UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

EFFET DU GEL ET DÉGEL SUR LES PROCESSUS DE DÉCOMPOSITION D'UN CORPS ET L'ENTOMOFAUNE NÉCROPHAGE

THE EFFECTS OF THE FREEZE AND THAW CYCLE ON THE PROCESS OF DECOMPOSITION AND ENTOMOLOGICAL SUCCESSION

MÉMOIRE PRESENTÉ

COMME EXIGENCE PARTIELLE

MAÎTRISE EN SCIENCES DE L'ENVIRONNEMENT

PAR

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DÉCEMBRE 2023

Acknowledgements

This research was only possible with the assistance of many people. First, I would like to thank one of my directors Dr. Shari Forbes for providing me the opportunity to do this project. Next, I would like to thank my other director Julie-Éléonore Maisonhaute. She has taught me how to collect insects, rear them to adulthood, and so much more. Without their guidance and knowledge, this project would not have been possible. I would also like to thank R. Christopher O'Brien for being on my thesis committee and for inspiring me to pursue my education in Quebec. Thank you to Eric Lucas for being the final member of my committee and for providing useful entomological insight during this project. Next, I would like to thank Gabrielle Harvey, Sophie Morel, Darshil Patel, Rushali Dargan, and Marc-Antoine Perreault for helping me to collect the samples and data in the field and their assistance in the lab. I would also like to thank any other members of the UQTR staff for assisting me.

I also must thank the organizations that financed this project. The first one being le Fonds de Recherche du Québec. The second organization was the Canada 150 Research Chair of Forensic Thanatology. This financial assistance was essential in the completion of this project.

It is also important to thank the two donors who have donated their bodies to the laboratoire d'anatomie de l'UQTR and their families. Without this generosity this project would have never been possible. The staff of the laboratoire d'anatomie de l'UQTR were also helpful in this project so, thank you for your assistance. Finally, thank you to my coworkers and friends at the REST facility for making the work at the facility fulfilling and enjoyable.

The last group of people that I must thank are my friends and family. Thank you to my parents, brothers, and other family members for their love, support and the occasional meal which inspired me to finish this project. Thank you to my friends for providing support and helpful distractions to make these past three years enjoyable.

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Abstract

The study of insects in a legal context is known as forensic entomology. This field has been well studied in many parts of Canada but there have only been a few studies conducted in Quebec. This province has cold winters that can last several months and there have been few forensic entomology studies conducted on bodies after they were exposed to a winter. The objectives of this study were to observe how pig and human bodies decompose during a Quebec winter and which insects will colonize the bodies following the freeze and thaw cycle that occurs. The study was spilt into two trials. The first trial was performed from the winter of 2020 until the autumn of 2020 using three pig carcases placed in a wooded area on the campus of the Université du Québec à Trois-Rivières. The second trial used two human donors placed in the autumn of 2020 until the summer of 2021 at the site for Research in Experimental and Social Thanatology (REST). This is the first taphonomic site in Canada that is dedicated to the study of human decomposition. During both studies, the sites were visited periodically, with observations and photos made of the decomposition characteristics and insects, as well as collection of adult arthropods and fly (Diptera) larvae. The larvae were reared to adulthood so that they could be identified along with the captured adult arthropods. This study revealed that winter temperatures delayed the decomposition process of pig carcasses and human donors. We also discovered that the human donors continued to decompose even when buried in snow and exposed to subzero temperatures. Notably, one of the donors never entered the bloat stage. Fly larvae were found to colonize the pig carcasses and human donors in two main successive groups. For the pig carcasses, the first group (“early colonizers") consisted of members of the Calliphoridae family, *Cynomya cadaverina, Calliphora livida, Calliphora vomitoria, Calliphora vicina,* and *Luciila illustris.* This group was present from the fresh stage until the active decay stage. The second group that colonized the carcasses later in the decomposition process consisted of the Piophilidae family larvae *Prochyliza xanthostoma* and *Stearibia nigriceps.* Immatures of *Phormia regina* and *Protophormia terraenovae* were seen throughout the whole study on pig carcasses. For the human trial, the first succession group consisted of *L. illustris* (found during the autumn only), *C. livida*, *C. cadaverina*, and *C. vomitoria* (the three species being found in autumn and spring), and *C. vicina* (found in spring only). The second succession group for the human donors consisted of *P. regina, P. xanthostoma*, and *S. nigriceps,* that were present during the bloat stage or later stages of decomposition. *Protophormia terraenovae* was seen throughout the entire spring portion of the human cadaver trial. The only succession information determined for beetles (Coleoptera) was that when beetle larvae were present, the pig carcasses were in the later stages of decomposition, while no clear pattern was observed on human remains. This project provides a foundation of insect succession after Quebec winters and can form the basis for future studies that examine decomposition in cold climates and for determining the minimum Postmortem Interval.

Key Words: Forensic entomology, winter, decay, Diptera, Calliphoridae, Coleoptera, colonisation, insect succession, minimum Postmortem interval

Résumé

L'étude des insectes dans un contexte juridique est connue sous le nom d'entomologie médico-légale. Ce domaine a été bien étudié dans nombreuses régions du Canada, mais seulement quelques études ont été menées au Québec. Cette province connaît des hivers froids qui peuvent durer plusieurs mois et peu d'études ont été menées sur les cadavres après qu'ils ont été exposés à un hiver. Les objectifs de cette étude étaient d'observer comment des cadavres de cochon et humains se décomposent lors d'un hiver québécois et quels insectes colonisent les corps suite au cycle de gel et dégel qui se produit. L'étude a été divisée en deux essais. Le premier essai a été réalisé de l'hiver 2020 à l'automne 2020 sur trois carcasses de cochon placées dans un boisé du campus de l'Université du Québec à Trois-Rivières. Le deuxième essai a utilisé deux donneurs humains placés entre l'automne 2020 et l'été 2021 sur le site de Recherche en Sciences Thanatologiques Expérimentales et Sociales (REST[ES]). Il s'agit du premier site taphonomique au Canada dédié à l'étude de la décomposition humaine. Au cours des deux études, les sites ont été visités périodiquement, avec des observations et des photos faites des caractéristiques de décomposition et des insectes, ainsi qu'une collecte d'arthropodes adultes et de larves de mouches (Diptera). Les larves ont été élevées jusqu'à l'âge adulte afin de pouvoir être identifiées, avec les autres arthropodes adultes capturés. Cette étude a révélé que les températures hivernales retardaient le processus de décomposition des carcasses de cochon et des donneurs humains. Il a également été découvert que les donneurs humains continuaient à se décomposer même lorsqu'ils étaient ensevelis sous la neige et exposés à des températures inférieures à zéro. Notamment, l'un des donneurs n'est jamais entré dans le stade gonflé. Des larves de mouche colonisaient les carcasses de cochon et les donneurs humains en deux groupes successifs. Pour les carcasses de cochon, le premier groupe (« premiers colonisateurs ») était constitué de membres de la famille des Calliphoridae, soit *Cynomya cadaverina, Calliphora livida, Calliphora vomitoria, Calliphora vicina* et *Lucilia illustris*. Ce groupe était actif du stade frais juste qu'au stade de décomposition active. Le deuxième groupe, qui colonisait les carcasses plus tardivement, était constitué des larves de la famille des Piophilidae, soit *Prochyliza xanthostoma* et *Stearibia nigriceps*. Des immatures de *Phormia regina* et *Protophormia terraenovae* étaient observés tout au long de l'étude sur les carcasses de cochon. Sur cadavres humains, le premier groupe colonisateur était constitué de *L. illustris* (trouvé au cours de l'automne uniquement), *C. livida*, *C. cadaverina* et *C. vomitoria* (les trois espèces trouvées en automne et au printemps), ainsi que *C. vicina* (trouvé au printemps uniquement). Le deuxième groupe colonisateur pour les donneurs humains était constitué de *P. regina, P. xanthostoma* et *S. nigriceps*, qui étaient présents au stade gonflé et durant les stades ultérieurs de décomposition. *Protophormia terraenovae* a été observé tout au long de la partie printanière de l'essai sur les cadavres humains. La seule information sur la succession déterminée pour les coléoptères était que lorsque leurs larves étaient présentes, les carcasses de cochon étaient aux derniers stades de décomposition, tandis qu'aucune tendance claire n'était observée sur les restes humains. Ce projet a permis de fournir une base

sur la succession des insectes après un hiver québécois et peut constituer une base d'études futures qui examineront la décomposition dans les climats froids et pour déterminer l'intervalle postmortem minimum.

Mots clés : Entomologie médico-légale, hiver, décomposition, diptères, Calliphoridae, coléoptères, colonisation, succession des insectes, intervalle postmortem minimum

Chapter 1: Introduction

1.1 Decomposition Processes and Stages

Once an organism dies, its body commences the decomposition process. Decomposition is a continual process with multiple phases that starts immediately after death and is deemed to end when there is only a skeleton present (although technically bone degradation can continue). This process is complicated and is impacted by many abiotic and biotic factors such as temperature, bacteria, and/or the succession of insects (Anderson, 2011; Johnson et al., 2013; Matuszewski et al., 2014; Meyer et al., 2013; Roberts & Dabbs, 2015). The stages that are most associated with this process were originally defined as fresh, bloat, active decay, advanced decay, dry stage, and remains stage (Payne, 1965). Each stage is marked by distinct characteristics. The fresh stage starts at death and ends when the body distends. During this time, the body undergoes many changes such as discoloration due to livor mortis, and muscle stiffening due to rigor mortis (DiMaio & DiMaio, 2001). The second stage is the bloat stage which is marked by the distension of the body due to bacterial activity. This stage starts once the cells inside the body degrade, releasing gases which causes the body to inflate. As this stage progresses, the body will become more distended, and some fluid may purge from the mouth, nose, and other orifices. Once the body deflates, it enters the next stage of decomposition referred to as active decay. The indicators of this stage include soft tissue liquefaction and the rupturing of the abdominal wall (Payne, 1965; Sharanowski et al., 2008). As active decay progresses, the skeleton will begin to be exposed and the putrefactive liquids will seep into the environment. This transition is referred to as the advanced decay stage. This stage is characterized by the loss of most of the soft tissue although some tissue can become desiccated. The last two stages are dry stage and the remains stage. Theses stages are difficult to distinguish because they are both marked by the presence of only the dry remains which typically includes dry skin, cartilage, and exposed bones. The remains stage is marked when there is only hair, skin, bones, and teeth present. This stage can last for as long as the bones are present.

1.2 Factors Affecting Decomposition

Decomposition is a complex process that is affected by factors such as physiological characteristics, including age, body weight, and integrity of the body (Campobasso et al., 2001). There are also numerous environmental factors that impact decomposition including temperature, precipitation, solar radiation, scavenging, if the body was burnt, or if the body is indoors or outdoors, to name just a few (Anderson, 2011; Sharanowski et al., 2008).

One of the most important environmental factors to impact the rate and process of decomposition is ambient temperature. If the body is exposed to warmer temperatures, it will accelerate the rate of autolysis and putrefaction and increase the rate of development of insects (DiMaio & DiMaio, 2001), while if a body is exposed to freezing temperatures, it will slow down decomposition and may even halt the process (Micozzi, 1986). According to the Environment and Climate Change Canada website (Environment and Climate Change Canada, 2020), Quebec is known for having cold winters where the lowest temperature can be below -40°C, and can also have warm summers with a daily averaging temperature of 18°C. This continental temperature range encompasses freezing to warm temperatures which will greatly impact how a body will decompose throughout the year.

During the winter months in Quebec, it is hypothesized that the body will freeze, and decomposition will be slowed significantly. Sub-zero temperatures will also inhibit most of the bacteria, except for some Gram-positive species (Roberts & Dabbs, 2015). This leads to the preservation of the soft tissue. Once the body starts to thaw, it will appear to decompose from the “outside in” because it is believed that the organisms that participate in the decomposition process will be initiated from external sources only (Micozzi, 1986). During the winter months in Canada, most insects also become inactive. During the autumn when the weather becomes too cold, Diptera and other insects will enter diapause (Pitts & Wall, 2005; Vinogradova & Reznik, 2013). This lack of insect activity will prevent further decomposition of a body.

Arthropod activity is considered another major driver of soft tissue degradation. The first insects to arrive on the body in warmer temperatures are typically flies of the Calliphoridae family, commonly known as blowflies (Byrd & Castner, 2010). They are reported to arrive within minutes of death and will start to lay eggs in dark moist areas such as the mouth, nose, genitalia, and any open wounds. In some cases, ants and beetles appear to prey on the fly eggs and larvae (Payne, 1965). The bloat stage attracts additional species of blowflies but will also attract flesh flies (Sarcophagidae) and house flies (Muscidae). They will continue to lay eggs (except for Sarcophagidae) and once hatched, the larvae, commonly called maggots, start to feed on the body. Sarcophagidae are known to be ovoviviparous, which means that the eggs hatch inside the mother's body and the larvae are deposited (Pimsler et al., 2014). Some carrion beetles (Silphidae) appear and may prey on the maggots as well as feed on the body. Active decay is typically characterized by the body deflating due to maggots penetrating the skin and consuming soft tissue (Anderson & VanLaerhoven, 1996). The most numerous insects observed during that stage will be the blowflies and their larvae. The larvae start to actively feed on the carrion especially in the eyes, noses, ears, mouth, and genital region. They also start to form masses. This stage typically includes additional beetles (Histeridae and Staphylinidae), who prey on fly larvae. During advanced decay, most of the Diptera larvae migrate away from the body to begin pupation (Leblanc & Strongman, 2002). Various beetle families, such as dermestid beetles (Dermestidae), can be observed consuming the remaining desiccated tissue (Schroeder et al., 2002). As the body starts to dry out, many of the carrion related insects start to dissipate, and if there are any fly or beetle larvae, they will often not complete their lifecycle due to the lack of food. At this stage, all insects present, are assumed to normally inhabit the area surrounding the remains (Payne, 1965). Mites (Acari) can be seen throughout the whole process (Maisonhaute & Forbes, 2021a; Silahuddin et al., 2015).

If insect activity is excluded from a body, then the process is slowed down significantly as shown in a study using pig carcasses (Comstock et al., 2015). When insects are partly excluded (insects had access to the carcass for a short amount of time), alternative decomposition stages have been reported as fresh, bloat, localized tissue removal, dry remains, and desiccation (Comstock et al., 2015). The first two stages are the same as when insects are present. The localized tissue

removal stage occurs when larvae are confined to a specific area, such as the abdomen, where oviposition has occurred. They will not feed on the entire carcass because the insects did not have access to other parts of the body. When insects were completely excluded, the decomposition stages were reported as fresh, bloat, deflation, and dry remains (Comstock et al., 2015). The final stage, dry remains, shows few external physical changes but the body slowly loses moisture.

1.3 Forensic Entomology

Forensic entomology is a broad field in which the study of arthropods is applied in relation to the law (Hall & Huntington, 2010). It is often divided into three categories. The first category is called 'urban entomology' and deals with issues involving termites, cockroaches, and other insects causing problems with human environments, such as infesting living persons or animals (e.g. myiasis), and buildings and habitations in both urban and rural areas (Hall & Huntington, 2010). The second category is "stored products entomology". This is used, for instance, when a consumer makes a complaint about an insect or a part of an insect being in their food or other product. The third category and the most well-known is "medico-legal entomology". One common application of this field is to determine the minimum Postmortem Interval, PMI_{min}, of human or animal remains. This is the minimum amount of time that has passed since the discovery of the body. This can be done by rearing larvae collected from a body or by studying the sequence in which the insects appear on a decomposing body (Anderson, 2001). This information can help determine how long a person has died.

The first use of insects in a murder investigation was in the XIIIth century in China. In this case, a Chinese criminalist named Sung T'zu was able to determine which scythe was used in a homicide because flies were attracted to the small amount of blood present on the blade. This account was written in a book Washing Away of Wrongs which was translated into English in 1981 (Sung, 1981). In the mid-1800s, European entomologists started using their knowledge to investigate deaths. The first case to investigate a death using insect succession was performed by Bergeret in 1855. In 1894, Mégnin published his book La faune des cadavres: Application de

l'entomologie à la médecine légale, which detailed eight "waves" of insects that colonize a cadaver (Mégnin, 1894). This has provided the foundation for other succession studies, which have found different results (e.g. different number of stages, different timing and succession) in different regions of the world (Anderson & VanLaerhoven, 1996; Lefebvre & Gaudry, 2009).

Determining the minimum PMI of a victim is an essential part of medico-legal entomology and involves two methods. To perform this estimation using the first method, an entomologist needs to know the ambient temperature at the scene prior to the discovery of the body and requires samples of the fly eggs and/or larvae collected on and around the body, and determining PMI_{min} relies on the study of the oldest ones on the cadaver. These samples will be reared to adulthood to be identified, since fly larvae are difficult to identify. The species and accumulated degree days calculated (which is the sum of the average daily temperature) can help determine how long a body has been colonized (Niederegger et al., 2010). The second method uses insect succession, which were first identified by Mégnin (Figure 1.1). He reported that the first insects arrive within minutes after death and the last insects arrive a year after death (Mégnin, 1894). More recent studies have found that the number and duration of the waves differ according to each geographic area (Anderson & VanLaerhoven, 1996; Lefebvre & Gaudry, 2009). This method is also not the preferred method due to it being less precise but will be used if there are no other options.

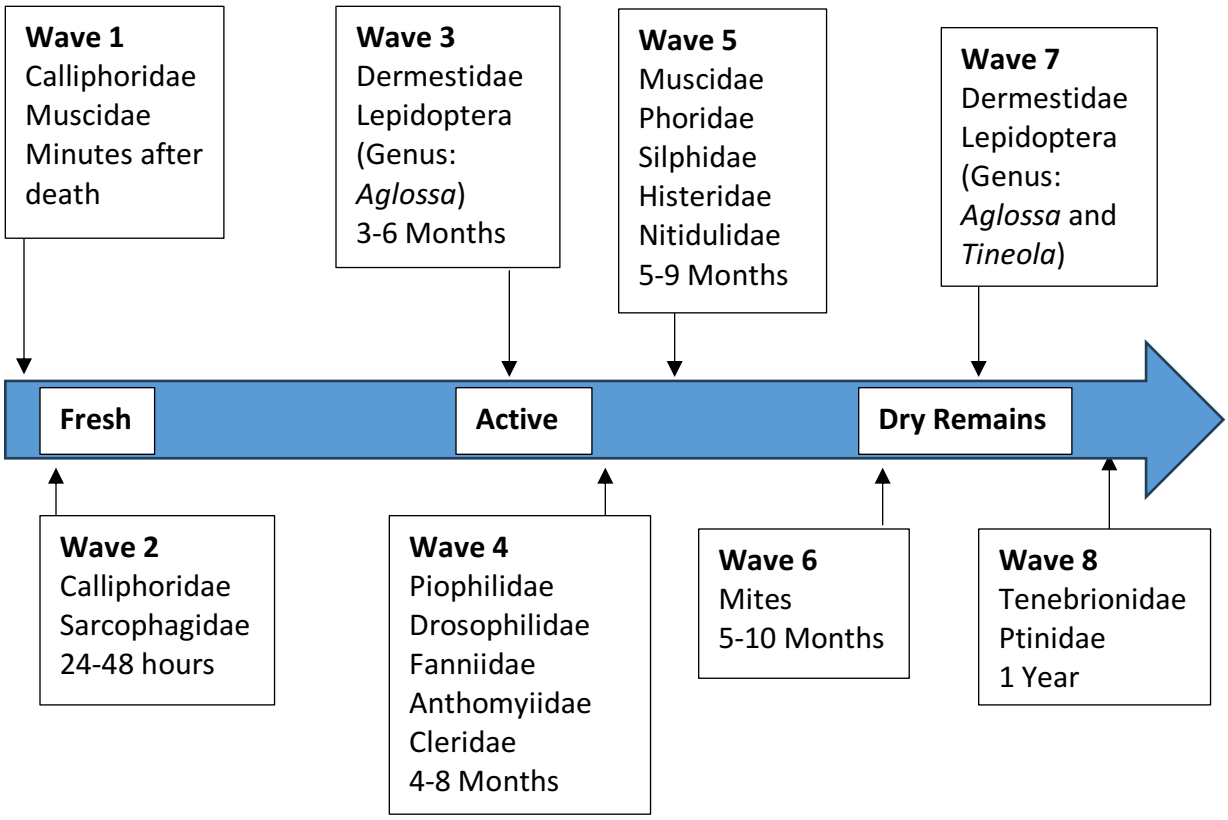


Figure 1-1: The eight waves of insect colonization on cadavers described by Mégnin (1894).

