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2 **Insects and associated arthropods analyzed during medicolegal**
3 **death investigations in Harris County, Texas, USA: January 2013-**
4 **April 2016**

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15 **ABSTRACT** -The application of insect and arthropod information to medicolegal death
16 investigations is one of the more exacting applications of entomology. Historically limited to
17 homicide investigations, the integration of full time forensic entomology services to the medical
18 examiner's office in Harris County has opened up the opportunity to apply entomology to a wide
19 variety of manner of death classifications and types of scenes to make observations on a number
20 of different geographical and species-level trends in Harris County, Texas, USA. In this study, a
21 retrospective analysis was made of 203 forensic entomology cases analyzed during the course of
22 medicolegal death investigations performed by the Harris County Institute of Forensic Sciences
23 in Houston, TX, USA from January 2013 through April 2016. These cases included all manner
24 of death classifications, stages of decomposition and a variety of different scene types that were
25 classified into decedents transported from the hospital (typically associated with myiasis or sting
26 allergy; 3.0%), outdoor scenes (32.0%) or indoor scenes (65.0%). Ambient scene air temperature
27 at the time scene investigation was the only significantly different factor observed between
28 indoor and outdoor scenes with average indoor scene temperature being slightly cooler (25.2°C)
29 than that observed outdoors (28.0°C). Relative humidity was not found to be significantly
30 different between scene types. Most of the indoor scenes were classified as natural (43.3%)
31 whereas most of the outdoor scenes were classified as homicides (12.3%). All other manner of
32 death classifications came from both indoor and outdoor scenes. Several species were found to
33 be significantly associated with indoor scenes as indicated by a binomial test, including
34 *Blaesoxipha plinthopyga* (Sarcophagidae), all Sarcophagidae including *B. plinthopyga*,
35 *Megaselia scalaris* (Phoridae), *Synthesiomyia nudiseta* (Muscidae) and *Lucilia cuprina*
36 (Calliphoridae). The only species that was a significant indicator of an outdoor scene was *Lucilia*
37 *eximia* (Calliphoridae). All other insect species that were collected in five or more cases were

38 collected from both indoor and outdoor scenes. A species list with month of collection and basic
39 scene characteristics with the length of the estimated time of colonization is also presented. The
40 data presented here provide valuable casework related species data for Harris County, TX and
41 nearby areas on the Gulf Coast that can be used to compare to other climate regions with other
42 species assemblages and to assist in identifying new species introductions to the area. This study
43 also highlights the importance of potential sources of uncertainty in preparation and
44 interpretation of forensic entomology reports from different scene types.

45 **KEYWORDS:** Post-mortem interval, Calliphoridae, Sarcophagidae, Decomposition,
46 Medicolegal Death Investigation

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48 INTRODUCTION

49 While forensic entomology has a broad scope, one of its most challenging applications is
50 to medicolegal death investigations. The application of the information provided by insects and
51 associated arthropods to death investigations can take many forms ranging from direct
52 association with cause of death (e.g. insect sting allergy), to information related to decedent
53 travel history (e.g. ticks traveling with their host decedents), to the estimation of time of insect
54 colonization (TOC) that can be used to approximate the post-mortem interval (PMI; [1]). The use
55 of insect colonization and development in estimating the PMI is one of the most well-known
56 applications of forensic entomology in the medicolegal setting [2].

57 Since its first recorded application to the death investigation of the infamous bloody
58 sickle [3] to the more recent application to the time of death of Danielle van Dam [4] forensic
59 entomology has had a strong association with homicide investigation and crime scene analysis
60 [1,2,5]. The recent addition of forensic entomology services to the medical examiner's office has
61 led to an even broader application of forensic entomology to medicolegal death investigations
62 across all manners of death [6]. When one starts to investigate the insects associated with a broad
63 range of decedents, scenes and manners of death one starts to appreciate the breadth of forensic
64 entomology casework opportunities. One of the first steps to understanding these cases is in the
65 identification of the insects involved in colonizing human remains in a given geographic
66 location, their seasonality and the characteristics of the scenes where they are found.

67 Forensic entomology surveys of different geographic locations are often performed as a
68 first step in establishing baseline data (e.g. [7–9]). However, these types of studies are often
69 based on trapping adult flies or using animal models and are not typically based on casework
70 information. In Texas, survey information specific to forensically important insects is rare with
71 only a few published surveys available from Central Texas [9,10]. Blow fly (Diptera:

72 Calliphoridae) trapping studies were prevalent prior to and immediately following the
73 screwworm eradication program but the focus of these efforts was mainly to detect *Cochliomyia*
74 *hominivorax* [11–13]. A series of experiments examining the interaction between the newly
75 introduced *Chrysomya* sp. into the United States also recorded the presence of several blow flies
76 in Central parts of Texas with traps as well [14,15] but the focus of these studies was not
77 primarily for survey purposes.

78 Species records and trends related to casework have been published in other geographic
79 areas. In North America these types of surveys have included the Hawaiian island of Oahu
80 [16,17] and Western Canada [18]. These types of data are rarely published but are exceedingly
81 important to understanding the local fauna important to casework in different geographic
82 locations especially when practitioners may be reliant upon old and restricted taxonomic keys,
83 which may not include all the species local to the area. To date no published data relating
84 casework and the local forensically important insects in Texas or specifically in Harris County
85 has been recorded. In this study the species, trends and seasonality of the forensically important
86 insects associated with casework are reported for Harris County, Texas, USA. These data will
87 aid in not only guiding identification and tracking of new species in the area but also in guiding
88 research questions into the species of importance in this area. This study also allows for
89 comparison with previous studies, particularly with respect to larger scale trends in insect
90 colonization patterns such as indoor and outdoor scenes, seasonality and introduced species.

91 **MATERIALS AND METHODS**

92 **Casework** – Cases involving insect or related arthropod specimens collected and
93 analyzed during the course of medicolegal death investigations performed by the Harris County
94 Institute of Forensic Sciences (HCIFS) for the period of January 2013 through April 2016 (N =
95 203) were included in this dataset. These cases involved the collection of specimens at the scene

96 or during the autopsy and occasionally at both locations (Figure 1). The procedure for specimen
97 collection follows standard operating procedures developed for the office. Briefly, this includes
98 collection of representative insect and/or related arthropod specimens from representative
99 locations on the body and scene representing the oldest identified life stages associated with the
100 body. Specimens are divided into representative portions, preserved by the most appropriate
101 method (e.g. hot water kill followed by 70% ethanol for larval fly specimens [19]) and reared to
102 the adult stage, if possible, for confirmation of identification.

103 Identification of specimens is based on morphology using life stage and taxon appropriate
104 taxonomic keys and literature, which are referenced in each individual report and the most
105 frequently used publications are presented in Supplemental Table 1. Specialized keys and
106 consultation with taxonomic experts are consulted as needed depending upon the specific case.
107 Dr. T. Whitworth (Washington State University) also generously provided the HCIFS with a
108 small adult reference blow fly (Diptera: Calliphoridae) collection in 2013 to facilitate
109 identification of adult Calliphoridae specimens.

