Arthropod Succession in Whitehorse, Yukon Territory and Compared Development of *Protophormia terraenovae* (R.-D.) from Beringia and the Great Lakes Region

By

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Abstract

Forensic medicocriminal entomology is used in the estimation of post-mortem intervals in death investigations, by means of arthropod succession patterns and the development rates of individual insect species. The purpose of this research was to determine arthropod succession patterns in Whitehorse, Yukon Territory, and compare the development rates of the dominant blowfly species (*Protophormia terraenovae* R.-D.) to another population collected in Oshawa, Ontario. Decomposition in Whitehorse occurred at a much slower rate than is expected for the summer season, and the singularly dominant blowfly species is not considered dominant or a primary colonizer in more southern regions. Development rates of *P. terraenovae* were determined for both fluctuating and two constant temperatures. Under natural fluctuating conditions, there was no significant difference in growth rate between studied biotypes. Results at repeated 10°C conditions varied, though neither biotype completed development indicating the published minimum development thresholds for this species are underestimated.

Keywords: Forensic entomology, decomposition, *Protophormia terraenovae*, minimum development threshold.

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ADH	Accumulated degree hours
ANCOVA	Analysis of Co-variance
HWK	Hot water killed (with reference to larvae)
PIA	Period of insect activity
PMI	Post-mortem interval

Chapter 1:

Introduction

1.0 Forensic Entomology

Entomology, in the broadest definition, is the study of insects (Castner, 2010). Forensic entomology is a branch of entomology that deals with the application of the study of insects to legal activities or investigations (Catts & Goff, 1992). Within forensic entomology there are three sub-fields: urban entomology, stored-product entomology and medicocriminal entomology (Catts & Goff, 1992). Urban entomology refers to insects within the human environment, such as household or garden pests (Catts & Goff, 1992; Haskell, Hall, Cervenka, & Clark, 1997). Stored-product entomology involves investigation into the source and time of insect contamination of bulk or commercially stored goods (Catts & Goff, 1992; Haskell *et al.*, 1997). These two sub-fields will often involve civil law actions or litigations (Catts & Goff, 1992). Medicocriminal entomology, the sub-field most commonly associated with the term forensic entomology (Anderson, 2001), involves the application of entomology to criminal investigations (Catts & Goff, 1992; Haskell *et al.*, 1997). These cases often involve death, and the most common application of medicocriminal entomology is in the estimation of the post-mortem interval (PMI) (Catts & Goff, 1992; Haskell *et al.*, 1997) or period of insect activity (PIA) (Amendt *et al.*, 2007; Kreitlow, 2010).

1.1 Post-mortem Interval and Period of Insect Activity

Post-mortem interval (PMI) is defined as the length of time between the moment of death and when remains are discovered (Amendt *et al.*, 2007; Tomberlin, Mohr, Benbow, Tarone, & VanLaerhoven, 2011). There are a number of different methods for determining PMI. Early on, a forensic pathologist may estimate the PMI using physical and chemical changes to the body's soft tissues (Amendt *et al.*, 2007; Goff, 2009). Some

common physical/chemical changes include livor mortis (settling of blood in lower regions of the body), algor mortis (approximation of ambient temperatures when the body stops regulating its temperature after death) and rigor mortis (stiffening of body muscles due to chemical changes in myofibrils of the muscles) (Goff, 2009). These changes begin within the first hours after death and reach their endpoints relatively quickly: livor mortis becomes fixed after 9-12 hours, ambient temperature is typically reached within 18-20 hours, and rigor mortis lasts 2-3 days before the muscles begin to gradually relax (Fisher, 2004). Beyond these points entomological evidence becomes a more accurate and reliable method of PMI estimation (Haskell *et al.*, 1997; Anderson, 2001; Amendt *et al.*, 2007).

Due to the influence of many variables involved in decomposition, there is no way to precisely determine the exact time since death (Amendt *et al.*, 2007; Goff, 2009). As a result, it has been suggested that PMIs determined using entomological evidence be expressed as a period of insect activity (PIA) (Amendt *et al.*, 2007). An estimated PIA refers to the time frame in which arthropods have colonized, or been associated with, decomposing remains (Amendt *et al.*, 2007; Tomberlin *et al.*, 2011). This estimate may not exactly correspond to the actual PMI as insect access to remains may have been delayed due to factors such as burial, wrapping, or other forms of concealment (Amendt *et al.*, 2007). A PIA estimate may also be longer than the PMI in cases where a victim was colonized by arthropod species while still living (myiasis) (Amendt *et al.*, 2007).

There are two methods of estimating a PIA: the pattern of arthropod colonization at decomposing remains, and the rate of development of arthropod larvae collected from remains (Catts & Goff, 1992; Anderson & Cervenka, 2002). Development rates are

useful only within the first life-cycle of the arthropod species used, whereas arthropod colonization patterns can be used for PIA estimations of up to months or years (Anderson & Cervenka, 2002). These methods are described in the following section.

1.1.1 Decomposition

Decomposition is a continuous process from the time of death until complete skeletonization (Goff, 2009). In biological terms, decomposition is broken down into three processes termed autolysis, putrefaction and diagenesis (Gennard, 2007). These processes are preceded by the early post-mortem changes described earlier.

Autolysis is the natural process of cellular breakdown through enzymatic digestion (Clark, Worrell, & Pless, 1997; Carter, Yellowlees, & Tibbett, 2007). Body tissues are invaded by microorganisms from the intestines and respiratory tracts, which deplete the tissues of oxygen and create anaerobic conditions (Carter *et al.*, 2007; Dent, Forbes, & Stuart, 2004). Anaerobic conditions are necessary for putrefaction, which is the bacterial breakdown and alteration of soft tissues, protein, carboydrates and fat constituents (Dent *et al.*, 2004). Diagenesis is the breakdown of skeletal material and hard tissues by physical breaking, decalcification and dissolution, and reduction to components of soil (Gennard, 2007).

Decomposition can also be broken down into a series of stages, defined largely by the perceived changes to the physical state of remains (Schoenly & Reid, 1987). There are five stages that are generally accepted in forensic research: fresh, bloat, active decay, advanced decay and dry/remains (Payne, 1965; Anderson & VanLaerhoven, 1996), although a variety of stages have also been described. The fresh stage of decomposition begins at the moment of death and persists until the first signs of abdominal bloat are

observed (Anderson & VanLaerhoven, 1996). It is in this stage that early post-mortem changes may be observed (Goff, 2009). The first insects to arrive at remains in the fresh stage are usually blowflies (Calliphoridae) (Payne, 1965; Anderson & VanLaerhoven, 1996; Gennard, 2007), which may be attracted within minutes of death occurring (Anderson & VanLaerhoven, 1996).

The bloat stage is evidence by abdominal distension due to the accumulation of gases created by the activity of anaerobic bacteria in the gut (Anderson & VanLaerhoven, 1996; Gennard, 2007). Due to internal pressure, fluids and feces may be purged from the body through natural orifices. At this stage adult blowflies continue to arrive and lay eggs (Anderson & VanLaerhoven, 1996). Predatory beetles, such as Staphylìnidae, may also be observed preying on Calliphoridae eggs (Anderson & VanLaerhoven, 1996; Gennard, 2007). The end of the bloat stage, and beginning of active decay, is marked by the deflation of remains due to skin rupture and the escape of internal gases (Anderson & VanLaerhoven, 1996; Gennard, 2007). Skin rupture may occur due to extreme internal pressure of gases, or by the feeding activity of Calliphoridae larvae (Payne, 1965; Anderson & VanLaerhoven, 1996). This stage is associated with a strong decomposition odour (Anderson & VanLaerhoven, 1996), which attracts a new assemblage of insects (Gennard, 2007). However, Calliphoridae larvae remain the dominant insect type at remains (Payne, 1965; Anderson & VanLaerhoven, 1996).

Very little remains of a body in advanced decay, as most tissue has been removed by the feeding activity of larvae in the active decay stage (Anderson & VanLaerhoven, 1996). At this stage the decomposition odour begins to fade (Payne, 1965; Anderson & VanLaerhoven, 1996), and Calliphoridae larvae begin to migrate away from remains in

order to pupate (Payne, 1965). The dominance of adult insect types shifts from blowflies to beetles (Payne, 1965; Gennard, 2007).

The final stage of decomposition is the dry stage, which may also be described as the remains stage or skeletonization. At this point nothing remains of a body with the exception of dried skin and cartilage, hair and bones (Payne, 1965; Anderson & VanLaerhoven, 1996; Gennard, 2007). Literature is inconsistent in the description of insect presence at this stage. Some authors describe more carrion insect-types associated with remains at this stage (Anderson & VanLaerhoven, 1996), while others state that there are no obvious insect associations with dry remains (Gennard, 2007). It is also described that insect assemblages present at remains undergo a shift and that there is an overlap between carrion-associated insects and the natural soil fauna (Payne, 1965). Payne (1965) describes this stage as two distinct phases, dry and remains. However, there is little distinction between these stages and it is therefore nearly impossible to distinguish between them (Payne, 1965).

1.1.2 Arthropod succession patterns

Decomposing remains represent a food resource that experiences biological, chemical and physical changes with time (Anderson & Cervenka, 2002; Anderson, 2010). As such, a large variety of insect taxa, not all of which feed on decomposing tissues (Campobasso, Di Vella, & Introna, 2001), are attracted to remains in a changing sequence that allows species to take advantage of the changing resources (Anderson & Cervenka, 2002; Anderson, 2010). This represents a "facilitation model of succession" where the activity of each species group changes the resource, making it less attractive for certain species and more attractive to others (Anderson, 2010). There is no apparent

relationship between the number of stages and the number of insect species observed (Goff, 2009). The insects associated with decomposing remains are referred to as carrion insects.

Carrion insects can be described by their ecological roles (Goff, 1993). There are four roles which are commonly described and accepted:

1. Necrophagous species
2. Predators and parasites of necrophagous species
3. Omnivorous species
4. Adventive species

Necrophages are those insects which feed directly on decomposing remains (Smith, 1986; Goff, 1993), and include a number of species of Diptera (true flies) and Coleoptera (beetles). As many necrophagous species complete their life-cycles on or near remains, they are considered to be the most important group for PMI estimations in the early stages of decomposition (Goff, 1993; Tabor, Fell, & Brewster, 2005). Omnivorous insects feed on both other carrion insects and the carrion itself (Smith, 1986; Goff, 1993). It has been shown that the actions of these insects can slow the rate of decomposition by removing the larvae of necrophagous species (Early & Goff, 1986), thereby affecting the accuracy of PMI estimates. The last group, the adventive species, are not necessarily attracted to decomposing remains (Smith, 1986; Goff, 1993). These insects use the remains as an extension of their natural environment (Smith, 1986; Goff, 1993), and may not have a significant role in the decomposition process (Goff, 1993).

It has been stated that the biogeoclimatic region is the most important variable influencing insect succession pattern (Anderson, 2010). The biogeoclimatic zone defines

the climate, vegetation, soil and meteorological conditions of an area, which in turn influences the insect fauna present (Anderson, 2010). Therefore the types and numbers of species attracted to remains will vary depending on geographic location (Haskell *et al.*, 1997; Anderson, 2010), though within an individual habitat the pattern of insect colonization is predictable (Haskell *et al.*, 1997). The arrival time of insects at decomposing remains may also be influenced by the geographic region (Anderson, 2010).

Another factor affecting insect succession patterns is season as many species are known to vary seasonally in presence, numbers and activity peaks (Anderson, 2010). Some insects may also display physiological or behavioural adaptations to different seasons, especially in temperate regions (Kreitlow, 2010). This is because insects are *poikilothermic*, or cold-blooded, making their behaviour highly temperature-dependent (Kreitlow, 2010). This creates a limitation in the use of known succession patterns, as patterns from another season, even within the same geographic region, should not be used for forensic PIA determination (Anderson, 2010). However, seasonal variation does not in itself preclude forensic usefulness of carrion-associated insects, as the natural presence or absence of certain species may assist in determining the season of death (Anderson, 2010).

Arthropod succession studies have been conducted all over the world, with distinct and varying patterns identified for each region. Succession research in Canada has been focussed in the southern regions, often in or just outside of a major urban centre. These include regions of British Columbia (Anderson & VanLaerhoven, 1996; Dillon & Anderson, 1995; Dillon, 1997), Alberta (Hobischak, VanLaerhoven, & Anderson, 2006),

Saskatchewan (Sharanowski, Walker, & Anderson, 2008), Manitoba (Gill, 2005), Ontario (Bygarski & LeBlanc, unpublished), New Brunswick (Michaud & Moreau, 2009) and Nova Scotia (LeBlanc & Strongman, 2002). Of these studies, the farthest north was conducted outside of Edmonton, Alberta (Hobischak *et al.*, 2006), which is located at approximately 53°32"N latitude. Thus far, no research has been conducted in the more northern regions of Canada.

1.1.3 Insect development

Development rate data is a more accurate method of estimating PIA than succession patterns, but is limited in usefulness to the early PIA period within the first life-cycle of the insect species used (Anderson & Cervenka, 2002). This is because the newly emerged adult, or imago, is indistinguishable from the originally colonizing adults once the exoskeleton has hardened and the wings have dried (Anderson & Cervenka, 2002). Development data is most often applied to necrophagous blowfly species for two reasons: they are often the first species to colonize decomposing remains (Anderson & Cervenka, 2002) and they have a predictable life cycle (Castner, 2010).

The blowfly life cycle consists of an egg stage, three larval stages (or instars), a pupal stage and an adult stage. Eggs are often laid in masses called rafts in or near natural orifices, as these locations provide protection and access to moisture and nutrients (Haskell *et al.*, 1997; Gennard, 2007). The first instar larvae hatched from eggs are the most difficult to distinguish, though slight movement is observable upon close examination (Haskell *et al.*, 1997). First instar larvae will moult into second instars, a larger larval stage in which feeding becomes more avid (Haskell *et al.*, 1997). The final larval stage is third instar, which are the largest and most voracious feeders (Haskell *et*

al., 1997). At this stage, tissue loss due to larval feeding is very evident (Haskell *et al.*, 1997). While there is a size difference between stages, larval instars are distinguished using the number of slits observed in the posterior spiracles: first instar larvae have a single slit, second instars have two, and third instars have three slits (Gennard, 2007).

Towards the end of the third instar larval stage, larvae enter a wandering or migratory phase (Haskell *et al.*, 1997; Gennard, 2007). During this phase larvae leave the food source in search of a suitable location to pupate (Haskell *et al.*, 1997; Gennard, 2007). Ideal pupation locations offer protection from the elements and predators (Haskell *et al.*, 1997). During migration, the crop gradually becomes less visible through the larval cuticle as stored nutrients are used (Gennard, 2007). Towards the end of migration the larval body begins to shorten until attaining a barrel-like shape at the beginning of the pupal stage (Haskell *et al.*, 1997).

The pupal case, or puparium, is formed from the outer cuticle of the larva. When time to pupate, the larval cuticle loosens and the larva secretes a number of substances that cause the cuticle to harden and darken (Anderson & Cervenka, 2002). The pupal case changes colour as a function of age, from white to reddish/brown to almost black (Haskell *et al.*, 1997). The new adult emerges from the pupal case by expanding the ptilinum, a blood-filled region on the head, which forces the end off the pupal case (Gennard, 2007). Newly emerged adult blowflies do not immediately resemble the original generation; they have long spindly legs, folded and crumpled wings, and their body walls are opaque and unpigmented until the exoskeleton dries (Haskell *et al.*, 1997; Anderson & Cervenka, 2002).

Of all the stages of blowfly development, the larval instars are the most useful for PMI estimations. Estimates are based on the oldest specimens present, making the egg stage useful only during the early PMI before the first larvae have eclosed (Anderson & VanLaerhoven, 1996). Similarly, newly emerged adults are only useful indicators until their wings and exoskeletons have dried and hardened, as until this point they are unable to fly and are therefore associated with remains (Anderson & Cervenka, 2002). New adults are often misidentified as spiders, and hence are not collected (Haskell *et al.*, 1997).

Certain assumptions are made when estimating a PMI using insect development. The first is that oviposition occurs almost immediately after death (Nabity, Higley, & Heng-Moss, 2006). Realistically, a number of factors may prevent insect access to remains and thereby delay ovipoisition. Such factors include wrappings or coverings, enclosure in a building or vehicle or meteorological conditions that reduce insect activity (Nabity *et al.*, 2006). The second assumption is that the largest larvae present at the remains represent the oldest specimens. In general, larvae do continuously lengthen throughout development. However, each species develops differently and different species will grow to different sizes. Because of this, a younger stage of one species could be larger than the older stage of another species.

Because insects are poikilothermic, their rate of development is highly temperature-dependent. Between the limits of extreme temperatures, rate of blowfly development is generally considered to increase linearly with an increase in ambient temperature (Haskell *et al.*, 1997; Nabity *et al.*, 2006; Higley & Haskell, 2010). Beyond these limits, each species of blowfly is subject to minimum and maximum development

thresholds, extreme temperature limits beyond which development is significantly reduced or halted (Higley & Haskell, 2010). These limits are often difficult to accurately determine, particularly as the upper threshold often occurs near the upper lethal temperature of a species (Higley & Haskell, 2010). The lower threshold is often estimated using development rate regressions (Grassberger & Reiter, 2002).

Development rates are often studied under controlled constant conditions, but unless a crime scene occurs in a temperature controlled location the thermal conditions will undoubtedly fluctuate (Wells & LaMotte, 2010). It has been predicted that the influence of fluctuating temperatures is more important with some species than others, and that differences in the thermal environment have the greatest impact on development (Higley & Haskell, 2010). While development rate is thought to be linearly related to ambient temperatures within the extremes, fluctuations in the upper- or lower-temperature portions of the development curve will cause development to progress either more slowly or more rapidly than predicted (Higley & Haskell, 2010). In recent years, researchers have begun looking at the effects of fluctuating temperature regimes in comparison with constant temperatures (Byrd & Allen, 2001; Clarkson, Hobischak, & Anderson, 2005).

Few studies have been performed to directly determine the effect of photoperiod on insect larval development (Nabity *et al.*, 2006). However, unpublished data from Nabity *et al.* (2006) suggest that photoperiod may influence development rates. Behaviours of adult Dipterans have also been shown to be altered by photoperiod (Grassberger & Reiter, 2002). However, there are as yet no conclusive studies on this factor.

When using development rates in forensic investigations it is important to consider the species of larvae being used, as it is well known and documented that each species develops at a unique and independent rate. Development rate data is available for a wide range of blowfly species including *Protophormia terraenovae* (Robineau-Desvoidy) (Kamal, 1958; Greenberg & Tantawi, 1993; Davies & Ratcliffe, 1994; Grassberger & Reiter, 2002; Clarkson, Hobischak, & Anderson, 2005; Warren, 2006), *Phormia regina* (Meigen) (Kamal, 1958; Anderson, 2000; Byrd & Allen, 2001; Nabity *et al.*, 2006), *Lucilia sericata* (Meigen) (Kamal, 1958; Davies & Ratcliffe, 1994; Anderson, 2000; Grassberger & Reiter, 2001; Gallagher, Sandhu, & Kimsey, 2010), *Lucilia illustris* (Meigen) (Anderson, 2000), *Calliphora vicina* (Robineau-Desvoidy) (Kamal, 1958; Davies & Ratcliffe, 1994; Anderson, 2000; Donovan, Hall, Turner, & Moncrieff, 2006; Hwang & Turner, 2009), and *Calliphora vomitoria* (Linneaus) (Kamal, 1958; Greenberg & Tantawi, 1993; Davies & Ratcliffe, 1994). Each species will also respond differently to thermal conditions and photoperiod. The majority of published research focuses on development under constant thermal conditions though more recent research is also beginning to recognize the need for development rates determined under fluctuating thermal conditions.

1.2 Diapause

Dormancy in insects has been defined as physiological adaptations that have evolved in order to covercome the adverse environmental conditions experienced in particular climatic zones (Mansingh, 1971). There are many states of dormancy, characterized by varying degrees of arrested growth and development (Mansingh, 1971). Diapause is the most highly evolved state of dormancy, used for overcoming cyclic, long-

term and extreme environmental conditions (Mansingh, 1971). This state allows synchronization of an insect's life cycle with favourable periods of the year, and in almost all insects is a seasonal adaptation (Danilevskii, 1965). Insect species which undergo overwintering in the adult stage are said to enter a form of diapause known as adult reproductive (also described as facultative or imaginal) diapause (Vinogradova, 1987).

Reproductive diapause is characterized, in female specimens, by failure of the ovaries to develop, often in the early stages (Stoffolano, 1974; Vinogradova, 1986). In addition to this developmental arrest, some species (*P. regina*, *P. terraenovae*) also undergo fat body hypertrophy (Vinogradova, 1986). Reproductive diapause may cause behavioural changes in individuals, including migration, reduced mating activity and changes in feeding behaviour (Greenberg & Stoffolano, 1977). As diapause is not a completely inactive stage and physiological changes continue to occur, in some cases of reproductive diapause adults will continue to feed actively (Danilevskii, 1965). This is especially true of flies in which reproductive diapause is a response to shortened photoperiods (such as those experienced in the fall) (Greenberg & Stoffolano, 1977).

Diapause is always induced well before adverse environmental conditions set in (Mansingh, 1971), at temperatures that permit continued development (Danilevskii, 1965). In the early stages of diapause induction, changes in the environmental conditions may reverse the physiological processes in some individuals (Mansingh, 1971). Mansingh (1971) puts forth that insects perceive the up-coming change in season through photoperiod, and that this initiates the preparation of diapause. However, others have stated that temperature is the main factor in inducing diapause and that photoperiodism is

secondary (Vinogradova, 1986). Danilevskii (1965) states that it is after the temperature falls below the threshold for active development when the next stage in diapause begins. There must be a special mechanism to maintain this condition of life cycle synchronization, as the agreement between time of diapause induction and the effects of external conditions are clear (Danilevskii, 1965).

Nearly all diapausing insects, under natural conditions, have the potential to terminate the condition of diapause after cold weather experiences of approximately three months (Mansingh, 1971). Similar to induction, termination of diapause is achieved through changes in environmental conditions, and there are a wide variety of natural and artificial stimuli which are capable of triggering diapause termination (Mansingh, 1971). Increasing the temperature was shown by Vinogradova (1986) to terminate diapause in all of the species studied, and changes in photoperiod triggered reactivation in some. However, the return of conditions favourable to development does not guarantee termination of diapause, or do so immediately (Mansingh, 1971). This action is dependent on the nature of the terminating stimuli and the activated condition of the individual, the physiological condition after individuals have achieved competency to restart development (Mansingh, 1971).

1.3 *Protophormia terraenovae* (Robineau-Desvoidy)

Protophormia terraenovae has a Holarctic distribution, and is thus commonly known as the Holarctic blowfly (Vinogradova, 1987; Byrd & Castner, 2010). The most cold tolerant of the Calliphorid species, characterized by a low temperature threshold (Vinogradova, 1987), it is most commonly found in Asia and Europe (Byrd & Castner, 2010) and is abundant in the Arctic (Grassberger & Reiter, 2002). The abundance and

dominance of *P. terraenovae* in the severe conditions found at high latitudes are likely a condition of some of its ecological characteristics, including winter adaptations and temperature reactions which determine summer and vital activities (Vinogradova, 1987). In North America, it is most frequently found in Alaska and Canada, as well as at altitudes above 3000 feet during the summer (Byrd & Castner, 2010). Within Canada *P. terraenovae* is an early spring species, with peak numbers observed in July (Byrd & Castner, 2010). *P. terraenovae* is considered to be the northern counterpart to *P. regina*; both display very similar behaviours, but the range of *P. terraenovae* extends considerably farther north (Byrd & Castner, 2010).

Adults of *P. terraenovae* are rather large, approximately 7-12 mm in length, with black legs (Byrd & Castner, 2010). The thorax is dark blue-black, upon which a series of longitudinal stripes may or may not be visible, and coated with a silver-gray powder (Byrd & Castner, 2010). The abdomen appears greenish-blue to blue (Byrd & Castner, 2010). In the Arctic and subarctic regions, *P. terraenovae* is found in both populated localities and in the wild (Vinogradova, 1987). Farther south, its association with human communities increases and it is considered a typical synanthropic species (Nuorteva, 1967, as cited by Vinogradova, 1987).

The larvae of *P. terraenovae* develop in fish, meat, and human wastes, carrion and feces (Vinogradova, 1987). Being a cold-adapted species, cooler weather favours larval development (Byrd & Castner, 2010). However, research into the development rate of *P. terraenovae* as a function of environmental factors is limited. Early research was conducted using only laboratory-controlled constant temperature regimes (Kamal, 1958; Greenberg & Tantawi, 1993; Grassberger & Reiter, 2002), though there has been

some research into the effects of alternating or fluctuating temperatures (Davies & Ratcliffe, 1994; Clarkson, Hobischak, & Anderson, 2005; Warren, 2006).

1.4 Beringia

Beringia is a unique biogeoclimatic region which extends across Siberia, Alaska and the Yukon Territory (Danks, Downes, Larson, & Scudder, 1997). During the Pleistocene era, Beringia remained unglaciated and provided a refuge for many plant and animal species (Danks *et al.*, 1997). Climate in Beringia at the last glacial maximum is thought to have been cold and dry (Hoffecker & Elias, 2003). Modern habitats are predominantly cold, though habitats in the Yukon are often warmer than those at similar latitudes elsewhere (Danks *et al.*, 1997). The Yukon territory has a sub-arctic climate, featuring cold dark winters, mild summers and temperatures which range between greater extremes than more southern regions (Government of Yukon, 2011). The severe climatic and ecological conditions of the Yukon may contribute to low insect diversity of the region (Danks *et al.*, 1997).

Insects within Beringia exhibit adaptations and properties that allow survival in extreme northern environmental conditions (Danks *et al.*, 1997). The insect groups which are best represented in the Yukon Territory are those which are widespread or have a northern distribution (Danks *et al.*, 1997). There have been at least three patterns of intraspecific genetic variation and differentiation identified in arthropod populations in the Yukon (Danks *et al.*, 1997):

1. Yukon populations of species with widespread distributions show no differentiation from populations elsewhere
2. Yukon populations show variations, but few geographic trends are recognized

3. Yukon populations show differences from those elsewhere at the population or sub-population level. Northern populations are not necessarily more or less variable than southern populations; the extent of variation in a population depends on the sub-population.

Differences between Yukon populations and similar species from more southern habitats may include taxonomic characters, size, colour and brightness, wing development and structure, and the extent to which variations are observed or developed (Danks *et al.*, 1997).

1.5 The Great Lakes Region

The Great Lakes region is located in a temperate zone of North America between 40° and 50°N latitude (Dunn, 1996). The Great Lakes watershed was formed by glaciation (Larson & Schaetzl, 2001). Unlike Beringia, evidence suggests that this region was glaciated multiple times (Larson & Schaetzl, 2001). The most important glaciation in the formation of the Great Lakes was the Wisconsinan, the last glaciation of the Pleistocene era (Dunn, 1996). The drainage basin of the five Great Lakes includes Michigan, major portions of New York, Ohio, Wisconsin and Ontario, and minor portions of Pennsylvania, Indiana, Illinois and Minnesota (Dunn, 1996).

After the glacial retreat, climate in the Great Lakes region was cooler than modern-day conditions (Dunn, 1996). The current climate of the region is continental, and temperatures in many parts are modified by effects of the Great Lakes (Dunn, 1996). The region sits within the westerly wind belt of North America, and the resulting mixing of contrasting air masses creates the potential for extreme weather conditions (Dunn, 1996). Evolutionarily, longer periods of climatic stability within a region will result in a

greater diversity of the flora and fauna which evolve within it (Dunn, 1996). The last glacial era was characterized by alternating glacial advances, retreats and short periods of stability (Dunn, 1996). During this time, the area up to 50 miles ahead of the glaciers was inhabited by large cold-adapted mammals and possibly a few cold-hardy arthropods (Dunn, 1996). While the final glacial retreat began approximately 10,000 years ago, the Great Lakes region has only been climatically stable for 7000 years and early insect recolonization was frequently disrupted by changing water-levels (Dunn, 1996). In comparison, tropical regions were not directly affected by glaciation which may in part explain the greater insect fauna diversity in these areas (Dunn, 1996).

Because the glaciers of the Pleistocene era removed nearly all records and evidence of preglacial inhabitants, there is little known of the insect fauna present in the Great Lakes region before the last glacial retreat (Dunn, 1996). All plant and animal species that exist currently in the region are thought to have established, or re-established, within the past 10,000 years, and since there has been little evolutionary change at the species level during this time frame nearly all modern insect fauna must have existed elsewhere at the time of deglaciation (Dunn, 1996). There are considered to be three major pathways by which insect fauna may have re-established in the Great Lakes region after glaciation (Dunn, 1996):

1. Insect species moved to the region from the great Appalachian forest of eastern North America
2. Insect species traveled up a prairie peninsula that extended up from central North America into northern Minnesota and western Ontario

3. Insect species dispersed to the east along the transcontinental boreal forests of Upper Canada. It is thought that many of these species may have originated in the unglaciated refuge of Beringia.

