

**Succession and Development Studies on Carrion Insects of Forensic
Importance**

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Kimberly Lane Tabor

Abstract

Forensic entomological field and laboratory studies were conducted to obtain data currently missing or conflicting in the literature. The first goal of this project was to identify and qualitatively assess the major taxa of forensic importance in southwest Virginia. Carcasses of the domestic pig, *Sus scrofa* L., were placed in field conditions and allowed to decompose until they reached the advanced stage of decay. Over 50 taxa were collected and identified, with *Phormia regina*, *Phaenicia coeruleiviridis*, *Phaenicia sericata*, *Calliphora* spp., *Sarcophaga utilis*, *Musca domestica*, *Hydrotaea leucostoma*, *Stearibia nigriceps*, *Prochyliza xanthostoma*, and *Meroplius minutus* among the most commonly observed fly species, and *Creophilis maxillosus*, *Platydracus maculosus*, *Aleochara lata*, *Oiceoptoma noveboracense*, *Necrodes surinamensis*, and *Necrophila americana* among the most commonly observed beetle species.

The second objective of this study was to analyze successional patterns of taxa collected and identified in the carrion-insect succession studies. Occurrence matrices were constructed for the successional patterns of insect taxa during 21 sampling intervals in the spring and eight intervals in the summer studies. Permutation analyses of the occurrence matrices showed that the successional patterns of insect taxa were similar between spring 2001 and 2002 ($P = 0.001$) and between summer 2001 and 2002 ($P =$

0.007). Results indicated that the successional patterns appear to be typical for the seasonal periods.

The third objective of this study was to analyze the effects of antemortem ingestion of ethanol by pigs on insect successional patterns and development rates. Pigs were dosed with a mixture of 95% ethanol and saline. Blood samples were collected immediately prior to euthanasia. The carcasses then were placed at an open

field site and allowed to decompose. Insect samples were collected from carcasses for ten days post-mortem and the collected data were used to develop occurrence matrices. Permutation analysis to test the null hypothesis of no similarity between successional patterns of taxa from treated and untreated pigs showed that the successional patterns were similar. Loin meat from the carcasses was used as a rearing medium for field development studies of the black blow fly, *Phormia regina*. Development rates of 3rd instar *P. regina* maggots feeding on meat from treated pigs were significantly different from development rates of maggots feeding on meat from untreated pigs. No significant differences were detected in 1st and 2nd instars.

Finally, the effect of relative humidity (RH) on egg hatch time and percent hatch rate of *Phormia regina* and *Phaenicia coeruleiviridis* was tested using saturated salt solutions. The global ANOVA of mean hatch times and percent hatch was found to be significant for *P. regina* at 20°C and 30°C and *P. coeruleiviridis* at 20°C among RH levels at $\alpha = 0.05$. *T*-test (LSD comparisons) results detected significant differences between some but not all RH levels within all three data series tested. Percent hatch observed varied widely, depending on the RH level. Of the levels tested, the lowest RH where hatch rate was observed was 53% (20°C) for *P. coeruleiviridis*, where only 1% of the eggs hatched. At 90+% RH, almost all of the eggs hatched for both species.

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1. Introduction

Forensic entomology involves the interaction of arthropods (mainly insects) with legal activity. Three principle areas of forensic entomology are currently recognized (Hall 2001): 1) *Stored products entomology* is primarily civil in nature and is concerned with insect infestation or damage of commercial commodities. 2) *Urban entomology* also deals mainly with civil issues involving the effect of insects on man-made structures, such as termite damage to buildings. 3) The third area of forensic entomology has both a criminal and a civil aspect. The criminal aspect is termed *medicolegal forensic entomology* and deals with the utility of arthropods in criminal investigations, particularly those involving wrongful or unexplained death. This specific area of forensic entomology has become synonymous with the phrase ‘forensic entomology’ and is the area in which this research is relevant.

Medicolegal forensic entomology deals primarily with insects and other arthropods which infest human remains. Insects lay eggs on or in human remains, as well as utilize the corpse for food or habitat. Insect development and successional patterns can be an indication of the postmortem interval when time of death is unknown. Insects also provide an alternate specimen for toxicological analyses when traditional specimens are no longer useful. Entomological evidence has also been implicated in cases of abuse or neglect, as well as in situations of death by suspected bee or wasp stings.

The use of arthropods in criminal investigations is not new. One of the earliest documented cases where insects were used to solve a crime occurred in 13th century China. A slashing occurred in a small village amidst a community of sickle-carrying farmers. Despite pleas of innocence among the farmers, the guilty individual was

identified when investigators noticed a cluster of flies surrounding his sickle. Although the weapon had been wiped clean, there was enough blood residue left on the blade to attract dozens of flies, thus identifying the suspect (McKnight 1981).

Megnin (1894) was one of the earliest researchers to observe that different insect species arrive at and lay eggs on a corpse at very specific times during decomposition. A cadaver has a very specific insect fauna which changes during the decay process. Therefore, by identifying the species present on a cadaver at different times, a reasonably accurate estimate of when death occurred can be obtained. Since 1894, arthropods have played a role in hundreds of forensic cases, although it was not until recent years that interest in the use of arthropods as forensic indicators increased (Goff 1993).

Data collected by forensic entomologists on the arthropod populations associated with a deceased individual can be used in a number of ways, for example to determine the place where death occurred and whether or not a body was moved after death, to establish the antemortem ingestion of toxins or drugs, and to identify wound sites. However, the most valuable use for entomological data is the estimation of the postmortem interval (PMI), or the time that elapsed since death (Hall 2001).

Pathologists can estimate the time of death based on several biological parameters: lividity, rigor mortis, postmortem cooling, changes in the chemical constituents of body, autolysis of tissue, and decomposition due to bacterial activity in the body. However, these parameters are not reliable beyond about 72 hours after death (Hennsge et al. 1995). The entomological method of determining PMI was found to be statistically more reliable and superior when compared to other pathological methods, particularly during the later stages of decay (Kashyap and Pillai 1989).

Entomological evidence is used in two primary ways to estimate the PMI of human remains. First, the degree of development of the oldest maggots feeding on the corpse indicates a close approximation to time-since-death because insects often lay eggs within minutes or hours of death (Catts and Goff 1992). Second, insect successional patterns are indicative of PMI because insects arrive at decomposing remains in predictable, successive waves based on the stage of decomposition (Reed 1958, Payne 1965).

Knowledge of additional information such as whether drugs or toxins were ingested prior to death and the effect of environmental conditions can improve the accuracy of PMI estimates based on entomological evidence as such conditions may affect insect development rates and succession patterns. In effect, drug or toxin use and relative humidity extremes may skew PMI estimates by hindering or speeding up development rates (including egg hatch). Therefore, knowing the extent to which relative humidity affects egg hatch rate and whether or not drugs or toxins were used prior to death provides valuable information in insect-based PMI estimation.

In succession-based PMI estimation, it is important to have knowledge of the baseline fauna for the area where a body is discovered because fauna are environmentally and location-specific (Anderson 2001). Forensic successional fauna are typically described in controlled studies using animal models. Currently, there are no published carrion arthropod successional studies for southwest VA, nor are there studies which test for the effect of antemortem ingestion of ethanol on maggot development rates. There are also few studies that analyze the effect of relative humidity on egg hatch of forensically important insects. Therefore, the goals and objectives of this research were:

1) To identify and describe the baseline insect fauna for southwest Virginia; 2) To analyze successional patterns of forensic insects and to determine whether these patterns are consistent year to year; 3) To test the effects of antemortem ingestion of ethanol on insect successional patterns and development, and 4) To test the effect of relative humidity on egg hatch time and percent hatch of the black blow fly, *Phormia regina* Meigen and the greenbottle fly, *Phaenicia coeruleiviridis* Walker.

2. Literature Review

2.1. Succession

Within the forensic context, succession is the timed, predictable progression of faunal specimens that visit a cadaver. The type and composition of fauna found on a corpse are indicative of its stage of decomposition (Megnin 1894, Reed 1958, Payne 1965), the environmental conditions, and length of exposure. Therefore, knowledge of successional patterns can help in estimation of the postmortem interval (PMI). Patterns of succession can be affected by a variety of factors, including temperature, relative humidity, rain fall, exposure to sunlight, and whether or not the body is covered or inside a structure. Information on the diversity of species, number of individuals of each species, life stages present, and the number of individuals of each life stage can be determined from succession studies. This information can be used to estimate PMI, particularly when the individual has been dead for only a couple of weeks (Keh 1985).

The sequence of arthropod colonization on cadavers remains fairly constant at the family level among locales (Early and Goff 1986). However, at the genus and species level, colonization is environmentally and location-specific based on the zoogeographic region (Payne 1965, Early and Goff 1986). Therefore, successional patterns at the genus and species level are only predictable within the parameters of the location where a cadaver is found (Anderson 2001, Rodriguez and Bass 1983). The composition of taxa found on a corpse at the time of discovery (corpse fauna) is compared with the composition of the arthropod assemblage at a given period of time derived from an animal model (baseline fauna) to estimate the PMI based on successional patterns (Schoenly et al. 1996). These baseline faunal data are generally obtained from controlled

experimental studies. Data from controlled studies provide a timeline of successional patterns characteristic of the area in which the study was conducted. When deriving PMI estimations from succession-based data, baseline fauna must come from studies conducted in an environment similar to that of the corpse location.

Carcass size and type can influence decomposition rates and successional patterns (Watson and Carlton 2003). Catts and Goff (1992) suggest that the most appropriate animal model for use in forensic successional studies is the domestic pig, *Sus scrofa* L, weighing \approx 23 kg. Twenty-three kg is desirable because it is approximately the same size as an average adult human torso. In addition, the domestic pig is the most accepted human model due to its skin type, gut bacteria, and relative lack of hair (Anderson 2001). It has been shown that the patterns of arthropod succession do not differ significantly between pig carcasses with moderate differences in size (Hewadikaram and Goff 1991). However, one study demonstrated that extreme size differences can greatly affect the rate of decomposition (Kuusela and Hanski 1982) and arthropod successional patterns.

A number of studies on the successional patterns of arthropods (based on arthropod visitation) have been conducted throughout the world in climactically different areas using various animal models. Bornemissza (1957) characterized the succession of organisms on guinea pig carcasses in the Mediterranean-type environment of Western Australia. Five stages of decomposition were identified (initial decay, putrefaction, black putrefaction, butyric fermentation, and dry decay) and these were correlated with the fauna that were present. In addition, a diagram of successional patterns was developed. It was also found that carrion decomposition affected the physical properties of the soil and of its arthropod population to a depth of 14 cm.

A comprehensive year-round study of insects associated with dog carcasses was conducted in Tennessee (Reed 1958). Forty-three carcasses were distributed among wooded and non-wooded areas and placed at study sites at different times throughout the year. Reed (1958) classified the arthropods into groups according to the stage of decomposition (also defined by the author) during which they were found most frequently. Arthropods in the *Fresh stage* of decomposition consisted primarily of Muscidae and Calliphoridae; those in the *Bloated stage*, included the families Silphidae, Histeridae, Staphylinidae, Muscidae, Calliphoridae, and Sarcophagidae; those in the *Decay stage* included families such as Silphidae, Histeridae, Piophilidae, Sepsidae, and Phoridae; and those in the *Dry stage* included the families Cleridae, Dermestidae, and Trogidae. This breakdown of insect taxonomic groups by stage was shown to vary seasonally, and differed in different land-types (wooded vs. non-wooded).

In another study, Payne (1965) described faunal successional patterns on newborn carcasses of the domestic pig, *Sus scrofa* L. in a hardwood-pine community in South Carolina. Six stages of decomposition were delimited for carrion exposed to arthropods. As in previous studies by Reed (1958), each stage was found to be colonized by a characteristic group of arthropods. Sarcophagidae and Calliphoridae deposited eggs during the *fresh stage* of decomposition. During the *bloated stage*, Calliphoridae, Muscidae, and Sarcophagidae species increased in number, while Piophilidae and Lonchaeidae were observed for the first time. Several beetle families (Scarabaeidae, Histeridae, and Staphylinidae) were also present. During the *active decay stage*, Staphylinidae and Histeridae increased in number and the first Silphidae were observed. Maggots fed actively on the carrion during this stage. During the *advanced decay stage*,

most of the flesh had been removed from the carcass. Dipterans greatly decreased in number, Trogidae arrived at the carcass at night, and Staphylinidae and Histeridae remained during the day. The final stage, the *dry stage*, was characterized by the absence of most dipterans, Histeridae, Staphylinidae, and Silphidae and by an increase in centipedes, millipedes, Dermestidae and Cleridae. A total of 422 insect species representing 11 orders, 107 families, and 283 genera were identified in this study (Payne 1965).

Early and Goff (1986) described arthropod succession patterns in exposed carrion in Hawaii using domestic cat carcasses. Stages of decomposition similar to those described by Bornemissza (1957) in Australia were described for the tropical climate in Hawaii, although the stages progressed more rapidly. The sequence of arthropod succession at the family and genus level was similar to that observed in studies conducted in other climates, while there was the expected local variation at the species level.

Rodriguez and Bass (1983) collected successional data in one of the few studies conducted on human cadavers in a decay research facility in Knoxville, Tennessee. The cadavers were placed outside during different times of year and daily observations were made. The data showed that there is a direct correlation between the rate of decay and the rate of succession of families and species found in association with the remains. Four separate stages of decay were described for un-embalmed, uncovered cadavers: fresh, bloated, decay and dry (Rodriguez and Bass 1983).

Smeeton et al. (1984) performed the first published succession study conducted in New Zealand using human cadavers. Most of the 50 corpses used in the study were allowed to decompose for two weeks or less, so most of the species identified from the

study were early-arrivers. Certain species were found to oviposit initially within a few hours following death, and thereafter throughout the study.

Anderson and VanLaerhoven (1996) conducted succession studies in British Columbia, Canada using pig carcasses. A database of insect successional patterns over time in an open, sunlit, rural area in summer was developed. The authors noted that some species of insects (e.g. Piophilidae) were collected earlier in the decomposition process than expected based on studies in other regions. They also found that the soil fauna under the carcass changed considerably in both species composition and the number of species observed over the study.

Watson and Carlton (2003) monitored black bear, white-tailed deer, alligator, and swine carcasses throughout decomposition in the spring/summer in Louisiana. A total of 93 arthropod species from 46 families were collected from the carcass. However, only 19 of the 78 insect species reported were collected on all four of the carcass types. These results clearly demonstrate the relevance of carcass type when performing succession studies using animal models.

Numerous other succession studies have been performed in a variety of locales throughout the world: Sukontason et al. (2003) in Thailand, Lane (1975) in England, Bharti & Singh (2003) in India, Archer and Elgar (2003) in Australia, Centeno et al. (2002) in Argentina, and Bourel et al. (1999) in France. However, there are currently no published forensic succession data for southwest Virginia.

2.2. Decomposition

Although the processes of decomposition and insect invasion are continuous, they are often described by discrete stages, which are characterized by the insect activity at

each point in decomposition. The stages can be described as followed: 1) *Fresh stage*. A corpse is considered to be in the fresh stage from the moment of death until the first signs of bloating. This is the stage during which the blow flies (Calliphoridae) and flesh flies (Sarcophagidae) arrive at the corpse and begin laying eggs or larvae. 2) *Bloated stage*. This stage marks the beginning of putrefaction. Anaerobic bacteria produce gases as the result of metabolic processes, which cause bloating. Bloating usually occurs first in the abdomen, although the corpse may later assume a fully inflated appearance. Calliphorid numbers usually peak during this stage. Also in this stage, the soil beneath the corpse tends to become alkaline, affecting the normal soil fauna. 3) *Decay stage*. This stage is thought to begin when gasses escape and the remains deflate. During this stage, dipteran larvae forming large maggot masses are predominant. Large numbers of coleopterans also begin to arrive. By the end of the decay stage, most of the flesh has been removed from the corpse and most of the Calliphoridae and Sarcophagidae have departed from the remains to pupate. 4) *Post-decay (dry) stage*. The coleopterans (particularly dermestids) usually are the predominant species during this stage when the remains have been reduced to skin, cartilage, and bones. 5) *Skeletal (remains) stage*. In this stage, the remains consist of only of hair and bones. Most of the previous taxa will have disappeared, leaving mainly mites as the useful indicators of the PMI during this stage (Early and Goff 1986, Goff 1993).

2.2.1. Arthropods Associated with a Corpse

Many insects occur on or near the corpse, but not all of them are useful in determining the PMI. Some insects that are collected are simply opportunistic and do not play a role in PMI estimation. Goff (1993) outlines four basic arthropod-corpse

relationships that have generally been accepted: 1) *Necrophagous species* include those taxa which feed directly on the corpse. These taxa include dipteran species, particularly those in the families Calliphoridae and Sarcophagidae and coleopterans such as those in the families Silphidae and Dermestidae. Necrophagous species are the most significant group for PMI estimation during the first two weeks of decomposition (Goff 1993). 2) *Predators and parasites of necrophagous species*, which include several beetle families (Silphidae, Staphylinidae, and Histeridae) and fly families (Calliphoridae and Stratiomyidae). 3) *Omnivorous species* include taxa that feed on both the corpse and arthropods associated with the corpse. Wasps, ants and some beetles are all representative of this group. 4) *Adventive species* include species of Collembola, Acari, centipedes and spiders that are opportunistic, e.g. use the corpse as an extension of their normal habitat (Goff 1993).

2.3. Fly Development

Developmental data for primary blow flies provide the most accurate means of estimating the PMI using arthropod information (Greenberg 1991). It is presumed that the first individuals that arrive at, and lay eggs in a corpse do so within hours after death (Catts and Goff 1992), provided the body is outside and there are no obvious barriers preventing egg-laying (such as environmental restrictions and whether the body is covered, buried or indoors). Therefore, time of death is assumed to be close to the time the first eggs are deposited.

In order for insect developmental analysis to yield an accurate PMI estimation, data must be available for the earliest colonizing species collected from the corpse at the time of discovery. Although there may be several early-arriving species, the oldest

individuals are the most relevant because they represent the first eggs deposited on the body. Because certain species can oviposit beginning a few hours following death and continuing for at least two weeks (Smeeton et al. 1984), it is crucial to determine which individuals are the oldest for each species, as well as to correctly identify the species of those individuals. There are a number of dichotomous keys available for identifying immature flies (Sanjean 1957, Greenberg and Szyska 1984, Liu and Greenberg 1989, Greenberg and Singh 1995, Wells et al. 1999). However, it is sometimes difficult to obtain the correct species identification of immature flies due to small or poorly preserved specimens, the absence of certain species in a key, or difficult morphological indicators. It is, therefore, customary for investigators to collect samples for rearing as well as preserving (Catts and Haskell 1990). A sub-sample of the eggs/maggots/pupae collected at the time of discovery of the corpse is reared in the laboratory until adult emergence when they can be more easily and accurately identified using one of the many keys available for adult flies (Hall and Townsend, Jr. 1977, Smith 1986, McAlpine 1987).

The rate of development is assumed to be a linear function of temperature, at least between upper and lower temperature thresholds (Arnold 1960). The developmental minimum (minimum threshold) is the lowest temperature at which development can proceed; the developmental maximum (maximum threshold) is the highest temperature at which development can proceed. Heat accumulation is commonly expressed in thermal units called degree-days. The total amount of heat accumulated is expressed as accumulated degree-days (ADD) or accumulated degree hours (ADH), which are the total number of degree-days (hours) required to complete development. The degree-day approach has been described in many studies (e.g., Arnold 1960, Allen 1976, Baskerville

and Emin 1969) and is commonly used to measure the effect of temperature on biological processes.

Accumulated degree-days are calculated by multiplying the total days to complete development by the difference between exposure temperature and the developmental minimum temperature for the insect. In forensic studies, the length and stage of maggots collected at a crime scene can be compared to the length and stage of maggots at known physiological ages (in ADD) based on rearing studies to determine the amount of ADD accumulated at the crime scene since oviposition. Once the required ADD for that stage of development is known, investigators can work backward from the time of corpse discovery to calculate the number of days (hours) of exposure to reach the required ADD based on weather data recorded at or near the crime scene. The resultant data are then used to develop an estimate of the postmortem interval.

Recent and historical weather data collected at weather stations across the U.S. are available and from the National Weather Service (www.nws.noaa.gov). While it is unlikely that a body is discovered in the exact location of a weather station, it is usually possible to obtain data from stations nearby that describe climatic conditions very similar to those at the crime scene.

Extensive work has been done on blow fly development and biology for many important forensic species. Greenberg (1991) described the biology and development time for several species of forensic blow flies, including *Phormia regina* Meigen, *Phaenicia sericata* Meigen, *Megaselia scalaris* Loew, *Calliphora vicina* Robineau-Desvoidy, *Cochliomya macellaria* Fabricius, and *Chrysomya rufifacies* Macquart. An

extended discussion of forensic applications is presented, with an emphasis on thermal effects of development.

In an earlier study, Kamal (1958) presented life history data for thirteen species of Calliphoridae and Sarcophagidae. Development data were obtained at constant temperatures and relative humidities for many forensically important fly species, including *P. regina*, *P. sericata*, *Sarcophaga* spp., and *Calliphora* spp. Constant temperature and humidity were found to accelerate the life cycle and shorten the adult life span in comparison with fluctuating conditions with the same average.

Ash and Greenberg (1974) studied the development of two sibling blow fly species, *P. sericata* and *Phaenicia pallescens*. *P. sericata* was found to develop at a slower rate and with more variability at each stage. In addition, *P. sericata* tended to diapause at both high and low temperatures.

Grassberger and Reiter (2001) studied the effect of temperature on *Lucilia sericata* (= *Phaenicia sericata*) under ten different temperature regimes. Data from these studies were used to construct a graph from which maggot age could be predicted based on maggot length. Time from hatching to 3rd instar feeding stage was plotted against temperature, with each line representing identical larval length at various temperatures. If temperature is constant, the age of the maggot can be read off instantly from its length based on temperature, provided the maggots have not begun migrating.

The effect of temperature on the development of *C. macellaria* (Byrd and Butler 1996) and on *C. rufifacies* (Byrd and Butler 1997) was studied under various temperature regimes. Growth curves for the eggs, larvae, and pupae were developed under both cyclic and constant temperatures. Anderson (2000) obtained minimum and maximum

development rates of five forensically important Calliphoridae species at several temperatures, including *P. regina*, *P. sericata*, *Calliphora vicina*, *Eucalliphora latifrons*, and *Lucilia illustris*.

2.3.1. Identification

Keys and diagnostic descriptions for egg, larval, and pupal stages have been developed for several flies of forensic importance (Liu and Greenberg 1989). Greenberg and Singh (1995) described species identification of calliphorid eggs. Greenberg and Szyska (1984) studied the biology of fifteen species of Peruvian Calliphoridae. The work included developmental rates, successional activity of highland flies in Montaro Valley and a key to the known third instars of Peru. A similar key to the third-instar of eight species of *Chrysoma* (Calliphoridae) likely to occur on carrion in the U.S. also has been developed (Wells et al. 1999).

2.3.2. Relative Humidity Studies

Most of the laboratory studies on the development rates of forensically important insects have focused on the effect of temperature, with relative humidity held constant (e.g. Greenberg 1991, Davies and Ratcliffe 1994, Byrd and Butler 1997, Anderson 2000, Byrd and Allen 2001, Grassberger and Reiter 2002).

However, a number of studies performed on egg hatch rate of various arthropods have shown that relative humidity also affects development. Adkisson (1959) demonstrated that egg hatch rate of the pink bollworm, *Pectinophora gossypiella* (Saunders), was significantly reduced by low relative humidities. Egg hatch of the bluegrass webworm, *Parapediasia teterella* (Zincken) was found to be significantly reduced by relative humidities within the range of 10-50%; however, consistently high

egg hatch was reported at relative humidities greater than 60% (Morrison et al. 1972). A low ambient humidity was found to have an inhibiting effect on egg development of the tropical horse tick, *Dermacentor nitens* (Despins 1992).

Few studies have examined the effect of relative humidity on egg hatch rates of forensically relevant insects. In an extensive study of the influence of the environment on the sheep blow fly, *Lucilia sericata* (= *Phaenicia sericata*) Evans (1934) found the relationship of humidity to egg development duration was to be linear, with low humidity retarding hatch rate. He also demonstrated that the eggs of *L. sericata* lose water readily under dry conditions, but will not absorb water if placed in a saturated atmosphere. Water loss was shown to be the probable cause of arrested development and mortality at different combinations of temperature and humidity. However, the relationship between humidity and survival is influenced by temperature and length of exposure. Low humidity brought about death at low and high temperatures, while it was tolerated at median temperature (Evans 1934).

