposition and insect colonization. However, they examined only one carcass in each habitat. Thus, their results may be anomalous, as they were not subject to replication. Shean *et al* (19) focused on blow fly activity, although the initial arrival of other important insects was reported. Dillon and Anderson (137) performed a similar

investigation to the present study. However, results from this study are still preliminary.

The present study examined three carcasses per habitat to avoid anomalous observations. Furthermore, the importance of coleopteran species in addition to dipteran taxa was highlighted. Similar to Shean *et al* (19), detailed visual observations on the decompositional process sacrificed the presentation of a more comprehensive species list. Although many other species were collected, their association with the carrion was incidental and not forensically significant. Incidental insects were not reported in the results.

8.1.1.1 Temperature

Similar to the findings of Shean *et al* (19), shaded site temperatures were typically higher in the evenings and fluctuated less than sun-exposed sites in all seasons. The differences in average ambient and internal carcass temperature are compared in Table 8.1. Only in the spring season did the ambient temperatures between sun-exposed and shaded habitats significantly differ ($p = 0.01$). This contributed to the greater differences in the decay rate between sun-exposed and shaded carrion in the spring season. In summer and fall, ambient temperatures in the two habitats demonstrated no significant differences ($p = 0.01$). The similarities between the ambient temperature readings of both habitats in fall may be due to the decreasing day length and exposure to sunlight as the season progresses. Thus, sun-exposed sites may not gain a significant level of heat over that of shaded sites.

Table 8.1: Comparison of habitat and seasonal differences in ambient and internal carcass temperatures.

	Ambient Average		Internal Average	
	Sun	**Shade**	**Sun**	**Shade**
Spring	17.8	15.4	26.0	18.2
Summer	19.5	19.1	21.6	25.5
Fall	9.0	9.4	10.0	not available

Shean *et al* (19) concluded that ambient air temperature was the chief factor influencing carrion decomposition. These findings are confirmed by the

present study, as the decay rate of sun-exposed carrion differentiated from shaded carrion only when the ambient temperature was different between the two habitats.

Enormous variation was observed in the seasonal difference of the internal carcass temperatures of both habitats (Table 8.1). In spring, the sun-exposed carrion had a 7.8°C higher average internal temperature than the shaded carrion. In contrast, the shaded carcasses in summer experienced higher internal temperatures, averaging a 3.9°C increase over sun-exposed carrion. Unfortunately, the internal carcass temperatures of shaded carrion in the fall season were lost, preventing comparison. Summer carcasses progressed through the stages of decay at the same rate, even though the shaded carrion had significantly higher internal temperature readings. Thus, internal carcass temperature is not highly related to the rate of decay. However, internal temperatures are a factor of larval heat generation and a factor for the development rate of larvae.

Several authors have confirmed that internal temperatures of carrion are elevated over ambient during decomposition due to bacterial metabolic reactions (13, 15, 16, 19). The movement and metabolic activity of maggots, when formed in masses, also generates thermal energy and contributes to the overall elevation of internal temperatures (3, 14, 42). In the present study, maximum internal temperatures without daily fluctuations always coincided with the presence of 3rd instar blow fly larvae. Subsequent drops in temperature coincided with the migration of post-feeding larvae. However, the presence of 3rd instar larvae did not always result in maximum temperatures for extended periods. Regardless of habitat, the initial peaks in internal temperature always occurred during the active decay stage of decomposition. These observations confirm the findings of Anderson and VanLaerhoven (13) and Early and Goff (12).

The level of heat generation is specific to each habitat. Large maggot masses were observed on shaded carrion in spring in the beginning of active decay. However, internal temperatures reached a maximum of only 6°C over ambient from the bloated stage until the first pupae were recovered. During this period the ambient temperature was often higher than the internal temperature,

also reaching a maximum of 6°C over internal. In contrast, the internal carcass temperature of sun-exposed carrion during this same period reached as high as 23°C over ambient. These observations confirm the findings of Turner and Howard (42), who also noted inconsistencies in the level of elevation of internal temperatures over ambient in different habitats.

