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ASSESSING ERROR IN PMI PREDICTION USING A FORENSIC ENTOMOLOGICAL COMPUTER MODEL
NIJ #2000RBCX0002

FINAL REPORT, REVISED OCTOBER 2004

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Summary

The study described herein details investigations into the thermal behavior of forensically important maggots, the effect of internal maggot mass heat generation on maggot development time, and the sources and magnitudes of error affecting a phenological computer model that predicts the likely post-mortem interval (PMI) of human remains found long after the person's demise. Field studies were performed in north Florida and northwest Indiana with 88 domestic pigs (*Sus scrofa*) allowed to become colonized naturally in a wooded setting by blowflies (Insecta, Diptera: Calliphoridae) other carrion-feeding flies, and other insects, while being protected from larger scavengers. Adult flies found around the corpses were captured and identified, and samples from maggot masses were characterized by species and life stage. Each maggot mass was also measured for dimensions and temperature. Some pigs were further monitored with a linear probe that continuously measured the internal temperature of the pig and masses in 12 locations along the probe

The computer model was revised to accept parameters for 3 species of fly: *Chrysomya rufifaces, Cochliomyia macellaria,* and *Phormia regina* using data from the Ph.D. thesis of J.H. Byrd. It was also enhanced with a maggot mass temperature submodel using data gathered from the linear probe, and individual temperatures of maggot masses. Using this expanded model, we ran simulations using weather data from the Florida test plots, and compared those PMI predictions to actual results found in the field trials.

With the few useable field trials, the time of death estimates have been found to be quite accurate in our limited testing, with preliminary error rates in the 1-2% range. The majority of the field trials were not useable at this time because we have no phenology data for the most common fly found, *Phaenicia coeruleiviridis*. When phenology is obtained for that species, we can run those trials and obtain a more robust set of error measurements. We also will perform validaiton using cold-case criminal data.

Sensitivity analysis showed that the most sensitive parameter in the model is the growth rate of the maggots. This is followed closely by air temperature, which becomes less important as the mass grows larger and has better internal temperature regulation. Other parameters were less sensitive, such as the rate at which heat contained in the air, soil, or body transfers to other locations. We also analyzed error rates brought about by sampling imprecision and found, unsurprisingly, that larger sample sizes lead to greater precision in the model.

INTRODUCTION

Forensic entomology is the broad field where arthropod science and the judicial system interact (Hall 2001). The field of forensic entomology has been divided into three areas: medicocriminal entomology (also referred to as medicolegal entomology), urban entomology and stored product entomology. Information gained from forensic entomology typically is used to determine time of death, place of death and other issues of medical or legal importance (Gordh and Headrick 2001). Urban entomology concentrates mainly on controversies involving termites, cockroaches, and other insect problems accruing to the human environment, whereas stored product entomology involves disputes over arthropods and arthropod parts in food and other products (Hall 2001).

When human remains are found, the most important questions are usually how, when, where and why the person died. Historically, determination of the postmortem interval has been estimated through observation and measurement of body conditions such as core body temperature (Nelson 1999), muscular flaccidity, rigor mortis, lividity, pallor of the skin and others (Smith 1986; Bass 2001; Byrd and Castner 2001). Entomological specimens in medicolegal death investigations can be reliable indicators for estimating the postmortem interval (PMI) in both early and advanced stages of cadaver decomposition (Nuorteva 1977; Smith 1986; Goff et al. 1988; Kashyap and Pillay 1989; Greenberg 1991; Byrd 1998).

Insects and other invertebrates feeding on carrion form a distinct faunal succession associated with the various stages of decay (Smith 1986). Recognition of the different immature stages of each species involved, together with the knowledge of their rates of development, can give an indication of the PMI (Smith 1986). A forensic entomologist can also determine the age of immature insects, based upon knowledge of the variables regarding insect invasion of human remains. Evaluation and interpretation of entomological evidence at a crime scene can address other complicated issues in addition to time of death, including: season of death, geographic location of death, movement or storage of the remains following death, location of specific sites of trauma on the body, sexual molestation and use of drugs (Haskell et al. 1997).

In case studies conducted in varying temperate and tropical climates, where human remains were exposed to the environment for 2.5 months or less, entomology-based PMI estimates differed by ± 48 hours when compared with the intervals determined by independent corroboration such as confessions and eyewitness testimony (Greenberg 1985; Goff, Omori et al. 1988; Lord 1990; Byrd 1998). Clearly, entomological evidence is the most reliable scientific means of estimating a PMI of 72 hours or more (Kashyap and Pillay 1989; Catts and Haskell 1990; Anderson 2001).

The study of insects important to forensic entomology has been conducted mainly through the use of non-human animal models. Decomposition studies worldwide have used a variety of different carcass types and sizes including: dogs (Reed 1958; Jiron and Cartin 1981; Early and Goff 1986), cats (Early and Goff 1986), voles (Lane 1975), rats (Greenberg 1990; Tomberlin and Adler 1998; Faucherre et al. 1999; Kocarek 2001), squirrels (Johnson 1975), foxes (Easton and Smith 1970; Smith 1975), pigs (Payne 1965; Tullis and Goff 1987; Haskell 1989; Anderson and VanLaerhoven 1996; Tessmer and Meek 1996; Richards and Goff 1997; deCarvalho et al. 1999; Shahid et al. 1999; Davis and Goff 2000; deCarvalho and Linhares 2001; Wolff et al. 2001; Tenorio et al. 2003), seals (Lord and Burger 1984), guinea pigs (Bornemissza 1956), mice (Putnam 1978; Blackith and Blackith 1989), lizards and toads (Cornaby 1974), raccoons (Joy et al. 2002), turtles (Abell et al. 1982), poultry (Hall and Doisy 1993; Tessmer,

Meek et al. 1995), sheep (Deonier 1940), rabbits (Denno and Cothran 1975; Tantawi et al. 1996; Bourel et al. 1999), elephants (Coe 1978), opossums (Goddard and Lago 1985), black bears (Anderson 1998), and impala (Braack 1981). The only faunal succession research on human remains was conducted in Tennessee (Rodriguez and Bass 1983; Catts and Haskell 1990).

Human cadavers are not easily obtainable for detailed decomposition studies. Pigs, *Sus scrofa*, are omnivorous, have similar gut fauna, are relatively hairless and have skin that is very similar to that of humans (Anderson and VanLaerhoven 1996). The putrefaction of pigs proceeds approximately at the same rate as for human bodies that are of the same torso weight (Campobasso et al. 2001). Haskell's 1989 study in Tennessee (unpublished) compared the insect community structure and decomposition rates between adult and infant human remains and that of pigs and found no significant difference in the composition of the insect communities in human and pig carcasses (Campobasso et al. 2001). Therefore, twenty-two kg pigs have been recommended as suitable human models for adult decomposition (Catts and Goff 1992).

Biology of Calliphorid Flies

Two major groups of insects are predictably attracted to cadavers and provide the majority of information in forensic investigation; the flies and the beetles (Castner 2001) . This study focuses on the Family Calliphoridae, commonly called the blow flies, which are the first to find and colonize human corpses. Experimental studies indicate that these flies arrive at carcasses within minutes of their exposure (Shean et al. 1993; Byrd and Castner 2001).

There are more than 1000 species of blow flies throughout the world. This family includes the green bottle flies (genus Phaenicia), blue bottle flies (genus Calliphora), the screwworm flies (genus Cochliomyia) and the black blow flies (tribe Phormiini). The common name is derived from the manner in which these flies deposit their eggs (Hall 1948). The family name means 'beauty bearer' in Greek (Greenberg and Kunich 2002).

Calliphorid flies have highly specialized sense organs on their antennae that are stimulated by putrefaction odors and gases that are released during post-mortem decomposition of organic matter. Studies indicate that some species of Phaenicia are attracted to various organic sulphur compounds, either alone or in combination with hydrogen sulphide, and also by ammonia (Cragg 1956; Cragg and Cole 1956; Ashworth and Wall 1994; Wall and Warnes 1994). Nilssen used insect flight traps baited with dimethyl trisulphide and found that the chemical was a strong attractant for some calliphorids (Nilssen et al. 1996). Odors from Proteus mirabilis, a bacterial infection that occurs in the fleece in sheep, are attractants to some calliphorid flies (Morris et al. 1998).

Landing behavior of calliphorids is also dependent on visual cues such as color (Wall et al. 1992, Hall et al.1995). Oviposition is elicited primarily by the presence of ammonia-rich compounds, moisture, pheromones, and tactile stimuli (Ashworth and Wall 1994) yet was rarely stimulated by chemicals used alone (Cragg 1956). Unfortunately, the complex interaction of semiochemical and visual cues used for resource location remains little studied in calliphorids (Wall and Fisher 2001).

Blow flies are heliotropic and usually rest at night. Eggs are not usually laid at night although clearly there are exceptions. Green (1951) observed that Calliphora deposited eggs at night under artificial light in slaughter houses. He wrote that "under laboratory conditions it has been found that Calliphora erythrocephala (now called C. vicina), Lucilia sericata and Phormia terrae-novae will all oviposit in total darkness, although Wardle (1930) asserts that blowflies do not oviposit in the complete absence of light". Greenberg (1990) observed Phaenicia sericata,

Phormia regina (Meigen) and Calliphora vicina (Robineau-Desvoidy) ovipositing a very small number of eggs on rat carrion. Singh (2001) pointed out that the flies in Greenberg's experiment probably were resting on a nearby bush and literally crawled over to oviposit on the rat carrion, thus indicating that blow flies were not actively searching for an oviposition site. Nocturnal oviposition has not been observed in large-scale studies in other areas (Greenberg 1990; Byrd 1997; Haskell et al.1997.

     Other factors that affect blow fly activity are temperature, size of the carcass, geographical location, humidity, light and shade, seasonal and daily periodicity, availability of food and competition, maggot mass temperature and manner of death.

Description of original "maggot model"

     Our model of forensic fly phenology was developed using the Matlab/Simulink software simulation package (Figure 1). Mathematically speaking, we use distributed delays (Manetch 1976, van Sickle 1977) to model the developmental delay process. MacDonald (1978) has called this method the 'linear chain trick'. For each biological stage (egg, larva1, larva2,…) a chain of differential equations is written as,

$$\dot{x}_1 = input - ax_1$$
$$\dot{x}_2 = ax_1 - ax_2$$
$$\dot{x}_3 = ax_2 - ax_3$$
$$\vdots \qquad \vdots$$
$$\dot{x}_n = ax_{n-1} - ax_n$$

where $\dot{x}_i = \dfrac{dx_i}{dt}$ is the rate of change of the ith substage in a linear cascade of n such substages. The output from the nth substage (the emergence) in response to an impulse of input (the 'impulse response') is a *distribution* of output over time having a mean delay time of τ and a variance over time given by $\sigma^2 = \dfrac{\tau^2}{n}$ where n is the number of stages in the delay chain above.

The rate of flow, a, for the appropriate emergence pattern is $a = \dfrac{n}{\tau}$, and thus everything necessary to construct this delay model is contained in the mean and variance, (τ) and (σ^2), which are commonly recorded with most data sets. For another stage a different set of values would be obtained and the flow rate would, in general, be different, say b, but the form of the equations would be the same as in (1) above. In the Maggot Model, a, the development rate, becomes a linear function of temperature (since τ is a function of temperature), and temperature is a 24 hr cycle whose mean and amplitude can be specified. The development rate differs from stage to stage, as does its dependence on temperature. We have parameterized the model for *Cochliomyia macellaria* (the secondary screwworm) (Byrd 1998, Byrd and Allen *in press*), and the output is illustrated in Fig.2. Stage abundance or proportion in each stage over time as predicted by this model is in good agreement with *C. macellaria* abundance in a pig carcass during field tests with the model (Byrd 1998).

MATERIALS AND METHODS

The use of human corpses for field studies in maggot development is generally not legal or practical, so a substitute decomposition subject was needed. As determined in other decomposition studies (Haskell 1989, Anderson and VanLaerhoven 1996, Campobasso et al. 2001) the rate decomposition and fly colonization in pigs is very similar to that of humans: therefore, dead pigs, *Sus scrofa* L, were used as animal models for this study.

