

Recognizing the Inherent Variability in Dipteran Colonization and Decomposition Rates of Human Donors in Sydney, Australia

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ABSTRACT

Introduction: Human decomposition is influenced by intrinsic and extrinsic factors including entomological activity, which can result in variability in the decomposition process. In death investigations, forensic entomology, the study of insects in a legal context, is the preferred method to estimate a post-mortem interval after pathologist methods are no longer applicable. The purpose of the current study was to document the primary dipteran colonization and rates of decay during the decomposition processes of human donors with known causes of death.

Methods: Five consenting human donors were placed in a forested area at the Australian Facility for Taphonomic Experimental Research (AFTER) in Sydney, Australia, and allowed to decompose in a natural environment. Temperature and humidity were monitored hourly while other factors like colonizers and decomposition stage were recorded at each visit to the site. Thermal summation, called Accumulated Degree-Days (ADD), was calculated to compare the rates of decay. **Results:** Results show that no two donors followed the same rate of decomposition. There were instances of delayed dipteran colonization, which resulted in slowed decomposition rates. Differences in rates of decay between donors could also have been influenced by intrinsic factors such as size, clothing and peri-mortem treatments.

Conclusions: This research supports the larger body of research involving the pre-colonization interval of insects, emphasizing the numerous variables that can affect colonization. Further research into the pre-colonization interval, and factors that affect it, should be performed using human donors to better understand how this knowledge can be applied to death investigations.

KEYWORDS: Forensic entomology, forensic taphonomy, stages of decomposition, blow flies, accumulated degree-days

INTRODUCTION

Human decomposition is a complex process affected by many intrinsic and extrinsic factors. The decomposition process can be studied, in specific regions of the world, using human donors. When human donors are not an option, animal carcasses such as domestic pigs can be used as an alternative source for vertebrate decomposition research, which can aide in making inferences about human decomposition [1–8]. Decomposition begins almost immediately after death and is highly dependent on many factors including temperature, relative humidity, and insect activity, which can influence, among other things, the rate of decomposition and tissue desiccation [9–15].

The post-mortem interval (PMI) is used to describe the time between death and discovery of a body, which is a vital component of death investigations [16]. Forensic pathologists can estimate PMI, or time of death, based on early post-mortem changes such as livor mortis, rigor mortis, and algor mortis; however, these methods are only useful within the first few hours since death and are not reliable beyond 72 hours after death [17]. Beyond this point, the PMI will most likely be inferred based on insect activity, if it is present.

Insect colonization is a major driver of the decomposition process, such that decomposition is slowed and/or halted when there is an absence of insect colonization [2,18–21]. Insects, specifically blow flies (Diptera: Calliphoridae) and other filth flies, colonize



the remains by laying eggs so that their subsequent immature stages may feed and seek shelter [11,22]. Colonization usually occurs in open orifices such as the mouth, nose, eyes, ears, anus, genitals, and any open wounds [10,18,23,24]. The life cycle of an insect includes four stages, namely egg, larval (1st, 2nd, & 3rd instars), pupal, and adult [25,26]. The rate at which a fly species will progress through these stages of life is based on the temperature in which they develop [26,27], and can be used to estimate the minimum time elapsed since death [28–30]. Descriptions of the time interval, from death to discovery, are based on insect behavior and carcass colonization referred to as pre-colonization interval (Pre-CI) and post-colonization interval (Post-CI) [31,32]. Using these descriptions, the PMI is encompassed by the Pre-CI and the Post-CI. The Pre-CI refers to the period of time between death and colonization and is difficult to estimate due to variability in foraging behaviours and lack of evidence of interaction between the insects and the remains [31]. The Post-CI refers to the time between oviposition and the departure of arthropods or time of discovery; the time when colonization occurs, the onset of Post-CI, will act as the minimum post-mortem interval (mPMI) and time of colonization (TOC) [31–33]. The minimum post-mortem interval (mPMI) describes the minimum time when the flies first laid eggs (or larvae) on the body until the body is discovered, providing the primary colonizing insects have been recovered [24]. Typically, a mPMI estimation will only encompass the Post-CI and not take into account the widely unknown Pre-CI, although research has attempted to fill this void [34–36]. More recently, TOC has also been used to describe this minimum time interval of when first eggs are laid [32,37,38]. While mPMI and TOC may be used interchangeably, in certain cases, for example, cases of myiasis (where colonization occurred before death), TOC would encompass the interval prior to death [32,37]. A reliable estimate of mPMI and TOC is ultimately limited by the accuracy of condition and development parameters which affect these determinations. These include, the weather station data closely representing the climate at the site of decomposition, published development datasets, the absence of myiasis, no other decomposing organism near the body, colonization occurs during the daytime

hours, insects have access to the remains, and faunal succession patterns can be used [47]. Finally, mPMI, TOC and Post-CI are all accepted terms to describe the time in which arthropods have colonized decomposing bodies, as long as the chosen form is suitably defined [37]. For the purposes of this publication, the term TOC will be used; however, mPMI would be equally as accurate as there were no instances of myiasis.

During the decomposition process, there are five recognized stages: fresh, bloated, active decay, advanced decay, and dry remains or skeletonization [23,39]. These stages are typically used to describe the process of soft tissue loss, particularly as a result of insect activity [18,25,40–42]. Insect communities and diversities change during each of these stages [10,18,23,43–45] and these are greatly affected by geographical location. In arid conditions with high solar radiation, desiccation of the upper surfaces of the deceased can occur, causing tough, leathery skin [5]. Desiccation begins in the early decomposition stages with the digits of the extremities and progresses towards full desiccation, dehydrated tissue over bone [46]. This typically occurs in the advanced decay stage [5,46].