One area of forensic entomology research is to determine the ecology of insects. This information can help determine if a body has been moved prior to discovery (Picard & Wells, 2010). This can be done because some insects prefer certain ecotypes such as urban or rural areas (Leblanc & Strongman, 2002). Other foci in the field of forensic entomology include entomotoxicology, the application of molecular biology methods, and the study of the biology of insects. Entomotoxicology is the study of toxicology applied to arthropods (Goff & Lord, 2010). This can be used to study: 1) how toxins, such as heavy metals and drugs will affect the development of larvae; 2) how toxins affect PMI estimations; and 3) how insects can be used to detect the drugs or other substances present in a body when there is no standard toxicological specimen present (Magni et al., 2016; Musvasva et al., 2001). Methods of molecular biology are being used to genetically identify insect species, when sequencing is available, and to recover a DNA profile of a body when it is too decomposed to find a viable tissue sample (Boehme et al., 2010; Wells &

Stevens, 2010). This can allow for more accurate estimation of the minimum PMI and could assist to identify victims and the potential offender. These three areas of research can potentially provide an investigator with information about a body and the insects associated with it.

1.4 Arthropods Associated with Decomposition

Insects are largely responsible for the decomposition of the soft tissue of a body. They can use the decomposing body as a food source (necrophagous organisms), as a site for reproduction, to prey on other insects, or a combination of all three. There are many orders of arthropods that are associated with cadavers, but the two main orders are Diptera (flies) and Coleoptera (beetles) (Byrd & Castner, 2010).

1.4.1 Diptera (Flies)

The Diptera order comprises over 86,000 species worldwide (Byrd & Castner, 2010). As holometabolous insects (complete metamorphosis), their lifecycle consists of four stages: egg, larva, pupa, and adult/imago (Figure 1.2). The life of most flies starts once the egg has been laid, although there are some members of the Sarcophagidae family that are ovoviviparous and give live birth to larvae (Niederegger et al., 2010; Pimsler et al., 2014). The larvae of necrophagous fly begin to feed on the soft tissue of a body and grow. This stage is divided into three instars (Pinilla et al., 2013). After the larvae enter the third instar they enter the post-feeding stage, also called the prepupa. This stage is marked by the larva migrating away from the body. It proceeds to bury itself in the ground and pupate. This is the beginning of the pupal stage during which the larva will pupate inside a puparium and become an adult. The final stage of the lifecycle starts once the adult emerges from the puparium. The fly then finds a mate and the gravid female will lay its eggs on carrion to start the process all over again. Many of the forensically important flies act as scavengers, and they encompass many different families.

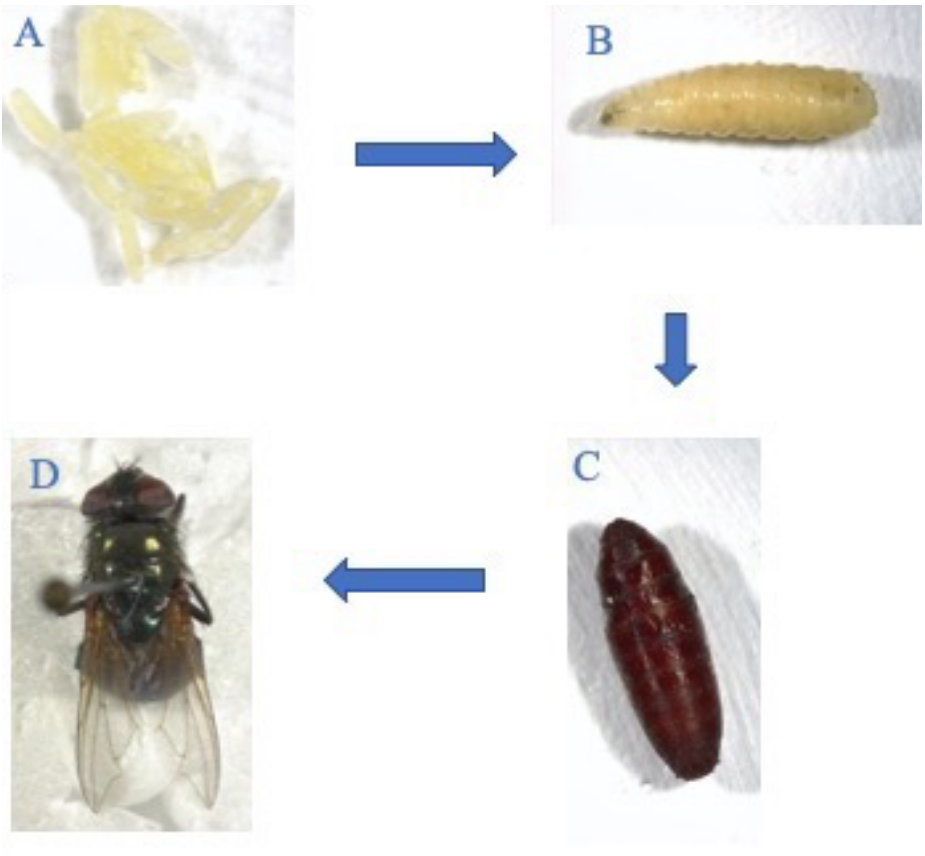


Figure 1-2: Lifecycle of Diptera. Life starts when an A) egg mass is laid, B) the egg hatches and becomes a larva, C) larva consume the soft tissue and then eventually become a pupa, D) finally the pupa becomes an adult to restart the whole process.

Photo: Pierre-Louis Arcand

1.4.1.1 Calliphoridae (Blowflies)

This family of flies consists of approx. 1,000 species worldwide and are usually the first insects to appear on a body. Some of the important species in Canada are *Phormia regina* (Meigen 1826), *Calliphora livida* (Hall 1948), *Calliphora vomitoria* L., *Protophormia terraenovae* (Robineau-Desvoidy 1830), *Lucilia illustris* (Meigen 1826), and *Cynomya cadaverina* (Robineau-Desvoidy 1830) (seen in Figure 1.3) (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). They are also some of the most important to estimate PMI_{min} (Byrd & Castner, 2010; Wallis, 1962). Blowflies are attracted to decomposing flesh, plant matter, and excrements, while other species of blowflies are attracted to wounds on living animals and

humans (known as myiasis) (Arias-Robledo et al., 2019). This ability to break down soft tissue on carrion makes them vital to nutrient recycling (Byrd & Castner, 2010). These flies can also cause myiasis and may be vectors of diseases.

Figure 1-3: *Cynomya cadaverina* (Calliphoridae) collected on April 29th, 2020 on pig 4 in the fresh stage. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

To find carrion, a blowfly uses their antenna to detect chemicals associated with decomposition (LeBlanc & Logan, 2009). They then conduct a visual search. Once the body is discovered, they walk on it to find a suitable site for oviposition, because Diptera have sensory organs located on their feet, legs, and body (Wallis, 1962). The usual target areas are natural orifices and sites of trauma. The female lays her eggs when she finds a suitable site. The eggs hatch to form a maggot mass which consumes the soft tissue of the carrion.

1.4.1.2 Other Forensically Important Diptera

The Sarcophagidae (Figure 1.5) family has approx. 2,000 species worldwide with 327 of these found in North America. Flesh flies usually appear on a body at the same time or slightly later than blowflies (Byrd & Castner, 2010). They have been seen flying in weather that would prevent other flies from flying. As larvae, they feed on flesh and excrement but as adults, they can consume decomposing plant and animal matter and sweet substances like nectar, sap, and honeydew. Flesh flies can cause myiasis and may be vectors of diseases.

The Muscoidea super family is comprised of Muscidae, Fanniidae, Anthomyiidae, and Scathophagidae (Balaban & Balaban 2004, Moisset 2004, Bayless 2007). These flies are closely associated with humans which makes them forensically important. They usually arrive on a body after the blowflies and flesh flies. Their larvae will feed directly on the carrion, but they have also been observed preying on other larvae and eggs as they mature. This behavior could affect the composition of other species of carrion-feeding flies. The biology and behavior of this superfamily of flies varies from species to species. Adults may feed on carrion, dung, decaying plant matter, pollen, and in some cases blood. Some species have been known to feed on garbage making them responsible for mechanically transmitting diseases (Byrd & Castner, 2010).

The family of Piophilidae consist of 69 species throughout the world (Byrd & Castner, 2010). These small flies are usually 2.5 to 4.5 mm long and are often associated with dry, protein-rich food. Some forensically important species are *Prochyliza xanthostoma* (Walker 1849) (Figure 1.4), *Stearibia nigriceps* (Meigen 1826), or *Piophila casei* (Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008; Taillefer & Giroux, 2021). They are commonly called skippers because some species' larvae can jump several centimeters to escape predators and in larval migration (Martin-Vega, 2011). They accomplish this by grabbing their anal protrusions with their mouth hooks and then releasing their grip. They also move in the same creeping manner as most larvae. Adults and larvae feed on the same food source. They are predominantly found on the body after active decay, but it is possible to see adults three to four days after death (Byrd & Castner, 2010).

Worldwide, there are about 240 species in the Sepsidae family (Byrd & Castner, 2010). Their larvae can be found on a variety of decaying plant matter and decomposing bodies (Nendick-Mason et al., 2004). The Sphaeroceridae family consists of 240 species in North America. The chance of these flies being present on human remains increases when there is excrement present. There has been little research done on the development time of these flies (Byrd & Castner, 2010).

Figure 1-4: *Prochyliza xanthostoma* (Piophilidae) collected as a larva on May 29th, 2020 on pig 5 in the active decay stage. Taken at UQTR (Quebec, Canada) Photo: Pierre-Louis Arcand

Finally, Phoridae is a large family of flies with 2,500 species worldwide and 350 of those are found in North America (Byrd & Castner, 2010). Larvae develop in decaying organic matter. Their pupa can be easily recognized because they are dorsoventrally flattened with a pair of horns at the anterior end. Some species are predacious and others parasitic. They are frequently associated with buried remains, and adults may congregate in large numbers over a gravesite. This behavior could help locate buried remains. The larvae of some species of Phoridae have been found on remains buried 30 to 100 cm deep (Boehme et al., 2010; Byrd & Castner, 2010).

Figure 1-5: Sarcophagidae collected as a larva on May 31st, 2020 on pig 5 in the active decay stage. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

1.4.2 Coleoptera (Beetles)

There are about 30,000 species in the order Coleoptera (Byrd & Castner, 2010). The lifecycle of the beetles within this order varies from family to family. Many of them start as eggs but there are some families, such as Staphylinidae, that experience ovoviviparity (Whitfield et al., 2013). The larvae become a pupa and the adults emerge following development, this complete metamorphosis makes them holometabolous. They have chewing mouth pieces which allow them to feed on soft tissue or be predators on insects that are present on decomposing bodies. The adults lay eggs on or near cadavers to start the process all over again.

1.4.2.1 Silphidae (Carrion beetles)

The Silphidae family of beetles has approx. 1,500 species worldwide, but only 46 are found in North America (Byrd & Castner, 2010). Adults of this family feed on fly eggs and larvae as well as on soft tissue. The larvae of these beetles typically appear during the active decay stage onward.

They feed on carrion, but also preys upon Diptera larvae if given the opportunity (Watson & Carlton, 2005).

Some species that are found in Quebec are *Necrophila americana* L.*, Oiceoptoma noveboracense* (Forster 1771) (Figure 1.6), *Nicrophorus tomentosus* Weber 1801, *Nicrophorus orbicollis* (Say 1825), and *Necrodes surinamensis* (Fabricius 1775) (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). The behavioral habits of these beetles have not been fully described (Dekeirsschieter *et al.* 2011). Most species are attracted to and will feed on carrion. Some of these, specifically *Nicrophorus* (bury beetles), have been known to bury small carcasses and lay their eggs on it. The larvae feed on the carrion, or prey upon the Diptera larvae. The adults are predators and consume fly larvae.

Figure 1-6: *Oiceoptoma Noveboracense* (Silphidae) collected on May 23rd, 2020, on pig 6 in the bloat stage. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

1.4.2.2 Other Forensically Important Coleoptera

There are approx. 123 species of the Dermestidae in North America (Byrd & Castner, 2010). Members of the genus *Dermestes* are forensically important because they are associated with the drier stages of decomposition (Byrd & Castner, 2010; Charabidze et al., 2014). Their larvae are usually found during the dry or skeletal stages of decomposition (Schroeder et al., 2002). The presence of these beetles and their excrement, also called frass, often indicates that a long time has passed since the time of death. The frass can also be used to detect the presence of illicit drugs and the DNA of the body (Miller et al., 1994).

There are over 47,700 species of Staphylinidae in North America making it one of the largest families. In a forensic context, there have only been four identified to the species level in Quebec, *Creophilus maxillosus* L. (Seen in Figure 1.7)*, Omalium rivulare* (Paykull 1789)*, Ontholestes cingulatus* (Gravehorst 1802)*,* and *Platydracus fossator* (Gravehorst 1802) (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). These beetles can be found in many different habitats and feed on carrion, plant debris, fungi, and prey on other insects (Byrd & Castner, 2010).

Figure 1-7: *Creophilus maxillosus* (Staphylinidae) collected May 29, 2020 on pig 5 active decay stage. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

The two species of Nitidulidae that have been reported in Quebec as being associated with cadavers are *Omosita nearctica* (Kirehtshuk 1987) (Figure 1.8) and *Glischrochilus quadrisignatus* (Say 1835) (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). Their larvae are found during the more advanced stages of decomposition, but as adults they have been reported starting from the bloat stage (Maisonhaute & Forbes, 2021a). The first species is associated with cadavers, but the other species is associated with fermented plant material (Williams et al., 1992). There have been few studies to determine the forensic importance of these beetles.

Worldwide, the Histeridae family, known as clown beetles, has over 3,000 species and more than 500 are found in North America (Byrd & Castner, 2010). They are commonly found on decaying animal tissue and excrement but have been known to feed on fungi and decaying plant matter. Clown beetles are usually active during the night and will hide in the soil under carrion during the day. Both the adult and larvae prey on fly larvae, fly puparia, and occasionally feed on dermestid larvae. Members of this order can be found from the fresh stage to the dry stages of decomposition (Cotinis et al., 2004; Maisonhaute & Forbes, 2021a).

Figure 1-8: *Omosita nearctica* (Nitidulidae) collected on May 24, 2020 on pig 4 in bloat stage. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

There are approx. 500 species of Cleridae (checkered beetles) in North America (Byrd & Castner, 2010), however, *Necrobia rufipes* (De Geer 1775) is the only member of this family to be identified, on pigs, to the species level in Quebec and reported throughout Canada (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). Both the larvae and the adult checkered beetle prey on fly eggs and larvae and they are also known for being necrophagous (Mayer & Vasconcelos, 2013). They are often found during later, drier stages of decomposition (Byrd & Castner, 2010). Another species to be that was identified in Quebec was *Enoclerus nigripes* (Say 1823) on human remains (Maisonhaute & Forbes, 2023). This species is known for being a predator (Lambdin et al., 2015).

1.4.3 Other Forensically Important Arthropods

There are other orders of arthropods that are important to forensic entomology but may only feed on carrion when available. Hymenoptera can also be important in the decomposition process, but they may not be associated with a particular stage of decomposition. Members of the Vespidae family have been observed cutting pieces of carrion and flying away with it (O'Donnell, 1995). Ants will often feed on carrion and prey on fly eggs and maggots. It is possible that their behavior can delay colonization by a day or two (Byrd & Castner, 2010). In indoor scenes, cockroaches (Order: Blattodea) have been known to feed on carrion and sometimes on living tissue. This is commonly seen in cases that involve neglected babies or elderly people in unsanitary conditions. A non-insect arthropod that is associated with cadavers are mites (Acari), and they can be seen on the body or on insects due to a phenomenon called phoresis (Gibbs & Stanton, 2001; Maisonhaute & Forbes, 2021a). This phenomenon is when a mite attaches itself to an insect, such as a Silphidae, for the purpose of travel. Many of them are known for being detritivores and consume dried meats. In outdoor scenes, it is possible to see large numbers of isopods (Order Isopoda) and some millipedes (Class Diplopoda) under the remains.

Other arthropods are important to forensic entomology because of the harm they do to humans rather than how they influence the decomposition process. These include for example spiders and scorpions. Their bites and stings can cause extensive damage and in some cases death (Byrd

& Castner, 2010). Members of the order Hymenoptera (bees, wasps, and hornets) are also social insects that have poisonous stingers. In many cases, death will only occur if the victim was stung by many individuals, or they have an anaphylactic reaction (Byrd & Castner, 2010).

1.5 Insect Overwintering and Diapause

Insects are poikilothermic animals that rely on their environment to regulate their internal temperature (Bale & Hayward, 2010). They can enter a state of prolonged dormancy regulated by hormones, which is called diapause (Whitfield & Purcell 2014). This is a planned genetic response to environmental conditions, such as temperature and precipitation, photoperiod, and seasonal change which can happen at any stage in an insect's lifecycle, and it varies between species (Bale & Hayward, 2010). This process is important for winter survival in areas with a cold climate such as Quebec.

There are many factors that will cause an insect to enter diapause. One of the common factors is the photoperiod. In temperate areas, the shortening of the daylight during the autumn causes the brain's endocrine system to release hormones that induce diapause (Whitfield & Pucell 2014). Another important factor is the temperature. Once the temperature drops to a certain range, e.g. 7°C - 9°C for *Calliphora vicina* (Robineau-Desvoidy 1830), then the insect will enter this state (Vinogradova & Reznik, 2013).

Diapause can happen at any point in an insect's lifecycle. Coleoptera of the Silphidae family often overwinter in the adult stage (Byrd & Castner, 2010). Conversely, members of the Diptera order overwinter in either the larval or adult stage depending on the family species (Vinogradova & Reznik, 2013). In Quebec, it was reported that members of the Piophilidae family, specifically *S. nigriceps*, have been seen to overwinter during the larval stage (Maisonhaute and Forbes (2021b), while members of the Calliphoridae family enter diapause either in the larval stage, which can occur by burying themselves, or in adult stage, depending on the family (Pitts & Wall 2005, Vinogradova & Reznik 2013).

Two factors that can affect the survival of the individuals overwintering in soil is the depth at which the individual buries itself and the amount of snow cover on the ground. In the United Kingdom, Pitts and Wall (2005) found that larvae that were buried closer to the surface had a greater chance of survival than if they were buried deeper (Pitts & Wall, 2005). The reason for this is that at deeper depths the soil temperature is greater than the temperature of the air. This means that individual buried deeper in the soil and exposed to higher temperature increases their metabolic rate, using up more of their nutrient reserves, and this decreases their chances of developing into adulthood. In a laboratory study by Sauders and Hayward (1998), *C. vicina* larvae were collected from Finland, Scotland, and Italy. Diptera from Scotland were able to survive at -4ºC and -8ºC in the larval stage, the larvae from Finland were more likely to survive at 0℃, and the larvae collected in Italy had no cold tolerance (Saunders & Hayward, 1998). The Scottish flies were able to survive at lower temperatures because they were not insulated by snow, and this exposes the Scottish flies to colder temperatures forcing them to adapt to this condition. During the winter in Finland, there is a prolonged snow cover on the ground which insulates the soil, making it warmer for the larvae in diapause. This results in them not being able to survive colder temperatures without the insulating snow. Prolonged snow cover is a characteristic of the wintertime in Québec, so it is possible that the larvae and pupae in the current study may benefit from this phenomenon as well.

