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Effects of Temperature and Tissue Type on the Development of *Megaselia scalaris* (Diptera: Phoridae)

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Abstract

The scuttle fly, *Megaselia scalaris* (Loew) (Diptera: Phoridae), is of medical, veterinary, and forensic importance. In the case of the latter, *M. scalaris* is commonly associated with indoor death or neglect cases of humans or household animals, and its larvae are useful in determining time of colonization (TOC). This study is the first to examine the effects of different temperatures and tissues from two vertebrate species on the growth rate and larval length of *M. scalaris*. A preliminary validation of these data was also conducted. Immatures of *M. scalaris* were reared on either bovine or porcine *biceps femoris* at 24 °C, 28 °C, and 32 °C. Temperature significantly impacted immature development time, including egg eclosion, eclosion to pupation, and pupation to adult emergence, to favor faster development at higher temperatures. From ovipostion to eclosion, development rate was 32.1% faster from 24 °C to 28 °C, 13.9% faster from 28 °C to 32 °C, and 45.5% faster from 24 °C to 32 °C. Development from eclosion to pupation displayed similar results with differences of 30.3% between 24 °C and 28 °C, 15.4% between 28 °C and 32 °C, and 45.2% between 24 °C and 32 °C. Development from pupation to adult emergence, likewise, displayed a 44.4% difference from 24 °C and 28 °C, 7.3% from 28 °C to 32 °C, and 51.2% from 24 °C to 32 °C. From oviposition to adult emergence, *M. scalaris* needed ~32.7% more hours to complete development when reared at 24 °C than 28 °C, 8.5% when reared at 28 °C rather than 32 °C, and 38.4% more time when reared at 24 °C over 32 °C. Tissue type did not significantly impact development.

A preliminary validation study was conducted in four indoor environments (two attics, a closet, and a bathroom) spanning two different buildings. Utilizing minimum and mean lengths, time of colonization estimates were underestimated in all instances. The predicted range encompassed the actual TOC for two of the four environments. On average, when using minimum length, time of colonization was underestimated by 45%, but overestimated by only 2% when using maximum development range. Data generated from this research could be useful when estimating a TOC of decomposing vertebrate remains. Future research will need to examine development for each stadium in order to increase precision of such estimates.

Key words: forensic entomology, development data, postmortem interval, coffin fly

Forensic entomology is the utilization of insects and other arthropods as evidence in both civil and criminal investigations (Smith 1986), as well as within noncriminal investigations of death or myiasis. The broad scope of this field can be broken into three subdivisions of urban, stored-product, and medicolegal forensic entomology (Archer 2007). Medicolegal forensic entomology relates to necrophagous arthropods colonizing and feeding on living and necrotic human and animal tissue. Insects found on vertebrate remains may be utilized to estimate the time of colonization (TOC; Amendt et al. 2007).

Forensic entomologists depend on development data from laboratory studies to estimate the TOC for insects, such as blow flies (Diptera: Calliphoridae) (Tarone and Foran 2006), flesh flies (Dipera: Sarcophagidae) (Singh and Sharma 2008), and phorids (Disney 2008) collected from decedents as well as the living (Anderson and Huitson 2004). Although similar development times have been documented in pre-existing data sets for a variety of blow fly species, some variations can be found within species (Tarone and Foran 2006, Gallagher et al. 2010, Owings et al. 2014). Variation could be due to a number of factors, such as experiment design, environment, and genetic variation (Tarone and Foran 2006).

Most development data utilized by forensic entomologists are obtained from studies examining the growth of larvae fed liver of various mammalian species (Clark et al. 2006). However, it has been demonstrated that the host species on which the larvae feed may significantly alter larval growth rate. For example, development

of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) fed lung, liver, and heart, from both cows and swine were compared (Clark et al. 2006). Larvae grew significantly faster on porcine than on bovine tissue. Furthermore, development varied when reared on lung and heart compared with liver of the same animal. For forensic entomologists, such results emphasize the need for development data sets across tissue types as well as across species tissue source. The importance of recording the location from which insect larvae are removed from a corpse was also demonstrated (Clark et al. 2006). Such information can allow for refined estimates of the TOC.

