ESTIMATION OF POSTMORTEM INTERVAL IN HUMAN CADAVERS USING TWO DIFFERENT QUANTITATIVE METHODOLOGIES

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DEDICATION

I would like to dedicate this thesis to my mother and father who have been instrumental in my success throughout this program. Without the support of my friends and family, I would not be where I am today.

ABSTRACT

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Postmortem interval (PMI) is defined as the time between death and discovery of the deceased. It is important in criminal investigations because it allows investigators to draw conclusions about the circumstances surrounding the death of a person. The postmortem interval is estimated using many different methods. Currently, the standard of the field is the use of insect succession or physiological age of insect larvae to determine PMI; however, quantitative scoring methodologies have become more common as they aim to allow non-professionals to efficiently estimate PMI in the field. Two quantitative methods of estimating PMI are Vass' universal PMI formula and Megyesi's total body scoring (TBS) system. However, the validity of these methods across different regions is unknown. We found that actual PMI and PMI estimated using Vass' universal formula were statistically different from one another ($p < 0.001$). There was not enough evidence to show that actual PMI and the TBS calculated PMI were statistically different from one another ($p = 0.208$). We know that decomposition is highly variable and dependent upon region/climate. From this, we can conclude that Vass' 'universal' PMI formula is not a reliable method of estimating PMI in Southeast Texas.

KEY WORDS: Postmortem interval, Total body score, Universal PMI formula, Forensic science, Forensic anthropology, Southeast Texas.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Introduction

Introduction to Forensic Taphonomy

The field of forensic taphonomy is defined as study of the events between death and discovery of an individual (Haglund & Sorg, 1997). Death is a process characterized by the cessation of all metabolic processes and cellular death. Immediately after death, the body will begin to undergo several physiological changes. The most notable taphonomic changes being pallor, algor, livor, and rigor mortis. Pallor mortis is widely described as being the first “stage” of death and refers to postmortem paleness (Schafer, 2000). Paleness of the body after death can be attributed to the lack of blood circulating through the capillaries (Schafer, 2000). Algor mortis is the cooling of the body postmortem (Eden & Thomas 2022). Algor mortis has been used as an early indicator of death for some time now but has since been renounced as an accurate estimation of post mortem interval, or time between death and discovery (Wardak & Cina, 2011) (Gelderman et al., 2017). Livor mortis occurs shortly after death, with the onset usually visible at around 2 hours postmortem (Clark et al., 1997). Livor mortis, also referred to as *lividity*, is commonly defined as the pooling of blood on the lower surface due to lack of circulation (Clark et al., 1997). In many cases, lividity becomes fixed between 4-6 hours after death, meaning that the redness on the lower surface of the body no longer vanishes upon pressure (Clark et al., 1997). The last of the major taphonomic changes to occur postmortem is rigor mortis. Rigor mortis is defined as the postmortem stiffening of muscles (Gill-King, 1997). The onset of rigor mortis usually occurs between 2-6 hours postmortem and can last for up to 84 hours (Gill-King, 1997). Contraction of the muscles is caused by the sliding of thick and thin filaments within the muscles, effectively shortening the sarcomeres (Sweeney & Hammers, 2018). This is an active process requiring ATP (Bate-Smith & Bendall, 1947). The lack of ATP contributes directly to the onset of rigor mortis (Erdös, 1943). At some point between 24-84 hours, the myosin cross-bridges break down eventually relaxing the muscles (Gill-King, 1997). The reactions above have been widely used in the past to estimate the time since death; however, they are highly variable, and the effects are fleeting. This has led investigators to develop other means of determining how long a person has been dead.

Stages of Decomposition

Human decomposition is generally characterized by four stages: fresh, early decomposition, advanced decomposition, and skeletonization (Megyesi et al., 2005). Each stage of decomposition has specific, defining elements that distinguish the stages. Despite this, different authors have different definitions for the stages of decomposition. Since variation exists within the definitions, the terms and vernacular following have been adapted from Megyesi's system (Megyesi et al., 2005). The first stage of decomposition happens directly after death has occurred and is characterized by a lack of discoloration of the skin (Megyesi et al., 2005). Early decomposition begins with significant discoloration of the skin in association with bloat and purge (Megyesi et al., 2005). Once bloating and purging of decomposition fluids have occurred, the remaining tissue and flesh will begin to cave in characterizing advanced decomposition (Megyesi et al., 2005). During this stage, moist tissues will begin to mummify and bone exposure may be seen (Megyesi et al., 2005). The last stage of human decomposition, skeletonization, describes bone exposure of more than half of the remaining cadaver. During skeletonization, one will usually see a progression of greasy, exposed bone of approximately 50% to mummified tissue and/or dry bone (Megyesi et al., 2005). Decomposition is a non-linear process that takes into account many unforeseen variables, so while each stage of decomposition can be generalized as mentioned above, it is important to acknowledge that some cadavers will spend more time in one stage than another or skip a stage altogether (Hyde et al., 2013; Vass, 2001).

The Postmortem Interval

In the event of an unattended death, it is crucial for investigators to determine the manner of death and the postmortem interval (PMI). PMI can be defined as the time between death of a person and discovery (Gelderman et al., 2017). PMI is a vital tool in legal investigations as it can aid in determining time of death of the decedent. An accurate estimate of PMI can lead to knowledge of circumstances surrounding death, which is useful in determining if an arrest needs to be made in the instances of violent crimes. PMI can be estimated using a number of different factors, such as insect activity, temperature/climate, and microbial succession. In order to determine an accurate and precise PMI, it is essential to use biological factors to holistically observe the process of decomposition.

Estimation of the Postmortem Interval

Estimation of PMI has been highly studied in the field of forensic science and new methodologies are developed constantly in an attempt to estimate this elusive interval more accurately. Currently, the standard of the field is to use qualitative taphonomic observations as well as accumulated degree days (ADD) to determine PMI. However, if entomologists are present, they will use insect succession and larval fly activity to determine the time since death. There are, however, limitations to using insects to gauge PMI as they age according to physiological time rather than chronological time, making them heavily reliant on temperature, climate, and other environmental factors. This means that fly larvae will take significantly different time to age depending on the region. There are also situations in which insects are absent from a corpse all together. Corpses that are discovered in air-tight or sealed environments such as the sealed trunk of a car or buried underground tend to lack significant insect activity (Gunn, 2006). Situations such as these result in physical barriers that do not allow insects to successfully colonize a body. In instances of burial, there is generally a decrease in insect colonization due to the lack of oxygen/nutrients in the soil, and the difficulty of access, although certain exceptions exist (Gunn, 2006). Limitations also exist for qualitative observations to estimate PMI. As mentioned previously, decomposition is highly variable and is dependent upon region, insect activity, and ambient temperature. These factors make relying upon qualitative observations such as stage of decomposition or rigor/algor mortis difficult in terms of PMI. Limitations like these have forensic scientists looking elsewhere to create a new standard methodology for determining PMI that doesn't rely on insect activity and is non-region specific. In the past years, several new techniques for estimating PMI have surfaced that allow for a quantitative approach to human decomposition.

Total Body Scoring System to Estimate PMI

One of the main approaches to predict PMI is to use ADD and a total body scoring system (TBS) to determine the correlation between the two and PMI (Megyesi et al., 2005). ADD is the sum total of average temperatures on the days since death in which the daily temperature exceeded the basal thermal temperature (Megyesi et al., 2005). This system relies mainly on accumulated degree days as a factor to standardize decomposition between regions with different temperatures. Since ADD represents the accumulation of thermal heat units, it allows for researchers to view decomposition as a temperature-dependent process as opposed to time-dependent (Hyde et al., 2013). The study which brought about this quantitative approach used a temperature of 0°C because temperatures below this will inhibit certain biological processes (Megyesi et al., 2005). So, to calculate ADD would be the sum of all average temperatures for the days that were above 0°C. This system enables the comparison of multiple cadavers placed in a different temperature setting. In addition to ADD, TBS is used to assign a numerical value to a specific substage of decomposition. Megyesi divides each stage of decomposition into multiple categories and provides a number point system to each category (Table 1-3). The body is also divided into 3 subsections: head and neck, trunk, and limbs. Since there are processes that only occur in specific regions of the body, subsections are necessary to optimize precision and accuracy (Megyesi et al., 2005). While the TBS system is a novel approach to categorizing decomposition, there are many limitations to this otherwise linear methodology. As mentioned above, decomposition is a process with many factors that can cause significant discourse in estimating PMI. This approach does not account for discoloration of skin of different races of people, nor can it account for individual variability in cadavers. The combination of TBS and ADD in estimating PMI do however provide for a more accurate PMI prediction than in previous studies. Validity studies have shown that there are significant differences in actual ADD and calculated ADD from the TBS equation despite the supposed standardization of region temperatures

(Wescott et al., 2018). The validity of this methodology demonstrates the need for new methodologies on PMI prediction.