110 Cases that require only identification of specimens, such as for stinging insect cases, may
111 not require further analysis, but a majority of cases require further analysis to estimate the age of
112 the collected specimens and to provide a time of colonization (TOC) estimate. Larval Diptera
113 and Coleoptera specimens are measured for length and each collected species is photographed
114 for representative identifying features using a Keyence VHX-600 digital microscope with an
115 attached VHX-S15E used as needed to stack images to enhance depth of field (Keyence Corp.,
116 Itasca, IL, USA). The estimated age of the larvae is calculated using the accumulated degree
117 hours (ADH) method [1,20] and taxon appropriate published development data for each species
118 using either published length data and/or development stage. The completed TOC estimate and

119 report for the specimens are included with the full autopsy report for each case involving the
120 collection of entomology specimens.

121 During the course of scene investigation, temperature and humidity recordings are made
122 using a combined thermometer-hygrometer (Fisher Scientific Education™, S66279, Fisher
123 Scientific, Pittsburgh, PA, USA). Scene conditions such as location of the body with respect to
124 shade or sun outdoors or thermostat settings indoors are also recorded to assist in the assessment
125 of temperature modifications at the scene that might influence differences in temperature from
126 the nearest weather station. Data is collected for ADH calculations from the nearest National
127 Oceanic and Atmospheric Administration (NOAA) quality controlled weather station for each
128 scene.

129 **Statistics** – Basic statistics were calculated for the forensic entomology cases where
130 insects were collected from 2013 through April of 2016 (two cases were excluded as they
131 reported on the absence of insects in the only two burial cases over the 3+ year period) for a total
132 of 203 cases. Basic statistics (e.g. mean, standard deviation, count) were calculated for scene
133 location (indoor, outdoor or hospital), month of scene investigation, length of TOC estimate,
134 ambient scene temperature (°C), ambient scene relative humidity and the presence of insect
135 species collected for each case (Table 1). Statistical comparisons of cases and case related factors
136 (e.g. temperature differences by case type and species presence indoors vs. outdoors) were
137 calculated using a Kruskal-Wallis X^2 test for continuous data (e.g. ambient scene temperature,
138 relative humidity and TOC estimate length) or an exact binomial test for categorical data (e.g.
139 scene location) for species which were collected in five or more cases. All statistical calculations
140 and tests were computed using Microsoft Excel® and R 3.2.4 (R Team 2016) using the R
141 Commander package [22].

142 **RESULTS AND DISCUSSION**

143 **Casework**– Perhaps the most important thing to consider when examining these data is
144 that they represent casework and not the results of planned ecological study, which imposes
145 certain limitations on the statistical analyses completed and the conclusions drawn. Of the 203
146 cases analyzed, six were from hospitals without an associated scene investigation (3.0%), 65
147 were from outdoor scenes (32.0%) and 132 were from indoor scenes (65.0%). Scene type
148 appears to play a significant role in not only the species of insects encountered (Table 1) but in
149 the conditions one may encounter during scene investigation (Table 2; Figure 1). It is also
150 important to note that the scene conditions reported here do not necessarily represent the
151 conditions experienced by the colonizing insects because these data were collected when insects
152 were collected after some period of development. These data represent the conditions in which
153 these insects might be found during the course of routine death investigations, which may be
154 valuable to understanding the conditions under which insects colonize human remains; a goal of
155 applied forensic entomology as it is used in casework.

156 **Scene Types**

157 **Hospitals** - Insect analysis involving hospital cases typically involved the identification
158 of insects or insect artefacts such as honeybee (*Apis mellifera* L.) stingers (Table 1) rather than
159 TOC estimation. In those cases where a TOC estimate was generated, the cases involved peri-
160 mortem myiasis wherein the decedent was colonized by insects, namely flies (Diptera; Table 1),
161 at some time point shortly before death. Estimation of TOC for decedents that come from the
162 hospital with fly larvae allows for the determination of colonization that can account for myiasis.
163 Otherwise without any extenuating circumstances, such as a significant discrepancy between the
164 stage of the insect larvae and the stage of decomposition or known medical history, myiasis can

165 be overlooked [20]. Even when myiasis is known the calculation of the TOC estimate can be
166 complicated by unknown factors related to which temperatures to use when calculating the
167 accumulated degree hours (ADH) required for development [20]. This can be due to health
168 related factors such as reduced circulation to extremities or due to unknown timing of demise
169 and the switch from the use of body temperature to ambient temperature in calculations [23].

170 Appreciating the complications associated with temperature, TOC estimation using
171 insects associated with wounds can provide useful information related to the timing of terminal
172 events. For example if a wound, which has been colonized by insects, is associated with a fall, a
173 TOC estimate may be a useful addition to the overall timeline of terminal events for the
174 decedent. Timing of injuries can be difficult for individuals that have a delayed death as without
175 scene details or witnesses the insects may provide the only timeline information available
176 regarding injuries.

177 Thus far, the insects and arthropods associated with decedent's coming from the hospital
178 have included honeybee, typical human parasites including lice and ticks, and blow flies (Table
179 1). There is a growing appreciation for the information that these organisms can provide. As an
180 example, a recent case demonstrated the potential of ticks to be useful to unraveling the travel
181 history and potentially the next of kin (NOK) information for a decedent. In this case, a decedent
182 came from the hospital emergency room with very little information, except that he had been
183 traveling and had been dropped off at the hospital by a friend. The decedent had a partially
184 engorged tick attached to his right ear that detached upon his arrival to the morgue and was
185 quickly collected. This particular specimen was identified as a nymphal *Amblyomma* sp. most
186 likely *A. cajennense* or *A. imitator*, which both have a known distribution in South Texas,
187 Mexico and Central America [24]. Once all the investigative information was put together, the

188 range for the tick and the decedent's travel history matched. One can imagine in the absence of
189 identification and/or NOK information that notice could be sent to consulate offices in the range
190 of the tick to help in the search or for a decedent that is from within the United States to law
191 enforcement agencies in the range of the tick species. One could also use the tick to test for tick-
192 borne disease, potentially for surveillance or in determination of cause of death. Insects and other
193 arthropods have the potential to help with a variety of other types of questions related to a
194 decedent's life and death; therefore, collection of specimens, no matter how trivial, can
195 sometimes prove to be incredibly useful to the investigation.

196 **Outdoor Scenes** –Cases involving specimens from decedents found outdoors primarily
197 involve estimating TOC for the purposes of estimating PMI. This is the classic scenario in
198 forensic entomology from which many of the protocols and standards were derived [25,26]. As
199 we integrate forensic entomology into routine medicolegal death investigations we can
200 appreciate the breadth of opportunities available for casework and applications of insects to
201 death investigations [6]. Figure 1 illustrates the official manner of death classifications for
202 HCIFS forensic entomology cases from January 2013 through April 2016. It shows that outdoor
203 scenes are outnumbered by those that occur indoors. A majority of all of the cases involve
204 natural manner of death classifications as the official manner of death. Initially these cases come
205 into the morgue as undetermined cases due to the difficulty of assessing trauma of decomposing
206 decedents. The number of cases involving undetermined, accident and suicide manners of death
207 that involve insects and occur outdoors are approximately equal to those that occur indoors.
208 These data suggest expanding forensic entomology tools and research to understanding insect
209 colonization indoors, which is an area of forensic entomology that has received comparatively
210 less attention [27,28]. Noticeably, there are a higher percentage of homicide cases involving

211 insects from decedents at scenes located outdoors than indoors. This is reflected in the initial
212 development of forensic entomology tools for the outdoor scene as the initial opportunities of
213 forensic entomology casework were to assist with homicide investigations [1]. It follows that
214 decomposing decedents have fewer delays in colonization related to insect access and more
215 opportunities for temperature modifications that may accelerate decomposition (e.g. direct sun
216 exposure).