8.1.1.2 Decomposition

There are few generalizations that can be made on the effect of shade or sunlight on decomposition in the Prairie Ecozone. Discrepancy in the rate of decay was only observed in spring and in the first two stages of decomposition in fall. Summer carrion, whether in shade or sunlight, decomposed at the same rate. These findings are contrary to Shean *et al* (19) who reported differential decomposition between shaded and sunlit pig carcasses in summer in Washington State. In addition to the different climates experienced in Washington compared to Saskatchewan, their summer study initiated three weeks prior to the start date of the present study and may also account for the different results. Preliminary results from British Columbia (137) are also contrary to the present study. Dillon and Anderson (137) observed a faster rate of decay in all sun-exposed carrion regardless of season or region.

Shaded carrion in spring and summer retained more abdominal skin and moisture than sun-exposed carrion. In summer, when the ambient temperatures were similar, these differences did not affect the rate of decay. Otherwise, there were no major differences in the pattern of decomposition, as carcasses in both habitats exhibited the same morphological changes, albeit at different rates in spring and fall.

8.1.1.3 Insect Succession

I theorized that the carrion fauna in the grassland ecoregion of Saskatchewan would be unique compared to other geographical regions. This hypothesis proved to be true, as many of the specific species collected in this study have not been reported on carrion elsewhere. The families of insects associated

with carrion and their time of arrival is somewhat similar to studies in other regions. For example, Rodriguez and Bass (16) report the arrival of silphid adults in the fresh stage, but most abundant in the bloated and decay stages. In the present study, carrion beetles were the first coleopterans to arrive on all carcasses, regardless of season or habitat. Rodriguez and Bass (16) also report immature silphids arriving in the decay stage and remaining abundant into the dry stage. This pattern was also evident in Saskatchewan with immature carrion beetles characteristic of active decay, advanced decay and dry/remains stages of decomposition. Larval silphids were never collected before active decay in any season or habitat. In direct contrast with the Saskatchewan faunal composition, several researchers (12, 13, 15) do not report the presence of any immature carrion beetles on carrion.

Habitat variations affected species diversity. Sun-exposed carrion attracted a greater diversity of species and a greater number of each species, compared to shaded carcasses in spring and fall. In spring, a total of 37 forensically significant species were collected from both habitats. Of the 37, 21 of the species were common to both sun-exposed and shaded habitats. The blow fly *C. vicina* was exclusive to sun-exposed carrion in spring, whereas *B. silvarum* was exclusive to shaded carrion. Both habitats were colonized by *Aphodius* sp. (Scarabaeidae), although the sun-exposed carrion also attracted the scarab *Phanaeus* sp.

In summer, insect diversity was reduced in both habitats. A total of 21 species were collected, 8 of which were common to both habitats. Shaded carrion attracted a greater diversity of insects in the advanced decay and dry/remains stage. This is likely a reflection of the higher levels of moisture that were retained on these carcasses. Both *C. maxillosus* and *Lobrathium sp.* were exclusive to shaded carrion. Most species demonstrated longer periods of colonization on shaded carrion. Interestingly, this was not a factor of a slower rate of decay, but of the potential of the carcass to remain an appropriate resource for insects.

In the fall, a total of 21 species were collected, although only 6 of these species were common to both habitats. Sun-exposed carrion had the greatest diversity of Calliphoridae colonizing the remains. However, several of these

species were rare visitors, including *P. sericata*, *C. vomitoria*, and *C. vicina*. The occasional visitation by these species is likely due to the seasonal changes, cueing several species for diapause. The majority of coleopteran species were identical in both habitats.