All dead pigs were purchased from a livestock market near to the study sites. Prior to purchase, the pigs were killed by a lateral, transverse shot into the top of the head with a .22 caliber rifle. This method resulted in instant death of the animal. Each dead pig was immediately double-bagged in a heavy-duty plastic trash bag and transported to the study site. The times of death and deposition were noted on the data sheet corresponding to each pig.

Study Sites and data collection

Two study sites, one in north Florida, and the other in northwest Indiana, were used to study maggot mass development and characteristics. The Florida study site was located in a wooded, 50 hectare parcel near Earleton, Florida. The Indiana study site was located in a wooded section of a 400 hectare farm near Rensselaer, Indiana. Pig carcasses were placed in partly-closed canopy areas where sunlight was somewhat restricted.

Trials were conducted from November 15, 2001, to March 5, 2004 (Table 1). Each trial consisted of 3 pigs labeled "a", "b" and "c", placed at least 18.3 meters apart. In Florida, a fourth pig, labeled "e" was used for continuous tempearture monitoring. Wire cages (86.5 cm long x 50.8 cm wide x 61 cm high), were placed over the pigs to protect them from large vertebrate scavengers. The cages were constructed of heavy wire mesh (5 cm x 5 cm), and were secured with bungee cords attached to at least four tent stakes driven into the ground around the cage (Photo 1). The cages were lifted off and set aside during sampling times. Observations and collections were made daily between the hours of 2:00 and 6:00 in the afternoon, when flies were most active, from the day of deposition until the first wave of maggots left the body for pupation. The pigs were not moved or disturbed in any way during the study, except for careful temperature readings taken from the exposed maggot masses. Scaled digital pictures of the pigs and of the expanding maggot masses were taken at each collection time. At the beginning of each collection time, a laminated identification sheet was placed in front of each pig, indicating the date, time and identification number.

On day 1, the pigs were removed from bags and placed in a location on the site, located for future reference with GPS (global positioning system). After all the pigs were in position, data were collected including pig number, time of death, date, time, sample number, ambient temperature, ground/pig interface temperature, ground temperature at 5 cm depth about 3 M from the pig, a brief description of the weather (sunny, cloudy, rain, etc.) and wind velocity in meters per second. An aerial collection of adult flies was made over each pig with side-to-side sweeping motions of a 12" aerial collection net until at least 10 adult calliphorid flies were captured, or for a maximum of 15 minutes. Flies were placed in vials with 70% isopropyl alcohol and later pinned and identified. A data logger (Figure 2-3) was affixed to a nearby tree or bush and the temperature probe was placed on the ground 1-2 m from each pig. Therefore, the ground temperature being recorded was similar to that which the pig was exposed.

After the first day, samples were made on a daily basis during warmer months, and every second or third day for cooler months. Adult aerial collections were made using similar methods

to those of the first day, and when larvae appeared, all visible maggot masses (defined as a continuous volume of closely-packed maggots) were photographed and measured for width with a cm-ruler. The depth of the maggot mass was measured by inserting the warmed stainless steel probe of a digital thermometer (described below) into the mass at several locations until the underlying flesh was detected. Larval samples of approximately 5ml (100-600 larvae, depending on size) from each maggot mass were collected, but only if the mass was large enough to sustain such depredation without more than an approximate 5% reduction in volume. The location where each sample was taken on the body area (or ground area) was noted on the collection sheet. Half of the sample specimens were boiled in water for about 2 minutes using a camp stove, and placed in vials with 70% isopropyl alcohol for preservation. These preserved larvae were separated into first, first-second transitional, second, second-third transitional and third instars. Only 3rd instar larvae were identified to species since there are no reliable taxonomic keys for first and second instar Calliphoridae larvae identification. The remaining half of the sampled larvae were placed in containers and reared to the adult stage.

At all sampling times, several temperature readings were taken for each pig with a Taylor 9841 digital thermometer (Forestry Suppliers, Jackson, MS) shielded from direct rays of the sun. These temperature readings included ambient air, ground/pig interface, external tissue, oral cavity of the pig, soil at 5cm depth 3 M from the pig, and soil at 5 cm depth under any maggot mass found on the ground. Maggot mass temperatures were taken by first warming the probe so that the maggots would not pull away from it, then thrusting the probe into the center of the mass and several nearby locations to ascertain the peak temperature.

Continuous monitoring of ground temperatures were taken with HOBO® H-8 thermister data loggers (Onset Computer Corp., Bourne, MA) set to record every 30 minutes (Photo 2). The temperature probe connected to each logger was set on the ground within 1-2 m of each pig (Figure 2-4).

Rearing Procedures

Maggot rearing pouches were constructed from aluminum foil and placed into ½ l plastic snap-top containers half-filled with substrate. The container lids each had ten 2 mm holes drilled for air circulation, and a paper towel was placed under each lid to prevent larvae/adult flies from escaping through lids and also to prevent other flies from getting into the containers (Photo 4).

Each foil pouch was filed with 100-200 g of calves' liver. The containers of live maggot were kept outside—except when temperatures were below 4.5ºC—on shelving placed in a sheltered, screened enclosure (Photo 5). The maggots were checked daily. Once the maggots migrated off the liver, the pouch was removed from the container. On occasion, the maggots would not move out of the foil pouch. When this occurred, a small amount of fresh liver was placed directly onto the substrate, and the maggots were gently moved from the foil pouch onto the liver. The liver would be removed a day or two later after the maggots migrated into the substrate.

At adult emergence, the containers were placed into the freezer for a few minutes to kill the flies. Adults were put in labeled vials of 70% isopropyl alcohol, then later pinned and identified. The keys used to identify calliphorids were by Wells, Seago, Dodge, White, and Furman (White et al. 1940, Dodge 1953, Seago 1953, Furman and Catts 1982, Wells et al. 1999). Gary Steck, dipterist at DPI (Florida Department of Plant and Industry, Gainesville, FL), verified the identifications of representative samples of the flies. All data were entered into a Microsoft Office XP Excel version 2002 spreadsheet (Microsoft Corp.). The data are presented in

Appendix 1. Voucher specimens are being kept in lab 2209 at the University of Florida Department of Entomology and Nematology and will be deposited with DPI.

Rearing Substrate

The substrate (which they do not eat) used to rear fly larvae consisted of about 90% dried laurel oak leaves (Quercus laurifolia) and branches, rye grass (Lolium perenne) cuttings, magnolia leaves (Magnolia grandiflora) and sandy soil. The plant mixture was ground up with a Troy-Bilt® Model 4731-10 HP chipper/shredder with a mulching blade. The substrate was stored in a 200 L Rubbermaid trash container with a tight fitting top. On most occasions, I was able to successfully rear the larvae in containers half-filled with substrate.

Table 1: Dates of pig deposition, approximate pig weight, and time each pig was placed on site for sampling dates from November, 2001 to March, 2004.

Date of Pig Deposition	Mean pig weight (kg)	Approx. deposition time (h)
Earlton, FL		
15-Nov-01	20	1730
23-Dec-01	20	1500
1-Feb-02	25	1530
15-Mar-02	24	1545
29-Apr-02	30	1445
20-May-02	32	1630
22-Jul-02	20	1550
19-Aug-02	30	1445
23-Sep-02	20	1450
24-Oct-02	20	1730
27-Nov-02	23	1500
30-Dec-02	25	1530
28-Feb-03	21	1545
31-Mar-03	19	1445
25-Apr-03	28	1630
12-Jun-03	22	1550
8-Dec-03	28	1440
23-Jan-04	15	1545
5-Mar-04	28	1530
Rensselear, IN		
18-Jun-03	25	1445
21-Jul-03	39	1430
11-Aug-03	23	1430
22-Sep-03	21	1430

Continuous spatial temperature monitoring of maggot masses

An important piece of information missing in the forensic literature is the temperature dynamics of maggot masses through time. It is well known that large maggot masses produce their own heat through bacterial digestion of the cadaver flesh, but the exact amount of heating present in the maggot mass remains unknown, as does the change in temperature through time. It is also unknown whether the maggot mass temperature remains fairly constant, or fluctuates on a daily cycle.

We knew we needed a device to measure maggot mass temperatures continuously through time, and we were also interested in the spatial aspects of mass temperature, in order to better model the temperature environment of a typical maggot. We ended up designing our own device using a fiberglass probe and several small bead thermistors (Omega 44031, Omega Engineering, Inc. Stamford, CT). The thermistors (12 in all) were spaced 1.5 cm apart along the probe, and all were connected to continuously recording data loggers (Hobo H8, Onset Computer Corp, Bourne, MA; Photos 7 and 8).

In use, the probe was driven into the carcass of pig "e" in various directions at the time the body was deposited (Photo 9). The wound caused by inserting the probe quickly became a maggot colonization site, and the maggots generally stayed in the vicinity of the probe. The probe was generally not moved during the replicate, so the maggot behavior was not altered by disturbance. For a few replicates, the probe was placed later into an existing maggot mass to check whether the temperatures seen in the other replicates were artifacts of the wounding process. Because this was an intrusive operation, the pigs used for continuous maggot mass temperature recording were kept separate from the pigs used to record the spontaneously generated maggot mass temperatures and volumes.

RESULTS AND DISCUSSION

Data sources and disposition

The work described herein is part of a larger series of investigations in the behavior and modeling of maggots and the subsequent prediction of the post-mortem interval. Table 2 details work performed prior to this study, and the disposition of data products from this study.

Table 2: Sources of development and independent validation data for different parts of the forensic development model.

Model element	Source for development	Source for Validation
Maggot phenology parameters and basic model	Byrd 1998 (lab experimentation with 3 independent replicates)	All pigs a,b,c (preliminary validation), solved case data (final validation, to be completed)
Maggot mass thermal model	Florida Pig #4e (1 pig)	All other pigs labeled "e"from Florida (8 useable, all independent)
Maggot Mass Peak Temperatures	Florida pigs 1a,b,c – 10a,b,c (30 pigs, 10 independent replicates)	Florida pigs 11a,b,c – 19a,b,c and all Indiana pigs (39 Pigs, 13 independent replicates)

Continuous spatial temperature monitoring of maggot masses

The output obtained from this probe was enlightening and impressive (Figure 3). When properly displayed, output graphs clearly show the initial cooling off of the corpse, daily temperature cycling corresponding with solar heating, and cyclic heating of the maggot mass after several days. We gained two key insights into maggot mass heating from this probe: maggot mass heat production is cyclic, and corresponds to a peak temperature determined by maggot mass volume. The data recorder from existing maggot masses with the digital thermometer matched closely those from the fiberglass probe, indicating that the maggot mass thermal behavior that we recorded was robust and a product of the maggots' natural behavior.

Maggot mass temperature results and modeling

Knowledge of maggot mass temperatures through time is vital to the estimation of PMI, because maggots are able to increase their own temperature through extra-oral metabolic heat production, thus speeding their development. Failure to take this extra heat into consideration will result in a PMI estimation that is too long.

Possible sources of maggot mass temperature error:
- Not finding the highest temperature with the probe
- Mis-measuring the mass size (especially depth)
- Possible recent changes in maggot mass structure
- Maggots are spilled out along the ground (the maggots might develop slower than expected)
- Misjudgment of the peak temperature hour

In only one case (first set of pigs) did the temperature of the maggot masses decrease in the older samples. These later temperature readings were also of smaller maggot masses, so the relationship of volume to temperature held constant. This leads to questions about how accurately we can estimate the past volume of the maggot mass by its volume at the time of

measurement. It would be simple to assign a constant growth rate to the mass based on the number of maggots found and their individual growth rates, and indeed this is what we do in the model, but this does not take into account migration and mortality. The magnitude of these 2 variables may never be known for a given mass, but if significant they can change a PMI estimate by a large amount.

Combining data recovered from the temperature probe with readings of maggot mass volumes and peak temperatures taken from the other study pigs allowed us to determine a relationship between maggot mass volume and peak temperature (Figure 4). We found that once a maggot mass reaches a volume of approximately 20 cm³, it is essentially providing its own temperature environment regardless of the ambient conditions. Masses found on the soil were relatively cooler and more influenced by ambient temperatures, because of the greater cooling potential of the soil versus the body. (Figure 5).

With the peak temperatures determined by the mass volume and location, we looked at the results from continuous maggot mass temperature monitoring. We found that we were able to produce a mathematical model of maggot mass growth and heat production that matches well with the data (Figure 6).