Megaselia scalaris (Loew) (Diptera: Phoridae) is widely encountered in forensic entomology investigations; however, little is known about the biology of this species. Phoridae are commonly known as hunchback flies, scuttle flies, or coffin flies, due in part to their morphology, behavior, and food source, respectively. Cosmopolitan by nature, *M. scalaris* can be a scavenger (Disney 2008), parasite (Miranda-Miranda et al. 2011), or detritivore (Koch et al. 2012). Utilizing diverse habitats from tropical rain forests to heavily urbanized areas, the wide distribution of *M. scalaris* makes it both a residential pest, and an excellent candidate for forensic entomology casework (Disney 2012). This species has been encountered on human remains located indoors in a sealed building, where the small size of the phorids allowed them access to a corpse where access by blow flies was restricted (Reibe and Madea 2010).

Though development data exist for *M. scalaris*, these experiments are based on populations either from other countries besides the USA, such as Mexico (Miranda-Miranda et al. 2011) and Malaysia (Zuha and Omar 2014), or from regions in North America other than Texas, USA (Illinois: Greenberg and Wells 1998, Illinois: Prawirodisastro and Benjamin 1979, Virginia: Trumble and Pienkowski 1979). Likewise, there are concerns that genetic differences between populations may result in development differences and caution should be exercised when applying these data to cases outside these regions and climates. Such differences have already been recorded with oriental latrine flies *Chrysomya megacephala* (F.) (Diptera: Calliphoridae), where populations from different regions in China along the same latitudinal gradient within different environments demonstrated slowed or increased overall development times (Hu et al. 2010). The same has been determined for *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae) from different regions in Texas, USA (Owings et al. 2014) and *L. sericata* from different regions of the USA (Gallagher et al. 2010). Furthermore, there has been no research, as of the writing of this paper, comparing the relationship between both temperature and species-specific tissue on development of *M. scalaris*.

Currently, no data sets are available on the development of *M. scalaris* in Texas, USA. Due to the importance of *M. scalaris* in forensic entomology, specifically for the Harris County Institute of Forensic Sciences, where nearly 50% of indoor cases during 2013–2014 involved *M. scalaris* as a primary colonizer of human remains (Sanford 2015), an accurate developmental set using local populations followed by a validation study is critical to the understanding of *M. scalaris* and its growth rates. Thus, the objectives of this study were to 1) determine the impact of temperature and tissue type on development of *M. scalaris*, and 2) validate these data to determine their accuracy when estimating the TOC.

Materials and Methods

Adult Fly Colony

The *M. scalaris* colony used in this experiment originated from larvae collected from human female remains from a case assigned to the Harris County Institute of Forensic Sciences in October 2014. Larvae were provided 50 g of fresh bovine liver within their transportation container and allowed to pupate.

Resulting adult flies were housed in a 30- by 30- by 30-cm nylon mesh arthropod-rearing cage (BioQuip Productes Inc., Rancho Dominguez, CA) and provided a 50:50 sugar:powdered milk mixture and fresh water ad libitum. The room was maintained at 24–28 °C and a photoperiod of 12:12 (L:D) h in the Forensic Laboratory of Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University, College Station, Texas, USA. Approximately 50 g of bovine liver in a 140 mm wide by 20 mm deep petri dish was provided to the colony for three hours as an oviposition substrate. Resulting eggs and associated liver were placed in a 236.5-ml, wide mouth Kerr Mason jar (Kerr Group Inc., Lancaster, PA) and reared under the same conditions previously mentioned. Emerged adults were collected daily and placed in a cage similar to the one previously described.

Source

The *biceps femoris* muscle from remains of two bovines, *Bos taurus* (L.), and two porcines, *Sus scrofa* (L.), was utilized in this development study. All tissue was acquired from a local butcher shop (Readfield Meats and Deli, College Station, TX), and were free of barbiturates or other materials used for euthanization. Each muscle was cut into 50-g pieces varying in shape and stored frozen at −20 °C in appropriately labelled Ziploc bags (S.C. Johnson and Son Inc., Racine, WI). All protocols were approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC) and Animal Welfare Assurance Program.