8.1.2 Seasonal Differences

Research on the seasonal effect on decomposition and faunistic patterns is sparse in the literature. Rodriguez and Bass (16) performed a noteworthy study on the seasonal differences of decomposition and insect succession on human cadavers. However, replication was not possible with human bodies. Introna *et al* (22) used jars with beef liver as baits to examine seasonal differences in sarcosaprophagous fly activity. Thus, the present study is novel in that pig carcasses were investigated in three different seasons. Similar research was also performed in British Columbia (137), although the results from this research are preliminary.

8.1.2.1 Temperature

In spring and summer, internal temperatures remained relatively constant for extended periods during active decay. In fall, however, this plateau was not evident. As mentioned earlier, internal carcass temperatures are a product of larval and bacterial heat generation. In fall, internal temperatures mimicked ambient readings, although exhibiting less daily fluctuation. Thus, maggot mass heat generation was never significant in the fall experiment.

The internal carcass temperature was actually lower in sun-exposed carrion in summer than in spring. This lower temperature is likely due to the ambient temperatures being at lethal levels for insects in summer. Thus, larval development became inhibited and heat generation was not as significant. Furthermore, larval aggregations were not witnessed past active decay in summer, as underdeveloped larvae began to leave the carrion in search of new resources.

8.1.2.2 Decomposition

The majority of the studies have been conducted in only one season, usually in summer (13, 15, 16, 19, 40). In comparison of the decay rates in summer in Saskatchewan to those of Anderson and VanLaerhoven (13) in British Columbia, decomposition occurred at a much faster rate in Saskatchewan (Table 8.2). Anderson and VanLaerhoven noted that rainfall impacted insect activity and delayed the onset of decompositional stages. The climate in Saskatchewan is far more arid than B.C. and may account for the rapid dehydration of carrion in summer in Saskatchewan compared to pigs in B.C. Summer decomposition was also faster in Saskatoon than in Edmonton (40) (Table 8.2). Differences in the decay rates between Edmonton and Saskatchewan are likely the result of two factors. First, Edmonton is further north than Saskatoon and may experience cooler summer temperatures. Second, the research in Edmonton for summer began at the end of June, and likely exposed the carcasses to cooler temperatures. Ambient temperatures are typically the warmest in the months of July and August in both Edmonton and Saskatoon. Summer decay rates in Edmonton were actually similar to spring decay rates in Saskatoon. In fall, carcasses decayed at a slower rate compared to other seasons. This is similar to the findings of Rodriguez and Bass (16) who also noted the slowest decay of humans in fall, compared to summer and spring.

Table 8.2: Comparison of decay rates in various seasons and habitats in different regions in Canada.

Season, Province	Days postmortem in each stage of decay									
	Fresh		Bloated		Active Decay		Advanced Decay		Dry/Remains	
	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade
Spring, SK	0-1	0-2	2-12	3-15	13-30	16-35	31-42	36-45	42- 63+	46-63+
Summer, SK	0	0	1-4	1-4	5-11	5-11	12-25	12-25	26+	26+
Summer, B.C. (13)	0-1	_	2-10	_	11-16	_	17-42	_	43+	_
Summer, AB (40)*	0-1	0-2	2-10	3-14	11-16	15-25	17-25	26+	26+	n/a
Fall, SK	0-2	0-3	3-11	4-11	12+	12+	n/a	n/a	n/a	n/a

* Averaged numbers from several pigs in the mid-size range (36-80kg).

Inconsistencies were observed with respect to the number of identifiable decomposition stages. The five stages employed by Anderson and VanLaerhoven (13) were used as a comparative model. In spring, the pattern of decomposition was better suited to the six classifications outlined by Payne (15), with a separation of the final stage into two stages: dry and remains. The remains stage of Payne (15) is characterized by skeletonization. In the present study, the outer layer of skin of spring carcasses completely dehydrated and extended the dry stage. Unfortunately, the study was not long enough to observe the onset of the remains stage.