Davies (1947) studied the effect of fluctuating humidity on egg hatch and development of the *L. sericata* egg using saturated salt solutions to maintain humidity at constant levels (Winston and Bates 1960). Experiments were conducted at temperatures from 30–39°C with relative humidities from 0 – 95%. Results indicated that the minimum humidity for development at all was 50% RH at 37°C; however, survivorship varied from 11–92% among the fourteen experiments. Forty percent RH was the minimum humidity for development at 34°C, and 25% RH at 30°C with 68–70% of the eggs completing development. The minimum humidity of development for eggs at 38 and 39° C was 75 and 80%, respectively, with 95% of the eggs completing development

at 38 °C/ 75% RH and 10% of the eggs completing development at 39 °C/ 80% RH. Hatch rate was found to be slower with progressively lower humidities, and fewer eggs hatched. Similarly, Davies and Hobson (1935) found that *L. sericata* eggs required 90–100% RH at 37°C for rapid hatching.

Wall et al. (2001) examined the effects of humidity on egg hatch in *Lucilia sericata* (Meigen) using serial KOH dilutions to generate a range of humidity treatments: 32, 34, 51, 59, 85, and 98%. 100% RH was also generated using de-ionized water. All of the humidity treatments were maintained at 15, 20, and 30°C. The number of eggs that hatched after 48 h was recorded for each temperature/ RH treatment. Egg hatch increased significantly with increasing humidity, while no eggs hatched below 50%. Temperature was not found to have a significant effect on successful egg hatch for the temperatures tested (Wall et al. 2001).

It is clear from the results of these studies that relative humidity can be an important abiotic factor influencing the population dynamics and development of insects. Forensic implications of this include inaccurate PMI estimations based on insect development.

2.4. Phormia regina

The black blow fly, *Phormia regina* Walker, can be found throughout most of the United States and is commonly associated with decomposing remains during certain times of the year. Studies have found that it is present on carrion in many regions (Reed 1958, Watson and Carlton 2003). *Phormia regina* is known to enter diapause in the northeastern United States in October or November (Stoffolano 1974, Tatar and Yin 2001) where temperatures are generally cooler.

Adults are approximately 7–9 mm long and are identified readily by their dark-green, shiny body and a distinctive yellow-orange anterior spiracle (Byrd and Castner 2001). This species is most active during the spring and fall and, therefore, is considered a “cold-weather” fly (Byrd and Castner 2001). The adult female deposits her eggs during the early stages of decomposition and the larvae subsequently develop in the carrion. Eggs are approximately 1.2 ± 0.1 mm long and 0.03 ± 0.03 wide (Greenberg and Singh 1995) and are generally deposited in small clusters of about 12–80 eggs (Kamal 1958). Biological and developmental data have been collected in a number of studies for *P. regina* (Table 2-1). Kamal (1958) included *P. regina* in his study of the biology of thirteen species of Calliphoridae and Sarcophagidae. Feeding and oviposition habits in addition to other general behaviors were cited for the various species. Developmental data were collected under controlled conditions. Life history data were recorded for flies held at 80°F (12.4°C) and 50 ± 2 % RH (Table 2-1).

Table 2.1: Published laboratory developmental data for *Phormia regina* at various temperatures/relative humidities

Life stage	Temp °C (%RH)	Mean duration of stage (hrs)	Rearing medium	Reference
Egg ¹	10 (75)	----	center cut pork	Byrd & Allen (2001)
	15 (75)	70.3		
	20 (75)	21.2		
	25 (75)	18.9		
	30 (75)	17.4		
	35 (75)	15.7		
	40 (75)	9.8		
	45-35 (75)	9.1		
	35-25 (75)	17.9		
	25-15 (75)	21.5		
	26.7 (50)	16	beef liver	Kamal (1958)
	22	20	ground beef	Greenberg (1991)
	29	18		
	15	51.97	beef liver	Melvin (1934)
	17.8	34.42		
	20.6	24.14		
	23.3	18.10		
	26.1	14.32		
	28.9	11.42		
	31.7	9.51		
34.4	8.55			
37.2	8.13			
40	8.70			
42.8	No hatch			
1 st Instar	10 (75)	--	center cut pork	Byrd & Allen (2001)
	15 (75)	75		
	20 (75)	30		
	25 (75)	25		
	30 (75)	18		
	35 (75)	16		
	40 (75)	12		
	26.7 (50)	18		
	22	25	ground beef	Greenberg (1991)
	29	12		
	16.1	45.7 ²	beef liver	Anderson (2000)
	23	22 ²		
	2 nd Instar	10 (75)	--	center cut pork

	15 (75)	135		
	20 (75)	55		
	25 (75)	44		
	30 (75)	39		
	35 (75)	31		
	40 (75)	23		
	26.7 (50)	11	beef liver	Kamal (1958)
	22	25	ground beef	Greenberg (1991)
	29	15		
	16.1	166.5 ²	beef liver	Anderson (2000)
	23	81.9 ²		
3 rd Instar	10 (75)	--	center cut pork	Byrd & Allen (2001)
	15 (75)	218		
	20 (75)	82		
	25 (75)	95		
	30 (75)	96		
	35 (75)	66		
	40 (75)	60		
	26.7 (50)	36	beef liver	Kamal (1958)
	22	25	ground beef	Greenberg (1991)
	29	25		
	16.1	279 ²	beef liver	Anderson (2000)
	23	134.9 ²		
Prepupal	10 (75)	--	center cut pork	Byrd & Allen (2001)
	15 (75)	355		
	20 (75)	192		
	25 (75)	156		
	30 (75)	148		
	35 (75)	112		
	40 (75)	146		
	26.7 (50)	84	beef liver	Kamal (1958)
	22	125	ground beef	Greenberg (1991)
	29	110		
	16.1	392.2 ²	beef liver	Anderson (2000)
	23	202.5 ²		
Pupal	10 (75)	--	center cut pork	Byrd & Allen (2001)
	15 (75)	458		

	20 (75)	244		
	25 (75)	209		
	30 (75)	208		
	35 (75)	148		
	40 (75)	128 - no emergence		
	26.7 (50)	6	beef liver	Kamal (1958)
	22	116.5	ground beef	Greenberg (1991)
	29	99		
	16.1	483.3 ²	beef liver	Anderson (2000)
	23	242.9 ²		
Adult	16.1	789.9 ²	beef liver	Anderson (2000)
	23	402.5 ²		

¹ Time of egg hatch since deposit

² Time to reach stage

Melvin (1934) described the incubation period of eggs of certain muscoid flies (including *P. regina*) at eleven different constant temperatures ranging from 59°F to 109°F. Egg hatch time ranged from 8.7 hours to about 52 hours, with no egg hatch at 109°F (Table 2-1).

Anderson (2000) obtained minimum and maximum development rates of *P. regina* under constant temperatures. Data for larval development are reported for $16.1 \pm 0.01^\circ\text{C}$ and $23.0 \pm 0.02^\circ\text{C}$, but no egg hatch data are given. Greenberg (1991) summarized development time spent as egg, larva and pupa for *P. regina* and provided a chronology of development within the puparium (Table 2-1).

In a detailed study of the development of *P. regina* under various temperature regimes, Byrd and Allen (2001) measured the time to complete development for groups of flies under both constant and fluctuating temperature regimes. Development time for each life stage, including egg hatch and adult emergence, were described graphically. Egg hatch times for 25°C and below were similar to those reported by Kamal (1958) and Melvin (1934), but Byrd and Allen (2001) reported longer developmental times at 30°C and above than were reported by Melvin (1934). Both studies reported that 40°C approaches the upper lethal limit for the egg stage (Melvin 1934, Byrd and Allen 2001). Larval development times reported by Kamal (1958) were shorter than those reported by Byrd and Allen (2001) at 30°C and 25°C. Similar pupal duration times were reported by Byrd and Allen (2001) and Kamal (1958), but Bishopp (1915) gave a much shorter pupal duration time.

2.5. Entomotoxicology

Entomotoxicology is an area of investigation in which insects are utilized as toxicological indicators to detect ante- or perimortem ingestion of drugs, toxins or alcohol (Goff and Lord 1994). Because these factors can sometimes contribute to, or indicate the manner of death, it is important to know whether drugs, toxins and/or alcohol consumption occurred prior to death (Miller et al. 1994, Beyer et al. 1980). There has been an increase in drug-related deaths over the past 2 decades (Goff and Lord 2001). Traditionally, tissue, blood, or urine samples are analyzed for the presence of these substances. However, in many drug or alcohol related deaths, the decedents are often not discovered until the carcasses are in an advanced stage of decomposition when analysis of traditional specimens is difficult (Goff and Lord 2001).

Anthropophagic insects provide investigators with alternate toxicological specimens because such insects are usually present in decomposing remains long after tissue, blood, and urine become unsuitable for analysis. Drugs and toxins present in the decomposing tissue can often be identified in insect tissue or remnants (puparial cases, frass, excrement, etc.).

A study involving the extraction of a heavy metal from maggot tissue demonstrated that substances contained in the feeding medium could be identified in tissues of insects that fed on the medium. Nuorteva and Nuorteva (1982) found that mercury contained in food sources eaten by carrion-feeding insects could be detected at different levels in both immatures (including pupae) and adults. Calliphoridae larvae were reared on fish tissue containing known concentrations of mercury. The amount of mercury recovered from larval tissue was directly proportionate to the duration of the

feeding period of the larvae. Larvae feeding on mercury-containing tissue retained the heavy metal through pupation, and mercury was detected in the emerging adult flies (Nuorteva and Nuorteva 1982).

The use of fly larvae, pupae or insect remnants/remains as alternate toxicological specimens is well-documented in the literature. Beyer et al. (1980) recovered phenobarbital from homogenates of *C. macellaria* larvae collected during autopsy of a 22-year-old female who died of a suspected overdose. Larvae were homogenized with distilled water, and then a salt and sulfuric acid solution was added before analysis to precipitate the proteins. Phenobarbital was identified at a concentration of 100 ug/g.

Malathion was detected by gas chromatography at a level of 574 ug in 0.26g of pooled larvae from a sample collected from the decomposing remains of a 58-year-old male. Two species of flies common to the area, *Chrysomya megacephala* and *C. rufifacies* were identified from maggots collected from the body and analyzed. This case was the first where an organophosphate compound was detected in larvae feeding on decomposing tissues (Gunatilake and Goff 1989).

In a similar case, the remains of a 49-year-old male who had been dead 67 days were discovered in his house. Though the cadaver was completely putrefied, ample postmortem specimens including heart, liver, lung, spleen, kidney and bile, in addition to hundreds of calliphorid larvae, were collected and analyzed using the same high performance liquid chromatographic (HPLC) procedure. Five drugs, triazolam, oxazepam, phenobarbital, clomipramine, and alimemazine were identified in most of the tissues (triazolam was not recovered in spleen or kidney due to interferences in the assay), as well as the larval preparations (Kintz et al. 1990). Although the concentration

of drugs in the larval tissue was lower than in the cadaver tissues for most of the drugs, they were sufficient to yield positive results for all five drugs.

Amitriptyline and Nortriptyline were extracted from chitinized insect remnants (puparial casings, exuviae, and frass) collected from a residence in which mummified remains were discovered (Miller et al. 1994). It was estimated that the decedent had died over 2.5 years prior to discovery. The drugs were recovered from the remnants using both a strong acid extraction and a strong base extraction and the results suggested an acute amitriptyline overdose.

Detection of drugs and toxins in carrion-feeding insects is a growing area of investigation. It is critical to know the effect that antemortem ingestion of drugs and toxins have on insect development, particularly with regard to the PMI estimation. These effects must be considered when attempting to derive an accurate PMI estimation based on insect development. Several studies have been conducted to analyze the impact of antemortem ingestion of drugs and toxins on insect development and behavior (Goff et al. 1989, Goff et al. 1991, Goff et al. 1993, Hédouin et al. 1999). Flesh fly larvae (*Boettcherisca peregrina*) were reared on tissues from rabbits that received various levels of heroin prior to death (Goff et al 1991). Larvae feeding on tissue from treated rabbits were significantly larger than the larvae feeding on control tissue. Similarly, development rate was faster for larvae feeding on treated meat, although the concentration of heroin in the food medium did not affect development rate. However, the time required for pupation was much greater in pupae from colonies feeding on treated meat versus untreated meat. Goff et al. (1991) also reported that the differences in rates of development observed in the study were significant enough to alter PMI

estimates based on larval and pupal development by up to 29 h and 18–38 hr, respectively.

In a similar study, larvae of the flesh fly *Parasarcophaga ruficornis* were reared on tissue from rabbits that received 0.0, 37.5, 71.4, or 142.9 mg doses of methamphetamine antemortem (Goff et al. 1992). The rabbit receiving 142.9 mg died within two minutes of receiving the drug; the other rabbits were sacrificed in a CO₂ chamber within 10 minutes of receiving the drug. Larvae feeding on tissue from rabbits that were dosed with 71.4 and 142.9 mg of the drug developed faster from hours 30–60 than control larvae and larvae feeding on tissue from rabbits dosed with 37.5 mg of methamphetamine. Similarly, the pupal stage was significantly longer in colonies fed on tissues from treated rabbits versus the control colony. During the first instar (0–24 hr), rates of development were not significantly different among all experimental and control colonies. However, the maximum length of larvae from all colonies feeding on tissues containing methamphetamine was smaller than were those from the control colony. Goff et al. (1992) reported that the observed differences in development rates among experimental colonies were sufficient to alter PMI estimation based on larval development up to 18 h and up to 48 hours in PMI estimates based on pupal development.

Goff et al. (1993) also demonstrated the effects of amitriptyline on larvae of *P. ruficornis* (Fabricius) reared on tissues of rabbits that were administered different doses of the drug. Unlike the results observed in the heroin and methamphetamine studies, there were no significant differences in the rate of growth of colonies during the larval stage, whether feeding on tissue from rabbits given 300, 600, or 1000 mg of

amitriptyline. However, larval stages were observed to be longer in colonies fed the larger amounts of the drug. These differences were enough to skew PMI estimates based on the total duration of the larval stage under normal conditions by up to 30 h (Goff et al. 1993). The 600 and 1000 mg colonies required a significantly longer period of time to complete the pupal stage than the 300 mg and control colonies. This difference is similar to the results obtained in heroin and methamphetamine studies.

The effects of phencyclidine in decomposing tissues on the development of *P. ruficornis* were also analyzed (Goff et al. 1994). Larvae were reared on tissue from rabbits administered 3.7, 7.31, and 14.6 mg of phencyclidine via ear vein infusion. Although no significant differences in development were reported among colonies, larval mortality was observed to be directly related to the dosage of the drug administered. A mortality rate of 29% was reported for the colony feeding on tissue from the rabbit receiving 14.6 mg of phencyclidine, whereas mortality rates of 9.5%, 5%, and 0% were reported for the medium dose, low dose, and control colonies, respectively. Larvae in colonies feeding on tissue from rabbits receiving any amount of the drug experienced longer puparial durations than larvae in the control colony.

3. Insect Fauna Visiting Carrion in Southwest Virginia

3.1. Introduction

The major focus of medicolegal forensic entomology is on the use of insects to assist in criminal investigations, particularly in cases of unexplained or criminal death. These investigations attempt elucidate the cause and place of death, and also the postmortem interval (PMI) or time that elapsed since death (Keh 1985).

There are two main methods for estimating PMI using information on the insects that visit a corpse. The first method is development-based, in which the predominant development stage of the flies on the corpse is used to indicate the time since death. Flies usually are the first insects to lay eggs on a body, often within minutes in cases of unattended death (Catts 1990). Once the first colonizing species have been identified, PMI can be estimated by comparing the degree of development of the eggs, larvae, or pupae with laboratory data on their development times under a temperature regime similar to that of the period leading up to the discovery of the corpse.

The second method for estimating the PMI uses succession-based studies to gather information on the faunal progression or successional patterns of carrion-arthropods. By this method, the PMI is estimated by comparing the composition of taxa found on human remains at the time of discovery (corpse fauna) with the composition of insects obtained under controlled conditions at different time intervals on an animal model (baseline fauna) (Schoenly et al. 1996). The type and composition of taxa that are attracted to a carcass usually change in a predictable pattern as decomposition progresses (Smith 1986). Also, the pattern of succession of insects is specific to the location and environmental conditions in which a carcass occurs (Payne 1965). Because taxa can vary

greatly with locale, it is important for precise estimation of the PMI to identify the forensically important insects that are specific to an area (Anderson 2000).

Studies of carrion arthropods have been conducted in several regions of the world to determine species composition and their successional patterns (Bornemissza 1957, Payne 1965, Early and Goff 1986, Anderson 1996, Dillon 1997). However, there are no such studies on the forensically important insects in southwest Virginia. This chapter presents a qualitative assessment of the major insect taxa visiting and/or colonizing domestic pig carcasses during specific periods in spring, summer, and fall in southwest Virginia.

3.2. Materials and Methods

Studies on the occurrence of carrion-arthropods were conducted in Blacksburg (37°11`N, 80°25`W) in southwest Virginia. The area is ~608 m above sea level and has average ambient temperatures of 15, 20.7, and 15.5 °C in the spring, summer, and fall, respectively; average annual rainfall and snowfall are 102 and 71 cm, respectively (National Weather Service). Four studies (spring: April 25th – June 20th and summer: June 25- July 9 2001 and 2002) were conducted at Kentland Farm, an agriculture research facility of the Virginia Polytechnic Institute and State University (Virginia Tech). A fifth study was conducted in fall (October–November) 2002 at a second farm located ~16 km from Kentland Farm.

Two pig carcasses from the Virginia Tech Swine Center were used in each study. Carcasses used in the spring studies were generally larger (41–45 kg) than those in the summer and fall studies (23–27 kg). The pigs electrocuted and transported immediately to the field sites. Each carcass was placed inside a 92x92x153 cm cage constructed of

2.54 cm steel-welded tubing enclosed with 1.27 cm mesh hardware cloth. Cages were open on the bottom so that the carcasses were in direct contact with the ground. Cages also were staked to the ground to prevent disturbance of the carcasses by vertebrate scavengers.

The two cages used in each study were located ~90 m apart at the edge of an open field bordered by a thickly wooded area. Although both cages received direct sunlight during most of the day, one of the cages was more shaded during the afternoon. Two round pitfall traps (12 mm in diameter and 7mm deep) were installed ~8 cm from the abdomen of each carcass and a plastic cup filled with water and a few drops of liquid soap were placed in each trap to capture crawling insects.

Because decomposition of the carcasses progressed at different rates during the spring, summer, and fall studies, the sampling protocol was adjusted accordingly for each period. Sampling was conducted daily (between 1200–1500 hr) for 21 days of the spring studies and 8 days of the summer studies. In the fall, sampling was conducted at irregular intervals for ~ 28 days.

Sampling of adult insects was conducted with aerial net sweeps above and around the carcass, with pitfall traps, and by taking specimens directly off the carcass to assess species occurrence. An assessment was also made of the relative abundance of taxa identified to family based on visual observations and collected specimens. Fly eggs and maggots were collected, when present, and reared on ground pork to the adult stage for species identification. Beetle larvae were not collected at regular intervals.

Most of the specimens were identified to genus or species using taxonomic keys. Specimens were also sent to taxonomic specialists for verification and voucher specimens were placed in the Museum of Natural History at Virginia Tech.

Other data were recorded at the time of sampling, including ambient temperature ~16 mm above the carcass (using a digital thermometer), temperature in the center of the maggot mass (using a meat thermometer) (Figure 3.1), and rainfall ~16 mm from the carcass (using a rain gauge). A weather station on the grounds of the research farm provided additional hourly temperature and rainfall data (Kentland Farm Weather Station).

3. 3. Results

Tables 3.1 and 3.2 show the major necrophagous taxa (taxa actually feeding on the corpse), predators of necrophagous species (taxa feeding on the necrophagous species) and life stages collected from decomposing pig carcasses during 21 days of the spring, 8 days of the summer, and 12 intervals in the fall studies. Specimens were identified using taxonomic keys as indicated (Tables 3.1 and 3.2).

Table 3.1: Diptera identified from specimens collected from pig carcasses in southwest Virginia in spring and summer 2001 and 2002, and fall 2002

Family ^a	Genus/ Species	Study Periods ^b		
		Spring	Summer	Fall
Calliphoridae [Hall and Townsend 1977]	<i>Phormia regina</i> Meigen	A, I	A, I	A
	<i>Phaenicia coeruleiviridis</i> Macquart	A, I	A, I	A, I
	<i>Phaenicia sericata</i> Meigen	A, I	A, I	A, I
	<i>Lucilia illustris</i> Meigen	A, I	A, I	A, I
	<i>Cochliomyia macellaria</i> Fabricius		A, I	
	<i>Calliphora vomitoria</i> Linnaeus	A, I		A
	<i>Calliphora vicina</i> Robineau-Desvoidy	A		A
	<i>Pollenia rudis</i> Fabricius	A	A	
Sarcophagidae [Sanjean 1957]	<i>Helicobia rapax</i> Walker	A	A	
	<i>Sarcophaga</i> Meigen	A	A	A
	<i>Sarcophaga sinuata</i> Meigen	A		
	<i>Sarcophaga houghi</i> Aldrich	A		
	<i>Sarcophaga utilis</i> Aldrich	A	A	
	<i>Sarcophaga mimoris</i> Reinhard		A	
	<i>Sarcophaga bullata</i> Parker	A, I	A, I	
	<i>Ravinia</i> Robineau-Desvoidy	A	A	
	<i>Boettcheria</i> Parker	A		
	<i>Boettcheria cimbicis</i> Townsend	A		
	<i>Macronychia aurata</i> Coquillett	A		
	<i>Blaesoxipha</i> Loew	A	A	

	<i>Amobia oculata</i> Zetterstedt	A		
	<i>Spirobolomyia flavipalpis</i> Aldrich	A		
	<i>Oxysarcodexia ventricosa</i> Wulp	A		
	<i>Oxysarcodexia</i> Townsend		A	
Muscidae (Shinonaga and Kano 1971)	Unidentified Muscidae	A	A	A
	<i>Musca domestica</i> Linnaeus	A	A	
	<i>Hydrotaea leucostoma</i> Weidemann	A	A	
Sepsidae (Ozerov 2000, Pont 1979)	<i>Sepsis neocynipsea</i> Melander and Spuler	A	A	
	<i>Sepsis flavimana</i> Meigen	A	A	
	<i>Sepsis punctum</i> Fabricius	A	A	
	<i>Meroplus minutus</i> Wiedemann	A	A	
Piophilidae [McAlpine 1977]	<i>Stearibia nigriceps</i> Meigen	A	A	A
	<i>Prochyliza xanthostoma</i> Walker	A	A	A
Sphaeroceridae [McAlpine 1987]	<i>Lotophila atra</i> Meigen	A		

^aName in square brackets refer to taxonomic keys listed in references, which were used for the initial identification of taxa.

^bAn “A” indicates that the species was collected as an adult; an “I” indicates that the immature stage (eggs and/or larvae) were collected.

Table 3.2: Coleoptera identified from specimens collected from pig carcasses in southwest Virginia in spring and summer 2001 and 2002

Family ^a	Genus/ Species	Study Periods ^b	
		Spring	Summer
Staphylinidae	<i>Creophilus maxillosus</i> Gravenhorst	A	A
	<i>Platydracus maculosus</i> Gravenhorst	A	A
	<i>Ontholestes cingulatus</i> Gravenhorst	A	A
	<i>Aleochara lata</i> Gravenhorst	A	A
	<i>Philonthus sericans</i> Gravenhorst	A	A
Silphidae (Anderson and Peck 1985, Downie and Arnett 1996, Ratcliffe 1996)	<i>Oiceoptoma</i> Leach	A	A
	<i>Oiceoptoma noveboracense</i> Forster	A	A
	<i>Nicrodes surinamensis</i> Fabricius	A	A
	<i>Nicrophorus marginatus</i> Fabricius	A	
	<i>Nicrophorus orbicollis</i> Say	A	
	<i>Nicrophorus tomentosus</i> Weber		A
	<i>Necrophila americana</i> Linnaeus	A	A
Cleridae	<i>Necrobia rufipes</i> De Geer	A	A
	<i>Necrobia ruficollis</i> Fabricius	A	A
	<i>Necrobia violacea</i> Linnaeus	A	
Trogidae	<i>Trox</i> Fabricius	A	
Dermestidae	<i>Dermestes</i> Linnaeus	A	A
Histeridae	<i>Hister abbreviatus</i> Fabricius	A	A
	<i>Hister coenosus</i> Erichson		A

<i>Euspilotus assimilis</i> Paykull	A	A
<i>Margarinotus foedatus</i> LeConte	A	A
<i>Margarinotus</i> Marseul	A	

^a Numbers in square brackets refer to taxonomic keys listed in references, which were used for the initial identification of taxa.

^b “A” indicates that the species was collected as an adult.

Visitation of the carcasses by insects occurred within the first two days of each study, with the earliest arrivers being dipterans in the families Calliphoridae, Sarcophagidae, and Muscidae (Figure 3.1, Figure 3.2). Six families of flies were observed visiting or colonizing the pig carcasses in the spring and summer (Table 3.1). *Phormia regina* was the dominant dipteran species in the spring comprising >90% of the specimens collected. However, *P. regina* was co-dominant with *Phaenicia coeruleiviridis* in the summer. Other blow flies (*Phaenicia sericata*, *Lucilia illustris*, *Calliphora* spp.) were found less frequently. Eleven species of Sarcophagidae in nine genera were identified from the spring and summer samples, with *Sarcophaga utilis* and *Helicobia rapax* being the most frequently collected species. *P. regina*, *P. sericata*, and *Sarcophaga bullata* immatures collected in the spring were identified as adults after rearing. Similarly, *P. regina*, *P. sericata*, *L. illustris*, and *Cochliomyia macellaria* were identified from reared specimens originally collected in the summer.