Base Temperature and Accumulated Degree Days

Basal temperature, defined as the thermal minimum below which development cannot occur, is vital in calculating degree days (DD) and ADD (citation). Base temperature is an important variable is calculating ADH or ADD, which is often used to determine the PMI. This variable is species-specific, meaning each species of insect/microbe/plant may have different temperature thresholds for which they are able to function (Gennard, 2012). When determining a base temperature to use for estimation of PMI, it is important to determine species of indicator as well as life history. Selecting an inappropriate base temperature can result in inaccurate ADD and thus inaccurate PMI (Oliveira-Costa & Mello-Patiu, 2004). This portion of the study aimed to determine how the use of different base temperatures alters the calculation of ADD. Based on previous literature and definitions of ADD, I expected lower base temperatures to result in an increase in ADD over time.

'Universal' Formula for Estimating PMI Above-Ground

Another approach to quantifying the stages of decomposition is with the 'Universal' PMI formula (UF PMI) (Vass, 2011). This unique approach takes the form of an equation used to estimate the PMI in an aerobic (not buried) environment. The formula accounts for moisture, temperature, humidity, ADD, and the state of decomposition on a scale of 1 to 100 (Vass, 2011). To calculate the supposed PMI, examiners should calculate/determine all of the variables and simply input them into the equation. The output value will be PMI calculated in days. This specific system does not account for insect activity or scavenging and, in fact, is not applicable in cases where extreme scavenging has occurred (Vass, 2011). Both of these factors play an important role in the decomposition of human remains and the exclusion of either factor could significantly affect PMI estimation efficiency. The validity of this methodology has been examined at research facilities across North America and Australia, but no validation studies have been conducted in Southeast Texas (Cockle & Bell, 2015).

Comparative studies are a necessary part of decomposition research because decomposition of any cadaver is heavily dependent upon temperature and climate. These factors control many aspects of decomposition such as bacterial growth, scavenging, and insect succession/activity. There is a need for more accurate methods to estimate the postmortem interval of a human cadaver, but the difficulty lies in creating a simple model system that is able to characterize such complex and variable processes.

Research Hypotheses

H_0: Total body score postmortem interval will not differ from the actual postmortem interval.

H_A: Total body score postmortem interval will be significantly different from the actual postmortem interval.

H_0: Universal formula postmortem interval will not differ from the actual postmortem interval.

H_A: Universal formula postmortem interval will be significantly different from the actual postmortem interval

H_{A1}: Universal formula postmortem interval will overestimate the actual postmortem interval.

H_0: Total body score postmortem interval will not differ from the universal formula postmortem interval.

H_A: Total body score postmortem interval will be significantly different from the universal formula postmortem interval.

CHAPTER II

Methods

Data Collection

This study used photographs depicting human cadavers at various stages of decomposition. All photographs were taken as part of the ongoing research of the Bucheli/Lynne Lab. A total of 14 human remains were placed at the Southeast Texas Applied Forensic Science Facility (STAFS) and allowed to decompose under variable conditions. The cadavers were placed asynchronously over the course of 3 years at different timepoints (Table 1). Due to some cadavers missing photographs for certain timepoints, a total of 11 cadavers were used in statistical comparisons. All human subjects were donated to the STAFS facility and kept at cool or freezing temperatures until their placement day to slow decomposition before it can be observed. We considered placement day as the date of death for this study. On placement day, each cadaver was placed on the ground in a supine position completely unclothed. In an attempt to make sure the environment was as similar to outdoor crime scene environments, the cadavers were left uncaged, which allowed for scavenging. Most of the cadavers were placed asynchronously over the course of 3 years. Photographs of the cadavers were taken once per day for 30 days.

Historical Weather Data

For this study, historical weather data was collected from Weather Underground. The readings from each day were all taken from the Huntsville Municipal Station (KUTS). Minimum temperature (˚F), maximum temperature (˚F), precipitation (inches), humidity (percentage), pressure, and wind speed were all put into an Excel sheet for review. All temperatures were converted to Celsius, and averages were calculated for each day.

Table 1

List of Cadaver IDs and Dates of Placement

Cadaver ID	Date of Placement	T_0	T_{31}
2013-009	2013 FEB	2/26/2013	3/28/2013
2013-011	2013 FEB	2/26/2013	3/28/2013
2013-028	2013 NOV	11/8/2013	12/8/2013
2013-042	2013 NOV	11/8/2013	12/8/2013
2014-004	2013 APR	4/22/2014	5/22/2014
2014-028	2013 APR	4/22/2014	5/22/2015
2014-052	2014 JUL	7/23/2014	8/22/2014
2014-053	2014 JUL	7/23/2014	8/22/2014
2014-072	2014 NOV	11/11/2014	12/11/2014
2014-076	2014 NOV	11/11/2014	12/11/2014
2015-021*	2015 JUL	7/22/2015	8/21/2015
2015-062*	2015 JUL	7/22/2015	8/21/2015
2015-095	2015 NOV	11/10/2015	12/10/2015
2015-104*	2015 NOV	11/10/2015	12/10/2015

Note. List of all cadavers in the study including month/year of placement. T_0 refers to timepoint 0, or day of placement. T_{31} refers to timepoint 30, which is the last day photographs were taken. *Cadavers excluded from analyses due to lack of photographs.

Accumulated Degree Days

A basal temperature of 0℃ was used because freezing temperatures are known to severely inhibit key decomposition processes such as bacterial growth and insect activity (Megyesi et al., 2005). ADD for each cadaver was calculated 5 separate times using 4 different base temperatures: 0˚C, 4 ˚C, 6 ˚C, and 10 ˚C. These base temperatures were derived from the literature (Hyde et al., 2013; Megyesi et al., 2005). ADD can be calculated using a simple formula, where DD represents degree days:

$$ADD = DDx + DDx + 1 + DDx + 2 \ldots$$ (Hyde et al., 2013)

Estimating PMI using Total Body Score

The first part of the experiment consisted of quantitatively scoring decomposition using the Total Body Scoring system (TBS) and calculating accumulated degree days (ADD) (Megyesi et al., 2005). For this portion of the experiments, all cadavers were scored at each time point based on the photographs taken.

According to the Megyesi *et al.* total body scoring system, each stage of decomposition is given a numerical score, relative to each category of decomposition, to indicate the observed level of decomposition (Tables 2-4). The head and neck, trunk, and limbs are all scored separately; the summation of these independent scores make up the TBS. The head and neck are scored 1-13, the trunk is scored 1-12, and the limbs receive a score between 1-10 (Table 2-4) (Megyesi et al., 2005). Each category is broken up into smaller subcategories to indicate the actual stage of decomposition: fresh, early decomposition, advanced decomposition, and skeletonization. For example, head and neck appearing fresh with no discoloration would receive 1 point, indicating fresh/little decomposition, whilst a cadaveric head with the appearance of "dry bone" with little to no skin present would receive 13 points, indicating skeletonization (Table 2) (Megyesi et al., 2005). The cadavers used in this study were scored once per day for 30 days to accurately account for rapid changes in decomposition during early decomposition. In an attempt to eliminate bias, each cadaver was scored with little knowledge of the TBS for the day prior. Once a total body score is calculated for each day, the TBS was put into equation (2) for an ADD output.

$$\text{ADD} = 10^{0.002 * \text{TBS} * \text{TBS} + 1.81} \pm 388.16 \qquad \text{(Megyesi et al., 2005)}$$

Equation 2 was adapted from Megyesi's TBS regression model and has a standard error of 388.16 ADDs. The output of this equation results in ADD, which then was divided by 24. In order to assess the comparability and accuracy of this method, the output needs to be divided by 24 to get ADD back to calendar days. The UF PMI and actual PMI units are both already in calendar days.

Table 2

Megyesi Head and Neck

Category	**Description**
A. Fresh	
(1pt)	1. Fresh, no discoloration
B. Early Decomposition	
(2pts)	1. Pink white appearance, skin slippage, hair loss
(3pts)	2. Gray to green, some flesh relatively fresh
(4pts)	3. Discoloration/brownish shades at edges/extremities
(5pts)	4. Purging out of eyes, ears, nose, mouth, some bloating
(6pts)	5. Brown to black discoloration
C. Adv. Decomposition	
(7pts)	1. Caving in of eyes and throat
(8pts)	2. Moist, bone exposure less than 50%
(9pts)	3. Mummification, bone exposure less than 50%
D. Skeletonization	
(10pts)	1. More than 50% bone exposure with grease
(11pts)	2.Mummification, more than 50% bone exposure
(12pts)	3.Bones largely dry, retaining some grease
(13pts)	4. Dry bone

Note. Table represents points associated with decomposition of the head and neck. The letter headings represent the four stages of decomposition while the numerical subheadings refer to the events that may occur during specific stage. Adapted from "Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains," by M. Megyesi, S. Nawrocki, and N. Haskell, 2005, *Journal of forensic sciences*, 50(3), 618-626, http://dx.doi.org/10.1520/JFS2004017, Copyright 2005 by ASTM International.