Experiment Design

Methods were based on those of Boatright and Tomberlin (2010). Tissue samples were removed from the freezer and allowed to thaw and reach room temperature prior to use. Approximately 50 g of either bovine or porcine tissue was placed in a 236.5-ml wide mouth Kerr Mason jar filled a quarter way with sand (Quikrete Premium Play Sand, Quikrete Companies Inc., Atlanta, GA) to prevent pooling of decomposition fluids. Previous observations showed that 50 g of the respective tissues were more than adequate for rearing purposes, with much of the tissue remaining at all temperatures after pupation. Six jars were prepared for each tissue type, 12 in total per trial. Each jar was inoculated with 400 eggs from the colony (F7–F9 generations) and covered with a paper towel and secured with a metal ring (jar lid band). This method allowed airflow and prevented any emerging adults from escaping the enclosure. The jars were then randomly assigned by tissue, to one of the three 136LLVL Percival (Percival Scientific, Perry, IA) upright incubators set at 24.0 °C ± 1.5 °C, 28.0 °C ± 1.5 °C, or 32.0 °C ± 1.5 °C. Light cycle and humidity in each incubator was set to 12 L:12D, and 55% ± 1% RH, respectively. Three trials were conducted so that each incubator was used with each temperature to rear flies. During the study, four Onset HOBO U12-006 data loggers (Onset Co., Pocasset, MA) were placed within four separate parts of the incubator to test for variability between shelf height (top shelves versus bottom shelves) and shelf sides (left versus right). Furthermore, each unit was rotated within the sections of the incubators to measure against differences between the data loggers. No significant differences ($P > 0.05$) in temperature or relative humidity were determined for the different locations within the incubators. Each section averaged a ± 1.5 °C and ± 1% shift in relative humidity.

Life-History Traits

Development time was recorded between ovipositon to larval eclosion, eclosion to pupation, pupation to adult emergence, and eclosion to adult emergence. Eggs in each sample were checked hourly for hatch. Following eclosion, observations were conducted every 12 h, in which three larvae were subsampled from separate areas within the tissue. Each larva was placed within a Brew&Save™ reusable filter cup (Eko Brands, LLC., Mill Creek, WA) and parboiled in water at 100 °C for no more than 15 s. Each larva was measured for length in millimeters using a Meiji Techno EMZ-8TR microscope (Meiji Techno America, Santa Clara, CA) coupled with an INFINITY1-3 C digital camera and its corresponding software suite, INFINITY Analyze and INFINITY Capture (Lumenera© Corporation, Ottawa, Canada). Due to the small size and mass of *M. scalaris* larvae, accurate determination of stadium and weight is difficult to obtain before the third stadium, often requiring a scanning electron microscope (SEM) (Boonchu et al. 2004) and a sensitive digital analytical balance (Zuha and Omar 2014). As such, developmental weight was not measured due to the larvae weight at earlier stages being below the AdventurerPro AV64 weighing scale (Ohaus, Pine Brook, NJ) detection threshold.

Due to brittleness pupae were left undisturbed within their respective jars and were checked every 12 h for adult emergence. The number of adults emerging during each observation were tabulated and sexed.

Statistical Analysis

All statistical analyses were performed using JMP statistical software (SAS Institute Inc., Cary, NC) and Microsoft Office Excel version 2007 (Microsoft Corp., Redmond, WA). For analysis, a split plot design repeated measures ANOVA with tissue type as the whole plot treatment, temperature as the split plot treatment, and time as the repeated measures factor, and separate incubators as random blocks. Larval length and rate of development were recorded in both clock hours and accumulated degree hours (ADH). Degree hours are measurements of thermal input required for insect development in predictable patterns calculated by subtracting the minimum development threshold by the mean environmental temperature for that hour, or maximum developmental threshold if it is reached (Greenberg 1991). Minimum threshold temperatures have been hypothesized for *M. scalaris*, but never been tested. Furthermore, evidence suggests there may be multiple thresholds based on various life stages (Prawirodisastro and Benjamin 1979; Zuha and Omar 2014). Using as close to local data and methods as possible, 10 °C was used as the overall lower threshold limit in estimating ADD (Prawirodisastro and Beenjamin 1979; Michelle Sanford, *personal communication*). Tukey's honest significant difference test was utilized to separate least square means following a significant F test ($P < 0.05$).