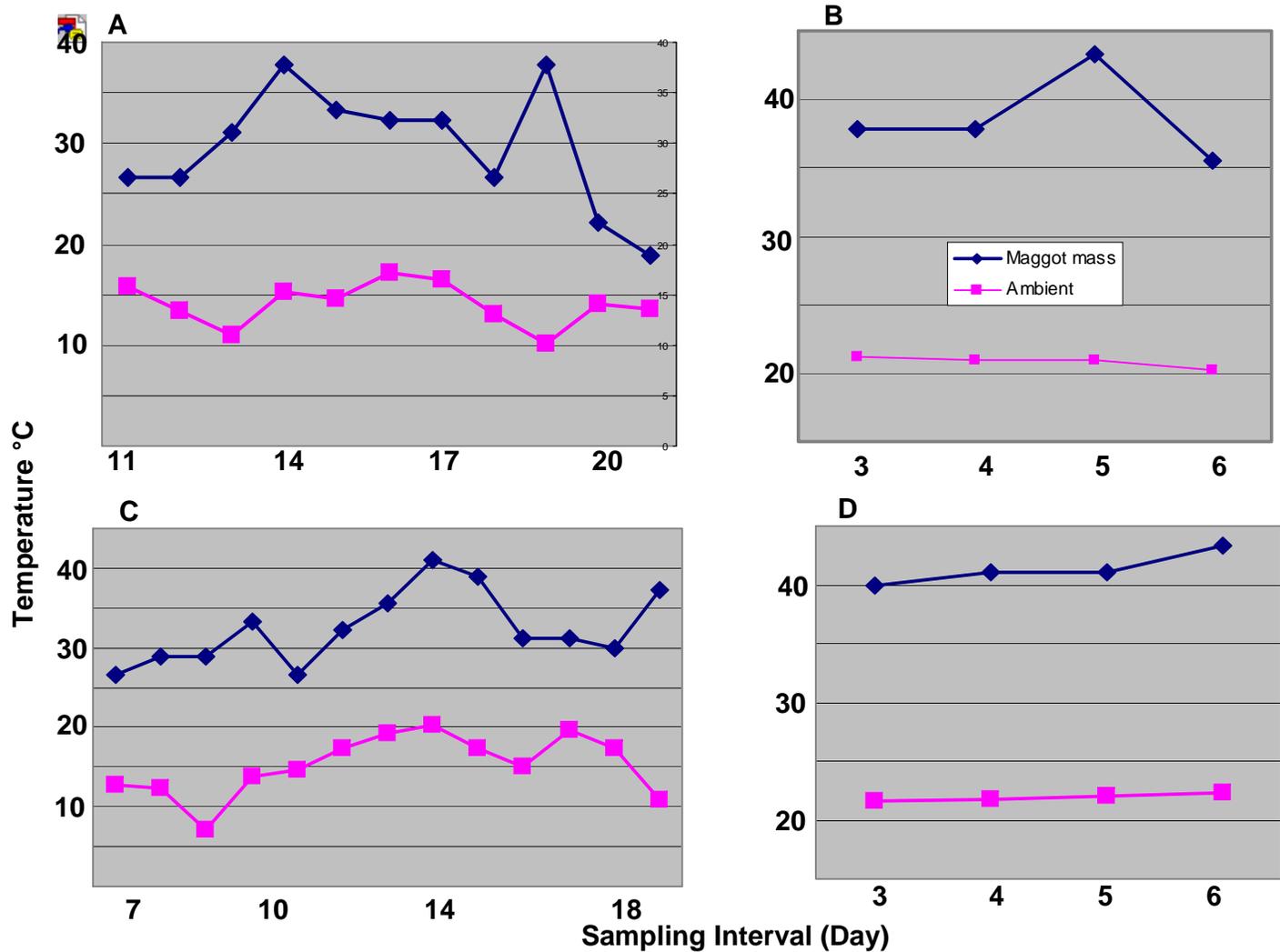


Figure 3.1: Maggot mass temperatures and corresponding ambient temperatures for succession studies in spring 2001 (A), summer 2001 (B), spring 2002 (C) and summer 2002 (D)

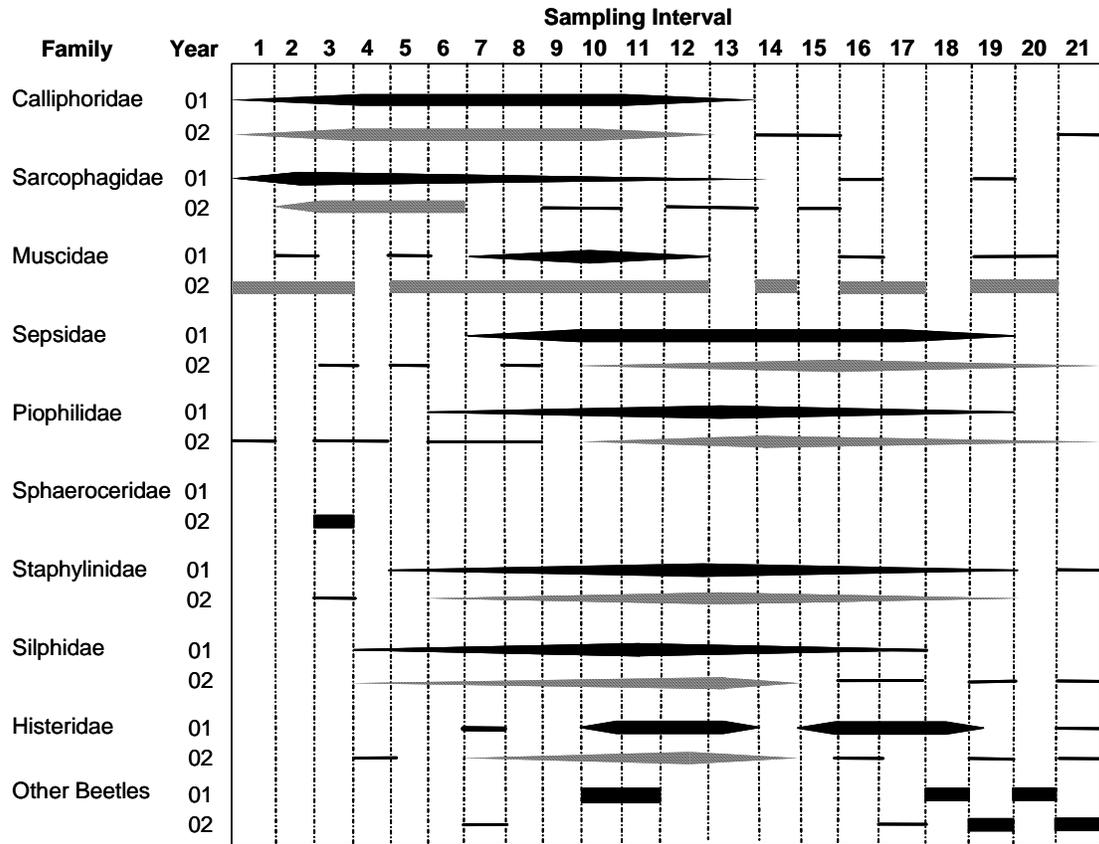


Figure 3.2: Occurrence and relative abundance (indicated by the thickness of bands) of families of insects found on pig carcasses in spring 2001 and 2002 in southwest Virginia. Sampling interval is one day.

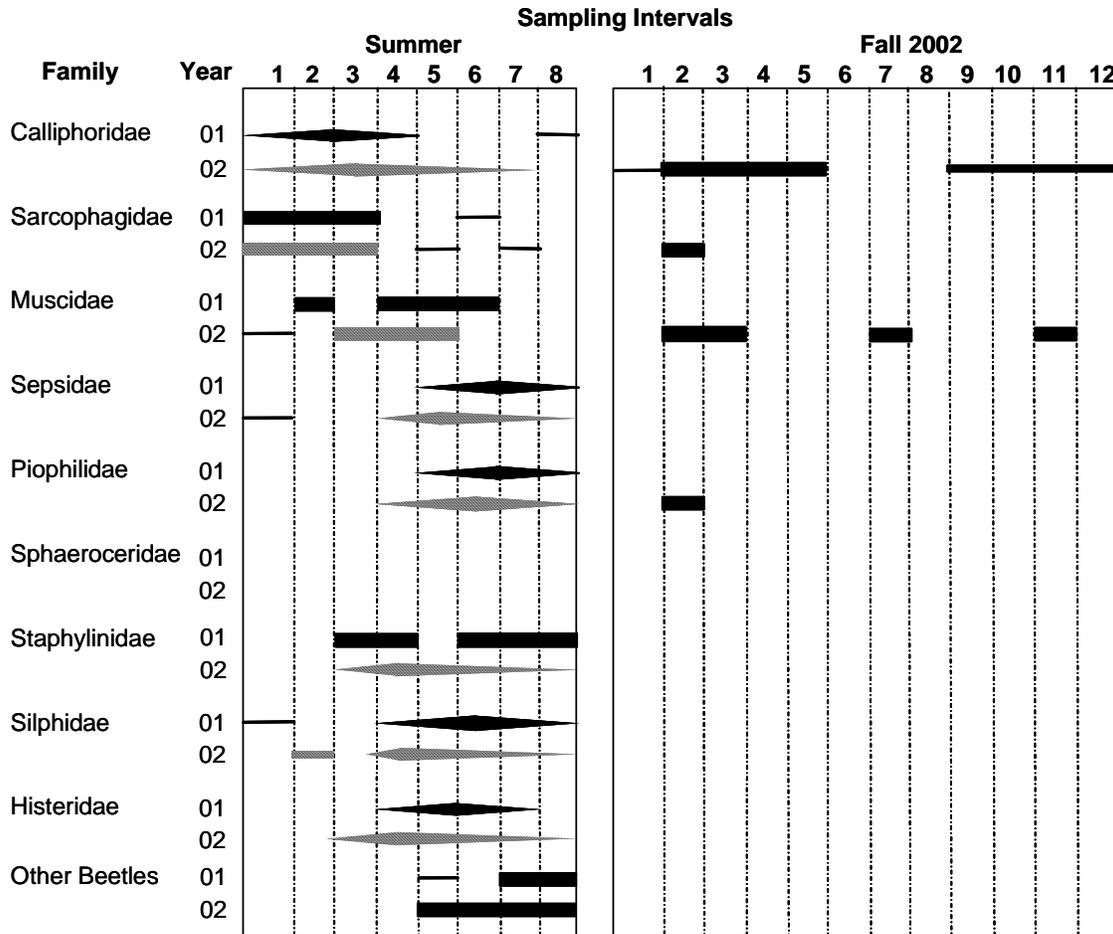


Figure 3.3: Occurrence and relative abundance (indicated by the thickness of bands) of families of insects found on pig carcasses in summer 2001 and 2002, and fall 2002 in southwest Virginia. Sampling interval in the summer is one day; samples were collected at irregular intervals in the fall. The “Other Beetles” group includes Cleridae, Trogidae and Dermestidae

Some of the muscids collected in the spring and summer were not identified beyond family (Table 3.1, “unidentified muscids”). This group of unidentified specimens, however, was thought to contain at least three additional species of muscids based on morphological characteristics.

Six families of beetles were collected (Table 3.2). The dominant species collected in the spring and summer included three staphylinids (*Creophilis maxillosus*, *Platydracus maculosus*, and *Aleochara lata*), three silphids (*Oiceoptoma noveboracense*, *Necrodes surinamensis*, and *Necrophila americana*) and two histerids (*Euspilotus assimilis* and *Hister abbreviatus*).

In the fall, visitation and colonization of the carcasses by insects was minimal during the first two weeks (Figure 3.2). *Calliphora vomitoria*, *L. illustris* and *P. coeruleiviridis* were the first and most dominant fly species to visit the carcasses, with only a few specimens of *P. regina*, *P. sericata*, *P. vicina*, and *Sarcophaga* spp. *Phaenicia coeruleiviridis*, *P. sericata*, and *L. illustris* also were identified from specimens that were reared. No adult insects were observed visiting the carcasses during the third and fourth weeks, and all of the maggots on the carcasses had died. Very few muscids and no beetles were collected in the fall due to cold temperatures (Table 3.3).

3.4. Discussion

Over 50 insect taxa were collected and identified during the spring, summer and fall studies. Taxonomic composition was similar in spring and summer, but slightly more species were collected in the spring. The total number of taxa reported in similar studies on vertebrate carcasses ranged from 30–522 (Reed 1958, Burger 1965, Payne 1965, Early and Goff 1986, Tullis and Goff 1987, Anderson and VanLaerhoven 1996, Richards and

Goff 1997, Carvalho et al 2000). Factors such as climate, location, surrounding habitat, sampling frequency, and use of different animal models may account for the wide range of taxonomic diversity reported in these studies.

The insects and other arthropods visiting a corpse can be classified into four groups based on their ecological role: Necrophagous species (taxa actually feeding on the corpse), predators and parasites of necrophagous species, omnivorous species (e.g. wasps, ants, and some beetles), and opportunistic species such as collembola, spiders, and centipedes, which exploit the corpse for habitat. This study reports only insect taxa falling into the first two categories.

When estimating PMI from development-based entomological data, the most significant taxa for use in PMI estimation are necrophagous species. These species are the first colonizers laying eggs on an intact carcass. Colonizing species of greatest importance in the early stages of decomposition usually are those from three dipteran families: Calliphoridae, Sarcophagidae, and Muscidae.

Flies in the family Piophilidae are sometimes collected as adults in early stages of decomposition (Easton and Smith 1970, Byrd and Castner 2001) but are recognized mainly as late colonizers (Easton and Smith 1970, Early and Goff 1986, Carvalho 2000). *Stearibia nigriceps* adults were collected on day 1 in spring 2002 and at non-consecutive intervals throughout the remainder of the study. However, in the summer studies, the earliest this species was collected was on day 4 (in 2002) and day 5 (in 2001) and, thereafter, at consecutive sampling intervals. *Piophila casei*, which occurs in southwest Virginia and has been reported to be an important forensic fly (Virginia Museum of Natural History) was not collected during the studies. Possible reasons for this might be

that *S. nigriceps* outcompetes *P. casei* in this area or, since the studies ended before the carcasses reached the dry remains stage of decomposition, *P. casei* had not arrived before the study ended. Late-arriving insects such as dermesids and trogids were also rarely encountered.

Some insects visit but do not colonize a carcass; rather, they exploit the carcass and developing maggots as food resources. These non-colonizing insects include predators and parasites of necrophagous species, such as beetles in the families Silphidae, Staphylinidae, and Histeridae, and are useful in succession-based PMI estimations (Anderson 2000). Most of the beetles that are collected during succession studies fall into this category.

The overwhelming majority of eggs, maggots and adult flies collected in the spring and summer were those of *P. regina*. This species is distributed widely throughout the U.S. and is considered a cold weather fly (Byrd and Castner 2001). It is not surprising, therefore, that *P. regina* was the dominant species recovered in the spring. Byrd and Castner (2001) reported that *P. regina* is typically not found during the hot summers of the southern United States. However, Dillon (1997) noted that *P. regina* was co-dominant with *L. illustris* in spring and summer studies in British Columbia, Canada where ambient temperatures were $>35^{\circ}\text{C}$ on certain days. In this study, *P. regina* was collected as frequently in the summer as in the spring.

Few adult specimens of *P. regina* were collected in the fall, even though the range of temperatures for the first two weeks of that study was similar to the early period of the spring studies (Table 3). *P. regina* is known to enter diapause in the northeastern United States in October or November (Stoffolano 1974, Tatar and Yin 2001) where

Table 3.3: Temperature and rainfall recorded during the spring, summer, and fall studies of insect occurrence on pig carcasses in southwest Virginia

Study Period	Mean (range)		Total (range) Rainfall (cm)
	Ambient (daytime) Temperature (°C)	Maggot Mass Temperature (°C)	
Spring 2001	18.3 (1.8–28.0)	32.0 (22.0–39.0)	8.2 (0.0–4.6)
Summer 2001	22.6 (13.2–29.1)	37.0 (31.0–42.0)	2.3 (0.0–0.7)
Spring 2002	17.7 (5.5–28.3)	36.0 (21.0–43.0)	6.7 (0.0–4.2)
Summer 2002	24.5 (15.9–31.7)	40.0 (37.0–43.0)	4.2 (0.0–2.5)
Fall 2002	8.8 (-3.1–20.8)	17.0 (11.0–29.0)	15.7 (0.0–2.6)

temperatures are generally cooler. Diapause is likely to have occurred for this species in southwest Virginia, despite the fact that this region is considered part of the south.

Interestingly, while *P. coeruleiviridis* was co-dominant with *P. regina* in the summer based on the number of adults collected, no individuals were identified from reared specimens. *P. coeruleiviridis* is difficult to rear under laboratory conditions compared with other blow flies (Hall and Doisy 1993, personal observation). Therefore, it is likely that this species also colonized the carcasses in the summer and was collected, but not reared successfully to the adult stage for identification.

Very few insects were collected during the first two weeks of the fall study, although the carcasses were colonized by *P. coeruleiviridis*, *P. sericata*, and *L. illustris*. During the third and fourth weeks of this study temperatures were consistently $\leq 10^{\circ}\text{C}$. At this point, adult insects disappeared and maggots that were present initially on the carcasses died before pupation.

Of the locations where succession studies have been conducted, Knoxville, TN (Reed 1958) is the most similar in proximity and climate to southwest Virginia. Not surprisingly, many of the same species were reported from carcasses in the two locations, although Reed (1958) reported a greater number of insect species (a total of about 217) than found in this study. Several factors might account for this difference. Reed (1958) used more carcasses (45 dog carcasses versus 10 pig carcasses in this study) and sampled in nine different areas (versus two for this study) throughout an entire year. In addition, Reed (1958) reported incidental, omnivorous and opportunistic species as well as necrophagous and predators of necrophagous species. Lastly, temperatures in the winter

months during the year of the Tennessee studies were much warmer than they generally are in southwest Virginia at the same time of year.

The differences between the study by Reed (1958) and this one highlight the need for succession studies in different areas due to dissimilarities in species composition. Succession-based PMI estimation requires the knowledge of local carrion fauna. It provides a complementary approach to PMI estimation using development-based data, which usually results in a narrower estimate of the PMI; however, this approach can only be used when the maggots and/or pupae are still present. Analyzing succession data for PMI estimation is most useful in later stages of decomposition. Although this method gives less precise PMI estimates, succession patterns can sometimes contribute critical clues to an investigation, such as in cases where the body has been moved from the site of death (Catts and Goff 1992). The diversity of species, type of species, number of individuals, life stages present, and the number of individuals of each life stage all can be derived from succession studies and provide useful clues about the circumstances surrounding the death of the individual.

4. Analysis of the Successional Patterns of Insects on Carrion in Southwest Virginia

4.1. Introduction

Medicocriminal or medicolegal forensic entomology is the area of forensic entomology that focuses on the use of arthropod evidence in criminal investigations, particularly those involving wrongful or unexplained death (Hall 1990, 2001). Data collected by forensic entomologists on the arthropod populations associated with a deceased individual can be used to determine the place where death occurred and to estimate the postmortem interval (PMI) or the time that elapsed since death of that individual (Hall 2001).

Information on the development and succession of arthropod species (mainly insects) are used in two primary ways to estimate the PMI of human remains. In cases where death was recent, the PMI can be estimated by analyzing the degree of development of early-arrival species that colonize the corpse (Goff 1993, Wells and Lamotte 2001). Data on the development of forensically important species derived from controlled studies (e.g., Bishopp 1915, Melvin 1934, Kamal 1958, Greenberg and Szyska 1984, Liu and Greenberg 1989, Greenberg 1991, Byrd and Butler 1996, Byrd and Butler 1997, Anderson 2000, Byrd and Allen 2001) are used to estimate the degree of development of similar species collected on the corpse. The PMI of a corpse also can be estimated from the successional patterns of carrion-arthropods. Because insects arrive at a corpse in a predictable manner specific to the location and environmental conditions under which the remains are found (Payne 1965), the time of death of the individual can be estimated using succession data when it is not possible to use the degree of insect development. In such cases, the composition of taxa found on a corpse at the time of

discovery (corpse fauna) are usually compared with the composition of the arthropod assemblage at a given period of time derived from an animal model (baseline fauna) to estimate the PMI (Schoenly et al. 1996). However, one should be aware that factors such as location, environmental conditions, whether or not the body is covered or indoors, the surrounding habitat, etc. can alter successional patterns.

The two approaches for estimating PMI (i.e., based on arthropod development or successional patterns) are complementary, although each may be better suited for use during different stages of carcass decomposition. The use of development data is most applicable in the early stages of decomposition when the immature stages of the first colonizers are present. This approach enables investigators to establish a minimum PMI because flies generally do not deposit their eggs on live individuals. Succession data are most valuable in the later stages of decomposition when the earliest colonizers are no longer present. In addition, succession data also can provide information on the diversity of species, number of individuals, life stages that are present, and the number of individuals in each life stage (Keh 1985).

Although the decomposing corpse represents a dynamic ecosystem that attracts different taxa as it changes (Goff 1993), the pattern of visitation on the corpse is somewhat predictable (Rodriquez and Bass 1983, Anderson 2001). The first and most significant insects to arrive at a corpse are typically species of necrophilous flies in the families Calliphoridae and Sarcophagidae. As decomposition progresses, the corpse becomes attractive to different taxa. Predators of fly maggots, including beetles in the families Silphidae, Staphylinidae and Histeridae, form another significant group of early-arriving carrion taxa, but these insects typically do not arrive until after the first maggots

appear (Goff 1993). Late arrivers such as larval beetle species in the family Dermestidae, invade a copse in the dry stages of decomposition after early colonizing taxa have already left the remains (Easton and Smith 1970, Rodriquez and Bass 1983). While succession of insects generally follows a similar pattern at the family level, there appears to be a lot of variation at the genus and species levels among locations. However, each location and type of environment tends to have a predictable and representative pattern of arthropod succession (Anderson 2001).

An understanding of the occurrence and successional patterns of forensically important taxa in the specific area (or a very similar area) in which decomposing remains are discovered is required if successional data are to be useful in estimating PMI (Anderson 2001). Studies on the successional patterns of arthropods (based on arthropod visitation) have been conducted throughout the world in climactically different areas using several different animal models (e.g., Bornemissza 1956, Reed 1958, Payne 1965, Rodriguez and Bass 1983, Early and Goff 1986, Anderson and VanLaerhoven 1996, Dillon 1997). However, there are still many regions for which no baseline successional data are available on forensically important arthropods. The purpose of this investigation was to collect data on carrion-arthropod succession (baseline fauna) for Montgomery County and nearby regions in southwest Virginia, which could be used for estimating PMI in cases of human death. The study describes the process of gathering these data on the successional patterns of carrion insects and examines the seasonal changes in these patterns through the analysis of occurrence matrices.

4.2. Materials and Methods

4.2.1. Study Site

Studies were conducted in spring (late-April to June) and summer (late-June to July) of 2001 and 2002 at Kentland Farm (37°11`N, 80°25`W, altitude 608 m), a research facility of Virginia Polytechnic Institute and State University (Virginia Tech) located in Montgomery County in southwest Virginia. Climate in the area is temperate with average temperatures in the spring and summer of 15.0 and 20.7° C, respectively. Average annual rainfall is 102 cm and average annual snowfall is 71 cm (National Weather Service, www.nws.noaa.gov).

4.2.2. Animals and Cages

Catts and Haskell (1990) suggested that the domestic pig (*Sus scrofa* Linnaeus) can be used as a model species for humans in decomposition studies. Therefore, commercially cross-bred pig carcasses obtained from the Swine Center at Virginia Tech were used in the successional studies conducted during the two periods (spring and summer) each year. Two pigs were used in each study. The animals in the spring studies were larger (\approx 41–45 kg) than those in the summer studies (\approx 23–27 kg). Each of the pigs was sacrificed by electrical shock early in the morning when temperatures were still relatively cool and fly activity was low. The carcasses were transported immediately to the test site at Kentland farm, which is \approx 13 km from the Swine Center. We examined the carcasses carefully for signs of fly oviposition before each was placed inside a test cage.

Each pig carcass was placed inside a wire cage with its legs pointing toward the hinged door on the front of the cage (Figure 4.1). The cages each measured 92x92x153 cm and consisted of a metal frame constructed with 2.5 cm steel-welded tubing, which was enclosed with 1.27 cm mesh hardware cloth. The bottoms of cages were open so that

the carcasses were in full contact with the ground. The cages also were staked to the ground to prevent disturbance of the carcasses by scavengers.