Table 3

Megyesi Trunk

<u>**Category**</u>	<u>**Description**</u>
A. Fresh	
(1pt)	1. Fresh, no discoloration
B. Early Decomposition	
(2pts)	1. Pink white appearance, skin slippage, marbling
(3pts)	2. Gray to green, some flesh relatively fresh
(4pts)	3. Bloating with green discoloration and purging.
(5pts)	Postbloating, discoloration from green to black
C. Adv. Decomposition	
(6pts)	1. Sagging of flesh/caving in of abdominal cavity
(7pts)	2. Moist, bone exposure less than 50%
(8pts)	3. Mummification, bone exposure less than 50%
D. Skeletonization	
(9pts)	1. Bones with decomposed tissue, body fluids and grease remain
(10pts)	2. Bones with desiccated or mummified tissue covering less than half area
(11pts)	3. Bones largely dry, retaining some grease
(12pts)	4. Dry bone

Note. Table represents points associated with decomposition of the trunk. The letter headings represent the four stages of decomposition while the numerical subheadings refer to the events that may occur during specific stage. Adapted from "Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains," by M. Megyesi, S. Nawrocki, and N. Haskell, 2005, *Journal of forensic sciences*, 50(3), 618-626, http://dx.doi.org/10.1520/JFS2004017, Copyright 2005 by ASTM International.

Table 4

Megyesi Limbs

Category	Description
A. Fresh	
(1pt)	1. Fresh, no discoloration
B. Early Decomposition	
(2pts)	1. Pink-white appearance, skin slippage on hands/feet
(3pts)	2. Gray to green, some flesh relatively fresh
(4pts)	3. Discoloration/brownish shades at edges/extremities
(5pts)	4. Brown to black discoloration, leathery
C. Adv. Decomposition	
(6pts)	1. Moist, bone exposure less than 50%
(7pts)	2. Mummification, bone exposure less than 50%
D. Skeletonization	
(8pts)	1. Bone exposure 50% or greater, some tissue and fluids remain
(9pts)	2. Bones largely dry, retaining some grease
(10pts)	3. Dry bone

Note. Table represents points associated with decomposition of the limbs. The letter headings represent the four stages of decomposition while the numerical subheadings refer to the events that may occur during specific stage. Adapted from "Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains," by M. Megyesi, S. Nawrocki, and N. Haskell, 2005, *Journal of forensic sciences*, 50(3), 618-626, http://dx.doi.org/10.1520/JFS2004017, Copyright 2005 by ASTM International.

Universal PMI formula

For the second portion of the experiments, the PMI for each cadaver was calculated using Vass' UF PMI formula. Formula I estimates PMI above ground by accounting for temperature and humidity (Vass, 2011). The equation for UF PMI is as follows (3):

$$PMIAerobic = \frac{1285\left(\frac{Decomposition}{100}\right)}{0.0103 * temperature * humidity} \qquad \text{(Vass, 2011)}$$

In equation 3, the number 1285 represents the ADD constant at which volatile fatty acid (VFA) liberation stops. The decomposition parameter in the above equation represents an estimated value of soft tissue decay on the cadaver at a certain timepoint (1-100) (Vass, 2011). The value of 0.0103 is a constant developed by Vass that represents the effect of moisture on the rate of decomposition. Temperature and humidity both represent the average temperature and humidity on either the day of recovery, or the average over a period of time. For this study, the $PMI_{Aerobic}$ was calculated for each cadaver at each timepoint. The average temperatures and humidity were adapted from historical temperature/humidity readings available from the historical weather data. The weather readings came from the Huntsville Municipal Station (KUTS). No temperature corrections were performed. The KUTS station is located approximately 8 miles from the STAFS facility, where the cadavers were placed. The level of decomposition used in the formula above was determined by myself and confirmed by the Principal Investigator. To determine the level of decomposition for UF PMI, the cadavers were assessed each day and scored as a percentage from 1-100%. Each portion of the body was given a certain number of points, based on size of the area totaling 100. If tissue was lost in certain areas, then points were deducted from the total. The summation of all points after tissue assessment represented the total percentage of soft tissue decomposition. This technique was not outlined in the original publication but was developed for this study as a means of standardization to eliminate bias.

Selection of Base Temperatures

For this study, I calculated ADD a total of 4 times, using a different base temperature each time. For the main portion of these experiments, ADD was calculated using the TBS system and formula, this ADD is excluded from this section. For the other 4 ADD calculations, I used base temperatures of 0˚C, 4 ˚C, 6 ˚C, and 10 ˚C. These temperatures were pulled from the current literature surrounding calculations of ADH/ADD (Megyesi et al., 2005; Hyde et al., 2013). 0°C was the base temperature Megyesi used when calculating ADD using TBS (Megyesi et al., 2005). This temperature was used because freezing temperatures inhibit biological processes, such as bacterial growth, which is integral in decomposition (Megyesi et al., 2005). Some studies suggest that putrefaction cannot occur at temperatures below 4˚C, or refrigeration temperature (Micozzi, 1991). The base temperatures of 6˚C and 10˚C are used as thermal minimums for key Dipteran species in decomposition (Hyde et al. 2013). In this study, the use of 4 different base temperatures allowed for a more robust analysis of how base temperature can affect ADD. For this portion of the experiments, historical weather data was pulled from Weather Underground for the Huntsville Municipal Station (KUTS) located approximately 8 miles from where the cadavers were placed. First, degree days were calculated each day for 30 days for each cadaver. Once all DD were calculated, ADD was then calculated for each cadaver. After ADD was calculated, the findings were represented in the form of line graphs to visualize trends and patterns over time.

Statistical Analyses

The data set was first assessed for normality using a Shapiro-Wilk test. To compare the methods of estimating PMI (actual PMI from placement, Megyesi TBS PMI, and Vass' universal formula) over nine timepoints (0, 1, 2, 3, 4, 5, 10, 15, 30), I used a two-way ANOVA without replication where each timepoint and method was represented by a single mean. A Bonferroni multiple comparisons procedure was used to assess the difference among PMI methods and timepoints. I regressed PMI against time for each method and tested the differences in slope and elevation between the UF PMI and TBS PMI to determine which methodology best approximates PMI. All statistical analyses were performed using SigmaStat® (v4.0) statistical software.

CHAPTER III

Results

TBS Image Analysis

Total body scores in this data set ranged from 3 to 30. No cadaver in this set exhibited a score higher than 30, meaning that decomposition did not progress to full skeletonization for any one cadaver during the course of this study. Cadavers assigned a TBS of 3 have little to no visible signs of decomposition (Megyesi et al., 2005) (Figure 1). Some cadavers were assigned a TBS of 3 on placement day, but many had at least some visible signs of decomposition or discoloration of head/abdomen. For example, STAFS 2013-009 was given a TBS of 3 because there was very little discoloration of the body (Fig. 1). The only visible signs of decomposition being slight discoloration of the head and pallor/livor mortis, which is not cause for increase in TBS. Only 1 cadaver in this study progressed to near full skeletonization. STAFS 2014-053 was given a TBS of 30 on day 30 of sampling (Figure 2). This cadaver was placed in July of 2014 and timepoint 30 occurred on August 22, 2014.

Bone exposure occurred rapidly for many cadavers in this data set due to scavenging. Most cadavers experienced at least some bone exposure within one week of placement, except 4: STAFS 2013-028, STAFS 2013-042, STAFS 2014-028, and STAFS 2015-095. STAFS 2013-028, STAFS 2013-042, and STAFS 2015-095 were all placed during the month of November. The first two mentioned were placed in 2013 and the last-mentioned was placed in the year 2015. During the first week of placement in November of 2013, average daily temperatures did not exceed 15˚C, or about 59˚F. STAFS 2013-028 experienced the first sign of bone exposure on day 10, while STAFS

2013-042 didn't experience any bone exposure until day 14. STAFS 2015-095, a 2015 November placement, did not exhibit bone exposure until day 12. Average daily temperatures prior to bone exposure were mild with temperatures not exceeding 23˚C, or about 74˚F. Lastly, STAFS 2014-028 did not exhibit bone exposure until day 7. This cadaver was placed in April of 2014. Temperatures were mild during this time, not exceeding 26˚C, or about 79˚F. STAFS 2014-004, placed concurrently with STAFS 2014-028 exhibited bone exposure on day 1. Many reasons could exist for this, but STAFS 2014-028 appeared to have a higher starting body mass than their placement counterpart (STAFS 2014-004). In this study, scavenging played a large role in decomposition, since the cadavers were placed outdoors with no barriers to deter scavenging. This was done purposefully as many corpses found decomposing outdoors by investigators will have at least some level of scavenging. Based on the results of this study, scavenging may cause severe tissue loss and result in a higher TBS earlier on in the decomposition process, though further studies may be required to test this hypothesis.