217 Analysis of factors associated with outdoor scenes in Harris County involves
218 significantly higher average temperature (28.0°C; Table 3; Figure 2) but few other significant
219 differences in the other factors observed when compared to indoor scenes (Table 3). The TOC
220 estimates for outdoor scenes are slightly longer on average but not significantly different from
221 those estimated from indoor scenes (Figure 3). Whereas the TOC estimates from hospitals were
222 significantly shorter from both (Figure 3), which is most likely due to these cases being
223 associated with known myiasis. Only one insect species was significantly associated with being
224 encountered at outdoor scenes, the green bottle fly, *Lucilia eximia* (Table 4). All other insect
225 groups, which were encountered in five or more cases, were either found to have a significantly
226 higher probability to be encountered at indoor scenes or to be encountered at both indoor and
227 outdoor scenes (Table 4). According to the binomial test, the probability of collecting *L. eximia*
228 indoors is significantly lower than at an outdoor scene based on the data currently available in
229 Harris County (Table 4). This suggests that finding this species on a decedent endorses outdoor
230 colonization of the decedent when considered alone. However, three cases involved the
231 collection of *L. eximia* at indoor scenes (Table 1) suggesting that under some circumstances this
232 species does colonize bodies located indoors. The combination of species encountered on a
233 decedent may suggest movement of the decedent if for example, when *L. eximia* is found

234 together with a strongly indoor colonizing species like *M. scalaris* (Table 4), but this may only
235 be of importance when other scene indicators suggest this possibility. The opposite scenario may
236 also be suggested if a strongly indoor colonizing species such as *M. scalaris* is found on a
237 decedent located outdoors. However, it was not analyzed with these data but the combination of
238 species found on the decedent may temper the strong association of certain species with outdoor
239 locations as suggested by the small number of cases where *L. eximia* colonized indoors. In the
240 casework analyzed here, it was actually more rare for bodies to be found to be colonized by a
241 single insect species with only 14.2% (28 of 197) of cases found to have only a single colonizing
242 species and the rest with more than one.

243 **Indoor Scenes** – Cases involving insects collected indoors also have a primary goal of
244 TOC estimation for the purpose of estimating PMI. A majority of the forensic entomology case
245 reports from Harris County over the analyzed period were from indoor scenes and they
246 encompassed every manner of death classification except fetal (Figure 1). The average ambient
247 scene temperature observed at indoor scenes (25.2°C) was significantly lower than that observed
248 at outdoor scenes (Table 3; Figure 2). As mentioned earlier the number of cases that involve
249 manner of death classifications involving accidents, suicides and undetermined classifications
250 involve scenes located indoors and outdoors suggesting a need for more tools for indoor scenes.
251 Indoor scenes can be complicated by multiple factors that affect insect colonization (e.g. delays
252 in colonization; [27]), temperature modification (e.g. air conditioning, heating), co-located
253 decomposing remains (e.g. decomposing pets; [29]), 24-hour lighting which may allow for
254 nocturnal oviposition (e.g. a TV or lamp left on at the scene;[30]), other sources of insects in the
255 residence that may reduce or eliminate a delay in colonization (e.g. hoarding of trash and food)
256 and a number of other case specific factors that may affect the TOC estimate. Many of these

257 complicating factors have as yet unknown effects on TOC estimation but have the potential to
258 have significant impacts on PMI estimation.

259 Another significant observation regarding indoor scenes is in the different insects found
260 at indoor scenes. Just as some insect species appear to have preference for outdoor scenes there
261 appears to be a strong association between indoor scenes and several insect groups including
262 *Megaselia scalaris*, Sarcophagidae including *Blaesoxipha plinthopyga*, *Synthesiomyia nudiseta*
263 and *Lucilia cuprina* as can be observed in the binomial test statistics presented in Table 4. This
264 suggests when each of these species, considered alone, may represent an indicator of indoor
265 colonization or at the very least allow for the consideration of an indoor scene from the insect's
266 perspective. A car in a covered carport or a storage container where a decedent was living may
267 not be what may typically be considered indoor scenes but from the insect's perspective, they
268 may fulfill colonization preferences. Therefore, scene indicators and context should all be taken
269 into account when considering insect specimens as indicators of body movement.

270 These results are similar to those observed by other researchers in other geographic
271 locations. On the Hawaiian Island of Oahu, Goff [31] found that three species were strong
272 indicators of indoor scenes including a species of the Phoridae *Megaselia scalaris*, the
273 Sarcophagidae *Bercaea haemorrhoidalis* and the Muscidae *Stomoxys calcitrans*. While not
274 exactly the same species encountered in the current study, a similar combination of
275 representatives of the same fly families was found with the same Phoridae *M. scalaris*, the
276 Sarcophagidae *B. plinthopyga* and the Muscidae *S. nudiseta* indicating indoor colonization. Goff
277 also found that there was a wider diversity of fly species indoors at least preliminarily. In the
278 cases analyzed in this study there was a similar, though small, result with an average of 1.9 (+/-
279 1.3 standard deviation) species of flies found from outdoor scenes and an average of 2.4 (+/-1.3

280 standard deviation) fly species encountered in specimens from outdoor scenes overall. The
281 Hawaiian study also reported a higher diversity of beetles encountered outdoors and this was also
282 a trend that was observed in the current study although beetles were more rarely collected (Table
283 1). In Western Canada Anderson [27] found that most of the blow fly species colonizing
284 experimentally placed pigs indoors and outdoors overlapped with several species appearing to be
285 restricted to the outdoors. Similar results were obtained in this study where the only species that
286 was strongly associated with outdoor scenes was *L. eximia* (Table 1 and 4). However, in an
287 analysis of casework Anderson [18] found that in British Columbia, Sarcophagidae and Phoridae
288 did not appear to be significant colonizers and did not appear to have the same dichotomy based
289 on scene location. When one considers the climate similarities and differences among the three
290 geographic locations, such as the temperatures, relative humidity (Table 2) and precipitation
291 encountered at scenes in Harris County along the Gulf Coast are perhaps more similar to those
292 encountered in Hawaii rather than British Columbia. Furthermore climate change may lead to
293 changes in species ranges overtime [32] or to differentiation in developmental progression in
294 different populations of the same species. This highlights the necessity and importance of
295 collecting and publishing these data for different geographic locations, different climatic regions
296 and different species assemblages rather than relying only on a handful of studies.