1.6 Research Aims and Objectives

This research was conducted in order to construct baselines of entomological data for use in post-mortem interval determination by law enforcement agencies in the Yukon Territory. There were three objectives of this study:

1. To determine the arthropod succession pattern on unclothed carcasses in the Whitehorse, Yukon Territory area, and identify differences from other regions in Canada. Currently the geographically-closest arthropod succession data available to investigators is from British Columbia. Given the known biogeoclimatic influence on succession patterns this gap in data availability could result in significant errors in PMI estimates in the Yukon Territory.
2. To identify the dominant blowfly species in the Whitehorse succession pattern and determine its rate of development under constant and fluctuating temperature conditions. The dominant blowfly in a region is likely to be encountered and collected by investigators. Therefore having accurate and relevant development rate data is important for PMI determination.
3. To compare development rates of two geographically distinct populations of the dominant blowfly species under experimentally identical conditions. Researchers have recently suggested that there may be intra-species variation in development rates between distinct populations. However, comparison between published studies may potentially introduce error due to variation in research and sampling

methods between researchers. The Great Lakes region was chosen as a comparison region due to the location of the research institute in Oshawa, Ontario in the Lake Ontario watershed.

Chapter 2:

Preliminary Research – Arthropod Succession in Whitehorse, Yukon Territory

2.0 Literature Review

Outside of Canada, forensic arthropod succession studies have been conducted world-wide. However, similar to Canadian research, very little has been published from northern regions. The farthest north data found thus far was collected in Western Poland (Matuszewski, Bajerlein, Konwerski, & Szpila, 2008) at approximately 52°31”N.

The city of Whitehorse, the capital city of the Yukon Territory, lies at a latitude of 60°43”N, just south of the biogeoclimatic region known as Beringia. Data from the Yukon Bureau of Statistics indicates that the local population, as of June 2009, was 25,636 (Yukon Bureau of Statistics, 2009). This represented 75.1% of the total population of the Yukon Territory at the time (Yukon Bureau of Statistics, 2009), and a 21.7% population increase from 1996 (The Yukon Government Executive Council Office, 1997). There were two reported homicides in Whitehorse during 2009, an extrapolated rate of 5.94 per 100,000 (national rate: 1.81 per 100,000) (Statistics Canada, 2009). The population of Whitehorse and the whole Yukon Territory has continued to increase. With an increased population, it is expected that crime rates (including homicide) will also increase (Sgt. J.J. Giczi, personal communication).

Forensic death investigations are not exclusive to cases involving homicides. The Yukon Territory is an increasingly popular tourist destination during the summer months. In the immense wilderness of the Yukon, it is easy to get lost if not familiar with the area. Therefore it is not unlikely for death investigations to involve remains of tourists and hikers who suffered accidents (Sgt. J.J. Giczi, personal communication). The vast expanse of the Yukon is also popular for hunting. Hunting accidents are a possibility in any region, and so too in the Yukon Territory.

Forensic investigations are not limited to human deaths; medico-criminal entomology can also be applied to cases involving wildlife (Anderson, 1999). Hunting is a common activity, and poaching is becoming an increasingly significant concern (Anderson, 1999). Research using wildlife species has shown that arthropod diversity is similar between black bear and white-tailed deer carcasses (Watson & Carlton, 2003), and that arthropod diversity and arrival patterns are also similar between clothed pigs (accepted human model) and furred carcasses (black bear and cougar) (Dillon, 1997).

2.1 Purpose

The purpose of this research trial was to determine the succession pattern of carrion-frequenting arthropods on unclothed pig carcasses in an area just northwest of Whitehorse, Yukon Territory, Canada. This was the first forensic entomology research of this type conducted in this region, and will therefore provide valuable entomological data to local investigators for use in death investigations. This project was conducted at the request of and in conjunction with the Royal Canadian Mounted Police (RCMP).

2.2 Materials and Methods

2.2.1 Experimental set-up

Field work and sampling for this project were adapted from Anderson & VanLaerhoven (1996), Sharanowski *et al.* (2008) and Michaud & Moreau (2009). Three domestic pigs (*Sus scrofa* L.) were obtained from a local farmer. Funding for the pigs was provided by the RCMP. The pigs were sacrificed on the morning of June 7, 2010 by a single .22 calibre shot to the head and transported to the research site in the back of a truck. Domestic pigs are the preferred models for decomposition studies as they are considered to be biologically similar to humans (Gennard, 2007). As both pigs and

humans are omnivorous, they share similar digestive systems and internal gut flora (Dillon & Anderson, 1995). Pigs also share similar skin tissues with humans and are relatively hairless (Dillon & Anderson, 1995). Catts & Goff (1992) recommend the use of pig carcasses weighing approximately 23 kg to approximate the decomposition of the average adult human. The pigs weighed approximately 35-40 kg; although the exact masses were not known and the three carcasses were not identical in size, they were comparable within this weight range.

The research site was at a secluded location within the Whitehorse Municipal Landfill, northwest of the city of Whitehorse (Figure 1). Access to the site was through two electric fences in order to prevent encounters with large carnivores during the study. The site was situated on a hill such that two of the carcasses were placed at a slightly higher elevation than the third. Earlier in the year, in preparation for the study, the individual sites had been dug slightly so that complete contact would be made with the soils. As such, there was no vegetation in direct contact with any of the carcasses. However, some carcasses were situated closer to various trees and scrub bushes than others, which acted to provide a variation in the amount of shade received at each carcass (Figure 2). The first pig carcass was situated south-east of some small trees so that it received almost no shade through the morning and afternoon. The second carcass was almost surrounded by trees and small bushes, but received full-sunlight from late-morning through the afternoon. The third carcass was near enough to a single tree that the head received shade by early afternoon.

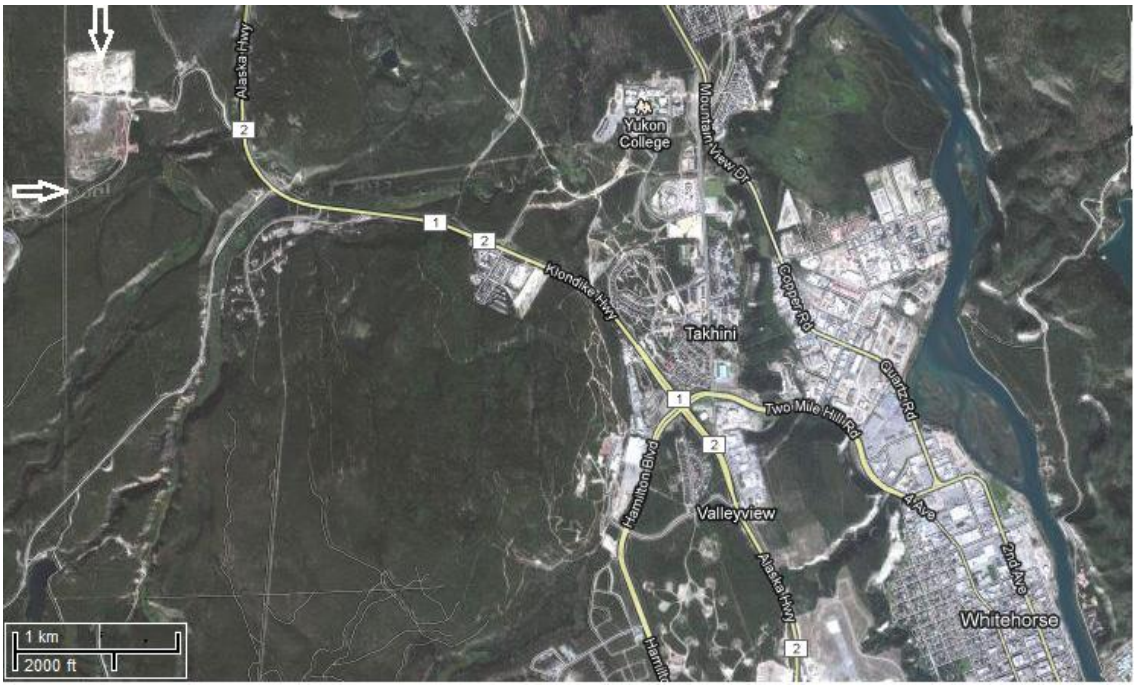

Figure 1. Satellite image of the region northwest of Whitehorse, YT. The main landfill location is indicated by a vertical arrow. The research location is indicated by a horizontal arrow. Image adapted from: http://maps.google.ca/maps?hl=en&tab=wl.

Carcasses were placed in their exact experimental locations within one hour of arrival at the facility on the first day of the study. This was the "day of death", or "day of placement", and is referred to as Day 0 (D0). All following days of the study are numbered sequentially from D0. All carcasses were placed on their left side. Carcasses were placed a minimum of 20 metres apart, as recommended by Anderson & VanLaerhoven (1996) in order to ensure independent olfactory orientation of attracted insects. Each carcass was covered with a cage constructed by Corporal J.J. Giczi, Forensic Identification Specialist with 'M' Division of the RCMP, using materials donated from Home Hardware. Cages were approximately 1.5 x 1.5 x 0.5 metres and consisted of a wooden frame covered with chicken wire. The purpose of the cages was to prevent access to the carcasses by scavengers and larger vertebrates which may remove large amounts of tissue, thereby accelerating decomposition.

Pitfall traps were placed at two locations around each carcass to collect arthropods present at carcasses between examination periods. Holes were dug in the ground at the snout of each carcass and again between the fore- and hind-limbs, approximately 30 cm from the abdomen. The holes were dug sufficiently deep so that the top of the pitfall traps lay flush with the surrounding ground surface. Two plastic cups were used for each trap, a total of six traps and 12 cups. The bottom cup was pierced so that the traps held their form. The top cup was fit snugly within the bottom cup. A small amount of water containing dish washing liquid was poured into the bottom of each trap. At intervals throughout the study, the traps were emptied and the insects caught within them collected. Fresh water and soap were added each time the traps were emptied. The collected insects were returned to the laboratory where they were preserved in approximately 85% ethyl alcohol in 20 mL scintillation vials. Figure 2 shows the experimental set-up of the three experimental carcasses.

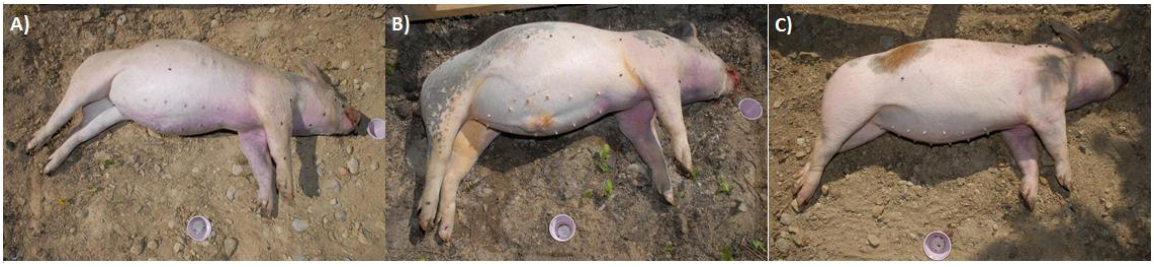

Figure 2. Experimental set-up and levels of sun/shade exposure of pig carcasses in the Yukon Territory at time of daily examination. A) Pig 1. B) Pig 2. C) Pig 3.

An independent pitfall trap was set-up at a control site away from the carcasses in order to monitor the natural entomo-fauna of the area. This trap was monitored and emptied throughout the study on the same schedule as the experimental traps. The control site also contained a data-logger, which monitored the ambient temperature and relative humidity on an hourly basis throughout the study. The data-logger consisted of a

temperature probe housed within a radiation shield, and was set on a large rock in order to prevent water-logging in the case of rains. Data was downloaded from the data-logger on a weekly basis, and analysed using HOBOWare Pro and Microsoft Office Excel Comma softwares. Figure 3 shows the set-up of the control site.

Figure 3. Control site set-up within the Whitehorse Municipal Landfill, with independent pitfall trap and data-logger.

A rain gauge and thermometer was set-up at the site for readings to be taken at each examination and used in conjunction with temperature readings taken by the data-logger. A stick was stood upright in a base of large rocks. The combination rain gauge/thermometer was inserted into the top of the stick and taped for support. Figure 4 depicts the rain gauge/thermometer set-up at the site.

Figure 4. Rain gauge and thermometer set-up at the research site within the Whitehorse Municipal Landfill.

2.2.2 Examinations

Examinations were conducted twice daily for the first five days of the study (D0-D4). Starting on D5 of the study examinations were dropped to once daily, as there had been no differences observed at the carcasses between twice daily visits. These daily examinations occurred between 1100 and 1330, and continued until D22. After this point

carcasses were examined every second day. Written and photographic documentation was kept of each examination.

Photographic documentation was taken using a Nikon D200 digital camera provided by the RCMP 'M' Division in Whitehorse, Yukon Territory. Documentation of each carcass began with six standard photographs. The first was a direct overhead of the carcass label, attached to the corner of each cage using plastic zip-ties. The next four were angled shots taken from the same position each day, straight out from each corner of the individual carcass site. The fifth was a direct overhead shot, taken from as close to perpendicular over the carcass as was possible. A perpendicular angle was not always achieved as a tripod was not used. Additional photographs were taken to document features of decomposition as well as the arrival, location and activity of adult and larval stage arthropods at each carcass.

Written documentation of each examination was taken using a standard template form to ensure consistency of data. A template of the form used can be found in Appendix A. Written notes documented the current weather conditions and ambient temperature, temperature of larval masses or other interesting features, stage of decomposition of each carcass, adult activity and arthropod dominance, and descriptions of the insect activity, pitfall traps and physical changes to the carcasses. Weather term descriptions were adapted from The Weather Network (The Weather Network, 2010). Level of cloud cover was divided into four categories: sunny (less than 10% cloud cover), scattered cloud (10-30% cloud cover), partial cloud (30-70% cloud cover) and cloudy (greater than 70% cloud cover). Temperature ranges were also divided into four categories: cold (less than 5°C), cool (5-15°C), warm (15-25°C) and hot (greater than

25°C). Level of cloud cover was subjective and was determined by the researcher at the time of examination. Ambient temperature readings were taken from the local thermometer at the site. Temperature of larval masses was taken using a digital meat thermometer, purchased from Canadian Tire.

The stages of decomposition were adapted from Payne (1965), Anderson & VanLaerhoven (1996) and Gennard (2007). Five stages were described: fresh, bloat, active decay, advanced decay, and dry/remains. Fresh stage was defined as the time of placement until the first signs of bloat. During the bloat stage the abdomen was distended, and there was characteristic skin marbling and many flies. Active decay was described as beginning when the skin had been pierced by feeding larvae and the carcass began to deflate. For the carcasses in this study, the beginning of the active decay stage was marked by deflation at the head and throat before the body of the carcass. Active decay was characterized by skin blackening and a strong odour. The advanced decay stage was characterized by the loss of most of the flesh leaving skin, bones and hair. In this stage, most Calliphoridae larvae migrate away from the carcass to pupate. According to Payne (1965), the dry and remains stages of decomposition are difficult to differentiate. Therefore, this study used a combined dry/remains stage as described by Anderson & VanLaerhoven (1996). All that remains in the dry/remains stage of decomposition was bones, cartilage and some dried skin. There is very little odour associated with the dry/remains stage.

Adult arthropod activity levels were recorded as a visual count of all adults present on the carcass in a 30 second period. Activity levels were classed in five categories: very low (less than 5 individuals), low (5-20 individuals), moderate (20-50

individuals), high (50-75 individuals) and very high (more than 75 individuals). A note was also made describing the dominant species or group at the carcass. Distinction was made between adult and larval dominance, and which family or group within each category was observed.

Live adult Dipteran specimens were collected from carcasses by placing a plastic collection cup over the individual, or individuals, and trapping them inside. Adults were then transferred to a plastic bag for transportation back to the laboratory. Coleopteran specimens, adult and larval, and larval Dipteran specimens were collected into plastic collection vials using plastic and metal forceps. A fresh picked leaf was included in jars containing Dipteran larvae in order to provide moisture until arrival at the laboratory. Where Coleopteran larvae were collected, a small number of Dipteran larvae from the same carcass were also collected as a food source.

Collected adult Diptera and Coleoptera specimens were killed by being placed in a standard kitchen freezer overnight. Samples were then either transferred to an open pillbox to be dried or pinned for future identification. Larval samples of both Diptera and Coleoptera were divided in half. Half of the samples were killed in boiled water and preserved in a 75% ethyl alcohol solution in 20 mL scintillation vials. The other half of the samples were reared in the laboratory until the adult form emerged. Larvae were reared in large glass jars containing five to eight centimetres of woodchips. A piece of cheesecloth or paper towel was held over the jars using elastic bands in order to contain samples while still providing fresh air access. A small dish made of tin foil rested on the woodchips and contained small amounts of raw liver or wild local game (provided by Clint Sawicki, manager of the Northern Research Institute). Larvae were fed on the raw

protein until migration into the woodchips. When the adult forms emerged they were captured in a plastic bag held over the tops of the jars and killed in a freezer. Emerged adults were kept dry in plastic collection vials for identification.

Pitfall traps were monitored throughout the study and collected every few days. Water was drained from the traps and the insects transferred to plastic collection jars using metal forceps. At the laboratory the collected insects were preserved in 20 mL scintillation vials containing 75% ethyl alcohol solution. Insects were preserved in ethyl alcohol to prevent degradation of samples due to mould and bacteria.

2.2.3 Identifications and analysis

Family and species level identifications were performed using a dissecting microscope and referencing entomological keys by Huckett (1965), Borror, DeLong & Triplehorn (1976), McAlpine (1981; 1987), Anderson & Peck (1985) and Whitworth (2010). Adult specimens of the beetle family Staphylìnidae were identified by Benoit Godin at Environment Canada.

Insect activity and identified specimens were recorded using Microsoft Office Excel 2010. Insect activity at each carcass, according to temperature range and cloud cover, was represented graphically using bar-charts created in Excel 2010. Presence of identified specimens collected from each carcass was graphed using SigmaPlot 12.0.

2.3 Results

2.3.1 Ambient conditions

Average daily temperature and relative humidity were calculated using 24 consecutive readings, taken between 000 and 2300 from the on-site data-logger, for the same calendar day. Daily averages for the first day of the study (D0) and the last day

(D61) used fewer readings due to the launch and final download times. Hourly temperatures ranged between -1.0°C and 29.2°C. The range of average daily temperatures was much narrower, from 7.9°C to 19.9°C (Figure 5). The overall daily average temperature was 12.6°C. Hourly relative humidity at the site ranged between 22.2% and 95.0%, and calculated daily averages ranged from 51.3% and 94.5% (Figure 6). The overall daily relative humidity was 66.6%.

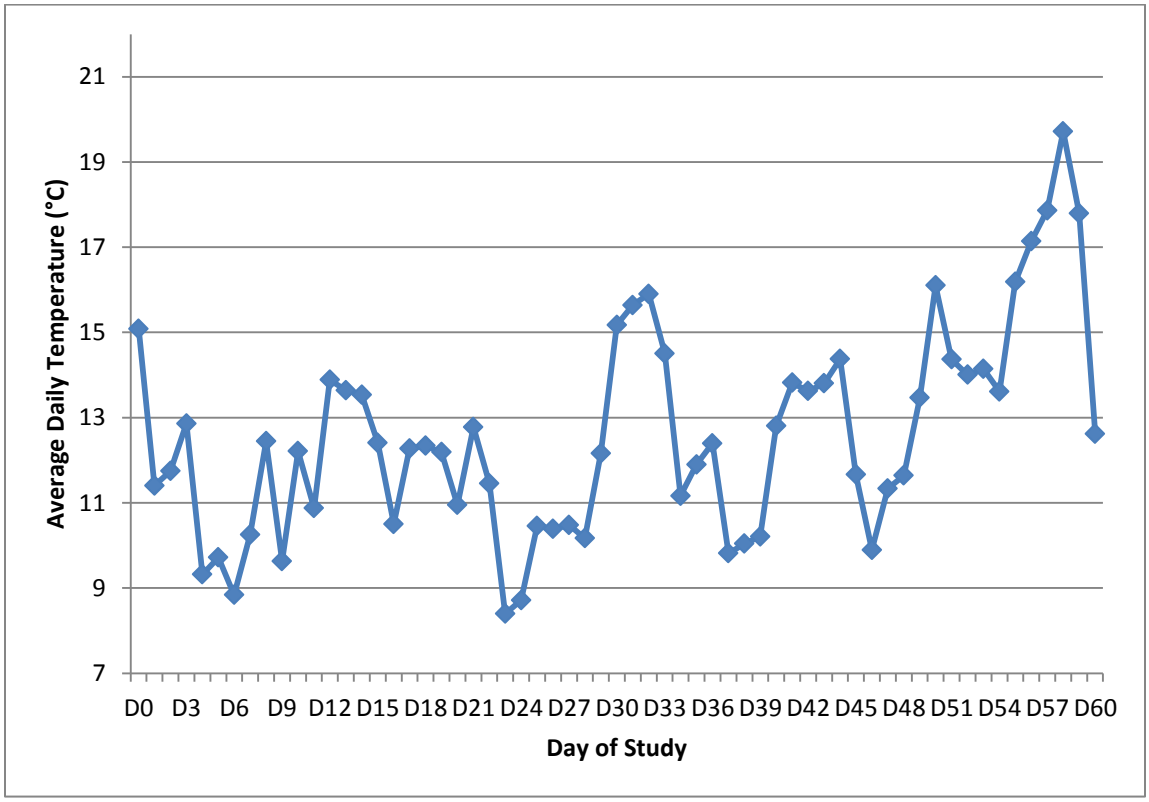

Figure 5. Daily average temperatures (°C) at the Whitehorse Municipal Landfill research site, calculated from hourly data-logger recordings.

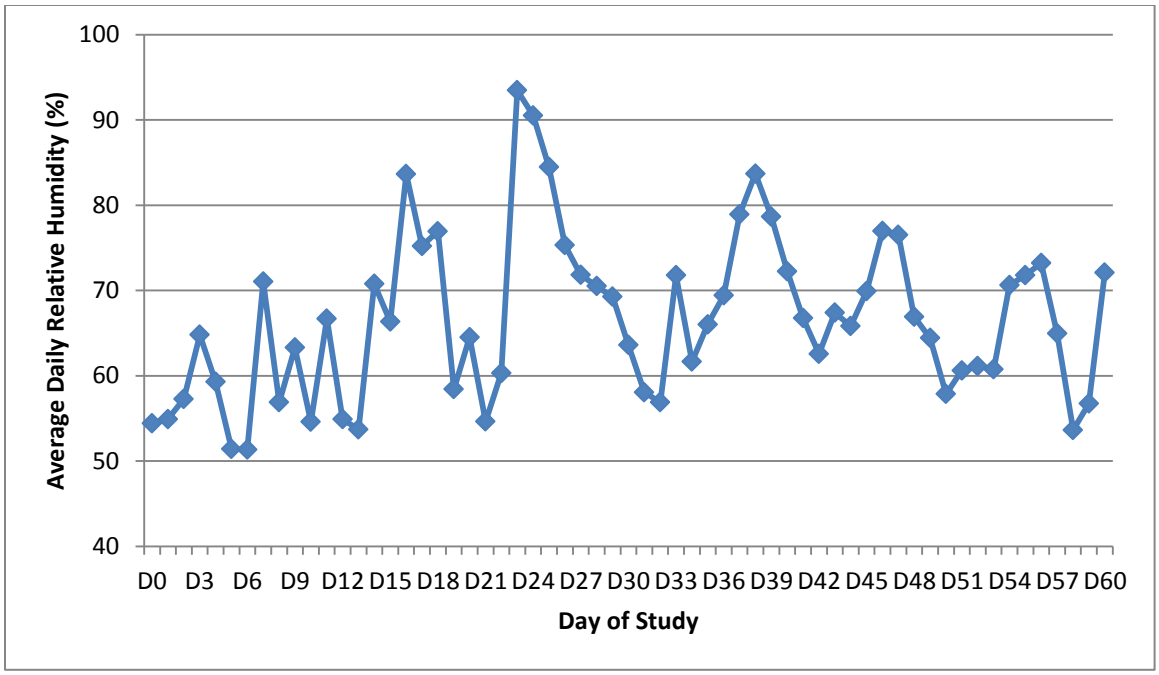

Figure 6. Daily average relative humidity (%) at the Whitehorse Municipal Landfill research site, calculated from hourly data-logger recordings.

2.3.2 Decomposition sequence

Decomposition was recorded over 61 days. Overall decomposition was similar between all three carcasses studied, and only four of the five described stages of decomposition were recorded at each carcass. All carcasses progressed through the fresh stage of decomposition quickly on the day of placement, D0 (Figure 7). The bloat stage lasted approximately 11.33 days ± 1.53 days (Figure 7). Active decay was the longest recorded stage at all carcasses, recorded for 27-34 days (Figure 7). The second carcass (Pig 2) progressed through active decay at the slowest rate. This carcass also received the most shade throughout the day. The first carcass (Pig 1), which received the most sun, entered the advanced decay stage six days earlier than either of the remaining carcasses. Advanced decay was recorded at this carcass for the last 23 days of the study, and for 15 days at the remaining carcasses (Figure 7). No carcass reached the dry/remains stage of decomposition within the duration of this study.

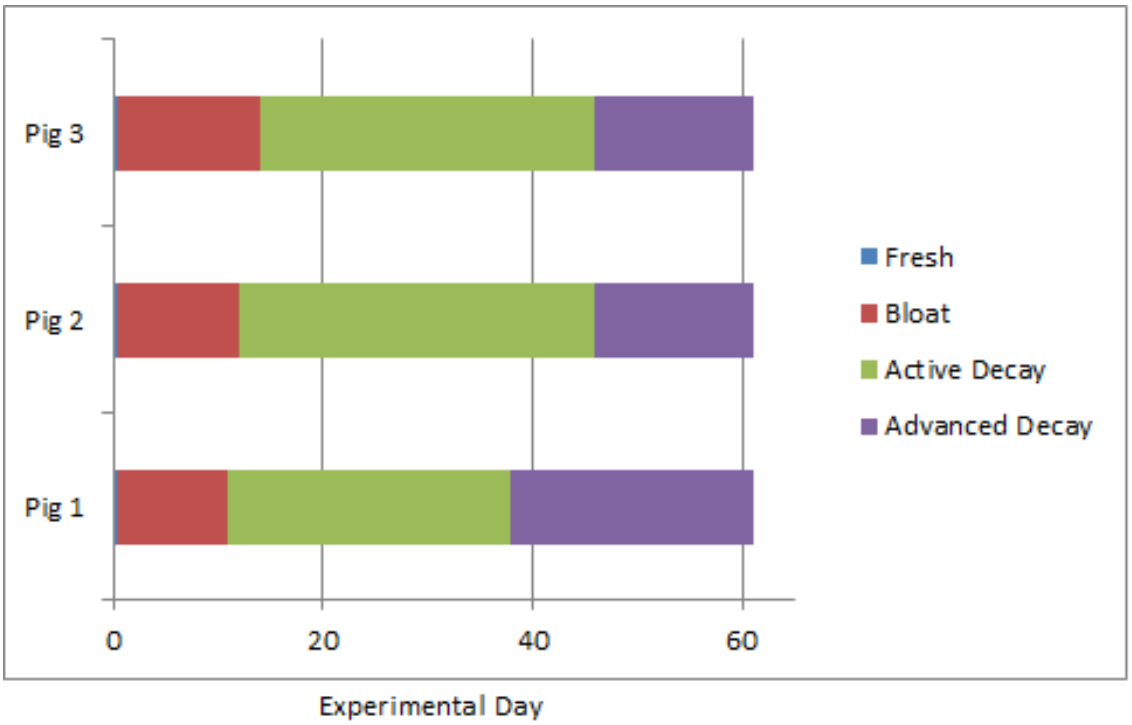

Figure 7. Comparison of the number of days spent in each stage of decomposition by each carcass. Total days = 61.

2.3.3 Insect activity

Each carcass was examined and documented a total of 45 times throughout the study. Overall, moderate levels of insect activity (20-50) were recorded for an average of 31.11 ± 10.18% examinations. Low activity levels (5-20) were reported at 25.19 ± 5.59%, and very high activity (>75) at 22.96 ± 3.39% of examinations.

The effects of ambient temperature and cloud cover on insect activity levels were studied individually. Ambient temperatures were read from the outdoor thermometer at the research site at the time of examination. Only two ranges of ambient temperature were recorded at examinations: cool (5-15°C) and warm (15-25°C). Under warm conditions, a zero-level of activity was not recorded at any carcass, and only two days of very low activity (<5) were reported at a single carcass (Figure 8). The majority of activity in this ambient range was recorded between low and high (50-75) levels (Figure

8). All activity levels were recorded at all carcasses during cool conditions, though the majority of activity levels were recorded in the moderate or low levels, respectively (Figure 8).