UF Image Analysis

The decomposition statistic for this data set ranged from 0.01 to 0.95, meaning that the cadavers ranged from 1% to 95% soft tissue loss. Cadavers exhibiting a D=0.01 were "fresh" and had little to no visible decomposition (Figure 1). Cadavers exhibiting a D = 0.95 were in skeletonization and had very little connective tissue present (Figure 2).

Figure 1

STAFS 2013-009 Day 0 Placement

Note. Image showing STAFS 2013-009 on placement day. Figure shows instance where UF decomposition variable is equal to 0.01. TBS for this cadaver is equal to 3 on this day.

Figure 2

STAFS 2014-053 Day 30

Note. Image showing STAFS 2014-053 on day 30 of sampling. Figure shows instance where UF decomposition variable is equal to 0.95. TBS for this cadaver on this day is equal to 30.

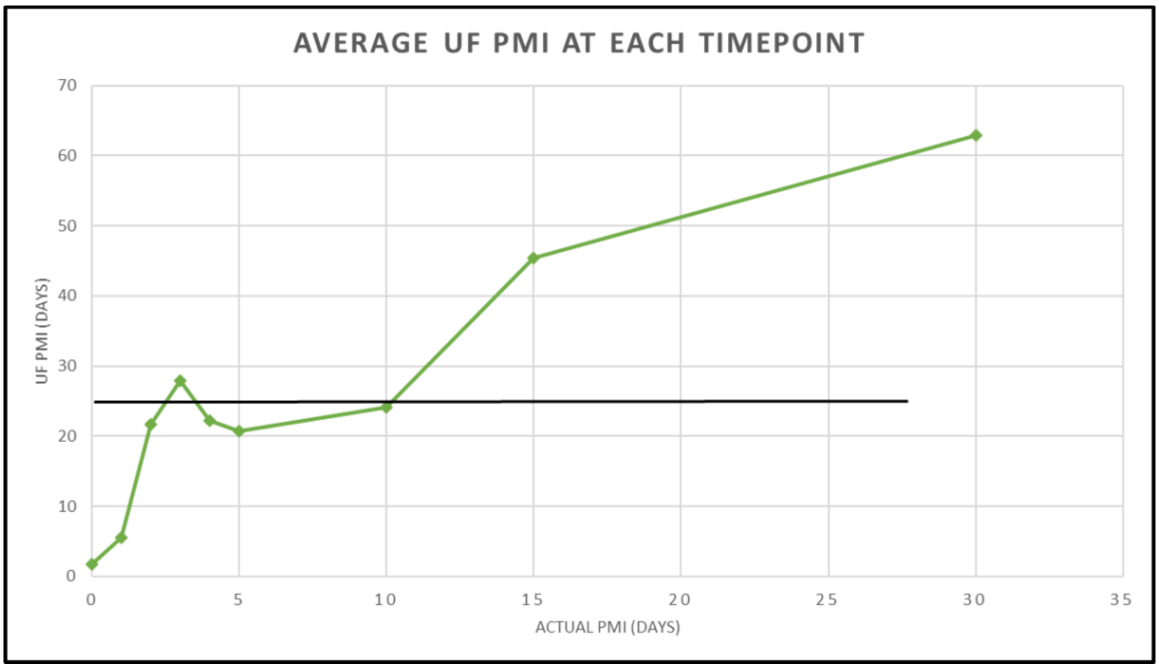
These instances depict separate decomposition events at varying levels throughout this study. Humidity also played a large role in calculating UF PMI, and in Southeast Texas during this study, did not drop below a daily average of 33.3%. The highest recorded humidity during this study was 96.4%. The mean humidity for all days in this study was 69.78% (n = 434).

In the UF method, temperature is one of the most important variables in determining PMI. As mentioned previously, this study took place during the months of February, March, April, May, July, August, November, and December in the years 2013, 2014, and 2015, meaning that temperatures varied greatly. The lowest temperature recorded was about -5˚C (23˚F), while the highest temperature recorded was approximately 31˚C (88˚F). The mean temperature recorded for all days was 18.64˚ (65.60˚F) (n = 434).

While estimations of TBS PMI continuously increased over the course of decomposition, estimates of UF PMI fluctuated over time with respect to actual days since placement (Figure 3).

Figure 3

Comparison of UF PMI to Actual PMI



Note. Line graph comparing average UF PMI to actual PMI in days. The horizontal line (y=25) represents actual PMI in days with a UF PMI output of 25 days.

The above graph is just one example comparing UF PMI to time since placement. As time progresses, decomposition should progress as well. The findings of this study indicate that decomposition regresses, meaning that the UF PMI is unreliable. Figure 3 shows that 2.5, 3.5, and 10 days postmortem all have a UF PMI output of 25 days. This means that there is not a singular UF PMI output for each actual PMI (Figure 3). This is forensically dangerous in that there cannot be more than one estimate per calendar day. Unreliable estimates have the potential to derail forensic investigations as well as hold the wrong person accountable for a crime. PMI is a measure of calendar time and therefore, should increase at a constant, positive rate (slope = 1).

Another observation of using UF to estimate PMI is that the formula does not work well with low temperatures. Since temperature as a variable is a denominator in the UF, a small number may have a great impact on PMI. For example, on December 7th, 2013, the average daily temperature was 0.28˚C, or 32.50˚F. For STAFS 2013-028, this was day 29 of sampling, the humidity was 87%, and the decomposition level was recorded as 0.58. When input into the UF, the PMI output was 2,994 days. The next day, day 30 for STAFS 2013-028, the decomposition level was still 0.58, the humidity was 89.3%, and the temperature was 3.8˚C, or 38.8˚F. The output for day 30 was only 214 days. This means that a 2% increase in humidity and a 3˚C increase in temperature resulted in a 2,780-day difference in PMI. Furthermore, when working in temperatures in the negatives (Celsius), the absolute value must be taken for the output PMI. Since time in unidirectional, a PMI of -100 days is not acceptable. This means that all other variables equal, a temperature of -10˚C (14˚F) and 10˚C (50˚F) would yield the same PMI.