Based on the individual temperature measurements and the linear probe data, we found that there were robust relationships among time of day, daily peak ambient temperature (ambient for the maggot mass, that is, the temperature of the surrounding flesh), volume of maggot mass, and its core temperature. What we found is that while the peak daily temperature of the maggot mass is independent of ambient temperature, its temperature at other times of the day is not.

The relationships that we found indicates that through some mechanism, the temperature output of the mass is reset each day, so that the peak temperature ends up at the same level based on the volume of the mass, but the nighttime temperatures will fluctuate according to the changing ambient temperatures. That is, if the ambient temperatures do not fluctuate, then temperature of the mass would not fluctuate, but as ambient temperature fluctuates, so do the maggots in the mass. This mechanism is apparently biochemical in nature, but we do not know whether it originates with the maggots themselves, or with the symbiotic bacteria that perform the extraoral digestion required for producing fluids upon which the maggots feed.

With these relationships defined, we can model the temperature of the maggot mass, **m**:

$$\mu(t) = \theta(t) + \left(\overset{*}{\mu}(t) - \overset{*}{\theta}(t) \right) \cdot \min\left[\frac{v(t)}{20} \quad 1 \right]$$

where **v** is the volume of the maggot mass in liters, and

$$\overset{*}{\mu}(t) = 3.892 \cdot \log(v(t) + 18.813 \,.$$

We validated this model for the Florida study site using independent pigs, and found a good fit to the measured core maggot mass temperatures. The rise and fall of the overall heat output of this particular maggot mass can be seen clearly, as the maggots coalesce and grow, then disperse to pupate in the soil some days later. Further validation should be undertaken in different geographical regions to ascertain whether the diurnal temperature patterns are robust for all locations.

Other modeling improvements

The original "maggot model" depended on visual examination of the output to determine the best estimated PMI on a case-by-case basis. We wanted to improve on this somewhat arbitrary methodology, so we developed an unbiased estimation based on Euclidean distance of the proportion of maggot life stages found in a sample from the changing proportions of life stages estimated in the model through time.

As the model progress is, the proportions of maggot life stages present are output at each hour of the simulation (Figure 7). Similarly, the numbers of each life stage counted in the sample taken from a corpse that you are trying to determine the PMI for can also be translated into a set of proportions. Measuring the multidimensional distance from the sample proportion to the model proportion at each time step gives a reading of the relative fit of the model to the sample at each time step (Figure 8).

To determine the most likely PMI, we measure the Euclidean distance between the proportion of insects found in each life stage and the proportion predicted by the model at each hour. For a system with k life stages (egg, larval stages, etc.), where $Y_{i\,s}$ is the proportion of insects in the ith stage found in the sample, and $Y_{i\,m}$ is the proportion of insects predicted by the model in the ith stage at time t, the distance between the sample and the model output is:

$$\varepsilon(t) = \sqrt{\sum_{i=1}^{k}\left(Y_{i\,m}(t) - Y_{i\,s}\right)^2}$$

The most likely PMI is the hour where this value is minimized. In practice, this value could be weighted to emphasize more important life stages or to exclude stages that are unreliably sampled, such as eggs or pupae.

Error measurement techniques and results

Sources of error

Sampling error has both the capacity to become the single largest source of error in the PMI determination, and the one that the investigator has the greatest opportunity to minimize. In a large enough maggot mass, we can assume an unlimited population relative to the small sample that is generally taken for analysis. In this case, the error inherent in sampling is simple enough to calculate, and is related to sample size. In general, the error will be smallest in samples with more individuals (larger n), and within samples, in those life stages that have either large or small proportional representation.

In all cases, the maggot mass must be well stirred before sampling to ensure an unbiased ratio of life stages. It seems likely that different size classes of maggots in the maggot mass would associate more with each other than with larger or smaller maggots, but we are unaware of any studies on this phenomenon. Also, due to interspecific predation, maggots of different species might associate more with conspecifics than with heterospecifics.

The sampling variance and distribution of a life stage proportion estimation (p) will approach normal for all samples where $0 \leq p \pm 2\sqrt{pq/n} \leq 1$ (Mendenhall et al. 1990). A normal sampling distribution is not essential, but it makes calculating confidence intervals due to sampling error much easier. For likely values of p, a sample size of approximately 80 will

suffice for this purpose. More important is the inherent sampling variability of the sample, and for reasonable error rate, the sample size should be larger than this anyway (Figure 9).

Sampling error is interesting in that it does not change the output of the model itself, but only the uncertainty of the results. As with most of the other sources of error, the effects of sampling error will vary through time. In this case, the effect of the error may be magnified or reduced based on the trajectories of the proportions of larval stages at that particular time. At times where the proportions are changing most rapidly, that is, when the life stages are at their maximum rate of metamorphosing to the next stage, any error in sampling those proportions has the least effect. At these points, there will be an error of as little as 0.75 hour per percentage point off. At times when the stages are stable the model can be off up to 4 hours per percentage point. This uncertainty can be calculated after the model run.

For small percentages of difference, the change in maggot stage proportions is fairly linear. Since the error in sampling is normal for large samples, the error in the PMI will be normally distributed as well, and will be a simple multiple of the sampling error. This multiple can again be calculated after the model run. With this information, it is possible to calculate a normally distributed post-test sampling error rate, and construct confidence intervals based on this rate.

A stroke of good fortune is that at the point of maximum uncertainty in the sampling proportions (any $p \approx 0.5$) those proportions are changing most quickly. Therefore, whenever the sampling error becomes large, the rate of change in the model is also large, minimizing the impact of the sampling error on the final prediction. For example, given a sample size of 100 maggots, we have a confidence interval that ranges from of±4% to ±10% of the predicted value, while under the same conditions, the effect that the error has on the final prediction ranges from 4 down to 0.75. Given these values, the theoretical maximum error would be ±40%, but in reality, it will range from a maximum of ±16% at times of stage stability, down to a minimum of ±7.5% at times of rapid stage change. To cut these confidence intervals in half, a sample size five times bigger (500 maggots) is required. These confidence intervals result from random errors in sampling only, and do not take into account errors inherent in the model itself, or environmental uncertainty.

Sensitivity of model

Key to the development of confidence intervals for a model, or even to know where you need to spend most of your parameter development effort, is sensitivity analysis. Because the output for this model is complicated, and changes through time, traditional sensitivity analysis could not be performed. Instead, we expanded traditional sensitivity analysis to include this time component into different ways. First, we plotted the output of the model against itself, assuming that the correct PMI increased by one hour each time we ran the model. The result of this plot resembled a valley, with the deepest part running diagonally, representing the correct output at each hour of the simulation. Other deep areas of the output plot represent inherently sensitive points in time. If these deep areas (dark areas on the plot) are narrow, then the model is insensitive at that point, resulting in a more accurate prediction. If they are wide, the model is very sensitive at that point, and any slight deviation in parameterization or model function would results in large deviations in the model output.

We also tested sensitivity to parameters through time both with constant temperature and daily cyclic régimes. We changed each parameter in the model by plus or minus 10 and 20%,

and plotted the output on a graph with a straight lines corresponding to a one-to-one ratio of parameter change to model output change.

Looking at the sensitivity basin with a constant temperature régime, we see that the model itself is not inherently sensitive. That is, it is robust to small deviations in parameters or sample proportions (Figure 10). Several of the parameters were tested for sensitivity using this constant temperature régime (Figure 11). By far, the most sensitive parameter in the model is (not surprisingly) the growth rate of the maggots.

The model was not sensitive at all to several parameters in the constant temperature régime, so we ran both the sensitivity basin analysis and the traditional sensitivity analysis using cyclic temperature and day/night data taken from a weather monitoring station in Falwell, Texas. Looking at the sensitivity basin in these conditions, we can see several areas where the basin is very wide, and hence very sensitive to error (Figure 12). These wide areas correspond to periods of darkness: they exist because flies generally do not lay eggs at night, and so if a body is deposited any time during the night, all of the maggot masses start their development the next morning at the same time. It is therefore difficult to determine exactly at what hour during the night the body was deposited.

We found that incorporating the daily cycling radically changed the sensitivity of the model to the parameters (Figure 13). In general, there were periods (again corresponding to the daily cycle) where the model was extremely sensitive (corresponding to deposition times during the night), or relatively insensitive (corresponding to deposition times during the day).

Validation of model

The maggot growth rate parameters were based on earlier work by Dr. J.H. Byrd, so all data collected here can be used for validation. We are still compiling validation model runs from the pig data, but the preliminary runs we performed are very encouraging, with estimations differing only a few hours from the true deposition times over a period of 100+ hours. We still need to obtain cold case data where entomological evidence was obtained for a PMI estimate, and where the true PMI was later independently established to further validate the model. We have a trip planned to Indiana for this purpose. The validation results will be reported in a future publication.

Applications and conclusions

The model as it stands will be most useful for cases where human remains are found in settings where they most likely have been undisturbed since the time of death, where the original eggs laid by early-arriving flies have developed into natural masses. These conditions are similar to other entomologically-based PMI estimation methods, where any unusual conditions such as delay in body deposition, movement of the body, freezing, or submergence in water will tend to invalidate simple, naive application of forensic models.

The most important advance brought by this model is that it provides a reliable estimate of its own imprecision, where the likely range of possible PMI's can be seen. In the face of increasing judicial requirements for all scientific estimates to include measures of error, this capacity will become essential. Another important advance is that this model will place hard figures at the hands of investigators, and given the appropriate assumptions and parameters, can increase the precision of PMI estimation using blow flies over commonly available techniques.

While not eliminating the need for expert entomologists, it can be used by non-entomologist forensic investigators to provide a rapid, reliable estimation of PMI.

Forthcoming manuscripts directly related to this grant:

Gruner, S.V. – Rearing adult carrion-feeding flies of forensic importance using natural leaf media

Slone, D.H. and S.V. Gruner – Estimating the temperature of carrion-feeding maggot masses by their volume, shape and location

Gruner, S.V., N.H. Haskell and D.H. Slone – Seasonality of Blow fly communities on carrion in north-central Florida (Masters thesis)

Slone, D.H., J.C. Allen, and S.V. Gruner - Field studies of carrion-feeding maggots related to a computer model for predicting time-of-death. (PMI of pigs and correlation to maggot mass data using model)

Slone, D.H., J.C. Allen, S.V. Gruner and N.H. Haskell – Validity and error rates of a model of carrion-feeding maggot development used to estimate time-of-death in criminal cases (uses pig data and cold-case data)