Figure 4.1: Cage (with an open-bottom) used to isolate pig carcasses during succession studies. Hinged door at the front of the cage provided easy access to the carcass during the study

In each study, the two cages with pig carcasses were placed ≈ 90 m apart at the interface of open pasture and a thickly wooded area. Because decomposition and colonization of a carcass by insects is affected by the placement of the carcass (Shean et al. 1993), the cages were positioned so that both carcasses received direct sunlight until mid-day, although one carcass usually was slightly shaded during the afternoon.

4.2.3. Sampling Protocol

A typical sample included adult insects obtained from aerial net sweeps above and around the carcass and immature stages collected, using forceps, directly off the carcass, whenever they were present. Two pitfall traps consisting of a plastic cup filled with

soapy water also were placed 8 cm from the abdomen of each carcass to collect crawling insects.

Sampling intensity in spring and summer studies was determined mainly by the rate of decomposition of the carcasses. In the spring, sampling was done twice daily for the first 10 d and, thereafter, once per day for 11 d; in the summer, sampling was done twice daily for the first 3 d and then once per day for the next 5 d.

Adult samples were placed immediately in individual 4.5 liter Hefty Onezip® plastic bags (Pactiv Corp. Lake Forest, IL) containing a piece of paper towel saturated with ethyl acetate. Approximately 50 maggots or eggs were placed directly into glass vials filled with KAA fixative (10 ml kerosene, 80–100 ml 95% ethanol, and 20 ml glacial acetic acid) (Catts and Haskell 1990), which was replaced with 80% ethyl alcohol within 24 h. Another group of 50 maggots or eggs was collected for rearing to the adult stage. Each group was reared in 500 ml MicroGourmet® SOLO cups (Solo Cup Co., Urbana, IL). The maggots were placed in pouches made with aluminum foil containing ground pork (Catts and Haskell 1990) and a wet paper towel to keep the insects and meat moist. Each pouch was then placed in a rearing cup containing approximately 7 cm of Hoffman® vermiculite (A.H. Hoffman, Inc., Lancaster, NY) in which migrating maggots could pupate. A large hole was cut in the lid of each cup to provide aeration and a piece of mesh cloth placed under the lid to prevent maggots from escaping. The rearing cups with maggots were placed in an incubator set at approximately 27° C. Adults that emerged were killed after a few days by placing the rearing cups in a freezer for 30 min. These adults and the specimens collected as adults were pinned, labeled, and identified to

species. Specimens also were sent to taxonomic specialists for verification and vouchers were placed in the Virginia Museum of Natural History at Virginia Tech.

4.2.4. Data and Statistical Analysis

Data collected from the two pigs in each study were combined to develop a diagram of succession of insect taxa and an occurrence matrix. Therefore, a total of four occurrence matrices were developed (for spring and summer 2001 and 2002). As shown in the hypothetical example in Figure 4.2 (A and B), 1 and 0 in the occurrence matrix represent the occurrence and absence, respectively, of a taxon within a sampling interval. For each of the occurrence matrices developed in this study, we derived Jaccard similarity coefficients to describe temporal changes in the between-sample similarities in composition of insects during the study period (Schoenly 1992). The Jaccard metric ranges from 0–1 representing complete dissimilarity between sampling intervals for any species to a perfect match of species between sampling intervals (Schoenly 1992, Krebs 1999). For each occurrence matrix, we then used the between-sample or pairwise similarities to calculate an overall or mean similarity (S_{gmean}) of insect species throughout the entire study period (i.e., among all sampling intervals).

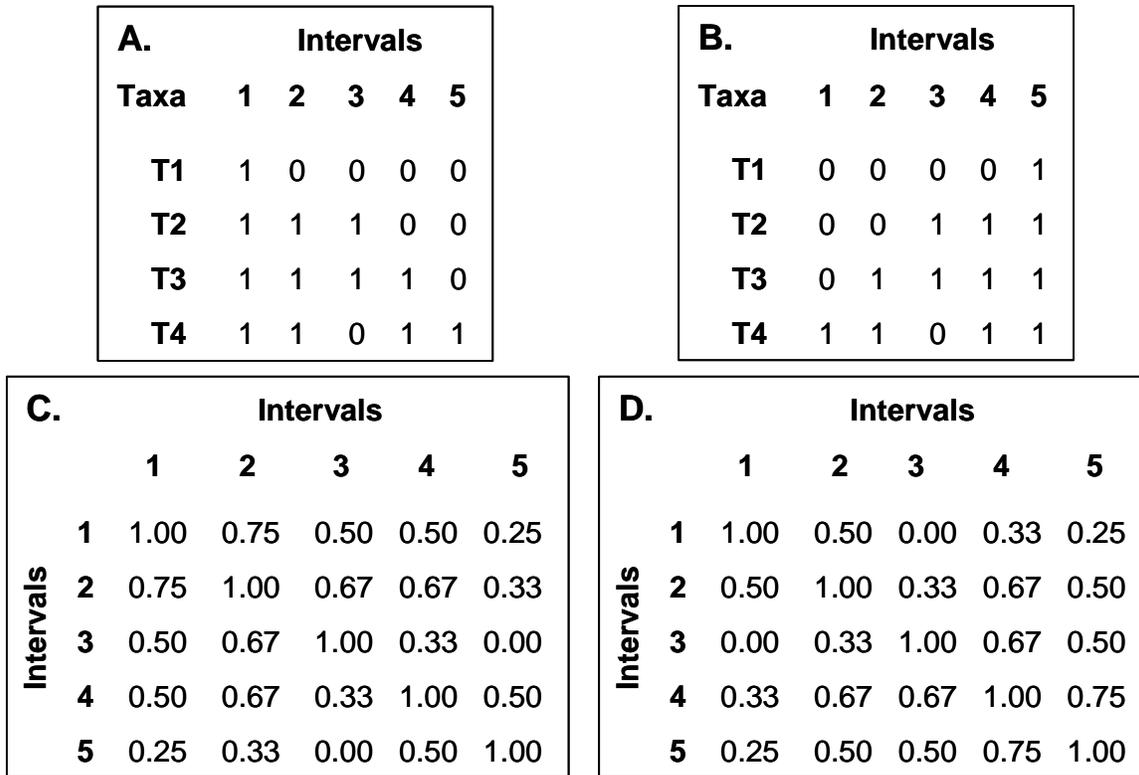


Figure 4.2: Hypothetical occurrence matrices (A and B) showing four taxa sampled over five intervals. C and D show the symmetric Jaccard similarity matrices for A and B, respectively. Average similarities for sampling intervals 1–5 in C are 0.50, 0.60, 0.38, 0.50, and 0.27, respectively; average similarities for sampling intervals 1–5 in D are 0.27, 0.50, 0.38, 0.60, and 0.50, respectively. S_{gmean} (overall similarity) of A = S_{gmean} of B = 0.45. Observed Pearson correlation (K_{obs}) between A and B is 0.48 with $P = 0.51$, the probability of obtaining a K -value ≥ 0.48 under the null hypothesis; the null hypothesis of no similarity in the patterns of occurrence in A and B cannot be rejected

The precision of S_{gmean} (i.e., $S_{gmean} \pm 95\%$ CL) was determined using the Jackknife procedure (Mayer et al. 1986, Manly 1997, Krebs 1999). By the Jackknife procedure, each of the n sampling intervals in an occurrence matrix was omitted one-at-a-time, with replacement, and recomputed the overall similarity of taxa (S_{-i}) for the new occurrence matrix with $n - 1$ sampling intervals. The Jackknife estimate or pseudo-value of overall similarity (PV_i) for the occurrence matrix with the i th sampling interval discarded was calculated as,

$$PV_i = nS_{gmean} - (n - 1)S_{-i} \quad i = 1 \dots n \quad (1)$$

where n is the number of sampling intervals in the original occurrence matrix, S_{gmean} is the overall similarity for the original occurrence matrix with n sampling intervals, and S_{-i} is the partial estimate of S_{gmean} when the i th sampling interval is discarded. Finally, the PV_i values ($i = 1 \dots n$) were used to compute the Jackknife estimate of S_{gmean} as

$$\hat{S}_{gmean} = \frac{1}{n} \sum_{i=1}^n PV_i \quad (2),$$

along with its standard error, and 95% confidence intervals.

Overall Jaccard similarity indices for two occurrence matrices with similar numbers of sampling intervals and taxa can be equal and yet the successional patterns of taxa in the matrices could differ (e.g., Figure 4.2). Therefore, S_{gmean} cannot be used with certainty to measure the degree of similarity between the successional patterns of species in two occurrence matrices of similar size. To determine the degree of similarity in species occurrence for corresponding periods in the two years (i.e., spring 2001 and 2002; summer 2001 and 2002) and hence the degree of consistency in the successional patterns of insects for those periods, a permutation approach based on Mantel's test was applied,

which has been used to compare distance or similarity matrices (Mantel 1967, Dietz 1983, Cheverud et al. 1989, Manly 1997). As is typical in this type of analysis (e.g., Cheverud et al. 1989, Manly 1997), the null hypothesis of no similarity between the patterns of succession in the occurrence matrices for similar study periods between years was tested.

In a few cases a species was observed to be present in only one sampling interval during a study period in one year, but was absent during all sampling intervals in the corresponding period of the other year. These species were excluded in developing the respective occurrence matrices so that the matrices would be of similar size. For each of the adjusted occurrence matrices, a Jaccard similarity matrix (e.g., Figure 4.2 C and Figure 4.2 D) was derived, in which the value of the elements, i,j represents the degree of similarity of taxa between the i th and j th sampling intervals. The matrix is square ($n \times n$) and symmetric because the similarity of taxa between sampling intervals i and j is the same as that between j and i and the similarity of taxa within an interval with itself (i.e., $i = j$) is 1.0. Next, the Pearson correlation coefficient (K_{obs}) between the two similarity matrices was calculated. A permutation distribution of K -values then was developed by carrying out successive correlations after corresponding rows and columns of one of the similarity matrices were randomly permuted, simultaneously. That is, if for example, rows 1 and 4 of the similarity matrix were exchanged, then, columns 1 and 4 were also exchanged. This type of randomization had the effect of maintaining symmetry in the similarity matrix (Mantel 1967, Cheverud et al. 1989, Manly 1997). In all cases, 999 permutations were carried out to create a permutation distribution of 1000 K -values (i.e., 999 permutation values plus K_{obs}), which has been shown to be sufficient for testing

significance at the 5% level (Cheverud et al. 1989, Manly 1997). Statistical significance (P) of the observed correlation coefficient, K_{obs} under the null hypothesis of no similarity between the successional patterns of insect species in the two similarity matrices was determined by its position in the distribution of 1000 K -values. That is, the P -value is the proportion of K -values $\geq K_{obs}$, with a low value ($P < 0.05$) indicating that the successional patterns of taxa in the two similarity matrices are similar. All of the analyses on the occurrence and similarity matrices were carried out using MATLAB 6.5 (The MathWorks Inc., Natick, MA) with the Image and Signal Processing toolboxes.

Finally, temperature data collected on-site at Kentland Farm and available online at <http://www.vaes.vt.edu/colleges/kentland/weather/> was used to calculate the degree-days (DD) for both years of the study. Degree-days were accumulated from 1 April of each year using the modified single sine method (Allen 1976) and daily minimum and maximum temperatures. A lower developmental threshold of 10° C was used because this temperature has commonly been observed for many forensically important dipterans (Higley and Haskell 2001).

4.3. Results and Discussion

Arthropods that visit a corpse either can be classified as necrophagous species (i.e., those that feed on the corpse), predators and parasites of necrophagous species, omnivorous species (e.g., wasps, ants, and some beetles), or adventive species, such as spiders and centipedes that exploit the corpse as habitat. This study reports on the necrophagous and predatory/parasitic insect species observed on decomposing pig carcasses in southwest Virginia during the first 21 d in the spring and eight days in the summer, after placement in the field (Figure 4.3, Figure 4.4, Figure 4.5).

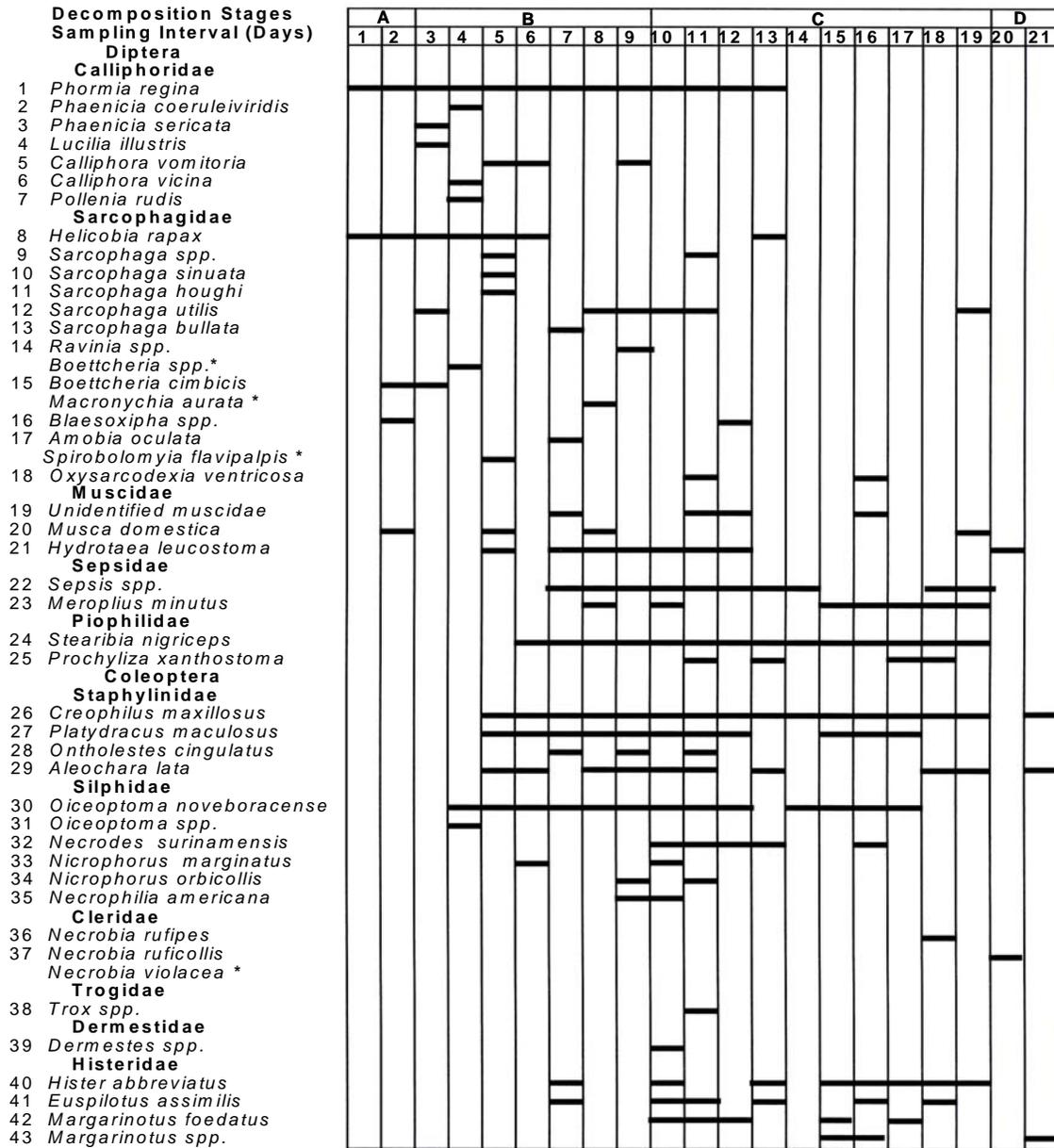


Figure 4.3: Succession diagram for insect species on pig carcasses during 21 sampling intervals in spring 2001 in Blacksburg, VA. Stages of decomposition are shown and are (A) Fresh, (B) Bloat, (C) Active Decay, and (d) Advanced Decay and Dry. Unnumbered (rare) species indicated by asterisk (*) were observed only in one sampling interval in the spring of one year, but not during any sampling interval in the corresponding spring study. These species were excluded in developing the occurrence matrix for spring 2001

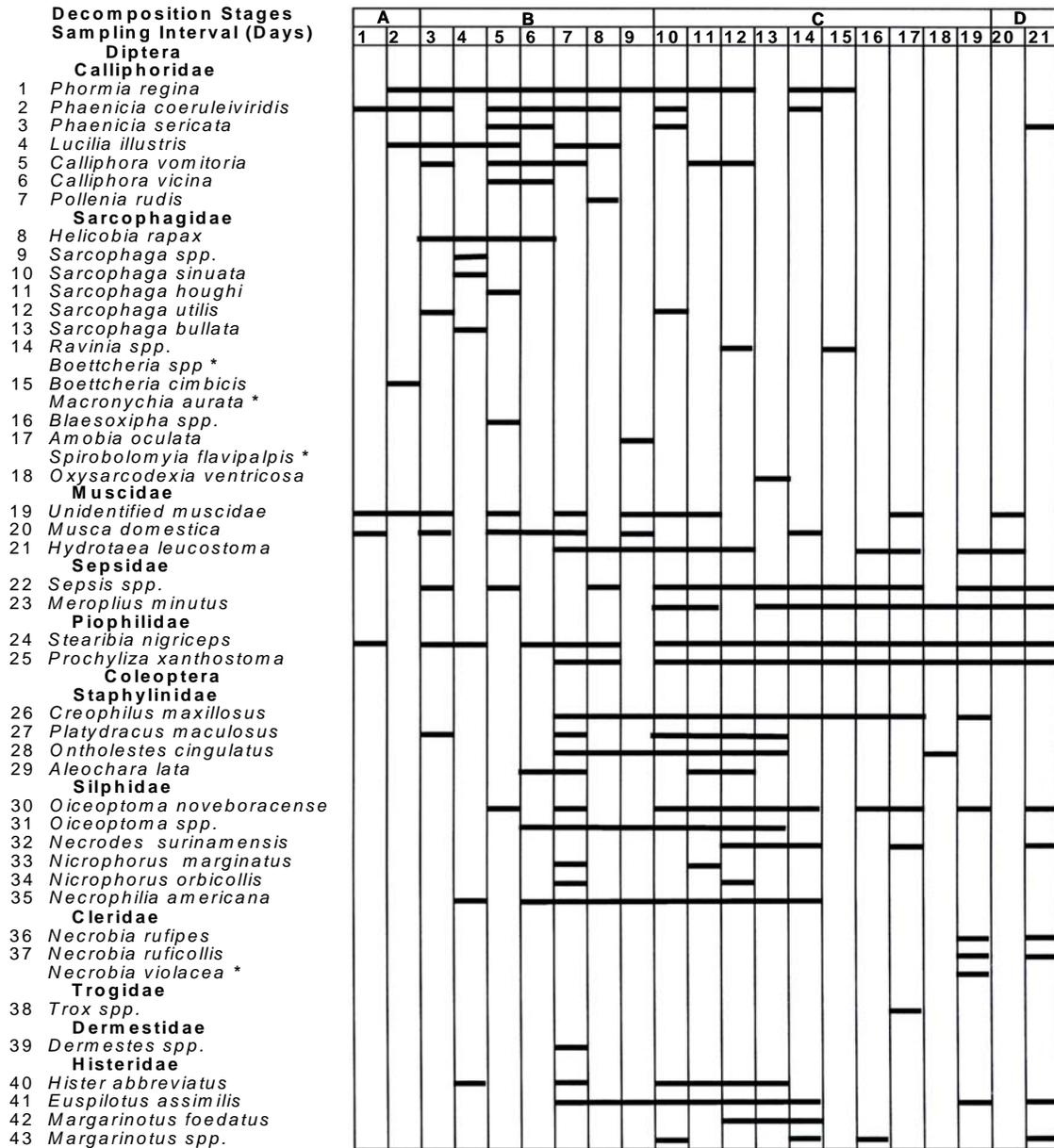


Figure 4.4: Succession diagram for insect species on pig carcasses during 21 sampling intervals in spring 2002 in Blacksburg, VA. Stages of decomposition are shown and are (A) Fresh, (B) Bloat, (C) Active Decay, and (D) Advanced Decay and Dry. Unnumbered (rare) species indicated by asterisk (*) were observed only in one sampling interval in the spring of one year, but not during any sampling interval in the corresponding spring study. These species were excluded in developing the occurrence matrix for spring 2002

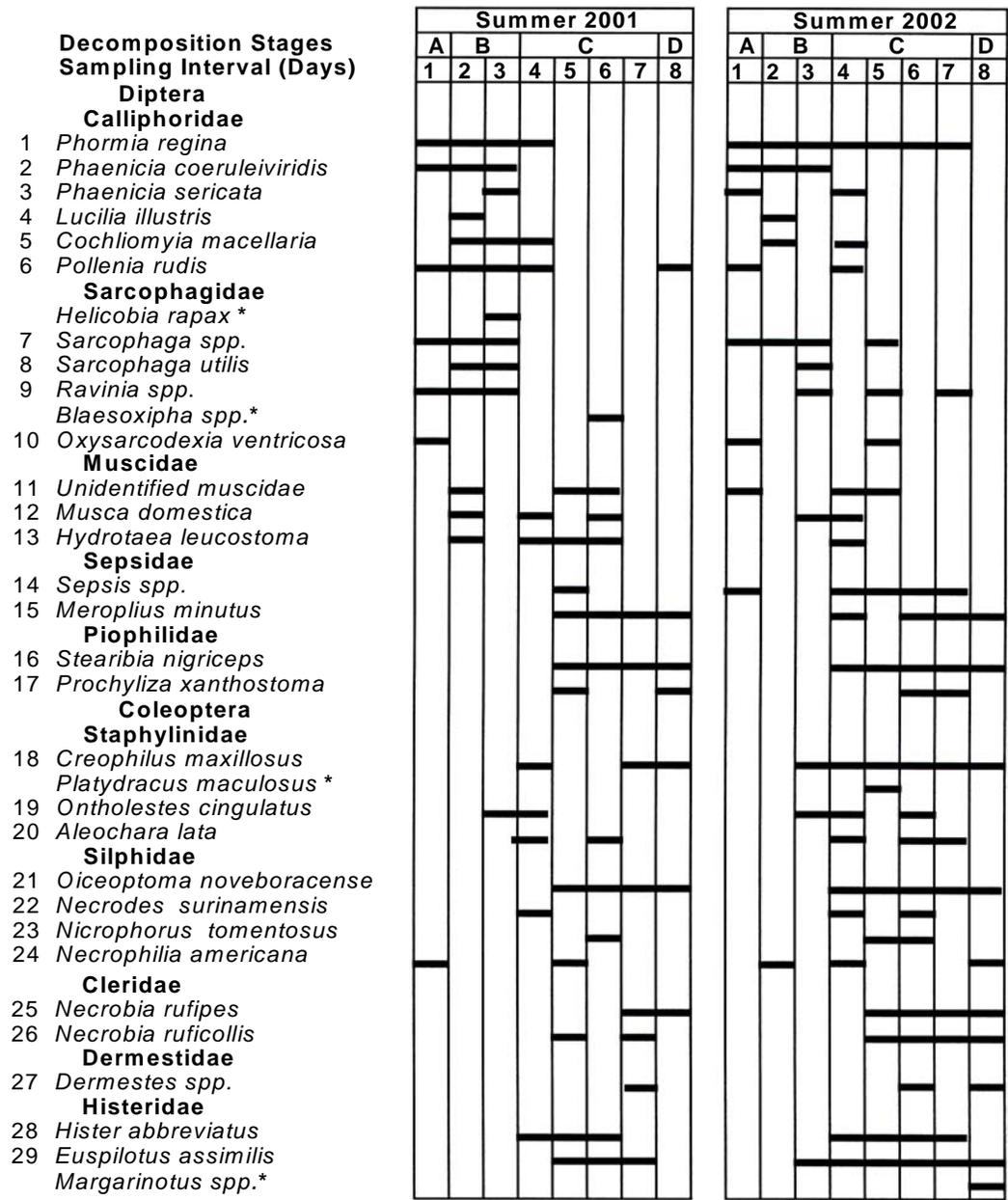


Figure 4.5: Succession diagrams for insect species on pig carcasses during 8 sampling intervals in summer 2001 and 2002 in Blacksburg, VA. Stages of decomposition are shown and are (A) Fresh, (B) Bloat, (C) Active Decay, and (D) Advanced Decay and Dry. Unnumbered (rare) species indicated by an asterisk (*) were observed only in one sampling interval in the summer of one year, but not during any sampling interval in the corresponding summer study. These species were excluded in developing the occurrence matrices for summer 2001 and 2002

More taxa were found in the spring study and in the summer. Forty seven species were observed in the spring and 32 species were observed in the summer during the two years (Figure 4.3, Figure 4.4, Figure 4.5). The number of taxa reported in similar studies on vertebrate carcasses was between 30 and 522 (e.g., Reed 1958, Burger 1965, Payne 1965, Early and Goff 1986, Tullis and Goff 1987, Anderson and VanLaerhoven 1996, Richards and Goff 1997, Carvalho et al. 2000). The large range of taxa found in these studies can be attributed to differences in climate, location, surrounding habitat, sampling frequency, number of animal models that were used, and the reporting of arthropods other than insects. These and other factors are known to affect insect successional patterns on carrion (Anderson 2001).