Preliminary Validation of Development Data

A preliminary, blind validation study was conducted utilizing the minimum and maximum lengths and ADH ranges of *M. scalaris* recorded in this development study. Validations were run in four different locations throughout two buildings of the F.L.I.E.S. Facility at Texas A&M University, College Station, Texas, USA, where the jars within the study were placed by a third party at unknown times.

Utilizing the same methods as for the development study, ~50 g of either bovine or porcine tissue was placed in a 236.5-ml wide mouth Kerr Mason jar filled a quarter way with sand. One hundred eggs < 3-h-old were placed in each of the two jars containing either bovine or porcine muscle and placed in four indoor locations. One jar containing beef and one containing pork was placed in two separate attics in Building/Attic A (beef) and Building/Attic B (pork). The dimensions of the rooms were, 29.87 m (L) by 12.19 m (W) by 3.15 m (H), and 18.29 m by 12.19 m by 3.15 m, respectively. Likewise, another placement of pork was located in a shower (0.64 by 0.71 by 2.49 m^3), and the final jar of beef was placed within a closet (1.14 by 2.06 by 2.06 m^3), both located in Building A. Each area contained a HOBO U12-006 data logger to record the temperatures within each environment. Jars were pulled from the "field" at random times prior to pupation. A range for TOC was determined upon collection of each sample using recorded ADH in combination with maximum, minimum, and average larval lengths. Temperatures in each site were closest to the lowest temperature (24 °C) utilized in the study; therefore, ADH and length was calculated based on development data collected for that temperature.

Results

Life-History Traits

A trial effect between incubators was not significant for length ($F_1 = 0.4427$, $P = 0.6446$) or ADH development time ($F_1 = 0.3974$, $P = 0.5352$). Larval length was significantly ($F_{2,\ 10.53} = 10.6060$, $P < 0.0051$) impacted by temperature (Fig. 1). However, tissue did not significantly affect larval length ($F_{1,\ 5.41} = 0.3486$, $P = 0.5787$) or larval development ($F_{1,\ 4.116} = 0.0058$, $P = 0.9430$). Degree hour (DH) data for *M. scalaris* development when reared on bovine and porcine tissue are shown in Table 1. There was no significant interaction between the temperatures (24 °C, 28 °C, 32 °C) and tissue (bovine and porcine) on larval length ($F_{2,\ 10.53} = 0.0852$, $P = 0.9189$) or development time ($F_{2,\ 5.564} = 0.0884$, $P = 0.9167$).

Degree hour data are presented in Table 1. In clock hours, time needed for egg hatch was significantly impacted by temperature ($F_{2,\ 23.24} = 157.0285$, $P < 0.0001$). From 24 °C to 28 °C, an average difference of 4.7 h was observed across all trials, 1.6 h between 28 °C and 32 °C, and 6.3 h solely between 24 °C and 32 °C. Likewise, development from hatch to pupation, as well as pupation to adult emergence, was significantly impacted by temperature (hatch to pupation: $F_2 = 173.2503$, $P < 0.0001$; pupation to adult emergence: $F_{2,\ 6.144} = 976.9159$, $P < 0.0001$). An average difference of 20 h was observed between developing larvae exposed to 24 °C and 28 °C. Larval development between 28 °C and 32 °C differed by 8 h, and by 12.3 h from pupation to adult emergence. Lastly, an average difference of 36 h was recorded between hatch to pupation for larvae reared in 24 °C and 32 °C, where those raised on 24 °C spent 112.3 h more as pupae before adult emergence. Table 2 displays a total percent difference in mean time of development between the temperatures for bovine and porcine separately. As tissue showed no significant effect, a cross-reference of the percentages between bovine and porcine was not estimated.