In summer, decomposition closely followed the stages outlined by Anderson and VanLaerhoven (13). The final stage of decay was a combination of semi-skeletonization combined with the preservation of remaining tissues. In fall, decomposition progressed through only three stages before sub-zero temperatures prevented further decay. The experiment did not last long enough to observe the continuation of decomposition in spring, preventing any direct correlation with previous research. However, fall decomposition may be similar to the four stages of Rodriguez and Bass (16), combining the active and advanced decay into one stage. Further research investigating the decomposition of carrion over winter is needed to confirm this assumption.

Several authors (12, 13, 15, 16) delineate the end of the bloated stage by the deflation of the carcass when larval feeding breaks the skin. The process is described as if deflation were instantaneous upon rupturing of the tissues. In Saskatchewan, regardless of season or habitat, deflation was a slow process. Deflation took place over a four-day period in summer, a 12-15 day period in spring, and a 10-11 day period in fall. Reed (24) marks the end of the bloated stage and beginning of the decay stage by the complete cessation of bloat in carrion. Use of this marker would be misleading in Saskatchewan, as the characteristics of decay occur simultaneously with deflation. Thus, in Saskatchewan, the end of the bloated stage and initiation of the active decay stage are best defined by the following combination of characteristics: presence of

liquefaction and associated froth, the burst of maggots outside of body cavities, and the initiation of deflation.

8.1.2.3 Insect Succession

The carrion fauna was notably impacted by season. The blow fly *P. regina* was present in all three seasons, but was not a common visitor on shaded carrion in summer and fall. The maximum threshold for *P. regina*, 45°C is relatively high compared to other species of blow flies (42). Thus, *P. regina* would have a competitive advantage over other species at sun-exposed carrion during periods of high temperatures. The blow fly *C. macellaria* did not appear until June 29, largely due to the seasonal abundance of this more southern latitude-inhabiting blow fly (105). There was a greater diversity of Diptera in the spring. The seasonal cues for diapause are species-specific and may have impacted which insects were present in which seasons.

Many insects have activity periods between May and October, although species may peak in abundance in specific months during this period. For example, *M. domestica* colonized carrion in summer only, corresponding with their peak activity in July and August (138). The first insects attracted to the remains were different in spring than in summer and fall. In spring, *C. cadaverina* arrived first whereas *C. macellaria* was the first colonizer in summer and fall. The abundance of *C. cadaverina* in spring agrees with the findings of Goddard and Lago (89). The presence of *P. regina* slightly later in spring and fall is also consistent with the findings of Goddard and Lago (89). In summer, *P. regina* was an early colonizer in B.C. (13) and the same was true for pig carrion in summer in Saskatchewan. Unlike other studies (12, 12, 15, 16, 19), members of Sarcophagidae were not overly common in Saskatchewan. Three different species were collected in various seasons, although their abundance relative to other dipterans was low. Furthermore, they were often collected in the vicinity of, but rarely on the carcasses proper.

The immediate arrival of the blow flies agrees with the findings of other studies, (13, 15, 16, 19) however, the specific species varied. In B.C. in summer

(13), *L. illustris* was the first to arrive, although Smith and Wall (116) report that this species is not adept at competing with other blow fly larvae. *L. illustris* was captured, but was not common in collected immature specimens except for shaded carrion in fall. This may indicate erroneous identification of immature specimens or that *L. illustris* is a poor competitor on carrion. Similar to the findings in B.C. (13), *P. regina* was a pioneer species, but only in spring. In summer and fall, oviposition by *P. regina* followed that of *C. macellaria*. In spring, *P. terraenovae* was a pioneer species in both habitats. The presence of *C. macellaria* is a novel finding, as this species has not been previously reported in Canada east of Ontario. Clearly the northern range of this species is being expanded and may impact insect succession of early colonizers in Canada.