Not surprisingly, the earliest visitors to the carcasses in the spring and summer of both years were dipterans in the families Calliphoridae, Sarcophagidae, and Muscidae (Hall 2001). Of the calliphorids, *Phormia regina* was the dominant species in the spring comprising >90% of the dipteran species that were observed. In the summer, however, *P. regina* was co-dominant with *Phaenicia coeruleiviridis*. The presence of *Cochliomyia macellaria* only during the summer was expected because this species is known to prefer warm humid climates (Byrd and Castner 2001). However, the presence of *P. regina* in the summer was somewhat surprising given that this species tends to favor cool weather conditions (Byrd and Castner 2001). Although *Calliphora vicina* favors urban habitats while *Calliphora vomitoria* is common in wooded rural and suburban areas, their biology and preference for shaded areas are similar and thus they were expected to occur together (Byrd and Castner 2001).

Eleven species of Sarcophagidae were identified from these studies. *Sarcophaga utilis* Aldrich and *Helicobia rapax* Walker were the most common. Hundreds of species of Sarcophagidae develop in decomposing matter (Smith 1986) so it is not surprising that we identified a large number of sarcophagid species relative to the number of individuals collected or seen on the carcasses (>5% of all flies were flesh flies).

Two piophilid species, *Stearibia nigriceps* and *Prochyliza xanthostoma*, were collected as adults but not as larvae. Most members of Piophilidae are scavengers; adults often frequent decaying carcasses in the early stages of decay, but do not colonize until the carcass is in advanced decay, the preferred stage for egg deposition and larval development (McAlpine 1977).

Hall (2001) analyzed succession data on arthropods on human cadavers from Rodriquez and Bass (1983) and showed that beetles are present mainly during the bloat and decay stages of decomposition. In this study, beetles appeared as early as the third day in the spring studies and on the first day in the summer studies. However, the majority of beetles were observed during the mid- to late-successional periods (Figure 4.3, Figure 4.4, Figure 4.5), which corresponds to the bloat and decay stages of decomposition.

Knoxville, TN is probably the closest in location and climate to southwest Virginia where studies of carrion-arthropod succession on an animal model were conducted (Reed 1958). In that study, Reed (1958) reported on approximately 217 insect species, many of which were also observed in our study despite the smaller numbers of species. Several factors might account for the difference between the two studies. Reed (1958) used a greater number of carcasses (45 dog carcasses versus 8 pig carcasses in this

study) and sampled in nine different areas (versus one in this study) throughout an entire year. In addition, Reed (1958) reported incidental, omnivorous and adventive species as well as necrophagous and predators of necrophagous species. Finally, temperatures during the winter months when Reed (1958) conducted his study were generally much warmer than are typical for southwest Virginia at the same time of year.

Rodriquez and Bass (1983) noted that there is a direct relationship between the rate of decay and the rate of succession of insect families and species arriving at a cadaver. In general, the succession patterns in this study were similar to those described by Reed (1958) and Rodriquez and Bass (1983) with minor differences in the time at which certain species begin frequenting the carrion. These differences might be explained by the different rates of decomposition exhibited by dogs (Reed 1958), humans (Rodriquez and Bass 1983), and pigs.

An occurrence matrix was developed for each of the succession diagrams in Figure 4.3, Figure 4.4, and Figure 4.5. The size (number of species and number of sampling intervals) of the occurrence matrices for corresponding study periods was equal after eliminating 'rare' species from the successional diagrams. Rare species were considered to be those that were present in only one sampling interval during a study period in one year, but was absent during all sampling intervals in the corresponding period of the other year. As such, three dipterans (*Boettcheria* spp., *Macronychia aurata*, *Spirobolomyia flavipalpis*) and one beetle (*Necrobia violacea*) were eliminated from the spring observations, and two dipterans (*Helicobia rapax* and *Blaesoxipha* spp.) and two beetles (*Platydracus maculosus* and *Margarinotus foedatus*) were eliminated from the summer observations. Mean pairwise Jaccard similarities for the successional patterns of

insect species in the occurrence matrices (without rare species) are presented in Figure 4.6.

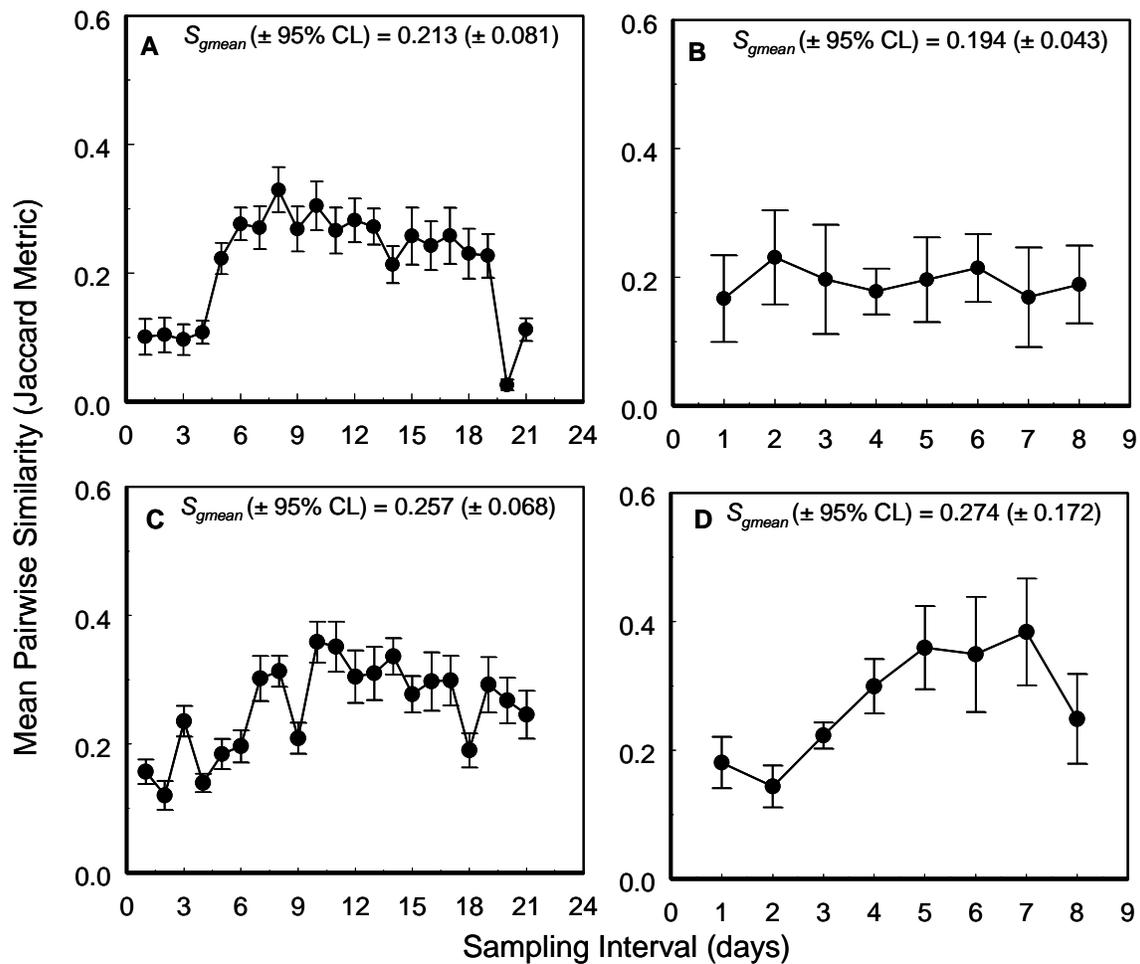


Figure 4.6: Jaccard similarities for the occurrence patterns of insect species on pig carcasses in Blacksburg, VA. Mean and S.E. (vertical bars) of the Jaccard coefficient is shown for the similarity between each interval and all other intervals. The plots are spring 2001 (A), summer 2001 (B), spring 2002 (C) and summer 2002 (D). Overall Jaccard coefficient in insect faunal succession ($\hat{S}_{gmean} \pm 95\% CL$) for each period is given. Observed correlation (K_{obs}) and significance value for the successional patterns of insects in the spring (A and C) are 0.57 and $P = 0.001$, respectively; K_{obs} and the significance value for the summer patterns (B and D) are 0.53 and $P = 0.007$, respectively

As is common for successional patterns of carrion arthropods (see Schoenly 1992), pairwise similarities in taxa were low at the start of decomposition (fresh stage), but increased to a maximum during mid-decomposition (bloating and active decay), and decreased toward the end of decomposition (advance decay and dry stages). The attractiveness of a carcass to specific species changes during decomposition, resulting in a typical horseshoe-shaped arch pattern of succession (Schoenly 1992). During the bloat and active decay stages of decomposition the carcass is more attractive to a wider range of species than in the fresh and dry stages (Hall 2001). Therefore, there is usually greater species richness in the mid-successional period, which results in higher pairwise similarities in taxa between samples and the horseshoe-shaped arch pattern. This pattern is evident in Figure 4.6, although it is much better defined in Figure 4.7 (top panel) for each of the study periods.

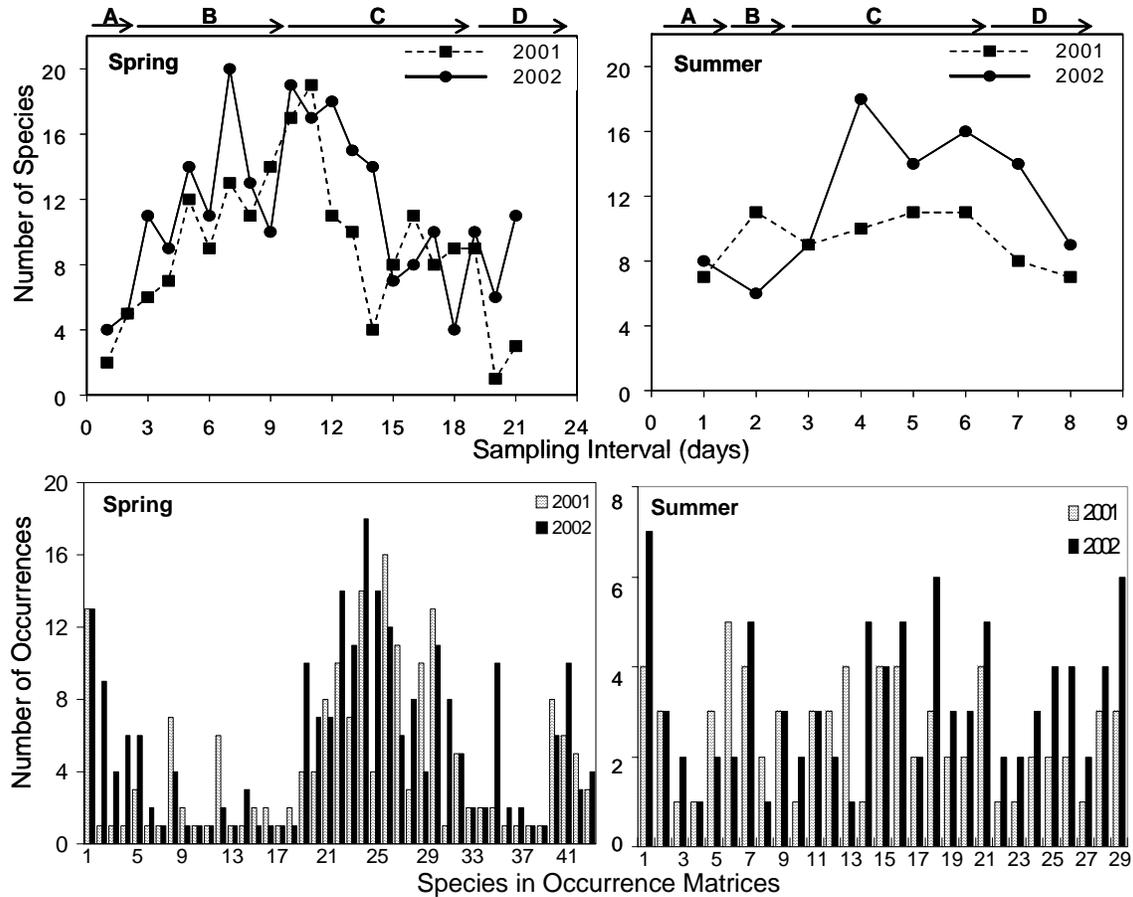


Figure 4.7: Number of species during each sampling interval (top) and number of occurrences for each species (bottom) during the succession of insect species on pig carcasses in spring and summer 2001 and 2002 in Blacksburg, VA. Graphs were developed from succession diagrams after elimination of rare species. The duration of stages of decomposition is shown above top panel and are, (A) Fresh, (B) Bloat, (C) Active Decay, and (d) Advanced Decay and Dry

Also to be expected in successional patterns of carrion arthropods are periods of taxonomic stasis in which there is very little change in the composition of taxa. Periods of taxonomic stasis can be recognized in plots of carrion-arthropod succession (e.g., Figure 4.6) by consecutive matching sample-pairs where the mean and variability in pairwise similarities are the same (Schoenly 1992). Based on this strict definition, none of the patterns in Figure 4.6 appear to contain periods of taxonomic stasis. However, if the definition was applied loosely to consider pairwise similarities to be equal when their confidence intervals overlap, then the successional patterns in Figure 4.6 would contain several periods of taxonomic stasis. By this loose definition, therefore, sampling intervals 1–4, 11–13, and 15–19 in Figure 4.6A would be considered periods of taxonomic stasis in insect composition in spring 2001. The same could be said for sampling intervals 3–6 in summer 2001 (Figure 4.6B). In general, only periods in which the pairwise similarities are equal for more than two consecutive sampling intervals might be thought of as taxonomic stasis. Therefore, although there are instances in spring 2002 (Figure 4.6C) and summer 2002 (Figure 4.6D) where pairwise similarities are equal (by our loose definition) for two consecutive sampling intervals, we do not consider these as periods of taxonomic stasis.

The lack of evidence for taxonomic stasis in spring and summer 2002 was likely due to the greater variability in species succession during these periods of the study compared with similar periods in 2001. From the data presented graphically in the top panel in Figure 4.7, the standard deviation (SD) in the number of species was calculated as the measure of variability in the number of species among sampling intervals. For the spring studies, the SD was 4.6 and 4.8 species/interval for 2001 and 2002, respectively,

and for the summer, the SD was 1.8 and 4.3 species/interval for 2001 and 2002, respectively. From the graphs presented in the bottom panel in Figure 4.7 12 species were found to occur more frequently in spring 2001 than in 2002, and 19 species occurred more frequently in spring 2002. The remaining ten species occurred with equal frequency in both years. Likewise, five species had greater residence times in summer 2001 than in summer 2002, and 18 species occurred more frequently in summer 2002. In addition to the higher number of occurrences for individual species in 2002, variability in species occurrences was generally higher in that year. The standard deviations of occurrences in spring 2001 and 2002 were 4.2 and 4.5 occurrences/species, respectively, and 1.2 and 1.6 occurrences/species in summer 2001 and 2002, respectively. Higher temperatures during the study periods in 2002 might explain the greater variability in the successional patterns. It was estimated that 107 and 115 DD accumulated between the beginning and end of the studies in spring 2001 and spring 2002, respectively. For the summer studies, ≈ 76 and 91 DD accumulated between the start and end of the studies in 2001 and 2002, respectively.

Jackknife estimates of overall Jaccard similarities and the precision of these estimates ($\hat{S}_{gmean} \pm 95\% \text{ CL}$) for each of the successional patterns were 0.213 ± 0.081 (spring 2001), 0.257 ± 0.068 (spring 2002), 0.193 ± 0.043 (summer 2001), and 0.274 ± 0.172 (summer 2002). These values of overall similarity are low, but are within the range of values (0.20–0.50) that have been derived from data on the successional patterns of carrion-arthropods in other studies (Schoenly 1992). The low values are indicative of the rapid turnover in species composition, which is expected for carrion arthropods.

The overlapping confidence intervals in overall Jaccard similarities for corresponding periods suggest that despite differences in the number of instances of taxonomic stasis, numbers of species occurring in each sampling interval, and in the occurrences of these species, the patterns of insect succession between corresponding periods might be similar. However, as was demonstrated with hypothetical data (Figure 4.2), the equality of S_{gmean} for two occurrence patterns does not imply that the successional patterns are similar. Therefore, using permutation analysis of the similarity matrices the null hypothesis of no similarity in the patterns of succession between the corresponding study periods in each year was tested. This hypothesis was rejected in all cases concluding that there was similarity between the similarity matrices for spring 2001 and spring 2002 ($P = 0.001$) and between those for summer 2001 and summer 2002 ($P = 0.007$). These results suggest that the successional patterns of the insects that were observed during the spring and summer studies are typical for the respective periods and that variations in these patterns from year to year should be minimal.

Estimation of postmortem interval (PMI) from succession data requires knowledge of local carrion fauna and their successional patterns at different times of the year (Anderson 2001, Wells and Lamotte 2001). These data show that such patterns can remain similar from year to year and thus can be used to provide the basis for PMI estimates. In addition, information on the diversity of species, type of species, number of individuals, life stages present, and the number of individuals of each life stage can all be derived from succession studies. This information can provide critical clues to a criminal investigation, such as in cases where the body has been moved from the site of death. As this study had demonstrated, the successional patterns of insect species can differ at

different seasonal periods of investigation even at the same site. These differences highlight the need for further studies of carrion-arthropod succession at different times and in different areas. This study represents the first step toward developing a knowledge-base of the successional patterns of carrion-arthropods for the southwest Virginia area.

5. The Effects of Antemortem Ingestion of Ethanol on Insect Development and Successional Patterns

5.1. Introduction

Entomotoxicology is a fairly new area of investigation, where entomological evidence is analyzed to determine whether or not drugs or toxins were used prior to death (Goff and Lord 2001). Entomotoxicology combines entomological and toxicological data for the purpose of establishing the manner or cause of death and estimating the postmortem interval (PMI) (Goff and Lord 2001). Drug / toxin use can contribute to or indicate the manner of death; therefore, it is important to know whether antemortem use of these substances (including alcohol) occurred (Beyer et al. 1980, Miller et al. 1994). Blood, tissue, or urine samples are traditionally analyzed for the presence of these substances; however, these specimens usually are not appropriate for analysis after about 72 hours following death (Hennsge et al. 1995). In many drug or alcohol-related deaths, the decedents are not discovered until they are in an advanced state of decay (Goff and Lord 2001).

Carrion-feeding insects are usually present on, or around decomposing remains long after tissue, blood, and urine become unsuitable for analysis. Drugs and toxins in the decomposing tissue can be identified in insect tissue or remnants (pupal cases, frass, excrement, etc.) as the result of continual feeding on the tissue (Sohal and Lamb 1977, 1979, Beyer et al. 1980, Nuorteva and Nuorteva 1982, Gunatilake and Goff 1989, Kintz et al. 1990) Toxicological analysis of entomological specimens can be carried out in a similar manner as traditional specimens, with procedures including radioimmunoassay (RIA), gas chromatography (GC), thin-layer chromatography (TLC), and high-performance liquid chromatography-mass spectrometry (HPLC-MS). The

entomotoxicological data from these analyses have been used successfully as indicators of cause and manner of death in a number of cases (Lord 1990, Nolte et al. 1992, Goff and Lord 1994). As such, the reliability of toxicological analysis of entomological specimens is well documented (Miller et al. 1994).

Studies have shown that antemortem use of various drugs and toxins affect maggot development rate, manifesting into an inaccurate PMI estimation based on insect development (Goff et al. 1989, 1991, 1993; Hedouin et al. 1999). Errors of up to 38 h can occur in PMI estimates with heroin (Goff et al. 1991), up to 48 h with methamphetamine (Goff et al. 1992), and up to 77 h with amitriptyline (Goff et al. 1993). Ethanol can potentially alter development and behavior of insects as well; however, there are currently no published studies that analyze the effect of antemortem ethanol ingestion on insect development rate or successional patterns.

This study has two major objectives: 1) to determine whether antemortem ingestion of alcohol affects postmortem insect successional patterns on *Sus scrofa* L. carcasses, and 2) to examine the effect of alcohol on maggot development of the black blow fly, *Phormia regina* (Meigen) under field conditions.

5.2. Materials and Methods

5.2.1. Animals and Alcohol Dosing

Two studies on the effect of antemortem ethanol ingestion on insect successional patterns and development were conducted in Blacksburg, Virginia from July 7–31, 2003. The area is ~608 m above sea level and has an average temperature of 20.7 °C in the summer (National Weather Service). Four commercially cross-bred pig carcasses obtained from the Swine Center at Virginia Tech were used in each study. The animals

weighed between 40–65 kg. Two animals were administered ethanol prior to euthanasia and two were not subjected to ethanol treatment and, therefore, served as controls. Ethanol was administered orally and intravenously. In order to simulate actual human ethanol consumption, test pigs in the first study were fed a mixture of 80-proof Aristocrat® vodka and fruit drink and allowed to drink until satiated. This amount corresponded to an intake of approximately 60 cc of vodka for the pigs in the first study. Because a pilot study showed that a larger volume of ethanol was required to achieve a desired blood-ethanol concentration between 0.10 - 0.30 % a second method of administering ethanol was employed. Thirty minutes after drinking ceased, a butterfly catheter was inserted into an ear vein and an intravenous catheter (IV) was used to administer 90 cc of 95% ethanol in 225–271 cc saline over a 30-min period (Figure 5.1). The amount of saline was adjusted for the weight of each animal.

In the second study, approximately 60 cc of vodka in saline was administered via oral gavage after the ethanol solution was given intravenously. An oral gavage was used



Figure 5.1. A, B: Intravenous ear catheter used for antemortem dosing of pigs with ethanol for succession and development studies

because the pigs in this study would not willingly drink the ethanol-fruit drink mixture as the animals had in the first study. Blood was collected from both treated and untreated pigs 15 minutes after dosing was completed, and euthanasia by electrical shock followed immediately. Approximately 1.36 kg of loin meat was excised from all pigs immediately following euthanasia to be used as the rearing medium in development studies. In addition, blood was collected from all of the carcasses 48 hours postmortem. All blood, tissue and maggot homogenate specimens were analyzed for ethanol by headspace gas chromatography (HSGC) utilizing a HP 7694 HS Sampler configured to an AgilentGC-6890 Plus™ with a flame ionization detector (FID). The column was a Restek Rtx-BAC1™ and n-propanol was utilized as the internal standard. Total run time was four minutes. The limit of quantitation (LOQ) was 0.01%.

The remainder of the carcasses was immediately transported to the test site at Moore Farm, a research facility of Virginia Tech approximately 4 km from the Swine Center.

5.2.2. Succession

The carcasses were placed under open-bottomed cages approximately 100 m apart at the edge of an open field bordered by a thickly wooded area and allowed to decompose for 10 days. Each cage measured 92 x 92 x 153 cm and was constructed out of 2.5 cm steel-welded tubing and 1.27 cm hardware cloth. The cages were staked to the ground to prevent disturbance of the carcasses by scavengers.

Samples of adult insects were collected from 10 aerial net sweeps above and around the carcass, pitfall traps, and directly off the carcass to qualitatively assess insect fauna. Adult samples were placed immediately in individual 4.5 liter Hefty Onezip®

plastic bags (Pactiv Corp. Lake Forest, IL) containing a paper towel saturated with ethyl acetate. In addition, several clusters of fly eggs and several hundred fly maggots were collected, when they were present, using soft-touch forceps. Half of each egg or maggot sample was placed directly into vials containing KAA fixative (80–100 ml 95% ethanol, 20 ml glacial acetic acid, 10 ml kerosene) (Catts and Haskell 1990). The remaining half of each egg or maggot sample was placed in an aluminum pouch containing ground pork (Catts and Haskell 1990). The pouches were then placed in 500 ml MicroGourmet® SOLO cups (Solo Cup Co., Urbana, IL) filled with about 7 cm of Hoffman® vermiculite (A. H. Hoffman, Inc., Lancaster, NY) in which migrating maggots could pupate. A large hole cut in the lid of each cup and covered with mesh cloth provided aeration while preventing maggots from escaping. The cups were placed into an incubator at 27°C, 75% RH where eggs and maggots were reared to adult. All collected and reared adults were pinned, labeled, and identified to genus or species.

5.2.3. Development

Twelve clusters of *Phormia regina* eggs were collected from wild-type flies. A carcass of the domestic pig, *Sus scrofa* L. was used to attract gravid females. The carcass was placed in a sunny area outdoors where *P. regina* is known to exist as the dominant blow fly species (Chapters 3 and 4). All of the egg clusters were collected within a 4-hour period on the second day postmortem. 170 grams of loin tissue taken from the four pigs was distributed into three 500 ml cups each for a total of 12 cups per study (6 treated, 6 controls). One egg cluster containing approximately 100-200 eggs was placed in each cup.