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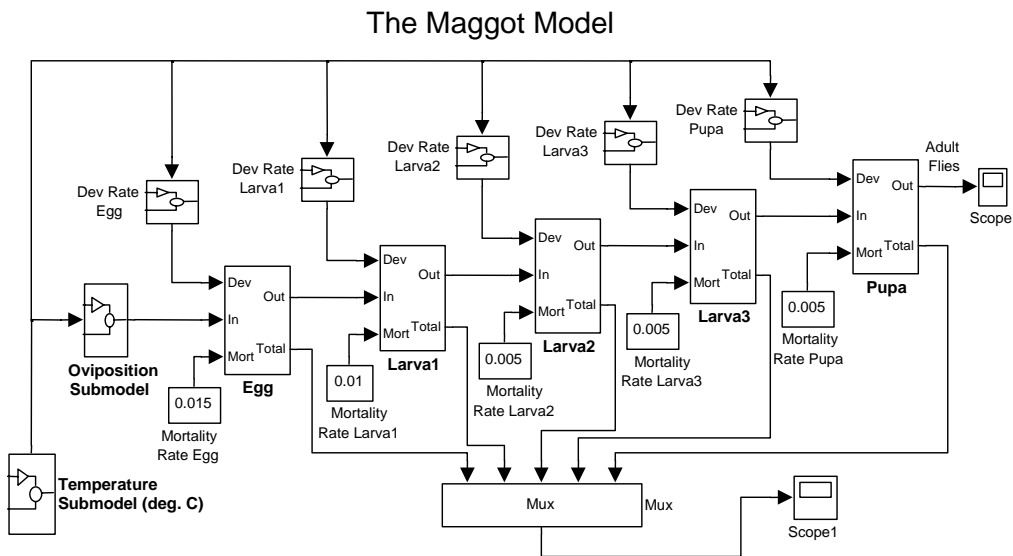
Figure 1: Simulink diagram of the original "maggot model", produced during the previous NIJ OJP grant.
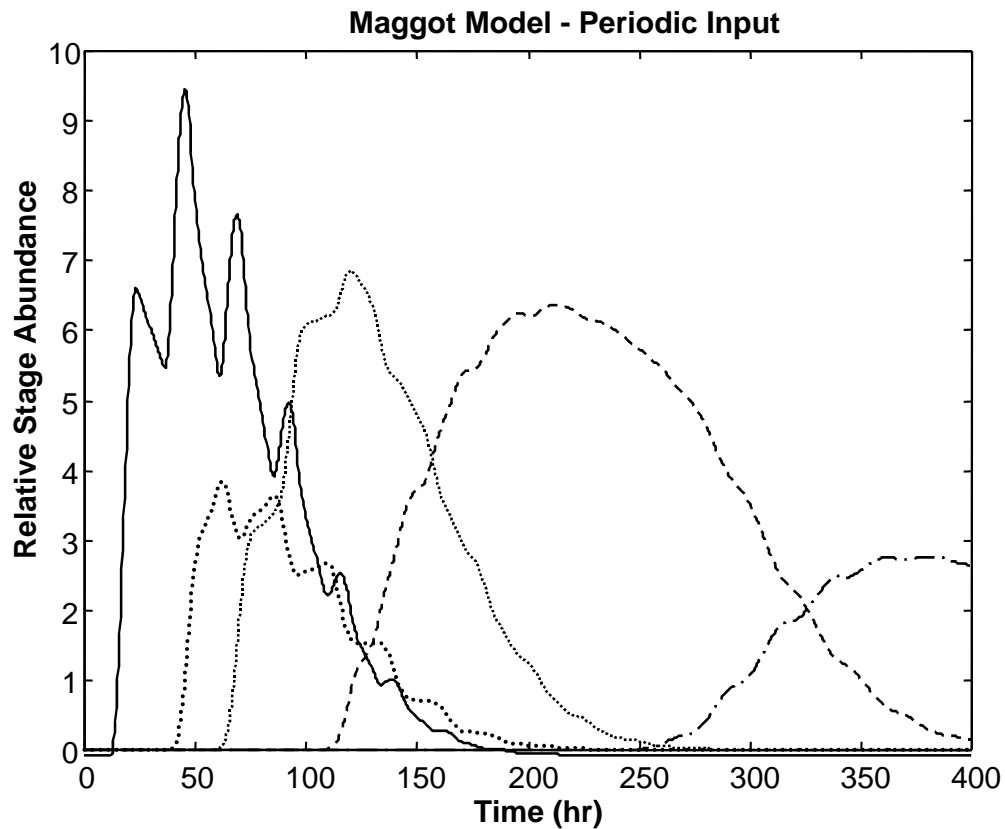
Figure 2: Output of the original "maggot model", showing all the life stages as