1.6 Forensically Relevant Insect Studies in Canada

Forensic entomology research has been carried out in most provinces of Canada, including the remote regions of the Yukon Territory (Bygarski & LeBlanc, 2013). All studies in Canada have been carried out using pig carcasses and have explored a range of variables, including the differences between biogeographical regions, seasons, the influence of burning on entomological evidence, and the effects of sun exposure (Anderson, 2011; Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Comstock et al., 2015; Leblanc & Strongman, 2002; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). All these research projects have provided useable information for forensic investigators. However, in Quebec, there has not been any published forensic

entomology research since the 1800's until recently. The first recent study was carried out from November 2012 to May 2013 and published in 2021 (Taillefer & Giroux, 2021). This was a small study that involved one pig carcass for one winter. This limits the scope of the study but still provides useful insight into the entomological activity and the impact of the freeze and thaw cycle on decomposition. During this study the pig never entered the bloat stage of decomposition, and this could be due to the freezing and thawing of the carcass. The second forensic entomology study to be published was carried out on three pig carcasses in summer 2019 (Maisonhaute & Forbes, 2021a). This study highlighted the importance of carrying out local studies because the insect succession can be different from other biogeographical regions (Anderson, 2011). The third study involved two cadavers being placed in the summer of 2020 (Maisonhaute & Forbes, 2023). This represented the first controlled study involving human donors in Canada. Forensic entomology is still a young field in Quebec and further study is needed to better understand the entomological community and how the climate impacts the decomposition of bodies.

The current study in forensic entomology in Quebec occurred at the Université du Québec à Trois-Rivières (UQTR). The pig portion of this study took place on the campus of UQTR, and the human portion took place at the site for Research in Experimental and Social Thanatology/ *Recherche en Sciences Thanatologiques [Expérimentales et Sociales]* i.e. the REST facility. This is the first human decomposition facility in Canada and allowed this study to be conducted using human cadavers, following optimization of the method using pig carcasses. The results are intended to provide forensic investigators with more accurate PMI information and allow us to better understand how the unique Quebec environment impacts decomposition, particularly during the winter months.

1.7 Objectives

The two objectives of this research are to document how the freezing temperatures of the Quebec winter impacts the process of decomposition and entomological succession. Temperature has a large impact on the rate of decomposition but there have been only a few

studies that examine how freezing impacts this process and the insects associated with it (Micozzi, 1986; Taillefer & Giroux, 2021). One hypothesis of how the cold will impact the body decomposition is that the process will take more time by slowing down the various biological, chemical, and insect activities. Insect activity is also impacted by temperature due to the fact that insects are unable to regulate their internal temperature (Bale & Hayward, 2010). Another hypothesis is that the cold will dry out a body. The final hypothesis is that the bodies won't be colonized by necrophagous insects during the winter but will be colonized by insects that emerge from diapause in early spring, once the temperature increase. These freezing temperatures could influence when insects are first seen, and which ones are the first to arrive. This is because some insects will emerge from diapause earlier than others while some insects are more cold-tolerant.

The study used three pig carcasses and two human cadavers. The three pig carcasses were used to optimize the method which was subsequently applied during the human portion of the study using two human donors. In Quebec, there has been no study that documents how the winter freeze impacts the human decomposition and entomological succession. This study will be the first to provide this important information to law enforcement, search and rescue, and other relevant agencies.

Chapter 2: Method

This project followed experimental designs like other insect succession studies in forensic science (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a; Payne, 1965). This design involves studying how pigs and humans decompose in a comparable experimental environment, collecting immature and adult insect specimens, and rearing larvae to adulthood for identification. The collected adults were also identified. Most studies have been carried out during spring, summer, and autumn but this study required that the experimental subjects be placed during the winter months. This study involved two trials. The first trial was performed in a wooded area of the Université du Québec à Trois-Rivières using pig carcasses to optimize the method. The second trial was performed at the REST facility in Bécancour with human donors.

2.1 Pig Trial

Three pig carcasses weighing approximately 70 kg each were used as human analogs for the method optimization part of the study. Pigs are often used as a human analog in these studies because they are easier to obtain, easy to replicate, their ethical considerations are more straight forward, they have a controllable cause and time of death, and they have a similar body mass range, anatomy, body composition, hair coverage, and gut microbiota to humans (Matuszewski et al., 2020). All pig carcasses were obtained from the Boucherie Côté at Ste-Eulalie (Québec, Canada) already deceased and therefore did not require animal ethics approval. The pigs were euthanized with a perforating gun in accordance with the protocol of the Ministry of Agriculture (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, MAPAQ). The carcasses were placed at the site on February 25th, 2020, within a few hours of their death. The experimental site is in a wooded area on the campus of the Université du Québec à Trois-Rivières. This area mostly consists of conifers and deciduous trees and as a result, the carcasses were predominantly covered by shade throughout the day. Once placed on the surface, one data logger (Hobo MX2302A Bluetooth temperature probes, Onset Computer Corporation) was inserted in the mouth and another in the rectum of each carcass. These were used to measure

the internal temperature of the carcasses as decomposition and insect activity progressed. Cages made of a metal frame with 2.54 cm (1 in) chicken wire were placed over the carcasses to prevent vertebrate scavenging as shown in Figure 2.1. Ambient temperatures were downloaded from the Environment and Climate Change Canada weather station that was located close to the site. The Accumulated Degree Days (ADD) was calculated by adding the average daily temperature, with all sub-zero temperatures referred to as zero. There were no pigs placed during the warmer time to serve as a comparison, but the results were compared to a study that was carried out during the summer of 2019 (Maisonhaute & Forbes, 2021a).

Figure 2-1: Pig carcass and cage set up for the winter trial at UQTR (Quebec, Canada).
Photo: Pierre-Louis Arcand

2.2 Human Donors Trial

Two human donors were used in this part of the study. Both donors were placed at the REST site, located in a young (30-50 years) mixed temperate forest dominated by maple and spruce trees in Bécancour, Québec (Pecsi et al., 2020). The high-security site is surrounded by an electric and chain linked fence that is topped with barbed wire to prevent unwanted visitors and large scavengers. The first donor of the trial (donor 4) was placed on October 5th, 2020. The donor was female, 79 years old, weighing 79 Kg, and 152 cm tall. The second donor of the trial (donor 5) was placed on November 2nd, 2020. The donor was male, 72 years old, weighing 72 Kg, and 180 cm tall. Medical and other information for each donor is presented in Table 2.1. There was a data logger (Hobo MX2302A Bluetooth temperature probes, Onset Computer Corporation) inserted in the mouths of each donor. An anti-scavenging cage similar to the pig carcass cages was placed over each donor to prevent avian scavengers. The mesh on the sides of the cage was 1.27 cm (0.5 in) and the mesh on the top was 2.54 cm (1 in). The difference in the mesh size was to allow snow to fall through the top of the cage naturally. A weather station (Hobo U30 Station NO Remote Communication, Onset Computer Corporations) at the site measured temperature and precipitation. In the calculation for the Accumulated Degree Days (ADD) all sub-zero temperatures were referred to zero.

Table 2-1: Physical and medical information about the donors studied at REST (Quebec, Canada) during the autumn of 2020 to the summer of 2021.

Information	Donor 4	Donor 5
Age	79	72
Sex	Female	Male
Height	152	180
Body mass (Kg)	79	72
Body mass index	34	26
Date of death	October 4th, 2020	November 1st, 2020
Cause of death	Stroke	Melanoma
Medication	Apo-Esomeprazole, Apo-Ramipril, Crestor, Pro-Glyburide, Pro-Metformin, Pro-Piolitazone, Victoza, Novolin	Bupropion xl, Apo-Clopidogrel, Metoprolol, Jamp-ASA-ec, Apo-Tamsulosin, Apo-Ezetimibe, Atorvastatin
Arrival at REST	October 5th, 2020	November 2nd, 2020

2.3 Visitations and Insect collection

At first, the pig site was visited once a week until the weather was above 5°C. Thereafter, the carcasses were visited on those days that the temperature was 5°C and above. Starting May 1st, 2020, (day 65, fresh stage), the pig site was visited every day. These daily visitations occurred until June 6th, 2020 (day 101, advanced decay stage), at which point the carcasses were visited every two days due to a decrease in insect activity, advanced decomposition progression, and changes in the decomposition or insect communities that occurred less rapidly. Starting on June 18th, 2020 (day 113, dry remains stage), the site was visited twice a week due to the carcasses entering the dry remains stage. Finally, on August 3rd, 2020 (day 157, dry remains stage) the carcasses were visited once a week until the last day of the trial on October 27th, 2020 (day 244, dry remains stage).

Visitations of the human donors followed a different pattern. The reason for this difference is that the humans were placed at a different time of the year, and they decomposed in a different manner than the pigs. During the Autumn of 2020, the donors were visited every day until the

first snowfall which occurred on November 11th, 2020 (day 37 for donor 4 and day 9 for donor 5, both fresh stage). The donors were not visited again until March 10th, 2021 (day 155 for donor 4 and day 128 for donor 5, fresh stage). At this point, they were visited once a week until April 5th, 2021 (day 181 for donor 4 and day 154 for donor 5, fresh stage). Thereafter, the cadavers were visited every day until May 1st, 2021 (day 208 for donor 4, fresh stage; day 180 for donor 5, bloat stage) when the donors were subsequently visited every two days. Twice weekly visits commenced on June 9th, 2021 (day 247 for donor 4 and day 216 for donor 5, both in active decay stage). From July 7th, 2021 (day 274 for donor 4 and day 248 for donor 5, both in advanced decay stage) to the last day of the trial on July 29th, 2021 (day 296 for donor 4 and day 274 for donor 5, both still in advanced decay stage), the cadavers were visited once a week.

During these visits for both the pig and human studies, the weather conditions, such as temperature and cloud cover, and the stage of decomposition was noted. For the pig carcasses, three photos were taken: one as an overview from the top, one close-up of the face, and one close-up of the rear. For the two human donors, six photos were taken: one as an overview from the top, then the face, both arms, and both legs. Photos of specific decomposition characteristics (e.g. livor mortis and marbling) and insect activity (e.g. egg or larval masses) were also taken when observed.

The process carried out during these visits is detailed herein. Initially, the stage of decomposition was described along with the presence, location, and development stage of insects before any disturbance at the site. A reference of the site visitation document is available in Appendix A. The cage was then removed, and photos were taken as described previously including photos of the maggot masses and the insect activity on the remains. Next, insects flying over the body were collected using an entomological net. This was done by passing the net in a figure eight shape over the body. The captured specimens were placed in container. Following, samples of eggs, larvae, pupa, and adults seen on and around the carcass were collected. This was done using forceps or gloved fingers. Once the final step was completed, the cage was replaced over the remains. Upon returning to the lab the containers of all the collect adult insects were placed in a freezer to kill and preserve them, for later identification. The Diptera egg and larvae were

collected reared to adulthood to for easier identification. This process was the same for each pig carcass and human donor.

2.4 Insect Rearing and Identification

The rearing of the insects took place in the forensic entomology lab at the Université du Québec à Trois-Rivières. The focus of the rearing was Diptera eggs and larvae. The rearing containers consisted of a Mason jar that was half filled with wood shavings and a cup made of aluminum foil containing a piece of pork liver (Figure 2.2). The maggots and eggs were placed directly on the liver. The rearing containers were placed into an environmental chamber (Thermo Scientific Precision Model 818 Incubator, model PR505755L; Thermo Fisher Scientific Inc.) programmed to a temperature of 23ºC, with a photoperiod of 12:12 of dark:light and the humidity was kept at a rate of 30% to 70%. This variation in the humidity is due to the environmental chamber not having the ability to maintain humidity levels; thus, it was maintained by watering the jar with a spray bottle and having water containers in the bottom of the chamber. The piece of liver was changed before it was too dry. Eventually, the maggots migrated away from the liver and buried themselves in the wood shavings to pupate. Once the adults emerged from their pupa, the rearing containers were taken out of the environmental chamber to collect the adults for identification. This was accomplished by placing a sleeve made of a muslin fabric over the rearing container to trap the adult flies. The sleeve was placed in the freezer to kill and preserve the trapped insects. The Coleoptera that were captured were placed in the freezer, to be killed and preserved until they were identified.

Figure 2-2: Diptera rearing jar. Mason jar with wood shavings, a cup made from aluminum foil, and liver used to rear Diptera. Photo: Pierre-Louis Arcand

The flies and beetles that were collected were identified using a stereomicroscope (Leica S9i) and identification keys (Jones et al., 2019; Marshall et al., 2011; Rochefort et al., 2015; Whitfield et al., 2013). The Calliphoridae and Piophilidae were identified to the species level (Jones et al., 2019; Marshall et al., 2011; Rochefort et al., 2015). Some Silphidae and Staphylinidae were also identified to the species level (Brunke et al., 2011; Mullins et al., 2013), while all other Diptera and Coleoptera were identified to the family level (Whitfield et al., 2013).

Chapter 3: Results

3.1 Pig Environmental Data

The pig study was conducted from February 25, 2020, to October 27, 2020. The maximum ambient temperature was 36°C (July 10, day 135), and minimum ambient temperature was –21°C (March 1, day 4), with a daily average temperature of 11 ± 10 °C (Figure 3.1) (Environment and Climate Change Canada, 2020). The internal temperatures of the pig carcasses are also reported in Figure 3.1. On day 0, the internal maximum temperature ranged from 27°C (pig 6 rear) to 34°C (pig 5 head), while the ambient temperature was -1°C. Within three days, the internal temperatures declined to 0°C. The carcasses remained frozen until day 33 (pig 6) or day 38 (pigs 4 and 5). The minimum internal temperature of pig 4 was -2°C (day 27), pig 5 was -1°C (day 30), while pig 6 was –3°C (day 27). Once the carcasses were thawed, their internal temperatures were very similar to ambient temperature until late May and early June, when the internal temperature increased above the ambient temperature. The maximum temperatures that the carcasses reached were 39°C (pig 4, day 98), 40°C (pig 5, day 100), and 44°C (pig 6, day 98), respectively. Note that some data points are missing due to a malfunction with batteries in the data loggers.

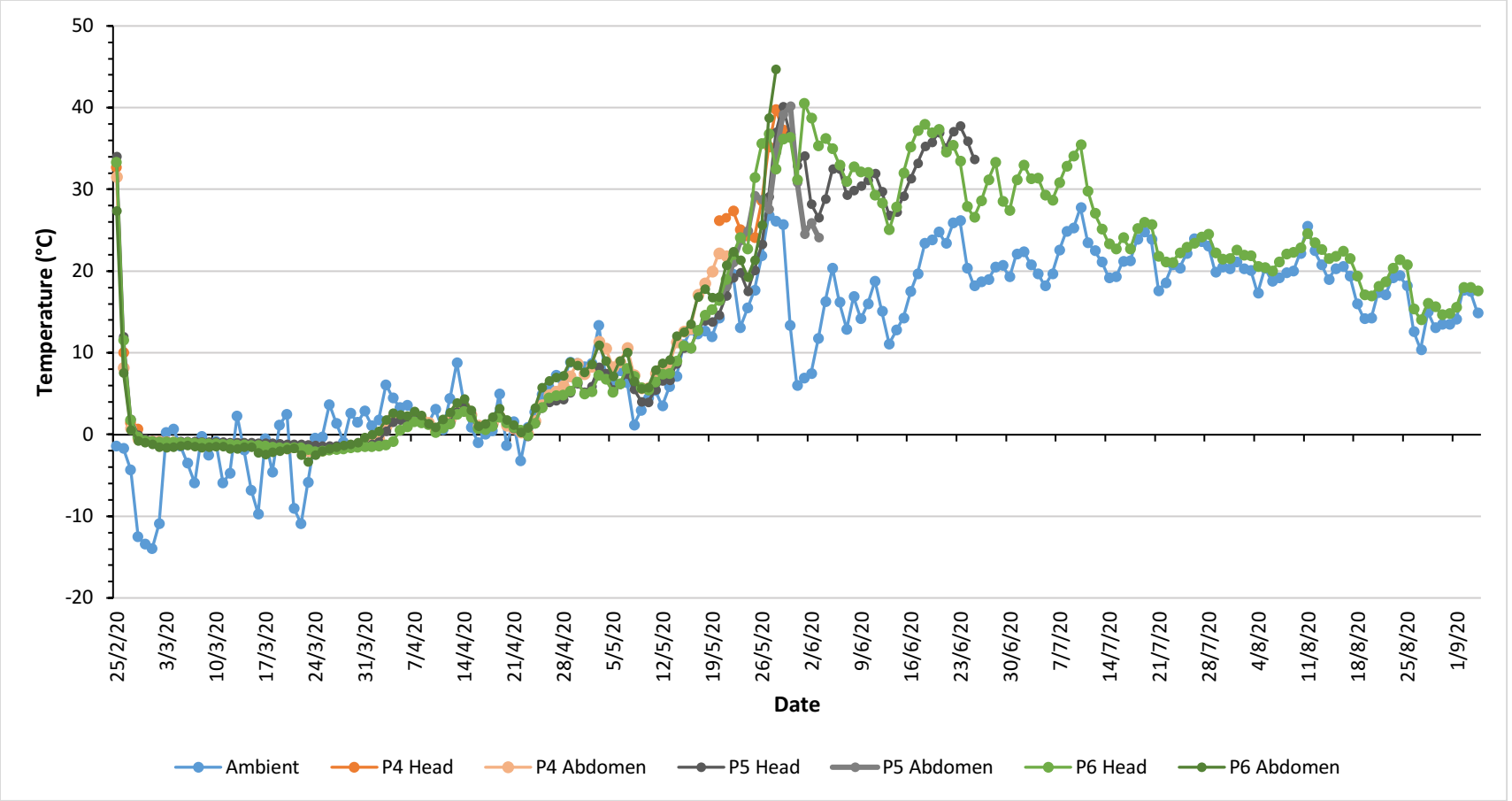


Figure 3-1: Mean daily ambient and internal pig carcass temperatures measured at Trois-Rivières (Quebec, Canada) during the pig study (recording for February 25-September 4, 2020). The values shown are an average of the daily hourly values. The ambient temperatures were obtained from Environment and Climate Change Canada.

The accumulated degree days (Figure 3.2) show that at the beginning of the study, the ADD value stayed the same due to the sub-zero temperatures. During the spring, the daily temperature started to rise slowly from 4 ADD to 764 ADD. During the summer there was a sharp increase from 789 ADD to 2491 ADD followed by a plateau at 2800 ADD during the autumn. There was significant snow covering the pig carcasses from day 0 to day 38. As the study started in late February, there was only 7 mm of snow recorded in that month. The month with the most precipitation was August with 196 mm and June had the lowest with only 21 mm (Figure 3.3).