Validation of Development Data

Validation results are shown in Table 3. Relative humidity was significantly greater ($F_3 = 3876.788$, $P < 0.0001$) than the measured RH during the development study in all areas except for the closet located in Building A. Relative humidity averaged 82% in Attic A, 75% in Attic B, and 74% in Building A shower room. Furthermore, temperatures across all sites averaged 22.0 °C ± 0.4 °C. As well, the temperature in Attic A fluctuated between 21.0 °C and 31.0 °C.

An entire range of corresponding minimum, maximum, and mean adjusted TOC (excluding tissue location and oviposition time)

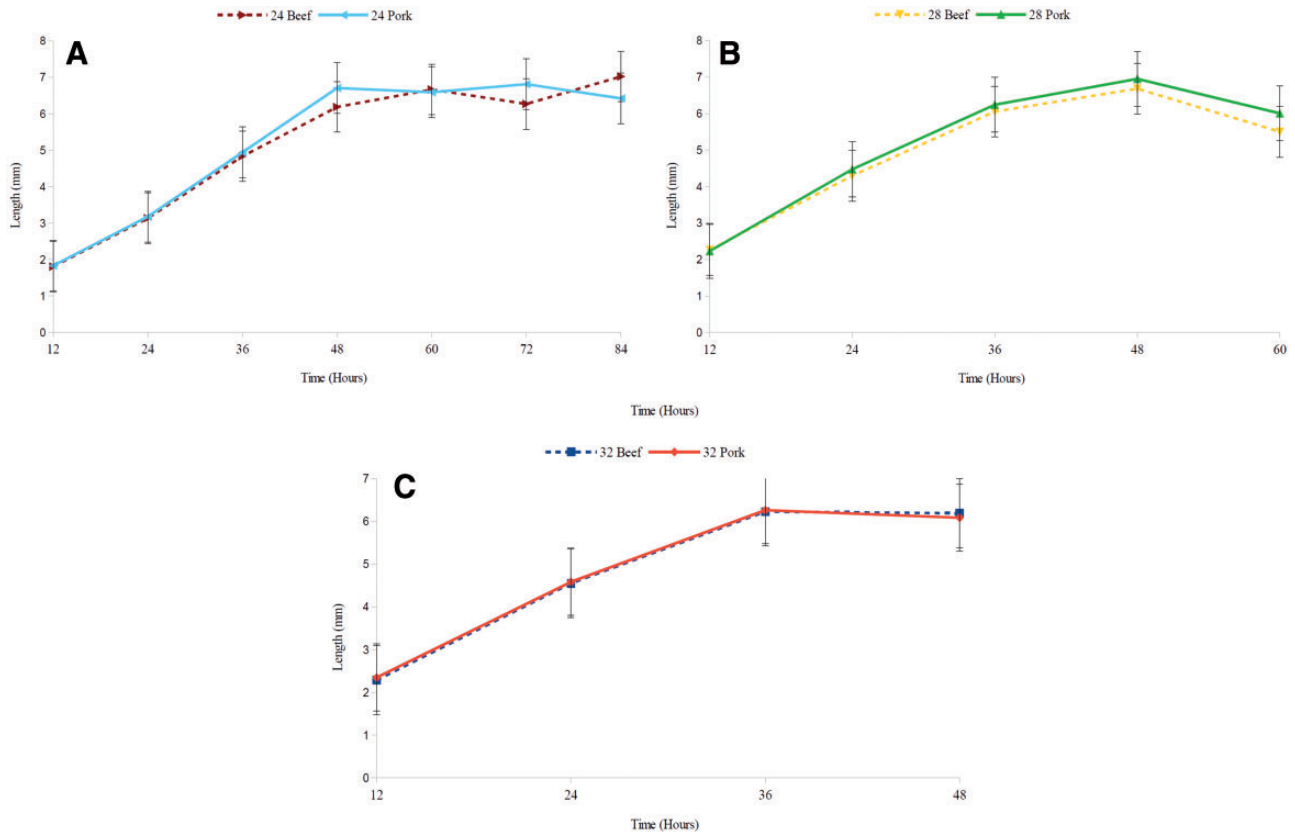

Fig. 1. Mean length (mm) ± SE of sampled *M. scalaris* larvae over time (h) in 12-h intervals between temperatures 24°C (**A**), 28°C (**B**), and 32°C (**C**) all ± 1.5°C and with two different tissues (beef and pork). Larvae were raised in 55 ± 1.0% RH at a photoperiod of 12:12 (L:D) h.