Eggs were observed hourly so that an initial hatch time ($t = 0$) could be determined for each cluster. At the onset of egg hatch, the cups with meat tissue and eggs clusters were transferred to gallon-sized wax coated containers (Sweetheart Cup Company, Owings Mills, MD) filled with 10 cm of Hoffman® vermiculite (A.H. Hoffman, Inc., Lancaster, NY). Holes were cut out of the cardboard lids, which locked into a lip on the inside of the container. A piece of gauze fabric was placed under the lid to cover the hole to prevent maggots from escaping. The containers were then covered with clear Saran Quickcovers™ (S. C. Johnson & Son, Inc., Racine, WI) to keep the humidity inside the cup at least 80% and to prevent rain from getting inside the cups. HOBO® Data Loggers (Onset Computer Corporation, Bourne, MA) were placed inside one cup so that temperature and relative humidity could be recorded every 30 minutes. The rearing cups were placed outside in partial sunlight under a cage similar to the ones used in the succession study to prevent scavenging. A plastic tarp was placed over the cage when heavy rain was expected.

Samples were collected every 8 hours until pupation, beginning at the onset of egg hatch. A sample consisted of 6 randomly selected maggots per cup for a total of 18 maggots per cup per day. The maggots were placed directly into KAA for at least 1 hr before measurements were taken. The fixative caused the maggots to extend fully so that they could be measured to the nearest 0.25 mm using a small ruler. Length and instar were recorded for each maggot.

Pupae were removed from the rearing cups as they appeared, and were placed in separate labeled containers. Maggot sampling continued until approximately 90% of the

individuals in the cup had pupated. The time to complete the pupal stage was also recorded.

5.2.4. Data and Statistical Analysis

The Jaccard similarity metric and Mantel's permutation test were used to test for differences in successional patterns of insect taxa on ethanol-treated and untreated pigs over 10 days, using the similarity matrices derived from species occurrence matrices. Within each study, data were combined for the two treated and two untreated pigs to develop two succession diagrams and corresponding occurrence matrices for each study (1 for treated, 1 for untreated), for a total of four occurrence matrices for the two studies (2 for treated and 2 for controls).

In an occurrence matrix, 1 and 0 represent the occurrence and absence, respectively, of a taxon within a sampling interval. For each of the occurrence matrices developed in this study, Jaccard similarity coefficients were used to describe temporal changes in the between-sample similarities in the composition of insects within the study period (Schoenly 1992). The Jaccard metric describes the degree of similarity between sampling intervals, with a value ranging from 0–1. A value of zero represents complete dissimilarity between sampling intervals for any species, while “1” describes a perfect match of species between sampling intervals (Schoenly 1992, Krebs 1999). Between-sample, or pairwise, similarities were used to calculate an overall grand mean (S_{gmean}) of similarity of insect taxa among all of the sampling intervals throughout the entire study for each occurrence matrix.

The Jackknife procedure was used (Mayer et al. 1986, Manly 1997, Krebs 1999) to determine the precision of S_{gmean} (i.e., $S_{gmean} \pm 95\% \text{ CL}$). With the Jackknife

procedure, each of the n sampling intervals in an occurrence matrix is omitted one-at-a-time, with replacement, and the overall similarity of taxa ($S_{.i}$) was recomputed for the new occurrence matrix with $n - 1$ sampling intervals. The Jackknife estimate of overall similarity (PV_i) for the occurrence matrix with the i th sampling interval discarded was calculated as,

$$PV_i = nS_{gmean} - (n - 1)S_{-i} \quad i = 1 \dots n \quad (1)$$

where n is the number of sampling intervals in the original occurrence matrix, S_{gmean} is the overall similarity for the original occurrence matrix with n sampling intervals, and S_{-i} is the partial estimate of S_{gmean} when the i th sampling interval is discarded. Finally, the Jackknife estimate of S_{gmean} was computed using the the PV_i values ($i = 1 \dots n$) where

$$\hat{S}_{gmean} = \frac{1}{n} \sum_{i=1}^n PV_i \quad (2)$$

along with its standard error, and 95% confidence intervals.

Because it is possible for successional patterns of taxa to differ even when overall Jaccard similarity indices for two occurrence matrices are equal, S_{gmean} cannot be used with certainty to measure the degree of similarity between the successional patterns of species in the occurrence matrices from alcohol-treated and untreated pigs. To determine the degree of similarity in the successional patterns of insects (based on similarity in species occurrence) for alcohol-treated and control carcasses, a permutation approach based on Mantel's test was applied. This approach has been used to compare distance or similarity matrices (Mantel 1967, Dietz 1983, Cheverud et al. 1989, Manly 1997). It is typical in this type of analysis test a null hypothesis of no similarity between the patterns of succession in the occurrence matrices being compared (e.g., Cheverud et al. 1989,

Manly 1997); therefore, the null hypothesis of no similarity between the patterns of succession in occurrence matrices of treated versus control carcasses was tested.

A Jaccard similarity matrix was developed for each of the occurrence matrices, in which the degree of similarity of taxa between the i th and j th sampling intervals was represented by the value of the elements i,j . The similarity of taxa between sampling intervals i and j is the same as that between j and i and the similarity of taxa within an interval with itself (i.e., $i = j$) is 1.0, therefore the matrix is square ($n \times n$) and symmetric. Next, the Pearson correlation coefficient (K_{obs}) was calculated between the two similarity matrices. A permutation distribution of K -values was developed by carrying out successive random permutations by simultaneously exchanging corresponding rows and columns of one of the similarity matrices while the other occurrence matrix was held constant. For example, if rows 2 and 5 were exchanged, the columns 2 and 5 were also exchanged. Symmetry, then, was maintained in the similarity matrix as the result of using this type of randomization (Mantel 1967, Cheverud et al. 1989, Manly 1997). A permutation distribution of 1000 K -values (999 permutation values plus K_{obs}) has been shown to be sufficient for testing significance at the 5% level (Cheverud et al. 1989, Manly 1997); therefore a total of 999 permutations were carried out. The position of the observed correlation coefficient K_{obs} in the distribution of 1000 K -values determined the statistical significance (P) under the null hypothesis of no similarity between the successional patterns of insect species in the two similarity matrices. That is, successional patterns of taxa in the two similarity matrices are similar if the P -value (the proportion of K -values $\geq K_{obs}$) is low ($P < 0.05$). All of the analyses on the occurrence

and similarity matrices were carried out using MATLAB 6.5 (The MathWorks Inc., Natick, MA) with the Image and Signal Processing toolboxes.

As measures of the variability in the succession among sampling intervals during each of the studies, the standard deviation (SD) in both the number of species and in the number of occurrences of each species was calculated.

For the development studies, maggot lengths were plotted against time for maggots feeding on tissue from ethanol-treated and untreated pigs. A second-order polynomial was fitted for each graph. A Student's *t*-test ($\alpha = 0.05$) was performed on the fitted data for each instar to test for similarity in development of maggots feeding on meat from treated and untreated pigs. Accumulated degree hours (ADH) were calculated for the time required to reach pupation by multiplying the developmental duration by the rearing temperature (obtained from the data loggers).

5.3. Results

Blood ethanol concentrations of antemortem blood, postmortem blood, and loin tissue collected from treated and untreated pigs are presented in Table 5.1. Succession diagrams for ethanol-treated and untreated pig carcasses during 10 sampling intervals in summer 2003 in Blacksburg, VA are shown in Figure 5.1.

Table 5.1: Antemortem and postmortem ethanol concentrations of blood and loin from pigs dosed with ethanol

Pig #	Antemortem Blood (% weight by volume)	Postmortem Blood (% weight by volume)	Loin Tissue (Treated and Control Pigs)
Treated 1	0.14	0.11	0.07
Treated 2	0.16	0.10	0.07
Control 3	ND	0.02	ND
Control 4	ND	0.01	ND
Treated 5	0.26	0.20	0.15
Treated 6	0.23	0.21	0.17
Control 7	ND ¹	0.03	ND
Control 8	ND	0.01	ND

¹ND = Not detectable

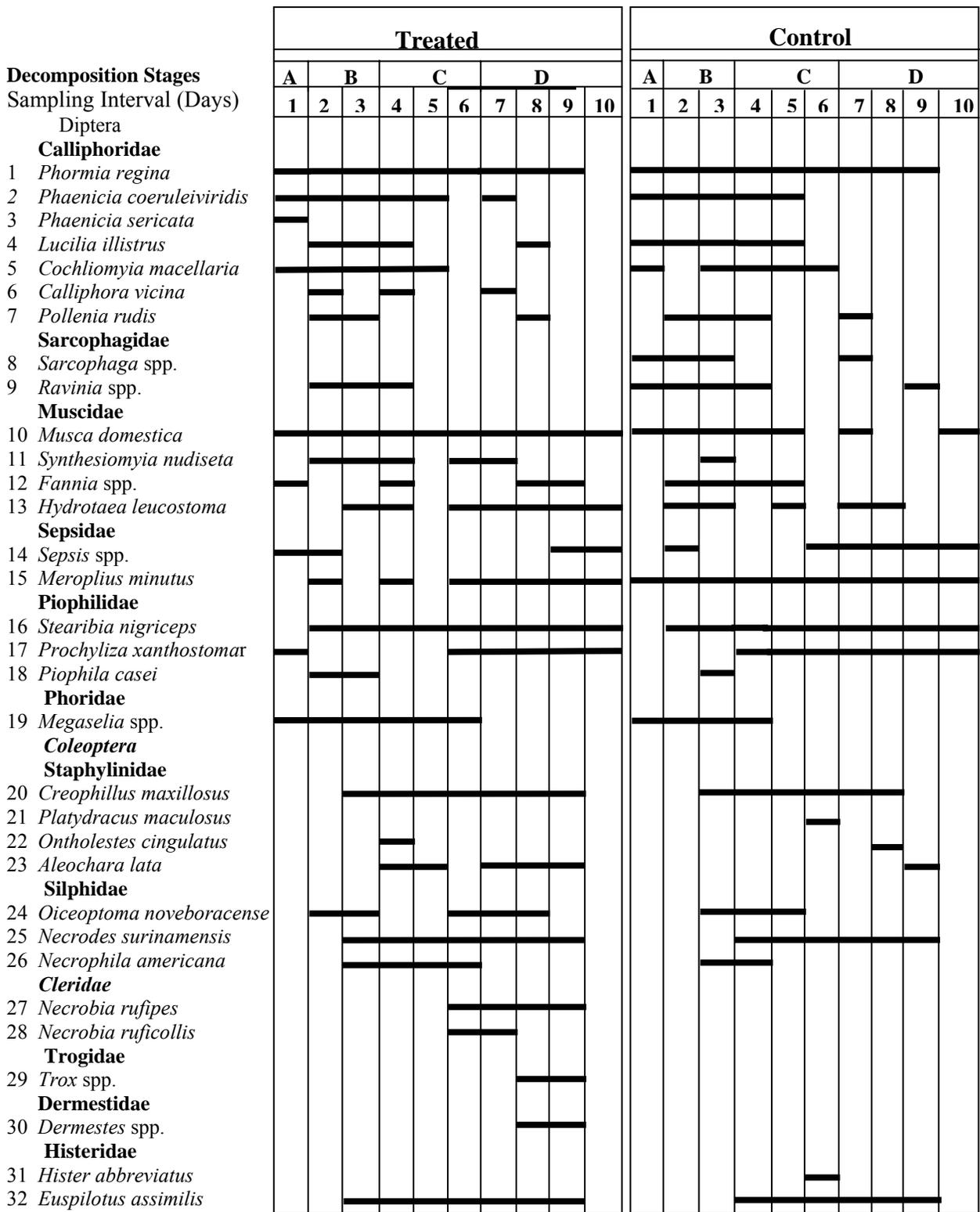


Figure 5.2: Succession Diagram for Ethanol-treated and Untreated pig carcasses during 10 sampling intervals in summer 2003 in Blacksburg, VA. Stage of decomposition are shown and are (A) Fresh, (B) Bloat, (C) Active Decay, and (D) Advanced Decay and Dry

Twenty-nine and 27 insect taxa were observed on treated and untreated carcasses, respectively. The earliest visitors to both carcass types were dipterans in the families Calliphoridae, Sarcophagidae, and Muscidae.

Dipterans were represented by 19 taxa in 6 families. All of the 7 blow fly species were collected from both treated and untreated carcasses, with *Phormia regina* (Meigen) (Calliphoridae) and *Phaenicia coeruleiviridis* (Macquart) (Calliphoridae) the most commonly collected species. Similarly, all of the 4 taxa of Muscidae, 2 taxa of Sepsidae, 3 species of Piophilidae, and 1 taxa of Phoridae were collected from both treated and untreated carcasses. However, only one of the two genera of Sarcophagidae was collected on both carcass types.

Twelve taxa of Coleopterans in 6 families were represented in the study. All 6 of the beetle families were represented by specimens collected from treated carcasses; however, only 3 of the beetle families were represented by taxa collected from untreated carcasses. Dominant beetle species on both carcass types were *Creophilis maxillosus* L. (Staphylinidae) and *Oiceoptoma noveboracense* Forster. Late-arriving taxa in the families Cleridae, Trogidae, and Dermestidae were collected only from treated carcasses. Jackknife estimates of overall Jaccard similarities and the precision of these estimates ($\hat{S}_{gmean} \pm 95\% \text{ CL}$) were 0.33 ± 0.117 and 0.28 ± 0.12 for the treated animals and 0.32 ± 0.098 and 0.30 ± 0.15 for the untreated animals in study 1 and study 2, respectively. Permutation analyses detected no differences between the similarity matrices for treated and untreated in study 1 ($P = 0.003$) and in study 2 ($P = 0.001$).

Growth under field conditions for *P. regina* larvae feeding on tissue from treated and untreated pigs is illustrated in Figure 5.2. The range of exposure temperature was

15–47°C, with a mean of 23°C. Relative humidity ranged from 50–100%, with a mean of 75%. The calculated ADH for the time required to reach pupation was 5304-7649 ADH. The fitted lines and stadia durations are presented in Figure 5.3. A significant difference was found in development of 3rd instars feeding on meat from treated and untreated pigs; however, no differences were detected in 1st and 2nd instars. Mean (\pm SD) duration of pupal stage for *P. regina* that fed on tissue from treated and untreated pigs was 147.0 (\pm 11.5) and 143.7 (\pm 7.9) hours, respectively.

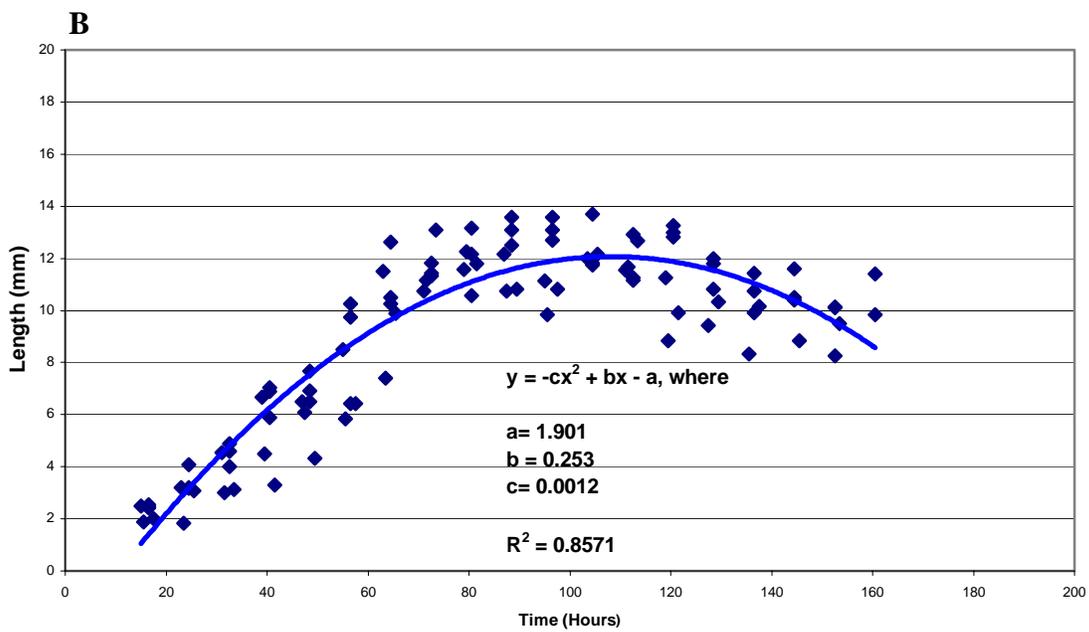
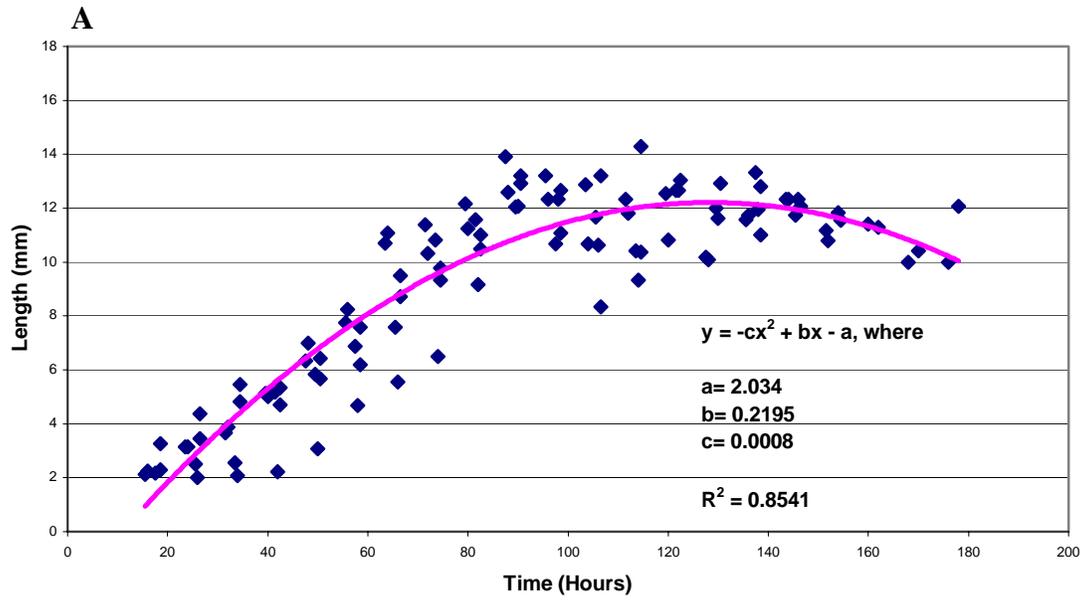


Figure 5.3: Fitted curves of lengths of maggots feeding on meat from treated (A) and untreated (B) pigs

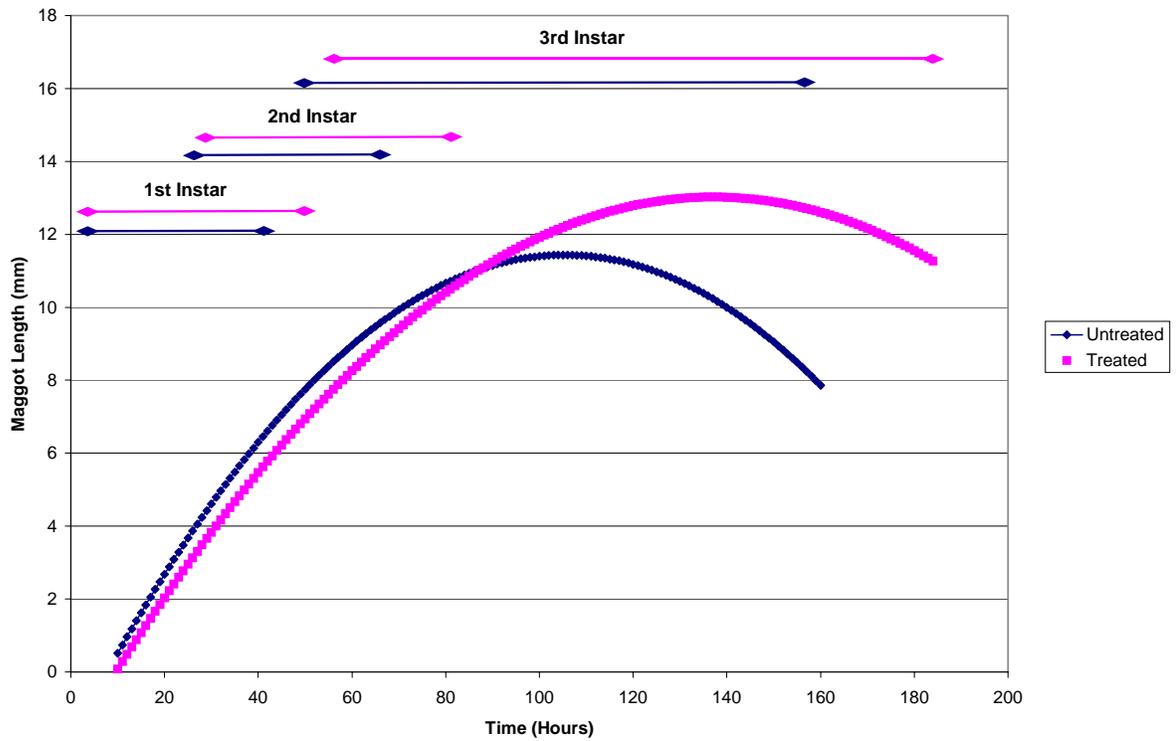


Figure 5.4: Combined fitted curves for length and stadia duration of maggots feeding on meat from treated and untreated pigs

5.4. Discussion

Taxa described in these studies were typical for carrion-succession studies performed in southwest Virginia (Chapters 3 and 4). The grand means of the Jaccard similarity coefficient for both treated and untreated animals were 0.33 and 0.28 for the treated animals and 0.36 and 0.31 for the untreated animals in the studies, indicating low similarity among sampling intervals within each study. These results are typical for decomposition studies, where one would expect the taxonomic composition to change according to the stage of decomposition (Reed 1958, Payne 1965). In hot weather, decomposition and, hence, taxonomic composition progresses more rapidly than in cooler weather.

Antemortem ingestion of ethanol by pigs does not appear to alter insect succession patterns on decomposing carcasses. A permutation test based on Mantel's test showed no lack of similarity among succession matrices for carcasses of treated vs. untreated pigs ($P < 0.05$). Similarly, there were no apparent differences based on visual observations in decomposition rates between treated and untreated animals. Insect visitation and colonization occurred at the same rate for treated and untreated pigs, even when antemortem blood ethanol concentration was as high as 0.25%, which is over three times the legal human limit in many states. Adult flies and eggs were collected from all carcasses by the end of the first day of placement in the field. By day 10, all of the carcasses in both studies had reached advanced decay.

Curiously, 3 of the 6 beetle families (Cleridae, Trogidae, Dermestidae) were collected from treated carcasses but not from untreated carcasses. With the exception of clerid beetles, which were collected from treated carcasses in both studies, specimens of

Trogidae and Dermestidae were only collected at 2 sampling intervals from treated carcasses in one study. The low number of occurrences of these late-arriving families was not sufficient to create statistical differences in occurrence matrices, as indicated by the results of the permutation test. However, with the exception of *Sarcophaga* spp., all of the fly genera collected in the studies had representatives in occurrence matrices of both treated and untreated animals.

In development-based postmortem interval estimation, it is assumed that insects feeding on carrion will develop at predictable rates depending on environmental conditions. However, a number of studies have demonstrated that antemortem ingestion of certain drugs and toxins can alter development rates of insects feeding on decomposed tissue from such individuals. Failure to account for such drug/toxin ingestion can result in an inaccurate PMI estimation. Goff et al. (1991) reported that the differences in rates of development of flesh fly larvae (*Boettcherisca peregrina*) reared on tissue obtained from rabbits that received graded levels of heroin prior to death were significant enough to alter PMI estimates based on larval and pupal development by up to 29 h and 18-38 hr, respectively. Similar results were reported for methamphetamine (Goff et al. 1992) and amitriptyline (Goff et al. 1993). The effect of ethanol on maggot development has not been previously reported for forensically important flies. Failure to account for antemortem ingestion of ethanol on insect growth rate could potentially yield inaccurate PMI estimations based on development rate.

Results from the Student's *t*-test ($P < 0.05$) indicated that growth of 3rd instar maggots feeding on loin tissue removed from ethanol-treated pigs was found to be significantly different from growth of maggots feeding on tissue from untreated pigs.

The BAC of the loin tissue used in the study was 0.15 and 0.17 for the treated pigs, and not detectable (ND) in the untreated pigs.

No significant differences were detected in development rate of 1st and 2nd instar maggots. These results are similar to a study by Goff et al. (1991) who demonstrated that the effect of heroin on maggot growth was significant during hours 18-96 in larval development, but not during hours 0-12.

In this study, the minimum accumulated degree hours (ADH) required to reach the pupal stage (5304) was within the range of what was reported by Anderson (2000) at 23°C and Byrd and Allen (2001) at 25°C. However, the maximum ADH calculation in this study (7649) was slightly higher than what Anderson (2000) reported, but within the range of what was reported by Byrd and Allen (2001). Both development studies were conducted under constant laboratory conditions, while our development studies were conducted in fluctuating field conditions. The lowest recorded temperature inside the rearing cups was 15°C, although at times, temperatures spiked to 47°C. The average temperature of exposure for the time to reach pupation was 23°C, with the average relative humidity 75±25%.