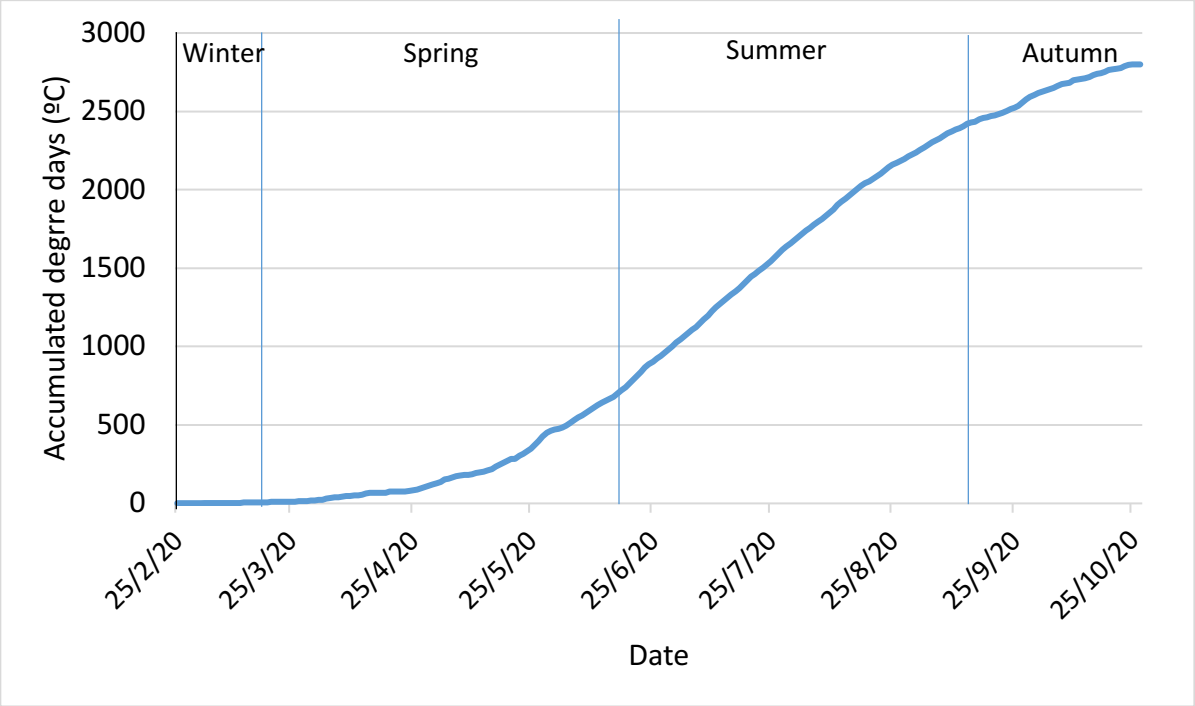


Figure 3-2: Accumulated Degree Days (°C) measured at Trois-Rivieres (Quebec, Canada) during the pig study (February 24th, 2020 - October 27th, 2020) (Environment and Climate Change Canada)

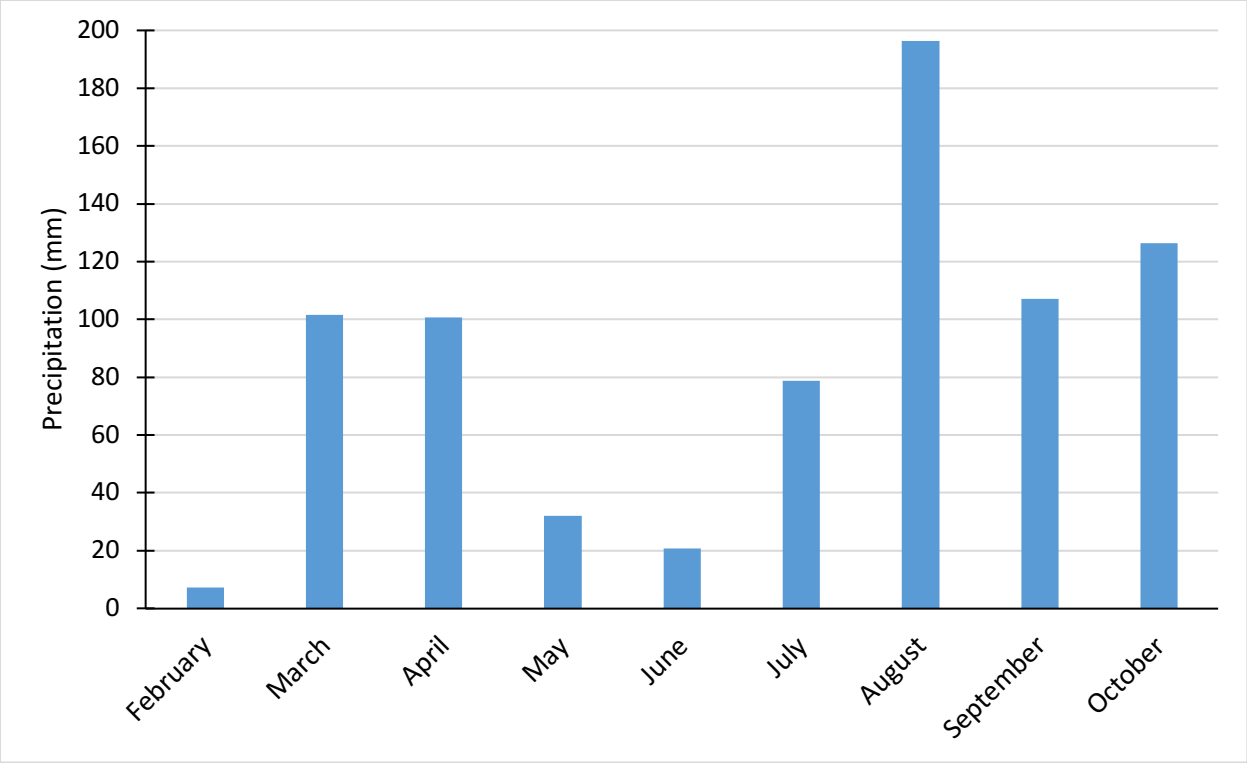


Figure 3-3: Monthly precipitation (combined rain and snow) data recorded at Trois-Rivières (Quebec, Canada) during the pig study (February 25th, 2020 - October 27th, 2020). Data was obtained from Environment and Climate Change Canada.

3.2 Pig Decompositions Stages

The timeline of the decomposition stages can be seen in Figure 3.4 and Figure 3.5. The pig carcasses remained in the fresh stage for 85 to 86 days. During this period, the pigs were frozen and covered in snow, which slowed down the decomposition process. The first sign of decomposition was seen on day 38 (April 4, 2020) with discoloration of the head and legs of all carcasses, and marbling was also visible on the rear legs of pigs 4 and 6. Pigs 4 and 5 entered the bloat stage on day 85 (May 21, 2020), and this stage lasted 8 days, while pig 6 entered the bloat stage on day 86 (May 22, 2020) and it lasted 7 days. This stage was marked by the abdomen of the carcasses becoming inflated. The pigs entered the active decay stage on day 93 (May 29, 2020), but it lasted only one day for pig 5, while it lasted 4 days for pigs 4 and 6. At this stage, the carcasses had all deflated and the abdomen of pig 6 had spilt. Pig 5 entered the advanced decay

stage on day 94 (May 30, 2020) and this stage lasted 19 days. During this time, the front half of the carcass began to desiccate. Pigs 4 and 6 entered the advanced decay stage on day 97 (June 2, 2020) and it lasted 16 days. The skull and the rear leg bones of pig 6 were exposed during this time. Finally, all the carcasses entered the dry remains stage on day 113 (June 18, 2020), which lasted until day 244 (completion of the trial). During this stage, the skin of the carcasses desiccated, there were large numbers of bones exposed, and there was very little soft tissue remaining. Images of the individual decomposition stages can be seen in Figure 3.5.

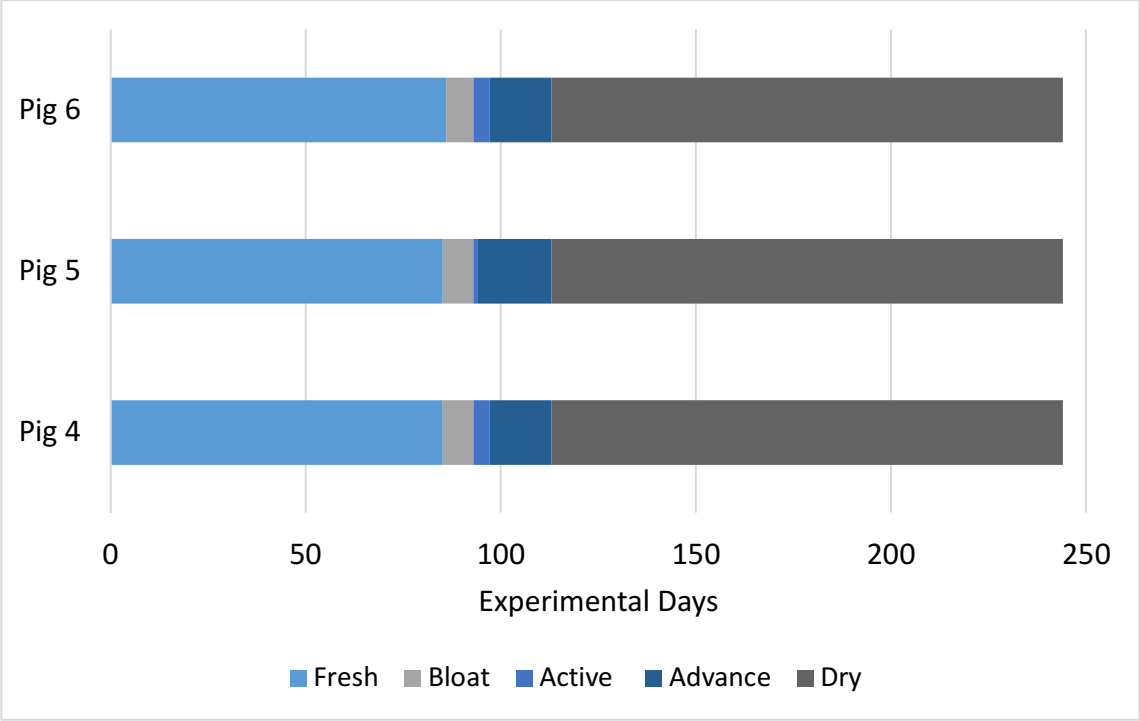


Figure 3-4: Decomposition stages of pig carcasses placed at UQTR site in Trois-Rivières (Quebec, Canada) from February 25th, 2020 to October 27th, 2020.

Figure 3-5: Stages of pig decomposition left to right: A) Fresh stage on day 0 B) Bloat stage on day 90, C) Active decay stage on day 96, D) Advanced decay stage on day 105, and E) Dry remains stage on day 110. Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

3.3 Fly Succession on Pig Carcasses

The data from observations, net sweeping, and rearing determined the succession of necrophagous flies and other flies on the pig carcasses. Table 3.1 shows the succession of Diptera throughout the study. There was no fly activity observed until day 30 (average daily temperature 2°C) when the carcasses were in the fresh stage. The first fly to be seen was on pig 5 and it belonged to the Sciaridae which is not a necrophagous fly (figure 3.6). The next fly to be observed on the carcasses belonged to the Dryomyzidae family and appeared on day 52 (0.5°C, fresh

stage). This family is often observed on decomposing carcasses in woodlands (Leblanc & Strongman 2002; Hwang & Turner 2005). Following this, individuals of the Calliphoridae family were observed on day 57 (1°C, fresh stage) and were present until day 175 (13°C, dry remains stage). They were not observed beyond this point. The first two adults of the eight species to be identified to the species level in this study were *C. cadaverina* and *C. livida* on day 58 (3°C, fresh stage). *Cynomya cadaverina* was observed as adults until day 67 (13°C, fresh stage) and *C. livida* until day 76 (4°C, fresh stage). The next species to be observed was *P. regina* on day 59 (5°C, fresh stage) until day 135 (28°C, dry remains stage). The first adult of *P. terraenovae* was observed on day 66 (9°C, fresh stage) and the last one was observed on day 159 (20°C, dry remains stage). They were not observed during the bloat and advance decay stage. On day 69 (5°C, fresh stage), specimens of *C. vomitoria*. were observed. The last of this species was observed on day 101 (12°C, advanced decay stage). Of the *Lucilia* genus, *L. sericata* Meigen 1826 was only observed on day 84 (14°C, fresh stage) and day 93 (26°C, active decay stage), while *L. silvarum* Meigen 1826 was only observed on day 86 (20°C, fresh stage) and day 87 (13°C, bloat stage). The last species to be observed in this genus was *L. illustris* on day 88 (16°C, bloat stage) on pig 5 and day 90 (22°C, bloat stage) on pig 4.

Figure 3-6: A specimen of Sciaridae seen on pig 5 on March 27th, 2020 (day 30, fresh stage). Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

The first Diptera egg mass was seen on day 52 (0.5°C, fresh stage) in the ear of pig 4. Possibly due to the cold temperatures, this mass produced no adults. The first collected egg mass to successfully rear adults belonged to *P. regina* and was collected on day 58 (3°C, fresh stage). The eggs were collected from a mass in the mouth of pig 6. The first larvae were observed on day 66 (9°C, fresh stage) around the nose of pig 4. These larvae were not identified to the species level since they did not survive the rearing process. Calliphoridae larvae started to migrate away from the carcasses on day 103 (17ºC, advanced decay stage). After this day fewer were observed and the last one was seen on day 146 (18°C, dry remains stage). On day 69, the first immatures of *P. terraenovae* and *C. vomitoria* were collected. The last *C. vomitoria* larvae were collected on day 101. On day 146, the last immatures *P. terraenovae* were collected. The first immatures of *C. livida* were collected on day 71 (6°C, fresh stage) and were present until day 98 (8°C, advanced decay stage). Likewise, the first immature specimens of *C. cadaverina* were collected on day 77 (6°C) and were present until day 95 (13ºC, advanced decay stage). Immatures of *C. vicina* were only collected from pig 6 on day 87 (13°C, bloat stage) and pig 5 on day 95 (13°C, advanced decay stage) but were never captured as adults using a net. Pig 4 was the only carcass to have immatures of *L. illustris,* on day 92 (26°C, bloat stage).

The first adult flesh flies (Sarcophagidae) were observed on day 84 (14°C), while the carcasses were still in the fresh stage. They were observed until day 149 (20°C) when the carcasses had progressed to the dry remains stage. The first larvae were collected on day 87 (13°C) during the bloat stage, and the last larvae were collected on day 146 during the dry remains stage.

The Muscoidea super family were present throughout the entire experiment, from the fresh stage to the dry remains stage. The first adult to be observed belonged to the Muscidae family on day 52 (7°C, fresh stage). The last adult was observed on day 227 (14°C, dry remains stage). Individuals of *Hydrotaea* were seen on day 71 (6°C, fresh stage) and day 191 (15°C, dry remains stage) was the last day they were observed. Adults of Anthomyiidae were observed from day 105 (16°C, advanced decay stage) to 113 (23°C, advanced decay stage). Muscidae larvae were first seen on day 89 (18ºC, bloat stage) on pig 4 and pig 5 while *Hydrotaea* larvae were also seen on pig 5 on day 92 (26°C, bloat stage).

Adults of skipper flies (Piophilidae) were first seen on pig 4 on day 67 (13°C, fresh stage) and until day 244 (-2°C, dry remains stage) on pig 5 and pig 6. The two most common species and the two that were identified to the species level were *P. xanthostoma* and *S. nigriceps*. *Stearibia nigriceps* larvae first appeared on day 93 (26°C, active decay stage) and remained until day 244 (-2°C, dry remains stage), which is when the study ended. The only *P. xanthostoma* larvae were seen on day 94 (13°C, active decay stage).

There were several families that were only observed as adults. The first family to be seen was the Phoridae on day 77 (6°C, fresh stage), which was seen until 183 (10°C, dry remains stage). The family observed the most was the Sepsidae family, which was present from day 87 (13°C, fresh stage) until day 191 (15°C, dry remains stage). Specimens of Heleomyzidae were seen during the whole process of decomposition and individuals of Sphaeroceridae were seen from the active stage until the dry remains stage. There were also adult Diptera that were observed simply because they were in the environment, which was a mixed forest. Mosquitoes (Culicidae) were observed flying around the site during the fresh and dry remains stage. Tachinidae was observed on pig 4 on day 71 (6°C, fresh stage), adult Chironomidae on day 124 (21°C, advanced decay stage), Mycetophilidae on day 105 (16°C, advanced decay stage) and Dolichopodidae on day 130 (18°C, dry remains stage). Finally, there was a variety of small flies that were not identified.

Table 3-1: Succession of Diptera on pig carcasses placed at UQTR (Quebec, Canada) from February 25th, 2020 to October 27th, 2020. Families are displayed following the same order as the colonization.

Families	Species	Fresh	Bloat	Active Decay	Advanced Decay	Dry Remains
		0-86	87-92	93-96	97-111	113-244
Culicidae	-	a				a
Tachinidae	-	a				
Sciaridae	-	a				
Dryomyzidae	-	a				
Calliphoridae		i,a	i,a	i,a	i,a	i,a
	Cynomya cadaverina	i, a	i	i		
	Calliphora livida	i, a	i	i	i	
	Phormia regina	i, a	i, a	i, a	i, a	i, a
	Protophormia terraenovae	i, a	i	i, a	i	i, a
	Calliphora vomitoria	i, a	i, a	i, a	i,a	
	Calliphora vicina		i			
	Lucilia sericata	a		a		
	Lucilia silvarum	a	a			
	Lucilia illustris		i, a			
Sarcophagidae	-	a	i	i, a	i, a	i, a
Muscidae		a	i, a		a	a
	Hydrotaea spp.	a	i			a
Piophilidae	-	a	a	a	a	a
	Prochyliza xanthostoma	a	a	i, a		
	Stearibia nigriceps		a	i, a	i, a	i, a
Phoridae	-	a	a		a	a
Heleomyzidae	-	a	a	a	a	a
Sepsidae	-		a	a	a	a
Sphaeroceridae	-			a	a	a
Anthomyiidae	-				a	a
Mycetophilidae	-				a	
Chironomidae	-					a
Dolichopodidae	-					a

a= Adult; i= Immature; Numbers indicate experimental days

3.4 Beetle Succession on Pig Carcasses

Data collected from visual observations allowed the determination of the beetle succession as shown in Table 3.2. The first beetles observed on the pig carcasses were the Silphidae *O. noveboracense* and the Nitidulidae *O. nearctica* on day 63 (9°C, fresh stage). *Omosita nearctica* was recorded on pig 4 and *O. noveboracense* was recorded on pig 6. Specimens of *O. nearctica* were seen throughout the whole process with the last day being day 240, while individuals of *O. noveboracense* were also observed during the boat and dry remains stage (day 113). Specimens of *O. noveboracense* were only observed on Pig 5 on day 78 (7°C, fresh stage). The next species of Nitidulidae to be observed was *G. quadrisignatus* on day 75 (5°C, fresh stage). It was only observed on this day on Pig 4. There were two Silphidae species collected in this study. The first was *Necrodes surinamensis* (Fabricius, 1775) which was only seen on day 86 (20°C, fresh stage) on Pig 4 and Pig 6. This species was also observed on day 87 (°C, bloat stage) and day 93 (26°C, active decay stage) for Pig 4. The second species to be observed belonged to *Nicophorus*, seen on day 89 (18°C, bloat stage) on Pig 6. This was the only time this insect was observed.

Adult rove beetles (Staphylinidae), clown beetles (Histeridae), and checkered beetles (Cleridae) were recorded throughout the decomposition process. The first Staphylinidae observed belonged to *C. maxillosus*. It was observed on day 67 (13°C, fresh stage) and remained until the active decay stage. The second Staphylinidae species observed was *O. cingulatus*, observed on day 84 (14 ºC, fresh stage) and day 88 (16 ºC, active stage). Several other non-identified Staphylinidae were observed until the last day of the trial (-2°C, dry remains stage). Cleridae were first observed on day 82 (13°C, fresh stage) and Histeridae on day 84 (14°C, fresh stage).

There were two families that were only present in very low numbers. Tenebrionidae were observed during the advanced decay and dry remains stages. They were first recorded on day 97 (7 ºC, advanced decay stage) and last seen on day 135 (27 ºC, dry remains stage). This family is not forensically important but is known for feeding on decomposing plant matter and can be used to clean animal bones (Rumbos & Athanassiou, 2021; Sizer et al., 2023) Dermestidae were only observed on day 75 (5 ºC, fresh stage) on Pig 4.

Silphidae, Staphylinidae and Nitidulidae were the only families of beetle to have larvae present on the carcasses. The Silphidae larvae were observed from day 92 (26 ºC, bloat stage) until day 124 (20 ºC, dry remains stage). Nitidulidae and Staphylinidae larvae were only seen during the advance decay and dry remains stage on day 116 (23 ºC) and day 124 (20 ºC), respectively.