Currently, the estimation of TOC and decomposition progression has involved the use of the Accumulated Degree-Days (ADD). ADD can be used to show a measurement of time that will integrate the effect of temperature on the rate of insect development and rate of decomposition [47,48]. The reason why ADD is so important to TOC determinations is that insects are ectothermic, meaning they rely on environmental conditions such as temperature and humidity [26]. Factors such as temperature and humidity are key in determining the extent to which remains are colonized by invertebrate scavengers, such as blow flies and beetles [15,16,49,50]. Colonizing insects require specific temperature ranges that will promote the continuation of their life cycle. Temperatures close to the upper or lower limit of insects' development threshold will delay or halt colonization and larval development and, in turn, delay soft tissue loss during the decomposition process [15,25]. Humidity also plays a large role in the rate of decomposition. Instances of high humidity will promote oviposition and high larval activity as well as accelerate autolysis and putrefaction causing an

increase in the rate of decomposition [1,5,18]. Alternatively, conditions with low humidity will cause the desiccation of eggs laid as well as the desiccation of tissue, making food unavailable to larvae and increasing insect mortality. This would slow the rate of decomposition considerably [5,18,51]. The use of ADD considers the influence that one of these factors, temperature, has on the development rate of specimens, and thus on decomposition, over time.

While there have been many publications on the forensically-relevant insects associated with carrion [25,52–56], there are comparatively few studies to date that have investigated the insect colonization and succession during the decomposition of human remains [38,55,57]. Specifically, none of these studies have included detailed information on the rates of decay and colonizing species on human remains in Sydney, Australia. As such, this research was conducted to investigate the decomposition process and blow fly colonization of five human donors by performing studies during the autumn months of 2017 and 2018 at the Australian Facility for Taphonomic Experimental Research (AFTER), the only human decomposition facility in the southern hemisphere.

MATERIALS AND METHODS

Australian Facility for Taphonomic Experimental Research (AFTER)

AFTER is a secure facility located in the rural outskirts of Sydney. It is approximately 12 acres of eucalypt open forest defined as a Cumberland Dry Sclerophyll Forest. Soils at AFTER are classified as sandy clay loam or gravelly sandy clay, with a pH of 5.5–6.5 [4]. The facility is located in the south-eastern region of Australia, classified by the Köppen Climate Classification System as a moist, mid-latitude (Cfb) climate where summers are warm to hot and winters are mild. This region does not represent the climate across all of Australia, however, results from AFTER can be applied to national and international regions with similar climates [58].

Experiment Design

These research experiments were performed in the Australian autumn season, from March to June of 2017 and repeated during the same period in 2018. The five human donors in this study were provided through the Body Donation Program overseen by the Surgical and Anatomical Science Facility (SASF) at the University of Technology Sydney (UTS). All donors provided consent to use their body for the purposes of research at AFTER, in accordance with the NSW Anatomy Act (1977) [4]. The research project was approved under the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15-0029). During both years of study, research began in early autumn (April) and ended in late autumn (June). Since donors were received as they became available, the observational time span for each donor study varied. The donors received in the early autumn were observed for a longer time span, from Experimental Day (ED) 0 to ED 32–58. Donors received during the late autumn were observed for a shorter time span of ED 0 to ED 18–27. It was not possible to observe the donors for the entire period of decomposition, rather this study focused on dipteran activity on human remains during a particular season. Information on each donor is available in Table 1.

Upon arrival to the facility, the donors were given an anonymous identity code that represents the year of donation and donor number; e.g. H18-09 was the 9th human donor received in 2018. For the purposes of this publication however, the donors will be referred to as Donors H1 to H5. Each human donor was refrigerated at 4°C until arrival at the AFTER facility. The date of placement was deemed ED 0. Upon arrival, the donor was placed on the ground either unclothed (H1, H2, H3) or clothed (H4, H5) in the centre of a 5x5m delineated plot. This means that donors were placed approximately 5m apart. This is less than the >50m recommended by Perez et al [59] and could not be avoided. As space needed to be used efficiently, plots were placed adjacent to each other, being careful not to place donors in the early stages of decay in proximity to each other. These donors were separated so that the primary colonizers of one donor would not influence the colonization of other donors nearby.

Table 1 A table with information for all donors that were observed during the study including date placed, season, sex, size, age, whether they were clothed, cause of death (COD) and the number of experimental days the donor was observed for.

Donor	Date Placed	Season	Sex	Size	Age	Clothed?	COD	Obs. Days
H1	17-MAR-17	Early autumn	M	Extra Large	74	No	Bowel perforation	54 days
H2	01-APR-17	Early autumn	M	Small	84	No	Kidney injury/renal failure Parkinson's disease	32 days
H3	03-MAY-17	Late autumn	M	Medium	21	No	Asphyxiation	18 days
H4	28-MAR-18	Early autumn	F	Large	61	Yes	Bacterial meningitis	58 days
H5	04-MAY-18	Late autumn	M	Small	57	Yes	Pneumonia, Liver Failure, Alcoholism	27 days

Information about the donor such as biological sex, age, cause of death (COD) and size, as well as any wounds or marks that were present on the remains were documented. The size of the donor was classified as small, medium, large or extra large, based the approximate clothing size (Australian) the donor would wear, since the weight of the donors was not available. In 2017, a data logger (HOBO, Onset, Ontario, Canada) was placed in the plot of Donor H1, which was in the vicinity of the plot of Donor H2. In 2018, the same logger was placed in the plot of Donor H4, near the plot of Donor H5. The logger was used to collect hourly recordings of ambient temperature and relative humidity in the area. Because of the limited number of data loggers, the hourly ambient temperature and relative humidity data for Donor H3 were collected from the weather station located at AFTER equipped with a HOBO U30 No Remote Communication data logger (OneTemp, Melbourne, Australia). ADD was calculated using average daily temperatures of 8-34°C, since Australian blow flies cannot complete their life cycle outside of this temperature range [25]. Note: Donor H3 was shared among researchers for differing studies. As a result, alterations were made to the donor with the insertion of four temperature probes into the torso of the donor. Two probes were inserted into the upper left and right sides of the chest and the other two probes were inserted into the lower left and right sides

of the abdomen. These were inserted approximately 10 cm deep.