The average pupation times (147.0 and 143.7 hours) were similar in maggot clusters that fed on tissue from treated vs. untreated pigs, respectively. These lengths of pupation times are within the range of those reported by Byrd and Allen (2001) at 25°C.

The ethanol concentrations detected in the loin meat (0.15 and 0.17%) was derived from animals with an antemortem blood ethanol concentration of 0.26 and 0.23%, respectively. The antemortem BACs detected in this study are consistent with postmortem determinations made in human specimens (Garriott 1996), demonstrating

that results obtained in this study are relevant to typical alcohol-related human death. Pathologists are often warned about interpreting BAC levels, as endogenous production of ethanol by microbial synthesis occurs *in vivo* in putrefying bodies, resulting in the detection of artifactual ethanol in toxicological analyses (Gilliland and Bost 1993). In this study, postmortem BAC for untreated carcasses yielded detectable levels of ethanol; however, the levels were much lower than those of the treated pigs (≤ 0.03 for untreated and between 0.10 - 0.21 for treated depending on antemortem level). Postmortem ethanol concentration of the loin tissue was not detectable in the untreated carcasses.

This study provides information on the effect (or lack of) of antemortem ingestion of ethanol on insect successional patterns and development rates of *Phormia regina*. Results indicate that while ethanol consumption had no apparent effect on successional patterns, a statistically significant difference in development was seen in 3rd instar maggots feeding on meat from ethanol-treated pig carcasses.

6. The Effect of Relative Humidity on Egg Development Time and Percent Hatch of *Phormia regina* Meigen and *Phaenicia coeruleiviridis* Macquart

6.1. Introduction

Developmental data provide the most accurate means of estimating the PMI using entomological information, particularly in early stages of decomposition (Smith 1986, Goff and Flynn 1991). Development is assumed to occur at a predictable rate based primarily on environmental conditions. The age of the oldest immature flies (eggs, larvae, pupae) collected from a body at the time of discovery approximates the time since death because flies can lay eggs within minutes of death (Smith 1986, Catts and Goff 1992).

Published development rates for the species of forensic flies that feed on a corpse are used to determine how much heat is required to reach a given point in development. Once weather conditions are known for the site where a corpse is discovered, the amount of heat to which the corpse (and hence the insects colonizing the corpse) was exposed can be determined. Working backward from the time of discovery, investigators can calculate the amount of time required for the “oldest individuals” to have been exposed to the required ADH to reach that stage of development (as determined in controlled development studies) based on data obtained from a weather station near the scene. Historical weather data can be obtained from one of hundreds of weather stations positioned throughout the U.S. (National Weather Service).

Laboratory and field development studies of numerous forensic fly species have been conducted under different temperature and humidity regimes. (Bishopp 1915, Melvin 1934, Kamal 1958, Greenberg and Szyska 1984, Liu and Greenberg 1989,

Greenberg 1991, Byrd and Butler 1996, Byrd and Butler 1997, Anderson 2000, Byrd and Allen 2001, Grassberger and Reiter 2001, Grassberger and Reiter 2002, Davies and Ratcliffe 1994). Data from such studies are indispensable to the field of forensic entomology, particularly in regard to estimating the PMI.

Development rate of the individual insects derived from the first eggs deposited on a corpse is, therefore, predictable based on environmental conditions. While temperature is of primary importance to growth rate, the effect of relative humidity (RH) may also be significant. Although the effect of humidity on egg hatch of other arthropods is well established in the literature (Morrison et al. 1972, Godfrey and Holtzer 1991, Adkisson 1959, Schausberger, 1998, Despins 1992), few studies have focused on the effects of RH on egg viability and egg hatch time of forensic flies. Evans (1934) conducted an extensive study on the effects of the environment on the life history of *Lucilia sericata* (= *Phaenicia sericata*) Meigen, including egg viability and hatch rate. Similar studies were performed on *L. sericata* by Davies (1947), Davies and Hobson (1935) and more recently, Wall et al. (2001). Little work has been conducted on the effect of relative humidity on other important forensic fly species. The purpose of this study was to test the effect of RH on hatch rate and egg viability of two common forensic fly species, *Phormia regina* Meigen and *Phaenicia coeruleiviridis* Macquart.

6.2. Materials and Methods

6.2.1. Sampling Protocol

Egg hatch time and egg viability (based on percent hatch) were recorded for egg clusters derived from the black blow fly, *Phormia regina*, and the green bottle fly, *Phaenicia coeruleiviridis*. Studies were conducted in environmental chambers (Percival

Scientific, Inc., Perry, Iowa) with relative humidity levels from 30 – 95% under constant lighting to prevent photoperiod induced emergence gating. Egg clusters were observed hourly so that newly hatched larvae could be counted and removed. Relative humidity and temperature data were logged every 20 min with HOBO[®] Data Loggers (Onset Computer Corporation, Bourne, MA). In addition, a Traceable[®] Jumbo humidity/temperature meter (Control Company, Friendswood, TX) was used to display temperature/ RH throughout the data collection. The percentage of eggs which hatched in each cluster was estimated by visual observation of the cluster after three hours passed with no further egg hatch from the time of the last hatched eggs. An average hatch rate was calculated for each replicate.

6.2.2. Egg Collection

Egg clusters were collected from both laboratory colonies and wild-type flies. Eggs were collected from *P. regina* colonies maintained at 25°C using ground pork that had been placed at room temperature for 24 - 48 h. When eggs were needed, 170 grams of meat was placed in the cages and observed until oviposition began. Clusters were removed with soft-touch forceps as they were deposited and placed in individual 207 ml plastic cups (Supervalu Inc., Eden Prairie, MN). The depth of the cups was adjusted to 6.35 mm by cutting the side of the cup down to the desired depth. Petroleum jelly was smeared around the rim of each cup to trap maggots as they hatched. Time of oviposition was noted for each cluster, as it took up to 4 hours to obtain all of the clusters (repetitions) for each experiment. With the exception of 20°C/48% RH for *P. coeruleiviridis* where only one cluster was collected for testing, the number of repetitions in all of the experiments ranged from 5-16. For each experiment, three clusters were

concurrently tested at 95% RH under the same temperature as the treatment clusters to serve as controls for the percent egg hatch variable. Over 90 percent of the eggs hatched in all of the controls, insuring that any drop in percent egg hatch was due to the RH as opposed to some other factor (e.g. infertility).

Wild-type *P. coeruleiviridis* eggs were collected with ground pork brought to room temperature. The meat was placed outside in an area where this species was known to be co-dominant with *P. regina* in the summer (see Chapters 3 and 4). The meat was continually observed to ensure that the clusters came from the correct species. When possible, gravid females were trapped under a small plastic cup while they were in the process of ovipositing, so that positive species identification could be made in the laboratory. When it was not possible to trap the adult fly, 10-20 eggs were randomly removed from each cluster and reared to 3rd instar to insure that the egg cluster was from the correct species. Egg clusters were placed in the incubator within 1 hour of being deposited.

6.2.3. Relative Humidity Chambers and Salt Solutions

Relative humidity chambers were constructed out of a series of Rubbermaid Servin'Saver[®] air-tight food storage containers (Rubbermaid Home Products, Wooster, OH), 9.5 mm Fisherbrand[™] vinyl tubing (Fisher Scientific, Pittsburgh, PA), and a 120 volt Apollo[™] aquarium pump (Apollo Enterprises, Ventura, CA) to form a closed-circuit system through which air circulated (Figure 6.1). To modify the pump to accommodate the need for a closed recycling air system, a barb splicer was attached with a glue gun over the exposed “out” hole on the side of the pump. This hole is the one through which air exited the pump and circulated through the circuit. A hole was then drilled into the

side of the plastic covering of the pump, over which another barb splicer was secured with glue. This second barb slicer served as the “in” hole through which air entered the pump from the circuit.

A hole was drilled in opposite sides of every container in the circuit half way up the sides from the bottom using a 6.3 mm drill bit. A 6.3 x 6.3 mm Watts AB nylon hose barb splicer (Anderson-Barrows Metal Corp©, Palmdale, CA) was inserted in each hole and secured with glue from a hot glue gun. Vinyl tubing was attached to each splicing barb to connect the containers. A rectangular 9 L container was used for the salt solution, a 4 L container for the egg chamber, and a 2.2 L container for the digital temperature/humidity meters and data loggers. A round 110 ml container filled with Frost King® fiberglass insulation (Thermwell Products Co., Inc., Mahwah, NJ) was used as an in-line filter. The air flow rate of the circuit was >1800 ml/min.

The path of the circulating air through the RH chamber was as follows: pump → salt solution chamber (A) → filter chamber(B) → egg chamber(C) → data logger chamber(D)→ pump(E) (Figure 6.1).



Figure 6.1: Relative humidity chamber used for collecting egg hatch data for *Phormia regina* and *Phaenicia coeruleiviridis* (A: salt chamber, B: filter chamber; C: egg chamber; D: data logger chamber; E: pump)

A series of RH levels was generated for each temperature using an appropriate saturated salt solution, as described by Winston and Bates (1960). The following salts were used to achieve a range of RH levels at each temperature:

Salt	20°C	30°C
Ca(NO ₃) ₂ * 4H ₂ O	30 - 53%	58%
NaNO ₂	66%	66%
NaCl	70 - 75%	70 - 75%
dH ₂ O	90% - saturation	not tested

For each RH chamber, approximately 2000 g of salt was placed in the chamber and mixed with enough water to produce a thick sludge. The system was then connected and allowed to equilibrate for 24 hours. While the salt solutions maintained a constant RH within $\pm 3\%$, the chambers equilibrated at slightly different RH levels during separate experiments for the same salt. For this reason, RH levels tested were not equal intervals apart, nor was it possible to exactly replicate RH levels between temperatures.

For the high RH levels (+90%), the eggs were placed directly into the incubator with the humidity set at 100%. For *P. regina*, eggs were placed in chambers with RH values of 47%, 49%, 66%, and 71% RH were tested at 20°C, and 30%, 42%, 43%, 58%, 70%, 75%, and 95% RH at 30°C. For *P. coeruleiviridis*, the effect of RH on egg development at 48%, 53%, 66%, 70%, 75%, and 95% RH was tested at 20°C, and an RH of 62% RH at 30°C.

6.2.4. Statistical Analysis. The relationship between RH and egg hatch time, and RH and percent egg hatch were analyzed for *P. regina* at 20 and 30°C, and for *P. coeruleiviridis* at 20°C. Averages for egg hatch time were obtained for individual replicates within a treatment. One replicate was an egg cluster consisting of 100-200 eggs, therefore averages of hatch time and percent hatch rate for each replicate within an experiment were based on 100-200 individual eggs. An average hatch rate was obtained for each replicate. To analyze the pooled data, a General Linear Model ANOVA with Fisher's protected least significant difference (LSD) (SAS, Proc GLM) was used to test the significance of differences in mean hatch time and percent hatch among RH levels within a temperature.

6.3. Results

The global ANOVA of mean hatch times and percent hatch was found to be significant for *P. regina* at 20°C and 30°C and *P. coeruleiviridis* at 20°C among RH levels at $\alpha = 0.05$. *T*-test (LSD comparisons) results detected differences between some but not all RH levels within all three data series tested (Table 6.1). For *P. regina*, significant differences in mean egg hatch and percent hatch were only observed at 20°C, 47% RH. At 30°C, mean hatch time was not significantly different between 70 and 75%, but 95% was found to be significantly different from 58, 70, and 75% RH. Mean percent egg data for all of the egg clusters (repetitions) within a given temperature/ RH combination were pooled, and are presented graphically in Figure 6.1-6.5. Egg hatch was observed over a span of as little as 3 h (for *P. regina* at 30°C, 75% RH) and as great as 10 h (for *P. coeruleiviridis* at 20°C, 75% RH). Very few *P. regina* eggs hatched at 20°C, 47% RH, whereas no egg hatch was observed at 30, 42, and 43% RH for at 30°C; Over 90% of all eggs hatched when tested at 70, 75, and 90% RH.

For percent hatch of *P. regina* eggs at 30°C, significant differences were observed only between 58% and all of the other RH levels tested (70, 75, and 95%). For *P. coeruleiviridis* at 20°C, significant differences were found between most of the RH pairs compared for mean hatch time and percent hatch (Table 6.1). The lowest percent egg hatch for *P. coeruleiviridis* occurred at 53%, where only about 1% of the eggs in each of the 10 egg clusters tested hatched. A higher hatch rate was observed for 20°C / 48% RH (10% hatch), although only one cluster was tested. However, 48 and 53% were not found to be significantly different for percent hatch for *P. coeruleiviridis* (Table 6.1).

Table 6.1: Mean hatch time and percent hatch data for *Phormia regina* and *Phaenicia coeruleiviridis* eggs. Means followed the same letter are not significantly different

Species: *Phormia regina*

Temp	RH	Hatch Time (Mean \pm SD)	% Hatch (Mean \pm S D)	<i>n</i>
20°C	47	22.9 \pm 1.0a	4.0 \pm 5.2a	6
	49	21.3 \pm 1.3b	50.6 \pm 19.9b	9
	66	20.8 \pm 1.0b	58.6 \pm 36.8b	7
	71	20.5 \pm .4b	47.5 \pm 7.1b	8
30°C	30	--	0	20
	42	--	0	6
	43	--	0	7
	58	12.5 \pm .6a, c	36.3 \pm 19.4	16
	70	11.5 \pm .4b, c	90.0 \pm 0a	10
	75	12.0 \pm .3a, b, c	90.0 \pm 0a	4
	95	13.4 \pm .6d	90.0 \pm 0a	7

Species: *Phaenicia coeruleiviridis*

Temp	RH	Hatch Time (Mean \pm S.D)	% Hatch (Mean \pm S D)	<i>n</i>
20°C	48	23.5 \pm 1.1a, b, d, e	10.0*a	1
	53	24.7 \pm 1.4a, b	1.0 \pm 0a	8
	66	20.4 \pm 1.0c, f	48.6 \pm 27.7b	14
	70	23.3 \pm .8a, d, e	90.0 \pm 0c	6
	75	23.0 \pm .7a, d, e	84.0 \pm 13.6c	11
	95	20.8 \pm .7c, f	76.0 \pm 14.3c	10
30°C	62	11.22 \pm 0.7	90.0	12

P < 0.05; Fisher's protected LSD

*: only one egg cluster tested

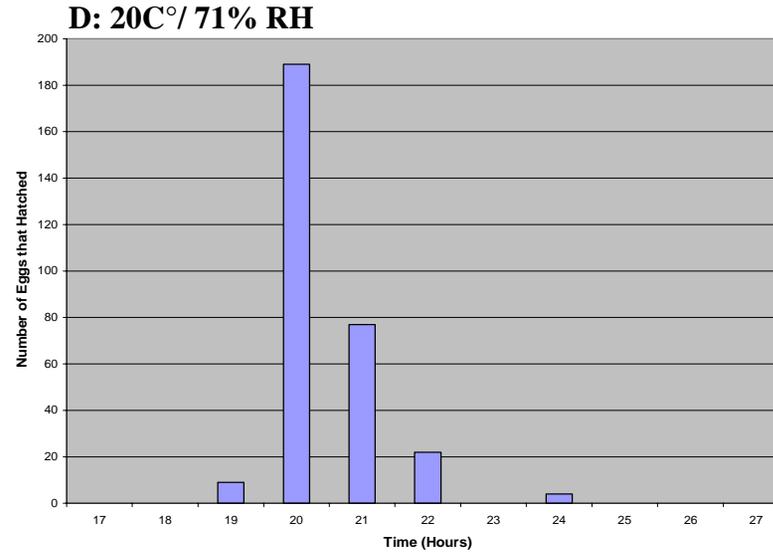
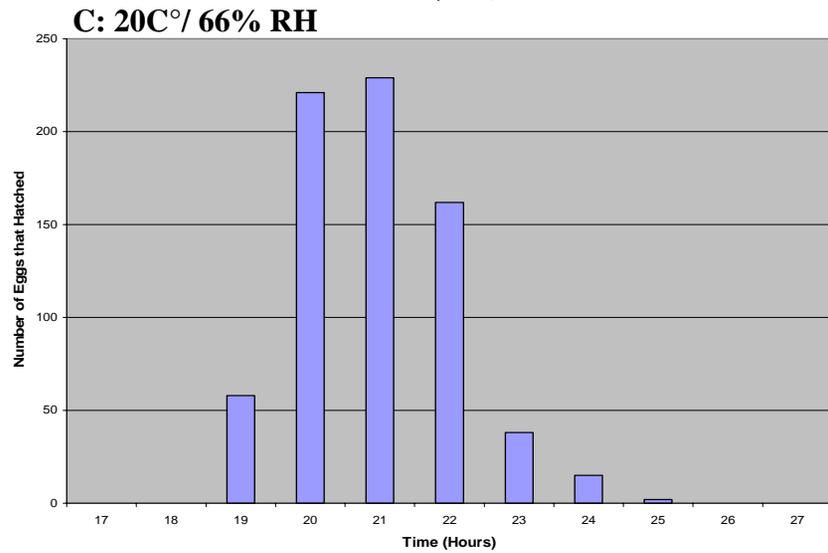
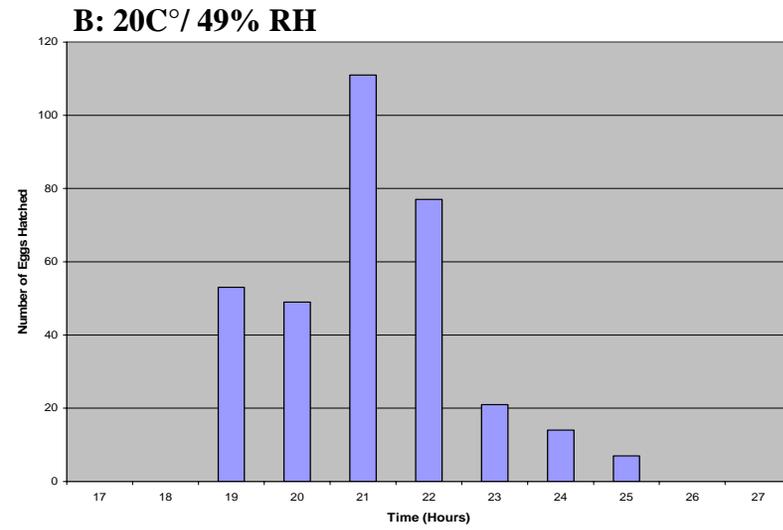
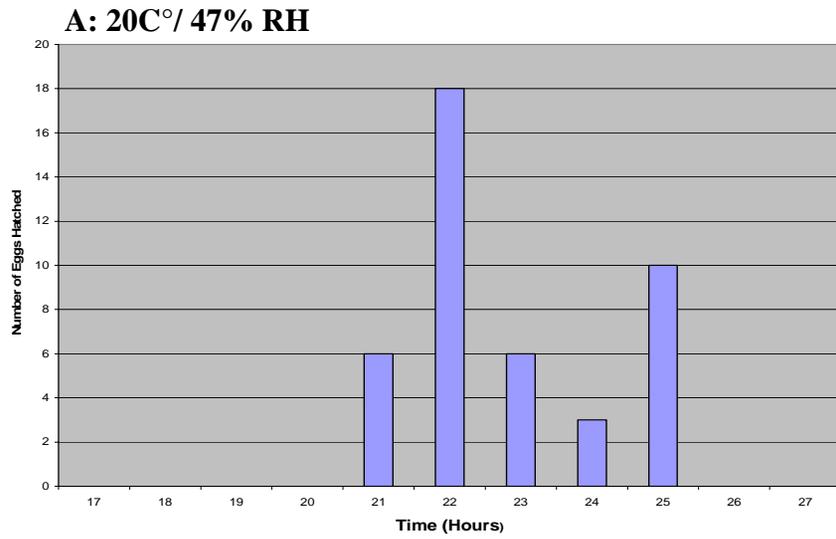


Figure 6.2: Total number of *Phormia regina* eggs hatched at 20°C, 47 (A), 49 (B), 66 (C), and 71 (D) % RH

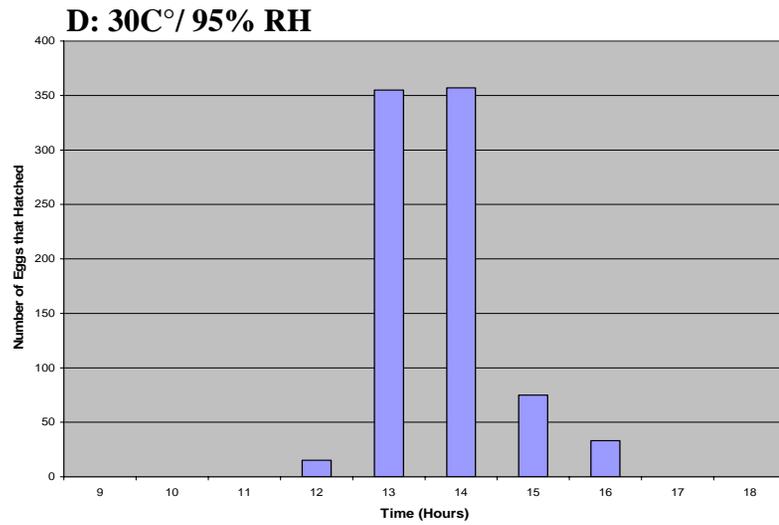
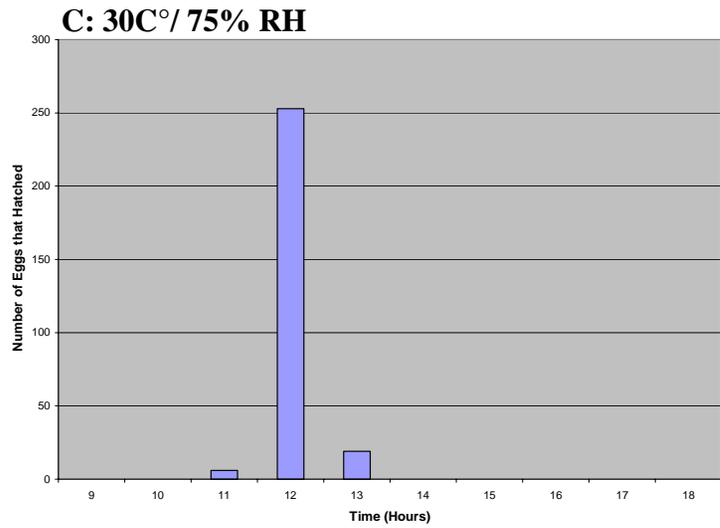
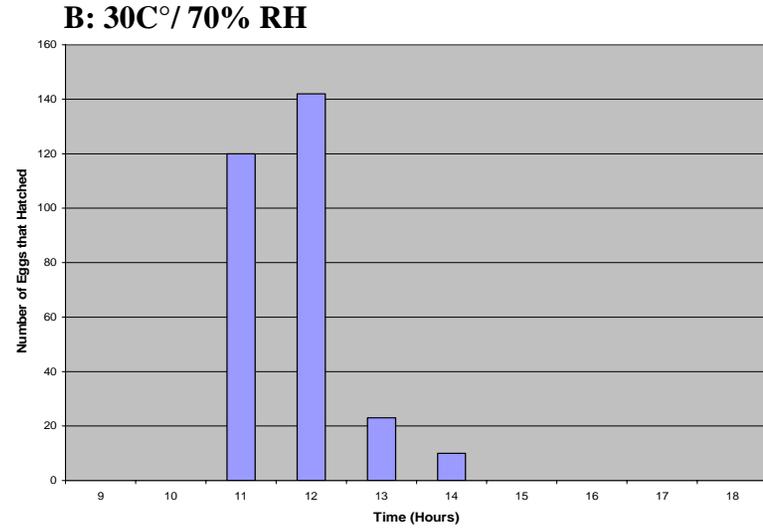
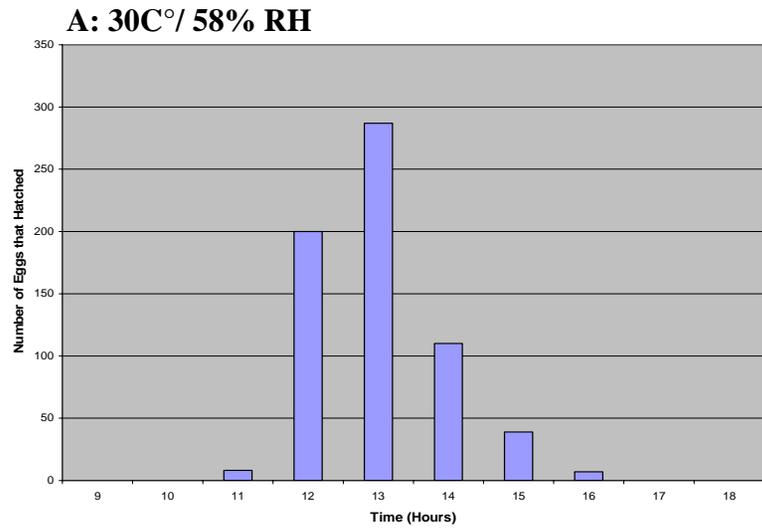