3.5 Other Arthropods Present on Pig Carcasses

Other insects and arthropods were observed throughout the decomposition process. They are also recorded in Table 3.2. On day 65 (8℃, fresh stage), individuals of Lampyridae were first seen on pig 6. This is a family of predatory Coleoptera that are not often associated with decomposition. They were also observed during the advanced decay stage and were last observed on day 127 (22°C) during the dry remains stage. A wasp (Hymenoptera: Vespidae) was observed on Pig 5 from day 71 (6℃, fresh stage) until day 175 (14℃, dry remains stage). Members of Formicidae were first observed on day 83 (12℃, fresh stage) for pigs 4 and pig 6, and on day 88 (16℃, bloat stage) they were first observed on pig 5. The last time they were observed was on day 240 (12℃, dry remains stage) on pig 5. Parasitoid wasps (Hymenoptera) were observed in the rearing jars on day 116 (23°C, dry remains stage) and were last observed on day 156 (20°C, dry remains). This observation occurred because infected fly larvae were collected. On day 156 (20°C, dry remains stage), one Hemiptera was observed on pig 4. Several specimens of Collembola were observed on all three carcasses on the day they were placed (-1℃, fresh stage) and their final observation was on day 30 (1°C, fresh stage). Mites (Acari) were observed on all three pigs. They were first observed on day 74 (5℃, fresh stage) for pig 4 and pig 6, and on day 75 (5℃, fresh stage) for Pig 5. They were recorded until day 240 (12℃, dry remains stage). On days 135 and 146 (18℃, dry remains stage), some individuals of Opiliones were observed on pig 4. On day 156 (20°C, dry remains stage), one Hemiptera was observed on pig 4.

Table 3-2: Succession of Coleoptera and other arthropods on pig carcasses placed at UQTR (Quebec, Canada) from February 25th, 2020 – October 27, 2020 Families are displayed following the same order as the colonization. a= Adult; i= Immature; Numbers indicate experimental days

Taxon	Species	Fresh	Bloat	Active Decay	Advanced Decay	Dry Remains
		0-86	87-92	93-96	97-11	113-244
Collembola		a				
Silphidae		a	i, a	i, a	i, a	i, a
	Oiceoptoma noveboracense	a	a			a
	Necrodes surinamensis		a	a		
	Nicrophorus spp.		a			
Nitidulidae		a	a	a	a	a
	Omosita nearctica	a	a	a	i, a	i, a
	Glischrochilus quadrisignatus	a				
Staphylinidae		a	a	a	i, a	i, a
	Creophilus maxillosus	a	a	a		
	Ontholestes cingulatus	a	a	a		
Dermestidae		a				
Cleridae		a	a	a	a	a
Histeridae		a	a	a	a	a
Tenebrionidae					a	a
Lampyridae		a			a	a
Vespidae		a	a		a	a
Formicidae		a	a	a	a	a
Parasitoid wasps						a
Acari		a	a	a	a	a
Hemiptera						a
Opiliones						a

3.6 Human Donors Environmental Data

The first donor (donor 4) was placed at REST on October 5, 2020, and the second donor (donor 5) was placed on November 2, 2020. This portion of the study ran until July 29, 2021. The minimum average temperature was -20°C (January 31, 2021) and the maximum average temperature was 28°C (June 7, 2020). The average daily temperature was 5 ± 11 °C (Figure 3.7). These temperatures were recorded from the weather station located on the site. A data logger was placed in the oral cavity of donor 4 on October 5 until November 19, 2020, when it was removed due to battery issues. The second donor did not have a data logger during this time due to the same issues. During the period recorded, the internal temperature was very similar to the ambient temperature. The data loggers were restarted to collect data on March 23, 2021 (donor 4) and on April 27, 2021 (donor 5). During the spring and summer, the ambient temperature and daily average internal temperatures were very similar (Figure 3.7). The maximum daily temperature for donor 4 was 23°C (day 266) and for donor 5 it was 27°C (day 214). While the minimum daily temperature for donor 4 was -2°C (day 45) and it was 11°C (day 180) for donor 5. The average temperature for donor 4 was 11 ± 6 °C and 19 ± 4 °C for donor 5. The highest temperatures for donor 4 was 26°C (274), while for donor 5 it was 60°C (218). This temperature only lasted an hour and could be due to a data logger issue. If this point was removed, then it was 34°C on day 219.

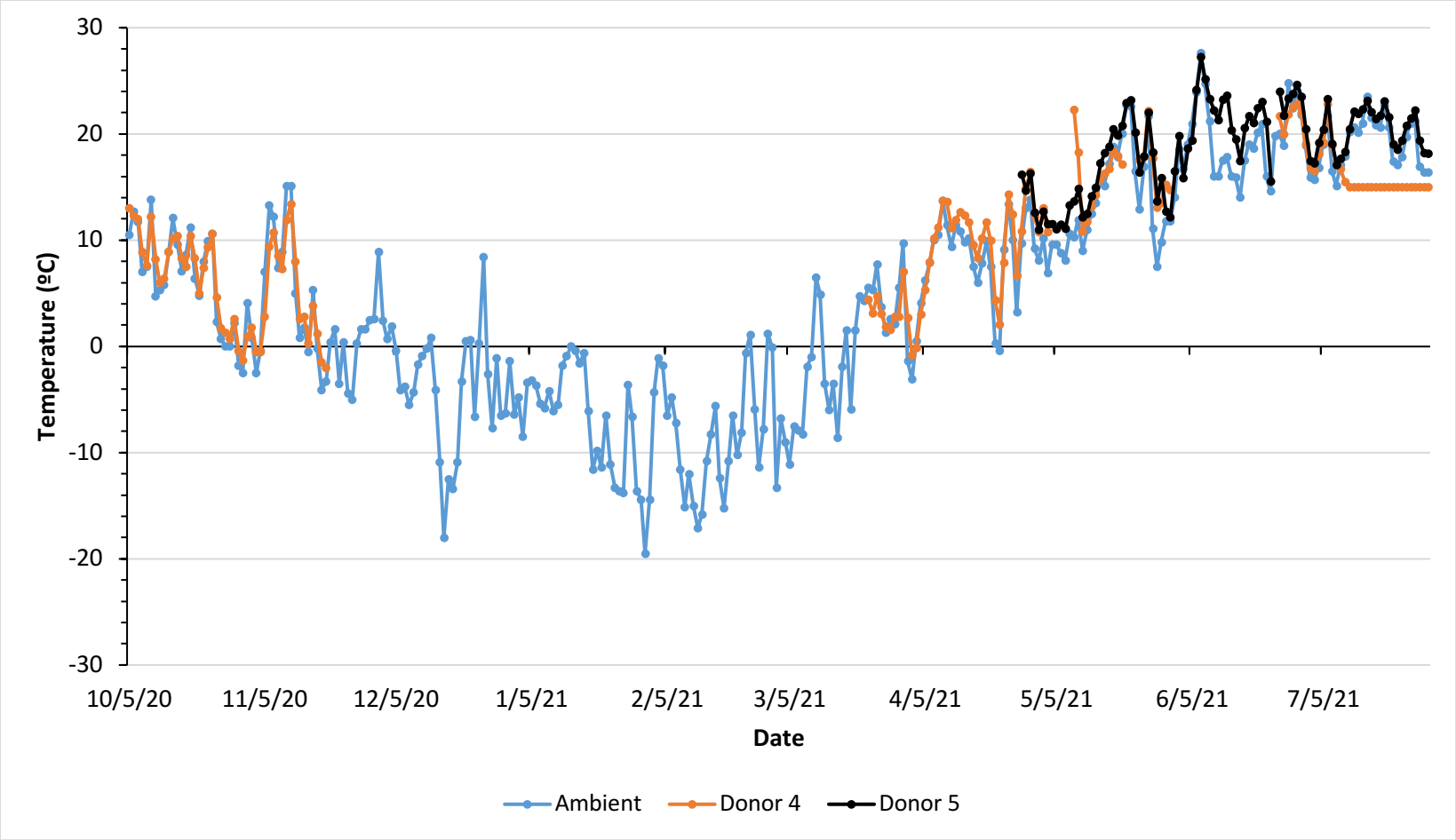


Figure 3-7: Mean daily ambient and internal donor temperatures measured at REST (Quebec, Canada) during the human study (October 5th, 2020-July 27th, 2021).

The accumulated degree days (Figure 3.8) show that there was a slow increase of temperature during the autumn months. The number of degree days associated to donor 4 increased from 10 ADD to 305 ADD, while it increased from 1 ADD to 119 ADD for donor 5. The temperature plateaued during the winter months due to sub-zero temperatures. During the spring and summer, the ADD increased rapidly for both donors, from 329 ADD to 2151 ADD for donor 4, and from 144 ADD to 1965 ADD for donor 5. The weather station at the REST site measured precipitation but it does not measure snowfall. The month of October experienced the most precipitation with 130 mm while February had the fewest with 2 mm of precipitation (Figure 3.9).

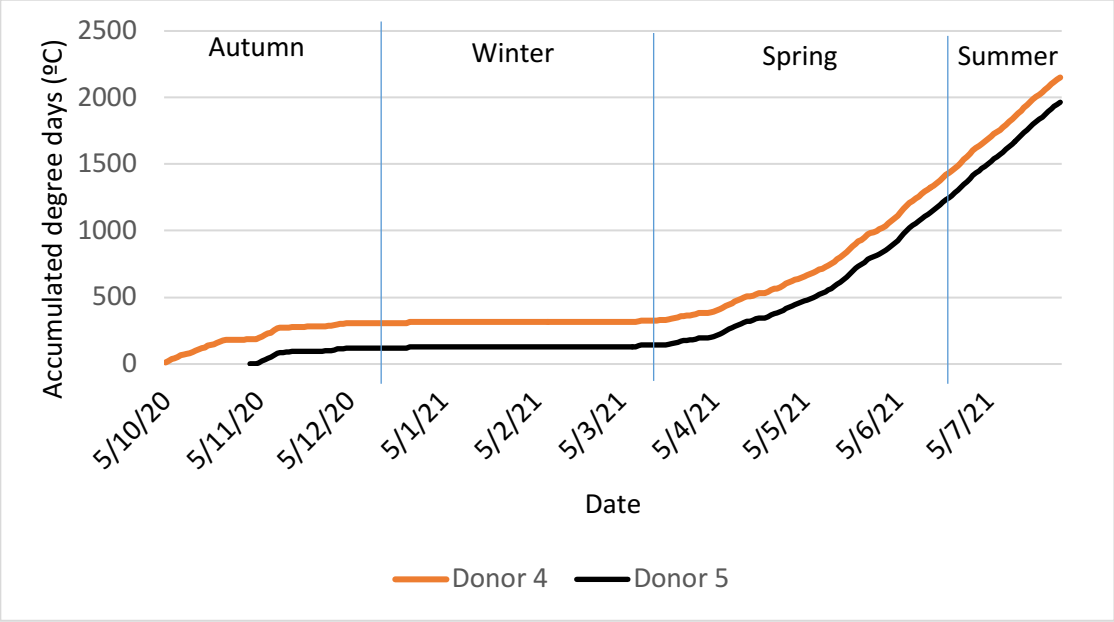


Figure 3-8: Accumulated Degree Days (°C) measured at REST (Quebec, Canada) during the human study (October 5th, 2020-July 27th, 2021). Data were obtained from the REST weather station.

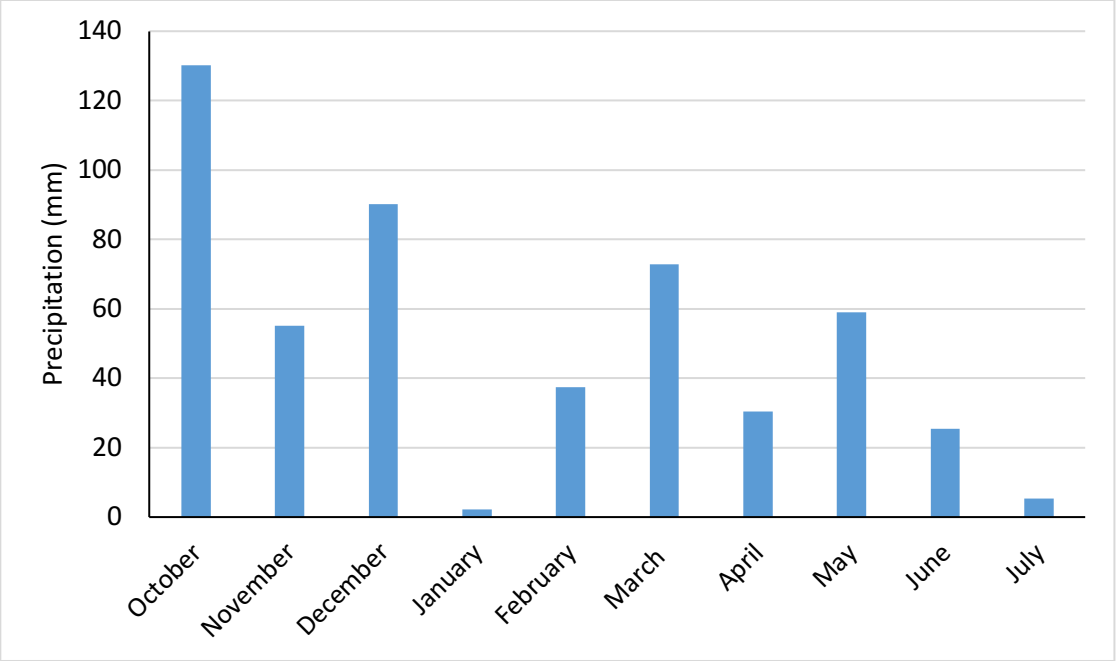


Figure 3-9: Monthly precipitation data recorded at REST (Quebec, Canada) during the human study (October 5th, 2020-July 27th, 2021). Data were obtained from the REST weather station.

3.7 Human Donors Decomposition Stages

The timeline of the decomposition process can be seen in Figure 3.10. During the autumn months (October and November 2020), both donors experienced very little change except for moderate color change, marbling, and some desiccation in the face, limbs, and torso. These can be seen in Figure 3.11 for donor 4 and Figure 3.12 for donor 5. When the bodies were re-visited in early March 2021 (spring) the skin had continued to desiccate and change color despite being covered in snow and exposed to sub-zero temperatures. Donor 4 never entered the bloat stage. This may have resulted from the fact that they were partly submerged in water during the early spring thaw. Donor 5 entered the bloat stage on day 177 (April 28, 2021) and it lasted 30 days. This was marked by the abdomen being slightly inflated. Donor 4 entered the active decay stage on day 209 (May 2, 2021), while donor 5 entered this stage on day 207 (May 28, 2021). This stage lasted 46 and 11 days for donors 4 and 5, respectively. Both cadavers demonstrated large amounts of desiccated skin, but there was still a significant amount of soft tissue present underneath. Donor 4 entered the advanced decay stage on day 255 (June 17, 2021) while donor 5 entered this stage

on day 219 (June 9, 2021). By this stage, both donors showed significant amounts of desiccation and minimal soft tissue. Donor 5 also presented large holes in the face, neck, and abdominal area. These were caused by insect activity and the drying of the skin. Neither of the bodies entered the dry remains stage by the completion of the experiment which ended on July 29, 2021. Figure 3.13 and Figure 3.15 shows the complete process observed for donors 4 and 5, respectively.

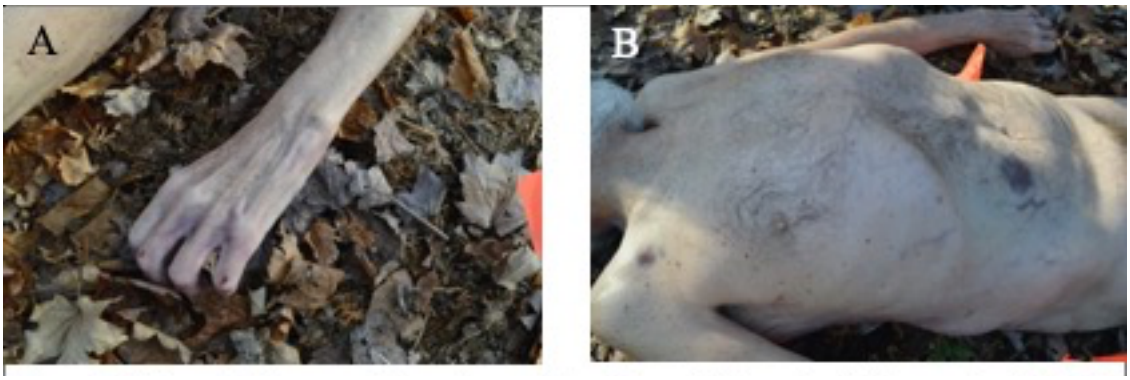


Figure 3-10: Signs of decomposition observed on donor 5 from day 5 (November 7th, 2020) to day 6 (November 8th, 2020). A) Discoloration of the left hand, B) Marbling and discoloration on the lower abdomen and chest (Quebec, Canada). Photo: Pierre-Louis Arcand

Figure 3-11: Stages of decomposition of Donor 4 from October 5th, 2020, to June 23th, 2021. Left to right A) Fresh Stage (Day 0, Autumn, October 5, 2020), B) Fresh Stage with Superficial Desiccation (Day 175, Spring, March 30, 2021), C) Active Decay Stage (Day 215, Spring, May 9, 2021), D) Advanced Decay Stage (Day 260, Summer, June 23, 2021). Photos taken at REST (Quebec, Canada). Photo: Pierre-Louis Arcand

Figure 3-12: Stages of Decomposition for donor 5 from November 6th, 2020 to June 16th, 2021. Left to right A) Fresh Stage (Day 4, Autumn, November 6, 2020), B) Fresh Stage with Superficial Desiccation (Day 143, Spring, 25 March, 2021), C) Bloat Stage (Day 197, Spring, May 18, 2021), D) Active decay stage (Day 216, Spring, June 9, 2021), E) Advanced decay stage (Day 222, Spring, June 16, 2021). (Quebec, Canada). Photo: Pierre-Louis Arcand

3.8 Fly Succession on Human Donors

The phenology of Diptera was determined from data collected by observations, net sweeps, and rearing of immature flies. This data is shown in Table 3.3 for donor 4. The first flies were seen during the autumn on donor 4 on day 1 (13°C, fresh stage), and include the fallowing families: Phoridae, Sciaridae, Simuliidae, and Trichoceridae. The first family listed is known for being associated with cadavers, but the last three families are not. Calliphoridae were first seen on day 4 (8°C, fresh stage). The first species to be reared from egg to adulthood were *C. cadaverina* and *L. illustris* and they were collected on day 5 (14°C, fresh stage). On day 6 (5°C, fresh stage), *C. livida* eggs were collected and on day 9 (9°C, fresh stage), *C. vomitoria* eggs were collected. All these flies were seen until day 35 (9°C, fresh stage) except for *L. illustris* which was only seen until day 18 (10°C, fresh stage). Day 9 was when the first Bibionidae and Piophilidae were observed, and they were present until day 37 (15°C, fresh stage). The adult Bibionidae are known for feeding on nectar, so they may have been seen on the cadavers because they were already present in the area (Skartveit et al., 2014), while the larvae are known for feeding on decomposing plant matter or leaf litter (Frouz & Šimek, 2009; Skartveit et al., 2014). On day 10, the first Muscidae was collected and was only collected one additional time on day 32. Dryomyzidae was first seen on day 7 and last observed on day 35 (Figure 25). Day 22 was the only time Anthomyiidae was observed during the autumn. The last family to be observed was Sphaeroceridae on day 35.