Each donor was visited once daily for the first week of decomposition. Visits were reduced to every second day for approximately 2 weeks thereafter and then further reduced to once per week until the end of the study. This frequency of visits was chosen so that more observations could be made during the early stages of decomposition when the rate of decomposition and dipteran attraction are generally high. As decomposition slowed, fewer visits were necessary to document changes in the bodies and insect activity. The time of day when visits occurred was between 10am-12pm with the exception of approximately 5 experimental days where the visit occurred in the early afternoon. During each visit, photographs were taken of the remains and observations were made on the stage of decomposition, and then entomological samples such as eggs, larvae and adult flies were collected. In between sample collection, anti-scavenging cages were placed over the remains to deter vertebrate scavenging (particularly avian) while still allowing invertebrate scavenging and exposure to natural environmental conditions.

Entomological Sample Collection and Rearing

During each visit, the donors were examined for new egg clutches and larval masses. Samples were collected

from any area with new eggs or young larvae (1st to 2nd instar). The instars of the larvae were estimated based on size, rather than number of spiracles, as there was no portable microscope on site. Specimens were collected using a small metal spoon or tweezers and placed in a cup with a small piece of moist paper towel until they could be transferred to the area designated for insect rearing. Care was taken not to collect too many samples so as not to interfere with the natural insect activity. All rearing was performed in a small cabin on land adjacent to the AFTER facility and as such the environmental conditions were similar to ambient conditions outdoors. All larval samples were reared to adulthood with kangaroo mince as a protein source. Rearing larvae were kept hydrated with sprays of water when needed and migrating larvae were given moist straw in which to pupate. Once emerged, all flies were identified using the LUCID Key to Australian Carrion Breeding Flies [60] and the LUCID Key to Australian *Sarcophaga* (sensu lato) (Diptera: Sarcophagidae) [61].

Statistical Analysis

Since ADD values are important to the determination of a TOC, a scatter plot of the Experimental Day (ED) and accompanying Accumulated Degree-Days (ADD) was generated to determine if there was a high correlation between ED and ADD. The scatter plot graph was created using Microsoft Excel 2019 including the trendline, equation of the line and R-Squared value functions.

Principal component analysis (PCA) was also employed to analyse and interpret the data. The prime purpose of PCA is to reduce the dimensionality of a multivariate data set and to clarify its interpretation by identifying a smaller number of variables to summarize the larger data set [62]. PCA reduces data by geometrically projecting them onto lower dimensions called principal components (PCs). The goal is to find the best summary of the data using the lowest number of PCs [63]. The principle component analysis (PCA) of the ADD values required to reach each stage of decomposition was performed using the PAST v4.02 statistical software available for free online [64].

RESULTS

Environmental Conditions

Since this current research is focusing on dipteran

colonization, the following temperature information is based on the first 3 weeks of decomposition where dipteran colonization occurred. The donors that were placed during the early autumn in 2017 experienced comparable average temperatures, ranging from 18-21°C; Donor H1 had an average of 21°C and Donor H2 averaged 18°C. Donor H4, placed in early autumn of 2018, averaged 23°C. The minimum temperatures, ranged from 10-12°C in 2017 where the minimum temperature for Donor H1 was 12°C and Donor H2 was 10°C. Donor H4 experienced a minimum temperature of 14°C. The maximum temperatures in the early autumn ranged from 37-47°C; where Donors H2 and H4 were exposed to a maximum of 37°C and Donor H1 to a maximum of 47°C. Two donors were placed in the late autumn. Donor H3 placed in 2017, experienced an average temperature of 14°C, with minimum and maximum temperatures of 6°C and 24°C, respectively. Donor H5 was placed in 2018, with an average temperature of 14°C, and minimum and maximum temperatures of 3°C and 28°C, respectively. The relative humidity (RH) averages were much higher in 2017, 81-91%, than the averages recorded in 2018, 65-66%.

Rates of Decomposition and Dipteran Activity

The rate of decomposition of the bodies varied and, importantly, no two bodies decomposed at the same observed rate (Figure 1). There were some aspects of the decomposition processes that were noticeably different between bodies. The rate of decomposition for the remains of Donor H1 progressed much faster through the early stages of decomposition than any of the other bodies, reaching active decay by ED 7 (182 ADD) (Figure 1). The higher temperatures and probable higher levels of solar radiation caused desiccation of the skin to occur and made the transition between the bloated stages and the active decay stages difficult to determine. Donor H1 began the bloated stage of decomposition quickly, ED 2 (66 ADD) and as the exposure to the environmental conditions continued, the skin of the torso became hard and leathery causing the bloated shape to be maintained. The active decay stage for Donor H1 occurred predominantly internally and was not as visually apparent since minimal tissue loss could be seen on the exposed surfaces. Shortly

after the onset of the active decay stage, Donor H1 showed desiccation on all exposed skin by ED 9 (229 ADD). Donor H1 reached the advanced decay stage on ED 26 (552 ADD) and, by the end of the observation period of 54 ED (1022 ADD), skeletonization had not occurred. Primary colonizers for Donor H1 included *Calliphora ochracea* (Schiner 1868), and *Chrysomya rufifacies* (Macquart, 1843) (Diptera: Calliphoridae)

who colonized in the fresh stage on ED 1 (42 ADD). Donor H1 was colonized by two more species in the bloated stage, *Chrysomya incisuralis* (Macquart, 1851) and *Calliphora augur* (Fabricus, 1775) (Diptera: Calliphoridae) (Table 2). Piophilidae (Insecta: Diptera) larvae were observed during the advanced decay stages of this donor.