Figure 6.3: Number of *Phormia regina* eggs hatched at 30°C, 58 (A), 70 (B), 75 (C), 95 (D) % RH

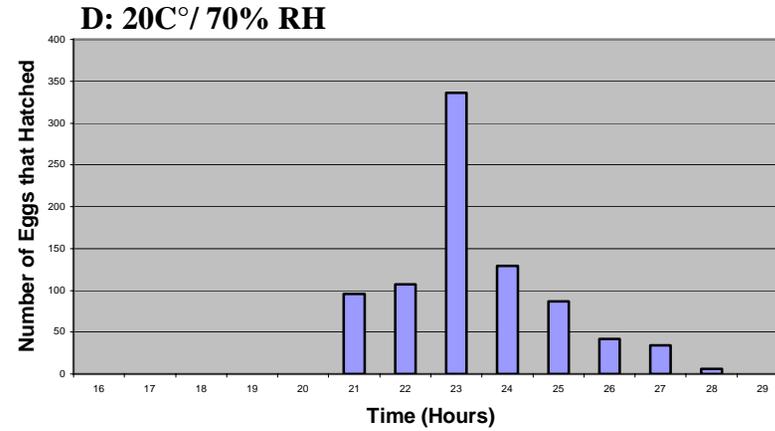
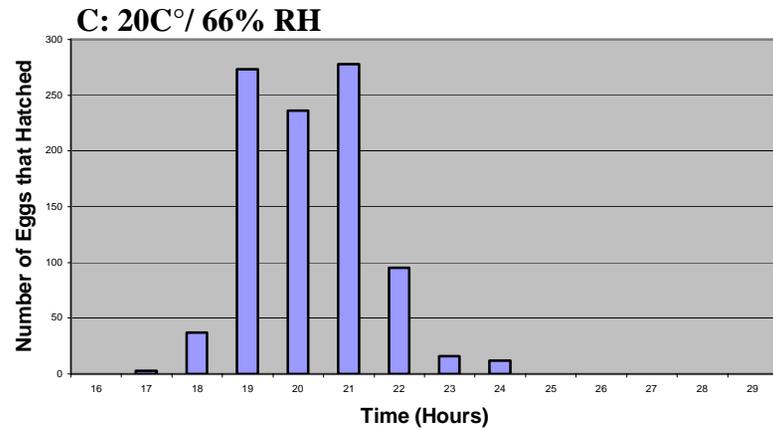
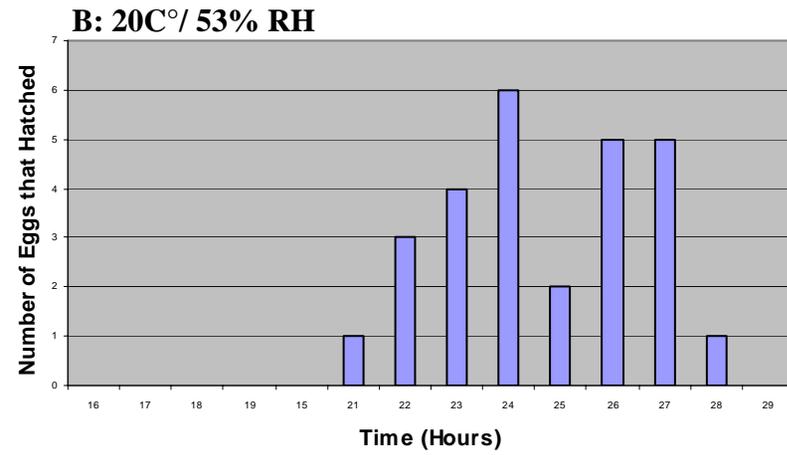
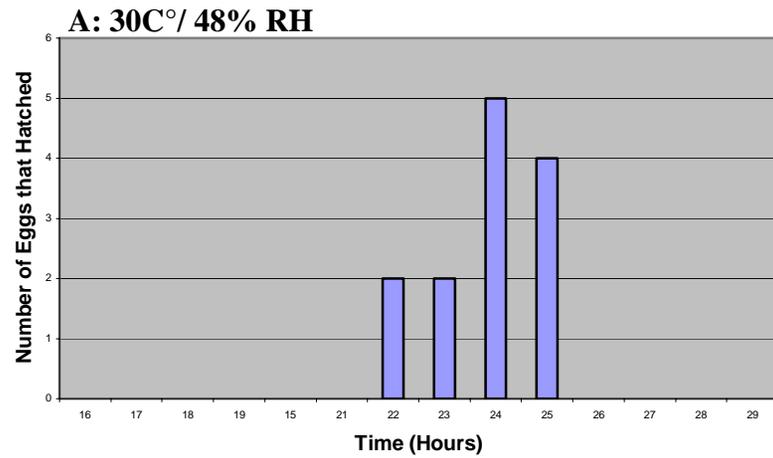


Figure 6.4: Number of *Phaenicia coeruleiviridis* eggs hatched at 20°C, 48 (A), 53 (B), 66 (C), and 70 (D) % RH

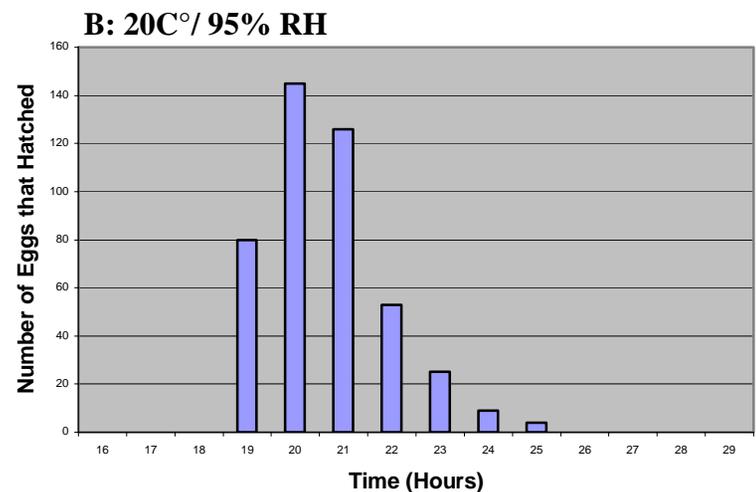
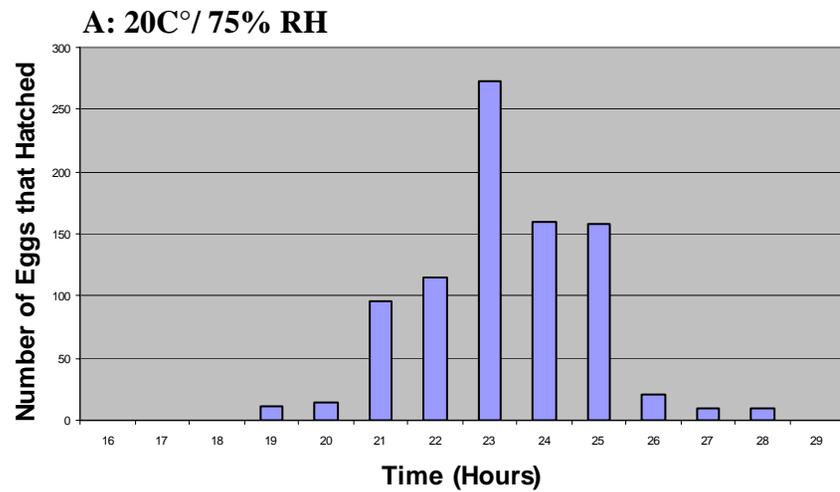


Figure 6.5: Number of *Phaenicia coeruleiviridis* eggs hatched at 20°C, 75 (A) and 95 (B) % RH

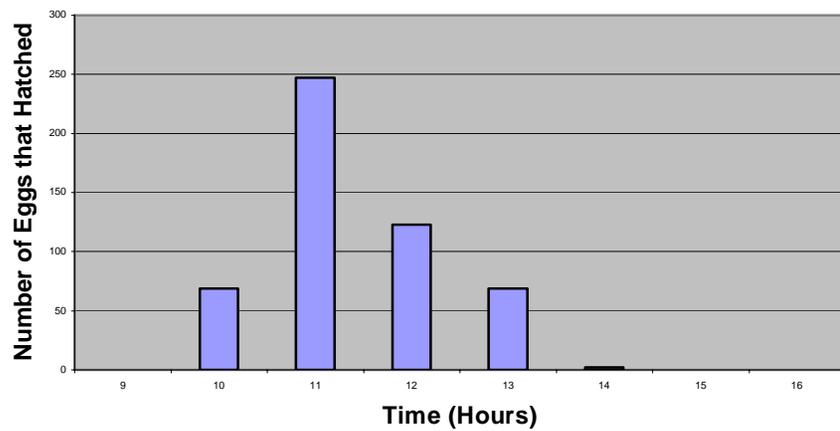


Figure 6.6: Number of *Phaenicia coeruleiviridis* eggs hatched at 30°C, 62% RH

6.4. Discussion

P. coeruleiviridis is known to be difficult to maintain in laboratory-reared colonies (Hall and Doisy 1993, Chapter 3) which is why it was necessary to obtain eggs from wild-type flies. These studies were conducted during late summer and fall when only a few visibly distinct species are common to this area. Fortunately, *P. coeruleiviridis* seemed to prefer fresh meat, whereas *P. regina* has been observed to lay eggs on meat a day or two old (Denno and Cothran 1976, Lord and Burger 1984), so targeting a species was not difficult. Ninety-nine percent of the clusters collected in this manner were found to be homogeneous.

Forty-seven percent was the only level tested for *P. regina* which was found to have a mean hatch time and percent hatch significantly different from the other three RH levels tested at 20°C. Mean hatch time was approximately 2 h longer than mean hatch times at 49, 66, and 71% RH. Percent hatch at 47% RH was only 4%, whereas at 49% RH percent hatch jumped to 50% (Table 6.1). These results are similar to those obtained for *P. sericata*, where no egg hatch was observed at RH levels below 50% (Wall et al. 2001).

The most accurate means of estimating the PMI based on entomological evidence is the use of development data for primary blow flies colonizing a corpse (Greenberg 1991). When death occurs in an outside environment, insects can arrive at and lay eggs on a corpse within hours or minutes of death, barring environmental or physical (clothing, shelter, etc) barriers. In such situations, the time of death is assumed to be close to the time the first eggs are deposited on a corpse. Eggs are considered to be the

least important life stage for forensic applications because eggs usually hatch within 24 h and before a body is discovered (Greenberg 1991). However, environmental factors (eg. temperature and relative humidity) have been shown to delay or prevent egg hatch (Evans 1934, Davies 1947, Wall et al. 2001), thus potentially skewing the PMI estimation based on development rate.

Although some of the LSD comparisons were not found to be significantly different among RH levels, RH level does appear to have a biological effect on hatch time (Figure 6.2 – 6.6). For *P. regina* at 20°C, the time at which the majority of eggs hatched decreased by approximately 1 h as the RH level increased. This same phenomenon was seen for *P. coeruleiviridis* at 20°C for RH levels 48, 53, 70, 75, and 95%, but not at 66%.

Results from this study indicated that the span of egg hatch times for same-aged eggs varies widely. We observed the range of complete egg hatch within single experiments to be as wide as 10 h (Figure 6.4a) and as narrow as 3 h (Figure 6.3c). Figure 6.4a is a normal curve with a mean hatch time of 24h, when approximately 32% of all eggs hatched. This value is in accordance with results from Byrd and Allen (2001), where egg hatch was observed for up to 13 h for eggs deposited within 20 m of each other, highlighting the potential for an error rate of half a day in PMI estimates based on egg hatch rate.

Water loss was shown to be the probable cause of arrested development and mortality of *L. sericata* eggs at different combinations of temperature and humidity (Evans 1934). Humidity was found to be more crucial to survival and development at high temperatures. Similarly, Davies (1947) reported that eggs transferred to a low RH

(30-40% RH) experienced 100% mortality after 9 h of exposure, even after the eggs were transferred back to 100% RH (Davies & Hobson 1935).

Several studies have been conducted on egg hatch rate of *P. regina* under different temperature regimes (Byrd and Allen 2001, Greenberg 1991, Kamal 1954, Melvin 1934). In two of these studies (Byrd and Allen 2001, Kamal 1954), relative humidity was held constant at 75% and 50%, respectively. In Greenberg and Melvin's studies, the relative humidity under which the experiments were conducted was not mentioned, but it is likely that it was held constant throughout the studies. Additionally, none of the studies included *P. coeruleiviridis*, although several of them reported on a very similar species, *Phaenicia sericata*.

The mean egg hatch rate and range of *P. regina* at 20°C / 71% RH reported in this study (20.5 h; 19.9 – 22.8 h) were very similar to those reported by Byrd and Allen (2001) at 20°C / 75% RH (20.5 h; 19-25 h). However, the mean hatch rate and range for 30°C/75% in this study (12.1 h; 11.5 -13 h) was lower than that reported by Byrd and Allen (2001) (17.5 h; 15-20-5) at the same temperature/RH. Melvin (1934) reported an even lower hatch rate at 31.7°C (9.51 h), which is the closest temperature to 30°C tested in that study. Greenberg (1991) reported an egg hatch time of 18 h for 29°C, which is within the range of what Byrd and Allen (2001) reported for 30°C.

Because development data for *P. coeruleiviridis* is rare, forensic entomologists customarily use data for *P. sericata* when estimating PMI based on development when *P. coeruleiviridis* is the species of interest (Byrd, personal communication). Davies (1947) found that the minimum relative humidity for development of *Lucilia sericata* (= *P. sericata*) eggs varied with temperature: 50% RH at 37°C, 40% at 34°C, and 25% at

30°C. Survivorship also varied from 11-92% according to the temperature and relative humidity of exposure.

Wall et al. (2001) examined the effects of humidity on egg hatch of *L. sericata* using serial KOH dilutions. Seven levels of RH were tested (32, 34, 51, 59, 85, 98, and 100%) at 15, 20, and 30°C. The number of eggs that hatched after 48 h was recorded for each temperature/ RH treatment. Egg hatch increased significantly with increasing humidity, while no eggs hatched below 50%. Temperature was not found to have a significant effect on successful egg hatch for the temperatures tested (Wall et al. 2001).

Only 2% of *P. regina* eggs hatched at 20°C / 47% RH (Figure 6.16), whereas the lowest RH at 30°C where any egg hatch was observed was 58%, where approximately 36% of the eggs hatched (Figure 6.17). No *P. regina* eggs hatched at 30%, 42%, or 43% RH at 30°C (Figure 6.17).

The effect of relative humidity on survivorship and hatch rates appears to become more pronounced at higher temperatures. In saturated air at 37°C, Davies (1947) found hatching to take 7.6-7.8 h, whereas at 50%, hatching did not occur until 11.8-14 hr (Davies1947). Evans (1934) demonstrated that low humidities caused retardation in development in *L. sericata* eggs. Davies and Hobson (1935) found that rapid hatching at 37°C required 90-100% humidity, whereas hatching was completely prevented at 50% RH. However, egg hatch did occur at 50% RH/ 37°C in larger, un-separated batches of eggs. In our studies, hatch rate was measured for individual eggs remaining in clusters, as deposited by the flies, whereas in some studies (Wall et al. 2001) individual eggs were separated and placed on a medium. The method of incubation used in egg hatch studies

is likely to have an effect on egg hatch rate and survivorship, which might account for variability of results among studies.

These studies were conducted under only 2 temperature regimes: 20 and 30°C. These temperatures were chosen because they are within the range of average summertime temperatures in southwest Virginia (National Weather Service). A greater survivorship increased proportionately with the level of RH at increasing temperatures. At greater percentage of survivorship was seen at 20 vs. 30°C, and egg hatch ceased completely at 43% RH and below. Similarly, egg hatch time decreased at 30°C.

7. Summary

The concept of using arthropods to help solve crimes is not new. One of the earliest documented cases involving forensic entomology dates back to 13th century China where a murder by slashing occurred in a small farming village. The guilty individual was identified because, although the weapon had been wiped clean, there was enough blood residue left on the blade to attract dozens of flies (translated by McKnight 1981). Since that time, there has been a great deal of major contributions to the field of forensic entomology. Bergeret (1855) was the first westerner to use insects as forensic indicators. A few decades later, Megnin (1894) observed that the cadaver has a very specific insect fauna based on the stage of decomposition therefore an estimate of when death occurred could be obtained by identifying the species present on a cadaver.

Further contributions to the field of forensic entomology include published descriptions and keys to adult and immature forensic flies (eg. Knipling 1939, Hall 1948, Sanjean 1957, Greenberg and Szyska 1984, Liu and Greenberg 1989, Greenberg and Singh 1995, Wells et al 1999) and development data for forensic flies (eg. Bishop 1914, Melvin 1934, Kamal 1958, Greenberg and Szyska 1984, Liu and Greenberg 1989, Greenberg 1991, Davies and Ratcliffe 1994, Byrd and Butler 1996, Byrd and Butler 1997, Anderson 2000, Byrd and Allen 2001, Grassberger and Reiter 2001, Grassberger and Reiter 2002). Additionally, several textbooks devoted to forensic entomology have been published (Smith 1986, Catts and Haskell 1990, Byrd and Castner, eds. 2001, Greenberg and Kunich 2002).

Entomological evidence can provide a great deal of information to an investigation, including whether or not the corpse has been moved from the location of

death, wound sites, and blood spatter analysis. The most important function of entomological evidence is its role in the estimation of the postmortem-interval (PMI). The two primary ways in which insects are used to estimate the PMI are 1) by analyzing the degree of development of the insects derived from the first eggs deposited on a corpse (i.e., the “oldest individuals”) and, 2) by analyzing successional patterns of insects visiting a corpse. The degree of development of the “oldest individuals” indicates the PMI because flies in the families Calliphoridae, Sarcophagidae, and Muscidae, often arrive at and lay eggs on a corpse within minutes following death (Catts and Goff 1992). Successional patterns also indicate the PMI because insects arrive at a corpse in predictable successional waves based on the stage of decomposition (Reed 1958, Payne 1965).

The use of insect development for PMI estimation requires development data for the species of flies collected from a corpse. In most locations, these species are flies in the families Calliphoridae, Sarcophagide, and Muscidae. A number of laboratory and field studies have been conducted on a variety of forensically relevant fly species (see above). For some species, studies are antiquated or rare, or data are conflicting in the literature.

In addition, although succession patterns are predictable at the order and family level, succession patterns are environmentally and location specific at the genus/species level. Therefore, it is necessary for successional patterns characteristic for an area (baseline fauna) to be known in order to provide a basis of comparison with the assembly of fauna collected on a corpse at the time of discovery (corpse fauna). In a typical forensic succession study, the carcass of a euthanized animal is placed in the field and

allowed to decompose for a given length of time. Arthropod sampling is conducted at regular intervals throughout the decay process so that taxa characteristic of each stage of decomposition can be identified. The resultant data provide baseline fauna for the area in which the study was conducted.

Succession studies are typically conducted on an animal model, although some studies have been performed using human corpses (Rodriguez and Bass 1983, Smeeton et al. 1984). The preferred animal model for such studies is the domestic pig, *Sus scrofa* L., because its physiology is similar to humans, it is easy to obtain, and it is relatively hairless. Succession studies using a variety of animal models have been conducted in a wide variety of regions throughout the world (Bornemissza 1956, Reed 1958, Payne 1965, Rodriguez and Bass 1983, Early and Goff 1986, Anderson and VanLaerhoven 1996, Dillon 1997, Watson and Carlton 2003).

To date, the baseline fauna for southwest Virginia has not been characterized. The first objective of this research was to identify and qualitatively assess the major taxa of forensic importance in this region. Carcasses of the domestic pig, *Sus scrofa* L., were placed in field conditions and allowed to decompose until they reached the advanced decay stage of decomposition. Over 50 taxa were collected and identified. The most dominant flies collected were two calliphorid species, *Phormia regina* and *Phaenicia coeruleiviridis*. *P. regina* was found to be the dominant fly species in the spring and co-dominant with *P. coeruleiviridis* in the summer. Both of these species were the most abundant in number as both adults, and as maggots developing on the carcasses. Other calliphorid species commonly collected in these studies included *Phaenicia (Lucilia) sericata*, *Lucilia illustris*, *Calliphora vicina*, *Calliphora vomitoria*, and *Pollenia rudis*.

Sarcophagidae was represented by 14 taxa in the spring, 8 species in the summer and 1 species in the fall, although the number of specimens of each species was minimal compared with the abundance of calliphorids. *Sarcophaga utilis* and *Helicobia rapax* were the most frequently collected sarcophagid species.

Several species of Muscidae were identified, although many specimens believed to represent 3-4 additional muscid species were collected but not identified beyond family. Of the identified Muscidae, *Musca domestica* and *Hydrotaea leucostoma* were the most commonly collected. Two Piophilidae species, *Stearibia nigriceps* and *Prochyliza xanthostoma*, and one Sepsidae species, *Meroplus minutus* were abundant in the mid- to late- stages of decomposition.

The primary beetle species collected in spring and summer included three Staphylinidae (*Creophilis maxillosus*, *Platydracus maculosus*, and *Aleochara lata*) and three Silphidae (*Oiceoptoma noveboracense*, *Necrodes surinamensis*, and *Necrophila Americana*). No beetles were observed in the fall study.

A second objective of this research was to analyze successional patterns of taxa collected and identified in the carrion-insect succession studies. Occurrence matrices were constructed for the successional patterns of insect taxa during 21 sampling intervals in the spring and eight intervals in the summer studies. Jackknife estimates (mean \pm 95% CL) of overall Jaccard similarity in insect taxa among sampling intervals in the occurrence matrices were 0.213 ± 0.081 (spring 2001), 0.194 ± 0.043 (summer 2001), 0.257 ± 0.068 (spring 2002), and 0.274 ± 0.172 (summer 2002). Permutation analyses of the occurrence matrices showed that the patterns of succession of insect taxa were similar between spring 2001 and 2002 ($P = 0.001$) and between summer 2001 and 2002 ($P =$

0.007). Results indicated that the successional patterns appear to be typical for the seasonal periods of the studies.

The third objective of this research was to test for effects of antemortem ingestion of ethanol by pigs on insect successional patterns and development. Pigs were dosed with a mixture of 95% ethanol and saline using an intravenous catheter, and orally by feeding and/or by oral gavage. Euthanasia immediately followed collection of antemortem blood samples and the carcasses were placed at an open field site. Insect samples were collected from carcasses for 10 days postmortem and the collected data were used to develop occurrence matrices. Permutation analysis to test the null hypothesis of no similarity between successional patterns of taxa from treated and untreated pigs showed that the successional patterns were similar ($P < 0.01$).

In addition, loin meat from the treated and untreated carcasses was used as a rearing medium for field development studies of the black blow fly, *Phormia regina*. Student's *t*-test results indicated that development (as measured by maggot length) of the 3rd instar maggots was significantly different between individuals feeding on tissue from alcohol-treated and untreated pig carcasses. However, no significant differences in development were detected in 1st and 2nd instars between treated and untreated pigs.

The final objective of this research was to test the effect of relative humidity (RH) on egg hatch time and % hatch on eggs of two common forensic Calliphoridae species, *Phormia regina* Walker and *Phaenicia coeruleiviridis* Macquart. Laboratory studies were conducted using saturated salt solutions to test a range of relative humidities at 20°C (47, 49, 66, and 71%) and at 30°C (58, 70, 75, 95%) for *P. regina* and at 20°C (48, 53, 66, 70, 75, and 95%) for *P. coeruleiviridis*. The global ANOVA of mean hatch times

and percent hatch was found to be significant for *P. regina* at 20°C and 30°C and *P. coeruleiviridis* at 20°C among RH levels at $\alpha = 0.05$. Significant differences were found between some but not all RH levels within all three data series tested. For *P. regina*, significant differences in mean egg hatch and percent hatch were only observed at 20°C, 47% RH. At 30°C, mean hatch time was not significantly different between 70 and 75%, but 95% was found to be significantly different from 58, 70, and 75% RH. For *P. coeruleiviridis* at 20°C, significant differences were found between most of the RH pairs compared for mean hatch time and percent hatch.

In conclusion, this project supplies relevant information to the database of forensic literature. A baseline fauna was described for southwest Virginia for spring and summer and was found to be consistent over two years. Additionally, it was determined that ante-mortem ingestion of ethanol affects 3rd instar maggot growth rate, thereby highlighting the potential of skewed PMI estimates based on development in death cases involving alcohol.

8. References

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9. Vita

Kimberly Lane Tabor was born on April 25, 1969 in Raleigh, North Carolina. She is the daughter of William and Robin Tabor and has a twin sister, Kellie Tabor-Hann and two older sisters, Tracy Mitchell and Kristen Tabor. She will be married to her fiancé, Kelly S. Kreitlow, in September, 2004. Kimberly attended North Carolina State University, from where she earned a Bachelor of Arts degree in Communication in 1992, a Bachelor of Science degree in Zoology in 1996, and a Master's degree in Entomology with a focus on Apiculture in 2000. She moved to Blacksburg, VA to pursue a Ph.D. in Entomology at Virginia Polytechnic Institute and State University.