Table 1. Minimum degree hours (DH) and accumulated degree hours (ADH) for development stages of *M. scalaris* between three different temperatures (32°C, 28°C, 24°C ± 1.5°C) and two different tissues (beef and pork, $n = 2$)

Temperature (°C) ± SE	Tissue	Oviposition to eclosion ± SE DH	Eclosion to pupation ± SE (DH/ADH)	Pupation to adult emergence ± SE (DH/ADH)
32.0 ± 1.5	Beef	251.2 ± 8.4	1,320.0 ± 33.9/1,571.2	3,582.3 ± 42.6/5,153.5
	Pork	253.0 ± 9.2	1,320.0 ± 37.1/1,573.0	3,590.4 ± 84.4/5,163.4
28.0 ± 1.5	Beef	246.0 ± 8.4	1,224.0 ± 33.9/1,470.0	3,150.0 ± 43.4/4,620.0
	Pork	246.0 ± 8.4	1,224.0 ± 33.9/1,470.0	3,159.0 ± 43.4/4,629.0
24.0 ± 1.5	Beef	257.8 ± 8.4	1,232.0 ± 33.9/1,489.8	3,864.0 ± 43.4/5,353.8
	Pork	261.3 ± 8.4	1,232.0 ± 33.9/1,493.3	3,843.0 ± 43.4/5,336.3

Larvae were raised in 55 ± 1.0% RH at a photoperiod of 12:12 (L:D) h.

Table 2. Percent difference of *M. scalaris* mean time of development (h) from egg to adult emergence of *M. scalaris* between three different temperatures (32.0°C, 28.0°C, 24.0°C ± 1.5°C) and two different tissues (beef and pork, $n = 2$)

Temperature (°C) ± SE (Tissue)	Pork			Beef		
	32.0 ± 1.5°C	28.0 ± 1.5°C	24.0 ± 1.5°C	32.0 ± 1.5°C	28.0 ± 1.5°C	24.0 ± 1.5°C
32.0 ± 1.5 (Pork)	0.0%	10.5%	3.4%	–	–	–
28.0 ± 1.5 (Pork)	10.5%	0.0%	13.5%	–	–	–
24.0 ± 1.5 (Pork)	3.4%	13.5%	0.0%	–	–	–
32.0 ± 1.5 (Beef)	–	–	–	0.0%	8.5%	38.4%
28.0 ± 1.5 (Beef)	–	–	–	8.5%	0.0%	32.7%
24.0 ± 1.5 (Beef)	–	–	–	38.4%	32.7%	0.0%

Larvae were raised in 55 ± 1.0% RH at a photoperiod of 12:12 (L:D) h.

Table 3. Minimum, maximum, and mean TOC estimates of *M. scalaris* within four locations in two buildings of the Texas A&M F.L.I.E.S.

Location	Number larvae sampled	ACH[a]/ADH[b] samples in field	Time of colonization estimates ACHE[c](PD[d])/ADHE[e]		
			Minimum length	Maximum length	Mean length
Building A: Attic A	49	67/862	28 (−58%)/386	66 (−1%)/848	47 (−30%)/617
Building B: Attic B	19	47/573	28 (−40%)/335	42 (−11%)/499	35 (−26%)/417
Building A: Shower	7	38/516	28 (−26%)/377	42 (11%)/574	35 (−8%)/475
Building A: Closet	45	61/785	28 (−54%)/366	66 (8%)/849	47 (−23%)/607
Average Over–Under Estimated	N/A	N/A	45%	2%	23%

[a]Accumulated clock hours; [b]accumulated degree hours; [c]accumulated clock hours estimated; [d]percent difference between estimate and actual time of colonization; [e]accumulated degree hours estimated.