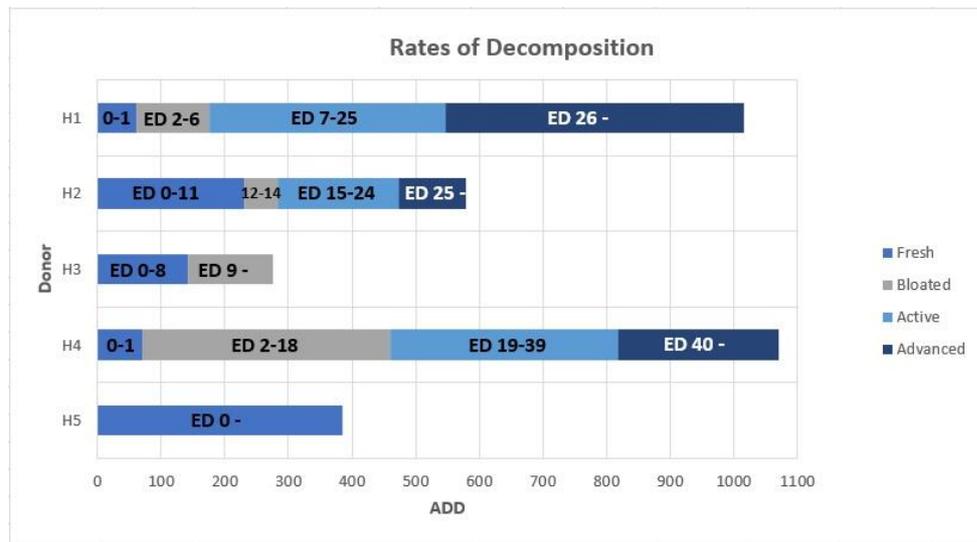


Figure 1 Rates of Decomposition. A bar graph of the rate of decomposition for each donor showing the ADD values on the x-axis and duration of each stage of decomposition in Experimental Days (black numbers found within each bar section)

Table 2 The colonizing fly species that were collected and reared to adulthood for each donor. Life cycle stages of collected specimens are listed beside the species name where (E) = Egg and (L) = Larva. A section of the column for Donor H5 is “Not Applicable” because the donor was not observed during this period

Stage	ED	H1 2017	H2 2017	H4 2018	H3 2017	H5 2018
Fresh	1	<i>C. ochracea</i> (E) <i>Ch. rufifacies</i> (E)			<i>C. hilli hilli</i> (L)	
	2	<i>C. ochracea</i> (L)				
	4		<i>C. augur</i> (L) <i>L. cuprina</i> (E) <i>C. stygia</i> (E)		<i>C. hilli hilli</i> (L)	
	8		<i>Ch. rufifacies</i> (L) <i>Ch. rufifacies</i> (L)			
	10		<i>Ch. incisuralis</i> (L) <i>C. ochracea</i> (L)			
	14					<i>S. impatiens</i> (L)
Bloated	3	<i>Ch. Incisuralis</i> (L) <i>Ch. rufifacies</i> (L)		<i>C. ochracea</i> (L)		N/A
	4	<i>Ch. rufifacies</i> (L)		<i>H. fergusonii</i> (L)		N/A

	5	<i>Chr. rufifacies</i> (L) <i>C. augur</i> (L)	<i>Ch. megacephala</i> (L) <i>Ch. rufifacies</i> (L)	N/A
	6		<i>C. stygia</i> (E) <i>C. ochracea</i> (E) <i>C. hilli hilli</i> (L) <i>Sarcophaga</i> sp.(L) *	N/A
	7		<i>L. cuprina</i> (L) <i>Ch. incisuralis</i> (L) <i>Ch. latifrons</i> (L)	N/A
	9		<i>Ch. megacephala</i> (L) <i>Ch. rufifacies</i> (L)	N/A
	12		<i>Ch. rufifacies</i> (L)	N/A
	16		<i>Ch. rufifacies</i> (E)	N/A
	19		<i>S. impatiens</i> (L)	N/A
Active Decay	21		<i>S. impatiens</i> (L)	N/A
	27		<i>S. impatiens</i> (L) <i>P. casei</i> (L)	N/A

* Sample was damaged and species identification was not possible through microscopic examination.

The early rate of decomposition for the remains of Donor H2 was slower than the majority of donors, with the fresh stage of decomposition lasting up to 12 ED (210 ADD) (Figure 1). In contrast, the bloated and active decay stages for Donor H2 were notably faster than Donor H1, even though Donor H2 was placed two weeks after Donor H1, lasting only 3 ED (54 ADD) and 10 ED (189 ADD), respectively. Donor H2 began to desiccate in the head and arms on ED 12 (230 ADD) and desiccation was complete by ED 21 (579 ADD). Donor H2 was colonized by 3 blow fly species, *C. augur*, *Lucilia cuprina* (Wiedemann, 1830), and *Calliphora stygia* (Fabricius, 1782) (Diptera: Calliphoridae), during the fresh stage but not until ED 4 (88 ADD). Colonization by three more species, *Ch. rufifacies*, *Ch. incisuralis*, and *C. ochracea*, occurred later in the fresh stage on ED 8 (160 ADD) (Table 2). Piophilidae larvae were observed during the advanced decay stages of this donor as well.

The fresh stage for the remains of Donor H3 lasted 9 ED (142 ADD). The bloated stage for Donor H3 was not completed during the observation period of 18 experimental days, likely due to the cooler temperatures in late autumn. The earliest colonizing species was *Calliphora hilli hilli* (Patton, 1925) (Diptera: Calliphoridae) which colonized on ED 1 (30 ADD). These were collected as 1st instar larvae,

indicating that oviposition occurred an undetermined number of hours prior to collection on ED 1 (30 ADD), since no development data is published for this species. The only other donor to be colonized as quickly was Donor H1, placed in the early autumn. Additional blow fly species, *C. stygia* and *C. ochracea*, colonized the remains of Donor H3 in the bloated stage on ED 6 (101 ADD). A *Sarcophaga* spp. (Diptera: Sarcophagidae) colonized Donor H3 on ED 6, but the sample was damaged and the species was not able to be determined.