297 Some species of insects and arthropods analyzed in Harris County casework have only
298 been found indoors thus far. The flesh fly *Blaesoxipha plinthopyga* is the only fly, which has
299 been identified exclusively indoors. This fly was not known to be a forensically significant fly in
300 the US until quite recently [33] and this is perhaps due in part to the difficulty in identification of
301 Sarcophagidae, which requires either prepared adult male flies [34] and expert morphological
302 expertise associated with male genitalia or expertise in the molecular systematics of

303 Sarcophagidae [35,36] and adequate laboratory capacity. Therefore, Sarcophagidae have often
304 been relegated to the Family level without further identification. Nevertheless, even with
305 identification there are significant gaps in the literature for detailed development data sets for
306 many of the forensically important Sarcophagidae species. The mite, *Myianoetus muscuarum*,
307 has only been found indoors but it has only been found in a few cases thus far ([37] and Table 1)
308 and it may be the case that the more it is looked for the more it may be found. The other species
309 that have been found exclusively indoors have very low case numbers and may have just not
310 been collected frequently enough to establish habitat or colonization preferences for these
311 insects/arthropods (Table 1).

312 **Insects/Arthropods**

313 The introduction of routine forensic entomology into the Medical Examiner's office has
314 allowed for the observation of several new associations between insects, arthropods and human
315 decomposition. One of those observations was the first report of what was determined to be
316 myiasis of a decedent shortly before his death by *L. eximia* and *C. rufifacies* for the first time on
317 a human in the US [38]. In another case, the blue blow fly, *Calliphora coloradensis* was
318 collected from an outdoor scene in February 2014, associated with a decedent in an advanced
319 state of decomposition and vertebrate scavenging. The specimen was reared from a pupa located
320 next to the decedent and it was the first time that this species had been collected from a decedent.
321 It is known to occur in Texas [15] but has not to the author's knowledge, been collected in
322 association with a decomposing human in Harris County. It has been previously recorded as an
323 adult associated with a human corpse found at high elevation in Colorado but was not observed
324 as a colonizer of the remains [39]. In another case the tawny crazy ant, *Nylanderia fulva* was
325 recorded on a decomposing human body in Harris County for what appears to be the first time
326 (Table 1).

327 Aside from new insect associations, other new arthropod associations are being made as
328 well. The mite, *M. muscuarum* was recently established to have a relationship with *S. nudiseta* at
329 indoor scenes [37] and has been found in additional cases since this publication was completed.
330 Mites continue to be encountered at scenes and with help of Dr. B. O Connor (University of
331 Michigan), identifications of the mite species are being made in order to develop a taxonomic
332 index and to be able to associate the mite fauna with timing in the decomposition process for this
333 geographic location. A nematode was recently found in association with decomposing decedents
334 found in advanced stages of decomposition located indoors. These nematodes were first
335 discovered in a rearing container from a case involving a decedent found in an advanced state of
336 decomposition in a hoarder home. The decedent was also colonized by Sarcophagidae,
337 Calliphoridae and *Hermetia illucens*. Rearing of the specimens to confirm identifications led to
338 the discovery of large numbers of nematodes in the rearing container. The identity of these
339 nematodes was difficult as they may represent a new species and their identity is still being
340 investigated by Dr. Y. Qing (Canadian National Collection of Nematodes).

341 The location of Harris county being on the Gulf Coast and near the southern US border
342 presents the opportunity to make observations related to new species introductions as well as
343 documenting new associations. The forensic entomologist can therefore serve in a capacity to
344 identify foreign species introductions and to notify relevant interested parties. This applies to not
345 only those insects that are used for TOC estimation but also those that may be relevant to public
346 health and cause of death determination as new arthropod-borne diseases are introduced to the
347 US.

348 **Seasonality**

349 The seasonality of the blow flies encountered in Harris County follows patterns generally
350 accepted for Calliphoridae in North America [40]. The typical cool weather blue bottle flies,
351 *Calliphora* spp. are found in the winter months and other species such as the black blow fly, *P.*
352 *regina*, and the Muscidae species, *S. nudiseta* are active in the spring months (Table 1). The
353 green/bronze bottle flies, *Lucilia* spp. are most often collected in the hot summer months but can
354 be encountered throughout the spring through the fall months (Table 1). The introduced species,
355 the hairy maggot blow fly, *Chrysomya rufifacies* and the oriental latrine fly, *Chrysomya*
356 *megacephala* appear to be active year-round in some years whereas in other years they can be
357 absent in the winter months (Table 1). It is a very rare occurrence for there to be snow in Harris
358 County and temperatures below 0.0°C rarely persist for more than a few days if at all [41]. These
359 climate characteristics probably help the introduced tropical Calliphoridae species persist
360 through the winter as freezing temperatures are thought to restrict their distribution in North
361 America [42].

362 The Sarcophagidae species, *B. plinthopyga*, and the Phoridae species, *M. scalaris*, are not
363 as seasonally restricted and appear to be collected in casework year-round (Table 1). It would
364 seem that this might be partially explained by their exploitation of indoor scenes. Currently no
365 data is available on the dispersal patterns of either of these fly species. Other Phoridae species
366 appear to be capable of transportation via wind and human modes of transport [43]. Preliminary
367 data suggests population structure exists within *M. scalaris* populations in the US even across
368 relatively small geographic distances [44]. Indoor environments with their cooler and less
369 variable temperature range (Figure 2) may offer refuge when outdoor conditions are unfavorable
370 allowing populations to persist locally during bad weather.

371 **CONCLUSIONS**

372 The integration of full time forensic entomology services to the medical examiner's
373 office in Harris County has revealed new observations, confirmed long-standing hypotheses and
374 opened up a wide range of new opportunities. The casework described here is not an endpoint
375 and new species observations and associations continue to be revealed. This study represents a
376 reference point from which to describe additional observations and trends and from which to
377 compare other geographic locations and species assemblages. These data also remind us that
378 depending upon generalizations and assumptions can be misleading and that keeping an open
379 mind and collecting something that may at first seem trivial may prove to be exceptionally
380 useful.

381 The data presented here also have illustrated several persistent issues in the application of
382 forensic entomology to casework. While molecular identification tools are being more widely
383 developed and applied to morphologically difficult groups (e.g. Sarcophagidae and Muscidae)
384 there are significant gaps in development data sets. A single development data set is available for
385 *B. plinthopyga* which was never intended for use in application to forensic entomology as it is
386 includes total generation time for specimens collected during a study of diapause [45]. Yet this
387 species is considerably important to casework in Harris County (Table 1). There are no known
388 published development data sets for *C. coloradensis*, *C. livida* or *D. maculatus* using a diet that
389 approximates human tissue. The suggested practice of using closely related species to
390 approximate development for those species that have missing data introduces unnecessary
391 uncertainty in an estimate of TOC and its application to PMI. Furthermore there is growing
392 awareness that different populations of the same species have different developmental responses
393 to the same temperatures over large [46] and relatively small geographic distances [47]. This
394 strongly suggests that using development datasets from populations not local to the collection

395 location for the case may introduce unwelcome uncertainty to a case that becomes difficult to
396 track and account for once the case has been filed. Several of the species that are common and
397 important to casework in Harris County lack local development data sets including *C.*
398 *megacephala*, *S. nudiseta*, *L. eximia* and *L. cuprina*.