Figure 2: Typical output from the original "maggot model", showing populations of each stage that developed in turn.
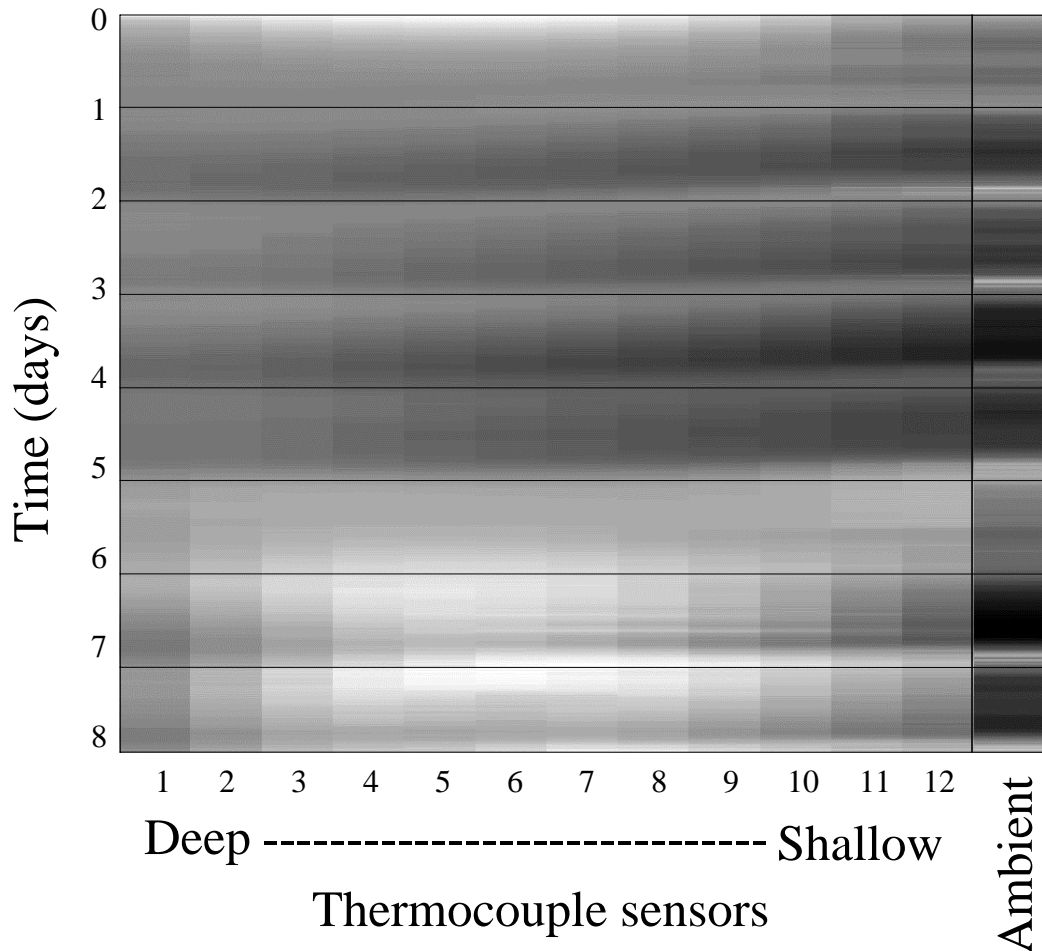
Figure 3: Typical output from temperature probe, showing low (dark) and high (light) temperatures through time along a depth gradient. Light areas near the bottom show cyclic heating of the maggot mass .
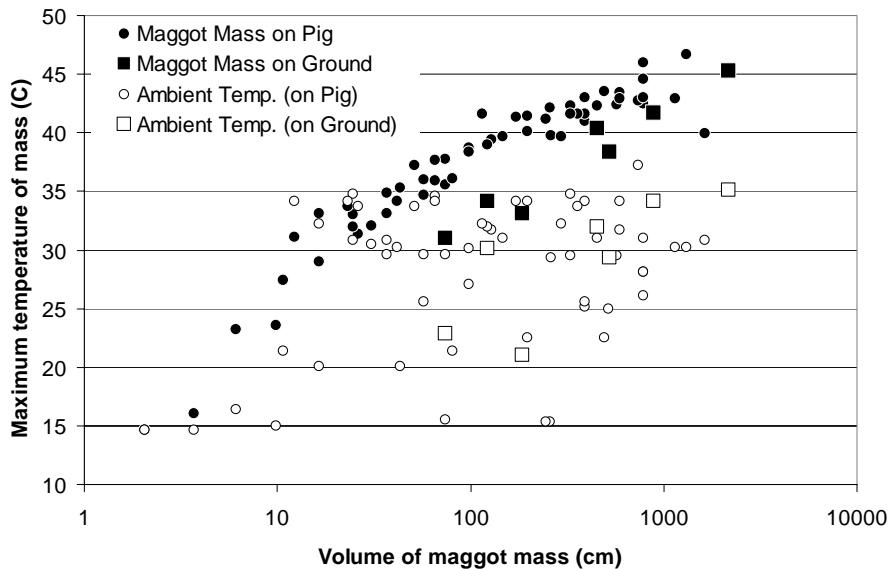
Figure 4: Peak temperature of maggot masses related to maggot mass volume. Ambient temperatures are shown in white, and the corresponding maggot mass temperatures are shown in black. On ground = maggot mass was found on ground, on pig = maggot mass was found on the pig carcass.
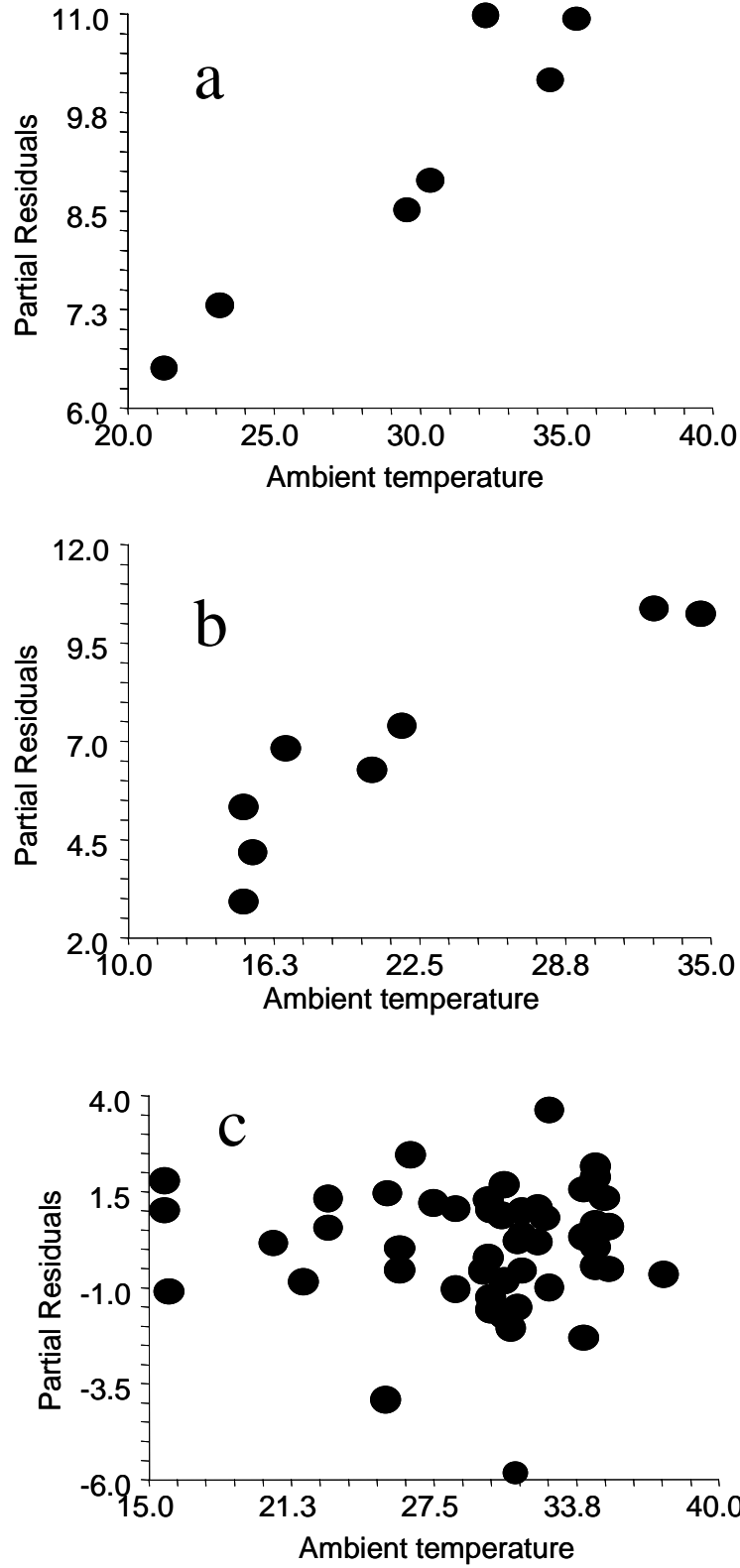
Figure 5: Partial residuals from regression of maggot mass temperature on ambient temperature, after removing the influence of maggot mass volume. a= small maggot masses (<=20cc), b= maggot mass found on ground, c= large maggot masses (>20cc).
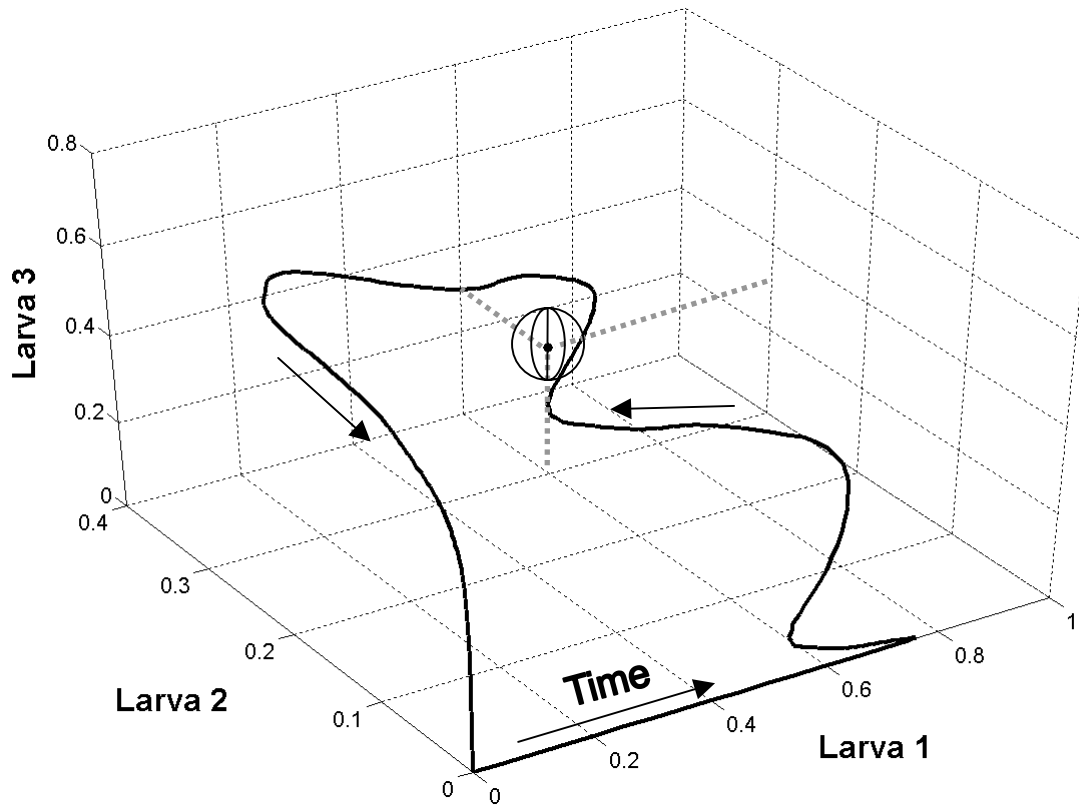
Figure 7: diagram of 3-D path taken by the proportions of three larval stages modeled through time, along with the point representing the maggot mass sample. The globe around the point represents the uncertainty in the sample proportions.
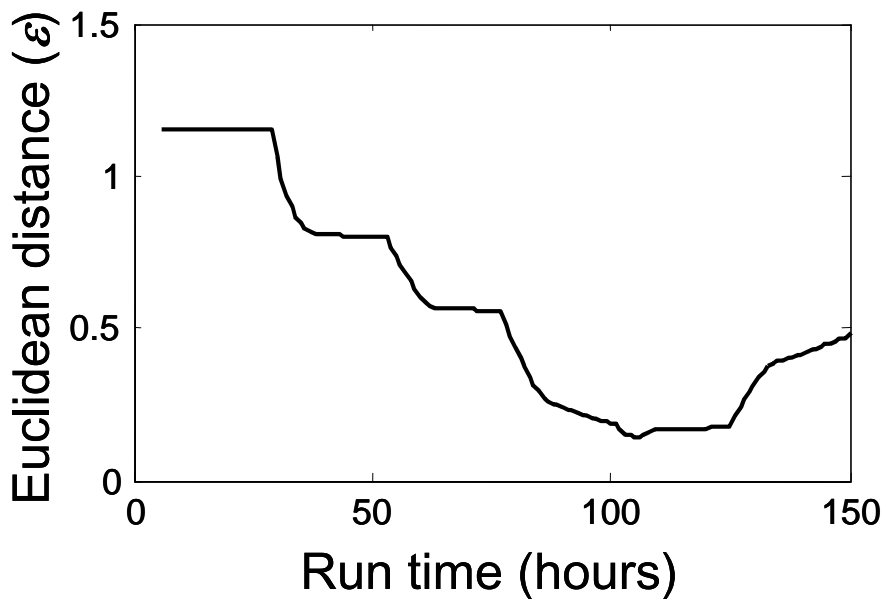
Figure 8: Diagram of the Euclidean distance measured in a typical model run from the proportions of maggot life stages found in the sample. The lowest point is the minimum Euclidean distance, and the predicted PMI.
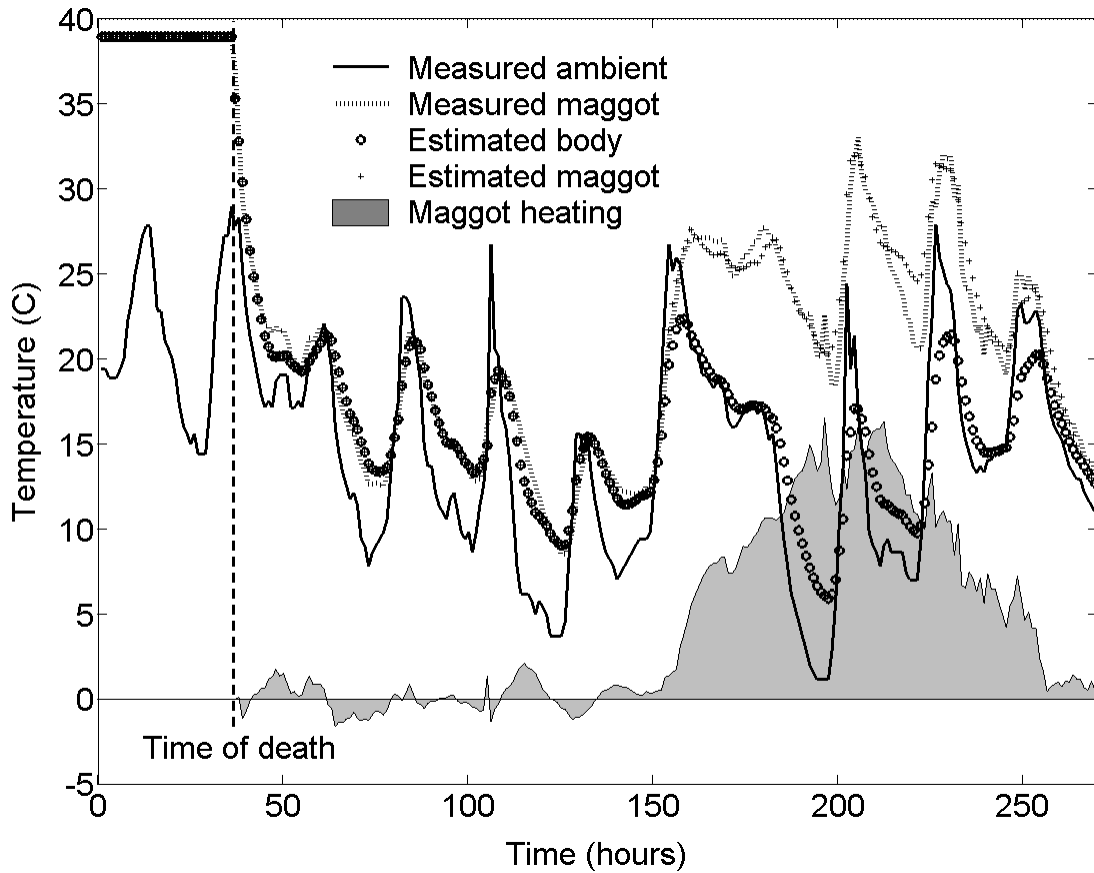
Figure 6: Output of the model combined with measured conditions from a typical pic replicate. Shown are measured ambient temperature from the hanging datalogger, measured maggot mass temperature from the temperature probe, and the related modeled temperatures of the body and the maggot mass. The modeled maggot mass heating (measured maggot temperature – modeled body temperature) is shown in gray.
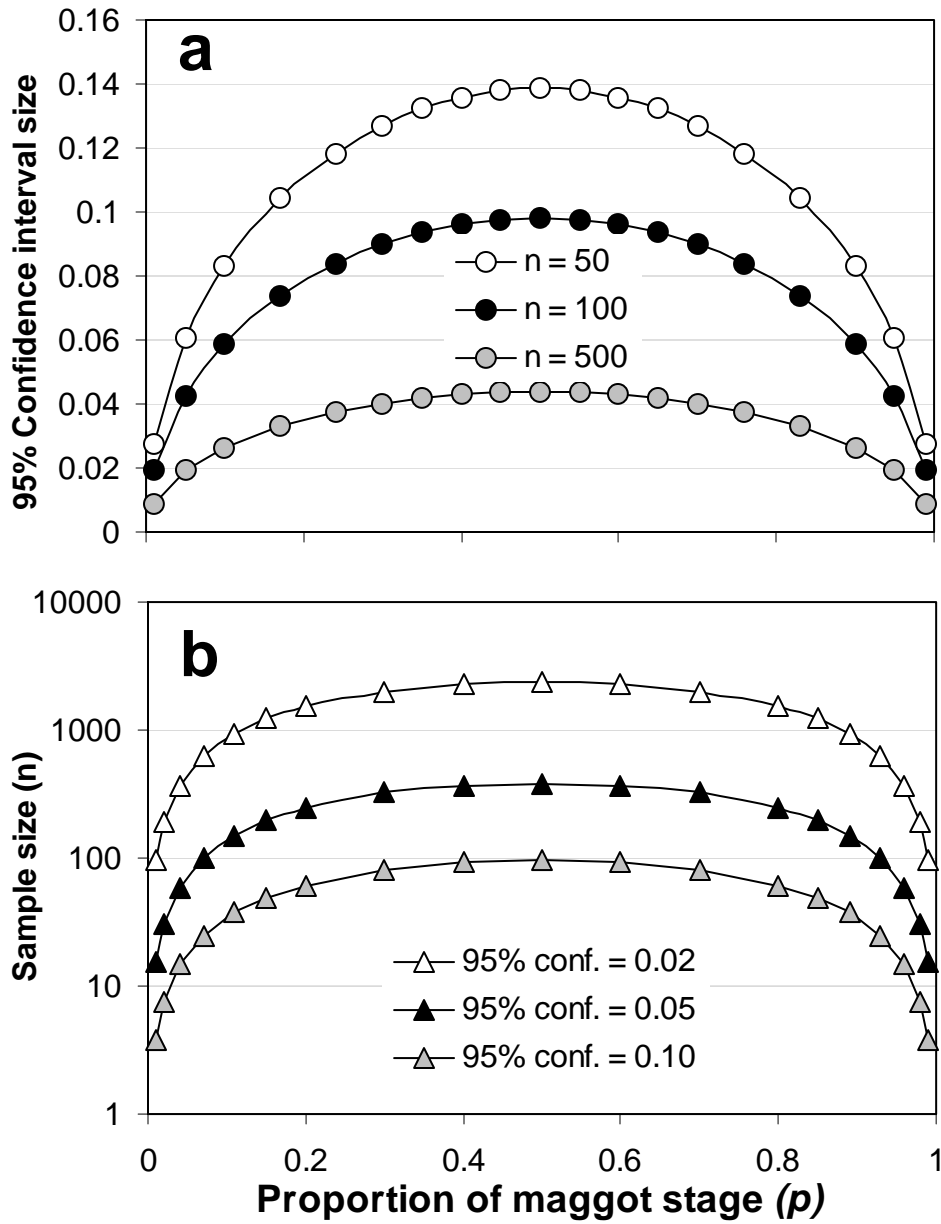
Figure 9: figures demonstrating (a) the 95% confidence interval size of larval instar proportions found in a sample for various sample sizes and proportions found, and (b) the sample size required to obtain a given 95% confidence interval at various proportions.
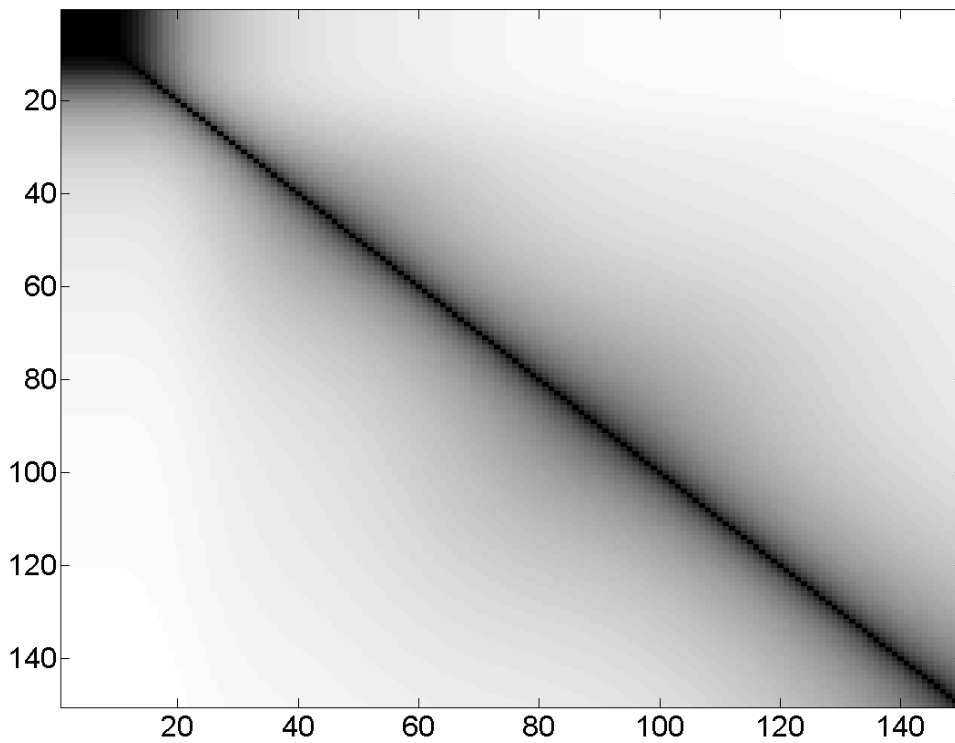