The remains of Donor H4 experienced a short fresh stage, lasting 2 ED (71 ADD). When comparing the body size and temperature conditions, this is comparable to the rate of decomposition for donor H1 in the early stages of decomposition. The bloated stage for Donor H4, however, was longer, lasting 17 ED (389 ADD). This was considerably longer than the bloated stage for Donors H1 and H2 who were also studied during the early autumn months. Early signs of desiccation could be seen on the face, hands, and feet of the body by ED 7 (184 ADD) and by ED 16 (391 ADD) desiccation was more pronounced, also affecting the skin under the clothing. The remains of Donor H4 were first colonized in the bloated stage of decay on ED 3 (94 ADD) by *C. ochracea*, showing a delay in colonization. The colonization of this donor also differed in that three

of the colonizing species, *Hemipyrellia fergusonii* (Patton, 1925), *Chrysomya megacephala* (Fabricius, 1794), and *Chrysomya latifrons* (Malloch, 1927) (Diptera: Calliphoridae), were not recorded on any other donors (Table 2). Colonization of *Piophilidae*, also known as the cheesekipper, was documented on ED 27 (611 ADD) during the active decay stage.

The fresh stage for the remains of Donor H5 lasted a minimum of 27 ED (384 ADD). This was much longer than the 9 ED (142 ADD) seen for Donor H3, who also decomposed in the late autumn. Due to the slow decomposition of the remains of Donor H5 as well as the completion of the time allotted for the research, the bloated stage was not reached during the observation period. This was the longest fresh stage observed in this study. The colonization of Donor H5 was delayed with little to no fly activity present until ED 14 (219 ADD). The body showed minimal decomposition and was still considered to be in the fresh stage at this time. First instar larvae were collected from the mouth and were later identified as *Sarcophaga*

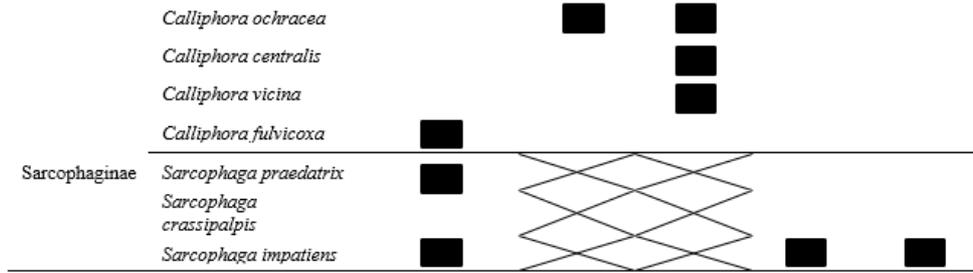
impatiens (Walker, 1849) (Diptera: Sarcophagidae) (Table 2).

Calliphora ochracea was one of the more prominent colonizers in this study and colonized four out of five bodies, during both the early and late autumn. Species that acted as both early and late primary, possibly secondary, colonizers were *Ch. rufifacies* and *S. impatiens* (Table 2). *Chrysomya incisuralis* and *Ch. rufifacies* were prominent colonizers in the early autumn while *C. hilli hilli* was only seen in the late autumn.

Many forensically relevant blow fly species were collected as adults during the early autumn, however, few of these forensically relevant species were collected in the late autumn (Table 3). Certain Diptera species were observed throughout the entire autumn season including *Ch. incisuralis*, *Ch. rufifacies*, *Chrysomya varipes* (Macquart, 1851), and *Chrysomya nigripes* (Aubertin, 1932) (Diptera: Calliphoridae). Adult species that were collected exclusively in the early autumn of this study included *Ch. megacephala*, *Ch. latifrons*, *H. fergusonii*, *C. stygia* and *C. augur* (Table 3).

Table 3 Adult Blow Fly Species Collected. Blow fly and other species that have been found to be forensically relevant are depicted as well as blow flies present in southeastern Australia during the summer and winter months. These results were taken from literature. Results of adult flies collected during this research are also shown (early and late autumn bush). A portion of the table is crossed out because Kavazos and Wallman did not discuss Sarcophagidae species in their 2012 publication. 1[25,55], 2[80], 3[78].

Sub-Family	Species	Forensically Relevant Species ^{1,2}	Summer Bush ³	Winter Bush ³	Early Autumn Bush	Late Autumn Bush
Chrysomyinae	<i>Chrysomya megacephala</i>	■	■	■	■	■
	<i>Chrysomya rufifacies</i>		■	■	■	■
	<i>Chrysomya incisuralis</i>		■	■	■	
	<i>Chrysomya latifrons</i>	■	■	■	■	■
	<i>Chrysomya varipes</i>	■	■		■	■
	<i>Chrysomya nigripes</i>	■				
	<i>Chrysomya saffrana</i>	■	■	■		
Luciliinae	<i>Lucilia cuprina</i>	■				
	<i>Lucilia sericata</i>	■				
	<i>Hemipyrellia ligurriensis</i>				■	
	<i>Hemipyrellia fergusonii</i>	■	■	■	■	
Calliphorinae	<i>Calliphora stygia</i>	■	■	■	■	
	<i>Calliphora augur</i>	■	■	■		
	<i>Calliphora hilli hilli</i>		■	■		



The most abundant adult dipteran species collected in the early autumn were *Ch. rufifacies* and *Chrysomya varipes*, the former was seen as a colonizer in this study while the latter visited the bodies but no immature stages were recovered (Table 2, Table 3). Fewer dipteran specimens were available for collection in the late autumn due to a lower abundance of flies and as such no specific species was observed to be more abundant over the other.

Experimental Day vs. ADD

Based on the scatter plot of Experimental Day vs. ADD values, the results show that there is a high correlation between the ED and ADD values. The R² value of the trendline for the data was 0.9744 and the equation for the line was $y=19.249x+17.35$ (Figure 2).

PCA Analysis of Duration of Stages in ADD

PCA analysis of the ADD values calculated for all

donors to reach each stage of decomposition shows the rate of decomposition of Donors H3 and H5 to be similar which may be explained by their placement during the later autumn months. These two donors are found at the intersection of the two axes of the graph and form a group. Donors H1 and H2 are clustered in the lower and upper left quadrants, respectively. Although there is a large gap between these two donors on the Component 2 axis, they are found at similar locations on the Component 1 axis which accounts for 71% of the variance in the data. This means that there is less variation between Donors H1 and H2 and they were more similar to each other than to Donor H4 who was also placed at AFTER in early autumn. Donor H4 is located in the upper right quadrant and at the edge of the positive end of the Component 1 axis (Figure 3). Donor H4 shows the most variation in decomposition rate from the other donors.