The first Diptera observed during the springtime belonged to the Muscidae and was observed on day 171 (8°C, fresh stage). Muscid flies were again seen on day 186 (14°C, fresh stage) and 247 (21°C, active decay stage). During the spring, individuals of Calliphoridae were first observed on day 182 (8°C, fresh stage). The first eggs to be reared to adulthood belonged to *C. cadaverina, C. vicina,* and *C. livida*, collected on day 186 (14°C, fresh stage). Specimens of *Calliphora livida* were observed until day 205 (14°C, fresh stage) and of *C. cadaverina* were observed until day 210 (7°C, active decay stage). *Calliphora vicina* was only observed on day 185. On day 219 (11°C, active decay stage), larvae of *P. regina* were observed and were seen until day 283 (24°C, advanced

decay stage). Immatures of *C. vomitoria* were observed on day 239 (18°C, active decay stage) and observed until day 260 (15°C, advanced decay stage). During the spring, the first Piophilidae was observed on day 184 (10°C, fresh stage). The first eggs to be reared to adulthood were *P. xanthostoma,* collected on day 224 (19°C, active decay stage). They were observed until day 290 (18°C, advanced decay stage). The first eggs of *S. nigriceps* were collected on day 226 (20°C, active decay stage) until day 274 (17°C, advanced decay stage) on donor 4. These two species were the most common ones to be collected, but there were others that were not identified to the species level. On day 244, a Phoridae larva was collected. This is the only day that a member of this family was collected. Sepsidae was first seen on day 185 (10°C, fresh stage), and then observed on day 204 (10°C, fresh stage), 216 (10°C, active decay stage), 219 (11°C, active decay stage), and finally 225 (19°C, advanced decay stage). On day 186 (14°C, fresh stage), specimens of Muscidae were observed for the first time during the spring, and they were also observed on day 247 (21°C, active decay stage). Anthomyiidae was observed on day 222 (16°C, active decay stage) while specimens of *Hydrotaea* were observed as adults on day 231 (13°C active decay stage). The next family to be observed was Sphaeroceridae on day 216. They were also seen on days 242 (19°C, active decay stage) and 249 (16°C, active decay). Day 242 (19°C, active decay stage) was the only day that Heleomyzidae was observed. The last family to be observed was Sarcophagidae, observed on days 244 (28°C, active decay stage), 246 (21°C, active decay stage), and 255 (19°C, advanced decay stage). Sarcophagidae was only observed as adult.

Table 3-3: Succession of Diptera on donor 4 at REST (Quebec, Canada) from October 5th, 2020 to July 29th, 2021. Families are displayed following the same order as the colonization.

Families	Species	Fresh Autumn	Fresh Spring	Active Decay	Advance Decay
		0-37	161-208	210-254	256-297
Phoridae		a	a	i, a	a
Sciaridae		a			
Simuliidae		a			
Trichoceridae		a			
Calliphoridae		a	a	a	a
	Cynomya cadaverina	i	i	i	
	Lucilia illustris	i			
	Calliphora livida	i	i, a		a
	Calliphora vomitoria	i		i, a	i
	Calliphora vicina		i		
	Phormia regina			i, a	i, a
Bibionidae		a			
Piophilidae		a	a	a	a
	Prochyliza xanthostoma		a	i, a	i
	Stearibia nigriceps			i, a	i, a
Sepsidae			a	a	
Muscidae/Fanniidae		a	a	a	
Muscidae		a	a	a	
	Hydrotaea spp.			a	
Anthomyiidae			a		
Dryomyzidae		a			
Sphaeroceridae		a		a	
Heleomyzidae				a	
Sarcophagidae				a	a

a= Adult; i= Immature; Numbers indicate experimental days

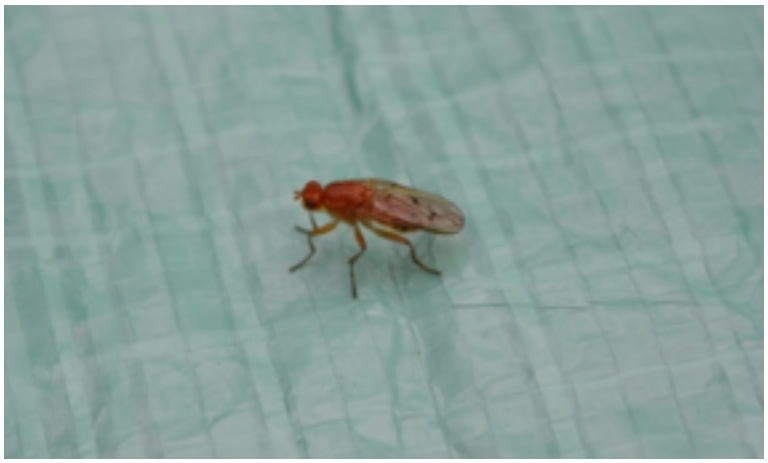


Figure 3-13: Dryomyzidae fly seen on donor 4 on October 19, 2020 (Day 14, fresh stage). Taken at UQTR (Quebec, Canada). Photo: Pierre-Louis Arcand

Table 3-4 shows the phenology of Diptera on Donor 5. During the autumn, there was a delay of four days in Diptera activity on donor 5. On day 4 (13°C fresh stage), the first Calliphoridae observed was *C. cadaverina* as eggs. The next Calliphoridae eggs were collected on day 9 (11°C, fresh stage) and they belonged to *L. illustris*. This is the only day this fly was collected at this time of year. On day 8 (15°C, fresh stage), Sphaeroceridae was observed as adults. This was the only time this family was observed on donor 5 during this time of year.

The first egg mass to be collected during the spring occurred on day 154 (6°C, fresh stage) and belonged to *C. cadaverina.* There was little to no activity before this because of the cold temperatures. This fly was only observed until day 177 (13°C, bloat stage). The next Calliphoridae specimens to be seen were *C. livida* and *C vicina* on day 155 (8°C, fresh stage). Specimens were collected as eggs. *Calliphora livida* was only observed until day 160 (9°C, fresh stage), while *C. vicina* was observed until day 167 (8°C, fresh stage). On day 161 (11°C, fresh stage), *P. terraenovae* eggs were collected. Specimens were observed two more times, on day 214 (28°C, active decay stage) and 252 (24°C, advanced decay stage). The last Calliphoridae to be collected was *P. regina* on day 178 (14°C, bloat stage) and specimens were collected until day 268 (18°C, advanced decay stage). On day 148 (4°C, fresh stage), the first Piophilidae was observed until day 274 (16°C, advanced decay stage), the final day of collection for the trial. The first eggs of this

family to be collected on day 197 (19°C, bloat decay stage) belonged to *S. nigriceps*. This species was observed in their immature form (larvae) until day 252 (24°C, advanced decay stage). A second Piophilidae species, *P. xanthostoma*, was collected on day 211 (19°C, advanced decay stage) as larvae. Once again these were the two most common species of this family, but few others were seen but they were not identified to the species level. Specimens of Dryomyzidae were observed on day 141 (6°C, fresh stage) and day 143 (8°C, fresh stage), along with Phoridae and Sphaeroceridae on the latter day. Sphaeroceridae specimens were seen until day 219 (21°C, advance decay state). The next family to be observed was Muscidae, on day 157 (10°C, fresh stage). This family was observed until day 214 (28°C, active decay stage). Sepsidae was first observed on day 162 (11°C, fresh stage) and was observed on a regular basis until day 205 (22°C, bloat stage), with occasional observations until day 263 (18°C, advanced decay stage). On day 180 (8°C, bloat stage) Simuliidae was first observed and seen regularly until day 191 (11°C, bloat stage). Sarcophagidae was observed on day 203 (13°C, bloat stage) and on day 214 (28°C, active decay stage). On day 205 (22°C, active decay stage), *Hydrotaea* and Scathophagidae were first observed. The last time *Hydrotaea* was observed was on day 255 (24°C, advanced decay stage). The last family to be observed on donor 5 was Fanniidae, also on day 255 (24°C, advance decay stage).

Table 3-4: Succession of Diptera on donor 5 at REST (Quebec, Canada) from November 11th, 2020 to July 29th, 2021. Families are displayed following the same order as the colonization.

Families	Species	Fresh Autumn	Fresh Spring	Bloat	Active Decay	Advance Decay
		0-9	141-176	177-206	207-218	219-269
Calliphoridae		a	a	a	a	a
	Cynomya cadaverina	i	i	i		
	Lucilia illustris	i				
	Calliphora livida		i			
	Calliphora vicina		i			
	Protophormia terraenovae		i		i, a	i, a
	Phormia regina		a	i, a	i, a	i, a
Sphaeroceridae		a	a	a	a	a
Dryomyzidae			a			
Phoridae			a			
Piophilidae			a	a	a	a
	Stearibia nigriceps			i, a	i, a	i, a
	Prochyliza xanthostoma				i, a	
Muscidae/Fanniidae			a	a	a	a
Hydrotaea				a		a
Sepsidae			a	a	a	a
Sarcophagidae				a	a	
Scathophagidae				a		
Simuliidae				a		

a= Adult i= Immature Numbers means Experimental Days

3.9 Beetle Succession on Human Donors

Beetle succession was determined using visual observation for the human donors. The beetles observed are reported in Table 3.5 for donor 4. During the autumn of 2020, the first Coleoptera

was recorded on donor 4 on day 1 (12°C, fresh stage). Unfortunately, this beetle could not be collected onsite and identified. The first specimens of Staphylinidae were observed on day 7 (5°C, fresh stage) and last seen on day 37 (15°C, fresh stage). The only Silphidae to be observed during the autumn was *N. surinamensis*, on day 35 (9°C, fresh stage). This day was also the last day that observations were made, and samples were collected during the autumn months. Observations were resumed in the spring once insect activity commenced again.

The first beetles to be seen on donor 4 during the spring and summer of 2021 were Staphylinidae, Dermestidae, and Nitidulidae, on day 184 (10°C, fresh stage) when the cadaver was still in the fresh stage of decomposition. This was the only day Dermestidae was recorded. There were two species of Nitidulidae throughout the trial. Specimens of *Omosita nearctica* were observed on day 184 until day 297 (16℃, advanced decay stage), the last day of the trial for this donor. *Glischrochilus quadrisignatus* was only seen on day 205 (13℃, fresh stage). Specimens of Staphylinidae were also observed from day 184 (10℃, fresh stage) until day 297 (16°C, advanced decay stage). *Creophilus maxillosus* was observed on days 239 (18°C, active decay stage), 242 (19℃), 261 (15℃, advance decay stage), and 297 (16°C), which was during the active and advanced decay stages. *Ontholestes cingulatus* was observed on days 249 (16°C, active decay stage) and day 256 (19℃, active decay stage). On day 227 (20°C, active decay stage), species from the Histeridae family were first seen. They were observed seven more times with their last observation occurring on day 283 (23°C, advanced decay stage). Specimens of the Carabidae family (which are frequently predators) were seen on days 225 (19°C, active decay stage) and 275 (17℃, advanced decay stage).

The first Coleoptera larvae were observed on day 219 (11°C, active decay stage) and belonged to the Silphidae family. They were present until the last day of the trial for donor 4. They were only present during the active and advanced decay stages of decomposition. Larvae of Nitidulidae were seen from day 256 (19℃, active decay stage) until the last day of the trial. There were no other larvae recorded.

Table 3-5: Succession of Coleoptera and other arthropods on donor 4 at REST (Quebec, Canada) from October 5th, 2020, to July 29th, 2021. Families are displayed following the same order as the colonization.

Family	Species	Fresh Autumn	Fresh Spring	Active Decay	Advance Decay
		0-37	156-208	210-254	256-297
Coleoptera		a	a	a	a
Staphylinidae		a	a	a	a
	Creophilus maxillosus			a	a
	Ontholestes cingulatus			a	a
Silphidae		a	a	i,a	i,a
	Oiceoptoma noveboracense		a	a	a
	Necrodes surinamensis	a	a	a	a
Dermestidae			a		
Nitidulidae			a	a	i,a
	Omosita nearctica		a	a	a
	Glischrochilus quadrisignatus		a		
Histeridae				a	a
Carabidae				a	a
Coccinellidae	*Harmonia axyridis*	a			
Hymeonoptera		a	a		
Formicidae		a		a	
Lepidoptera				a	
Acari		a	a	a	a
	Red Acari	a	a	a	a
	Pale Acari			a	a
Araneae		a	a		
Collembola		a			
Chilopoda		a			

a= Adult i= Immature Numbers means experimental days

Table 3.6 reports the observed beetles on donor 5. The first Coleoptera observed on donor 5 belonged to the Staphylinidae, observed on day 4 (13°C, fresh stage). These specimens were observed one additional time on day 9 (15°C, fresh stage). Once the snow had thawed and observations resumed, the first two specimens of Coleoptera to be observed during the spring were members of the Staphylinidae and Nitidulidae families on day 155 (8°C). Notably, the cadaver was still in the fresh stage even after several months of snow cover. Specimens of Staphylinidae were seen until day 269 (16°C, advanced decay stage), the last day of the trial. One species of Staphylinidae, *C. maxillosus,* was first recorded during the bloat stage on day 199 (9°C). It was observed one more time during the bloat stage on day 203 (13°C). It was not seen again until day 219 (advanced decay stage), but then it was observed four more times with the last time being on day 269 (16°C, advanced decay stage). Another species of Staphylinidae, *O. cingulatus*, was recorded on day 205 (14°C, bloat stage) and again on the last day of the trial. The only Nitidulidae species to be seen on donor 5 was *O. nearctica*. It was recorded from day 155 (8°C, fresh stage) until the last day of the trial. The first Silphidae was observed during the fresh stage on day 156 (10°C) and recorded until day 252 (24°C, advanced decay stage). The first species to be observed was *O. noveboracense* on day 158 (14°C, fresh stage) until day 252. The other identified species of Silphidae was *N. surinamensis*. This species first appeared during the active decay stage on day 212 (19°C). They were also observed during the advanced decay stage on days 240 (22°C), 247 (16°C), 255 (24°C), and 263 (18°C). Specimens of Cleridae were observed on day 158 (14°C) and then not again until day 205 (22°C), at the end of the bloat stage. They were last recorded on day 263 (18°C, advanced decay stage). Specimens of Histeridae were first observed on day 162 (11°C, fresh stage) and several additional times until day 255 (24°C, advanced decay stage). Finally, specimens of Lampyridae, which are not necrophagous, were recorded only on day 188 (10°C) during the bloat stage.

Silphidae, Staphylinidae, and Nitidulidae were the only families of Coleoptera to have larvae present on donor 5. The first larvae to be seen was a Nitidulidae on day 172 (9°C). They were present from the fresh stage until the advanced decay stage. The first Silphidae larvae were observed on day 191 (11°C, bloat stage) and were present until day 242 (16°C, advanced decay stage). They were observed on day 263 (18°C) and 269 (16°C). Staphylinidae larvae were seen on

days 207 (7°C, active decay stage), 212 (18°C, active decay stage), 214 (19°C, active decay stage), 217 (28°C, active decay stage), and finally 269 (15°C, advanced decay stage).

Table 3-6: Succession of Coleoptera and other Arthropods on donor 5 at REST (Quebec, Canada) from November 11th, 2020 to July 29th, 2021. Families are displayed following the same order as the colonization.

Families	Species	Fresh Autumn	Fresh Spring	Bloat	Active Decay	Advance Decay
		0-9	155-172	176-205	207-217	219-269
Coleoptera			a	a	a	a
Staphylinidae		i, a	a	a	i, a	i, a
	Creophilus maxillosus			a		a
	Ontholestes cingulatus			a		a
Nitidulidae			i, a	i, a	i, a	i, a
	Omosita nearctica		a	a	a	a
Silphidae			a	i, a	i, a	i, a
	Oiceoptoma noveboracense		a	a	a	a
	Necrodes surinamensis				a	a
Cleridae			a	a		a
Histeridae			a	a	a	a
Lampyridae				a		
Coccinellidae	*Harmonia axyridis*	a				
Vespidae		a				
Formicidae			a	a	a	a
Lepidoptera			a		a	a
Acari			a	a	a	a
	Red Acari		a	a	a	
	Pale Acari		a	a		a
Araneae		a	a	a		

a= Adult; i= Immature; Numbers are experimental days

Chapter 4: Discussion

4.1 Pig Decomposition

Since 1996, there have been many entomological studies that used pigs as human analogues in most provinces of Canada (Anderson, 2011; Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Cockle & Bell, 2017; Leblanc & Strongman, 2002; Sharanowski et al., 2008; VanLaerhoven, 2008). However, until 2020 there were no published studies carried out in Quebec (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). All these Canadian studies have investigated different factors, such as geography, shade, insect presence, etc., that impact the decomposition process. However, there is only one study that looked at how the winter months in Quebec impacts the process of decomposition and entomology succession, which means that there is a lack of important information for scientists and law enforcement agencies that operate in areas with cold winters, such as Quebec and the rest of Canada.

The pig carcasses in the current study were placed at the site during the winter months of February and subsequently remained in the fresh stage for 85 days. This delay in the decomposition process was expected due to the freezing temperatures (daily temperature during the winter months averaged 2°C). During spring and summer studies in other regions of Canada, the fresh stage will typically only last a day or two (Anderson, 2011; Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Comstock et al., 2015; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008), while it can last upwards of five days during autumn studies (Leblanc & Strongman, 2002). In the only other study conducted during the winter in Quebec, the fresh stage lasted 142 days (Taillefer & Giroux, 2021). In this study, a pig carcass was placed during the late autumn months and studied throughout the winter months. This study only had one replicate, which makes it more difficult to draw general conclusions, but it still provided a useful comparison. Had the pig carcasses in our study been placed earlier in the autumn or winter months, it is likely we would have seen a comparable period for the fresh stage. The results of the previous and our current study in Quebec therefore indicate that decomposition is delayed

due to the long freezing period experienced during winter. However, the low temperatures did not completely prohibit the decomposition process since there were postmortem changes observed in the spring, such as discoloration and marbling on the pig carcasses. These slight changes could be due to the increasing internal temperature, which would accelerate the chemical and biological processes of decomposition. The other winter study in Quebec made no mention of similar changes (Taillefer & Giroux, 2021).

In the current study, the pigs reached the bloat stage in spring when temperatures reached an average of 20°C in late May and it lasted seven to eight days. In the other winter study in Quebec, this stage was never observed (Taillefer & Giroux, 2021). One reason for this variation could be that their carcass was exposed to a longer freeze period than the carcasses in the current study, and the microbiome of the carcasses could have been altered in such a way that the bloat stage could not occur. This could also mean that the bloating might be a characteristic combined with the active decay stage rather than its own stage. During the warmer months of the year, this stage can last from three to twelve days, depending on the ambient temperature, insect activity and location. A study conducted in Saskatchewan during the spring months of 2000 found that the bloat period lasted ten and twelve days, respectively for the two pig carcasses used in the study (Sharanowski et al., 2008). This is interesting because even though the bloat stage occurred during the same time of year as the current study, this stage was shorter during our study. This could be due to the prolonged freezing of the carcasses in our study that weakened the skin and various membranes, which allowed the gas to escape the carcasses more rapidly. In a study done in Illinois (United States) it was also observed that freezing weakened the external barriers of the carcass (Roberts & Dabbs, 2015). Another potential reason was that our study had a warmer daily average ambient temperature of 20°C, than Saskatchewan, which was 15°C. The cooler temperature may have slowed down the process of decomposition, which could cause the prolongation of the bloat stage as reported. In contrast, this stage lasted only three days in a study conducted at the same site in Trois-Rivières as our study in the summer of 2019 (Maisonhaute & Forbes, 2021a). This comparison shows that the time of year does impact the length of the early stages of decomposition.

The active decay stage lasted one day for pig 5 and four days for the other two carcasses (pig 4 and 6). This difference could be the result of the difference in sun exposure that the carcasses were exposed to, in relation to where they were placed within the site since pig 5 was in an area with more shade than the other two. Unfortunately, no data was collected regarding the sun exposure in my study, but this factor has been shown to impact the decomposition process (Sharanowski et al., 2008). However, another study performed at REST did take this parameter into account (Perreault, personal communication). In other studies, in Canada, this stage has been reported anywhere between five to 43 days. During the summer Quebec study, a study in British Columbia, and the summer portion of the Saskatchewan study, this stage lasted five or six days (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). During the Yukon study and the autumn portion of the Saskatchewan study, this stage lasted from 27 to 43 days (Bygarski & LeBlanc, 2013; Sharanowski et al., 2008). It was notable that the active decay stage was so short in our study compared to other studies conducted in warmer seasons, since heat typically accelerates decomposition and insect activity. During the other winter study in Quebec, this stage lasted 64 days which is considerably longer (Taillefer & Giroux, 2021). This demonstrates that the prolonged freezing of a carcass can both lengthen or shorten the timeframe of active decay and thus, more research is needed to examine this discrepancy.

The advanced decay stage for pig 5 lasted 19 days, and for the other two carcasses, it lasted 16 days. In the study that took place at the same site in summer, this stage also lasted 19 days (Maisonhaute & Forbes, 2021a), while in the British Columbia study it lasted 25 days (Anderson & VanLaerhoven, 1996), and in the Saskatchewan study this stage lasted 9 or 11 days during the spring and 13 days during the summer (Sharanowski et al., 2008). The pigs placed during the autumn months of that study did not enter this stage due to cooler temperatures. Based on these comparisons, it suggests that the variation of the length of this stage can be more attributed to the geographical location of the studies, rather than the season. Indeed, different geographical areas may have different temperatures, relative humidity, and fauna (necrophagous species) that can impact the length of this decomposition stage.