Figure 10: Sensitivity "basin" of the model using constant temperature and lighting conditions. The narrow strip of dark color indicates the output is relatively insensitive, so that small errors will not cause large deviations in output.
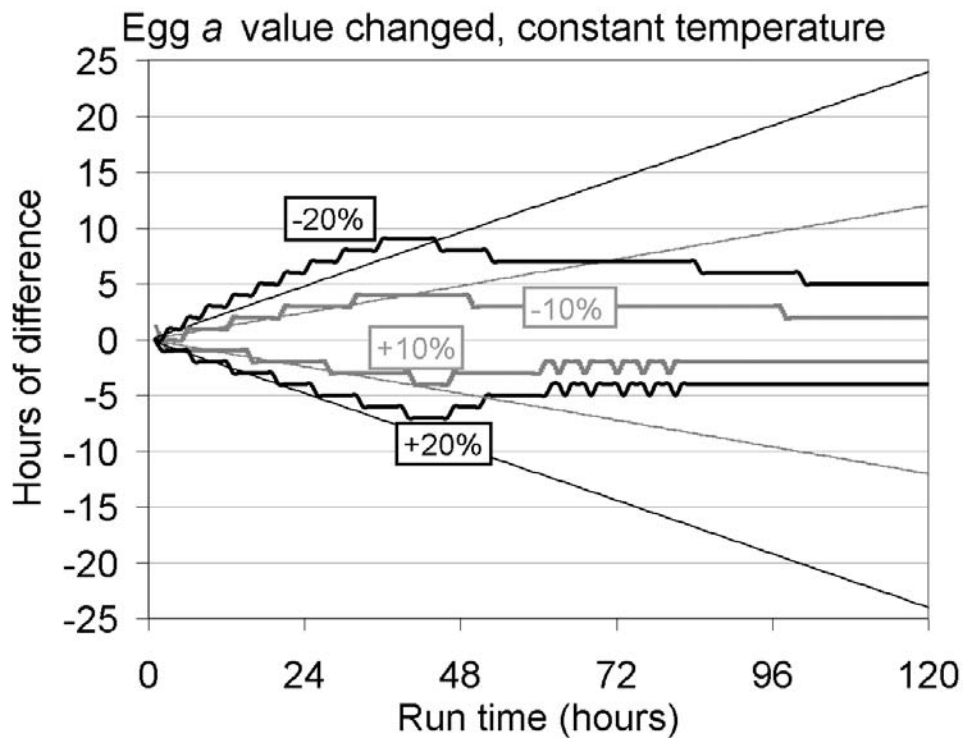
Figure 11a: If the egg development rate (a) is changed (measurement error), it changes the output at a 1:1 ratio, but only during the time the egg is present.
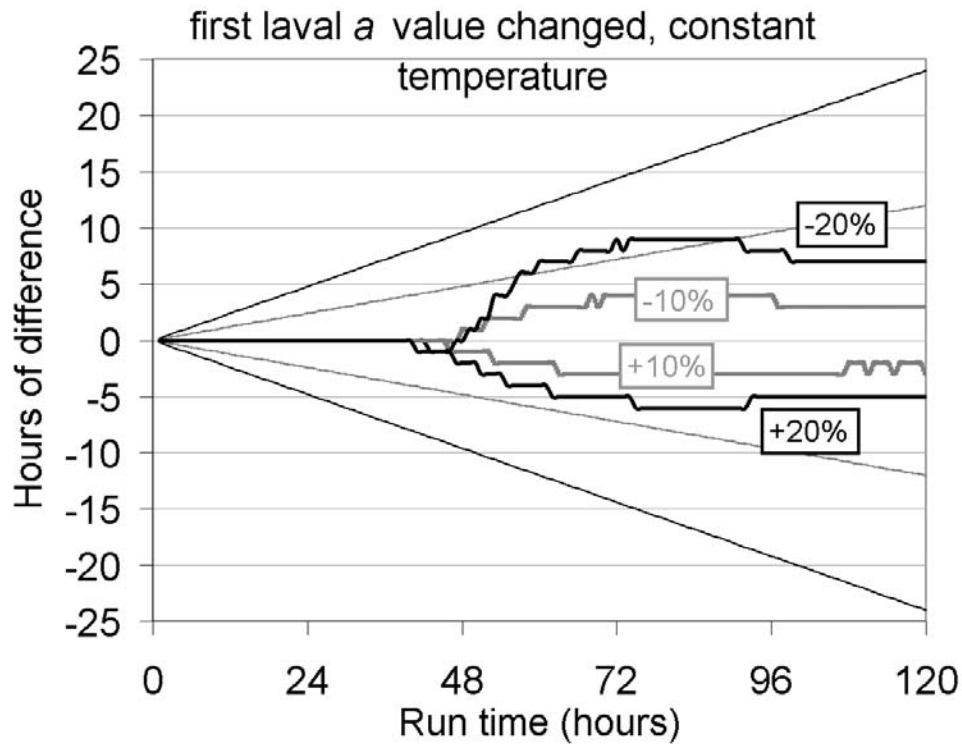
Figure 11b: Similarly, changing the first larval stage development rate (a) changes the output later, during the time that stage is present.
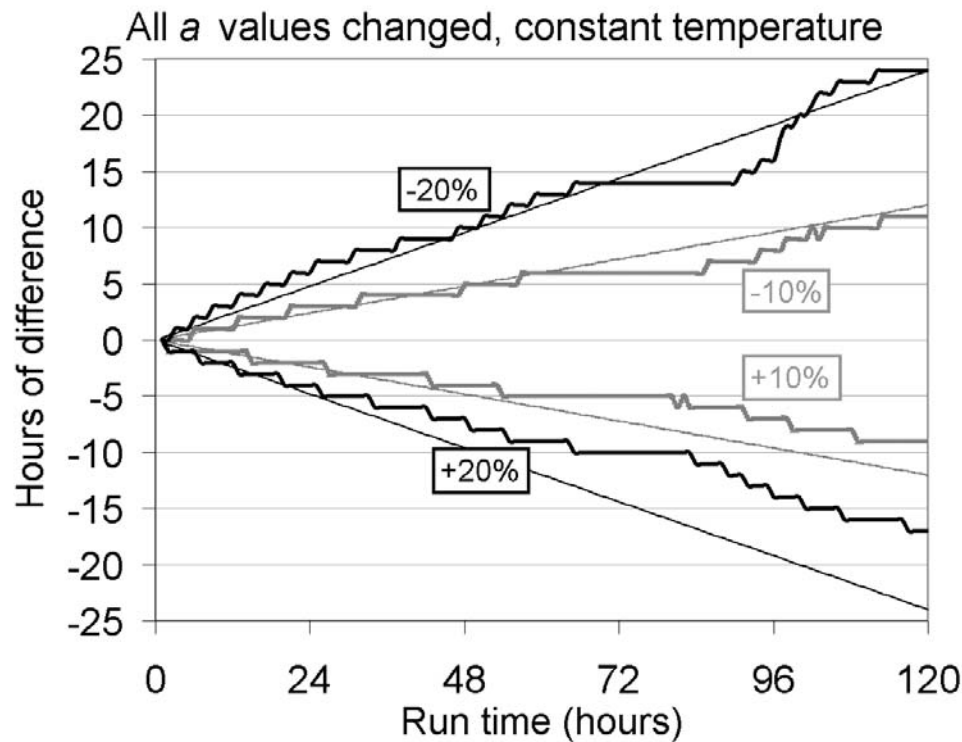
Figure 11c: If all the development rates (a) are changed, the entire model will be off by the amount of the error. The model is clearly most sensitive to these types of errors.
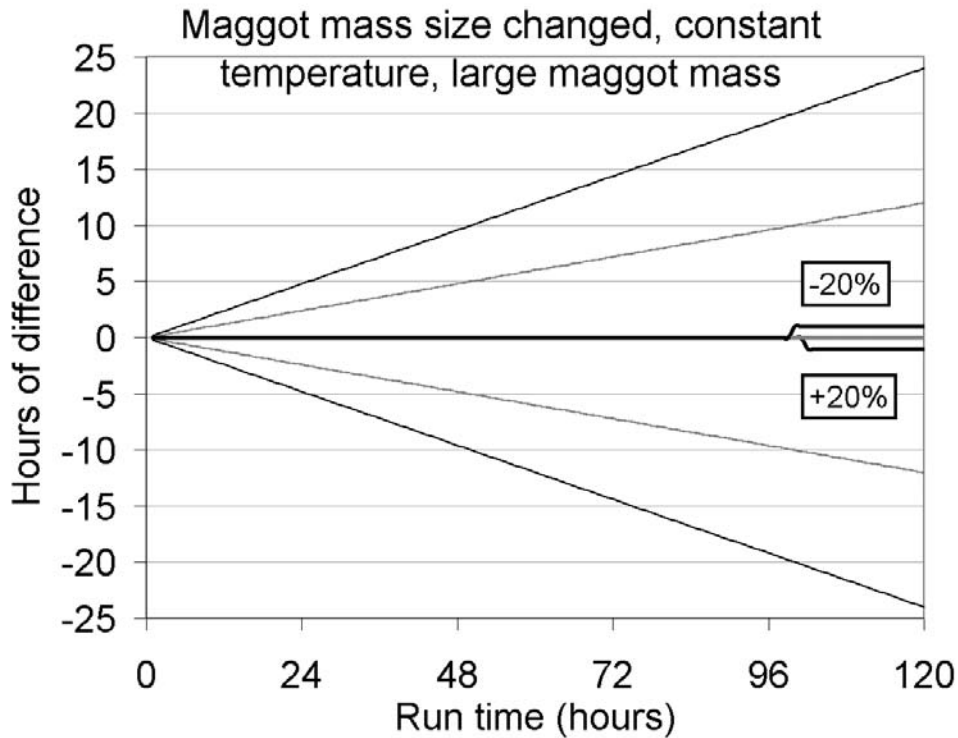
Figure 11d: By contrast, mis-measuring the volume of a large maggot mass has an insignificant effect on the model output. This is not a critical measurement.
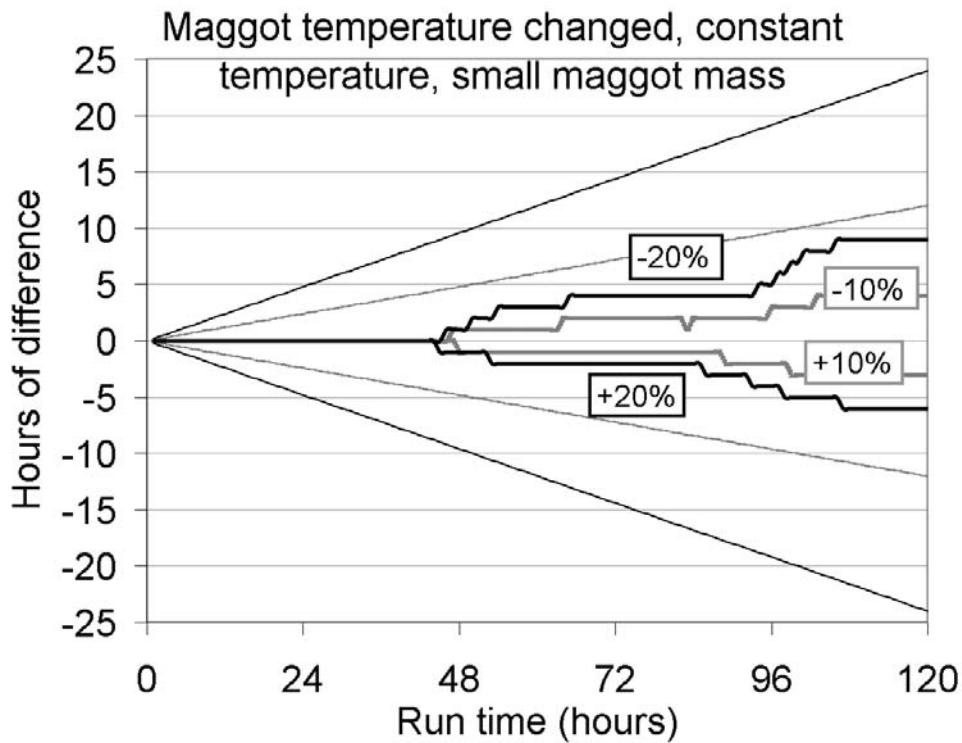
Figure 11e: In a smaller maggot mass, the measurement of the volume becomes more critical, because of the change in temperature associated with the changes in volume.
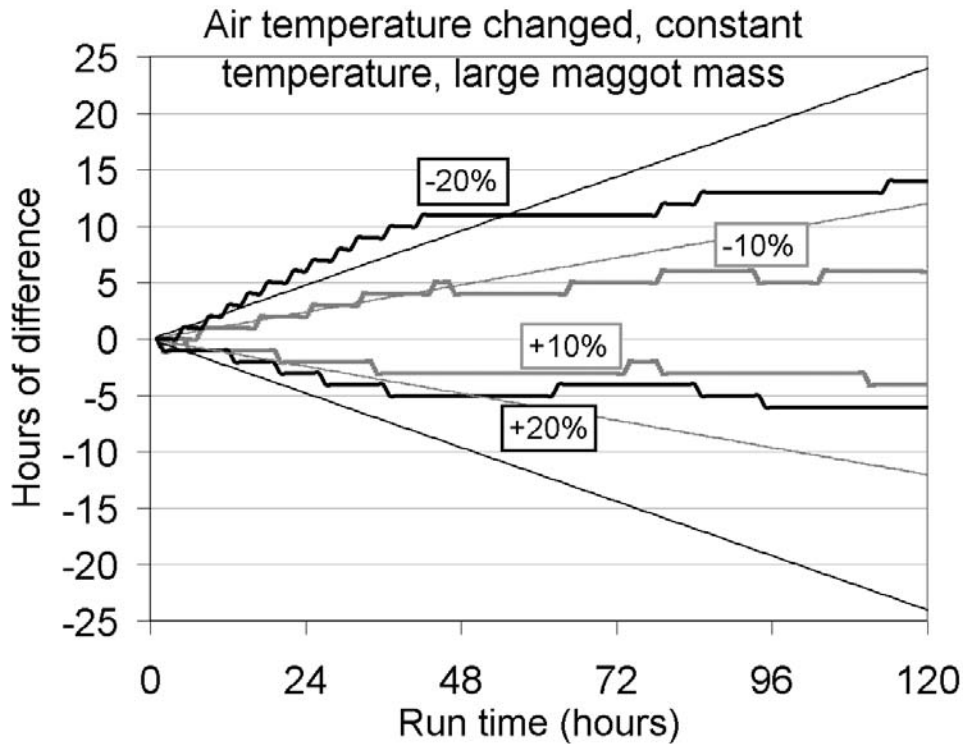
Figure 11f: Measurement of air temperature is important even with a large maggot mass, especially before the mass has grown large enough to determine its own temperature.
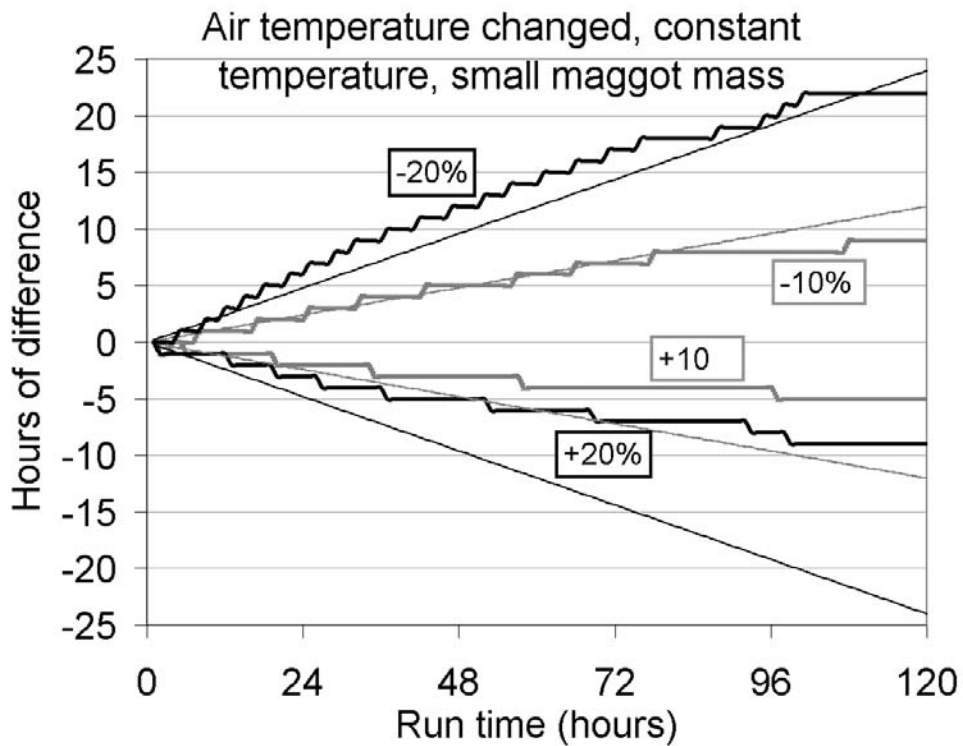
Figure 11g: Small maggot masses do not regulate their own temperature as well as large ones, and so the measurement of ambient temperature becomes more critical.
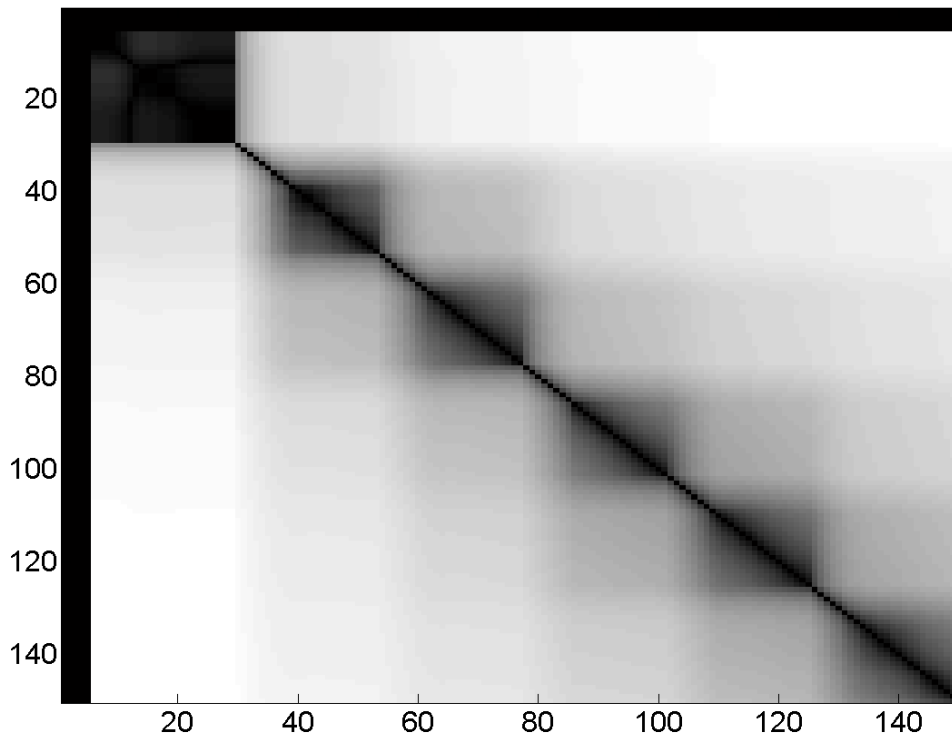
Figure 12: Compared to the basin under constant conditions (Figure 10), this basin produced with measured temperature and lighting from a site in Texas shows daily variation in the width of the dark areas. These areas correspond with night time, when PMI cannot be accurately determined
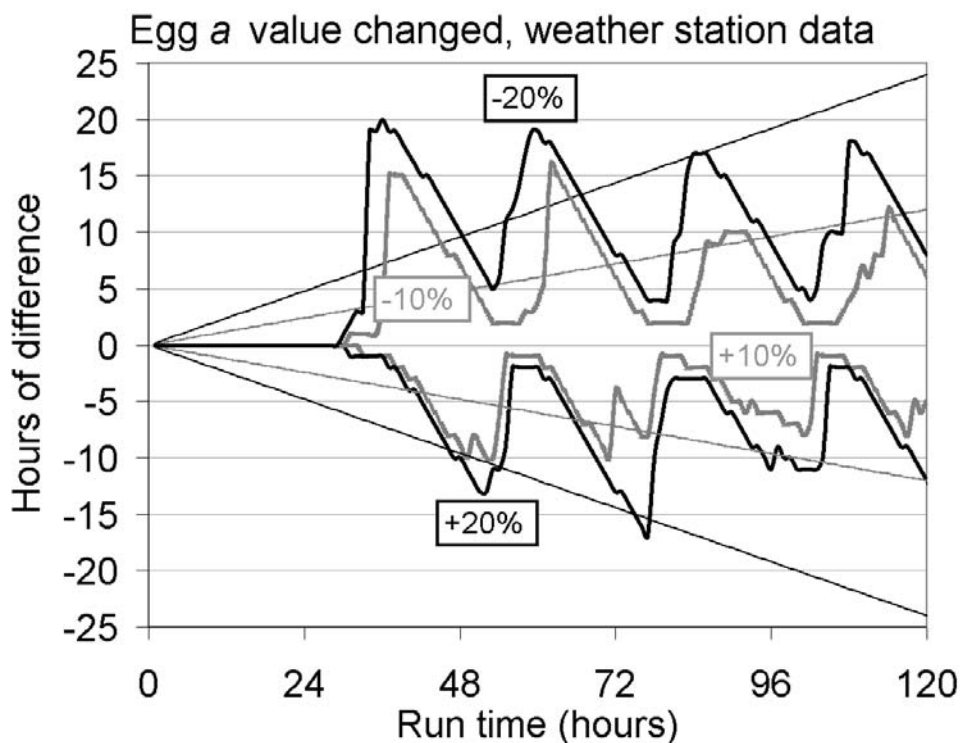
Figure 13a: When daily temperature cycling is taken into account, changing (mis-measuring) the egg a value can produce more or less than a 1:1 error in the model, depending on the final PMI that is predicted.
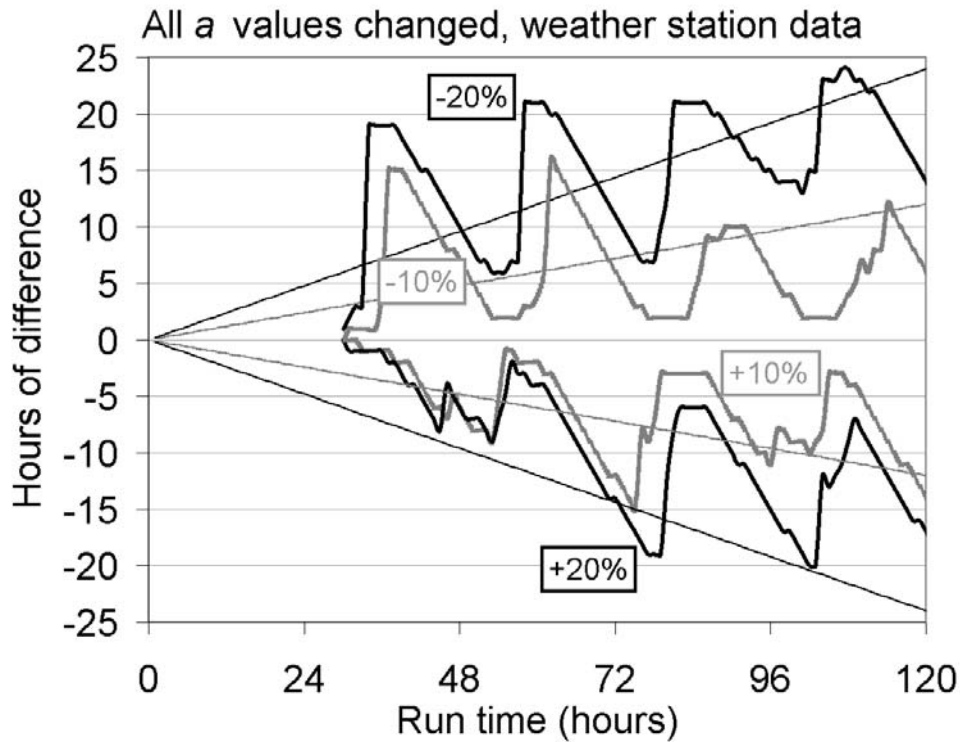
Figure 13b: Similarly, changing all a values produces drastic changes in the final model output at all times.
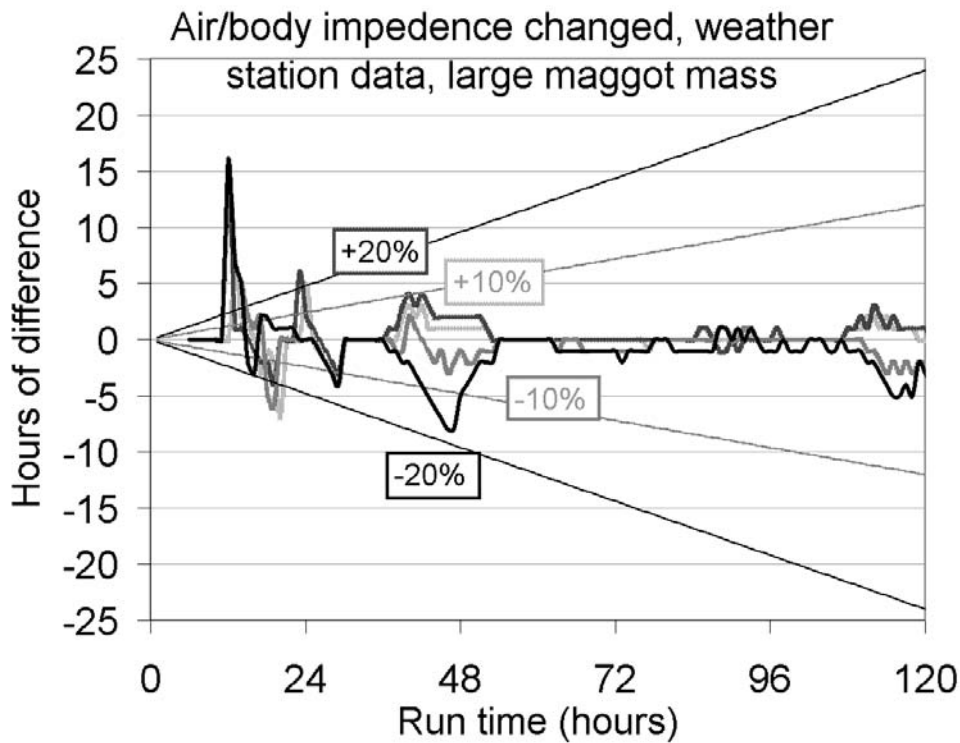