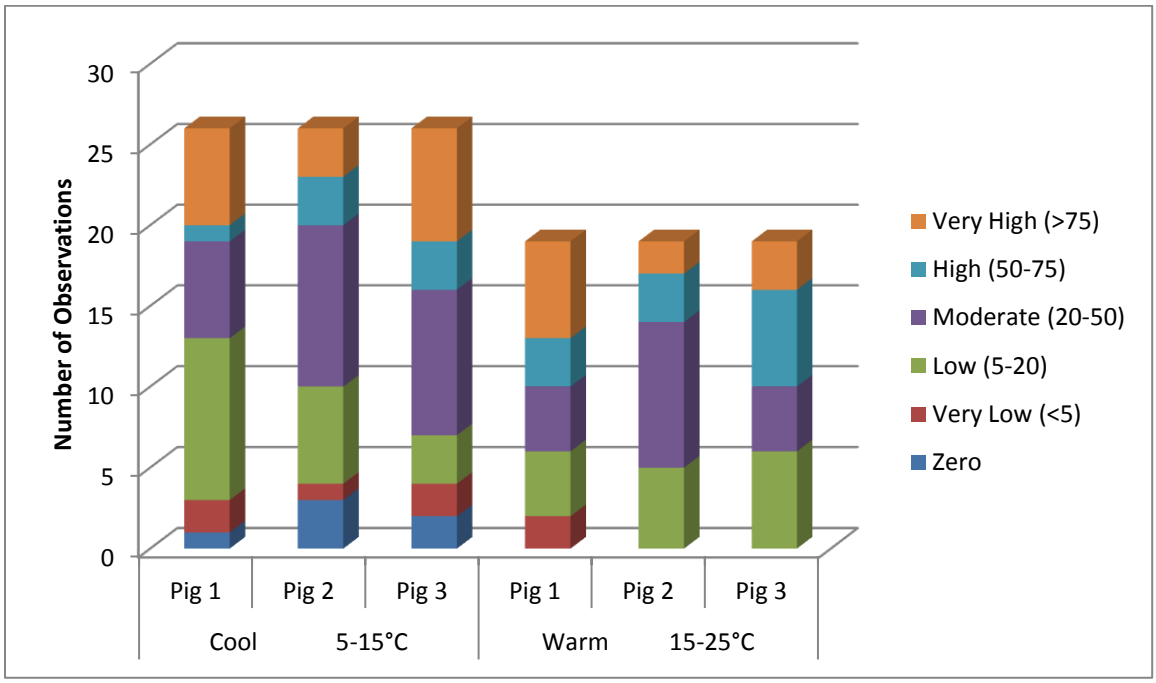

Figure 8. Comparison of the frequency of observation of each insect activity level at each carcass in two ambient temperature ranges. Total number of observations made at each carcass = 45; 26 at 5-15°C, 19 at 15-25°C.

Cloudy conditions (>70% cloud cover) were reported twice as often as any other cloud cover condition. Under this condition, low and moderate levels of insect activity were reported in nearly 60% of examinations (Figure 9). This condition also showed the most recordings of zero insect activity (Figure 9). Activity under partial cloud cover conditions (30-70%) was variable, but tended towards moderate and higher levels (Figure 9). Moderate activity levels were reported at over 60% of examinations with scattered cloud cover (10-30%) (Figure 9). Under sunny conditions (<10% cloud cover) the majority of activity level recordings were high or very high (Figure 9). An activity level

of zero was only reported once (single carcass) in any condition of <70% cloud cover (Figure 9).

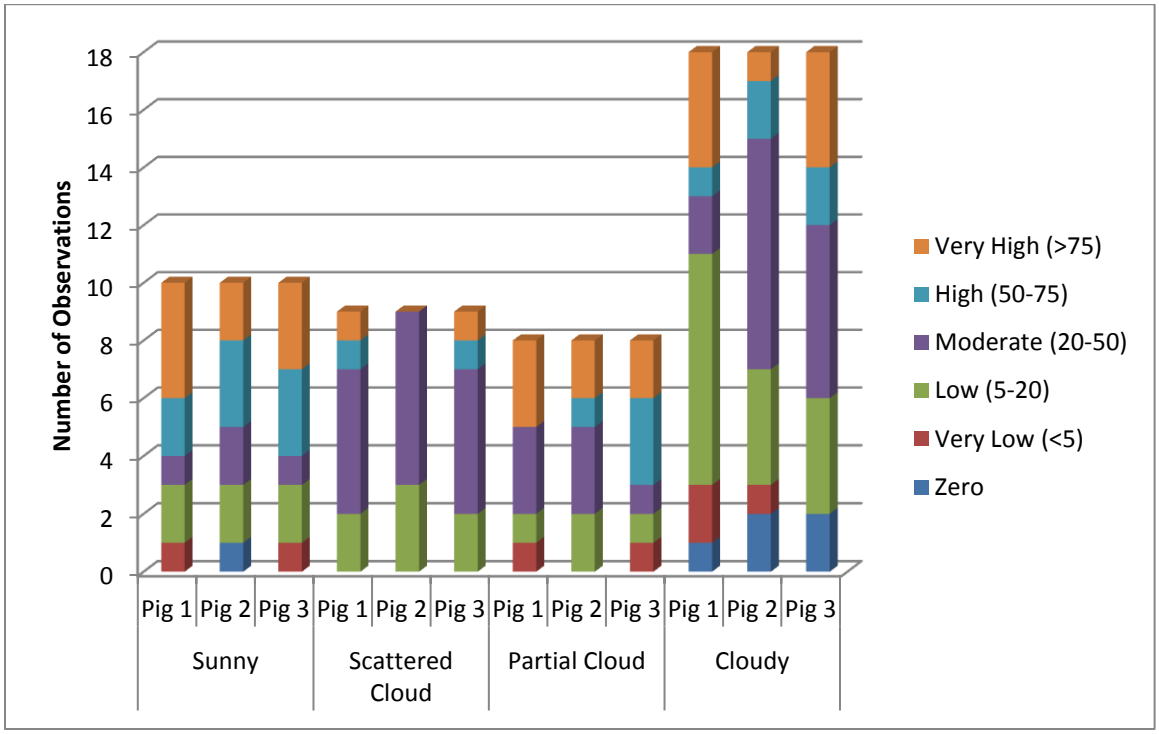

Figure 9. Comparison of the frequency of observation of each insect activity level at each carcass under given levels of cloud cover. Total number of observations made at each carcass = 45; 10 at <10%, 9 at 10-30%, 8 at 30-70% and 18 at >70% cloud cover.

2.3.4 Insect succession

The arrival patterns and frequency of collection of insects identified at carcasses during this study are illustrated in Figures 10 (Pig 1), 11 (Pig 2) and 12 (Pig 3). Adult blowflies were attracted to remains immediately after placement. The only species to be collected from all three carcasses on D0 was *Protophormia terraenovae*. Other blowfly species identified from D0 collections include *Calliphora vicina* and *Cynomya cadaverina* (Robineau-Desvoidy). The first eggs were observed on D0, the initial oviposition sites being in the nose and mouth, and behind the ears of all carcasses. After rearing, these were identified as *P. terraenovae* (two carcasses) and *Lucilia illustris*.

These were the only immature specimens of *L. illustris* collected throughout the study, and adults of the species were not collected from carcasses until D1.

Additional egg masses, occurring at later points, were observed along the carcass backs and at the anus. The first migration of larvae away from carcasses occurred in the active decay stage. Not all larvae migrated away from the carcasses, and at the point of termination there were larvae still feeding within remains.

Adults of the species *P. terraenovae* were dominant at carcasses until the mid-point of active decay. Specimens continued to be collected after this point, but in lower numbers. The larval form was dominant on carcasses during the bloat stage. Teneral adults, identified to be *P. terraenovae,* were first collected in the early advanced stage of decomposition (D40). By D46, teneral adults had been collected from all carcasses.

Adults of four species of bluebottle blowfly were collected in this study: *Calliphora terraenovae* (Macquart), *C. cadaverina*, *Calliphora vicina* and *Calliphora vomitoria*. *C. terraenovae* and *C. cadaverina* are the only bluebottles which were collected from all three experimental carcasses. The only larval specimens collected were identified as *C. cadaverina* and *C. vicina*. A single species of greenbottle blowfly, *L. illustris*, was collected in the adult form from all carcasses in the early bloat stage. This species was not frequently collected in subsequent stages of decomposition. The black blowfly *Phormia regina* was collected from two experimental carcasses on three separate occasions. No immature specimens of this species were identified.

The genera *Hydrotaea* and *Fannia* were the predominant representation of the family Muscidae, beginning in the bloat stage of decomposition. Though adult numbers peaked through the late bloat and active decay stages, at no point was this family group

considered a dominant feature of the arthropod community. This group was not identified from any immature specimens collected throughout the study.

Adults of the small-fly family Piophilidae arrived in mid-bloat, by D5, and their numbers increased through the active decay stage. Piophilidae were the dominant adult specimens at carcasses from active decay through study termination. The family Piophilidae is commonly referred to as "cheese-skippers" due to a defense mechanism of the larval form: when threatened the larvae will pinch their body in a "C" and then release, resulting in a "skipping motion" to avoid predators. Despite adult dominance, no immature specimens were observed at carcasses in this study.

The dominant species of beetle identified at all carcasses was the Sílphid *Thanatophilus lapponicus* (Herbst). Adults arrived in late-bloat/early active decay stage, though observed numbers decreased through advanced decay. Two additional species of Sílphidae were also observed at the carcasses, *Thanatophilus coloradensis* (Wickham) and *Nicrophorus hybridus* (Hatch & Angell). Larval forms of Sílphidae were observed in peak numbers in mid- to late-bloat stage. Only two adults were successfully reared from collected larvae, both identified as *T. lapponicus*.

Two additional families of Coleoptera were collected from carcasses, Staphylìnidae and Cleridae. Adult Staphylìnidae were observed from late-bloat through early-active decay stages. Due to small size and quick speed, adult specimens were infrequently collected. A total of eight genera were identified, but only three genera were collected as live specimens from carcasses. Larval Staphylìnidae were observed infrequently and not collected for rearing. Adult Cleridae were collected regularly from the third pig carcass (Pig 3) at the beginning and end of active decay, but were

infrequently observed mid-stage. Across carcasses the arrival of adult specimens varied more than other insect groups, ranging from late-bloat through early-advanced decay. No larvae of this family were observed.

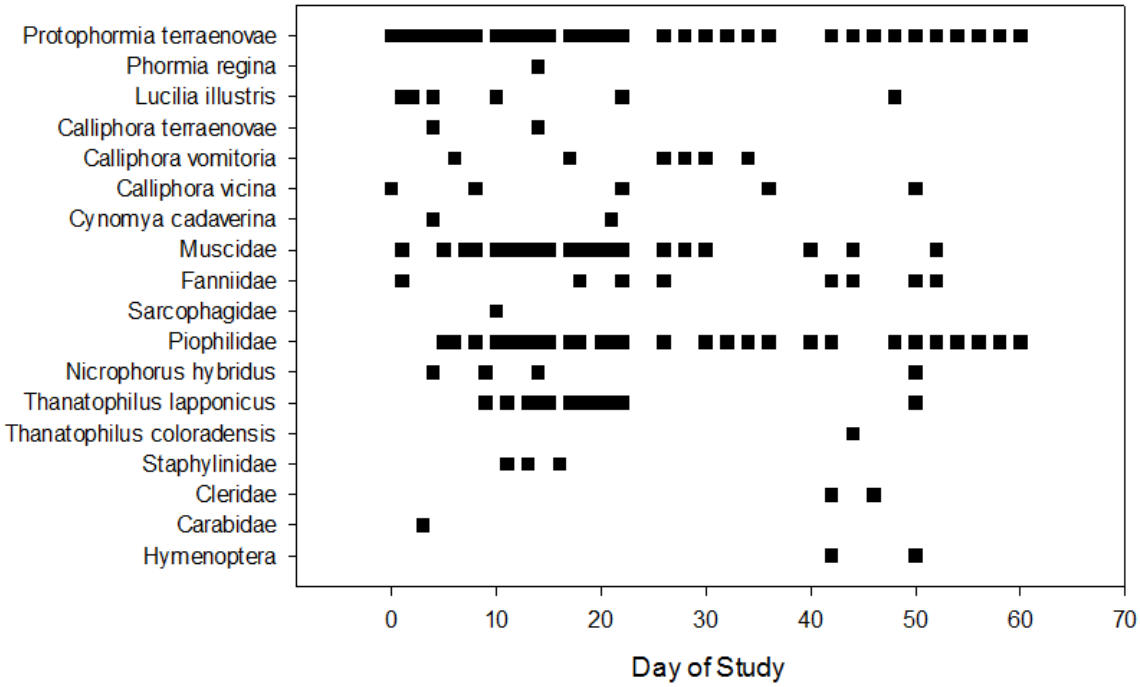

Figure 10. Arrival and frequency of collection of adult arthropods on Pig 1. Gaps indicate days when the species or family groups were not collected.

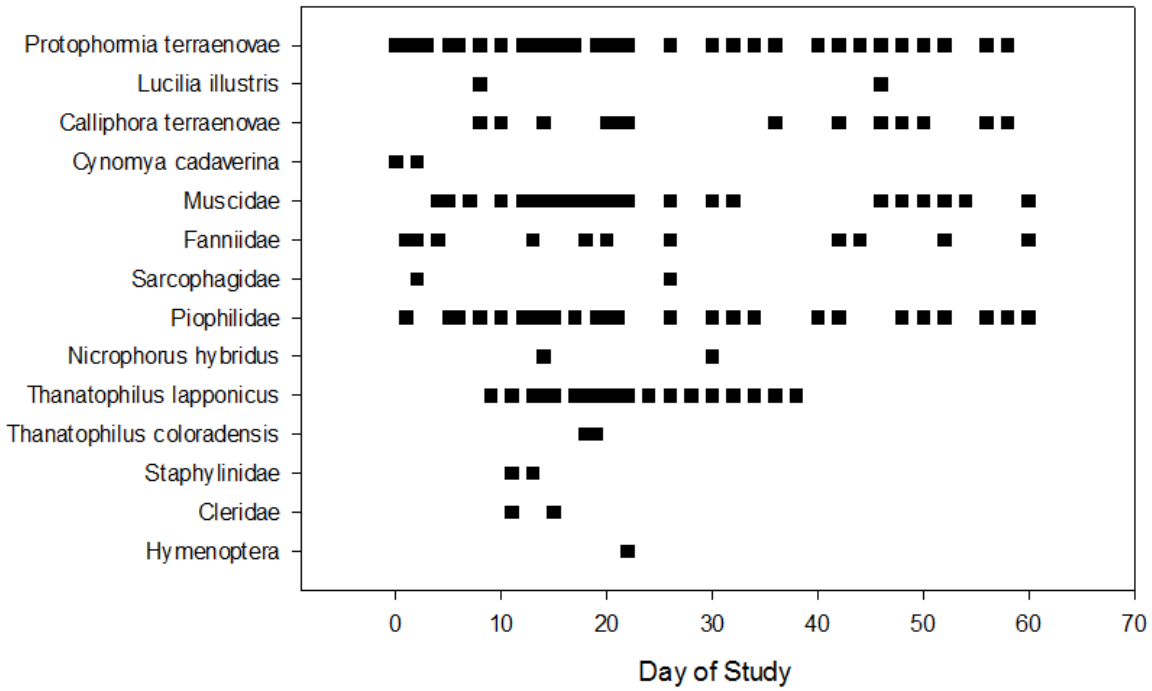

Figure 11. Arrival and frequency of collection of adult arthropods on Pig 2. Gaps indicate days when the species or family groups were not collected.

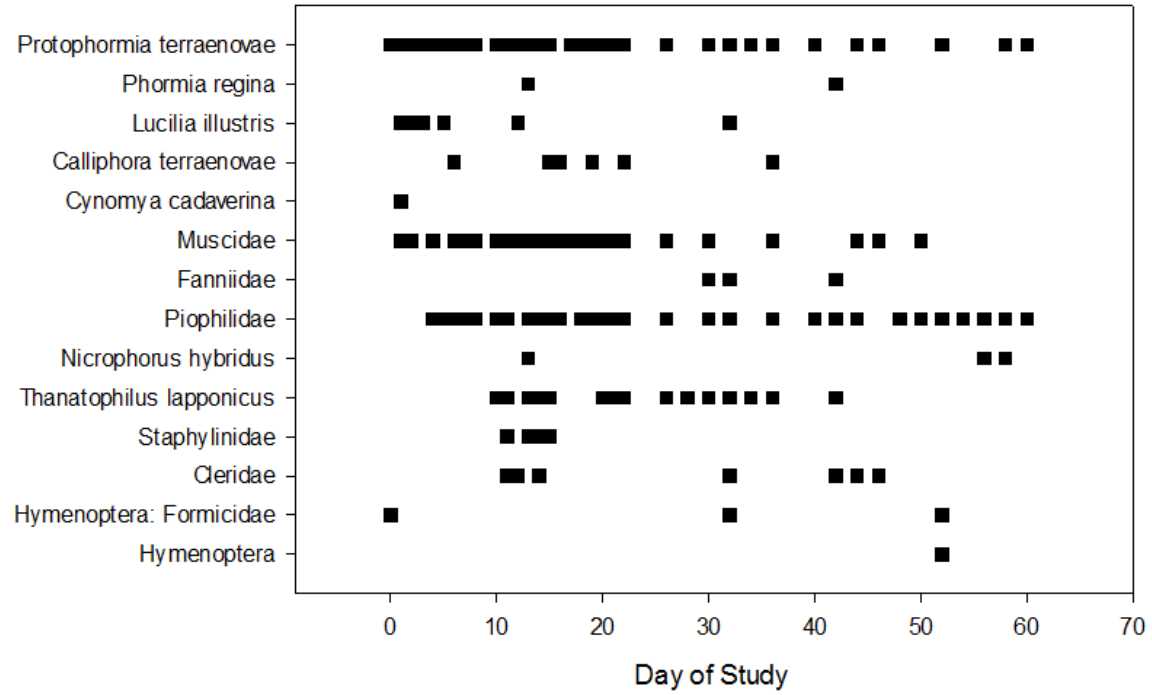

Figure 12. Arrival and frequency of collection of adult arthropods on Pig 3. Gaps indicate days when the species or family groups were not collected.

2.4 Discussion

2.4.1 Ambient conditions

Though maximum temperatures at the research location reached nearly 30°C, corresponding minimum temperatures resulted in an average daily temperature of 12.6°C. This is low when compared to values published in previous research conducted in the summer season (Payne, 1965; Centano, Maldonado, & Oliva, 2002; Tabor, Brewster, & Fell, 2004; Tabor *et al.*, 2005; Sharanowski *et al.*, 2008; Wang, Li, Chen, Chen, & Yin, 2008; Michaud & Moreau, 2009; Voss, Spafford, & Dadour, 2009; Michaud, Majka, Prive, & Moreau, 2010). However, geographically Whitehorse is located much farther north than any previously published research and the extreme northern latitude may account for cooler daily average temperatures during the summer. The daily average calculated for this study are more comparable to values published in research conducted during the fall (LeBlanc & Strongman, 2002; Martinez, Duque, & Wolff, 2007; Sharanowski *et al.*, 2008; Michaud & Moreau, 2009; Michaud *et al.*, 2010).

According to Environment Canada (1993) the historical daily mean temperatures in Whitehorse, Yukon Territory for June, July and August are 11.6°C, 14.0°C and 12.3°C, respectively. Using these values, the calculated daily average across the three months is 12.63 ± 1.23°C. The overall daily average calculated for the duration of this research (June 7 – August 6, 2010) is not statistically different from the historical mean, indicating that the climatic conditions of the region have not varied significantly since 1961 and that the current research is a valid approximation of a standard summer in the Yukon Territory.

2.4.2 Decomposition

Only four of the described stages of decomposition were recorded at all carcasses in this study (Figure 7). It is not unheard of for carcasses to not reach the dry/remains stage in decomposition research, though it is more often reported in fall studies (LeBlanc & Strongman, 2002; Eberhardt & Elliot, 2008; Sharanowski *et al.*, 2008). Only one Canadian study reports carcasses failing to progress to the dry/remains stage in the summer (Hobischak *et al.*, 2006). However, many summer-season studies report complete decomposition in less than a month (Payne, 1965; Grassberger & Frank, 2004; Horenstein, Linhares, Rosso, & Garcia, 2007; Sharanowski *et al.*, 2008). The fact that the average daily temperature in Whitehorse is more comparable to fall than summer may explain the slower observed rate of decomposition, as it is generally shown that decomposition progresses faster in warmer seasons than cooler ones (Rodriguez & Bass, 1983; Centano *et al.*, 2002; Horenstein *et al.*, 2007; Sharanowski *et al.*, 2008; Voss *et al.*, 2009).

Carcasses progressed through the fresh stage of decomposition very quickly (Figure 7), showing signs of bloat by the afternoon of D0. This is faster than many published summer rates, even from warmer climates (Payne, 1965; Anderson & VanLaerhoven, 1996; Eberhardt & Elliot, 2008; Sharanowski *et al.*, 2008). Contrarily, the bloat stage of decomposition progressed at a much slower rate than many previous studies (Payne, 1965; Tabor *et al.*, 2004; Sharanowski *et al.*, 2008). Geographically, the previously studied regions which are closest to Whitehorse are British Columbia and Alberta. A study by Anderson & VanLaerhoven (1996) out of BC reported bloat stage duration of nine days, which is up to 4.5 days shorter than the duration observed in

Whitehorse. These authors attributed the slower rate of progression to heavy rains which washed away previously laid eggs and delayed colonization. However, no such explanation can be offered here. Similar to temperature trends, the current study appears to demonstrate a stronger correlation of decomposition rates through the bloat stage with published research from cooler seasons (Martinez *et al.*, 2007; Sharanowski *et al.*, 2008).

The longest stage (of those observed) at all carcasses was active decay (Figure 7). During this stage the calculated average daily temperatures at the research site was 12.1°C, which is lower than the overall calculated average and may be a factor in the slow rate of progression through active decay. As no carcass reached the dry/remains stage, termination of this study occurred while carcasses were in advanced decay. Therefore, it is not possible to predict how long advanced decay would have endured if allowed to progress normally.

One carcass (Pig 1) decomposed faster than the others and reached a state of advanced decay a total of six days earlier (Figure 7). This carcass was located at the highest elevation of the research site and received almost no shade throughout the day. Currently published research is inconclusive regarding whether decomposition, during the summer season, progresses at a faster rate when carcasses are sun-exposed or shaded (Centano *et al.*, 2002; Hobischak *et al.*, 2006; Sharanowski *et al.*, 2008). Contrarily, it is generally agreed that the active decay stage will progress at a slower rate when shaded and sheltered (versus sun-exposed) during the spring and fall (Centano *et al.*, 2002; Horenstein *et al.*, 2007; Sharanowski *et al.*, 2008).

2.4.3 Insect activity

There was only one major difference in adult activity levels distinguished between ambient temperature ranges: examinations under cooler temperatures showed increased documentation of zero or very low adult activity levels (Figure 8). However, the majority of activity levels recorded under both conditions fell into the low or moderate levels, and both supported adult activity at a high or very high level.

When comparing cloud cover conditions (Figure 9), an inversely proportional relationship was identified between the level of cloud cover and the level of adult activity observed at carcasses. Days recorded as cloudy showed the majority of zero activity, while the majority of activity recorded under sunny conditions was in the high or very high ranges. It was therefore determined that cloud cover had a greater overall effect on adult activity levels than ambient temperature. A single observation of zero activity was recorded for any condition other than cloudy; this examination day fell into the cool temperature range, and no carcass was recorded with an activity level above low (5-20 adults).

2.4.4 Insect succession

The arctic blowfly *Protophormia terraenovae* was the singularly dominant insect, in both the adult and immature forms, collected from all three carcasses, and the only species collected from all carcasses in the fresh stage of decomposition (Figures 9-11). It was further shown to be a primary colonizer as adult specimens reared from the first eggs collected were also identified as the same species. The blowfly *P. terraenovae* is not described in the literature as a primary colonizer or a dominant species at decomposing remains. Many summer season studies do not record *P. terraenovae* as being present at

all throughout decomposition, even in temperate regions (Reed, 1958; Payne, 1965; Anderson & VanLaerhoven, 1996; Sharanowski *et al.*, 2008). Adult *P. terraenovae* have previously been reported as fresh-stage colonizers in the spring (Grassberger & Frank, 2004; Sharanowski *et al.*, 2008) and late-summer/early-fall periods (Grassberger & Frank, 2004), as well as being a common component of bloat stage communities in rural Canada (Hobischak *et al.*, 2006; Sharanowski *et al.*, 2008).

Laboratory rearing of eggs collected during the fresh stage yielded two species of blowfly in addition to *P. terraenovae: Lucilia illustris* and *Cynomya cadaverina*. However, *L. illustris* and *C. cadaverina* were only collected from a single carcass (Pig 2). Adult *C. cadaverina* were also collected from this carcass on D0 (fresh stage) (Figure 11), though adult *L. illustris* were not collected from any carcass until at least D1 (Figures 11, 12). The fresh stage was the only time either *C. cadaverina* or *L. illustris* were collected as egg or immature specimens. The first collections of *Calliphora vicina* occurred on D4, when eggs were collected from the third pig carcass (Pig 3). This was the only collection at the egg stage, through this species was later identified from adults emerged from samples of later-stage larvae collected from all three carcasses. However, *P. terraenovae* remained the dominant species identified from egg and larval collections.

Bluebottle blowflies are common at decomposing remains in temperate regions (LeBlanc & Strongman, 2002; Grassberger & Frank, 2004; Matuszewski *et al.*, 2008; Michaud *et al.*, 2010). Certain species of bluebottle have been reported as dominant components of the arthropod community in the early stages of decomposition, though the individual species varies by region and season (LeBlanc & Strongman, 2002; Grassberger & Frank, 2004; Sharanowski *et al.*, 2008). Though four species of bluebottle blowfly

were collected/identified in this study, only *Calliphora terraenovae* and *C. cadaverina* were present at all three experimental carcasses. Collection of bluebottle blowflies was most common in the bloat and active decay stages of decomposition, and no species was considered to be dominant at any stage.

A single species of greenbottle blowfly, *L. illustris*, was identified in this study. Though eggs were collected during the fresh stage, first collection of adults did not occur until the bloat stage and it was not frequently collected at later stages. The literature supports the early arrival of this species (Anderson & VanLaerhoven, 1996; Michaud & Moreau, 2009; Michaud *et al.*, 2010). The commonly reported black blowfly *Phormia regina* was rarely collected in the adult form, was not identified from larval rearings, and was not reported at all carcasses. Previous studies demonstrate a significant relationship of this species with the bloat stage of decomposition (LeBlanc & Strongman, 2002; Matuszewski *et al.*, 2008; Michaud & Moreau, 2009).

As this research was conducted within a landfill facility, there were concerns regarding co-attraction of certain species of "landfill flies". Studies have shown that common species present in landfill ecosystems are *Musca domestica* (Linneaus) (Howard, 2001; Lole, 2005) and *Fannia spp.* (Lole, 2005). However, distribution research showed that predicted landfill-associated species are more commonly found in the inner rings near the landfill working face, and are less frequently collected at greater distances from the central point (Howard, 2001; Lole, 2005). In a comparison of landfill sites, Goulson, Hughes and Chapman (1999) found that there is little overlap or movement of landfill species between the working face and external sites within 500m. The research presented here was conducted a minimum of 1.5km (1500m) from the

nearest landfill activity, nearly three times the distance studied by Goulson *et al.*, and it is therefore unlikely that landfill activities significantly altered the insect community composition at the studied remains. It is noted that there are no published studies documenting landfill "nuisance flies" in the Yukon Territory, and therefore further conclusions cannot be made at this time.

Arrival of adults of the family Piophilidae in the mid-bloat stage is supported in the literature by previous findings from Canadian succession studies conducted during the summer season (Anderson & VanLaerhoven, 1996; Sharanowski *et al.*, 2008). However, this result highlights the importance of succession research conducted in various biogeoclimatic regions as research conducted from additional locations globally indicate the family is more commonly associated with later stages of active and advanced decay (Reed, 1958; Grassberger & Frank, 2004; Matuszewski *et al.*, 2008).

Species richness with respect to Coleoptera was low. Three families of beetles were collected/identified from all carcasses: Sílphidae, Staphylìnidae and Cleridae. The dominant family, for both adult and larval forms, was Sílphidae and the dominant species was the carrion beetle *Thanatophilus lapponicus*. The first adults of *T. lapponicus* were collected in the later period of the bloat stage. The species *T. lapponicus* is not specifically discussed in much of the literature, and in many publications it is not reported at all (Reed, 1958; Payne, 1965; LeBlanc & Strongman, 2002; Hobischak *et al.*, 2006; Michaud & Moreau, 2009; Michaud *et al.*, 2010). Sharanowski *et al.* (2008) described the species as "elusive". Publications which do discuss *T. lapponicus* report presence at decomposing remains in late-spring/early-summer (Anderson & VanLaerhoven, 1996; Grassberger & Frank, 2004; Sharanowski *et al.*, 2008) or fall

(Matuszewski *et al.*, 2008; Sharanowski *et al.*, 2008; Michaud *et al.*, 2010). *T. lapponicus* was the only species that was reared from larval specimens, which were first observed in the active decay stage. Arrival of larvae at this point is supported by previous publications which report larvae as a major component of the later stages of decay (Reed, 1958; Grassberger & Frank, 2004; Sharanowski *et al.*, 2008). Two additional species of Sílphidae were collected, but were not observed with the same abundance or frequency as *T. lapponicus* and neither was reared from larvae collected from the remains.

The majority of adult Staphylìnidae, in numbers and diversity, were collected from pitfall traps. Staphylìnidae are often reported to arrive in the bloat – active decay stages (Payne, 1965; Anderson & VanLaerhoven, 1996; Grassberger & Frank, 2004; Matuszewski *et al.*, 2008; Sharanowski *et al.*, 2008), and in this study were first observed in the early-active decay stage. Staphylìnidae are predatory on Dipteran larvae (Payne, 1965; Anderson & VanLaerhoven, 1996), and their arrival around active decay may be attributed to the increased population numbers of their prey at this time.

A single genus of the beetle family Cleridae was collected throughout the study from all carcasses, though arrival time between carcasses was more varied than any other identified group (Figures 10-12). The last carcass to be colonized was Pig 1, which received the most sun throughout the day. It has previously been shown that Cleridae frequency of presence is different between sun-exposed and shaded carcasses, particularly in the early and later decomposition stages, but that they may arrive as early as the fresh stage in both environments (Hobischak *et al.*, 2006).