Facility, when using larval length data generated in the laboratory at 24°CPercentages shown are differences (h) between the estimate and the actual time in field.

was cross-referenced with larval lengths (Table 3). Maximum length was the closest in all observations to the actual TOC. Within Attic A, all measures resulted in an underestimation of actual TOC by 58% (39 h), 1% (1 h), and 30% (20 h) (minimum, maximum, and mean, respectively). TOC calculations for specimens from Attic B were underestimated by 40% (28 h), 11% (5 h), and 26% (12 h) (minimum, maximum, and mean, respectively). For the bathroom, length underestimated TOC by 26% (10 h), overestimated by 11% (4 h) and, underestimated 8% (3 h) (minimum, maximum, and mean, respectively). Lastly, the closet space produced similar over–under estimations as the shower room, in that minimum underestimated actual TOC by 54% (33 h), maximum overestimated actual TOC by 10% (5 h), and the average underestimated actual TOC by 23% (14 h). These ranges are likely caused by the age of the eggs in general, as it is assumed, in this case, the longest larva is the oldest. Following a bell curve, the average length would correspond to when most of the eggs hatched, marking the maximum and minimum lengths as statistical outliers.

Discussion

To date, there is no other published research examining the impact of temperature and tissue type on the development of *M. scalaris*. Furthermore, this study is the first to provide an assessment of the accuracy (validation) of these data for determining the TOC.

Environmental conditions used in development studies are rarely the same as those experienced during casework. Often, many abiotic and biotic factors impact development patterns of arthropods associated with decomposing remains, much more than those in controlled laboratory studies. Thus, a validation study is useful for determining the accuracy of the lab-generated data when applying them in casework. Furthermore, these validations should be expanded beyond the localized area of the study in order to assess regional variability.

The most recent development study on *M. scalaris* was conducted in Malaysia (Zuha and Omar 2014). Overall, development times between oviposition and adult emergence for *M. scalaris* in the present study were different than those found by Zuha and Omar (2014). Ranges in development were from 381.125 h at 24 °C in this study, to 417.5 h at 23 °C and 304 h at 25 °C in Zuha and Omar (2014). Likewise, average time (h) for completing development at 32 °C was 249.2 h in Zuha and Omar (2014), but only 234.7 h in the present study. These differences in development in *M. scalaris* across studies could be due to a number of factors involving population genetic differences, the use of different equipment, experimental techniques, and food differences.

One of the most important and often overlooked factors impacting arthropod development is food quality. In an earlier study with *M. scalaris*, immature development rates were enhanced when fed raw beef liver rather than an artificial diet of liver agar. As much as an 8-h difference was found when reared on these two diets at 27 °C, 10 h at 30 °C, and 15.4 h at 33 °C (Zuha et al. 2012). For the blow fly *L. sericata*, development of immatures varied depending on tissue type reared on: brain, lung, liver, kidney, heart, minced meat, or intestine (El-Moaty and Kheirallah 2013). Larvae raised on brain and lung grew ∼35% faster than those raised on intestine; were 1.5 mm larger (>16%) and had a higher mean adult survivorship (85%) than of those raised on liver, heart, or minced meat (≤60%) (El-Moaty and Kheirallah 2013). Similar results stemmed from an earlier study on *L. sericata* fed bovine or porcine lung, liver, and heart tissue, either liquefied or as a solid (Clark et al. 2006). Overall, larvae grew faster and larger on pork versus beef and on lung and heart versus liver. Likewise, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) and *Calliphora augur* (Fabricius) (Diptera: Calliphoridae) took two days longer to develop on sheep liver versus muscle tissue and brain matter (Day and Wallman 2006).