Figure 13c: The model is less sensitive to changes in the air/body impedance, which models how fast heat transfers from one medium to the other.
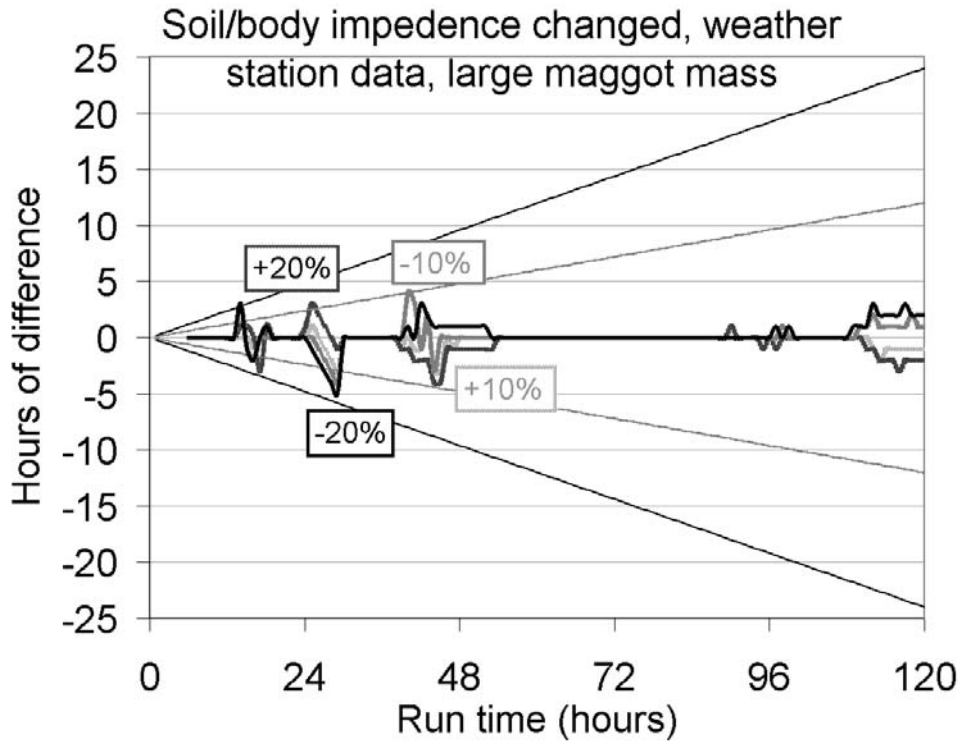
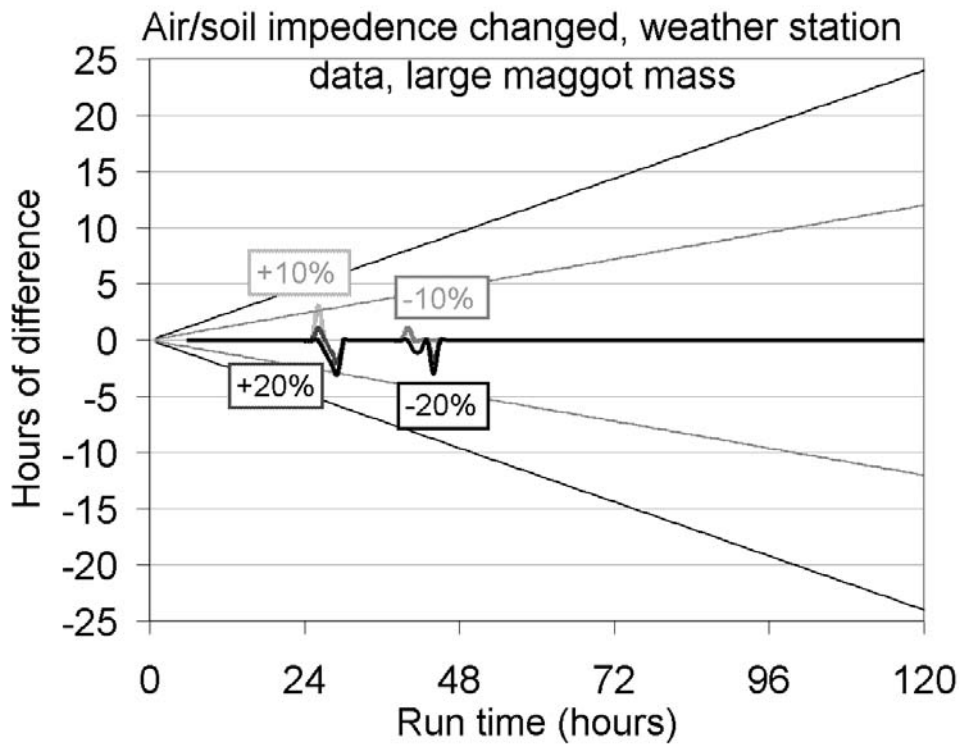Figure 13d: The soil/body impedance was a very insensitive parameter.