399 While missing development datasets can be generated, other issues continue to haunt
400 forensic entomology casework that are not and will not be as easy to remedy. In 1992, Catts [48]
401 outlined several complications in using insects to estimate postmortem interval. These issues,
402 such as maggot mass heat, insect access and entomotoxicology, continue to be sources of
403 uncertainty for the application of forensic entomology to casework. It has only been relatively
404 recently that there has been an increased awareness for the need to account for uncertainty and
405 sources of error in forensic entomology within the context of all forensic sciences in the US [49].
406 In addition, while identifying potential sources of error is more widely appreciated accounting
407 for how these errors and influences impact individual TOC and PMI estimates has not yet been
408 resolved. Tackling these challenges in forensic entomology will require strong collaboration
409 between academic and practitioner parts of the community but the results will make forensic
410 entomology an even more powerful tool in understanding the post-mortem interval.

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429

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568

	<u>N</u>		<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>
Scene Type: Hospital	6													
Species														
Diptera: Calliphoridae	3													
- <i>Chrysomya rufifacies</i>	1							A						
- <i>Lucilia eximia</i>	2							A	B					
Hymenoptera: Apidae														
- <i>Apis mellifera</i>	2					A						B		
Pthiraptera: Pulicidae														
- <i>Pediculus humanus</i>	1							A						
Acari: Ixodidae														
- <i>Amblyomma imitator</i> or <i>cajennense</i> (nymph)	1									A				
Scene Type: Scene	<u>N</u>	<u>N</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>
	<u>In</u>	<u>Out</u>												
Diptera: Sarcophagidae	85	8	ABCD	ABCD	ABCD	ABCD	ABC	ABC	BC	ABC	ABC	ABC	ABC	ABC
- <i>Blaesoxipha plinthopyga</i> ¹	37	0 ¹	BC	BC	BCD	C	AB	C	C	BC	AC	AC	AB	A
Diptera: Phoridae	60	2												
- <i>Megaselia scalaris</i>	59	2	ABC	ABCD	ABC	ACD	AB	ABC	BC	AC	ABC	ABC	ABC	ABC
- <i>Dorniphora cornuta</i> ²	1	0												B
Diptera: Calliphoridae	64	56	AD	ACD	ABCD	ABCD	ABC							
- <i>Phormia regina</i>	12	8	D	ACD	ABCD	ABCD		B	C			C		ABC
- <i>Cochliomyia macellaria</i>	17	20	D	A	D	ABCD	BC	ABC	ABC	C	BC	B		C
- <i>Chrysomya rufifacies</i>	34	36	AD	AC	ACD	D	AC	ABC	ABC	ABC	ABC	AC	BC	ABC
- <i>Chrysomya megacephala</i>	21	19		A	AD	ACD		ABC	ABC	AC	ABC	ABC	A	ABC
- <i>Lucilia eximia</i>	3	17			D	B	AC	AB		C	AB		ABC	B
- <i>Lucilia cuprina</i>	8	1			C	ABD	A					B		A
- <i>Lucilia sericata</i>	1	0				A								
- <i>Calliphora vomitoria</i>	0	2		A										B
- <i>Calliphora livida</i>	0	1	D	C										
- <i>Calliphora coloradensis</i>	0	1		C										
- <i>Calliphora vicina</i>	1	0			D									

	<u>N</u> <u>In</u>	<u>N</u> <u>Out</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>
Diptera: Muscidae	20	14	B	C	AB	ABC	AB	A	BC	B	C	C	BC	AB
- <i>Synthesiomyia nudiseta</i>	9	1	BD	CD	D		AB		C	B		C	BC	
- <i>Musca domestica</i>	4	1			A	AB	A							A
- <i>Hydrotea</i> sp.	7	10	D	CD	BD	ABC		A	BC	B	C	C	C	B
- <i>Fannia scalaris</i>	0	1						A						
Diptera: Piophilidae														
- <i>Piophila casei</i>	2	4				BD	A	A	C				C	
Diptera: Stratiomyidae														
- <i>Hermetia illucens</i>	2	6		A					BC			C		BC
Diptera: Anthomyiidae	0	1	A											
Diptera: Sepsidae	0	1	A											
Diptera: Drosophilidae	1	0												C
Diptera: Chironomidae	0	1												
- <i>Goelichironomus holoprasinus</i>	0	1									B			
- <i>Polypedilium flavum</i>	0	1									B			
- <i>Chironomus</i> sp.	0	1									B			
Diptera: Tachnidae	0	1									C			
Diptera: Psychodidae														
- <i>Psychoda</i> sp.	0	1							C					
Coleoptera: Dermestidae	8	11	AD	A	D	BD	AB	A	ABC	BC		C	BC	A
- <i>Dermestes maculatus</i>	6	7				D	AB	A	ABC	B	C	C	C	A
Coleoptera: Nitidulidae														
- <i>Omosita</i> sp.	0	5						A	C			C	C	A
Coleoptera: Histeridae ³	1	3			A		A	A		A				
Coleoptera: Silphidae ³	0	2		A										B
Coleoptera: Cleridae ³	3	0						A						
Coleoptera: Staphylinidae	0	1		D										
Coleoptera: Tenebrionidae	0	1					A							
Coleoptera: Carabidae	0	1								B				
Coleoptera: Elateridae	0	2							C					B

	<u>N</u> <u>In</u>	<u>N</u> <u>Out</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>
Hymenoptera: Apidae														
- <i>Apis mellifera</i> ³	0	1		B										
Hymenoptera: Formicidae														
- <i>Solenopsis invicta</i> ³	1	2							AB	C				
- <i>Nylanderia fulva</i> , ³	0	1				D								
Hymenoptera: Chalcididae														
- <i>Brachymeria fonscolombei</i>	1	0							B					
Hymenoptera: Pteromalidae														
- <i>Nasonia vitripennis</i>	0	1		D										
Dictyoptera: Blatellidae														
- <i>Blatella germanica</i> ³													B	
Lepidoptera	1	2							C		C		B	
Orthoptera: Gryllidae ³	0	1								C				
Orthoptera: Cicadellidae ³	0	1												B
Nematoda	1	1		C		D			B					
Acari: Astigmatidae	0	3		C										C
- <i>Myianoetus muscuarum</i> ⁴	0	2		C										
Acari: Macrochelidae	0	1												A
Heteroptera: Cimicidae														
- <i>Cimex lectularis</i> ³	1	0								C				
Annelida ³	0	1												C
Diplopoda ³	0	1		C										
Isopoda ³	0	3				C			C					B

Table 1. A list of insect and arthropod species collected during selected medicolegal death investigations handled by the Harris County Institute of Forensic Sciences from January 2013 through April 2016. The month of recorded collection is indicated by the letters A for 2013, B for 2014, C for 2015 and D for 2016. ¹Confirmation of this species is based on the appearance of male genitalia from reared adult males and this could be an underestimate of actual abundance for this species because reared males may not always be obtained. ²Identification of this specimen is based on a single larva and is tentatively identified to the species level. ³These species may be regularly observed at scenes but not regularly collected and reported upon because they are not typically related to time of colonization estimation. ⁴This species was recently associated with *Synthesiomyia nudiseta* [37].