Chapter 3:

Development of

Protophormia terraenovae

(Robineau-Desvoidy)

3.0 Literature Review

3.0.1 Temperature-dependent development

Temperature-dependent development trials looking specifically at *Protophormia terraenovae* have previously been conducted in Austria (Grassberger & Reiter, 2002) and British Columbia, Canada (Clarkson *et al.*, 2005; Warren, 2006), though researchers have also studied it in conjuction with a number of other species (Kamal, 1958; Greenberg & Tantawi, 1993; Davies & Ratcliffe, 1994; Marchenko, 2001). Under constant temperature conditions, and using larval length as an indicator of growth, the development rate of *P. terraenovae* has been shown to increase linearly with temperature within the range of 15-30°C (Grassberger & Reiter, 2002). The same authors also found that within the range of 15-30°C, development rates of the population from Austria did not differ significantly from those published by Marchenko (2001) in Russia. Research in British Columbia shows the development rates of the local population at a constant 15°C do not differ significantly from either the Russian or Austrian data (Warren, 2006), but are somewhat faster at 20°C (Clarkson *et al.*, 2005). Though there have not been many significant differences determined between published studies, Grassberger & Reiter (2002) have proposed that larger differences between populations may not necessarily be due to extrinsic experimental methods, but that geographic adaptations may play a significant role in the differences observed in temperature-dependent development.

Most crime scenes are located in areas exposed to natural fluctuating environmental conditions, though there are few publications which have studied development accordingly. Three previous studies using *P. terraenovae* have been conducted to compare larval development under fluctuating conditions to constant

temperatures which approximate the mean of the fluctuations. Two of these studies have found that larval development occurs faster in fluctuating conditions than at the corresponding constant temperature (Davies & Ratcliffe, 1994; Warren, 2006). The fluctuating regimes in each study were different, though the mean constant temperature used by Warren (2006) was 1°C different than one of the mean values used by Davies & Ratcliffe (1994). In contrast, a third study described larval development as occurring more slowly in fluctuating conditions (Clarkson *et al.*, 2005). These authors used the same parent colony as Warren (2006), and the mean constant temperature used was the same as one trial conducted by Davies & Ratcliffe (1994). However, the fluctuating temperature trial was conducted outdoors under natural conditions (Clarkson *et al.*, 2005), rather than in laboratory-controlled settings between pre-determined high- and low-temperatures (Davies & Ratcliffe, 1994; Warren, 2006). Regardless of differences, these studies highlight the potential error in using development rates determined under constant temperatures for PMI estimations.

3.0.2 Incidence of diapause

P. terraenovae has been shown to overwinter in a stable and prolonged condition of imaginal diapause, though the stability and long-term inhibition of reproductive activity varies between biotypes (Vinogradova, 1987). Behavioural changes in *P. terraenovae* individuals associated with diapause include reduced activity levels and the tendency of adults to form compact clusters in folds of parent colony chambers or cages (Vinogradova, 1986).

While temperature plays a major role in the induction, it appears that diapause is controlled by photothermic reactions (Vinogradova, 1987). Vinogradova (1986) found

that at 20°C, all *P terraenovae* individuals entered diapause, and even at 25°C there were only "some" non-diapausing individuals. In these cases, diapause was terminated in 20% of female individuals within four months (Vinogradova, 1986). Diapause was found to be terminated early by increased temperatures (Vinogradova, 1987). Transference of diapausing individuals from 20°C to 26°C resulted in oviposition within 5-7 days, and a decrease in the incidence of diapause to 40-50% within two weeks (Vinogradova, 1987). Increasing temperature to 30°C resulted in a more accelerated rate of reactivation (Vinogradova, 1987). However, increasing the temperature was also found to increase the incidence of mortality on individuals unable to terminate diapause (Vinogradova, 1987).

More recent studies suggest that diapause in *P. terraenovae* is temperature independent, and induced by changes in photoperiod (Greenberg & Stoffolano, 1977; Numata & Shiga, 1995; Shiga & Numata, 1997). However, these results also show a correlation with rearing temperature. In 1995, Numata & Shiga found that the incidence of diapause in individuals was significantly higher when reared at a photoperiod of 12:12 (L:D) than when reared at 18:6 (L:D). Incidence was increased with lower temperatures, as it was found that over 95% of females entered diapause when reared below 22.5°C with a photoperiod of 12:12 (Numata & Shiga, 1995). While these studies found that at photoperiods of 18:6 (L:D) more than 90% of females developed normally, later research indicates that even typical long-day photoperiods (18:6) may not guarantee the prevention of diapause (Shiga & Numata, 1997). Constant light, regardless of intensity, was shown to prevent diapause, creating an incidence rate of less than 24% at low intensity levels (Shiga & Numata, 1997).

Insects which have invaded warmer regions from the arctic or subarctic may adapt the ability to reproduce without the intervention of diapause, producing two or more generations per year (Numata & Shiga, 1995). Intraspecific variation in incidence of diapause is an indication of adaptability of a species to a range of climatic conditions (Vinogradova, 1986). Thus far, research indicates that geographic variation in the incidence of imaginal diapause of *P. terraenovae* is weakly expressed and most biotypes tend toward monocyclism (Vinogradova, 1986), though forms in the southern part of its distribution may be polycyclic (Vinogradova, 1987).

3.1 Purpose

The purpose of this research was to compare temperature-dependent development rates of populations of *P. terraenovae* collected in Whitehorse, Yukon Territory (Beringia) and Oshawa, Ontario (Great Lakes Region) under constant and fluctuating temperatures. *P. terraenovae* was chosen to study as it was previously determined to be the dominant blowfly present at decomposing remains in the Yukon Territory (Chapter 2). As development of both populations-types was to occur simultaneously, under near-identical conditions, this research will allow for direct comparison of development rates between biotypes.

3.2 Optimization of Rearing Vessels

Two optimization trials were run to test the appropriateness of rearing vessels for use in development trials. Trial 1 measured the temperatures created by feeding larval masses, as well as approximated the stage of larval development through migration and adult emergence. The purpose of this trial was to test for any adverse effects, in the form of high larval mass temperatures or delayed development, created by either of the rearing

vessels. Trial 2 measured the length of migrating larvae (over the first two days of migration), as well as the length and colour of pupae from the first observation until the first adult emergence. Larval mass temperatures were also recorded. The purpose of this trial was to test for any end-point development differences between the test vessels. Optimization trials were run using only the Beringia colony, as the Great Lakes colony had not been established by this point

3.2.1 Methods and materials

The two optimization trials tested larval development in two rearing vessels: a 1L glass mason jar (vessel 1), and a large round plastic container (diameter = 20cm) (vessel 2). Each vessel contained woodchips as a dry substrate for larval pupation. The mason jar contained approximately 9cm of woodchips, and the plastic container contained approximately 5cm. Larvae were reared on raw beef liver, added *ad libitum*, in tin-foil dishes placed on top of the woodchips. Rearing vessels were covered with a mesh fabric to allow air-flow circulation while preventing the escape of larvae. The fabric was secured in place using the outer ring of the mason jar cap, or by elastic bands (vessel 2).

To collect eggs, liver was removed from the parent colony for three days before being re-introduced. The fresh liver was checked every 15 minutes for two hours, then hourly until the first eggs were observed. In the first trial, no eggs were obtained by the end of the day (~1800hrs), and the liver was left *in situ* overnight. Eggs were observed at 0800hrs the following morning and collected at 1550hrs. As there were insufficient eggs for both test vessels, the 1L mason jar was started first. The liver was left in the colony overnight again, and sufficient eggs were collected for the second rearing vessel by

1040hrs. Both vessels were started simultaneously in the second trial, ~7.5 hours after liver was re-introduced to the parent colony.

Vessels and eggs were placed within an environmental chamber as soon as possible after collection. Environmental chamber parameters are listed in Table 1. Vessels in each trial were examined daily at approximately 24 hour intervals. When vessels were placed mid-afternoon, examinations began the following morning and continued at 24 hour intervals from this point.

Table 1. Environmental chamber parameters of development optimization trials using the Beringia biotype of *Protophormia terraenovae*. L = light phase of the photoperiod (lights on), D = dark phase of the photoperiod (lights off).

Trial	Set Temperature (°C)	Actual Temperature (°C)	Photoperiod (L:D)	Relative Humidity (%)
1	20	20.270 ± 0.343	16:8	24.310 ± 7.316
2	L: 22 D: 20	L: 22.651 ± 0.606 D: 20.520 ± 0.442	17:7	33.218 ± 9.291

In the first trial, the stage of larval development was estimated visually. Temperature measurements of larval masses were recorded whenever masses large enough to obtain accurate readings were observed. Temperature readings were recorded in degrees Celsius (°C) using a digital meat thermometer. The probe of the thermometer was held within the mass so that it was surrounded by larvae until the digital read-out remained constant.

Two larval samples of 10 specimens each were taken from the rearing vessels in the second trial, one on each of the first two days of observed larval migration. Larvae were killed in hot water, and measured for length (mm) and preserved in 75% ethanol. Length measurements were taken under a stereo-microscope using a standard ruler. Pupae were collected daily, in samples of 10 specimens, from the first observation until

the first adult emergence. Each pupa was measured for length (mm), and pupal case colour determined using The Globe Soil Colour Book. In order for more accurate calculations of adult emergence/survival rates, all collected pupae were returned to rearing vessels within one hour of collection. To ensure the same pupae were not continuously sampled, specimens were always returned to the same location within the vessel, marked by the placement of the vessel label, and successive pupae were collected from elsewhere in the vessel.

Newly emerged adults were collected from vessels in large, clear plastic bags, counted, and returned to the parent colony. At the end of each trial any remaining pupae and pupal cases were sorted from the woodchip substrate, counted, and recorded for emergence rate calculations.

3.2.2 Results and discussion

Larval mass temperatures from each vessel were compared using a Wilcoxon Rank Sum test with $\alpha=0.05$. Comparison was conducted for each trial. In both trials the calculated 2-sided p-value was greater than 0.05, indicating that there were no statistical differences in larval mass temperatures between vessels (Table 2).

Table 2. Wilcoxon Rank Sum test statistical values, comparing larval mass temperatures between glass mason jar and plastic rearing vessels.

Trial 1	
S-value	21
Z-value	0.245
2-sided p-value	0.810
Conclusion	Do not reject H_o: no significant differences between vessels
Trial 2	
S-value	4
Z-value	1.162
2-sided p-value	0.246
Conclusion	Do not reject H_o: no significant difference between vessels

*α=0.05

Length values of migrating larvae from each vessel over the two sample days were pooled and compared using a student's t-test. Population variances were found to be equal, and there was no significant difference calculated between vessels (Table 3).

Table 3. T-test statistical values comparing lengths (mm) of migrating larvae between glass mason jar and plastic rearing vessels.

$\mathbf{t_{calculated}}$	1.876
$\mathbf{t_{df=38}}$	2.024
Conclusion	Insufficient evidence to conclude there is a significant difference between vessels

*α=0.05

Pupal lengths are not expected to change throughout development. Therefore all length measurements recorded for each vessel were pooled for comparison. Using a student's t-test, there was no statistical difference found between pupal lengths from each of the tested rearing vessels (Table 4).

Table 4. T-test statistical values comparing lengths (mm) of pupae between glass mason jar and plastic rearing vessels.

$\mathbf{t_{calculated}}$	0.350
$\mathbf{t_{df=138}}$	1.96
Conclusion	Insufficient evidence to conclude there is a significant difference between vessels

*α=0.05

The percent of adult emergence was calculated as the number of adults collected versus the total number of pupae and empty pupal cases remaining in the woodchip substrate at the end of each trial. Both vessels had a greater than 80% emergence rate in Trial 1, with less than 5% difference between vessels, and greater than 60% emergence in Trial 2, with less than 2% difference between vessels (Table 5). As temperature and photoperiod parameters were different between each trial, inter-trial comparisons were not conducted.

Table 5. Adult emergence rates from glass mason jar (vessel 1) and plastic (vessel 2) rearing vessels in each optimization trial.

Trial 1	
Emergence, Vessel 1	86.32%
Emergence, Vessel 2	82.28%
Difference	4.907%
Trial 2	
Emergence, Vessel 1	64.46%
Emergence, Vessel 2	63.22%
Difference	1.949%

Small differences were identified between vessels with regards to development to individual stages. In Trial 1, a 1-day difference in development to the 2nd instar and pupal stages was identified between vessels, but no significant overall differences were reported (Figure 13). In Trial 2, a 2-day difference in development to the pupal stage was recorded (Figure 13). However, similar to Trial 1 this delay did not affect development rate to subsequent stages. In both cases, where differences were identified the slower rate was reported for Vessel 1 (mason jar).

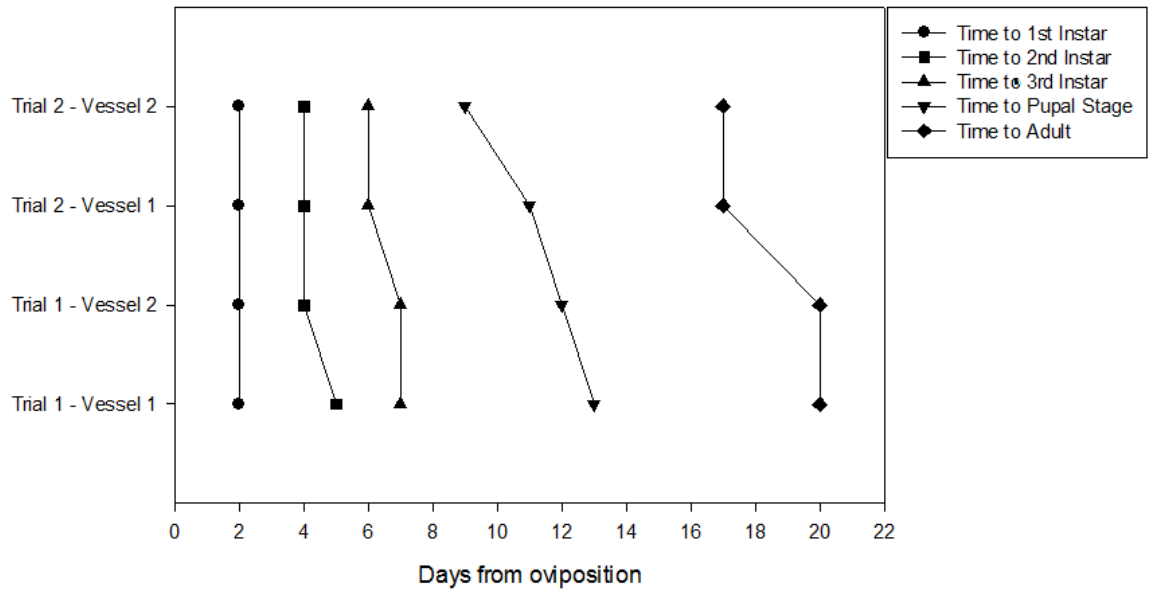

Figure 13. Isomorphen diagram showing the rate of larval development to the major development stages in vessels tested in optimization trials. Development times (days) were compared within the same trial only.

As there were no significant adverse effects detected in either vessel, and neither vessel showed a significant delay in overall rate of development, choice of rearing vessel was made based on ease of use. The plastic vessels had a wider opening which allowed for easier access for feeding and sampling, as well as easier collection of emerged adults. Therefore, plastic rearing vessels were utilized for subsequent development trials.

3.3 Methods and Materials

3.3.1 Colony collection and maintenance

The parent colony from Beringia was collected in Whitehorse as larvae during succession pattern research in the summer of 2010. Larvae were reared to the adult stage, identified, and maintained as an adult colony. Additional larvae were provided from Whitehorse in the spring of 2011. Larvae were reared to the adult stage and identified before being added to the laboratory parent colony.

The parent colony from the Great Lakes Region was collected as adults at the decomposition facility of UOIT during the spring and summer of 2011. Difficulties were incurred in collecting this parent colony as *Protophormia terraenovae* is a cool-temperature adapted species and the spring and early summer seasons of this year were very hot. Due to low adult numbers, no research trials could be conducted until at least the second generation of this population had emerged in mid-July.

Adult colonies were maintained in 60 x 60 x 60 cm cubic net cages under constant laboratory conditions. Colonies were maintained and fed three times weekly. Adults were fed on water, granulated sugar and powdered milk, provided *ad libitum*. Dead flies were removed from the colonies regularly.

Fresh beef liver was provided for oviposition purposes. Liver was provided in a tin-foil dish sat on top of woodchips, which acted as a pupation substrate. Larvae were originally reared within the parent colonies. However, due to difficulties with obtaining sufficient eggs for research purposes the larvae were removed from the colony and reared externally until the adult stage. Larvae were reared on beef liver, provided *ad libitum*. Woodchips within rearing vessels were collected and returned to the appropriate parent colony.

3.3.2 Development trials

Egg collection

Liver was removed from the parent colonies in the days before development trials, as a period of deprivation. Deprivation periods were utilized in an effort to maximize oviposition activity when a suitable oviposition medium (liver) was introduced. Because of the difficulties obtaining eggs, a variety of combinations of

deprivation and exposure periods, as well as light stimuli, were tried (Appendix B). When eggs were required, fresh liver was re-introduced into parent colonies by 0800hrs. Liver was checked hourly until the first eggs were observed, then left for 3-5 hours for sufficient egg numbers to be oviposited. If sufficient egg numbers were not obtained after 5 hours, eggs were removed and the attempt was started over. For consistency in age and development, no eggs older than 5 hours were used for research purposes. If sufficient egg numbers were not obtained by 1800hrs, the attempt was terminated. No eggs were placed after 1900hrs, during the dark period of the photoperiod cycle. Though it has been shown the blowflies can and will oviposit after dark (Greenberg, 1990; Singh & Bharti, 2001; Baldridge, Wallace, & Kirkpatrick, 2006; Amendt, Zehner, & Reckel, 2008), the likelihood and egg numbers are significantly reduced as compared to during daylight (Singh & Bharti, 2001).

Development trials

A total of four development trials were completed. A fifth was attempted, but was abandoned due to lack of sufficient eggs. Completed trials include one under the natural summer climatic conditions of the Great Lakes Region (Oshawa, Ontario) and three at laboratory controlled constant temperatures: 30°C and 10°C (10°C condition was tested twice). Extreme difficulties were incurred in obtaining eggs from the Great Lakes Region colonies. For this reason, the Beringia population was studied under extreme constant temperature conditions first. All constant temperature trials were conducted under a photoperiod of 14:10 (L:D). As complete development was not achieved at a constant 10°C in the first trial attempt, these trial conditions were repeated to verify

original results. Trial parameters (temperature, photoperiod and relative humidity), data-logger outputs and set-up photos can be found in Appendix C.

When sufficient egg numbers were obtained for research they were divided into approximately equal numbers, by weight, in each of three replicate rearing vessels for each population. To determine weights, sets of 20 eggs were counted and weighed, and the calculated weight per egg was averaged across all sets. Sets of 20 eggs were used as the weight of an individual egg was below 0.1mg (readability limit for the analytical balance). Rearing vessels consisted of plastic containers, as determined by optimization trials, with 4-6 centimetres of dry woodchips as a pupation substrate and covered by mesh netting held in place by elastic bands. Eggs were added to fresh beef liver in a tin-foil dish placed on the woodchips. Larvae under research conditions were provided with fresh beef liver *ad libitum*, and woodchips were misted daily to prevent desiccation.

It has been stated by Nabity *et al.* (2006) that within a temperature-controlled chamber not all locations experience the same temperature, and that the set-temperature of a chamber may not be the same as that actually experienced within. It is recommended that for each replicate within the chamber, separate "treatment conditions" should be recorded to account for within-chamber variation (Nabity *et al.*, 2006). Limited data-loggers were available; therefore replicate vessels were randomized within the chamber (or natural trial conditions) so that all replicate vessels of either population were not purposely placed together. A data-logger was included with replicate vessels to monitor actual chamber conditions. An exception to this set-up strategy was in Trial 5 (repeated 10°C condition) when lack of sufficient eggs resulted in biotypes being studied from different start dates. In this case all replicates from a single biotype were placed on

the same shelf within the environmental chamber. Shelves within the chamber were placed equi-distance from light sources, and separate data-loggers were included on each shelf to account for any differences in ambient temperature. Temperature and relative humidity were recorded hourly, and these values used to calculate accumulated degree hours (ADH) for the purposes of growth rate determination. ADH is traditionally calculated by multiplying the time required to reach each development stage by the rearing temperature (Anderson, 2000). To avoid the assumption of constant rearing conditions, hourly temperature measurements from data-loggers were summed over the development period(s).

Five eggs from each vessel were weighed, measured and preserved in ethanol as a base-line for larval development. All remaining eggs were placed under research conditions. Vessels in the natural conditions trial were checked every two hours for eclosion. This was increased to four hours for subsequent trials due to length of time between placement and eclosion. During the repeated 10°C trial, eggs were checked for eclosion every 12 hours due to the length of time required in the initial trial.

The first larval collections were made once approximately 10% of eggs in the rearing vessle had eclosed. After 10°C eclosion, larvae were checked and sampled every six hours, with the exception of the repeated 10°C trial (Trial 5) which was sampled at 12 hour intervals. Samples were killed by immersion in boiling/hot water, as suggested by Tantawi & Greenberg (1993) and Adams & Hall (2003). Hot water killed (HWK) larvae undergo maximum extension, producing measurable lengths comparable to the maximum extension of a live larva (Tantawi & Greenberg, 1993). However, it has been shown that over the first 12 hours after preservation, HWK larvae lengthen significantly (Adams &

Hall, 2003). Therefore HWK larval samples were immediately weighed (grams), measured for length (millimetres), and analyzed for stage of development using the number of slits in the posterior spiracles. Samples were then preserved in ethanol. As a preservative, Tantawi & Greenberg (1993) recommend 70% ethanol while Adams & Hall (2003) recommend 80%. The original preservation solution used was diluted 75% ethanol, but it was observed that in this solution larval samples became discoloured. Subsequent samples were later preserved in a stock solution of 85% ethanol.

When 10% of research larvae had pupated, sampling was reduced to every 12 hours. Similar to larval collections, at each sampling 5 pupae were removed from each replicate vessel and analyzed for length, weight and pupal case colour. The first 5 pupae from each vessel were set aside and allowed to complete development under trial conditions. These were analyzed *in situ* for pupal case colour until adult emergence. Pupal sampling from each vessel was continued until a total of 100 adults had been collected. Adult collections continued at 12 hour intervals, and 10% emergence was determined retroactively. All collected adults were counted and returned to the appropriate parent colonies.

Analysis

Growth was measured as larval length and larval weight as a function of ADH. The linear portions of each growth curve were statistically compared using as student's t-test to determine the effect of temperature on growth rates, as well as the differences between populations reared under experimentally identical conditions. To compare the mean, the slopes of the linear regressions of each replicate vessel were averaged. Linear growth of pooled values, minus outliers (1.5x the standard deviation from the mean),

were compared using analysis of co-variance (ANCOVA). Length and weight were statistically compared between biotypes when both types were studied under the same conditions, and between trials within a single biotype. Length/weight regressions were conducted for all matching larval points, excluding all previously calculated outliers, and compared across biotypes and rearing conditions.

The stage of development, determined from the posterior spiracles, was analyzed using isomorphen diagrams. The mean minimum time, as ADH, to reach each stage of development was analyzed statistically using a student's t-test by averaging the ADH recorded from each replicate vessel. Minimum time was determined to be when 10% of larvae in a rearing vessel were identified at a given stage of development.

3.4 Results

3.4.1 Growth measured as larval length

Biotype comparison within a trial condition

When reared under the natural summer conditions of Oshawa, Ontario (Trial 1), there was no significant difference detected in the rate-of-change of larval length between Beringia and Great Lakes biotypes over the linear portions of replicated growth curves (Figure 14) using a student's t-test for independent populations (Table 6). This was confirmed using ANCOVA, where biotype was the independent variable. From ANCOVA the measure of association was calculated to be 0.000174 (Table 6), indicating that biotype accounted for approximately 0.0174% of the total variance in change of larval length when controlling for the effect of ADH.

Table 6. T-test and ANCOVA statistical values, comparing rate-of-change in larval lengths of Beringia and Great Lakes biotypes reared under natural summer conditions in Oshawa, ON.

T-test		
Variable	**Value**	**Conclusion**
$t_{calculated}$	1.441	Do not reject H_o ($\mu_1 = \mu_2$), insufficient evidence that population means are not equal
$t_{df=4}$	2.776	
ANCOVA: Homogeneity-of-regression assumption		
F(1,403)	2.508	Interaction between covariate and independent variable is not significant
p	0.114	
ANCOVA: Between-Subject Effects, Independent Variable		
F(1,404)	2.645	Insufficient evidence to conclude the population means are not equal
p	0.105	
Measure of Association		
ω^2	0.000174	0.0174% of total variance in dependent variable is attributed to the independent variable

*α=0.05, all tests

†ANCOVA: dependent variable = larval length; independent variable = biotype; covariate = ADH.

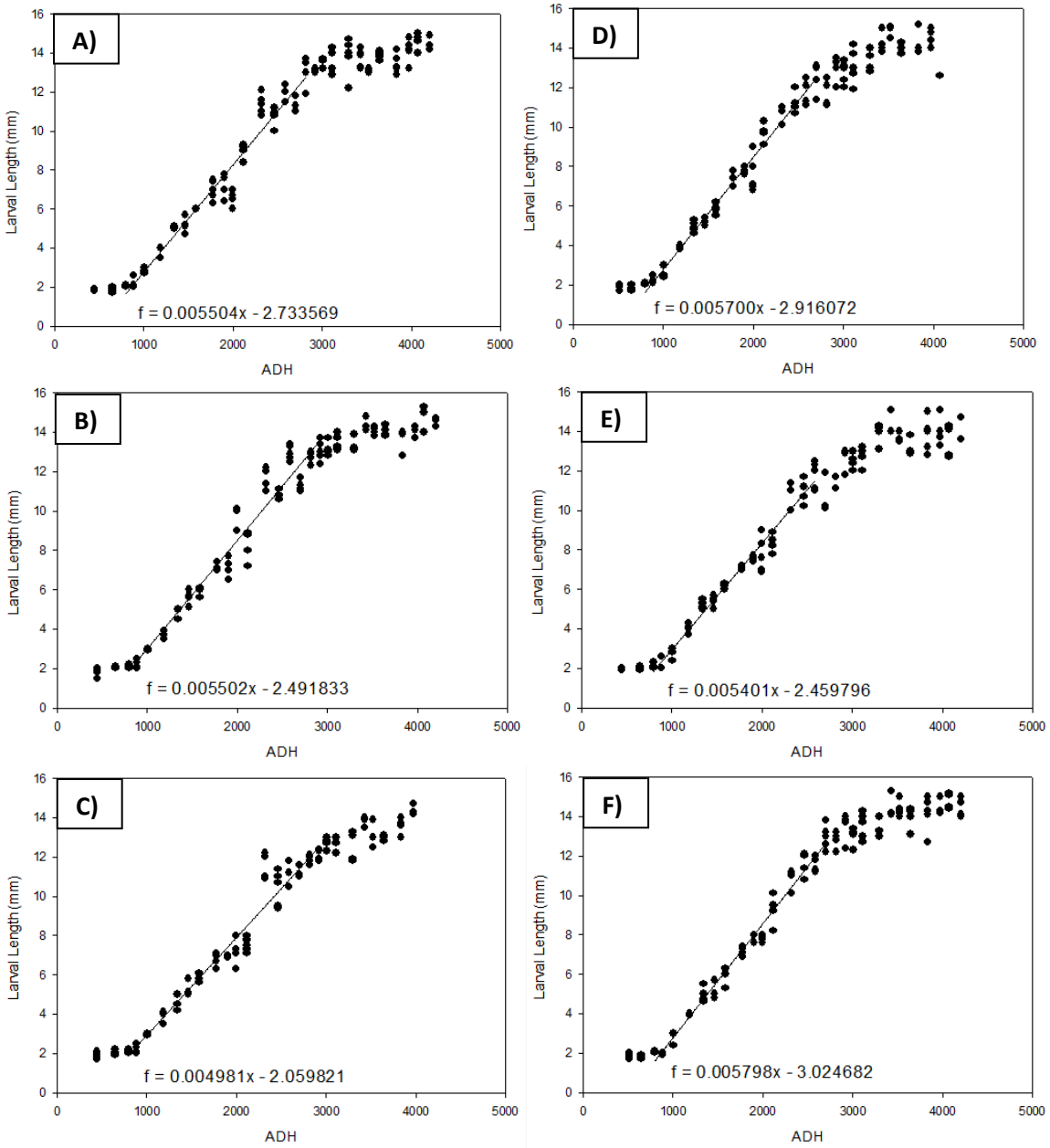

Figure 14. Growth curves, including linear regression, showing larval length as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia and Great Lakes biotypes reared under natural summer conditions of Oshawa, ON. A-C) O-1 – O-3 (respectively), D-F) W-1 – W-3 (respectively). O = Oshawa; W = Whitehorse.

Only one constant temperature trial was run using both biotypes, at 10°C. Comparison under these conditions included comparison of both biotypes in the repeated trial as well as comparing the Beringia biotype growth rates collected from the initial 10°C trial (Trial 4) with the Great Lakes biotype growth rates from the repeated trial

(Trial 5). Under low temperatures, most larval growth followed a linear pattern throughout (Figure 15, 16); in this case all growth data was used for computing linear regressions. Complete development was not achieved in either trial, for either biotype, as all larvae in experimental replicates died before reaching pupation, and only larvae of the Beringia biotype reared in Trial 5 reached 3rd instar. Therefore statistical results should be interpreted with caution.