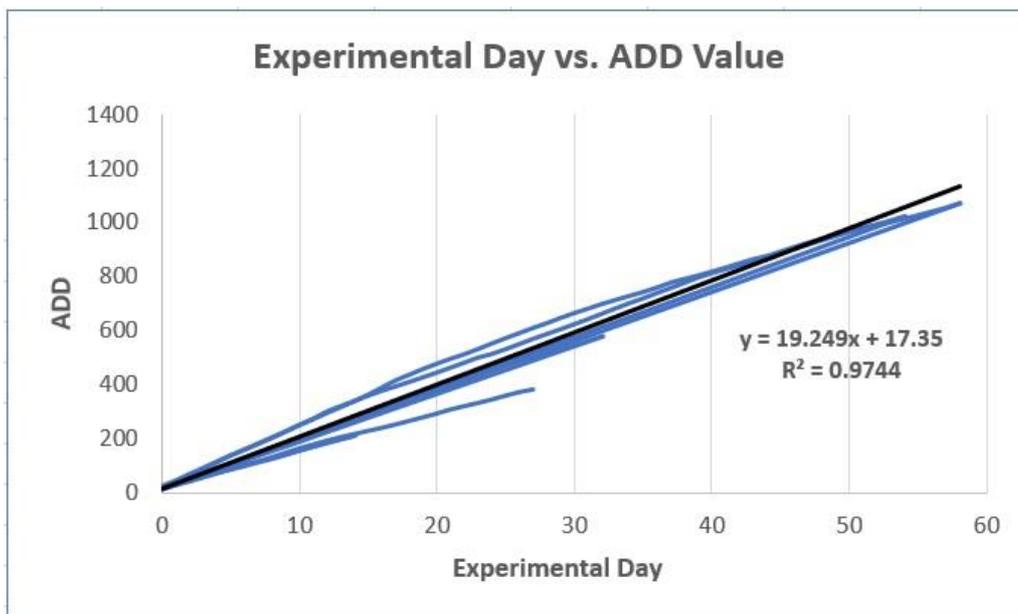


Figure 2 ED vs ADD. A scatter plot of the Experimental Day versus ADD values that includes data from all donors. This scatterplot includes the Equation of the Trendline and R² Value for the data

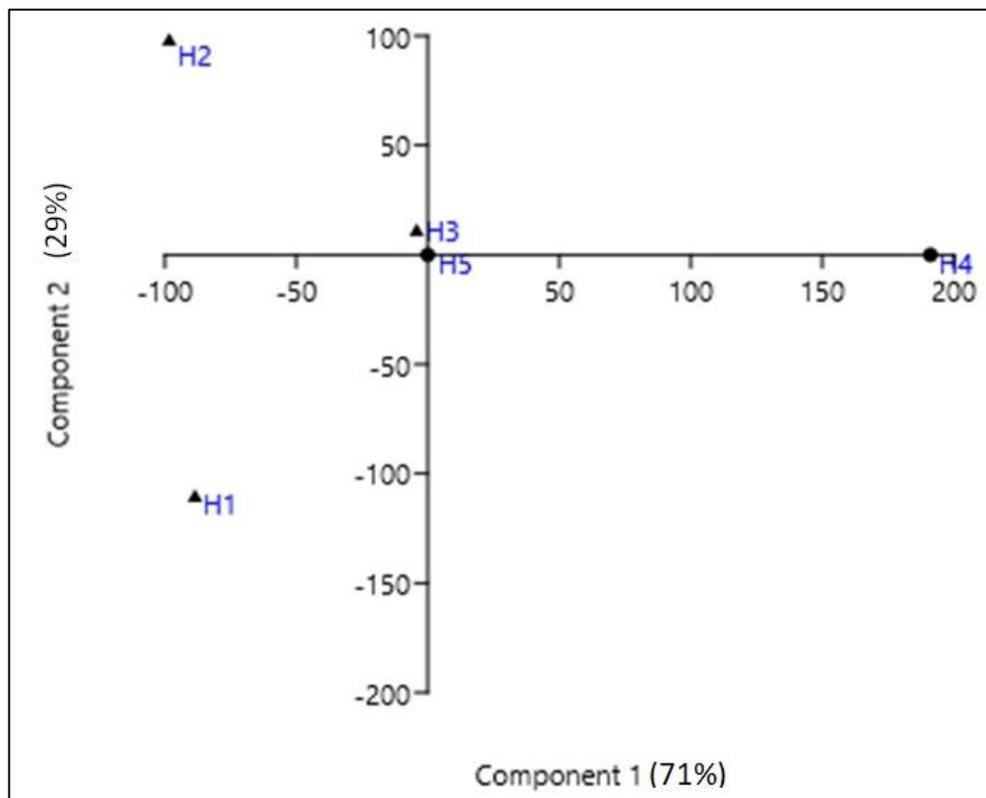


Figure 3 PCA of Rates of Decay. A principle component analysis of the ADD values required for each stage of decomposition to commence. The donors with black triangles are those that were unclothed and the donors with the black circles were clothed

DISCUSSION

While TOC provides the best estimate of time since death after pathological methods can no longer be applied, it does not take into consideration an extended Pre-CI. It is the Pre-CI that is largely responsible for the difference between the actual time of death and the TOC, making the determination of the Pre-CI incredibly important if any delays in colonization occurred [31,33]. Variations in decomposition rates and colonization have been reported based on variables such as clothing [7,53,65,66], carcass size [7,67,68] and perimortem antibiotic usage [69]. It is important to explore the impact of each of these variables to insect colonization and subsequent mPMI and TOC estimations.