Species/group	<u>N</u> <u>In</u>	<u>N</u> <u>Out</u>	<u>Avg. Ambient</u> <u>Temperature</u> <u>(+/-st.dev.)</u>	<u>Avg.</u> <u>Ambient</u> <u>Relative</u> <u>Humidity</u> <u>(+/-st.dev.)</u>	<u>Avg. TOC</u> <u>(days)</u> <u>(+/-st.dev.)</u>
Diptera: Sarcophagidae	85	8	25.8°C (5.1)	50.8% (14.8)	9.7d (12.1)
- <i>Blaesoxipha plinthopyga</i> ¹	37	0 ¹	25.7°C (4.4)	49.6% (13.7)	9.8d (9.5)
Diptera: Phoridae	60	2	24.1°C (4.5)	53.0% (16.5)	8.8d (7.6)
- <i>Megaselia scalaris</i>	59	2	24.1°C (4.5)	53.0% (16.5)	8.8d (7.6)
- <i>Dorniphora cornuta</i> ²	1	0	16.6°C (unk)	56% (unk)	12d (unk)
Diptera: Calliphoridae	64	56	26.9°C (7.2)	53.5% (19.1)	11.3d (18.2)
- <i>Phormia regina</i>	12	8	22.4°C (7.2)	47.4% (18.0)	8.3d (10.8)
- <i>Cochliomyia macellaria</i>	17	20	29.2°C (6.7)	54.3% (16.5)	4.9d (6.5)
- <i>Chrysomya rufifacies</i>	34	36	27.7°C (7.8)	52.4% (21.7)	14.5d (21.2)
- <i>Chrysomya megacephala</i>	21	19	28.0°C (6.6)	55.6% (19.6)	3.6d (3.4)
- <i>Lucilia eximia</i>	3	17	29.2°C (6.6)	56.3% (21.8)	2.2d (2.8)
- <i>Lucilia cuprina</i>	8	1	27.4°C (7.5)	45.1% (10.9)	4.7d (1.9)
- <i>Lucilia sericata</i>	1	0	unk	unk	2.0 (unk)
- <i>Calliphora vomitoria</i>	0	2	21.2°C (10.3)	44.5% (4.9)	9.5d (3.5)
- <i>Calliphora livida</i>	0	1	15.2°C (8.8)	47% (12.7)	0 (unk)
- <i>Calliphora coloradensis</i>	0	1	6.1°C (unk)	75% (unk)	30d (unk)
- <i>Calliphora vicina</i>	1	0	25°C (unk)	36% (unk)	8d (unk)
Diptera: Muscidae	20	14	24.9°C (8.7)	52.2% (22.8)	19.6d (21.4)
- <i>Synthesiomyia nudiseta</i>	9	1	22.7°C (6.8)	50.6% (21.4)	17.4d (22.9)
- <i>Musca domestica</i>	4	1	26.4°C (8.2)	33.8% (20.3)	8.8d (8.2)
- <i>Hydrotea sp.</i>	7	10	26.7°C (11.2)	57.7% (25.4)	21.1d (22.8)
- <i>Fannia scalaris</i>	0	1	30.6°C (unk)	64% (unk)	11d (unk)
Diptera: Piophilidae					
- <i>Piophila casei</i>	2	4	26.2°C (9.3)	53.0% (21.1)	21.1d (37.6)
Diptera: Stratiomyidae					
- <i>Hermetia illucens</i>	2	6	23.3°C (10.0)	57.6% (20.6)	53.0d (33.3)
Coleoptera: Dermestidae	8	11	25.5°C (5.5)	57.5% (16.4)	29.9d (25.6)
Coleoptera: Nitidulidae					
- <i>Omosita sp.</i>	0	5	25.6°C (4.7)	67.0% (30.4)	36.8d (21.5)
Coleoptera: Histeridae ³	1	3	32.0°C (6.5)	54.4% (25.6)	5.2d (4.3)
Coleoptera: Silphidae ³	0	2	16.8°C (4.1)	63.0% (21.2)	5.0d (2.8)
Coleoptera: Cleridae ³	3	0	27.5°C (unk)	83% (unk)	10d (unk)

Species/group	<u>N</u> <u>In</u>	<u>N</u> <u>Out</u>	<u>Avg.</u> <u>Temperature</u> <u>(+/-st.dev.)</u>	<u>Avg.</u> <u>Relative</u> <u>Humidity</u> <u>(+/-st.dev.)</u>	<u>Avg. TOC</u> <u>(days)</u> <u>(+/-st.dev.)</u>
Hymenoptera: Apidae					
- <i>Apis mellifera</i> ³	0	1	29.8°C (unk)	39% (unk)	n/a
Hymenoptera: Formicidae					
- <i>Solenopsis invicta</i> ³	1	2	34.7°C (2.3)	42.7% (13.3)	2d (unk)
Hymenoptera: Chalcididae					
- <i>Brachymeria fonscolombeii</i>	1	0	31.1°C (unk)	60% (unk)	16d (unk)
Hymenoptera: Pteromalidae					
- <i>Nasonia vitripennis</i>	0	1	34.9°C (unk)	32% (unk)	43d (unk)
Acari: Astigmatidae	0	3			
- <i>Myianoetus muscuarum</i> ⁵	0	2	19.2°C (2.0)	51.3% (30.5)	22.3d (2.1)
Heteroptera: Cimicidae					
- <i>Cimex lectularis</i> ³	1	0	33.5°C (unk)	54% (unk)	18d (unk)
Isopoda ³	0	3	28.5°C (17.6)	47.5% (37.5)	40.3d (51.1)

570 **Table 2. Number of cases coming from indoor and outdoor scene investigations with the**
571 **average observed ambient scene temperature (°C) relative humidity (%) and the average**
572 **maximum estimated time of colonization (TOC) for selected species collected in Harris**
573 **County from January 2013 through April 2016 (N=203). ¹Confirmation of this species is**
574 **based on the appearance of male genitalia from reared adult males and this could be an**
575 **underestimate of actual abundance for this species because reared males may not always be**
576 **obtained. ²Identification of this specimen is based on a single larva and is tentatively**
577 **identified to the species level. ³These species may be regularly observed at scenes but not**
578 **regularly collected and reported upon because they are not typically related to time of**
579 **colonization estimation. ⁴This species was recently associated with *Synthesiomyia nudiseta***
580 **[37].**

Comparison	Kruskal-Wallis X² Test Statistic	d.f.	P-value
Ambient scene temperature by scene location ¹	8.1531	1	0.00430*
Relative humidity by scene location ¹	1.0947	1	0.2954
Length of TOC estimate by scene location	7.9093	2	0.01917*
Ambient scene temperature by stage of decomposition	2.0434	4	0.7278
Scene relative humidity by stage of decomposition	2.1554	4	0.7072
Length of TOC estimate by stage of decomposition	77.682	4	>0.0001*

581 **Table 3. Kruskal-Wallis Chi-Squared statistical comparisons for ambient scene**
582 **temperature, relative humidity and length of the TOC estimate (days) by scene location**
583 **and stage of decomposition.** ¹**Temperature and relative humidity comparisons did not**
584 **include hospital cases, as these measurements were not made at the hospital. *indicates a**
585 **significant difference at the $\alpha=0.05$ level.**