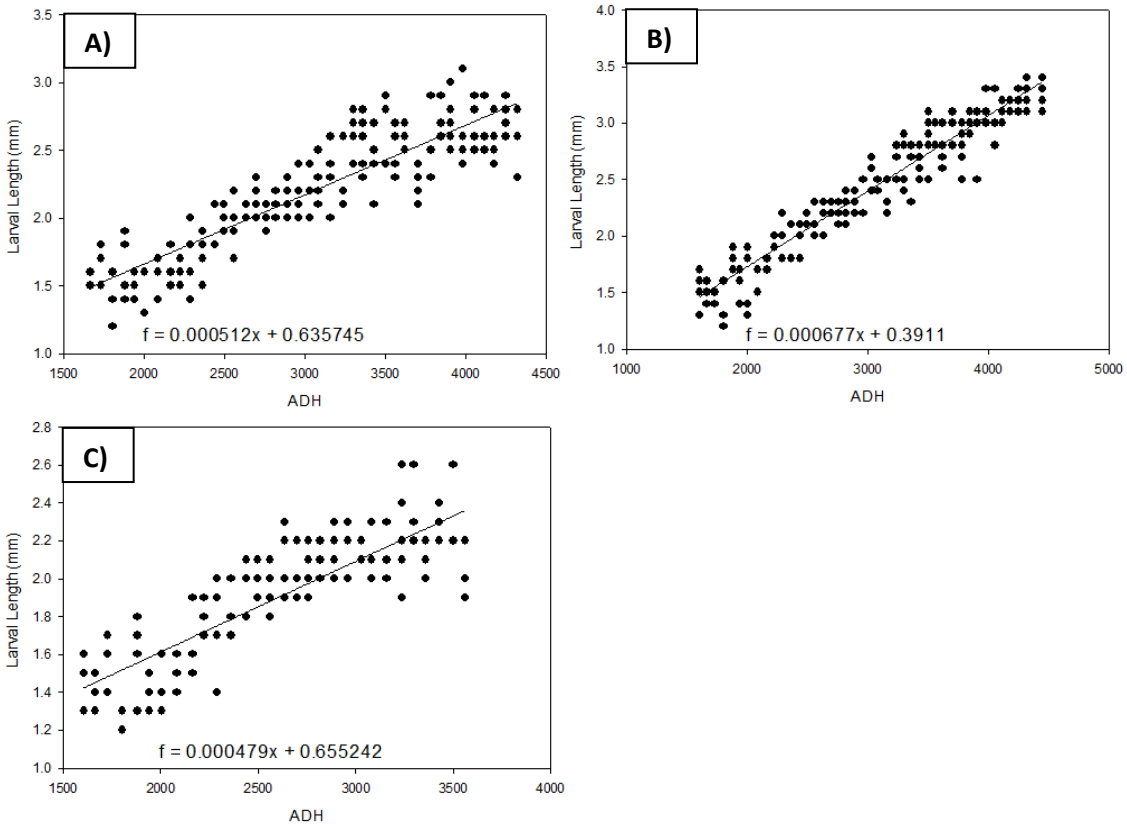

Figure 15. Growth curves, including linear regression, showing larval length as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia biotype reared under constant 10°C conditions. A-C) W-1 – W-3, respectively. W = Whitehorse.

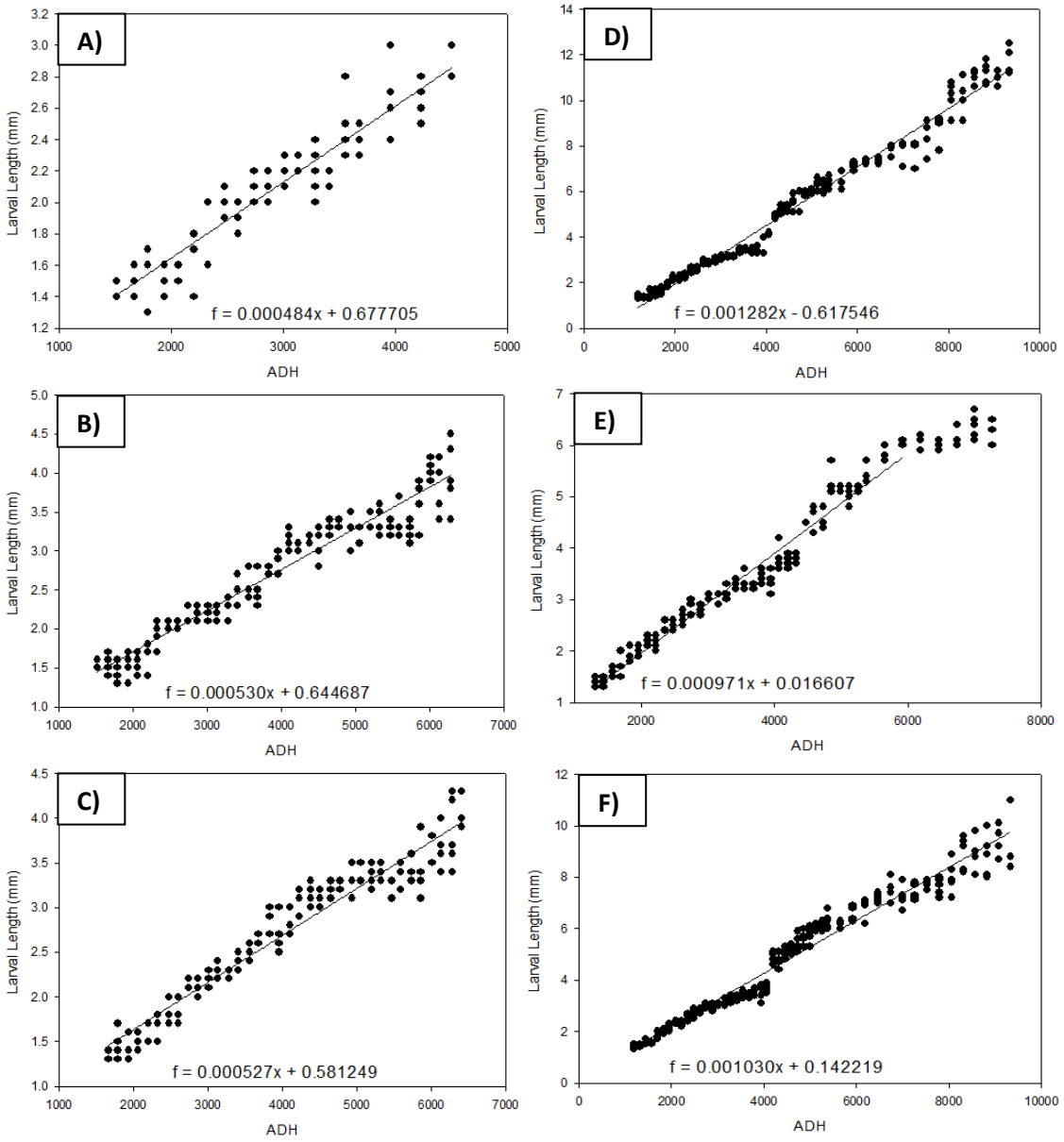

Figure 16. Growth curves, including linear regression, showing larval length as a function of accumulated degree hours (ADH) for each replicate vessel of Beringia and Great Lakes biotypes reared simultaneously under constant 10°C conditions. A-C) O-2 – O-4 (respectively), D-F) W-2 – W-4 (respectively). O = Oshawa; W = Whitehorse.

When compared within the same trial (replicates reared in the same incubating chamber simultaneously), a statistical difference was detected in the change in larval lengths between biotypes using a student's t-test (Table 7). However, when biotypes were compared across trials there was no significant difference detected (Table 7).

Neither result was confirmed using ANCOVA as the interaction between the covariate and the independent variable was significant in both cases. This interaction made it impossible to distinguish the individual effects of either variable, rendering any ANCOVA results meaningless.

Table 7. T-test and ANCOVA statistical values comparing rate-of-change in larval lengths of Beringia and Great Lakes biotypes reared under constant 10°C.

Biotypes compared under simultaneous conditions		
T-test		
$t_{calculated}$	6.016	Reject H_o
$t_{df=3}$	3.182	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,1011)	986.445	Interaction between covariate and independent
p	< 0.05	variable is significant
Biotypes compared across 10°C condition		
T-test		
$t_{calculated}$	0.6717	Do not reject H_o
$t_{df=4}$	2.776	($\mu_1 = \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,906)	24.343	Interaction between covariate and independent
p	< 0.05	variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval length; independent variable = biotype; covariate = ADH.

Single biotype growth rates compared between trials

Using a student's t-test, no statistical difference was detected in the rate-of-change in larval lengths of the Beringia biotype when reared under natural Oshawa summer conditions (daily average temperature: 21.6 ± 2.40°C) (Figure 17) compared with constant 30°C (Table 8). The same test did detect a significant difference in the change in larval lengths of this biotype when reared under constant 30°C versus 10°C conditions (Table 8). Neither of these results was confirmed using ANCOVA as there was a significant interaction between the covariate and independent variable (Table 8).

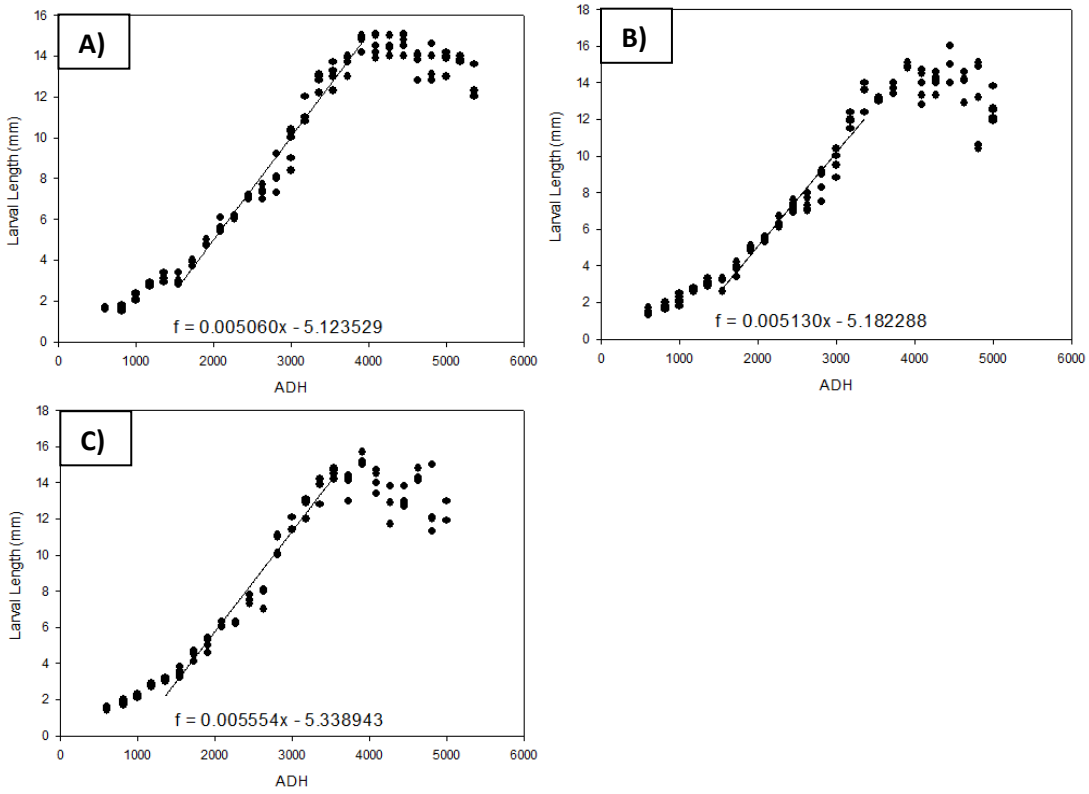

Figure 17. Growth curves, including linear regression, showing larval length as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia biotype reared under constant 30°C conditions. A-C) W-1 – W-3, respectively. W = Whitehorse.

Table 8. T-test and ANCOVA statistical values, comparing rate-of-change in larval lengths of the Beringia biotype.

Natural conditions vs. constant 30°C		
T-test		
$t_{calculated}$	1.973	Do not reject H_o
$t_{df=4}$	2.776	($\mu_1 = \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,350)	21.286	Interaction between covariate and
p	< 0.05	independent variable is significant
Constant temperature comparisons		
T-test: Constant 30°C vs. 10°C (Trial 4)		
$t_{calculated}$	28.26	Reject H_o
$t_{df=4}$	2.776	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,656)	28.26	Interaction between covariate and
p	< 0.05	independent variable is significant
T-test: Constant 30°C vs. 10°C (Trial 5)		
$t_{calculated}$	22.90	Reject H_o
$t_{df=4}$	2.776	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,761)	2153.184	Interaction between covariate and
p	< 0.05	independent variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval length; independent variable = rearing conditions; covariate = ADH.

A statistical difference was detected, using a student's t-test, in the change in larval lengths of the Great Lakes biotype when reared under natural Oshawa summer conditions compared with a constant 10°C (Table 9). This was not confirmed using ANCOVA as there was a significant interaction between the covariate and independent variable (Table 9).

Table 9. T-test and ANCOVA statistical values, comparing rate-of-change in larval lengths of the Great Lakes biotype.

T-test		
$t_{calculated}$	27.57	Reject H_o
$t_{df=3}$	3.182	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,617)	7255.099	Interaction between covariate and
p	< 0.05	independent variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval length; independent variable = rearing condition; covariate = ADH.

3.4.2 Growth measured as larval weight

Biotype comparison within a trial condition

When reared under the natural summer conditions of Oshawa, Ontario, there was no statistical difference detected in rate-of-change in the weight of larvae between the Beringia and Great Lakes biotypes over the linear portions of replicated growth curves using a student's t-test for independent populations (Figure 18, Table 10). This was not confirmed using ANCOVA as there was a significant interaction between the covariate and independent variable (Table 10).

Table 10. T-test and ANCOVA statistical values, comparing rate-of-change in larval weights of Beringia and Great Lakes biotypes reared under natural summer conditions in Oshawa, Ontario.

T-test		
$t_{calculated}$	1.254	Do not reject H_o
$t_{df=4}$	2.776	($\mu_1 = \mu_2$)
ANCOVA: Homogeneity-of-regression		
F(1,346)	23.682	Interaction between covariate and
p	< 0.05	independent variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval weight; independent variable = biotype; covariate = ADH.

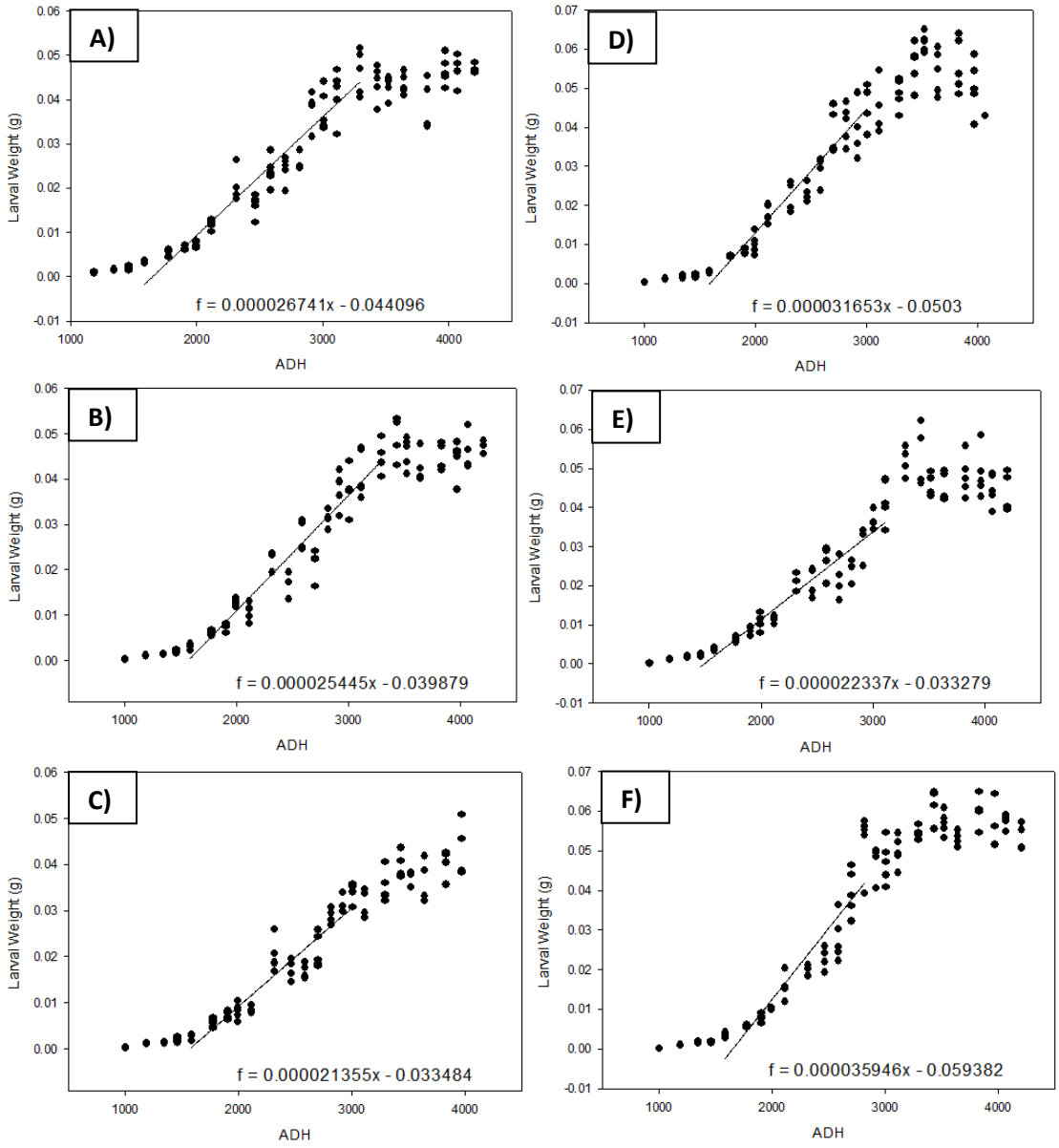

Figure 18. Growth curves, including linear regression, showing larval weight as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia and Great Lakes biotypes reared under natural summer conditions of Oshawa, Ontario. A-C) O-1 – O-3 (respectively), D-F) W-1 – W-3 (respectively). O = Oshawa; W = Whitehorse.

Weight data collected under constant 10°C conditions was compared within Trial 5 only (Figure 19). When compared under simultaneous rearing conditions, there was no statistical difference detected between biotypes using a student's t-test, but this was not confirmed using ANCOVA due to a significant interaction between the covariate and

independent variable (Table 11). Analysis was not conducted with Beringia replicates reared in Trial 4 as the weight data collected was sporadic and did not display any recognizable pattern with ADH. As such, it was not possible to accurately distinguish any linear growth for regression analyses. All weight data collected from the Great Lakes biotype was used in regression analysis as all growth followed a linear trend. Weight data collected from the Beringia biotype more closely followed the S-shape typical of growth curves, displaying a curvi-linear portion at both the upper and lower tails with a linear relationship through the middle. Therefore, only the linear growth portion of the Beringia biotype replicates was used for regression analysis. Only two replicate vessels of the Great Lakes biotype reared under constant 10°C were used to calculate the mean growth as only sporadic weight data was collected from the third before all larvae died.

Table 11. T-test and ANCOVA statistical values comparing rate-of-change in larval weights of Beringia and Great Lakes biotypes reared under constant 10°C.

T-test		
$t_{calculated}$	2.441	Do not reject H_o ($\mu_1 = \mu_2$)
$t_{df=3}$	3.182	
ANCOVA: Homogeneity-of-regression		
F(1,566)	226.394	Interaction between covariate and independent variable is significant
p	< 0.05	

*α=0.05, all tests
†ANCOVA: dependent variable = larval weight; independent variable = biotype; covariate = ADH.

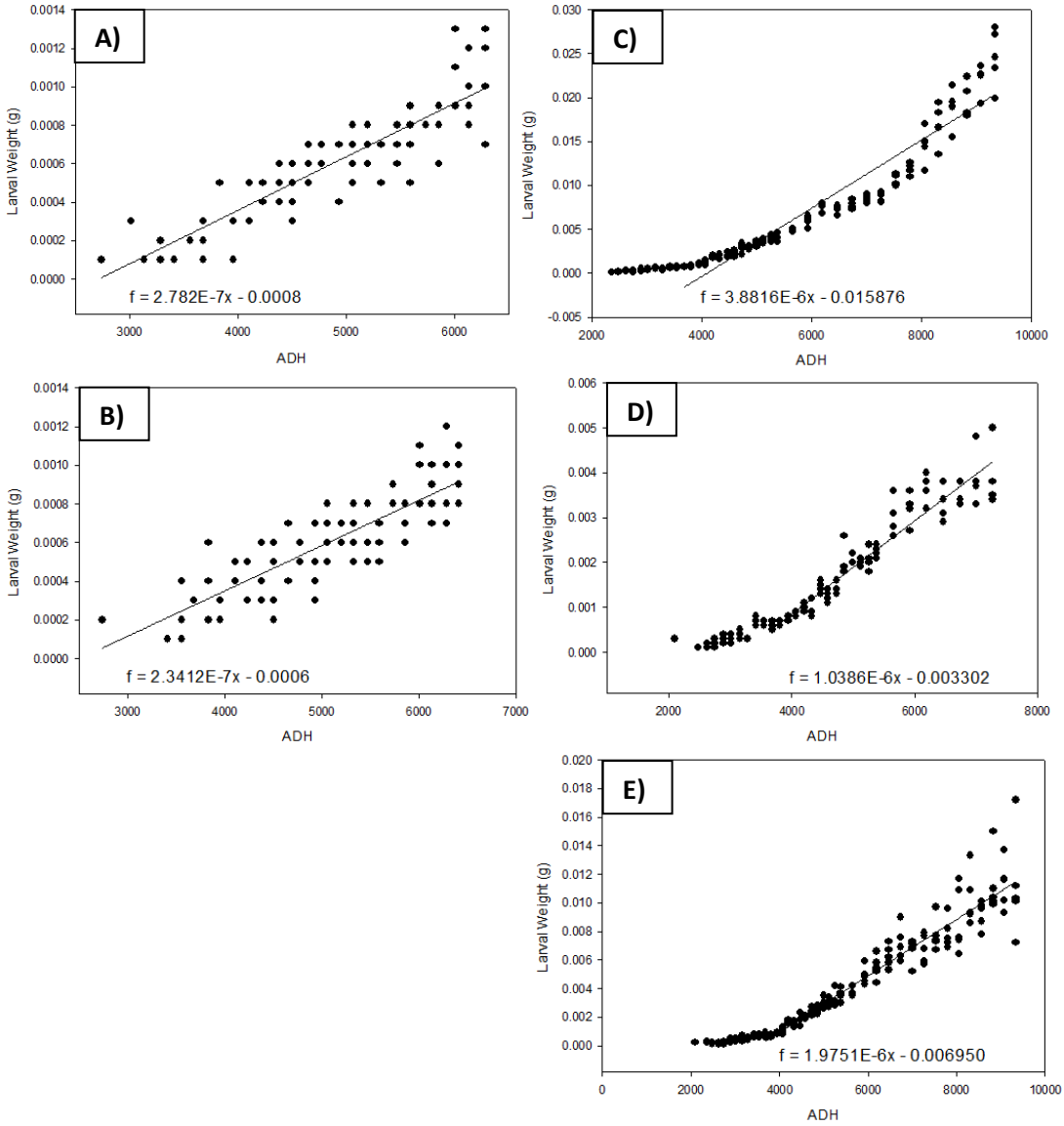

Figure 19. Growth curves, including linear regression, showing larval weight as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia and Great Lakesbiotypes reared simultaneously under constant 10°C conditions. A-B) O-3 – O-4, C-E) W-2 – W-4 (respectively). O = Oshawa; W = Whitehorse.

Single biotype growth rates compared between trials

Statistical comparison within the Beringia biotype was not conducted against data collected during Trial 4 as there was insufficient data obtained for regression analysis. T-test comparison within the biotype showed a significant difference in growth rates between rearing under natural summer condition (daily average temperature: 21.6 ±

2.40°C) and constant 30°C (Figure 21), as well as between constant 30°C and constant 10°C condition (Table 12). Neither was confirmed using ANCOVA as there was a significant interaction between the covariate and independent variable.

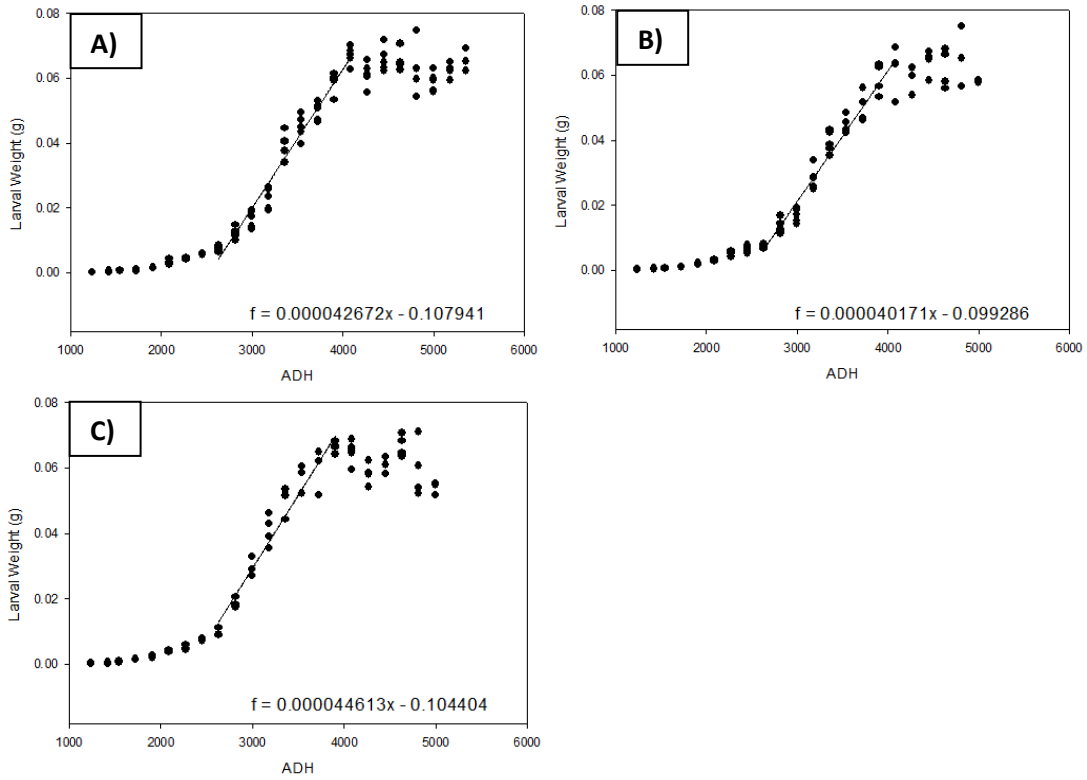

Figure 20. Growth curves, including linear regression, showing larval weight as a function of accumulated degree hours (ADH) for each replicate vessel of the Beringia biotype reared under constant 30°C conditions. A-C) W-1 – W-3, respectively. W = Whitehorse.

Table 12 T-test and ANCOVA statistical values, comparing rate-of-change in larval weights of the Beringia biotype.

Natural conditions vs. constant 30°C		
T-test		
$t_{calculated}$	2.872	Reject H_o
$t_{df=4}$	2.776	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,275)	48.201	Interaction between covariate and
p	< 0.05	independent variable is significant
Constant temperature comparison		
T-test		
$t_{calculated}$	26.20	Reject H_o
$t_{df=4}$	2.776	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,474)	2754.865	Interaction between covariate and
p	< 0.05	independent variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval length; independent variable = rearing conditions; covariate = ADH.

A statistical difference in larval weights was detected within the Great Lakes biotype between natural conditions and constant 10°C, using a student's t-test of independent populations (Table 13). This result was not confirmed using ANCOVA as there was a significant interaction between the covariate and independent variable (Table 13).

Table 13. T-test and ANCOVA statistical values, comparing rate-of-change in larval weights of the Great Lakes biotype.

T-test		
$t_{calculated}$	13.65	Reject H_o
$t_{df=3}$	3.182	($\mu_1 \neq \mu_2$)
ANCOVA: Homogeneity-of-regression assumption		
F(1,397)	2505.288	Interaction between covariate and
p	< 0.05	independent variable is significant

*α=0.05, all tests
†ANCOVA: dependent variable = larval length; independent variable = rearing conditions; covariate = ADH.

3.4.3 Length/weight regressions

There was a great amount of overlap of length/weight regressions, both between biotypes within a trial condition and between trial conditions within a single biotype (Figure 21). Length/weight ratios from natural conditions and constant 30°C showed a greater linearity than at 10°C. Data from 10°C tended to show a more broad range of length/weight ratios at the lower end, though these narrowed as both lengths and weights increased (Figure 21). The regression of Beringia biotype data collected in Trial 5 showed the greatest linear similarity to natural and constant 30°C conditions as larval lengths and weights reached the greatest values of any 10°C measurements.