Factors affecting Decomposition

As previously mentioned, the difference between the actual time of death and an estimated TOC is largely attributed to the Pre-CI [31]. There are many factors that influence the duration of Pre-CI, making this interval highly variable. In the current study, there were multiple instances where there was a prolonged period

before insect colonization, which occurred in both early and late autumn. A similar delay was reported by Dawson et al. [57] at the AFTER facility. The authors described delays in colonization of human remains placed both in summer and winter seasons, with more prominent delays recorded in the winter season [57]. This was also observed in the current study, where delays were seen regardless of date of donor placement, however, delays occurring during cooler temperatures were longer. As the length of the Pre-CI is crucial to determining an accurate PMI, this will be discussed first. Donor H1 and H3 each had a Pre-CI of 1 ED (30-42 ADD). In comparison, Donors H2, H4 and H5 all showed a prolonged Pre-CI of 4 ED (74-117 ADD) or later. Since colonization can occur minutes after death [24,70], these are considered important delays. Two of these three donors also showed a slowed decomposition rate in the early stages of decomposition. Relevant literature reports that a delay in primary colonization of remains does have an effect on the rate of decomposition, slowing the rate considerably [21,71-73]. The variation seen between the donors can be explained by various factors, both biotic and abiotic, that could have affected the Pre-CI and/or microbial

activity of the remains. The main abiotic factor is temperature while the biotic factors to be discussed include donor size, clothing and peri-mortem medication. Variations between donors could be attributed to more than one factor, offering competing hypotheses on why certain aspects of the decomposition process may or may not have occurred.

The remains of Donors H1 and H4 were placed earliest in the autumn seasons, in their respective years, when temperatures were highest. As a result, both of these bodies had the shortest fresh stages as well as earliest times of colonization. Alternatively, the remains of Donors H3 and H5, who decomposed in cooler conditions, had long fresh stages and reduced insect activity. This supports the findings by George, Archer and Toop [36] who state that temperature is a positive predictor variable for blow fly colonization, meaning that higher temperatures yield a higher probability that calliphorids will colonize a carcass. It should be noted, however, that the decomposition of Donor H2, exposed in the early autumn, did not follow this pattern. Donor H2 was not colonized until ED 4 (88 ADD). The average temperature for Donor H2 was only a few degrees lower than those of Donors H1 and H4, which may have accounted for the difference in colonization time. However, further research is required before coming to any conclusion.

Studies indicate that, generally, larger masses of carrion decompose slower than smaller masses [67,68]. Matuszewski et al. [7] reported that larger carcasses tend to take longer to decompose due to the large amount of organic material that needs to be anaerobically broken down and consumed by microbiological and entomological processes. Despite this, a larger mass will go through autolysis and begin putrefaction more efficiently, presenting as a quick onset of the bloated stage followed by a prolonged bloated stage [7]. According to Matuszewski et al. [7], larger carcasses were seen to have a less efficient active decay stages where tissue loss and the onset of advanced decay were delayed. Similar observations were reported in the current study when comparing donors who decomposed in both early and late autumn. Beginning with the early autumn, the fresh stage of decomposition was shortest for Donors H1 and H4, the two largest donors in the study. As seen in Matuszewski

et al. [7], both Donor H1 and H4 of this study also had prolonged bloated and active decay stages. The remains of Donor H2, one of the smaller donors, had a fresh stage that lasted much longer than the larger donors. Additionally, the body of Donor H2 showed the fastest overall decomposition rate, indicating that the observations seen in Matuszewski et al. [7] were also witnessed in this study. Comparing donors who decomposed in the late autumn, Donor H3 was a medium-sized donor while Donor H5 was very thin, almost emaciated. Donor H3 reached the bloated stage by ED 9 while Donor H5 had not reached the bloated stage during the observation period of 27 ED. It should be noted, however, that emaciated individuals often transition from fresh stage directly to desiccation without passing through the more traditional stages of decomposition such as bloated (S. L. Forbes, pers. comm). This phenomenon was also reported by Knobel et al. [4] at the AFTER facility and involved the observation of two human donors in the summer and two in the winter. Observations included the absence of characteristic bloating features in one of the winter donors, and active decay stages were noted to have occurred internally and were only apparent in the head and neck of all four donors. While Knobel et al. [4] may show there is a possibility that Donor H5 entered into the active decay stage without any signs of bloat, the near lack of larval activity as a whole with this donor would make this difficult to determine as larval activity is one of the main aspects of the active decay stage.

Although two of the donors in this study were clothed (H4 and H5), Donor H4 demonstrated a comparable rate of decomposition to the unclothed donors (H1-H3). Donor H4 experienced the longest bloated stage but then progressed through active and advanced decay similar to Donor H1, who was unclothed. While published research performed in western Australia shows that the active decay stage of decomposition can be extended with clothed remains [53], the length of the bloated stage was not discussed in the referenced study. Other publications have documented that clothing causes an extension in moist decomposition and insect activity [53,65]. Based on this, clothing could be responsible for the prolonged insect activity as well as the length of the bloated stage in Donor H4. It is possible that clothing is the reason

for the high variation seen between Donor H4 and other donors in the PCA analysis. While a lengthy fresh stage was recorded with Donor H5, the other clothed donor, there was almost no insect activity reported, which does not corroborate the prolonged insect activity seen with clothed individuals in the literature. The remains of Donor H4 also showed the highest variety of colonizing dipteran species. Research investigating the colonization of clothed remains shows that clothing allows for more areas for female blow flies to oviposit [66]. It is probable that the clothing on Donor H4 provided the opportunity for the colonizing community to become more diverse with less competition for resources.