586

Groups with ≥ 5 cases	N	Probability of success	95% confidence interval of success		P-value
			min	max	
<i>Blaesoxipha plinthopyga</i>	39	0.9744	0.8652	0.9994	1.46E-10*
<i>Megaselia scalaris</i>	67	0.9701	0.8963	0.9964	<2.20E-16*
<i>Lucilia cuprina</i>	11	0.9091	0.5872	0.9977	0.01172*
Sarcophagidae	103	0.9029	0.8287	0.9525	<2.20E-16*
<i>Synthesiomyia nudiseta</i>	18	0.8889	0.6529	0.9862	0.001312*
<i>Musca domestica</i>	5	0.8000	0.2836	0.9949	0.375
<i>Phormia regina</i>	31	0.5806	0.3908	0.7545	0.4731
Calliphoridae	134	0.5448	0.4565	0.6310	0.342
<i>Chrysomya rufifacies</i>	78	0.5000	0.3846	0.6154	1
<i>Cochliomyia macellaria</i>	46	0.4783	0.3289	0.6305	0.883
<i>Hydrotea</i> sp.	21	0.4762	0.2571	0.7022	1
<i>Dermestes maculatus</i>	22	0.4091	0.2071	0.6365	0.5235
<i>Piophilidae casei</i>	7	0.2857	0.0367	0.7096	0.4531
<i>Hermetia illucens</i>	8	0.2500	0.0319	0.6509	0.2891
<i>Lucilia eximia</i>	22	0.0000	0.0000	0.1544	4.77E-07*
Nitidulidae	5	0.0000	0.0000	0.5218	0.0625

587 **Table 4. Exact binomial test results indicating the probability of success as defined by**
588 **encountering a particular insect group indoors. The 95% confidence interval and P-value**
589 **for each test are also provided. *indicates a significant difference at the $\alpha=0.05$ level.**
590 **Statistics were only calculated for cases where each insect group was encountered five or**
591 **more times during the study period.**

592

593 **Figure Legend:**

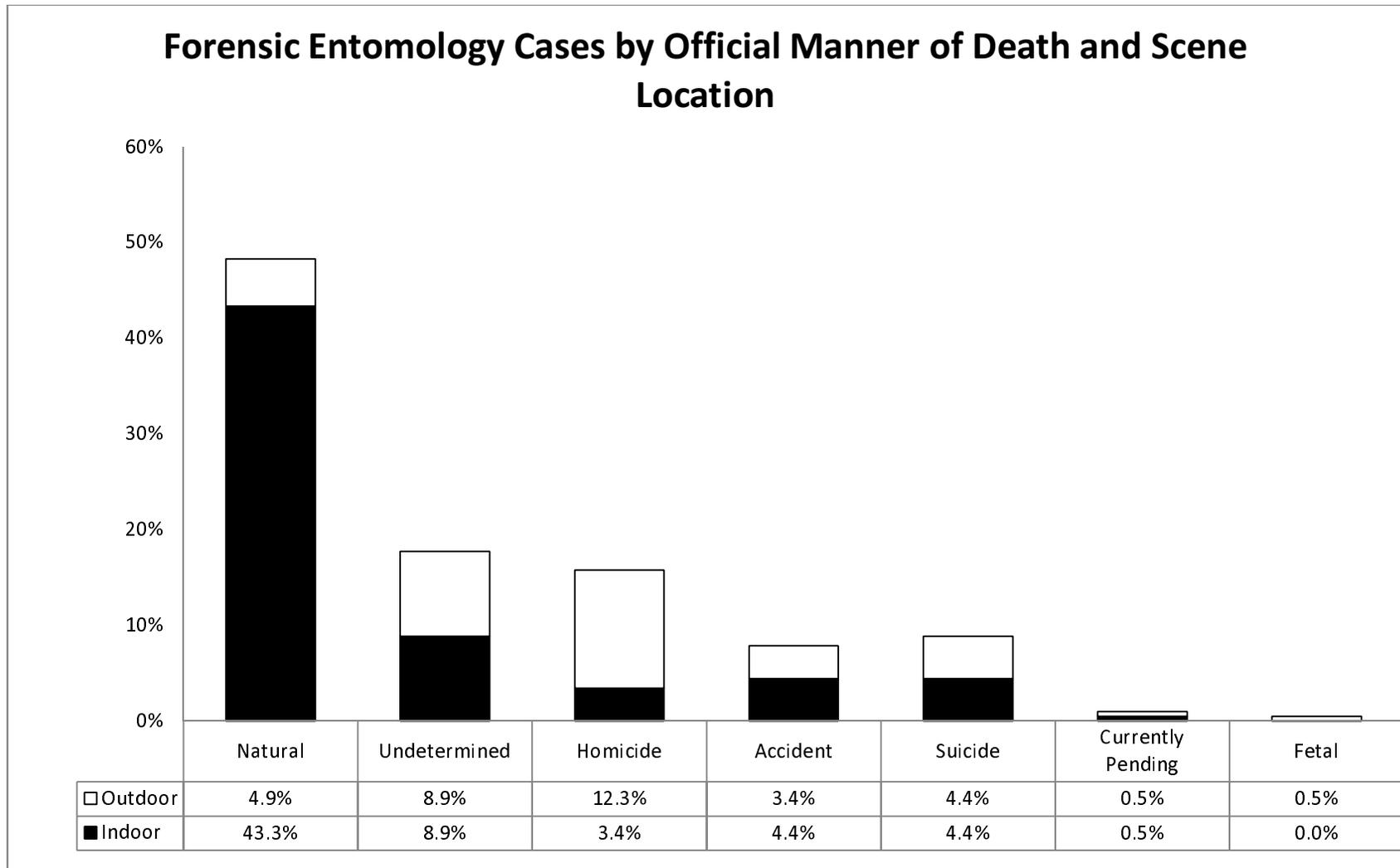
594 **Figure 1:** Percentage of forensic entomology cases over the study period (January 2013 - April
595 2016) by official manner of death classification, showing the proportion of cases where the scene
596 was located indoors (blue) or outdoors (red). (N=203).

597 **Figure 2:** Average (+/-standard deviation) ambient air temperature (°C) recorded near the
598 decedent at scenes located indoors and outdoors for forensic entomology cases over the study
599 period (January 2013 – April 2016). (N=203).

600 **Figure 3:** Average (+/-standard deviation) time of colonization (TOC) estimate (days) by scene
601 location for forensic entomology cases over the study period (January 2013 – April 2016).
602 (N=203).