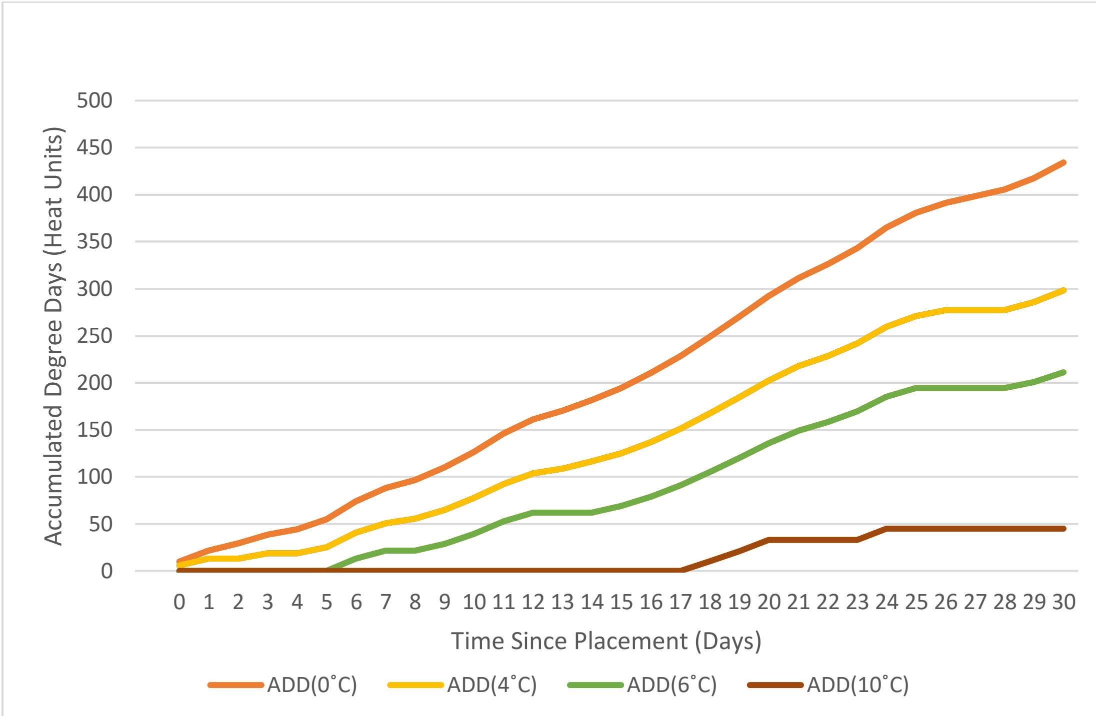
Effects of Base Temperature on ADD

I found that over the course of 30 calendar days, ADD increased, with the exception of some plateaus due to temperatures below the acceptable threshold (Figures 4-7). As expected, each line graph shows that ADD increases at a faster rate the lower the base temperature (Figures 4-7). Consistently, a base temperature of 0˚C had the steepest slope, followed by 4˚C, 6˚C, and 10˚C, respectively (Figures 4 -7). Moreso, during the warmer months in Southeast Texas (April, May, July, and August), the slopes were greater than the cooler, winter months (February, March, November, and December) (Figures 4-7). This occurrence is due to the fact that the temperatures during the cooler months are not exceeding the base temperature, thus degree days are not accumulating meaning that ADD is remaining the same day to day (Figure 5). Figure 5 depicts the effects of base temperatures on ADD and represents trends that occur during the cooler months (November and December). During this sampling period, degree days are not accumulating from day 13 through day 25 using a base temperature of 10˚C (Figure 5). This means that during this time, ambient temperatures did not surpass 10˚C, or 50˚F. However, when using a base temperature of 0˚C, the only plateau in ADD was from day 19 to day 21, where average temperatures were below 0˚C, or 32˚F. The figures for November/December 2014 and 2015 look similar to the graph for November/December 2013 and thus were excluded from this section (Figure 4).

While there is still much debate on which base temperature to use in specific instances, the significance of base temperature should not be in question. Figure 7 depicts a vertical line (x = 23) that represents 23 days since placement of a cadaver in July of 2014. This line intersects with all 4 ADD calculations with different base temperatures (Figure 7). An actual PMI of 23 days will result in ADD of approximately 670, 570, 520, or 410 degree days depending on the selection of base temperature (0°C, 4°C, 6°C, 10°C, respectively) (Figure 7).

Figure 4

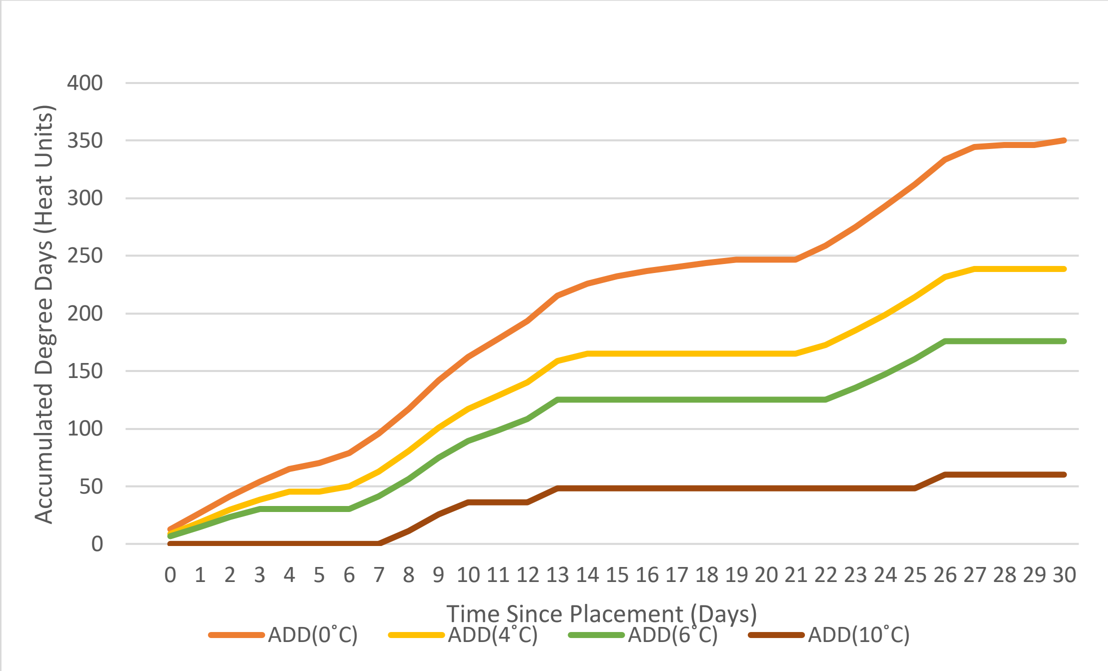
Base Temperature on ADD in February/March 2013



Note. Effect of base temperature on ADD during the months of February and March of 2013.Represents cadavers STAFS 2013-009 and STAFS 2013-011.

Figure 5

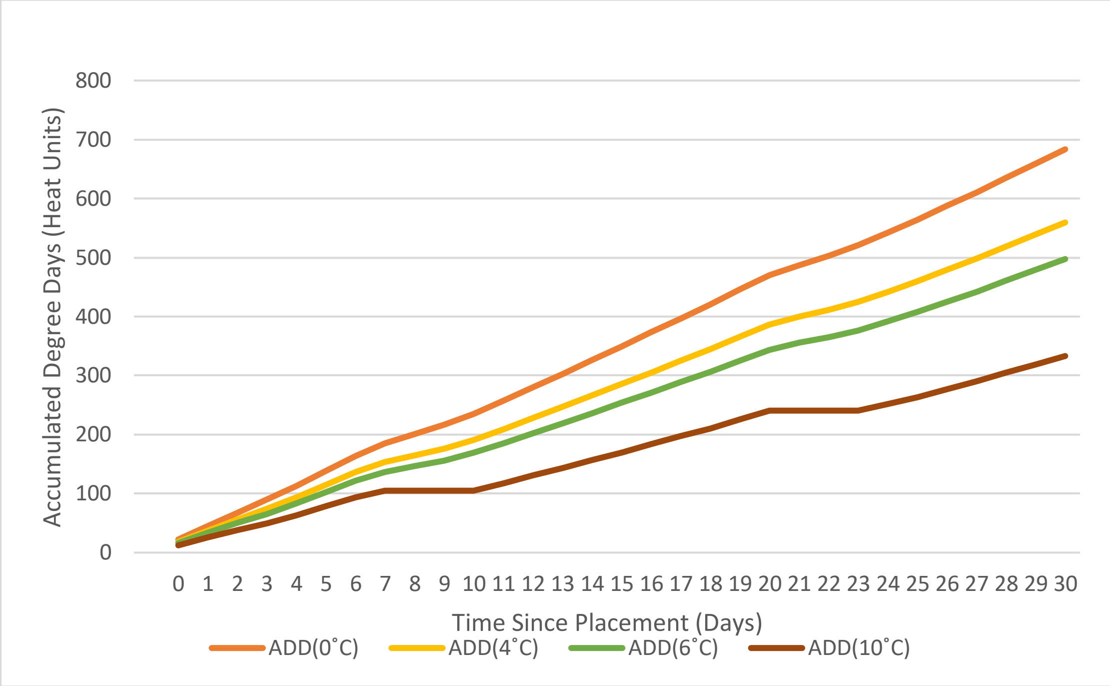
Base Temperature on ADD in November/December 2013



Note. Effects of base temperature on ADD in November and December of 2013. Represents cadavers STAFS 2013-028 and STAFS 2013-042. The line graphs for November/December for 2014 and 2015 look similar, so they were excluded. Those figures represent cadavers STAFS 2014-072/STAFS 2014-076 and STAFS 2015-095, respectively.

Figure 6

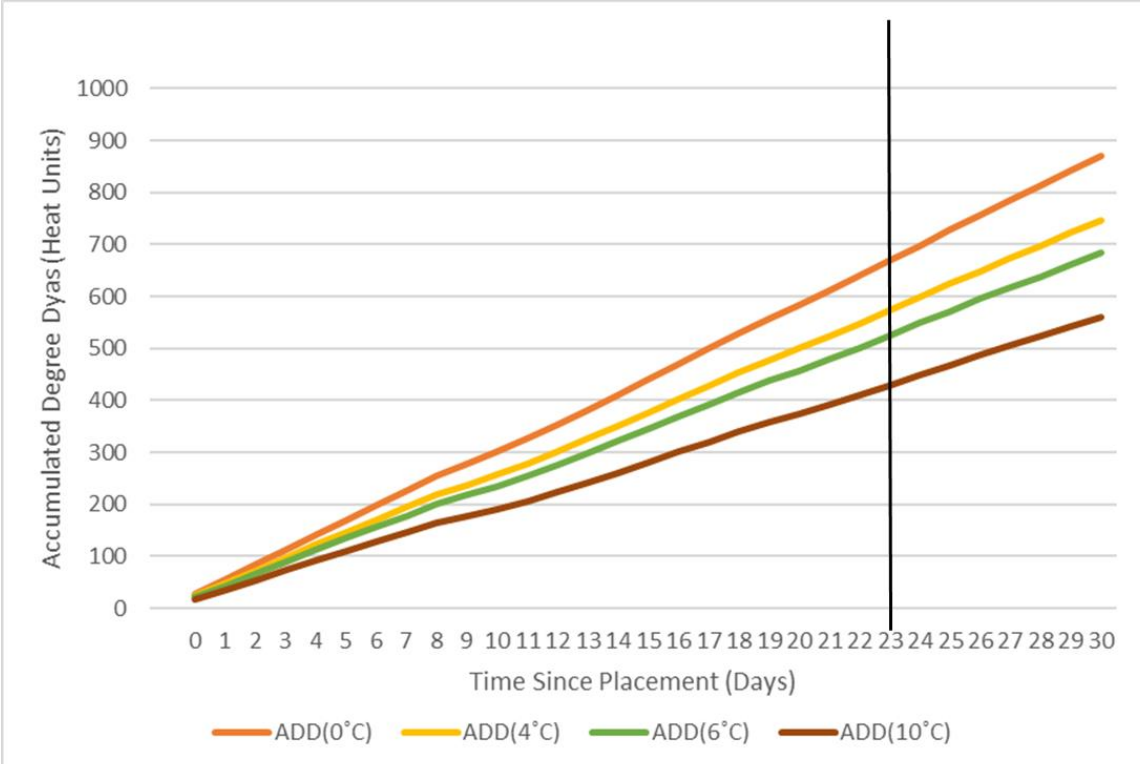
Base Temperature on ADD in April/May 2014