Similar to the reports of Early and Goff (12) and Rodriguez and Bass (16), the Cleridae did not arrive on carrion until the dry/remains stage. Members of the family Dermestidae were nearly absent from the collections. This may suggest that the observational periods were too short to observe their arrival. The arrival of Staphylinidae early in decomposition agrees with the findings of others (12, 13, 15, 16, 19). However, larvae were collected much earlier than in other studies. In B.C. (13), larvae were not collected until 78 days postmortem in summer. Larval staphylinids were collected as early as 16 days postmortem in shaded carrion in summer in Saskatchewan. The arrival of the Histeridae in bloat in spring coincides with the findings of Rodriguez and Bass (16). However, in summer, the Histerids did not arrive until the dry/remains stage. These findings correspond with the summer decay study of Anderson and VanLaerhoven (13) and Early and Goff (12). Members of the family Scarabaeidae were frequent colonizers of carrion in active decay in spring and fall. Rodriguez and Bass (16) reported a later colonization for scarab beetles, with peak abundance in the dry stage. Species of Scarabaeidae may be good indicators of active decay in spring and fall, when groups of 5 or more are present on a carcass at a given time. Scarab beetles were absent from the summer collections, similar to the findings of several authors (12, 13, 15). The sap beetles (Nitidulidae) were relatively uncommon in the collections and had intermittent periods of colonization in various stages. This is

contrary to the findings of other authors (13, 16), who note a continual succession and increase in abundance of Nitidulidae from the bloated stage onward.

8.2 Clothed Versus Nude Carrion

Most publications on forensic entomology do not mention clothing as a factor in the pattern of arthropod succession (4, 12, 13, 15, 17, 19). Additionally, publications on the effects of clothing have been conflicting. Mann *et al* (41) reported that clothing accelerated decay, providing protection for developing larvae and thus, enhancing the rate of tissue consumption. Others reported that decomposition is decelerated and preservation is enhanced in the presence of clothing and other fabrics (35, 52). The present study theorized that clothing would alter the microclimate by providing shady conditions on a carcass that is otherwise in full sunlight. It was also hypothesized that clothed carrion would demonstrate a different pattern of insect succession than nude carcasses due to differential decomposition. Finally, it was assumed that clothing would provide protection for developing larvae from the lethal temperatures experienced in summer.

8.2.1 Temperature

In the first 11 days and three stages of decomposition, internal carcass temperatures were nearly identical between clothed and nude carrion. During the active decay stage, as in other experiments, the maximum recordings of internal temperature corresponded with the development of 3rd instar blow fly larvae. The major differences between the internal carcass temperatures of clothed and nude carrion occurred in the advanced decay stage. Clothed carrion experienced more frequent peaks in temperature and less daily fluctuation in the later stages of decay. The reason for these differences is based in the higher moisture level on clothed carrion, providing more optimal development conditions for Calliphoridae larvae. Thus, maggot masses were sustained for longer periods of time and were more frequent in later stages of decay.

8.2.2 Decomposition

Clothing was shown to decelerate decay by 7 days in summer. The fabric absorbed decompositional fluids, preventing the dehydration of tissues. The outer tissues of nude carcasses were completely desiccated by day 12 and inner tissues were dry by day 26. Several underdeveloped larvae were forced to leave the carcasses and many were found dead on or near the carrion. By day 12, 3rd instar blow fly larvae were still able to develop at optimal rates on clothed carrion. Dillon and Anderson (59) reported that clothing provided more ovipositional sites for blow flies due to the retention of moisture in the fabric. These findings are true in the present study after the bloated stage. More eggs were observed on clothed carrion after day 5. Before the end of the bloated stage, there were no differences between the two types of carrion, as there was significant moisture present in the tissues of nude carrion. Furthermore, clothing was able to absorb precipitation, further preventing the desiccation of tissues.