This study is the second study to document how pig carcasses will decompose when exposed to a long freezing period, specifically in Quebec. These two studies can provide important preliminary information that can be potentially used by law enforcement, forensic scientists, and ecologists, but they also show that there is a considerable lack of information. Further studies are required to investigate how insects, microbes, and fungi are impacted by cold temperatures within the body, and to determine whether bloating should be considered a characteristic of the active decay stage rather than its own separate stage. More studies are needed to give law enforcement and scientists a more complete picture of how the extended freeze and thaw cycle impacts the decomposition process in Canada.

4.2 Pig Diptera Succession

In Canada during the warmer months of the year, Diptera are usually first observed within a few hours to a few days after a pig carcass has been deposited at the experimental sites (Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Leblanc & Strongman, 2002; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). This is to be expected because flies are active during this time of year. During the entomological studies previously reported in Canada, the first families to arrive were Calliphoridae, Muscidae, Anthomyiidae, Sarcophagidae, Piophilidae, and Dryomyzidae, depending on the location of the study. During the winter study in Quebec by Taillefer and Giroux (2021), the Diptera colonization was delayed by five days compared to other warmer studies, and the carcass was colonized by Antomyiidae, Calliphoridae, Heleomyzidae, and Phoridae. This delay is because the pig was placed during the autumn months, when the ambient temperature was becoming colder, which makes the flies less active. The families that visited the carcass were also different when comparing the Taillefer and Giroux (2021) winter Quebec study to the other warmer studies (referenced above), because Heleomyzidae and Phoridae that appeared only in the winter study are known for being more active at lower temperatures than the other families (Anton et al., 2011; Taillefer & Giroux, 2021). In my study, the first Diptera to be observed was a Sciaridae during the fresh stage. This family is not usually associated with decomposition, and it is assumed that the insect landed on the pig carcass by

chance. The first carrion associated family to be observed was Dryomyzidae, also during the fresh stage. This family is known to feed on carrion and decaying fungi (Barnes, 1984). In Poland, this family is reported to colonize pig carcasses in large numbers (Bajerlein et al., 2022; Jarmusz et al., 2020), but in Canada, they have only been previously reported in an autumn study in Nova Scotia (Leblanc & Strongman, 2002) and in a study that used human cadavers in Quebec during the summer months (Maisonhaute & Forbes, 2023). Although these flies are associated with carrion, more research is needed to determine their significance to forensic entomology in Quebec.

The first Calliphoridae were also observed in the current study during the fresh stage. While their arrival was greatly delayed compared to other studies, they were still one of the first families to colonize the carcasses, which is consistent with literature. One of the first species to be identified was *C. cadaverina*. This fly was also reported in studies in Saskatchewan, New Brunswick, the Yukon territory, and Alberta (Anderson, 2011; Bygarski & LeBlanc, 2013; Michaud et al., 2010; Sharanowski et al., 2008), as well as being reported in the other winter study in Quebec (Taillefer & Giroux, 2021). All these studies included the spring months, and *C. cadaverina* was observed at this time. The exception to this was the Yukon study that took place during the summer (Bygarski & LeBlanc, 2013). These studies had an average temperature of 12°C-18°C. This indicates that *C. cadaverina* may preferred cooler temperatures and may be one of the first Calliphoridae to emerge from diapause in spring. This allows it to be one of the first Diptera to find and colonize carrion when temperatures are cool during the spring season. In most of the Canadian studies cited here, this species arrived during the fresh stage except for the Alberta study, which was colonized during the bloat stage. This indicates that *C. cadaverina* is attracted to carrion in the early stages of decomposition. *Calliphora livida* was also one of the first species to be observed. This fly was seen during the summer study at the same site in Quebec (Maisonhaute & Forbes, 2021a), but not during the winter study in Quebec in a different location (Taillefer & Giroux, 2021). Their winter study had a pig that was placed in Sainte-Anne-de-Bellevue (Quebec) which is further west than Trois-Rivières (location of the present study). This could mean that this species is important in the Trois-Rivières region of Quebec, but it could also be important in other regions of the province, although more research is needed to confirm this.

Calliphora livida was also reported in the New Brunswick study which borders Quebec to the east (Michaud et al., 2010). The authors of this study (which had a range of temperatures of 3°C-10°C during the spring portion) report that this species had an affinity for the spring and our winter study supports this hypothesis.

Phormia regina was first observed during late April and during the fresh stage of our study. In the other Quebec winter study, this species was also observed in late April (Taillefer & Giroux, 2021). *Phormia regina* have been reported in many Canadian studies throughout the year and always arrive during the fresh stage (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). In Nova Scotia (autumn study), the Alberta, and the Yukon studies, this species was first observed during the bloat stage (Anderson, 2011; Bygarski & LeBlanc, 2013; Leblanc & Strongman, 2002). The authors of the Nova Scotia study state that this delay was due to the season (Leblanc & Strongman, 2002), while the delay of this species in the other two studies could be attributed to other Diptera colonizing the carcass first (Anderson, 2011; Bygarski & LeBlanc, 2013). The Authors of the Nova Scotia study also stated that the species composition showed seasonal changes every 4-6 weeks, but the factors involved are unknown (Leblanc & Strongman, 2002). *Protophormia terraenovae* were first observed during the fresh stage in early May. This species was also reported in the summer Quebec study at the same site (Maisonhaute & Forbes, 2021a), but was not reported in the other Quebec winter study (Taillefer & Giroux, 2021). In Saskatchewan, *P. terraenovae* was one of the first flies to colonize the carcasses during the spring months of the study and arrived during the active decay stage in the autumn months but notably, was not observed during the summer months (Sharanowski et al., 2008). The summer portion of the study started in mid-July. In Canada, this species is known to be active in early spring, and activity will peak in July (Bygarski, 2012). Our winter study, the summer Quebec study and the Saskatchewan study seem to confirm this statement. *Calliphora vomitoria* were also seen in both the summer and the other winter Quebec studies. In the other winter study, it was only reported during two visits, one in late April and one in mid-May (Taillefer & Giroux, 2021), which is comparable to our findings. In the summer Quebec study at the same site, this species was not recorded as an adult but was collected as larvae during the first eight days of the experiment (Maisonhaute & Forbes, 2021a). This fly was also seen during the autumn months of

the Saskatchewan, Nova Scotia, and the summer months of the Yukon studies (Bygarski & LeBlanc, 2013; Leblanc & Strongman, 2002; Sharanowski et al., 2008). These observations show that this fly is present throughout Canada and prefers the cooler months of the year (Greenberg & Tantawi, 1993).

Lucilia sericata is known for its preference towards urban environments (Simpson & Strongman, 2002). This species was not reported in the other two Quebec studies (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021) but was recorded in our study during the fresh stage. The other winter Quebec study took place in a rural site, which is not the preferred habitat for this species, whereas the summer Quebec study, as with our winter study, took place in a wooded site in the middle of an urban environment. It is likely that *L. sericata* were present at the site, but not in high numbers which makes them less likely to be observed. More studies are needed to determine how wooded forests in urban areas impact necrophagous and carrion insect population dynamics. *Lucilia silvarum* and immatures of *C. vicina* were also observed during the bloat stage. These species were not seen in either of the other Quebec studies (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). *Calliphora vicina* is known for preferring urban areas. In the Nova Scotia study, the authors found that this species was observed more in urban areas when compared to a forested park (Leblanc & Strongman, 2002). This observation suggests that the location of our study, and the summer Quebec study, is not the ideal habitat for *C. vicina*. The location of this study was a wooded forest in an urban area, which seems to represent an area that *C. vicina* can occupy but is not ideal for this species. The last Calliphoridae to be observed in this study was *L. illustris*, during the bloat stage. This fly was seen in November and April during the other winter Quebec study (Taillefer & Giroux, 2021) and during the summer Quebec study, several immatures were collected (Maisonhaute & Forbes, 2021a). Since this fly was only seen a few times during the winter studies but was seen more often in the summer study, it could indicate that this species prefers warmer temperatures, as suggested by Wang et al. (2016).

Another family to arrive during the fresh stage was Sarcophagidae, and in other Canadian studies, flies of this family usually arrive within the first few days of the pig carcass placement (Bygarski

& LeBlanc, 2013; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). Interestingly, this family was never observed during the other winter Quebec study (Taillefer & Giroux, 2021). The authors state that this is due to the members of the family being more active during the late spring, which was outside of the experiment timeframe. Our findings support this statement because the first adult and larvae were observed or collected in late May.

The first member of the Muscoidea super family to be observed in the current study was Muscidae, observed on the same day as the first Calliphoridae. In the other winter Quebec study, this family was also first observed during the same time of year (Taillefer & Giroux, 2021). In the summer Quebec study, this family, along with *Hydrotaea*, arrived on day 1 (Maisonhaute & Forbes, 2021a). This indicates that, regardless of whether the body has been exposed to cold temperatures or not, the Muscidae will be one of the first families to colonize a carcass. Specimens of *Hydrotaea* were only first observed on day 71 of our winter study which was during the fresh stage and was not reported at all in the other winter Quebec study (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). This might indicate that certain members of this family are more active during the spring as the temperatures are rising.

The first adult Piophilidae in this experiment was observed during the fresh stage in early May. The two species that were identified were *P. xanthostoma* and *S. nigriceps*. In the other Quebec winter study, only *P. xanthostoma* was identified and they were observed in late April (Taillefer & Giroux, 2021) which is comparable to our study. The adults of this fly are usually first observed during the bloat or early active decay stage (Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Sharanowski et al., 2008). One exception to this was the summer Quebec study where this family was first seen during the fresh stage (Maisonhaute & Forbes, 2021a). The summer Quebec study did not report *P. xanthostoma* but did report *Mycetaulus bipunctatus* (Fallen, 1823) which was not seen in our winter study. This could indicate that *P. xanthostoma* prefers carcasses that were exposed to winter, while the *M. bipunctatus* prefers carcasses that were placed during the summer.

When examining the succession of immature Diptera, there is a pattern that emerges. The blowfly species *C. cadaverina, C. livida, C. vomitoria, C. vicina,* and *L. illustris* were laid during the early stage of decomposition, between the fresh and the active decay stage, while immature skipper flies, namely *S. nigriceps* and *P. xanthostoma,* were present from the active decay stage until the dry remains stage. This is useful information because it shows that there are early succession flies and late succession flies that are forensically relevant in Quebec. These findings are similar to the findings in the summer Quebec study (Maisonhaute & Forbes, 2021a). The authors stated that *C. livida, C. vomitoria,* and *L. illustris* were also present from the fresh stage to the active decay stage. The Piophilidae larvae were observed from the end of the advanced decay stage until the dry remains stage. This is different than my winter study because the immature Piophilidae arrived during the active decay stage and during the summer study they arrived during the later stages of decomposition. Another difference is in the composition of the early succession species. The presence of immature *C. cadaverina* seems to indicate that the body was exposed during the spring. This can provide important information to assist with a more accurate PMI calculation.

4.3 Pig Coleoptera Succession

Due to the cold temperatures experienced in our winter study, the arrival of the Coleoptera was delayed. During warmer times of the year, they can arrive within the first 24 hours after a carcass has been deposited (Anderson & VanLaerhoven, 1996; Bygarski & LeBlanc, 2013; Maisonhaute & Forbes, 2021a; Seguin et al., 2021; Sharanowski et al., 2008). This delay is caused by Coleoptera entering diapause, which can occur in the adult stage of development (Hodek, 2012). In our winter study, the first Coleoptera arrived at the end of April (9°C), while in the other Quebec winter study, the first beetle was observed at the end of March (5°C) (Taillefer & Giroux, 2021). While there is a month difference between this study and the other winter study, it does confirm that the colder temperatures experienced in the Quebec winter will delay the arrival of beetles. The first winter study seems to have had a warmer month of March (average ambient temperature of 1.5°C) compared to this study (the average ambient temperature of -3°C), which

could explain why the first beetles was seen earlier in the first winter study (Taillefer & Giroux, 2021).

In the current study, the first Coleoptera to be observed were the Silphidae *O. noveboracense* and the Nitidulidae *O. nearctica* during the fresh stage. They were also seen early in the decomposition process during the other two Quebec studies (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). During the other winter study, both species arrived in late April and they arrived after the first Diptera, which is consistent with our findings (Taillefer & Giroux, 2021). During the summer Quebec study, *O. noveboracense* and *O. nearctica* arrived during the bloat stage or active stage instead of the fresh stage (Maisonhaute & Forbes, 2021a). The long fresh stage of our study provides both species more of an opportunity to arrive during this stage when compared to the summer study. Another possibility is that the extreme freezing created conditions that make the fresh carcasses more attractive, but more research is needed to confirm this. *Oiceoptoma noveboracense* was also reported in the Alberta study from May until July and in the Nova Scotia study during the summer months (Anderson, 2011; Simpson & Strongman, 2002). In the Nova Scotia study, this species was observed more often in wooded sites, similar to the one in this study, versus urban sites (Simpson & Strongman, 2002). A further study in Nova Scotia that took place during the autumn months, did not report this species (Leblanc & Strongman, 2002). This could indicate that *O. noveboracense* is not active during the autumn months, which is consistent with what is found in the literature since. This species is known to overwinter as an adult (Majka, 2011). *Necrodes surinamensis* was first observed during the bloat stage in our winter study. This species was not observed during the other winter Quebec study but it was seen during the summer Quebec study, during the active decay stage (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). The larvae of this family are often observed from the active decay stage until the dry remains stage (Anderson, 2011; Bygarski & LeBlanc, 2013; Maisonhaute & Forbes, 2021a; Sharanowski et al., 2008). In our study, they were observed during the bloat stage. This could indicate that once a body has experienced a long freezing period, it makes it more susceptible to being colonized by Silphidae larvae than if it was not.

In this study and the summer Quebec study, adults of *Omosita* sp. were observed during the fresh and bloat stage, respectively (Maisonhaute & Forbes, 2021a). In the British Columbia study, the adults were first observed during the advanced stage of decomposition (Anderson & VanLaerhoven, 1996). In that study, the authors state that this family arrives during the later stages of decomposition, but my study and the summer study disagrees with this statement. In both my study and the summer study the larvae of this family were both first observed during the later stages of the decomposition process which is consistent with the British Columbia study (Anderson & VanLaerhoven, 1996; Maisonhaute & Forbes, 2021a). This could have important implications in determining PMI_{min}. Another species of Nitidulidae that was observed once during this winter study was *G. quadrisignatus.* It was not observed in the other Quebec studies (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). This species is known for being attracted to fermented plant matter rather than carrion, which explains why they were not observed very often (Williams et al., 1992). This species may be present during decomposition, but it does not seem to be important to the estimation of PMI_{min}.

The Dermestidae family is reportedly commonly seen during the late stages of decomposition (Charabidze et al., 2014). All three Quebec studies either observed this family once or not at all (Maisonhaute & Forbes, 2021a; Taillefer & Giroux, 2021). This is consistent with the Yukon, Alberta, Saskatchewan, and two Nova Scotia studies (Bygarski & LeBlanc, 2013; Leblanc & Strongman, 2002; Sharanowski et al., 2008; Simpson & Strongman, 2002) In fact, the only study that observed this family was the British Columbian study (Anderson & VanLaerhoven, 1996). This seems to indicate that this family is relevant at very late stages of decomposition that were not reached in Canadian studies, or they may not be relevant to forensic entomology in Canada.

There were several families of predators that visited the carcasses. The first family that observed early in the decomposition process was Staphylinidae. The first species to be recorded was *C. maxillosus* in early May during the fresh stage. This species was seen at the end of April in the other winter Quebec study (Taillefer & Giroux, 2021), and during the early stage of decomposition in the summer Quebec study (Maisonhaute & Forbes, 2021a). Both findings are consistent with our study. The next species to be seen was *O. cingulatus* in mid-May during the

fresh stage. This species was also observed in both Quebec studies. For the summer study, it was present during the fresh stage, and for the winter study it was seen in early May. In several Canadian studies, larvae of this family can be seen throughout the process of decomposition (Hobischak et al., 2006; Maisonhaute & Forbes, 2021a), while there are other studies that only reported these larvae during the later stages (Anderson, 2011; Anderson & VanLaerhoven, 1996). In our study, they were first observed during the dry remains stage. More research is needed to determine their significance. One potential area of research for this family is their interaction with the Diptera populations and how they can impact Diptera colonization. Since they prey upon fly larvae, a high abundance of rove beetles could be related to a higher consumption of larvae, which could delay colonization.

Another predatory family is Histeridae, and it was first observed during the fresh stage of our study. Members of this family were also seen during the bloat stage of the summer Quebec study at the same site (Maisonhaute & Forbes, 2021a), during the bloat stage of the Saskatchewan study in spring and the dry remains stage in the summer, but they were not observed during the winter study (Taillefer & Giroux, 2021). According to Taillefer and Giroux (2021), the reason for the lack of this family is that there was a lack of their prey (fly larvae) on the carcass during the experiment even though these beetles enter diapause in the adult stage. The forensic relevance of this family needs to be further studied.

Specimens of Cleridae were seen during the month of May and during the fresh stage for both our study and the other winter Quebec study (Taillefer & Giroux, 2021). This arrival during the early portion of decomposition is shared by the spring portion of the Saskatchewan, British Columbia, and Yukon studies (Anderson & VanLaerhoven, 1996; Bygarski, 2012; Sharanowski et al., 2008). During the summer Quebec study, it was first observed at the beginning of the advanced decay stage (Maisonhaute & Forbes, 2021a), which is later than our winter study. This arrival in the later stages was also observed in the summer portion of the Saskatchewan study and the Alberta study (Anderson, 2011; Sharanowski et al., 2008). This wide variation between the different regions needs more research to determine its relevance.

When examining the adult Coleoptera, there is no clear pattern of succession. They all arrived during the fresh stage and most of them stayed until the dry remains stage. The only exceptions to this are *C. maxillosus, O. cingulatus,* and *Necrodes surinamensis*. They were only present from the fresh stage until the active decay stage. One notable observation that was made is that the larvae of *Omosita* sp. and Staphylinidae were only present from the advanced decay stage onwards. This indicates that if these larvae are present then the carcasses are in the later stages of decomposition.

4.4 Human Donors Decomposition

This study represents the first to investigate how human bodies decompose when they are exposed to a Canadian winter in a semi-controlled environment. This means that the bodies were visited on a regular basis and there were certain variables that were excluded, such as large scavengers. Interestingly, our results highlight that the bodies had very long fresh stages, the cadavers continued to decompose throughout the winter, the process of decomposition was unique between the two bodies, and they never became skeletons. Only two prior Canadian studies have examined human remains, but both studies used retroactive police cases (Cockle & Bell, 2017; Komar, 1998). While these studies provide important information, they do not provide a detailed timeline of how the bodies decompose. The first of these studies was carried out in Alberta using 20 historical cases. The author reported that a body that was exposed to outdoor winter conditions can skeletonize in four months (Komar, 1998). The two human donors in my study were decomposing for a period of eight and nine months without resulting in skeletonization, which is longer that reported in the study in Alberta. One of the potential reasons for this acceleration in the Alberta study is that those bodies were exposed to large scavengers, while the cadavers of my study were protected from vertebrate scavenging (Komar, 1998). The second retroactive study involved 96 historical police cases from across Canada. The authors found that the cold temperature in winter delays the decomposition process. In my study, the fresh stage of decomposition lasted between 177 and 210 days depending on when the donor arrived at the facility, which demonstrates a considerable delay compared to the three days the

fresh stage lasted in warmer temperatures in the same environment (Maisonhaute & Forbes, 2023). While the two retroactive studies provide some information about human decomposition after experiencing a Canadian winter, it is very difficult to compare those studies to my study due to differences in climate, geographical location, and method.