Note. Effects of base temperature on ADD during the months of April and May of 2014. Represents cadavers STAFS 2014-004 and STAFS 2014-028.

Figure 7

Base Temperature on ADD in July/August of 2014



Note. Effects of base temperature on ADD during the months of July and August of 2014. Represents cadavers STAFS 2014-052 and STAFS 2014-053. Vertical line (X = 23) represents 23 days since placement of cadaver.

Statistical Analysis

Initially, I planned on performing a two-way repeated measures ANOVA with replication. However, upon preliminary testing, the data set did not meet the assumptions of normality and equal variance with attempts of transformation (e.g., log, reciprocal, and Box-Cox transformation) and therefore could not be analyzed using a two-way repeated measures ANOVA with replication. The data was then collapsed to single means, thus meeting assumptions of normality (Shapiro-Wilk test) and equal variance. After collapsing the data set, the data met the assumptions for a two-way ANOVA without replication. I found that there is a significant difference in estimated PMIs between different timepoints ($p < 0.001$) (Table 5). Upon further analysis using a Bonferroni multiple comparisons procedure, I found that almost all of the PMIs estimated at each timepoint were different from one another except for 30 vs. 15 and 15 vs. 4 (Table 6). Most of the comparisons showed to be highly significant from one another ($p < 0.001$), except for the above mentioned, which showed no significance at higher p values ($p < 0.075$, $p < 0.070$ respectively) (Table 6).

Table 5

Two-way ANOVA without Replication

Source of Variation	DF	SS	MS	F	p
Time	8	4527.805	565.976	17.318	<0.001
Methods	2	1547.274	773.637	23.673	<0.001
Residual	16	522.889	32.681	-	-
Total	26	6597.968`	253.768	-	-

Note. Two-way ANOVA without replication table. $p < 0.001$ for time and $p < 0.001$ for methods. No interactions exist between time and methods.

Similar to time levels, the methods were shown to be significantly different from one another (Table 5). Actual PMI, determined by placement day, differed significantly from UF PMI ($p < 0.001$) (Table 7). TBS system also differed significantly from UF PMI ($p < 0.001$) (Table 7). Finally, there was no significant difference between actual PMI and TBS PMI ($p = 0.208$) (Table 7).

Table 6

Bonferroni Multiple Comparisons Procedure for Time

Comparison	**Diff of Means**	**t**	**p**	**p < 0.050**
30.000 vs. 0.000	44.107	9.449	<0.001	Yes
30.000 vs. 1.000	42.354	9.074	<0.001	Yes
30.000 vs. 2.000	36.431	7.805	<0.001	Yes
30.000 vs. 4.000	34.380	7.366	<0.001	Yes
30.000 vs. 5.000	34.211	7.329	<0.001	Yes
30.000 vs. 3.000	33.649	7.209	<0.001	Yes
30.000 vs. 10.000	29.311	6.280	<0.001	Yes
30.000 vs. 15.000	17.121	3.668	0.075	No
15.000 vs. 0.000	26.986	5.781	0.001	Yes
15.000 vs. 1.000	25.232	5.406	0.002	Yes
15.000 vs. 2.000	19.309	4.137	0.028	Yes
15.000 vs. 4.000	17.258	3.697	0.070	No

Note. Table represents the Bonferroni MCP for factor of timepoint.

Table 7

Multiple Comparisons Procedure for Methods

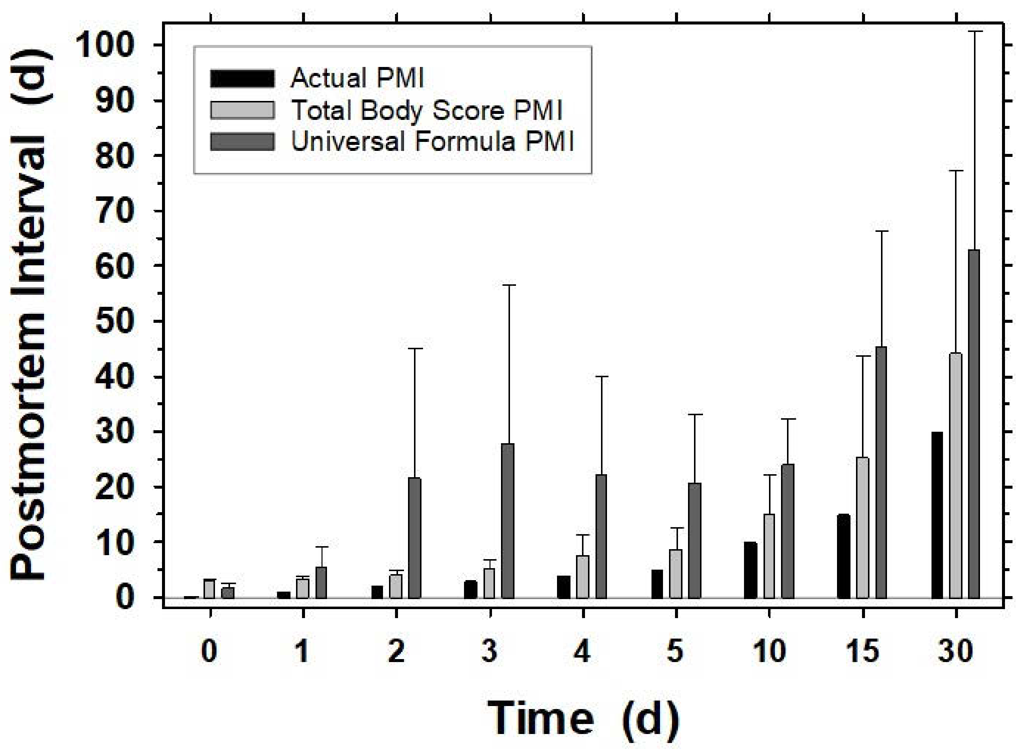
Comparison	**Diff of Means**	**t**	**p**	**p < 0.050**
UF vs. Actual	18.026	6.689	<0.001	Yes
UF vs. TBS	12.779	4.742	<0.001	Yes
TBS vs. Actual	5.246	1.947	0.208	No

Note. Table represents MCP for factor of method.

Upon graphing the findings from the data set, the distribution of PMI at each timepoint seemed to be significant (Figure 8). Timepoints 1 and 2 show little variation within/between methods as the errors bars seem negligible (Figure 8). At timepoint 3, the data began to shift and UF PMI separated significantly from the actual and TBS PMIs (Figure 8). This was also where the standard error increased significantly within the UF PMI data (Figure 8). Another notable characteristic of the calculated UF PMI was that PMI increased and decreased inconsistently, unlike the TBS PMIs, which increased daily (Figures 8-9).

Figure 8

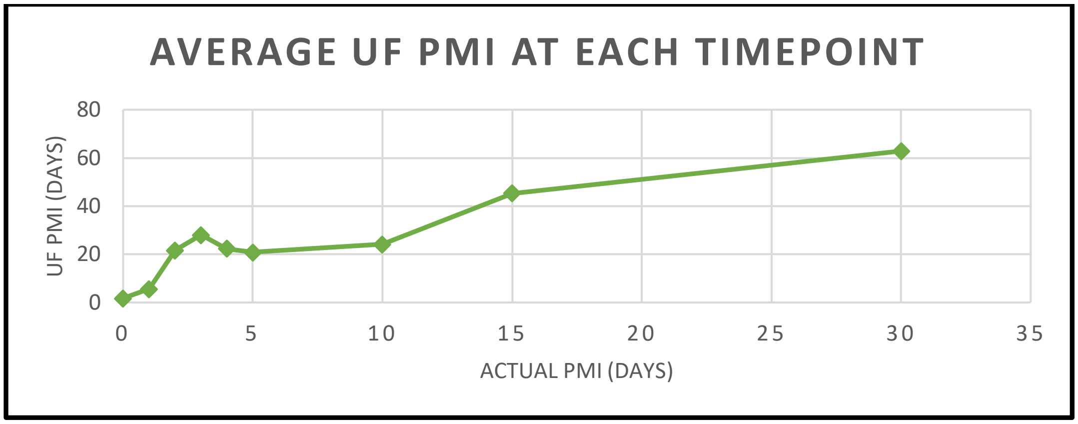
Distribution of Average PMI at Each Timepoint



Note. Bar chart representing average PMI at each timepoint (n=11).