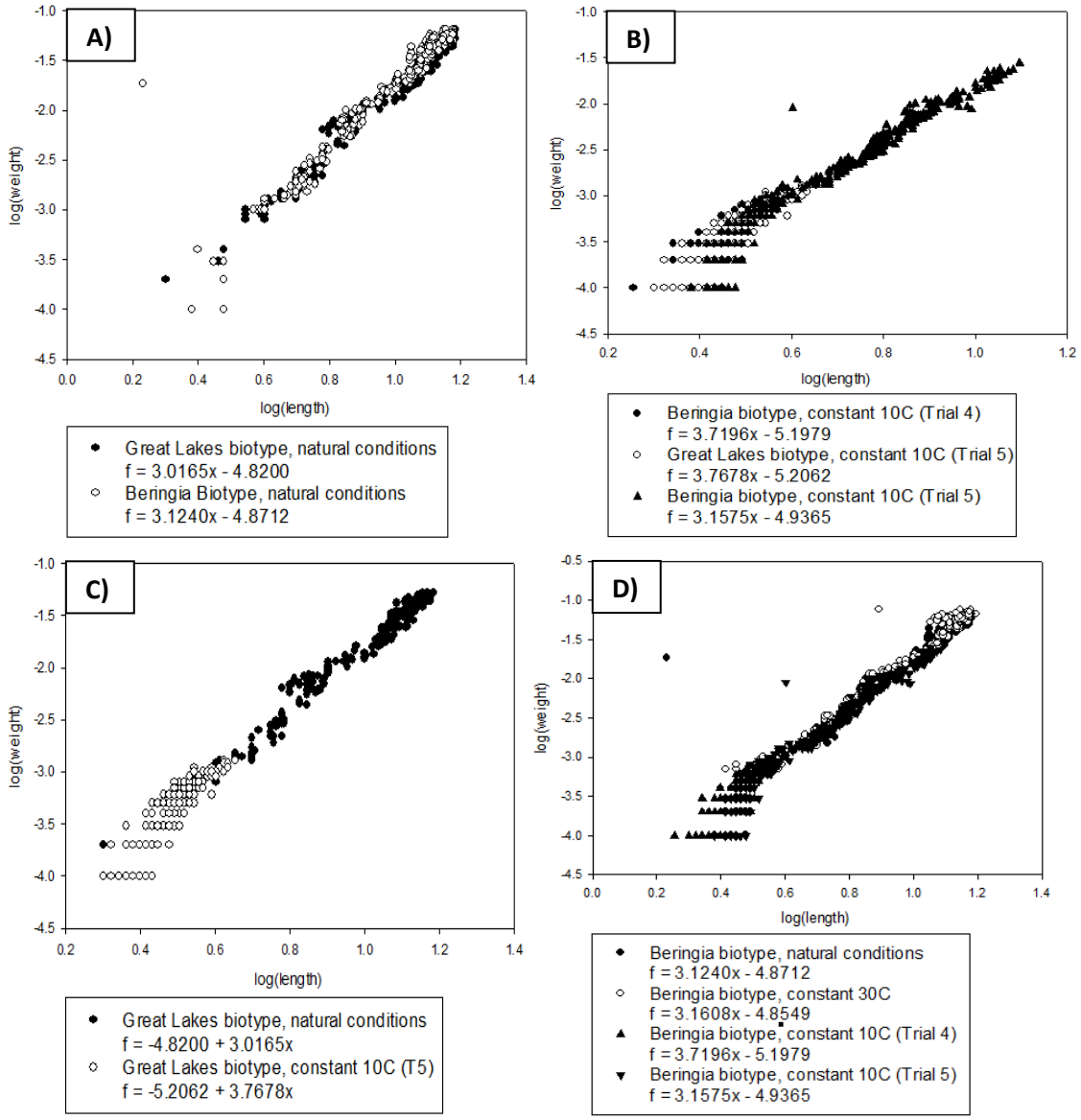

Figure 21. Length/weight regressions (logarithmic transformed). A) Both biotypes within natural summer conditions (Trial 1); B) Both biotypes within constant 10°C conditions (Trial 4, Trial 5); C) Great Lakes biotype; D) Beringia biotype.

3.4.4 Stage development and isomorphen growth

Mean development times to each major stage for both biotypes under all trial conditions are shown in Figure 23. Statistical analysis by student's t-test was conducted on development times between biotypes (within a trial condition) and between trials

(within a biotype) where stage identifications of replicate vessels were different and allowed for mean and standard deviation calculations.

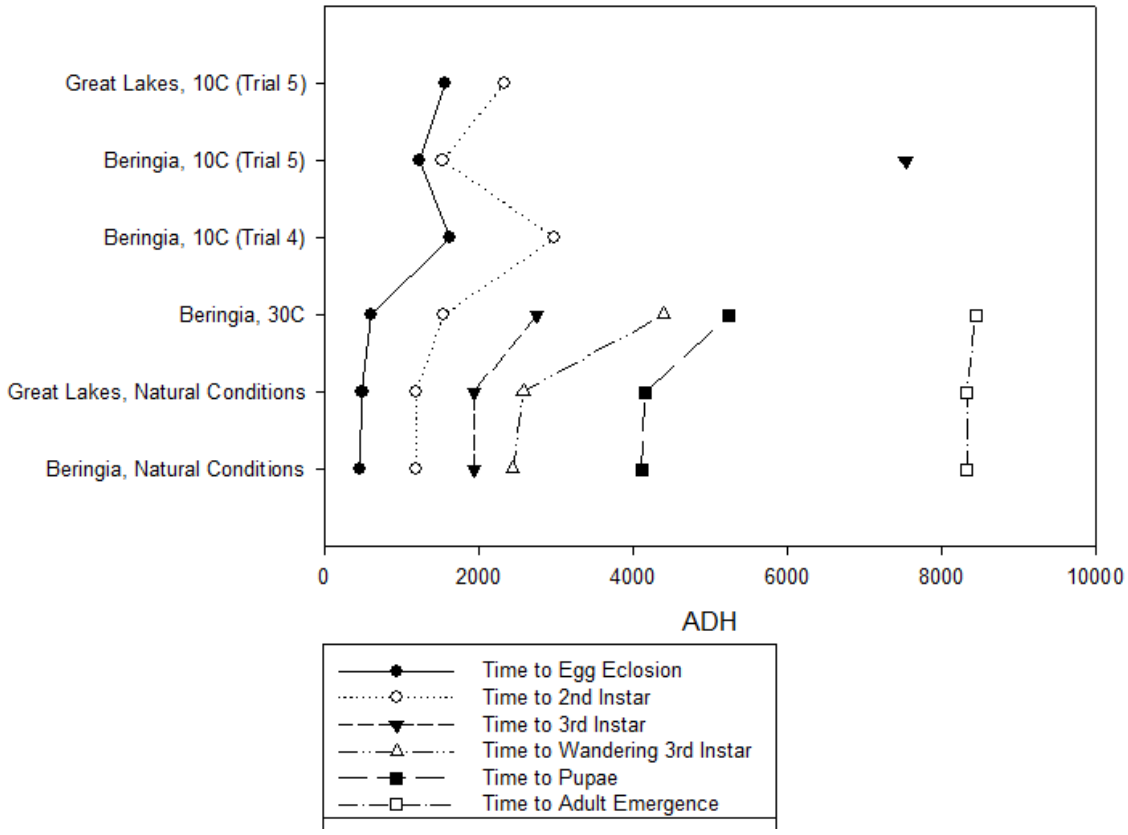

Figure 22. Isomorphen diagram of mean required accumulated degree hours (ADH) to reach each major development stage in each biotype for each rearing condition.

Under natural conditions, there was no statistical difference detected between biotype in growth time to any major stage of development. Under constant 10°C, there was a statistical difference calculated between biotypes to egg eclosion and 2nd instar when replicates were reared simultaneously (Table 14). When compared across trials within the constant 10°C condition there was no statistical difference between the Great Lakes biotype and the previously tested Beringia replicates in development time to egg eclosion. There was a significant difference determined between biotypes for development to 2nd instar, as well as within the Beringia biotype (between trials) to both

stages (Table 14). No analysis was conducted between biotypes reared at constant 10°C for development to 3rd instar as this stage was only reached by Beringia larvae reared in Trial 5.

Within the Beringia biotype, a statistical difference was determined for development to the 3rd instar, wandering 3rd instar and pupal stages between natural summer and constant 30°C conditions (Table 14). However, no difference was detected to the adult stage. When comparing constant 30°C and 10°C conditions, development at the cooler temperature required a much greater ADH to reach a given development stage than at 30°C (Figure 22). There was one exception to this trend: the required ADH for development to the 2nd instar at 10°C in Trial 5 was nearly identical to the required ADH for the same stage at 30°C (Table 14). Within the Great Lakes biotype development at 10°C also required a much greater ADH than determined for natural conditions (Table 14).

Table 14. T-test statistical values comparing mean accumulated degree hours (ADH) required for growth to major development stages.

Biotype	Trial Condition	Development Stage	Test Statistic (t)
Beringia and Great Lakes	10°C (Trial 5)	Egg Eclosion	5.031
Beringia and Great Lakes	10°C (Trial 5)	2nd Instar	8.918
Beringia and Great Lakes	10°C (Trial 4, Trial 5)	Egg Eclosion	1.107
Beringia and Great Lakes	10°C (Trial 4, Trial 5)	2nd Instar	7.124
Beringia	10°C (Trial 4, Trial 5)	Egg Eclosion	8.281
Beringia	10°C (Trial 4, Trial 5)	2nd Instar	22.32
Beringia	Natural vs. 30°C	3rd Instar	11.76
Beringia	Natural vs. 30°C	Wandering 3rd Instar	8.395
Beringia	Natural vs. 30°C	Pupal	8.347

*$t_{df=4}$=2.776, α=0.05.

Larvae were staged by the number of slits observed in the posterior spiracles at the time of sampling. It is possible to stage larvae in this manner as at each moult between larval instars an additional slit is gained within the spiracle, and the number of slits present matches the development stage (1^{st} instar = 1 slit, 2^{nd} instar = 2 slits, 3^{rd} instar = 3 slits) (Gennard, 2007). In normal development the size of the posterior spiracles is also proportional to the size of the larva, and the largest size difference occurs after the moult between 2^{nd} and 3^{rd} instar (personal observation). During the repeated 10°C trial (Trial 5), larvae from both biotypes displayed a unique development during the second instar stage. Spiracles and the slits within them were small at the beginning of the trial, proportional to the size of the larvae. After moulting from 1^{st} to 2^{nd} instar, it appeared that a larger set of spiracles was forming beneath the surface pair. When surfaced, these larger spiracles also contained only two slits (Figure 23). As larvae were beginning to moult into 3^{rd} instar, it was also observed that some larvae sampled from the Beringia replicates possessed spiracles with an unequal number of slits (Figure 24). This was observed in two replicate vessels, and occurred in up to three larvae of each rearing vessel sample set once the third instar stage was reached (sample set = 5 larvae).

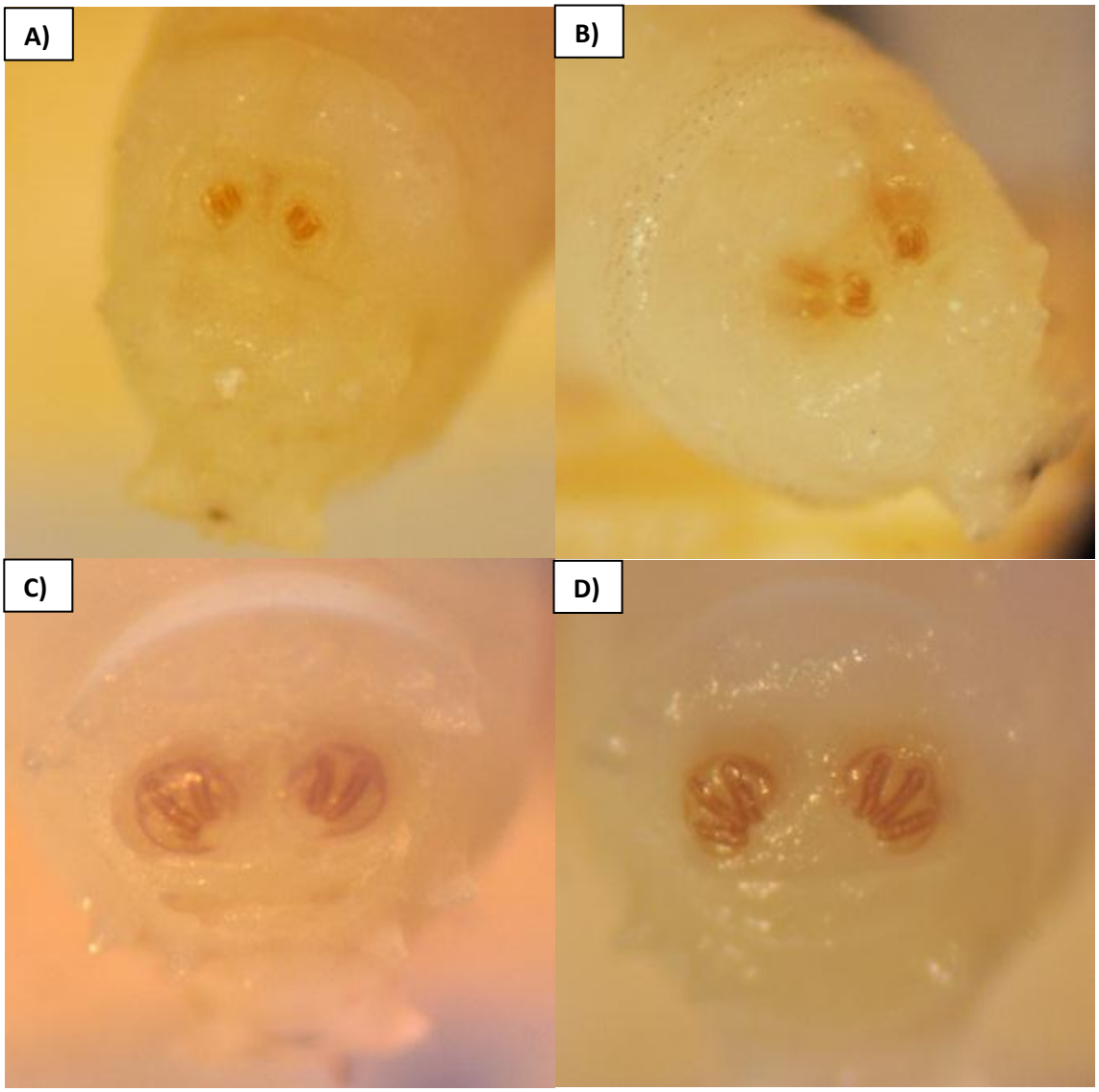

Figure 23. Progression of posterior spiracle development in Beringia larvae reared under constant 10°C (Trial 5). A) 2nd instar larva with small spiracles; B) 2nd instar larva with larger spiracles apparent beneath cuticle; C) 2nd instar larva with large spiracles; D) 3rd instar larva with large spiracles.

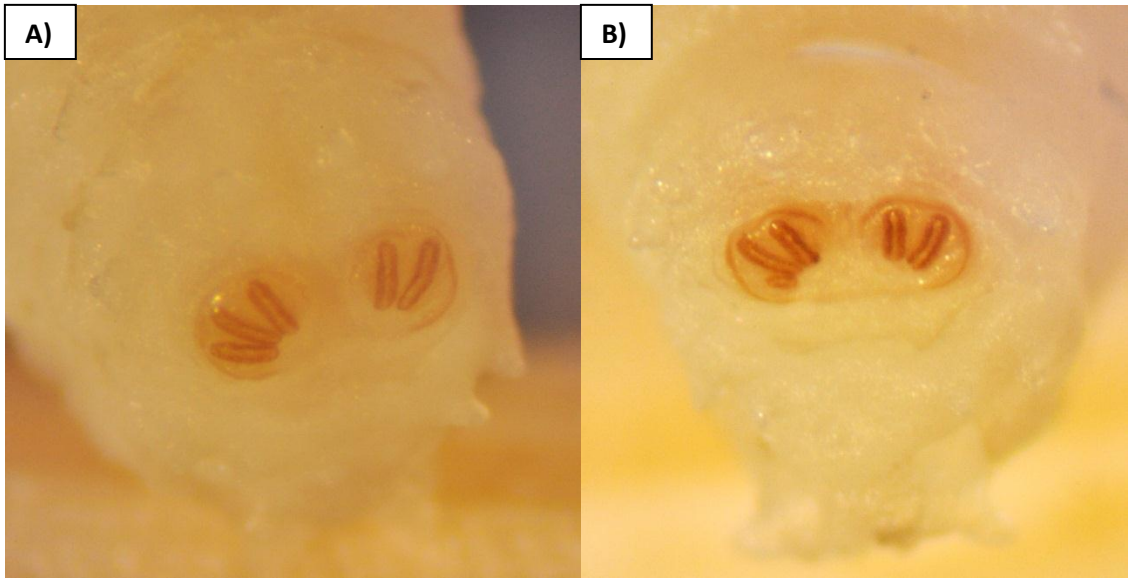

Figure 24. Unequal slits in posterior spiracles of Beringia larvae reared under constant 10°C (Trial 5). A) Larva displaying 3 slits in one posterior spiracle, 2 slits in the other; B) Larva displaying 2 slits in one posterior spiracle, 2 complete slits and a third, smaller slit in the other.

3.5 Discussion

3.5.1 Rearing difficulties

Extreme difficulties were experienced in rearing and maintaining laboratory parent colonies, resulting in delays between development trials. As adults within the colonies failed to oviposit over long periods of time, adult numbers within parent colonies were significantly reduced. This in turn resulted in significantly fewer sexually mature adults available to oviposit and as a result eggs in sufficient numbers for research were not obtained for many weeks. A complete list of methods used to induce oviposition, as well as their success, can be found in Appendix C.

Because of the complete cessation in oviposition by parent colonies of both biotypes, maintained at laboratory conditions, it was hypothesized the adult stages may have entered a period of diapause. Diapause is a seasonal adaptation (Danilevskii, 1965) designed to overcome adverse or extreme environmental conditions in particular climate

zones (Mansingh, 1971). As a Holarctic species, *Protophormia terraenovae* is known to overwinter in the adult stage, in what is known as adult reproductive diapause (Vinogradova, 1987). Differences have been noted in the stability and long-term inhibition of reproduction between biotypes (Vinogradova, 1987), although both of the biotypes reared for this research were observed to undergo reproductive inhibition. Key changes in the rearing environments at the time inhibition was first noted may have contributed to the induction of diapause.

Reproductive inhibition was first noted after parent colonies were moved between research buildings at the beginning of September, 2011. Though this time of year is still generally warm, the day was cool and there was a significant difference in indoor and outdoor temperatures at the time of moving. Temperature is known to play a role in the induction of diapause in *P. terraenovae*, even as high as 20°C (Vinogradova, 1986). Vinogradova (1986) found that diapause terminated naturally in 20% of the laboratory colony within four months, and could be terminated early by increasing the ambient temperature. However, as laboratory temperature was externally controlled it was not possible to significantly increase ambient temperature conditions during the course of this research.

Another environmental change experienced by laboratory colonies which may have assisted in the induction of reproductive diapause was the shortening of the natural daily photoperiod through the fall season. Multiple authors have found that changes in photoperiod, in correlation with rearing temperature, can induce diapause in *P. terraenovae* (Greenberg & Stoffolano, 1977; Numata & Shiga, 1995; Shiga & Numata, 1997), and that even typical long-day photoperiods cannot guarantee prevention (Shiga &

Numata, 1997). However, Shiga & Numata (1997) found that the incidence of diapause could be reduced to below 24% when colonies were kept under 24-hour photoperiod conditions. As light intensity was not found to cause variation in diapause prevention (Shiga & Numata, 1997), each parent colony was provided with 24-hour artificial light for the duration of the research period. While difficulties were still incurred in obtaining sufficient eggs for research purposes, oviposition was found to resume within days of increasing the ambient photoperiod (personal observation).

3.5.2 Comparison of biotypes

Only two trials were conducted where growth and development of both target biotypes of *Protophormia terraenovae* were able to be compared. Under natural, fluctuating summer conditions in Oshawa, Ontario, there was no significant difference in the rate-of-change of either larval length or weight between biotypes, and ANCOVA analysis determined the biotype was responsible for <1% of the total variation in growth measured as larval length. Minimum times (ADH) to each major development stage, determined as 10% of larvae, were also not statistically different. Length/weight regressions of both biotypes showed significant overlap, also indicating similar growth ratios between biotypes.

Development of *P. terraenovae* under fluctuating conditions has been studied previously by Davies & Ratcliffe (1994), Clarkson *et al.* (2005) and Warren (2006), though these studies tested a single population of the species and did not directly compare between biotypes. Though contradictory, all studies found significant differences in growth rates between fluctuating temperatures and constant temperature trials, suggesting that applying growth rates obtained under constant laboratory conditions may not be

accurate for PMI estimations if applied to larvae previously developed in the more natural environments of most crime scenes. Given this, the next question to be addressed is whether there is intraspecies variation, and whether development data obtained for one population can be accurately applied to another for fluctuating temperature development.

Despite extreme differences in the originating geographic regions, there is statistically no difference in growth rate between the Beringia and Great Lakes biotypes of *P. terraenovae* when reared under the same natural conditions. However, the minimum development times (ADH) obtained in this research were found to be significantly faster (α=0.05) than those presented for the same species under natural fluctuating conditions in British Columbia for all major development stages (overall mean temperatures: 21.6°C ± 2.40°C, 19.7°C ± 0.1°C, respectively) (Clarkson *et al.*, 2005). Both biotypes tested here also resulted in significantly faster rates of development to all major stages when compared with insects from British Columiba reared under chamber-controlled fluctuating regimes (overall mean temperature: 16°C). (Warren, 2006). Given daily temperature differences, this result suggests that variation in development rate under natural fluctuating regimes is more greatly affected by environmental conditions (which may include temperature, relative humidity, photoperiod, etc.) than by biotype. Warren (2006) noted that, due to the rate summation effect, larvae may develop faster when fluctuating temperatures are climbing from a lower temperature to a much greater temperature. This would in part explain the significant differences stated above, as the overall temperature range experienced by Beringia and Great Lakes larvae was much greater than either study in British Columbia.

Contrary to natural condition results, larvae reared simultaneously at a constant 10°C showed statistically significant differences in growth rates for length and weight measurements, as well as the minimum ADH required to reach each identified stage of development. In both cases, growth rates of the Great Lakes biotype were slower than the Beringia biotype. However, when comparing rates-of-change of larval length between constant 10°C trials, the Beringia larvae reared in Trial 4 and the Great Lakes larvae from Trial 5 were not statistically different, though the Beringia larval lengths between trials were. Differences within the Beringia biotype between trials could be an effect of low temperatures, as the set-condition was approaching the minimum development threshold as proposed by Grassberger & Reiter (2002). Despite differences in growth rates, length/weight regressions between biotypes at 10°C showed significant overlap and similar slopes. This suggests that the ratio between individual larval length and weight remains constant, regardless of growth rate differences.

Both the Beringia and Great Lakes biotypes spent a much longer period of time (hours) in the egg stage than that reported by Greenberg & Tantawi (1993) for specimens reared at 12.5°C, though as 10°C the duration of the first instar stage was up to 10 times shorter than at 12.5°C. Further comparison was not achieved against any Great Lakes samples as all larvae died before reaching 3rd instar. Comparison with Beringia larvae reared in Trial 5 showed a 2nd instar duration of over twice as long as reported for 12.5°C by Greenberg & Tantawi (1993) (544 ± 34 hours, compared to 240.0 at 12.5°C). Duration of the egg stage at 10°C was up to twice as long as that determined at 15°C (Grassberger & Reiter, 2002). Marchenko (2001) presents development data conducted

at 11°C, though gives only the required time period (days) to develop from egg to puparium and imago. Neither of these stages was reached by either biotype at 10°C.

Development data collected nearest to the set-constant temperature 10°C is from Warren (2006), who looked at development at 9.8°C (published minimum development threshold). Comparison with this data revealed inconsistencies in development time variations between biotypes. Beringia replicates from Trial 4 and Great Lakes replicates from Trial 5 took longer to reach minimum egg eclosion (10%) than reported from British Columbia, but Beringia replicates from Trial 5 showed a minimum development time which was not significantly different from that presented by Warren (2006). Conversely, British Columbia minimum development times to the 2nd instar (Warren, 2006) were significantly slower than either biotype studied simultaneously. However, Beringia replicates from Trial 4 showed no significant difference in development to this stage when compared with Warren's minimum requirements. Only two Beringia replicates in the repeated condition reached third instar, but took a much longer period (28.7 ± 1.41 days) than the 21.0 days presented by Warren (2006).

The proposed minimum development threshold for *P. terraenovae* has been published as low as 7.8°C (Marchenko, 2001). Using regression analysis, Grassberger & Reiter (2002) put forth a minimum threshold for development from oviposition to pupariation as 9.8°C (~10°C), and a threshold for total immature development as 8.9°C (~9°C). However, when reared under a set-constant 10°C (~11.1 ± 1.34°C) neither of the studied biotypes of *P. terraenovae* completed immature development or reached the pupariation stage. This was also found by Warren (2006), who determined that at 11°C *P. terraenovae* would not develop beyond the 3rd instar. Low temperature research

conducted here and by Warren (2006) seems to indicate that larvae tolerate lower temperature better in the earlier stages of development. The implication of these results is that lower development thresholds of blowfly species should be determined experimentally as opposed to mathematically from the regression analysis of development curves determined for high temperature regimes. Grassberger & Reiter (2002) tested five temperatures from 15°C - 35°C inclusive, and fit a regression line with r=0.99 to determine their proposed minimum development threshold. Yet based on current data, this threshold is under-estimated. Warren (2006) also found that linear-regression analysis of experimentally determined growth rates consistently underestimated lower development thresholds. This would suggest that below ~15°C, the relationship between temperature and the rate of development (1/time) becomes less linear.

Few studies have been conducted directly comparing larvae of the same species from geographically distinct regions and none have directly compared distinct populations of *P. terraenovae* reared simultaneously. The prediction that there is intraspecies variation in development rates has been supported for the blowflies *Calliphora vicina* (Hwang & Turner, 2009) and *Lucilia sericata* (Gallagher *et al.*, 2010). From constant 10°C development data collected here it appears that *P. terraenovae* may also follow this trend, though no differences were detected under natural conditions.

3.5.3 Effect of temperature within biotypes

Due to difficulties in obtaining eggs for research trials, only the Beringia biotype was tested at both extreme constant temperature regimes as well as under the natural summer conditions. Within the Beringia biotype all statistical comparisons were

significant except two: rate-of-change of larval lengths showed no statistical differences between the natural and 30°C conditions, and the averaged minimum ADH required for development to the 2nd instar stage was nearly identical between the 30°C and repeated 10°C (Trial 5) trials. As the rate of blowfly development is generally shown to be linear with increased temperature, it is expected that development at a low temperature such as 10°C would proceed at a much slower rate than at the opposite extreme. However, without additional development rates determined for constant conditions within the extremes, the exact relationship between temperature and growth rate is impossible to accurately calculate.

Development of *P. terraenovae* at constant 30°C has previously been studied for biotypes from Leningrad/Lithuania (Marchenko, 2001), Austria (Grassberger & Reiter, 2002), and British Columbia (Warren, 2006). Duration of each stage was also compared to data for a population from eastern Washington, USA reared at 29°C (Greenberg & Tantawi, 1993). In this case the current data showed a significantly longer duration of all immature development stages with the exception of the post-feeding (wandering) 3rd instar, which progressed much more quickly at 30°C, and the pupal stage which was sustained for nearly identical time periods between studies. When compared to European populations at the same temperature overall development from oviposition to adult emergence proceeded at almost the same rates in all cases. Minimum times to egg hatch and pupariation were significantly longer in the Beringia biotype than those reported from Austria (Grassberger & Reiter, 2002), though development to pupariation was identical to rates reported for Leningrad/Lithuania (Marchenko, 2001).

As development rates to each stage of development at 30°C were presented by Warren (2006), a more complete comparison of biotypes was possible between British Columbia and Beringia. Development of Beringia larvae was not seen to be statistically different from British Columbia for minimum time required to egg eclosion, 2nd instar and pupal stages. However, minimum development times were slower in the Beringia biotype to the 3rd instar, post-feeding (wandering) 3rd instar and adult stages. Differences seen may be attributed to how the minimum was calculated in each case, as Warren (2006) determined the minimum development as when a single larva had progressed to the next stage. Minimum development was determined in this research as 10% of sampled larvae, though as the largest larvae were sampled at each period this was often over-achieved.

Despite intermediate development differences, minimum times to both pupariation and adult eclosion for the Beringia biotype under constant 30°C conditions are accurately calculated, within the experimentally determined standard deviation, using linear regressions determined by Grassberger & Reiter (2002). This supports the accuracy of the linear relationship between rate of larval development and (constant) ambient temperature, though it brings into question the significance of development differences between biotypes as these particular regressions were determined using a population from Austria (Grassberger & Reiter, 2002). However, further constant temperature development trials must be conducted to confirm or refute the accuracy of the proposed regressions across biotypes.

Similar to the biotype comparisons, length/weight regressions of the Beringia biotype only showed a considerable amount of overlap between trial conditions. The

exception was the early measurements in the 10°C condition(s), which had a more broad distribution of ratios. These distributions are seen to become narrower as lengths and weights increase, and appear to follow the linear trend seen in warmer trial conditions.