The first Canadian human decomposition study conducted in a semi-controlled environment at the REST facility was published online in 2022, and was performed in the late summer of 2020 and overlapped with my study (Maisonhaute & Forbes, 2023). While both studies took place during different times of the year, their procedures were similar and can be compared. The authors of the summer study reported that the decomposition of the bodies could not be described by the traditional stages namely, fresh, bloat, active decay, advanced decay, and dry remains (Maisonhaute & Forbes, 2023), which is in agreement with my observations. For example, one of the donors went through the bloat stage while the other did not. Additionally, both donors had significant amounts of dried skin and soft tissue present during the active decay stage. This suggests that the decomposition stages need to be redefined in Quebec. The donors in both studies also decomposed differently. During the summer study, both bodies entered the bloat stage on day three (Maisonhaute & Forbes, 2023), while during the winter study, one of the donors took 177 days to enter the bloat stage and the other donor did not undergo bloating. Furthermore, after 16 to 18 days, both donors in the summer study were mummified (Maisonhaute & Forbes, 2023). Even after 296 days, neither donor in the winter study entered this stage or the dry remains stage. These differences demonstrate that exposure of the remains during the winter months does cause cadavers to decompose differently.

4.5 Diptera Succession on Human Donors

During the autumn portion of this study, the succession of the Diptera was different between both donors. The first flies were observed on day 1 for donor 4 but on day 4 for donor 5. This delay could be due to the rapidly reducing temperature since the average ambient temperature of day 1 was 13°C for donor 4, and donor 5 did not experience similar temperatures until day 4. The average ambient temperature for donor 5 on day 1 was 1°C. The first families that were observed

on donor 4 were Phoridae, Sciaridae, Simuliidae, and Trichoceridae, the only one to be associated with cadavers was Phoridae. These families were not seen on donor 5 during the autumn portion of the study. The first family to be observed on donor 5 was Calliphoridae as *C. cadaverina* and *L. illustris.* These two species also visited donor 4 along with *C. livida* and *C. vomitoria*. Bibionidae, Muscidae, Dryomyzidae, and Antomyiidae were also recorded on donor 4 but not on donor 5. These differences could be due to donor 4 having 32 more days to decompose and more time to be colonized by insects than donor 5 during the autumn portion of the experiment. During this period, insects have started their process of overwintering.

The first Diptera to be observed during the spring (late March) was a Muscidea specimen on donor 4. This suggests that this family can emerge from diapause during the early spring. This trend was also reported in Nebraska, USA (Taylor et al., 2007). The first eggs that were collected in spring (first week of April) were on donor 5 and belonged to the family Calliphoridae. Eggs did not appear on donor 4 until later in the study and this could be due to the donor being partly submerged in water due to the snow melt during the month of March which did not present ideal oviposition conditions until later in the trial. *Calliphora vomitoria* was only observed on donor 4 during the active decay stage. This species was also collected only once during the summer study in the same location (Maisonhaute & Forbes, 2023). This could indicate that this species of fly prefers to colonize other sources of carrion, such as animals, rather than human cadavers. The preferred habitat of this species is forested areas (Leblanc & Strongman, 2002), the same habitat found at REST, so their diet preference seems to be a reasonable conclusion to draw as to why they were not seen very often in this study or the summer study. *Protophormia terraenovae* were not collected on donor 4 or during the summer study (Maisonhaute & Forbes, 2023), but they were collected from donor 5. The authors of the summer study state that the lack of observation of this species could indicate that it prefers animal carrion over cadavers. This species was only observed on one cadaver and the larvae were only collected several times during the winter study suggesting that *P. terraenovae* will colonize cadavers, but they may prefer animal carrion. During the spring and summer portion of my winter study, there were no members of the *Lucilia* genus either collected or observed, but they were observed and collected during the autumn on both cadavers. They were also collected during the early stage of decomposition in the summer study

(Maisonhaute & Forbes, 2023). This could indicate that once this genus emerges from diapause during the spring, there is too much competition from other fly larvae. In one study, the authors stated that *P. regina* was able to outcompete *L. sericata*, which supports my statement of the *Lucilia* genus emerging from diapause and not being able to compete with other Calliphoridae already present (MacInnis & Higley, 2020). This information could be important to investigators in determining if a body has been exposed to the winter months.

Piophilidae were first observed in late March on donor 5 and early April for donor 4. As previously noted, this delay in colonization could be due to the donor 4 being partly submerged until the beginning of April. The first eggs to be collected on both cadavers were *S. nigriceps* in late May. It is interesting to note that the arrival of the adults of this family were staggered between both donors, but the eggs were laid within a few days. Immature forms *of P. xanthostoma* were also collected in late May and early June, but the immatures of this species were not collected during the summer study (Maisonhaute & Forbes, 2023). This could indicate that this species prefers cadavers that have experienced a freeze/thaw cycle over those that have not. A potential reason for this is that the freezing temperature caused the flesh of the cadavers to desiccate and dry flesh is the preferred food for the larvae of this species (Byrd & Castner, 2010).

Sarcophagidae was observed on donor 4 in early June and late May for donor 5. This family is known for being observed shortly after the arrival of Calliphoridae (Byrd & Castner, 2010), and during the summer study the Sarcophagidae arrived the day after the blowflies (Maisonhaute & Forbes, 2023). In the winter study, this family arrived almost a month after Calliphoridae while adult Piophilidae arrived before Sarcophagidae, which is surprising. No Sarcophagidae larvae were collected during the winter study, but they were collected during the summer study (Maisonhaute & Forbes, 2023). The lack of the collection of flesh fly larvae during this winter study shows that cadavers that experience a freeze/thaw cycle may be less appealing than bodies that are decomposing during the warmer times of the year.

When examining the larval colonization of Diptera, there is a pattern that emerges, with two distinct groups of colonizers over the time. First, there are immatures of Calliphoridae that were found during the fresh stage of decomposition only, specifically *L. illustris* (found during the

autumn only), *C. livida* (found during the autumn and spring), and *C. vicina* (found in spring only). These species could be considered as the first group of colonizers or "early" colonizers. Immatures of *C. cadaverina* and *C. vomitoria* that were also some of the first immature flies to be present during the fresh stage in the autumn and were recorded until the active decay stage in the spring could also be included in this group. The second group of colonizers consisted of larvae of *P. regina, P. xanthostoma*, and *S. nigriceps,* that were present during the bloat stage or later stages. Finally, immatures of *P. terraenovae* were present during the fresh stage during the spring and were recorded until the active decay stage, making them non-specific colonizers. The summer study noticed a pattern of early colonizers, late colonizers, and non-specific colonizers (Maisonhaute & Forbes, 2023). The early colonizers consisted of *L. silvarum, C. livida, Cochliomyia macellaria* (Fabricius 1775)*, L. illustris*, and Sarcophagidae. These immature flies were seen within the first two weeks of decomposition when the cadavers were in the early stages of decompositions. Notably, of these species, the only immature Diptera to be seen during the winter study was *L. illustris* and *C. livida,* while *C. macellaria* was never observed and Sarcophagidae was only observed as an adult later in decomposition process. This means that if an immature *C. macellaria* is collected on a cadaver, it means that the body has not experienced a prolong cold period. The late colonizers of the summer study were larvae of Piophilidae, Muscidae, and Heleomyzidae (Maisonhaute & Forbes, 2023). This stage was restricted to when the donors were experiencing desiccation due to being in the later stages of decomposition (2-3 weeks or later). The "late" colonizers in the present winter study consisted of immatures of Piophilidae and Calliphoridae, specifically *P. regina* (the latter that was not part of late colonizers in the summer study). This difference could be the result of the "late colonizer group" of the summer study started when the donors were undergoing mummification, while the second succession group of this winter study started during bloat stage. During the summer study the bloat stage was covered by the early colonizers. It is thus of prime importance to clearly specify which stages of decomposition refers to "late decomposition" since it can differ between the studies. Overall, these differences in the fly colonization provide important information to estimate PMI_{min} when cadavers are exposed to a winter period.

4.6 Coleoptera Succession on Human Donors

During the autumn portion of the winter study, one of the first beetle families to colonize both the bodies was Staphylinidae, that doesn't include necrophagous species but predators. They were present when their prey (fly larvae) was present. Donor 4 was also colonized by Silphidae, specifically *N. surinamensis*. This family was not observed on donor 5, which could be due to the reduced temperatures at the time of placement since cooler temperatures would cause the adults of this family to enter a state of diapause.

In Spring, the first Coleoptera arrived in the first week of April, and both donors had members of the Staphylinidae and Nitidulidae families at that time. The first species of Staphylinidae to be identified was *C. maxillosus* and it was first observed in late May for donor 5 and early June for donor 4. This species may have colonized donor 5 first because there was more prey available at that time. Both donors were colonized by a species of Nitidulidae called *O. nearctica* for the entirety of the spring and summer. This species is saprophagous (Williams et al., 2021) and was also reported throughout the summer study (Maisonhaute & Forbes, 2023). *Glischrochilus quadrisignatus* visited donor 4 once in late April but was not observed on donor 5. This species is known for being attracted to fermented plant matter (Williams et al., 1992). Surprisingly, this insect was observed several times during the summer study (Maisonhaute & Forbes, 2023). This suggests that cadavers may not be an important food source for this species, but it will still consume soft tissue if the opportunity arises. Also, since *G. quadrisignatus* were seen several times during the summer study but only once during this winter study (Maisonhaute & Forbes, 2023), this suggests that this beetle prefers the more rapid soft tissue decomposition in summer compared to winter. Donor 4 was also visited by one member of the Dermestidae family. This family is known for colonizing bodies in drier states of decomposition (Byrd & Castner, 2010), however donor 4 was in the fresh stage of decomposition. It is possible that this family was attracted to the body because it was showing some signs of superficial desiccation.

There were two species of Silphidae that visited both cadavers. The first one was *O. noveboracense* on donor 5 in early April. Specimens of *N. surinamensis* were observed again

during mid-May on donor 4 and then in early June on donor 5. This species is known for being a necrophage and to prey on Diptera larvae (Popescu et al., 2023) so, they arrived on the cadavers when there was a large amount of prey available and when there was decomposition flesh still available. During early April, donor 5 was colonized by Cleridae. This family is known for preying on fly larvae and occasionally being scavengers (Zanetti et al., 2015). Donor 4 was not colonized by this family because donor 4 was partly submerged. This limits the amount of available flesh and the amount of prey, so the beetles are less likely to colonize it.

The beetle larval composition of the two cadavers was also different. Donor 4 only had Silphidae larvae present during the active and advanced decay stages, and Nitidulidae larvae present during the advanced decay stage. Donor 5 had Staphylinidae larvae and Nitidulidae larvae present throughout the entire study, and Silphidae larvae from the commencement of bloat until the end of the experiment. These differences suggest that examining beetle larvae is not a reliable method of determining PMI_{min} until further studies are conducted with more donors.

Chapter 5: Conclusion

5.1 Objectives and Summary of Results

There have been very few studies that examine how the winter impacts the decomposition of pigs and humans and the insects that colonize them. This study was performed to provide the beginnings of such information. The first objective of this study was to observe how pigs decompose after being exposed to the winter and to determine which insects colonize the carcasses. The second part of this study was to observe how human bodies decompose when exposed to a winter, and the insect succession on the bodies.

The first part of this project was carried out using three pig carcasses placed on the campus of UQTR with a goal of optimizing the method for the human portion of this study. This optimized method assisted in determining decomposition characteristics, insects, and characteristic of the Quebec winter and spring. One of the first observations was that the freezing weather of the winter delayed the decomposition noticeably. The first signs of decomposition were not seen until a month after the carcasses were deposited. The carcasses entered the bloat stage on day 85 or 86 followed by active decay 7 to 8 days later, and finally entered the dry remains stage, 16 to 19 days after that.

There was a clear pattern of succession that emerged when examining the immature Diptera on pig carcasses. The first group of colonizers (early colonizers) were members of the Calliphoridae, specifically *C. cadaverina, C. livida, C. vomitoria, C. vicina,* and *L. illustris,* which were collected between the fresh and active/advanced decay stage. From the active decay stage to the dry remains stage, immatures of Piophilidae, namely *S. nigriceps* and *P. xanthostoma,* emerged as the late colonizers. *Cynomya cadaverina* was present during the spring months but were not observed during summer months, which makes it an indicator species for a cadaver that was exposed to cooler temperatures, such as early spring. A species that can be used as an indicator for a cadaver that was not exposed to the winter could be *L. illustris*. This species did visit the pig

carcass, but they were not observed as often as studies that took place during the summer. When examining Coleoptera, the only succession pattern that was observed were that the Nitidulidae and Staphylinidae larvae were present during the advanced decay and dry remains stage. The adults of this order were seen throughout the decomposition process making them less useful to determine PMI_{min}.

The human portion of this study took place at the REST facility. During the autumn months the donors stayed in the fresh stage of decomposition. Once the snow melted, signs of superficial desiccation were present on the bodies and notably, only one of the bodies entered the bloat stage. This suggests that bloating may not be its own stage but rather a sign of active decay commencing. It is also possible that one body did not bloat because they were partly submerged in water during the early spring. Notably, even after nine to ten months, depending on the placement of the donors, neither of the bodies entered the dry remains stage.

On human remains, once again, when looking at the immature Diptera a pattern was observed. The first group of colonizers consisted of *L. illustris* (found during the autumn only), *C. livida* (found during the autumn and spring), and *C. vicina* (found in spring only). *Cynomya cadaverina* and *C. vomitoria* are in this group because they were first seen in the autumn and then they reappeared during the spring and were present until active decay stage. The second group of colonizers are *P. regina, P. xanthostoma,* and *S. nigriceps* because they arrived during the bloat and later stages. Finally, the larvae of *P. terraenovae* were seen throughout the process meaning that they are non-specific. When examining the Coleoptera there was no clear pattern of succession, which makes them unreliable when determining a PMI_{min}.

5.2 Limitations of this Study

While this study does provide much needed information, it still has limitations. The first limitation of this study is that it was an observational study. This means that there was no statistical analysis conducted due to the limited sample size, although a good baseline of information for future projects was produced. The second limitation is the lack of abundance data. This could have

provided more detailed information about the succession of Diptera and Coleoptera. In both parts of this study, vertebrate scavengers were excluded from the carcasses and cadavers producing an unnatural alteration to the decomposition process. Vertebrate scavengers were excluded from the pig portion of the study to optimize the method and the human portion of the study mainly for ethical reasons. However, it is known that these scavengers can consume decomposing bodies which accelerates the rate of soft and hard tissue loss and could impact when and what insects colonize the bodies. This study was also limited because neither the carcasses nor the donors were visited during the wintertime. It would have been interesting to observe how the bodies decomposed under the snow, but this could not be conducted for safety and logistical reasons. Due to the timing of commencement of the pig study, the carcasses were only placed during the late winter. If they were placed during the autumn like the human donors, they would have experienced a longer freeze period. This could have influenced how the carcasses decomposed and the subsequent insect succession. It was not possible to study human decomposition in other regions of Quebec because the REST facility is the only human decomposition facility in Canada, hence limiting the geographical location of this study. The sample size (n=2) for the human study was also restricted and both bodies had very different heights and body mass index (BMI). Additionally, one of the donors was submerged under water for a portion of the study which altered the decomposition process. Therefore, it is recommended that these studies be repeated with a larger sample size for both pig carcasses and human remains.

5.3 Perspectives

This project demonstrates that there is still more research that is needed to examine the decomposition process and insect succession in relation to the freeze/thaw cycles that occur in Quebec. Increasing the number of cadavers studied would provide more information on the how bodies decompose when exposed to freeze/thaw cycles and the insects associated with the process. Conducting studies in different geographical regions of Canada with cold winters would also provide more information to entomologists and ecologists who operate in those areas and

add to their knowledge base for those environments. While replicating the human portion of the study may be difficult due to the lack of appropriate facilities in Canada, pig carcasses can be used instead. A third area of investigation would be to clothe or cover the bodies. These coverings can impact when insects colonize cadavers, since during the wintertime people wear more clothes than during the summertime, which can impact when and where the insects colonize the body and how it decomposes.

Appendix A

FEUILLE DE DONNÉES – SUIVI DES POPULATIONS D'INSECTES SUR CORPS HUMAIN
UQTR - CHAIRE DE RECHERCHE EN THANATOLOGIE FORENSIQUE

Date _____ **Corps #** _____
Heure _____ **Jour de décomposition** _____
Observateur : _____

Météo				
□ Soleil	□ Pluie fine	□ Faible vent	□ Humide	□ Frais
□ Soleil-Nuage	□ Averses	□ Venteux	□ Orageux	□ Tempéré
□ Nuageux	□ Pluie soutenue	□ Rafales de vent	□ Chaud	

Stade de décomposition

□ **Frais :** immédiatement après dépôt du cadavre, peu de changement, pas d'odeur forte

□ **Gonflé :** corps détendu, peau avec marbrures, beaucoup de Diptères, odeur

_____

□ **Décomposition active :** corps dégonflé, peau noircie, forte odeur, apparence humide, Diptères présentes

_____

□ **Décomposition avancée :** chair presque absente, larves de Calliphoridae ayant migré ou sont en train de le faire

_____

□ **Sec/Restes :** os, cartilage, reste de peau, faible odeur, Diptères (petites) et Coléoptères

_____

Megyesi's body score

Tête/cou	□ 1	□ 2	□ 3	□ 4	□ 5	□ 6	□ 7	□ 8	□ 9	□ 10	□ 11	□ 12	□ 13
Tronc	□ 1	□ 2	□ 3	□ 4	□ 5	□ 6	□ 7	□ 8	□ 9	□ 10	□ 11	□ 12	
Membes	□ 1	□ 2	□ 3	□ 4	□ 5	□ 6	□ 7	□ 8	□ 9	□ 10			

Remarques :

_____

___

_____

_____

_____

_____

Activité des Diptères adultes (observation pendant 30 secondes)

Heure 1 : _____

_____

Nb observés 1 : _____

Niveau d'activité 1 :
- ☐ Très faible (<10)
- ☐ Faible (10-50)
- ☐ Modéré (50-100)
- ☐ Élevée (100-200)
- ☐ Très élevée (200+)

Heure 2 :

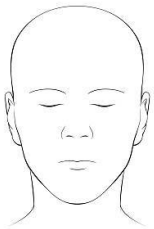
Nb observés 2 : _____

Niveau d'activité 1 :
- ☐ Très faible (<10)
- ☐ Faible (10-50)
- ☐ Modéré (50-100)
- ☐ Élevée (100-200)
- ☐ Très Élevée (200+)

Observations des insectes

Heure :

Durée :



Diptera (adulte)
- ☐ Calliphoridae
  - ☐ *Lucil* ☐ *Calliph.* ☐ *Phormia*
- ☐ Muscidae

_____
- ☐ Fanniidae
- ☐ Hydrotaea
- ☐ Sarcophagidae

_____
- ☐ Piophilidae
- ☐ Sepsidae

Coleoptera (adulte)
- ☐ Silphidae
  - ☐ *Necrophila* ☐ *Nicrophorus* ☐ *Oiceoptoma*
- ☐ Histeridae

- ☐ Staphylinidae
  - ☐ *O. cingulatus* ☐ *Creophilus* ☐ Autre _____
- ☐ Nitidulidae

- ☐ Cleridae
- ☐ Dermestidae

Autres
- ☐ Formicidae
- ☐ Hymenoptera :

- ☐ Araneae
- ☐ Opiliones
- ☐ Acari :

- ☐ Lepidoptera
- ☐ Heteroptera

□ Psychodidae □ Scarabaeidae □ Autres :
□ Phoridae □ Autres : _____
_____
□ Sphaeroceridae
□ Scatopsidae
□ Culicidae □ Autres : _____

Larves □ Diptera □ Calliph. □ Pioph. Localisation : _____ □ Migration
□ Coleoptera □ Staphylinidae □ Silphidae_____ □ Nitidulidae □ Autre _____
Localisation : _____
□ Autres : _____ Localisation : _____
Pupes Diptera □ Localisation : _____
Œufs Diptera □ Localisation : _____

Échantillons collectés :

5.3.1 # Échantillon	Lieu de collecte	Description	Note

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