The final biotic factor to be considered is peri-mortem medications. Peri-mortem medications is an example of a factor that can make the decomposition of humans unpredictable, since they vary ante-mortem and are often unknown. In certain cases in this study, the possible peri-mortem use of antibiotics was suspected to have influenced the rate of decomposition and possibly the Pre-CI. Donor H4 died of bacterial meningitis. Treatment of bacterial meningitis includes strong antibiotics [74,75] because the bacteria that cause meningitis can be antibiotic resistant [75]. Similarly, Donor H5 suffered from alcoholism and died due to liver failure. Complications can arise in individuals that have cirrhosis of the liver such as pneumonia, which was also an aspect of this donor's death. If his pneumonia was bacterial, it would have been treated with antibiotic drugs [76]. The result of antibiotic use is that the beneficial bacteria in the body is killed along with the pathogenic bacteria [77]. A publication by Hayman and Oxenham [69] described the decomposition process for an individual who was treated with cytotoxic drugs as well as 5 antibiotics prior to death. The authors outlined a slowed decomposition process, the absence of bloated stage features like marbling and an extended abdomen and the absence of insect activity [69]. One possible explanation for these results would be a reduced load of microbial communities caused by antibiotic medications [69]. Since both Donor H4 and H5 were likely treated with antibiotics, a change in the microbial flora could have impacted the onset of the bloated stage of decomposition [69]. Donor H4 had a prolonged bloated

stage whereas Donor H5 did not show marbling or reach the bloated stage at all in 27 ED (384 ADD). Less beneficial bacteria in the system would theoretically delay or slow the bloat process as well as the marbling effect. The detection of volatiles by blow flies could have been delayed due to low concentrations caused by diminished bacterial communities. This, in turn, could have affected the Pre-CI and thus the TOC. While it is not suggested that antibiotic treatment alone is responsible for these results [69], it is suspected to be an influencing factor.

PCA Analysis

While this PCA analysis shows the decomposition rate of H3 and H5 to be similar, the rate of decay shown in Figure 1 suggests otherwise. It is suspected that these Donors were both found in the center of the plot, at the junction between the two axes, because of the lack of data beyond the fresh and bloated stages, showing a similarity that is not reflected in other results.

Experimental Day vs. Accumulated Degree-Days

Evaluating the rate of decomposition based on changes in stage of decomposition in relation to Experimental Day is comparable to evaluating based on ADD value, since a scatter plot shows there is a high correlation between ED and ADD values in this study. The equation of the trendline for this data could be useful in estimating the rate of decay for humans in similar environmental conditions by calculating the expected ADD values for specific experimental days. Attempting to predict the rate of decay of donors using this data would aid in the understanding of the relationship between time and temperature relating to decomposition. These results, however, are specific to the Sydney, Australia climate and would not be applicable elsewhere. Conducting further human research at the AFTER facility and adding further ED and ADD values to the dataset would serve to strengthen the correlation between ED and ADD and improve the accuracy of ADD estimations.

Dipteran Community

Few publications exist which discuss primary colonizers in Sydney, Australia [78]. During this study,

primary colonizing species in early autumn include *C. hilli hilli*, *C. ochracea*, and *Ch. rufifacies*. These are expected colonizing species in southeastern Australia [78]. Kavazos et al. [78] reported *Ch. rufifacies* to be one of the most abundant species in the summer while *C. hilli hilli* and *C. ochracea* were both most abundant in the winter months. *Calliphora augur*, *L. cuprina*, and *C. stygia* were recorded as delayed colonizers in the early autumn. These three species are considered important colonizers [25,55] and are a mix of species expected during the summer and winter months [78], which is unsurprising as these donors were placed during April-May and observed for the entire autumn season.

As for colonization during the late autumn, *C. ochracea*, *Ch. megacephala*, *Ch. rufifacies*, *H. fergusonii*, *L. cuprina*, *Ch. incisuralis*, and *Ch. latifrons* were all documented as delayed primary colonizers on Donor H4. These are a mix of species common in the summer and winter months [78]. A previous publication on blow fly community composition in Sydney, Australia found that *H. fergusonii* was present in high abundances but were only observed in urban and suburban areas [78]. In contrast, this study identified *H. fergusonii* as a colonizer in a Sydney bush environment. This may be the first record of this species in such an environment. *Chrysomya megacephala* and *Ch. rufifacies* acting as colonizers is expected as they are some of the most abundant species to be observed in Sydney, Australia and are both considered forensically relevant [25,55,78]. *Chrysomya incisuralis* and *Ch. latifrons* were not among the list of expected species to colonize remains and, in fact, *Ch. latifrons* is reported to be an uncommon species as it constitutes less than 1% of the total blow flies caught in the Sydney community study [78]. *Chrysomya incisuralis* and *Ch. latifrons*, however, have been reported in the Sydney bush areas in summer and winter months [78].

Species *S. impatiens*, was documented as both a primary and secondary colonizer in this study. This species is larviparous [79], which explains why they were not detected in egg form. *Sarcophaga impatiens* was observed as a secondary colonizer on Donor H4, which agrees with published literature indicating Sarcophagidae are generally recorded as secondary colonizers in Australia [53,54]. In the case of Donor

H5, however, *S. impatiens* acted as a delayed primary colonizer. Only one publication supports this finding; in one mortuary case in Queensland, Australia, *S. impatiens* acted as a primary colonizer whereas the same species was seen as a secondary colonizer in three other mortuary cases [55]. Very little is known about the development of *S. impatiens*, even though the species has been shown to be forensically relevant [80]. More recent studies have begun exploring the thermobiology of *S. impatiens* to aid in the estimation of the PMI [55].

CONCLUSION

Decomposition research involving human remains occurs less frequently than animal decomposition studies due to the limited human decomposition facilities available. The locations of human decomposition research facilities do not cover the many different environmental climates of the world and as such cannot contribute to all death investigations. Previous PMI estimation models have relied on data collected from research performed on human proxies, such as the domestic pig, however recent research shows that the rates of decay between humans and pigs differ [6,81,82]. This study has shown that human decomposition and initial colonization was highly variable among the 5 human donors observed. Our findings suggest that it is possible for extrinsic factors such as temperature and intrinsic factors such as body size, clothing, and peri-mortem medications, specifically antibiotics, to influence the processes of colonization and decomposition. Therefore, it is extremely important to continue research using human donors to understand how variation in human physiology and ambient environment can affect decomposition and insect colonization. It is important that we explore the various factors that may delay colonization, thereby affecting minimum time since death estimations.

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Conflict of Interest

Authors declare none.

Author's Contribution

All authors contributed to the results analysis for this publication. Angela D. Skopyk acted as researcher and author. Shari L. Forbes and Hélène N. LeBlanc both acted as supervisors and editors in addition to providing funding for the research.

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