Figure 13e: Changing the air/soil impedance had almost no effect on the model output. This is not surprising since this heat transfer is one step removed from the body, and 2 steps removed from the maggot masses.

Photo 1: A datalogger protected in a plastic container was suspended near each pig carcass to record ambient temperature for the duration of each trial The temperature probe extends from the box to hang in the open air.

Photo 2: Each pig carcass was covered with a heavy wire cage to exclude larger scavengers such as vultures and coyotes, but easily permit insects to colonize.

Photo 3: A portable stove was used to boil water for preserving the maggot samples.

Photo 4: Fully-grown larvae were transferred to pupation containers filled with partially composted leaves and a small foil tent

Photo 5: A set of shelves was erected inside a screen cage to allow the maggots to pupate under natural conditions.
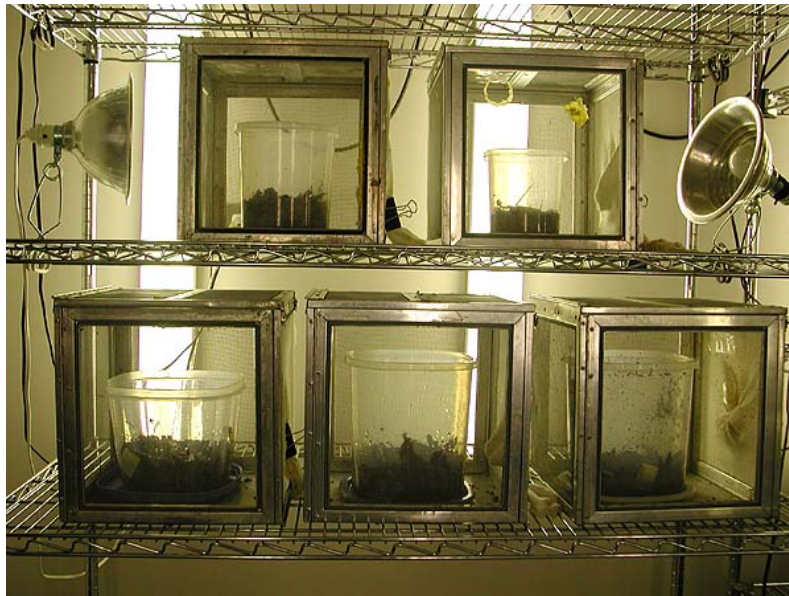

Photo 6: Emerging adults were captured in isolation boxes to keep the samples separated.

Photo 7: A fiberglass probe was impregnated with a row of thermistors attached to dataloggers to continuously record maggot mass temperatures through time and space.

Photo 8: A close-up view of some of the temperature probe thermistors. They were spaced 1.5cm apart.

Photo 9: The temperature probe is seen here inserted into a pig carcass. Because of the wound that is made, the probe site becomes a focus of maggot colonization.