The Great Lakes biotype was only tested under two trial conditions: natural summer conditions and constant 10°C. Between trial conditions there was a significant difference in the rate of change for both larval length and larval weight, with development proceeding much more slowly at 10°C. Minimum ADH required for growth to major development stages was also seen to be much greater at 10°C than under natural conditions. Once again, this is to be expected as 10°C approximates the published minimum development threshold of the species, and the daily average temperature from the natural conditions trial was much higher than 10°C. However, growth rate comparisons against natural climate conditions cannot be accurately quantified from the current research as development was not studied at a constant temperature approximating the average daily mean of the fluctuating condition.

3.5.4 Stage determination under low temperatures

It is well accepted that as blowfly larvae age, at each moult between instars they gain an additional slit within the posterior spiracles that mimics the stage of development (Gennard, 2007). Throughout development trials conducted at higher temperatures (natural conditions, constant 30°C), the spiracles observed in sample larvae followed this accepted progression and grew in size as the larvae grew – therefore, 3rd instar larvae had larger spiracles than 2nd instar and so forth (personal observation).

During the repeated trial at 10°C (Trial 5), two unique phenomena were observed. Within both biotypes, beneath the set of small 2nd instar spiracles a larger set could be

observed beneath the surface of the larval cuticle. In previous trials, this observation was an indication of approaching moult into 3rd instar. However, when moult occurred these larger spiracles also contained only the two slits indicative of 2nd instar. Descriptions of this phenomenon have not been found in the literature, and as this research was looking only at the rate of development and not the underlying physiology and biochemistry it is unknown exactly why this may have occurred. Borror *et al.* (1976) briefly describe the biochemical control of immature growth in terms of three hormones: a brain hormone, a juvenile hormone, and a moulting hormone (ecdysone). According to these authors, the brain hormone signals the prothoracic glands to produce ecdysone, which signals the larvae to grow and moult. The juvenile hormone works to promote larval development and prevent moulting or pupariation (Borror *et al.*, 1976). It is possible that at temperatures approaching and/or approximating the minimum development threshold, the biochemical control of immature growth and development is interrupted. From the observations in this research, it seems likely that while the larva may have been signalled biochemically to moult the low ambient temperature has prevented or reduced the physical ability of the larva to progress through the stages of development.

The second phenomenon was observed in the Beringia biotype only, as no Great Lakes larvae reached the same stage of development at the low temperature. In this phenomenon, the posterior spiracles in larvae approaching the 3rd instar were observed to have unequal numbers of slits between them. Once again, this phenomenon has not been found described in the literature. As the number of slits in the posterior spiracles increases with larval stage of development, it could be another indication of a disruption

in the physiological and/or biochemical growth signalling pathway(s) due to the low ambient rearing temperatures.

In addition to low-temperature effects, the unanticipated spiracle observations may be the result of genetic mutation due to in-breeding and genetic "bottle-necking" caused by severely reduced adult numbers in the parent colonies. Severe adult die-off and reduced numbers occurred in parent colonies of both biotypes before commencement of 10°C trials, which may have introduced genetic mutation with regards to successful larval development. If these phenomena were observed in subsequent research trials conducted at temperatures above 10°C mutation may be a more likely explanation than low ambient temperatures, especially as spiracle abnormalities have not been reported in previous studies conducted at low temperatures. The constant 30°C trial was also conducted after a period of severe parent colony die-off, but no abnormalities were reported. However, as the 10°C conditions were studied after 30°C it is possible that any genetic mutations developed at a later period.

Chapter 4:

Conclusions and Recommendations

4.0 Arthropod Succession

Geographic region has a major effect on insect arrival times and types of species attracted to remains, and it is therefore not recommended to apply insect succession patterns gathered in one region to PMI or PIA estimations in another (Anderson, 2010). Decomposition in the Yukon was slow and did not reach skeletonization, which may be attributed to a much lower daily average temperature than many regions farther south; although remains were exposed to an extreme range of temperatures over 24-hour periods, the daily average temperature of the research site through June, July and early August was a mere 12.5°C. Because the decomposition rate in Whitehorse was much slower, the use of decomposition data from more southern regions would more than likely result in a severe underestimation of the PMI if applied to remains in the Yukon Territory. This is especially true if the data used was from the same season in a region which experiences much greater daily average temperatures.

The majority of arthropod succession research conducted uses unclothed pig carcasses for determining decomposition rates and succession patterns of attracted insects. However, in real-life the majority of decomposing remains encountered by law-enforcement are often clothed or wrapped in some fashion (Dillon & Anderson, 1995). This is especially true in areas such as the Yukon Territory where homicide numbers are comparatively low and remains encountered may frequently be attributed to tourists/hikers/hunters that experienced unfortunate and fatal accidents in the wilderness. Future research including clothed carcasses is therefore required, especially as earlier research comparisons have shown that a greater diversity of insect species are attracted to clothed remains (Dillon & Anderson, 1995; Bygarski & LeBlanc, unpublished). As the

current research was the first of its kind in Canada's north, unclothed carcasses were used for the purposes of creating baseline succession patterns which in future research can be used as comparisons for control carcasses and to determine the stability of the pattern(s) within the region.

A major distinction between arthropod succession in the Yukon Territory and more southern regions was the dominant species of blowfly and beetle identified, as well as the near complete dominance of these species (both adult and larval forms on all carcasses. This, along with the reduced rate of decomposition, confirms the need for local arthropod succession patterns for PMI-estimate applications. Whereas decomposition rates from more southern regions may underestimate the PMI, use of far-removed succession patterns may in-fact over-estimate the PMI of remains in the early stages of decomposition due to the much earlier arrival of the dominant blowfly *Protophormia terraenovae* in the Yukon. It is therefore vital that forensic research be continued in regions such as the Yukon Territory to ensure local law enforcement has access to the most applicable and accurate data for death investigations.

4.1 Development

Despite extreme differences in geographic origin, there were no significant differences detected between the two chosen biotypes of *P. terraenovae* reared in the natural summer conditions of Oshawa, Ontario. This result is limited as it is drawn from a single research trial. The natural conditions are also only applicable to the Great Lakes biotype. Therefore results should be verified both in the same environment as well as under additional fluctuating patterns. Most development research utilizing fluctuating temperature regimes use controlled environments to cycle temperatures between pre-

determined high and low "extremes". While this method may allow for more accurate comparisons of development rates to a constant mean temperature conditions, and allows for control of non-temperature factors (photoperiod, humidity, etc.), it does not necessarily correspond to the natural conditions of a given region. This is highlighted by Warren (2006), who found there were differences in development rate of larvae reared under laboratory-controlled fluctuating conditions when compared to previously published results of larvae from the same parent colony reared outdoors. As most crime scenes involving decomposing remains are outdoors, and not subject to controlled conditions, error may be introduced to PMI estimations if there are significant differences in the development rates between natural fluctuating conditions and laboratory-controlled fluctuations within the local biotype.

Comparison between constant temperatures was only possible within the Beringia biotype, which appears to conform to the accepted relationship between ambient temperature and rate of development. Development at the high-temperature 30°C was significantly faster than at the low-temperature 10°C with the exception of development to the 2nd instar stage in the repeated low-temperature trial (Trial 5). Development in the natural conditions trial (daily average ~21.6°C) also preceded much faster than at 10°C. As the daily average temperature was more than twice the ambient temperature of the low-temperature trial(s), this result is expected according to the accepted linear growth/temperature relationship. However, further research at a constant temperature approximating the daily mean temperature of the fluctuating conditions would be required to confirm development rate differences.

An unexpected result was the decreased rate of development at 30°C when compared to the natural fluctuating conditions, which had a lower mean daily temperature. According to the accepted relationship, development occurs faster with increased ambient temperatures (within extremes). From this it would be expected that development would occur faster at 30°C than around the mean temperature of 21.6°C. This result is likely due to the rate summation effect, whereby the increased rate of development caused by fluctuations above a mean exceeds the decrease in rate caused by fluctuation below the mean (Higley & Haskell, 2010). However, as none of the environmental factors were controlled for in the natural conditions trial it is possible that one or more factors affected the observed development rates.

Previous research by Warren (2006) has questioned the accuracy of published minimum development thresholds for *P. terraenovae*, though results of low-temperature studies were limited by small sample sizes of surviving larvae. Repeated low-temperature trials and use of two additional biotypes has confirmed that previously determined minimum thresholds for both complete development and development from oviposition to puparation are underestimated. Though rates of development to each major stage of development may vary between biotypes and research trials, the most advanced stage of development reached in low-temperature trials was 3rd instar (confirming development determined by Warren (2006)).

Although minimum development thresholds mathematically determined from linear development regressions have been shown to be underestimated, the validity of the linear regression within the given range of 15-35°C has been confirmed. When calculated from the regression equation, development rates at 30°C fell within the

standard deviation of the experimentally-determined rates of the Beringia biotype. However, outside of the experimentally tested range of constant ambient temperatures the validity of the proposed relationship is questioned. Development rates calculated for ambient temperatures outside of this range, at either extreme, should therefore be interpreted with caution.

4.2 Limitations

Development research was significantly limited by adult availability, ability to obtain sufficient eggs from parent colonies, and access to environmentally-controlled chambers. As *P. terraenovae* is a cold-adapted species, the extreme heat experienced in Oshawa, Ontario during the spring and summer of 2011 made it difficult to capture sufficient adult numbers to build a parent colony. The original Beringia colony was started using larvae obtained during succession research in 2010, but due to distance was limited in the ability to introduce new individuals. Few larvae were obtained in the summer of 2011, but due to delays in shipping many specimens died before reaching the laboratory. As few new individuals were introduced to either parent colony during the course of research, it is possible that the apparent lack of developmental differentiation between biotypes may have been in part due to lab adaptation. Research under natural outdoor conditions was conducted within the first three generations of the Great Lakes Region parent colony, reducing the probability of lab-adaptation in this biotype at this time.

Both parent colonies experienced extreme reduction in adult numbers after the natural conditions trial due to significant die-off and cessation of reproduction. This would have resulted in extreme genetic "bottle-necking", which may in turn have led to

genetic mutation through in-breeding. No gross morphological mutations were observed in adults or larvae of either biotype. Lab-adaptation, as a result of reduced population and genetic variation, may have occurred.

Access to incubators was limited due to laboratory space and movement, as well as unforeseen difficulties with the incubators themselves. Originally, the fluctuating conditions trial was to be conducted in an environmentally-controlled chamber owned by the biology department within the Faculty of Science. The day after trial conditions were programmed, this chamber was found to be mal-functioning and was therefore not used for research. Subsequent research was conducted after repairs had been made. A chamber owned by the research laboratory was obtained by February of 2012 and utilized for low-temperature trials.

4.3 Recommendations

From the results and observations of this research, three major recommendations have been identified:

1. Laboratory rearing conditions of *Protophormia terraenovae* should be optimized to avoid future problems of severe colony die-off, reproductive diapause, and inability to obtain eggs for research purposes. Preliminary observations in rearing success have been made and included in Appendix B for future work and expansion.
2. Research into arthropod succession patterns in northern regions should be continued in order to provide the most accurate data for local law enforcement agencies when estimating PMI. Future research should include conditions most often encountered in the region(s), including the use of clothed carcasses and

research in seasons when accidental deaths may be seen to increase (tourist and hunting seasons, etc.).

3. Development research for *Protophormia terraenovae* should be expanded, particularly with regards to the determination of the minimum development threshold and development under natural outdoor conditions. This is particularly significant in areas such as the Yukon Territory, where not only is there an increased probability of remains found outdoors under uncontrolled environmental conditions, but the natural daily temperature fluctuations have been shown to be extreme and the daily average in the summer months approaches the current published minimum threshold.

Chapter 5:

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5.0 References

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Chapter 6:

Appendices

Appendix A:
Succession study field-note template

Master's of Science, Applied Bioscience – UOIT
Whitehorse, Yukon Decomposition Project – Summer 2010

Weather:

O Sunny	O Hot sun	O Rain	
O Clear skies	O Scattered cloud	O Partially cloudy	O Cloudy
O Cold	O Cool	O Warm	O Hot
O Breezy	O Windy	O Humid	

O Other/Notes: _____

Stage of Decomposition:

P1	P2	P3
O	O	O Fresh: Immediately after placement; few changes, no strong smell
O	O	O Bloat: Body distended, skin marbling, many flies, smell
O	O	O Active Decay: Body deflated, skin blackening, strong smell, still wet, flies still present
O	O	O Advanced Decay: Most flesh removed
O	O	O Dry/Skeletal: Bones, cartilage, some skin, little odour, small flies and beetles

Notes: _____
_____
_____

Temperatures (°C): Time _____ **Weather (if changed):** _____

P1
Maggot mass: _____ Maggot mass:_____
Other: _____ Other:_____

P2
Maggot mass: _____ Maggot mass:_____
Other: _____ Other: _____

P3
Maggot mass: _____ Maggot mass:_____
Other: _____ Other: _____

Activity

Adult Count (/30s): **P1** _____
P2 _____
P3 _____

Activity Level:	P1	P2	P3	
	O	O	O	Very low (<5)
	O	O	O	Low (5-20)
	O	O	O	Moderate (20-50)
	O	O	O	High (50-75)
	O	O	O	Very High (>75)

Dominance:
P1: O Adults – Species _____
O Larvae – Family _____
P2: O Adults – Species _____
O Larvae – Family _____
P3: O Adults – Species _____
O Larvae – Family _____

Time **Insect Observations**

Pitfall Traps

Other Observations

Appendix B:

Parent colony rearing attempts and successes

Table B. Parent colony rearing attempts and successes.

Deprivation/Exposure	Date of egg collection attempt	Result
Overnight deprivation (1 night)	2011/07/29	No eggs
	2012/01/28	No eggs
	2012/03/10	~80-100 eggs (Beringia only)
	2012/03/11	~20-30 eggs (Beringia only)
	2012/03/17	No eggs
	2012/03/18	~450 eggs (Beringia only)
	2012/03/24	>1800 eggs (Beringia only)*
	2012/04/23	~80-100 eggs (Beringia & Great Lakes)
	2012/04/29	Small egg masses (not quantified)
	2012/05/04	Small egg masses (Great Lakes only)
	2012/05/11	Small egg masses (not quantified, insufficient for research)
	2012/05/17	~300 eggs (Great Lakes) ~125 eggs (Beringia)
	2012/05/23	No eggs
	2012/05/24	>3000 eggs (Beringia)*
Overnight deprivation (1 night), all laboratory lights turned off	2012/03/04	No eggs
Overnight deprivation (1 night), large amount of liver re-introduced	2011/07/30	>2000 eggs (Beringia & Great Lakes)*
	2012/05/10	~500-600 eggs (Beringia)
Liver left in colonies overnight (1 night), all laboratory lights turned off	2012/01/29	~200 eggs (Beringia, 2 colonies)
	2012/02/04	(<100 eggs laid overnight) 10-30 eggs
	2012/02/05	(<130 eggs laid overnight) No eggs
	2012/03/03	<100 eggs (Beringia only)
	2012/05/05	~100 eggs (Beringia only)
	2012/05/18	(large egg masses overnight, Beringia) ~100 eggs (Beringia) >2700 eggs (Great Lakes)*
1-2 days deprivation	2012/01/27	No eggs
	2012/03/02	No eggs
	2012/03/16	No eggs
	2012/05/07	Small egg masses (not quantified, Beringia)
	2012/05/09	~10-15 eggs (Beringia)

>2 days deprivation	2011/07/28	~100 eggs (Great Lakes)
	2012/02/03	No eggs
	2012/04/28	Small egg masses (not quantified)
	2012/05/03	Small egg masses (not quantified)
	2012/05/22	No eggs
Combination deprivation/exposure over a series of days (e.g. 1 day exposure, 1 day deprivation, third day collection attempt)	2012/02/10	(many eggs laid overnight) >1500 eggs (Beringia)*
	2012/03/09	No eggs
	2012/03/14	(large egg masses laid overnight) No eggs
	2012/05/16	(small and large egg masses overnight) ~100 eggs (Great Lakes)

*Indicates attempts where sufficient eggs were obtained to begin research (using biotype stated in parentheses)

Appendix C:
Development trial condition parameters and set-up

Trial 1: Natural outdoor summer conditions, Oshawa, Ontario

Table C-1. Trial 1 (natural conditions) parameters.

Average Temperature (°C)	21.6 ± 2.40
Relative Humidity (%)	84.84 ± 20.58
Photoperiod	Uncontrolled

*all values calculated from data-logger readings.

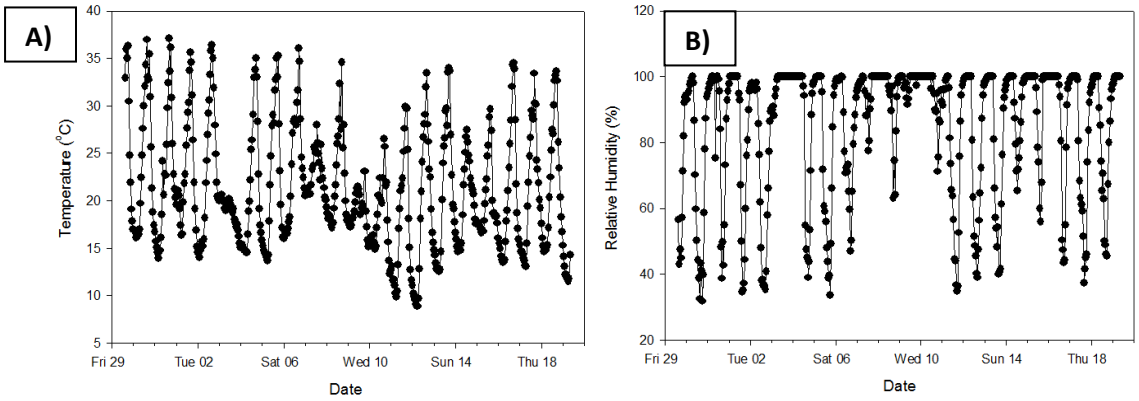

Figure C-1. Data-logger outputs of hourly temperature (°C) and relative humidity (%) at the research site for Trial 1 (natural outdoor conditions). A) Temperature; B) Relative humidity.

Figure C-2. Trial set-up for Trial 1 (natural conditions). A) research site; B) Rearing vessels and data-logger placed within research set-up.

Trial 3. Constant 30°C

Table C-2. Trial 3 (constant 30°C) parameters.

Set Temperature (°C)	30
Actual Temperature (°C)	30.28 ± 0.4745
Relative Humidity (%)	20.71 ± 3.209
Photoperiod (L:D)	14:10

*Actual temperature and relative humidity calculated from data-logger readings.

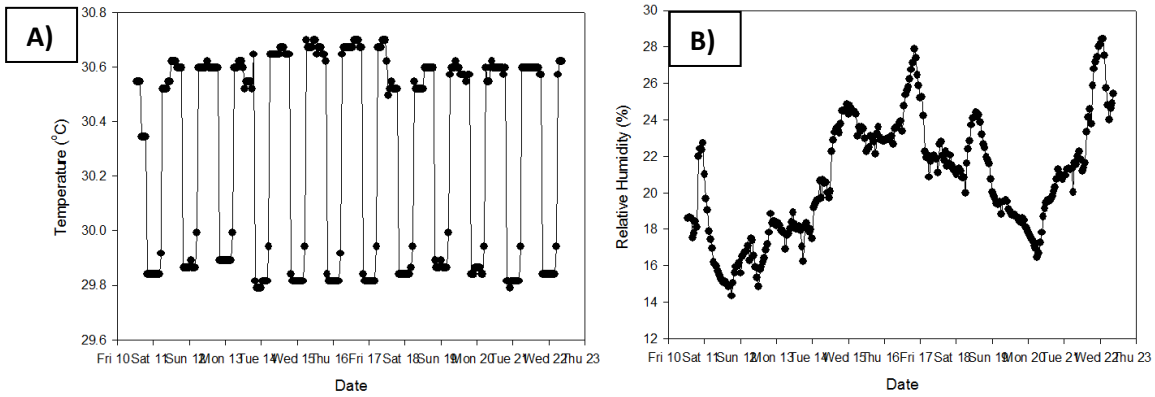

Figure C-3. Data-logger outputs of hourly temperature (°C) and relative humidity (%) within environmental chamber site for Trial 3 (30°C). A) Temperature; B) Relative humidity.

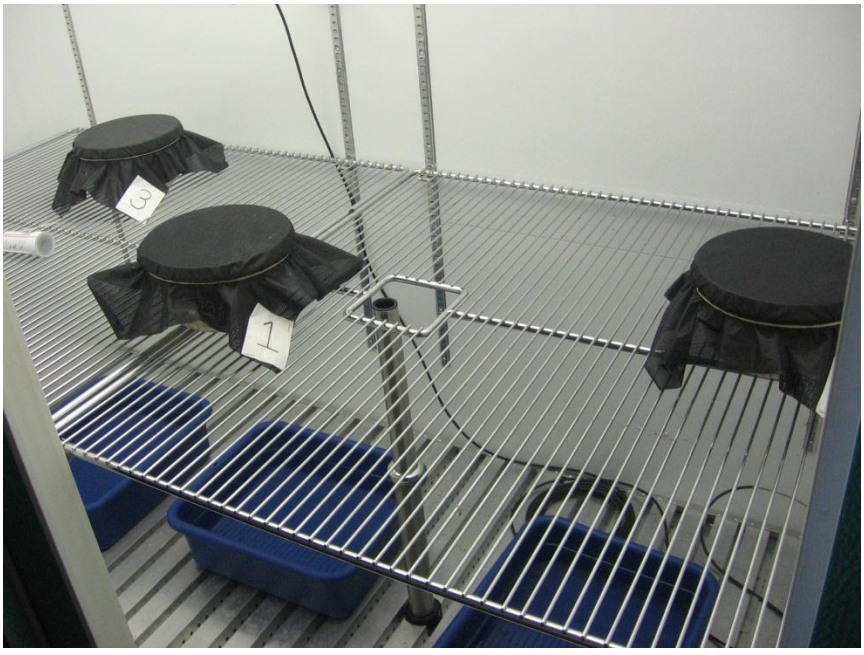

Figure C-4. Rearing vessels and data-logger placement within environmental chamber Trail 3 (30°C).

Trial 4. Constant 10°C

Table C-3. Trial 4 (constant 10°C) parameters.

Set Temperature (°C)	10
Actual Temperature (°C)	11.08 ± 1.313
Relative Humidity (%)	76.48 ± 8.393
Photoperiod (L:D)	14:10

*****Actual temperature and relative humidity calculated from data-logger readings.

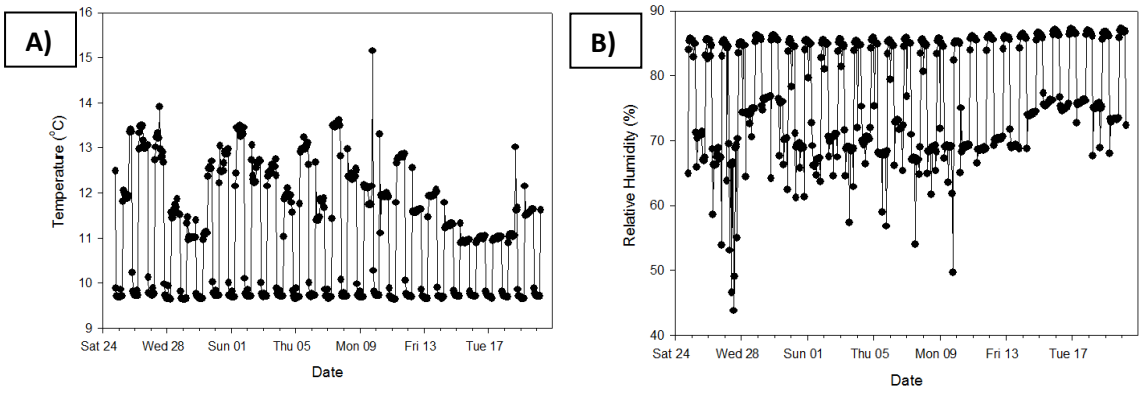

Figure C-5. Data-logger outputs of hourly temperature (°C) and relative humidity (%) within environmental chamber site for Trial 4 (10°C). A) Temperature; B) Relative humidity.

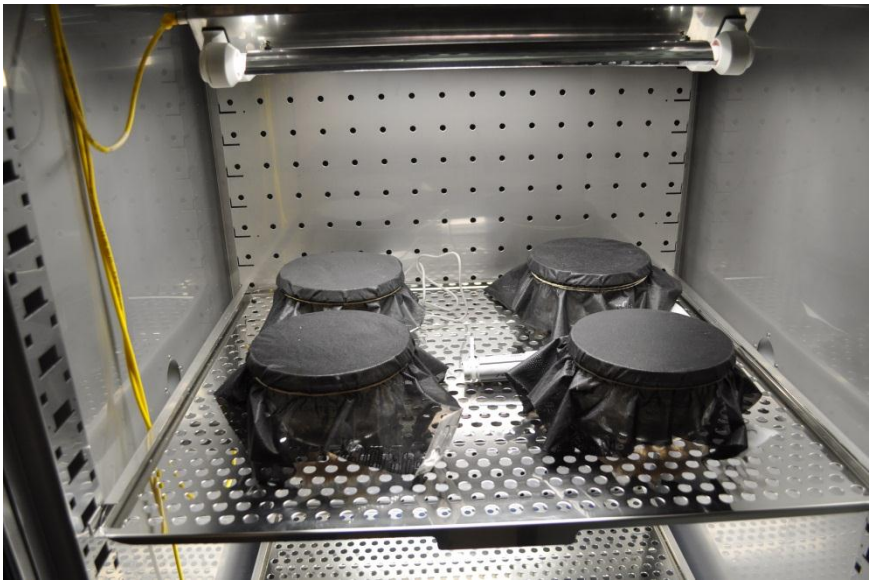

Figure C-6. Rearing vessels and data-logger placement within environmental chamber, Trial 4 (10°C).

Trial 5. Constant 10°C

Table C-3. Trial 4 (constant 10°C) parameters.

Set Temperature (°C)	10
Actual Temperature (°C) – Great Lakes	11.32 ± 1.489
Actual Temperature (°C) – Beringia	10.91 ± 1.225
Relative Humidity (%)	78.44 ± 8.229
Photoperiod (L:D)	14:10

*****Actual temperature and relative humidity calculated from data-logger readings.

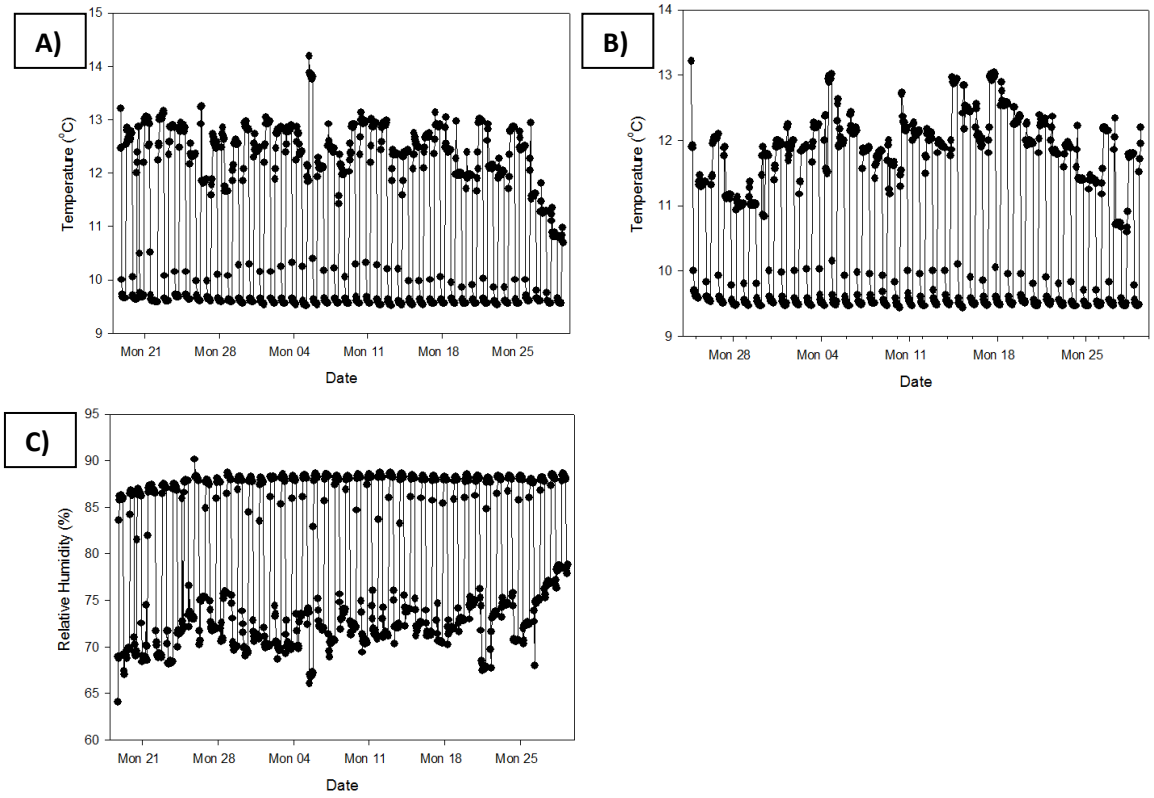

Figure C-7. Data-logger outputs of hourly temperature (°C) and relative humidity (%) within environmental chamber site for Trial 4 (10°C). A) Temperature, Great Lakes biotype; B) Temperature, Beringia biotype; C) Relative humidity.

Figure C-8. Rearing vessels and data-logger placement within environmental chamber, Trial 5 (10°C). Top shelf: Great Lakes biotype replicates and observation vessel; Bottom shelf: Beringia biotype replicates and observation vessel.