Figure 9

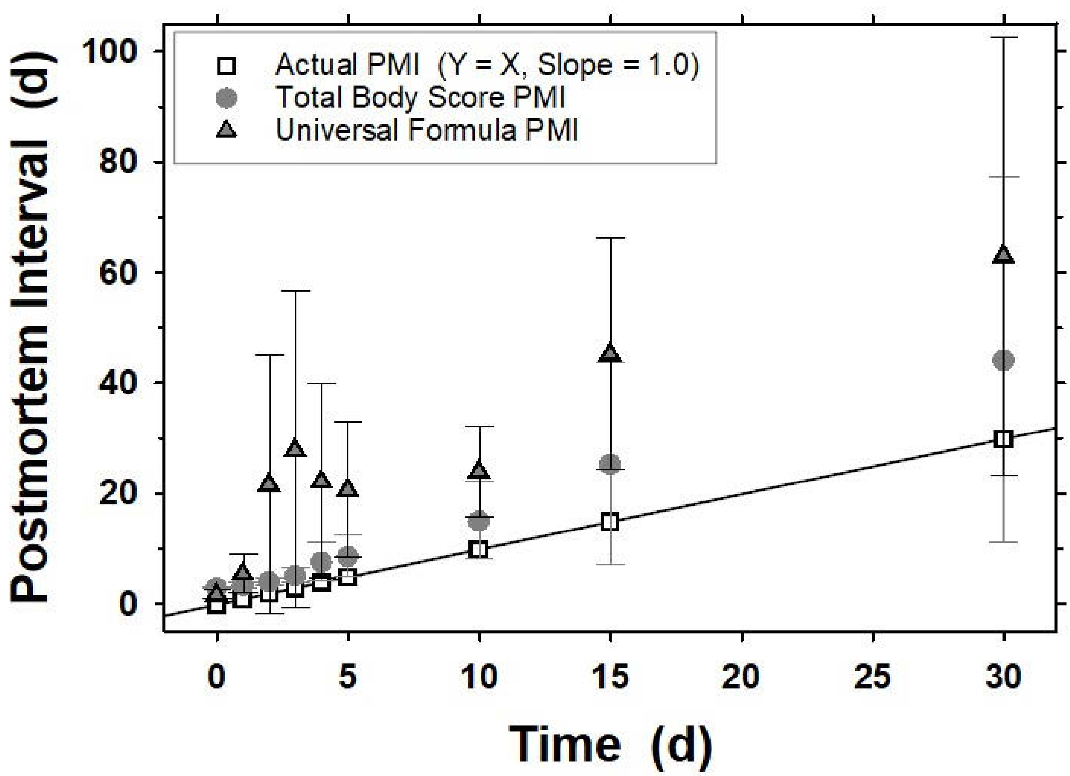
Average UF PMI at Each Timepoint



Note. Scatterplot comparing the calculated mean UF PMI (n=11) to the actual PMI at each timepoint.

Figure 10

Variation of Each Method from Actual PMI



Note. Line graph representing variation of each method from the actual PMI.

Preliminary assessments were made on the above figure, and it was determined that much variation exists not only within the UF PMI data set, but also between other methods (Figure 10). On days 0-1, it appears that both methods of estimating PMI are relatively close to the actual PMI (Figure 10). However, starting on day 2, the UF PMI data began to stray significantly from the actual PMI (Figure 10). While there is a drastic difference between the UF PMI data and the actual PMI data, TBS PMI appears to remain relatively close to the actual PMI on each day, beginning to stray further from the actual PMI around day 15 (Figure 10).

Regression analyses were performed on the TBS PMI and UF PMI. I found a significant relationship between Total Body Score PMI and days since placement ($F = 47.90$; $df = 1, 97$; $p < 0.001$) where time explained 33.1% of the variation in TBS PMI (Table 8). I also found a significant relationship between Universal Formula PMI and days since placement where ($F = 27.84$; $df = 1, 97$; $p < 0.001$) where time explained 22.3% of the variation in UF PMI (Table 9).

Slope and elevation were assessed for both methods based on the above figure (Figure 10). There was no significant difference in slopes between the TBS and UF methods ($t = -0.93513$, $df = 194$, $p = 0.350882$). This means that the regression lines for TBS PMI and UF PMI are parallel to one another. The next analysis run was an elevation test to assess differences in intercepts between the two methods. I found there to be a very highly significant difference in elevation between the TBS PMI and UF PMI ($t = -3.5419028$, $df = 195$, $p = 0.0004969$).

The y-intercept for TBS PMI is 1.94, whereas the y-intercept for UF PMI is 11.80 (Figure 11). This means that at 0 days postmortem, the TBS PMI output is 1.94 days, whereas the UF PMI output is 11.80 days. In order to calculate PMI accurately in forensic investigations, the y-intercept should be as close to 0 as possible, when x = 0. The difference in intercepts between methods is significant and shows that the Total Body Scoring system is a better estimate of PMI rather than the Universal Formula (Figure 11). I found that not only does TBS PMI more accurately predict the correct PMI but will provide more precise estimates as well (Figure 11). Much variation exists within the UF method as the average PMIs calculated each day stray from the regression line (Figure 11).

Table 8

Regression Analyses TBS PMI

REG	Coefficient	Std. Error	t	p
Constant	1.937	2.464	0.786	0.434
Time	1.430	0.207	6.921	< 0.001

ANOVA	DF	SS	MS	F	p
Regression	1	16548.500	16548.500	47.898	< 0.001
Residual	97	33513.136	345.496		
Total	98	50061.636	510.833		

Note. Tables depict regression analyses for TBS PMI. TBS PMI = 1.43t + 1.94. Rsqr value for TBS PMI and time is 0.331, standard error is 18.588 (N = 99).

Table 9

Regression Analyses UF PMI

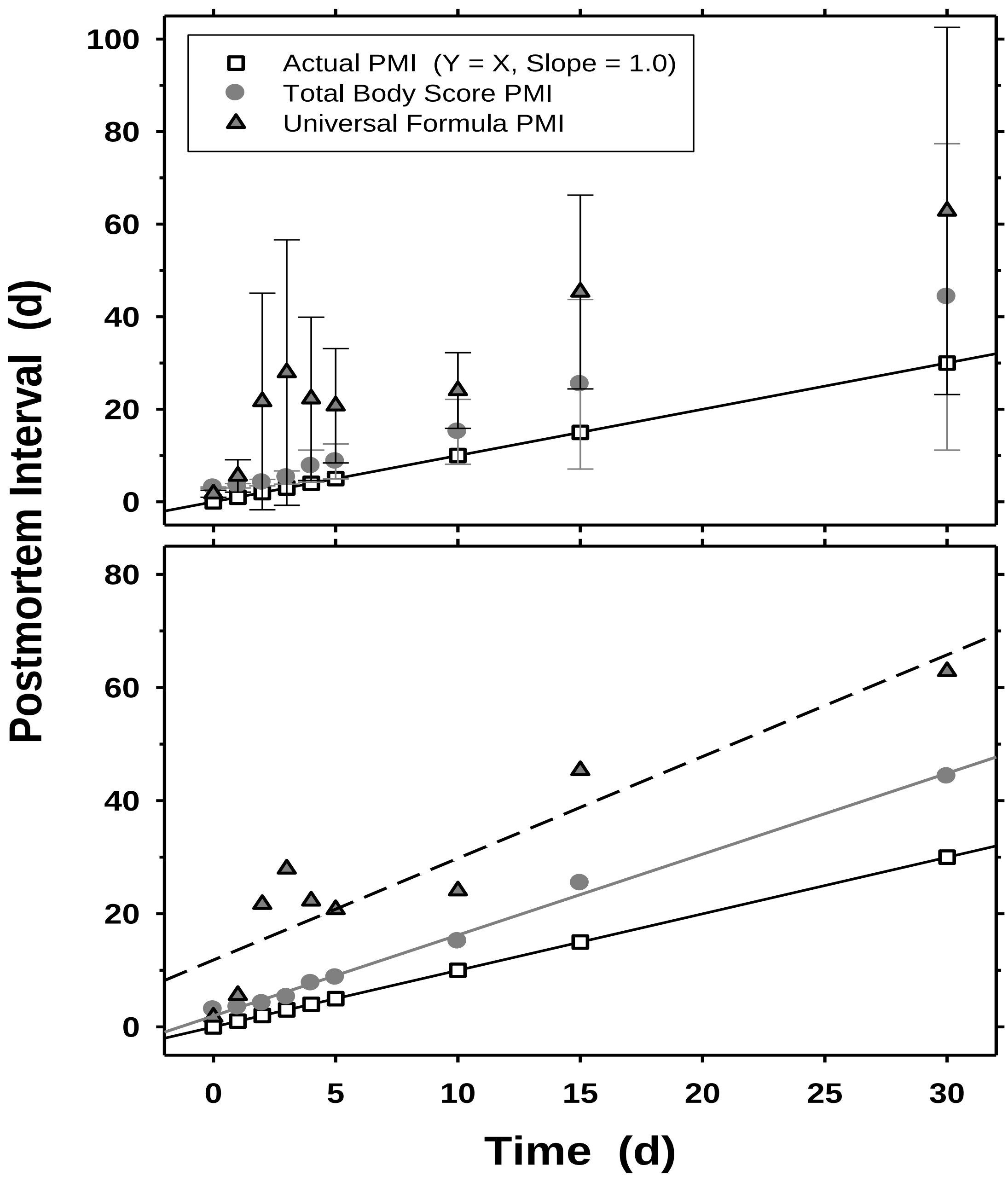
REG	Coefficient	Std. Error	t	p
Constant	11.802	4.067	2.902	0.005
Time	1.799	0.341	5.276	< 0.001