603

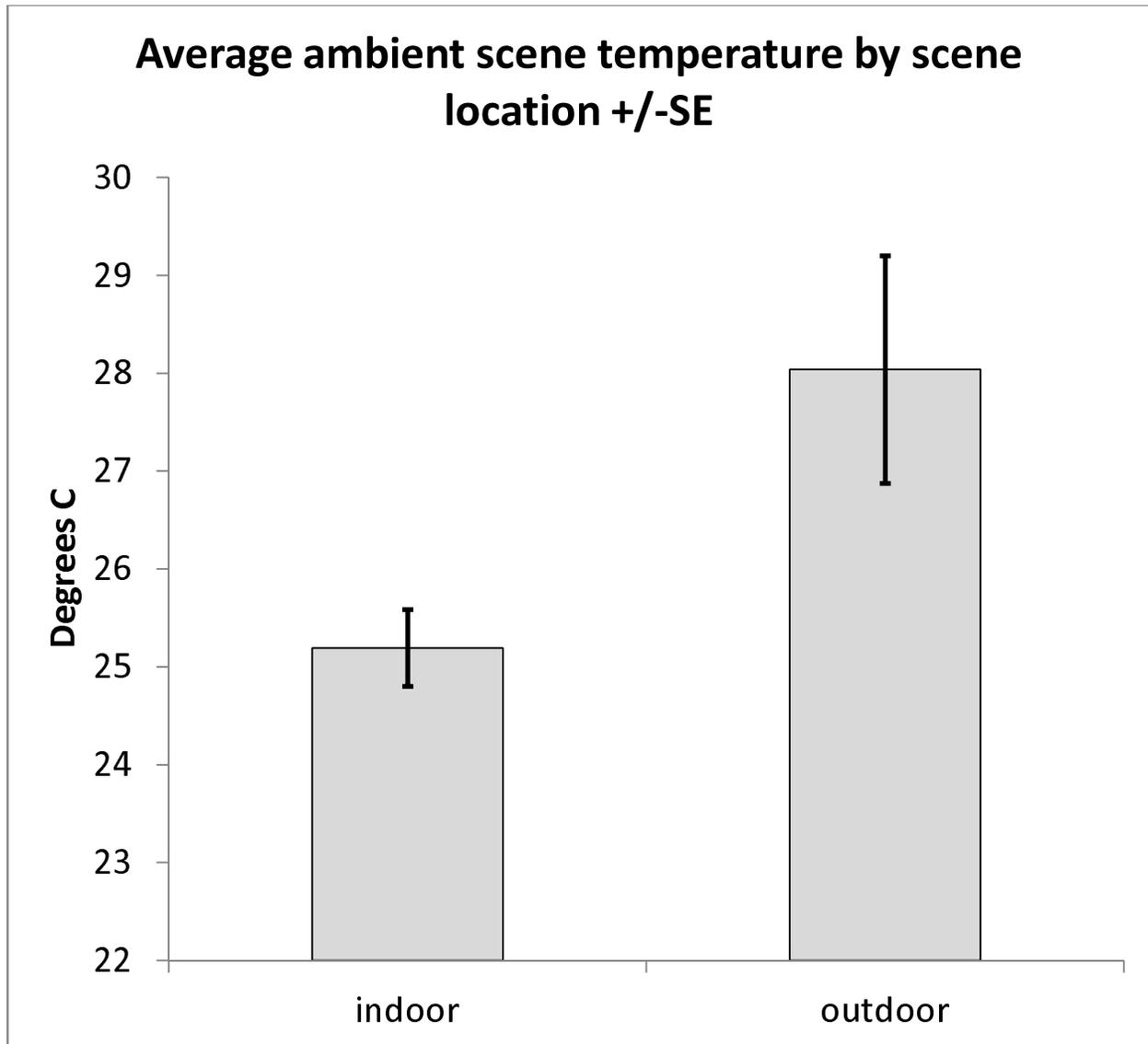
604 **Figure 1:**



605

606

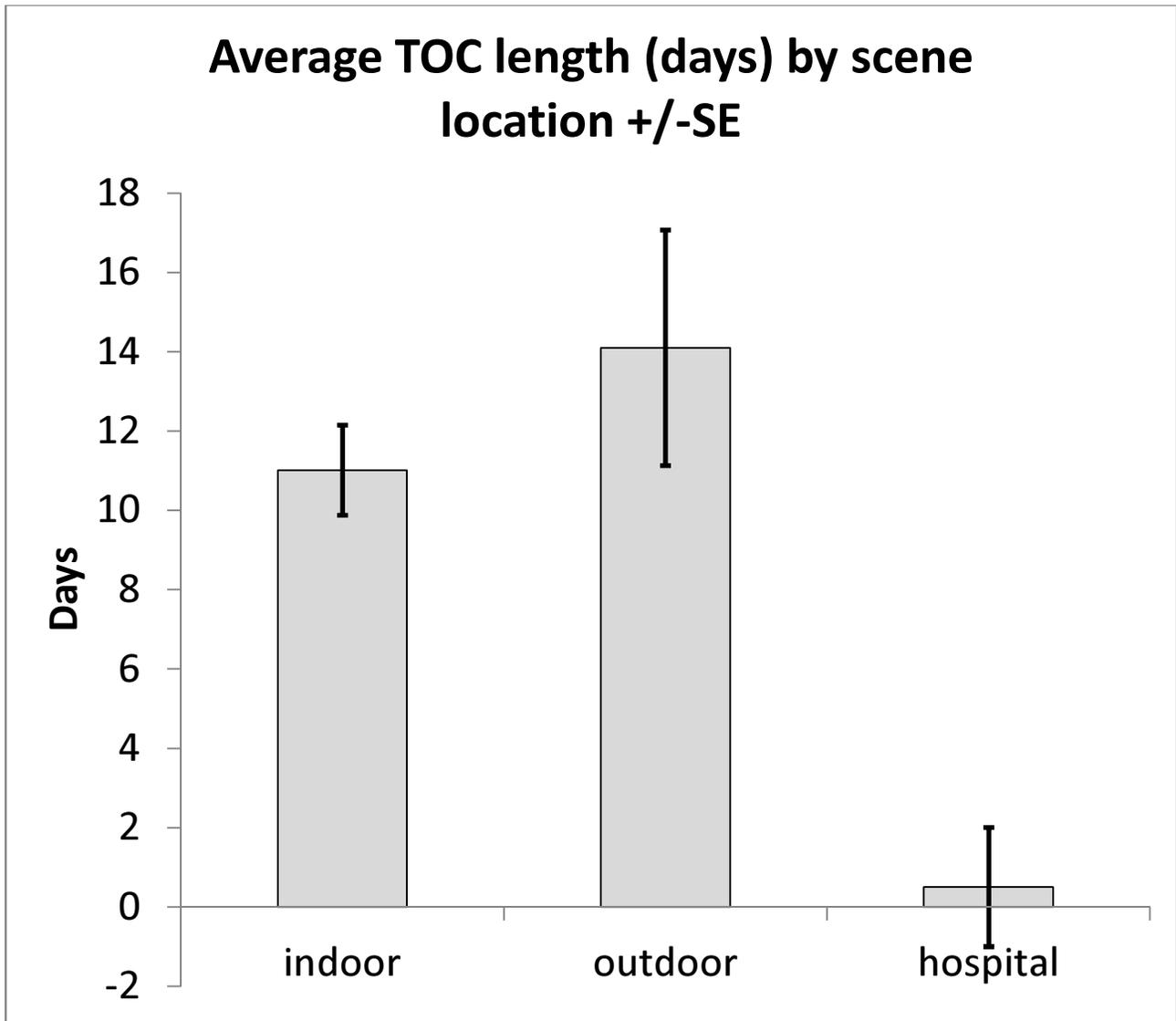
607 **Figure 2:**



608

609

610 **Figure 3:**



611