Appendix D: Publication-Decomposition and arthropod succession in Whitehorse, Yukon Territory, Canada

Decomposition and arthropod succession in Whitehorse, Yukon Territory, Canada

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The research presented within this manuscript has previously been presented at the 57th Annual Meeting of the Canadian Society of Forensic Science (November 29 – December 2, 2010), the 2nd Annual Graduate Student Conference at the University of Ontario Institute of Technology (May 18-20, 2011) and has been accepted for presentation at the 9th Annual North American Forensic Entomology Association meeting (July 20-22, 2011). Publication at each of these meetings/conferences is restricted to the abstract.

ABSTRACT:

Forensic arthropod succession patterns are known to vary between regions. However, the northern habitats of the globe have been largely left unstudied. Three pig carcasses were studied outdoors in Whitehorse, Yukon Territory. Adult and immature insects were collected for identification and comparison. The dominant Diptera and Coleoptera species at all carcasses were *Protophormia terreanovae* (R-D) (Fam: Calliphoridae) and *Thanatophilus lapponicus* (Herbst) (Fam: Sílphidae), respectively. Rate of decomposition, patterns of Diptera and Coleoptera succession and species dominance were shown to differ from previous studies in temperate regions, particularly as *P. terraenovae* showed complete dominance among blowfly species. Rate of decomposition through the first four stages was generally slow and the last stage of decomposition was not observed at any carcass due to time constraints. It is concluded that biogeoclimatic range has a significant effect on insect presence and rate of decomposition, making it an important factor to consider when calculating a postmortem interval.

KEYWORDS: Forensic science, forensic entomology, decomposition, arthropod succession, Whitehorse, northern environment

In criminal death investigations, accurate determination of the post-mortem interval (PMI) is of great importance (1). Within the first 72-84 hours, this interval can be estimated using physical/chemical means such as livor mortis, rigor mortis and algor mortis (2). However, beyond this time frame these methods often can no longer be relied upon, and entomological methods become the most dependable method of calculating PMI (1; 3). It has also been suggested that this calculation be referred to as PIA, or period of insect activity (4; 5). This period encompasses the time interval between initial insect association and colonization of remains until discovery (5), and may not correspond exactly with minimum PMI in cases involving delayed insect access to remains or myiasis (4).

There are two entomological methods by which PMI is calculated: larval growth along with development rates, and the succession patterns of arthropods (6). The former is relevant for estimates within the first lifecycle of the insect, which can range from days to over one month depending on the larval species, whereas the latter is useful over much longer periods of time (7). The number and type of insects present will vary depending on the region in which decomposing remains are located (8). Therefore, succession studies have been conducted world-wide to determine the baseline succession patterns in a wide variety of geographical regions (9; 10; 11; 12; 13; 14; 15). Within Canada, succession data has been published from many of the provinces including British Columbia (16), Alberta (17), Saskatchewan (18), Manitoba (19), New Brunswick (20) and Nova Scotia (21). However, there are no studies that have previously been conducted in the territories, in regions beyond 60°N latitude.

Beringia is a unique biogeoclimatic region that extends across Siberia, Alaska and most of the Yukon Territory. The region was not glaciated during the Pleistocene, providing a refuge for a variety of flora and fauna (22). Beringian climates during the last glacial maximum are thought to have been cold and dry (23). Climate in the Yukon Territory today is sub-arctic with cold dark winters, mild summers and extreme temperature ranges (24). The region is relatively dry, and precipitation is split between rain and snow (24).

Severe climatic and ecological conditions may contribute to low insect diversity in the Yukon Territory (22). Survival in extreme northern environmental conditions requires a variety of adaptations and has thus resulted in differential representation of insect taxa, the best represented groups being widespread or more northern (22). Presence of many widely-distributed northern insect groups shows a wide overlap between the Yukon Territory and the adjacent American state of Alaska (22). Comparatively, there are twice as many of these insect groups present in the more southern province of British Columbia (22).

The capital of the Yukon Territory is the city of Whitehorse. According to the Yukon Bureau of Statistics (25) the population of Whitehorse was 25,636 as of June 2009, accounting for 75.1% of the population of the territory. This represents a 21.7% population increase from the Whitehorse city population reported by the 1996 Census of Canada (25; 26). Of the total population of Whitehorse, 75.4% are classified as the "working age" population, including persons aged 15-65 (25). This is significant as, according to statistics presented by Statistics Canada in 1996, over 70% of persons accused of violent crimes in Canada fall between the ages of 14 and 37 (27). Between

1996 and 2009, the Yukon Territory experienced a 39.9% increase in violent crimes (27; 28).

The city of Whitehorse lies to the south of Beringia. Because of the uniqueness of the region and surrounding areas, and the necessary adaptations of arthropod fauna necessary for survival in a northern environment, it is logical to hypothesize that the insects present may be different from more southern regions or display different successional behaviours. The present research was undertaken as an investigation into the arthropod succession pattern(s) which can be expected at decomposing remains in the southern region of the Yukon Territory.

Materials and Methods

This study was conducted from June to August, 2010 on land owned by the Whitehorse Municipal Landfill. This location was selected as it offered protection from large carnivores such as grizzly bears in the form of two high voltage electric fences surrounding the facility. The nearest landfill activities to the research site were compost and recycling of tires and sheet metal, beyond which was the disposal of household wastes. However, the research site was a minimum of 1.5km away from any landfill activities to ensure no cross-attraction occurred.

Three pig carcasses (*Sus scrofa* Linneaus), obtained from a local pig farmer, were used as these are considered the closest human analogs for decomposition studies (6). Each carcass was approximately 20-30 kg in weight. Pigs were killed by the farmer using a bolt-gun to the top of the head on the morning of placement at the facility, and transported to the landfill in the back of a pick-up truck. Carcasses were placed at the research site with a minimum of 20 meters separation, as recommended by Anderson and

VanLaerhoven (16) to ensure independent olfactory attraction of carrion insects. Cages consisting of a wooden frame covered with chicken wire were used to prevent access by large scavengers.

Two pitfall traps were used for each carcass, one each at the snout and abdomen. These locations were chosen as the most likely locations for collection of early colonizing arthropods as well as migrating larvae. Rocky terrain conditions prevented the use of additional pitfall traps. An independent trap was also used to collect the natural fauna, and was placed at a control site away from the carcasses.

Carcasses were examined twice daily for the first five days of the study, once daily until day (D) 24, and every second day for the remainder of the study (until D60). Adult arthropods were recorded and collected from above, on, inside and below each carcass as observed. Immature specimens were collected as new aggregates were observed. A new aggregate was defined as a collection of eggs or larvae in a previously uncolonized location of the carcass. New aggregates, as opposed to former aggregates, were sampled to increase probability of sampling new colonizing species and avoid the continuous re-sampling of the same population. Half of each immature collection was reared on wild game muscle tissue in the laboratory. The other half was killed in hot water upon arrival at the laboratory and preserved in 75% ethanol. Identifications were conducted in the laboratory and taxonomists consulted for verification.

Written field notes and a photographic record were maintained for each examination. Notes included local meteorological conditions, and ambient temperature and daily rainfall as read from a thermometer/rain gauge placed at the site. Hourly temperature and relative humidity were recorded throughout the study using a

'HOBOWare' data logger placed at the control site. Temperature and humidity data was downloaded on a weekly basis.

Results

Ambient conditions

Average daily temperature and relative humidity values (Figure 1, Figure 2) were calculated by averaging all hourly readings from 000 – 2300 for each calendar day of the study (24 measurements daily). Recordings at 2400 were labelled as 000, and therefore used in the next day's average calculations. Calculations for the first and last days of the study use fewer readings in the average calculations due to the timeframe when the data logger was launched and the last data download. Hourly temperature ranged from -1.0°C to 29.2°C. Daily temperature averages ranged between 7.9°C and 19.9°C, with an overall daily average calculated at 12.6°C. Hourly relative humidity ranged between 22.2% and 95.0%. The daily relative humidity average ranged between 51.3% and 94.5%, with an overall daily average calculated at 66.6%.

Decomposition

Four stages of decomposition were recorded from June to August 2010. Carcasses remained in the fresh stage of decomposition only until the afternoon of the day of placement, D0 (Figure 3). The bloat stage lasted until D10 on one carcass, D11 on the second, and D13 on the last carcass (Figure 3). Active decay was the longest stage recorded, lasting between 27 and 34 days (Figure 3). The slowest rate of progression through this stage was recorded at the pig carcass which received the most shade throughout the day (Pig 2). Two carcasses were recorded in the advanced stage of decomposition for the last 15 days of the study; the third carcass was recorded in this

stage for 23 days. The final stage was not reached during the time of this experiment. Due to slow decomposition and the time constraints of the project, the study was terminated before any carcass reached the final stage of decomposition.

Insect Activity

The first eggs were observed on D0 of the decomposition study. Initial oviposition occurred in the nose and mouth, and behind the ears of all carcasses. Subsequent egg masses appeared along the back of the carcasses and at the anus. Larvae were first observed to migrate in the active decay stage, though some larvae were still feeding at the termination of the study.

The blowfly species *Protophormia terraenovae* (Robineau-Desvoidy) were collected in the fresh stage (Table 1), and reared from the first eggs collected on two carcasses. Adults of this species were dominantly collected through the first half of active decay, and it was the dominant species of larvae collected in the bloat stage. The first teneral adults were collected on D40 in the early advanced decay, and had been collected from all carcasses by D46.

Four species of bluebottle blowfly (Calliphoridae) were collected during the study: *Cynomya cadaverina* (Robineau-Desvoidy), *Calliphora terraenovae* (Macquart), *Calliphora vicina* (Robineau-Desvoidy) and *Calliphora vomitoria* (Linneaus) (Table 1). However, only the first two species listed were collected from all three carcasses. Of the four species, only two were collected as both larvae and adults: *C. vicina* and *C.cadaverina*. The greenbottle *Lucilia illustris* (Meigen) (Table 1) were collected from all carcasses in the early bloat stage, but were infrequently seen in active and advanced decay. The only immature specimens were collected as the first eggs on a single carcass.

Adults of the family Muscidae, predominantly in the genera *Hydrotaea* and *Fannia*, were collected at all carcasses starting in the bloat stage (Table 1), but no immature specimens were collected or successfully reared. Adult Muscidae numbers peaked in the late bloat and active decay stages but this insect group was not considered dominant.

Adults of the family Piophilidae were collected starting D5, in the mid-bloat stage (Table 1). Attraction increased in the active decay stage, and dominance from this point was divided between Calliphoridae larvae and adult Piophilidae. Immature specimens were not observed or collected.

Three species of Sílphidae were identified, arriving in the late bloat stage of decomposition (Table 1). The species *Thanatophilus lapponicus* (Herbst) was the dominant beetle collected in the early active decay, though its presence decreased towards the advanced decay. Larval presence peaked in the mid- to late-bloat stage, and two adults of *T. lapponicus* were successfully reared.

Adult Staphylìnidae were observed between the late-bloat and early-active decay stage but were rarely collected. No larval specimens were collected. Adult Cleridae were most commonly collected at a single carcass at the beginning and end of active decay, but not often mid-stage. Arrival times of Cleridae varied from late bloat to early advanced decay.

Discussion

The average daily temperature of 12.6°C recorded at the research site is low in comparison to previously published studies conducted in the summer season (10; 13; 18; 20; 29; 30; 31; 32; 33), and is more comparable to fall studies (15; 18; 20; 21; 33; 34). The geographic location of Whitehorse is 60°43’N, farther north than any published

study available for comparison. The extreme northern latitude of the study location may account for the cooler overall temperatures, even in the summer.

Four stages of decomposition, as described by Anderson & VanLaerhoven (16), were recorded. Decomposition progressed slowly through most stages, as studies in temperate regions have reported entering the remains stage inside four weeks (10; 18). All carcasses had left the fresh stage by the afternoon of D0, faster than most studies even in warmer climates (10; 16; 18; 35). Studies involving carcasses exposed in different season, decomposition generally proceeds faster in warmer summer temperatures than in cooler season (15; 18; 29; 32; 36). As the daily average temperature was cool for summer, this may explain the slower rate of decomposition.

Carcasses remained bloated for a longer period that previously reported in summer studies (10; 13; 18). Studies conducted in British Columbia (BC) (16) and Alberta (AB) (17), Canada, are geographically the closest to Whitehorse. The length of time carcasses spent in the bloat stage in BC was reported as 9 days (16), which is only 1.5-4.5 days shorter than the time reported in this study. However, the rate in BC was attributed by authors to three days of heavy rains which washed away eggs and delayed immature stage colonization of remains. In Alberta, Hobischak *et al.* (17) reported a slower rate of decomposition in all stages, and carcasses remained bloated for 2-3 times longer than carcasses in Whitehorse. This study was begun in the mid- to late-spring, indicating that, similar to temperature trends, rate of decomposition through at least the bloat stage demonstrates a strong correlation with published data from cooler seasons (18; 34).

The longest stage recorded at all carcasses was the active decay stage (Figure 2). However, as no carcass reached the dry/remains stage it is unknown how long the advanced decay stage would have persisted had decomposition been allowed to continue. Average temperature at the research site during the active decay stage was 12.1°C, centered on the cool overall daily average temperature at the site (Figure 1), and may have been a factor in the slow progression through this stage.

One carcass decomposed significantly faster than the other two, reaching the advanced decay stage 6 days earlier (Figure 2). This carcass was located at the highest point of terrain in the research facility and received almost no shade during the day; the remaining two carcasses received partial- to full-shade for parts of the day. Published data does not conclusively agree on whether sun-exposed or shaded carcasses will decompose at a faster rate during the summer (17; 18; 29), though studies agree that progression through the active decay stage is slower in shaded or sheltered carcasses compared to sun-exposed during spring and fall (15; 18; 29).

The dry/remains stage was not reached by any carcass during the time of this study. This trend has been previously reported, most often in studies conducted in the fall as temperatures cool towards winter (18; 21; 35). Only one study conducted within Canada during the summer reports carcasses not reaching the dry/remains stage (17), though many studies report this stage within four weeks (10; 15; 18; 37).

The dominant adult at all three carcasses through most of decomposition was the blowfly *P. terraenovae*. This was the only species collected from all three carcasses during the short fresh stage, and eggs collected from two carcasses on D0 confirm its presence as a first colonizer. Eggs collected from the third carcass at the same time did

not yield adult *P. terraenovae*, verifying the presence of *C. cadaverina* and *L. illustris* in the fresh stage. Adults of *C. cadaverina* were only collected from one carcass on D0, while *L. illustris* was not collected until D1. The blowfly *P. terraenovae* is described as having an Holarctic distribution and is common in cooler global regions (38). However, even in temperate regions it has not previously been reported as a singularly dominant species or first colonizer at decomposing carrion, and is often not reported at all in summer studies (10; 16; 18; 39). The species has been observed in the fresh stage in some spring (18; 37) and late summer/early fall studies (37). It has also been reported as a common component of insect assemblages associated with the bloat stage (17; 18). In addition to being the single most dominant blowfly species collected in adult form, *P. terraenovae* was also the most dominant species collected as immature specimens. Laboratory-reared larval specimens yielded adults of the species from collections made as late as D32 in the active decay stage.

Representation of other Calliphoridae was low in comparison. A significant relationship has been previously reported for the black blowfly *Phormia regina* (Meigen) with the bloat stage of decomposition (20; 21; 40) but adults of this species were rarely collected in Whitehorse and no larval collections yielded adults when reared. Arrival of the greenbottle *L. illustris* in the early bloat stage is supported by previous research reporting the species as an early colonizer (16; 20; 33).

Four species of bluebottle blowflies were collected, but adults of only two species appeared at all three carcasses: *C. terraenovae* and *C. cadaverina*. Bluebottles are often identified as common at carcasses in temperate regions (21; 33; 37; 40) and certain species have been reported as dominant within the early stages of decomposition, though

the species identified vary by region and season (18; 21; 37). The bluebottle species in this study were most commonly collected in the bloat and active decay stages, but no species was dominant in either the larval or adult form.

Adults of the family Piophilidae arrived approximately mid-way through the bloat stage, which supports previous findings from studies conducted in Canada (16; 18). However, the family group is more commonly associated with the active and advanced decay stages of decomposition in other global regions (37; 39; 40).

Coleoptera species richness was low in this study, with only three families identified at all carcasses. Sílphidae was the dominant family, and *T. lapponicus* the dominant species. The first adults were collected towards the end of the bloat stage, and the first larvae were observed in active decay. This species is not specifically discussed in many published succession studies, if reported at all (10; 17; 20; 21; 33; 39), and has been described as "elusive" (18). When reported, *T. lapponicus* arrives in the bloat stage and are most often seen in the late spring or early summer seasons (16; 18; 37). It has also been reported in select habitats during the fall (18; 33; 40). Larvae are reported as a major component of later decay stages (18; 37; 39), which was supported in this study.

Two additional species of Sílphidae were collected from the carcasses, though not all were in the same abundance or frequency as *T. lapponicus*. Neither of these species were reared from collected larvae.

Few adult Staphylìnidae were collected from the carcasses; the majority of specimens came from pitfall traps. The first observation of Staphylìnidae occurred in the early active decay stage. Staphylìnidae are often described as arriving in the bloat or active decay stages (10; 16; 18; 37; 40). Adults are usually observed feeding on Diptera

larvae (10; 16), explaining the presence of these predatory beetles in decomposition stages dominated by Calliphoridae larvae.

Only one genus of Cleridae was collected in this study. Specimens were first collected from two carcasses in the late bloat stage of decomposition, but not from the third until early advanced decay. The later colonized carcass received the most sun of the three, and it has been shown previously that the presence of Cleridae adults is different between sun-exposed and shaded habitats (17).

Studies of landfill insect fauna have shown that one of the most common "landfill fly" species present in landfill ecosystems is *Musca domestica* (Linneaus) (41; 42), though *Fannia spp.* are also commonly found (42). However, these species are more commonly collected from the landfill working face and inner rings of study than from farther out from the central point (41; 42). Site comparison studies have shown that there is little overlap or movement of common landfill species between the landfill face and external sites, even as close as 500m (43). As the site used in this study was a minimum of 1.5km from the nearest landfill activity, it is unlikely that the landfill played a significant role in the presence of identified insect species. Studies documenting nuisance flies in the Yukon have not been published; therefore, further conclusions cannot be made at this time.

Insect succession is a useful tool in forensic investigations for the determination of PMI, but is precisely correlated to each geographical region (8). This study provides insect colonization and dominance patterns for the Whitehorse region, under almost complete sun-exposure and summer meteorological conditions, and has shown that these patterns are notably different from those published elsewhere. Further work is required

to confirm patterns across successive years, as well as establish patterns in different seasons and subject to variables such as clothing and scavengers.

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Additional Information and Reprint Requests

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In response to the reviewer's comment on the use of rural studies for confirming the presence of *P. terraenovae*: The references cited in this section include both rural and urban (Vienna) locations. Lack of research has restricted the references available for use.

Table 1. Insects* collected from three pig carcasses at the research site in the Yukon Territory.

Stage	Order	Family	Genus and species	Carcass 1	Carcass 2	Carcass 3
Fresh	Diptera	Calliphoridae	*Protophormia terraenovae*	x	x	x
			Calliphora vicina	x		
			Cynomya cadaverina		x	
	Hymenoptera	Formicidae	*Formica spp.*			x
Bloat	Diptera	Calliphoridae	*Protophormia terraenovae*	x	x	x
			Phormia regina			x
			Lucilia illustris	x	x	x
			Calliphora vicina	x		
			Calliphora vomitoria	x		
			Calliphora terraenovae	x	x	x
			Cynomya cadaverina	x	x	x
		Muscidae	*Hydrotaea [Hydrotaea] spp*	x	x	x
			Hydrotaea [Ophyra] spp	x		
			Neohydrotaea			x
			Fannia spp	x	x	
			Muscina spp	x		x
		Sarcophagidae		x	x	
		Piophilidae		x	x	x
	Coleoptera	Carabidae		x	x	
		Cleridae			x	x
		Silphidae	*Nicrophorus hybridus*	x		
			Thanatophilus lapponicus	x	x	x
		Staphylinidae			x	x
Active Decay	Diptera	Calliphoridae	*Protophormia terraenovae*	x	x	x
			Phormia regina	x		x
			Lucilia illustris	x		x
			Calliphora vicina	x		
			Calliphora vomitoria	x		
			Calliphora terraenovae	x	x	x
		Muscidae	*Hydrotaea [Hydrotaea] spp*	x	x	x
			Hydrotaea [Ophyra] spp	x	x	x
			Fannia spp	x	x	x
			Spilogona spp	x	x	x
		Piophilidae		x	x	x
		Sarcophagidae			x	
	Coleoptera	Cleridae			x	x
		Silphidae	*Nicrophorus hybridus*	x	x	
			Thanatophilus lapponicus	x	x	x
			Thanatophilus coloradensis		x	
		Staphylinidae		x	x	x
	Hymenoptera	Argidae			x	
		Formicidae	*Formica spp.*			x

Advanced Decay	Diptera	Calliphoridae	*Protophormia terraenovae*	x	x	x
			Lucilia illustris	x	x	
			Calliphora vicina	x		
			Calliphora terraenovae		x	
		Muscidae	*Hydrotaea [Hydrotaea] spp*	x	x	x
			Hydrotaea [Ophyra] spp	x	x	
			Fannia spp	x	x	
		Piophilidae		x	x	x
	Coleoptera	Cleridae		x		x
		Silphidae	*Nicrophorus hybridus*	x		x
			Thanatophilus lapponicus	x		
			Thanatophilus coloradensis	x		
	Hymenoptera			x		
		Formicidae	*Formica spp.*			x

*Indication of the presence of adult insects at carcasses as collected throughout study.

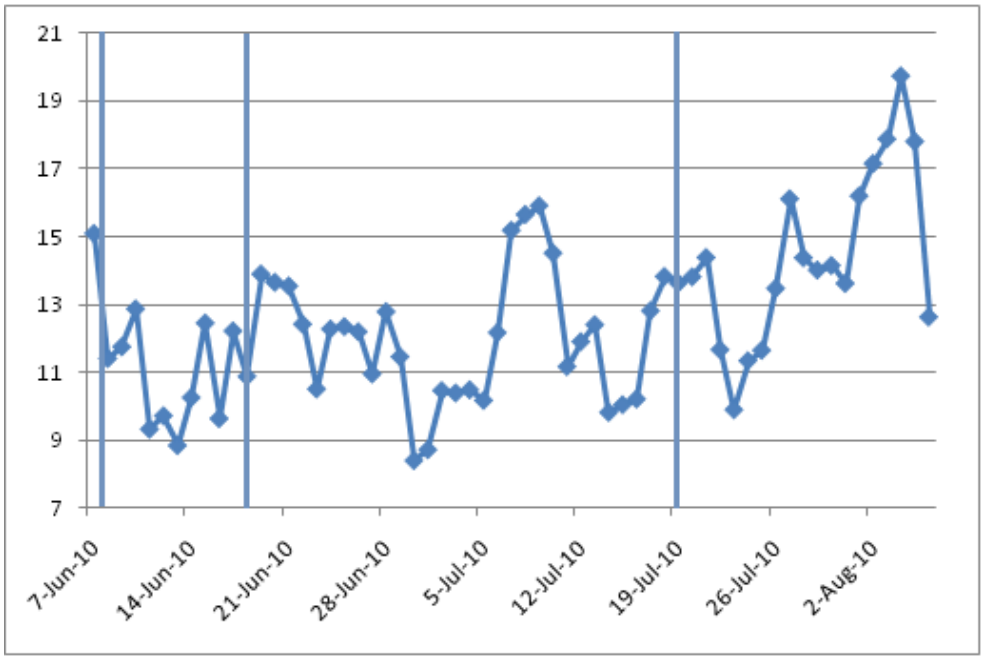

Figure 1. Daily average temperature (°C). Values calculated by averaging hourly temperature readings taken between 0000 and 2300 (inclusive) for each calendar day ($\bar{x}$ = 12.59°C, σ = ± 2.435). Vertical bars indicate new stage of decomposition. The first bar denotes the beginning of the bloat stage, the second bar denotes the beginning of active decay stage, and the final bar denotes the beginning of advanced decay stage.

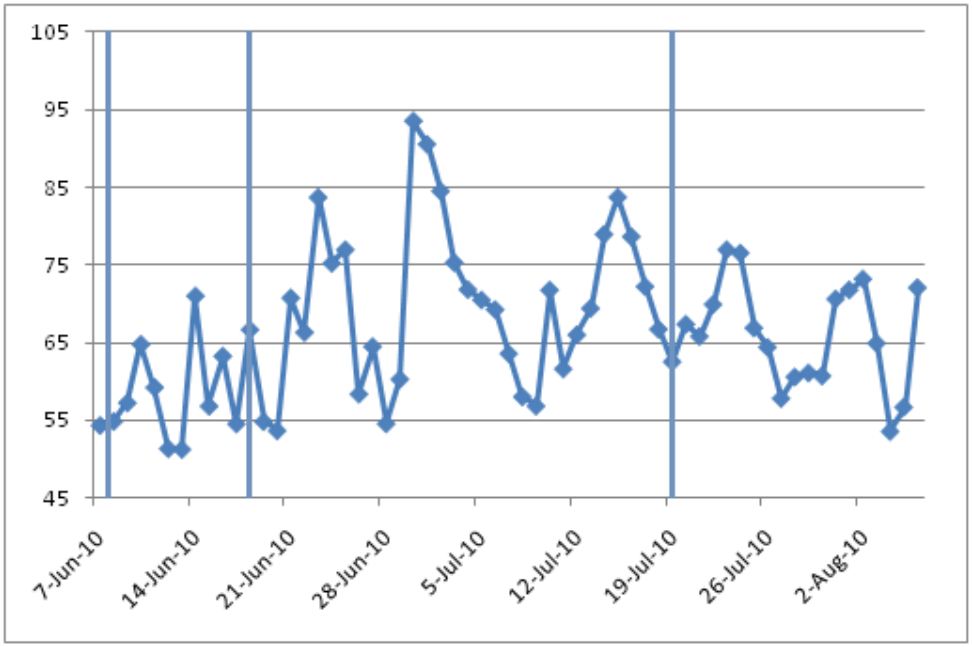

Figure 2. Daily average relative humidity (%). Values calculated by averaging hourly RH readings taken between 0000 and 2300 (inclusive) for each calendar day ($\bar{x}$ = 66.60°C, σ = ± 9.661). Vertical bars indicate new stage of decomposition.

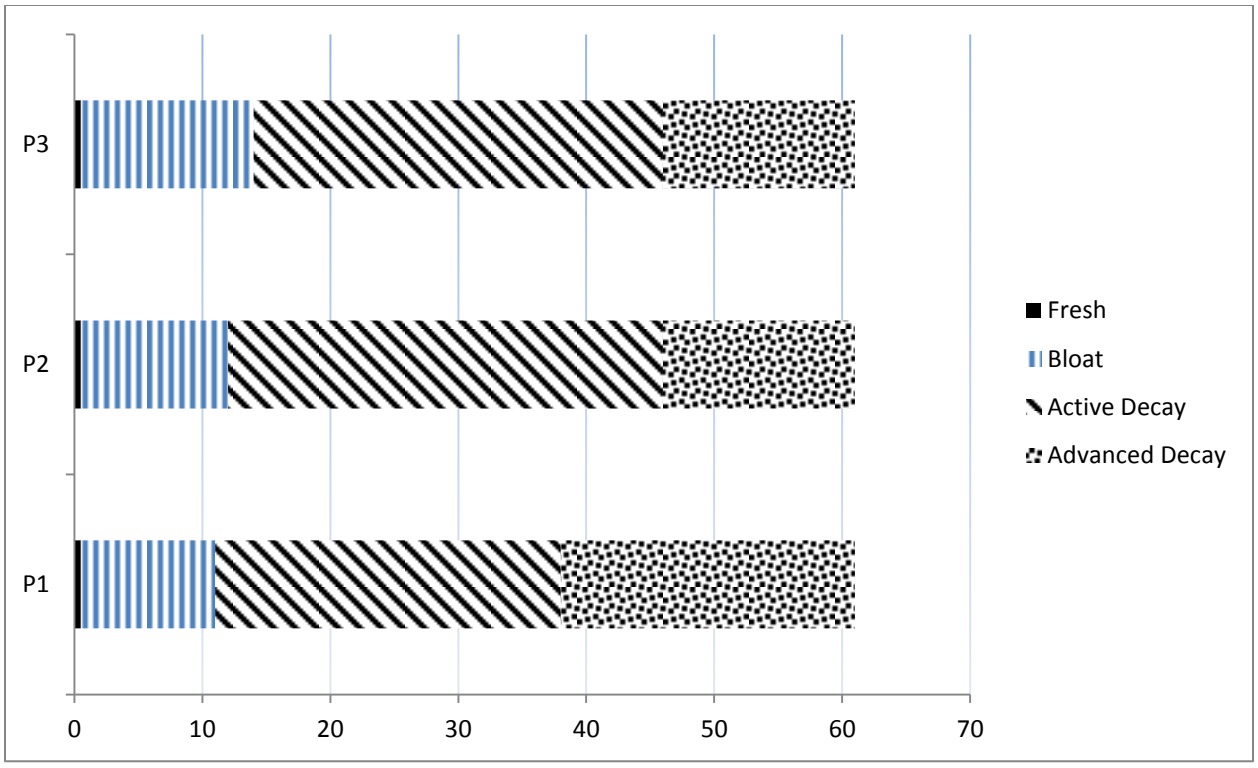

Figure 3. Progression of each carcass through four (4) stages of decomposition according to day of study. D0 is June 7, 2010.