Population differences due to location of study have been shown to be a factor impacting development. Phenotypic plasticity has been examined for the development of *C. macellaria* from different regions in Texas, USA (Owings et al. 2014). Due to differences in climate within south central (San Marcos), northeast (Longview), and southeast (College Station) regions, genetic differences developed between the strains, and the resulting larvae displayed significantly different development times, mass, and survival rates, despite being the same species. Furthermore, mean development time by cool versus warm environments, and year of observation made an impact as well. The population from Longview, TX, displayed shorter development times by up to 17 h when compared to those from College Station, TX (a distance of 290 km), and San Marcos, TX (470 km), during the cool seasons, and up to 18 h during the warmer months. However, the following year had Longview populations with an increased time of development by 9 h during the cooler months and 16 h in the warmer seasons. Similar results were found for *L. sericata* collected from three locations (Sacramento, CA, San Diego, CA, and Easton, MA) and reared at three temperatures (16, 26, and 36 °C; Gallagher et al. 2010). The authors found a significant difference in growth rates across all populations, especially for those from San Diego, CA, reared at 16 °C. Larval development time was 185 h as opposed to 207.5 h for the Sacramento, CA population and 210.5 h Easton, MA population. However, when raised at 36 °C, larvae of the San Diego, CA population took the longest to develop (74 h) while the other two needed 64.5 h (Sacramento, CA) and 66.5 h (Easton, MA). These

results suggest climate impacts developmental progression at different temperatures even within the same fly species over generations. For this reason, having multiple data sets for the same species across different climates and geographic locations is critical to achieving an accurate TOC.

Variation in the methods employed across the studies has also been shown to be a source of variation in development of forensically relevant arthropods. Developmental plasticity has been analyzed between field and laboratory studies (Tarone and Foran 2006) where it was found that developmental discrepancies exist between *L. sericata* raised under variable laboratory conditions, and those reared in field conditions, as well as those raised under laboratory conditions of varying materials and methods.

Although the validation study was preliminary, some appreciation for the accuracy when applying the generated development data to case studies was determined. It is important to note that despite the abiotic differences within each validation environment and the study itself, all four validation means were underestimated, but accurate within 20 h, with the largest difference being that in Attic A, where temperatures fluctuated and RH levels were much greater than those in the laboratory study. The lowest difference of 3 h was in the shower area of Building A, despite having similarly-high humidity and being collected at varying stages of larval development. This suggests RH may more strongly effect development times and lengths during the later stages of growth over the larval instars immediately following egg hatch.

Although this study is informative and useful, there are some limitations. Primarily, the lack of weight measurements and the inability to determine stadium of the developing larvae are critical weak points in this study. In the case of *Chrysomya megacephala*, stadium data were more effective than size for estimating the age of young larvae; however, after 72–120 h, neither size nor stadium could give an accurate age determination (Wells and Kurahashi 1994). As well, even though the colony population originated from a local case, multiple populations or an addition of local, wild populations would be useful. Periodically introducing wild adults from different locations within the immediate area could not only eliminate generation effects, but laboratory colony effects and increase genetic diversity (Anderson 2000). Finally, as previously stated, the validation study was preliminary. Though there were four samples taken from four different areas, the study lacked replication. Multiple replicates and trials should not be limited to lab work alone, and should be included when planning a validation study to test data sets when at all possible. A final note on validations considers the use of human and companion animal cadavers as the best test of accuracy.

Development studies serve as a primary method for estimating the TOC in forensic case studies, criminal or otherwise. A thorough understanding of the data sets regarding forensically important arthropods and their varying factors is critical to obtaining the most accurate estimate of TOC. Laboratory trials should attempt to replicate these effects as closely as possible either within the controls themselves, or by applying the data to a field study incorporating such factors. Using data collected from both temperature and tissue type, one could effectively create a more useful data plot to estimate the TOC in any case, criminal or otherwise, when compared with data of either tissue or temperature alone. Besides tissue comparison between different vertebrates, one should consider where larvae are sampled on the body; some insects, such as *C. vicina*, develop differently on various organs and tissues due to nutrition content (Kaneshrajah and Turner 2004). Hopefully this research will inspire similar study variations involving many biotic and abiotic factors and multiple arthropod species of forensic importance.

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