The stages of decomposition were readily identifiable in clothed carrion, contrary to the findings of Dillon and Anderson (137). Mann *et al* (41) stated that clothing provided protection for developing larvae. These findings are confirmed in the present study. However, Mann *et al* (41) reported that this protection served to accelerate decay. The present study contradicts these findings, as decay was decelerated in clothed carrion. However, in summer in Saskatchewan, moisture levels were a significant factor in determining the rate of decay and preservation. Dehydration of nude carrion accelerated the onset of decay stages more so, than the ability of larvae to consume tissues in clothed carrion. In spring and fall, moisture was never completely lost in the inner tissues until very late in decomposition. Thus, moisture levels were not a factor in other seasons for the development of larvae and hence, the consumption of tissues. Thus, the findings of Mann *et al* (41) would likely be true in other seasons. More research on the effect of clothing in different seasons of the year is clearly needed.

8.2.3 Insect Succession

Core maggot mass temperatures on nude carrion were typically 5-7°C higher than masses underneath the fabric of clothed carrion. This resulted in a greater mortality rate of insects at nude carrion and a slower rate of development as temperatures approached the maximum threshold for *C. macellaria*. Pupae were often recovered from the underside of clothing, demonstrating that clothing provides a suitable pupation substrate for dipterans.

Dillon and Anderson (137) reported that clothing enhanced the diversity for insects in spring in British Columbia. In the present study, nude carrion attracted slightly more insects. A total of 17 species were collected from both types of carrion, and 12 were common to both habitats. These findings reflect the overall lack of diversity on carrion in the summer season. Dillon and Anderson (137) also reported that clothing altered the pattern of insect succession. In the present investigation, insect succession was nearly identical on clothed and nude carrion. The majority of species arrived on the same day on both types of carrion, including *T. lapponicus* (Silphidae), *C. macellaria* and *P. regina* (Calliphoridae), and *M. domestica* (Muscidae). Other species arrived within 1-2 days of each other. The only exception was *N. rufipes* (Cleridae), which colonized nude carrion 29 days earlier than clothed carrion. The earlier arrival of this clerid on nude carrion is a reflection of the affinity for this insect to drier tissues.

The present study agrees with the findings of Dillon and Anderson (137) that clothing results in longer periods of insect colonization. Longer colonization intervals were noted for blow fly species, including *P. regina* and *C. macellaria* and for larval *P. casei* (Piophilidae). Coleopterans and other dipterans demonstrated similar colonization periods.

CHAPTER 9: CONCLUSIONS

This investigation demonstrated that the patterns of decomposition and insect succession are unique in the Prairie Ecozone of Saskatchewan. These patterns are different in varying seasons of the year. Ambient temperature is a critical factor in the determination of the rate of decay in various seasons. Furthermore, the seasonal distribution of insects significantly impacts the species that are recovered from carrion in different times of the year.

The effect of habitat on decomposition is significant in spring. Sun-exposed carrion in spring decay at a faster rate and have shorter periods of insect colonization. Habitat is not a significant factor in the decompositional rate of carrion in summer and fall. However, several indicator species are exclusive to either the shaded or sun-exposed habitat in all seasons. The best indicators for postmortem interval based on successional patterns are insects other than species of Calliphoridae. These insects arrive in a predictable sequence, although the pattern varies in different times of the year and in different habitats.

Clothing decelerates decay in summer in Saskatchewan. Clothing provides shelter for developing larvae and thus, allows a longer association with clothed carrion. Clothing does not impact the general faunistic sequence on carrion. However, the arrival times of species that prefer dry tissues, or predate insects that prefer this type of resource, may be delayed. Research on the effect of clothing in different seasons and different habitats would be a prudent follow-up to the results presented in this study.

The data generated from this research are now available for the nation-wide database on insect succession patterns on carrion and for homicide investigations in Saskatchewan. Further research is needed on the biological and ecological characteristics of the particular species associated with carrion in the Prairie Ecozone. The development rates of Saskatchewan strains of Calliphoridae

needs to be investigated, as insects inhabiting the Prairie region may be more acclimatized to severe daily fluctuations in ambient temperature. Internal carcass temperatures were also shown to fluctuate immensely throughout the day. Additionally, Saskatchewan strains of some species have varied from identification characteristics. A key to the Saskatchewan species of forensic importance would be of great value to researchers continuing the investigation of this subject.

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