ANOVA	DF	SS	MS	F	p
Regression	1	26199.202	26199.202	27.835	< 0.001
Residual	97	91298.757	941.224		
Total	98	117497.960	1198.959		

Note. Tables depict regression analyses for UF PMI. UF PMI = 1.80t + 11.80. Rsqr value for UF PMI and time is 0.223. The standard error is 30.679 (N = 99).

Figure 11

Regression Analysis of TBS PMI and UF PMI



Note. Regression analysis showing regression lines for TBS PMI and UF PMI. Actual PMI (null) also included.

CHAPTER IV

Discussion/Conclusion

Total Body Score PMI

This analysis aimed to determine if these methods were able to be applied in regions that they were not developed for, namely, Southeast Texas. Through analysis of the findings, I concluded that the TBS system accurately determines PMI throughout the course of this study, though some error exists within this method. Contrary to some literature on the validity of the TBS method, the findings of this study showed consistent, reliable predictions (Cockle & Bell, 2015).

Universal Formula PMI

Future studies are needed in Southeast Texas to expand the field and develop more accurate methods of estimating PMI. The main takeaway from this research is that there is a need for accurate and efficient methods of estimating PMI. Ideally, a formula/system would exist that encompasses all variables and is applicable in all regions. However, from the results of this study and previous validity studies, we know that is nearly impossible given how variable the process of decomposition is (Vass, 2001). It is impractical to focus energy and resources into developing a 'universal' method of estimating PMI across all regions. This study shows that Vass' UF is inaccurate and not applicable in Southeast Texas as a means of estimating PMI . The variation that exists within the UF data as well as between this method and actual PMI is too great to be used reliably by investigators and other law enforcement agents. Although this method was developed as a way for investigators to obtain a preliminary PMI before forensic scientists can develop a more formal hypothesis of PMI, it can still become misleading under certain conditions. I hypothesize that the variability within this system exists because of the use of temperature. While decomposition is temperature-dependent, the formula may be more practical if it used ADD rather than temperature in Celsius, so it can account for the days when cold temperatures inhibit decomposition processes (Hyde et al., 2013). This would solve the issue mentioned previously in which multiple temperatures (both below and above 0˚C), resulted in the same PMI output.

The UF system also has limitations or requirements in order to be able to use this formula (Vass, 2011). One of the main requirements for this formula is that temperature must not drop below 0˚C or it may cause inaccuracies within the formula (Vass, 2011). This was made clear in the study. Most of the historical temperatures in this study were above 0˚C, except for a few. While there were problems with temperatures under 0˚C, many problems occurred for low temperatures above 0˚C, as mentioned above. A way to correct this could be the use of ADD.

Furthermore, PMI estimated using the UF method does not accurately, or reliably, determine PMI at each timepoint. Moreso, this method consistently overestimated PMI, which will cause significant error during field applications of this technique. The data presented here supports some findings of other validation studies using the same UF method (Cockle & Bell, 2015).

Effects of Base Temperatures on ADD

While ADD may be a solution to estimating PMI in a temperature-dependent system, it is important that correct base temperature is chosen. This study assessed the effects of different base temperatures on the calculation of ADD. Results showed that ADD increases at different rates depending on base temperature chosen. Previous research suggests the use of an incorrect base temperature when analyzing ADD may lead to inaccurate estimates of PMI, which is supported in this study (Gennard, 2012). If base temperature is too low, degree days will not accumulate on the days when ambient temperatures are low, bringing estimated PMI further from actual PMI. If the selected base temperature is too high, ADD will accumulate consistently, which could also alter PMI. It is important that more research be done on the topic of base temperature, especially in Southeast Texas. Potential research on selecting proper base temperatures for insects as well as microorganisms is vital to the field.

Other Factors Affecting PMI

There are many aspects of decomposition that the systems used in this study don't necessarily account for. The TBS system, while not statistically different from actual PMI could be improved upon to make more applicable to people of color as well as account for more of the daily taphonomic observations. Some studies have attempted to rework the TBS system to include more descriptors for each region (Bytheway, 2020). In the future, developing a more reliable, region-specific method of estimating PMI using TBS and ADD should be the goal. While the TBS system does have its own issues, ADD increases consistently with time, whereas UF PMI increases and decreases according to ambient temperature. This issue causes problems during investigations as estimated PMI may not be accurate depending on historical temperatures at the crime scene.

While TBS does accurately reflect actual PMI in this study, there were some limitations on how well this system portrays decomposition in the presence of mummification. Mummification is the opposite of decomposition in that is the preservation of tissues at any point during decomposition (Schotsmans et al., 2011). We know that decomposition can happen at any point during the process of decomposition, but in Southeast Texas, mummification can happen quickly after placement. In this study, many of the cadavers mummified in the few days following placement. According to the TBS system, mummification occurs during advanced decomposition (Megyesi et al., 2005). Since we know that TBS is a linear system, that means that mummification is only accounted for near the end of the decomposition process. While mummification stops the physiological clock during decomposition, actual PMI is increasing with each passing day. The below figures represent two cadavers, one mummified and one not mummified, used in this study that have similar ADDs, but different calculated TBS PMIs. STAFS 2014-028, a non-mummified cadaver on May 09, 2014, had an ADD value of 397 (base temperature 0°C) (Fig. 12). STAFS 2015-062, a mummified cadaver on August 04, 2015, had an ADD value of 417.78 (base temperature 0°C) (Fig. 13). Both cadavers had an approximate PMI of 16-17 days based on ADD calculations. Upon calculating PMI using TBS, STAFS 2014-028 had a TBS of 19, whereas STAFS 2015-062 had a TBS of 24. This puts the TBS PMIs at 14.18 and 38.18, respectively. This means that while both cadavers had similar actual PMIs, due to mummification, the resulting TBS PMIs were vastly different since the TBS system places mummification in later stages of decomposition. When establishing PMI in the field, it is vital to remember that mummification can happen at any point during decomposition. Using a system that does not account for this fact introduces error into the PMI estimate, which can have severe consequences. So, while TBS PMI was not significantly different from actual PMI in this study, it does not accurately account for the amount of variation within decomposition as a whole.

Figure 12

STAFS 2014-028 Non-mummified Remains

Note. STAFS 2014-028 April placement date. Photograph taken on May 09, 2014. Cadaver has an actual PMI of 17 days and a Megyesi TBS of 19.

Figure 13

STAFS 2015-062 Mummified Remains

Note. STAFS 2015-062 July placement day. Photograph taken on August 04, 2015. Actual PMI of 13 days and a Megyesi TBS of 24.

Vass mentioned that postmortem dismemberment by large carrion animals may also cause inaccuracies within the system (Vass, 2011). However, scavengers play a large role in decomposition and determining PMI. The exclusion of scavengers for the purpose of estimating PMI creates too controlled of an environment to be applicable in a natural setting. Studies done with uninhibited feeding by scavengers simulate natural crime scenes and are important for determining PMI in an uncontrolled setting such as that of a crime scene. This being said, due to the nature of this research, high rates of scavenging may have altered PMI calculated using Vass' UF method, though no dismemberment of any cadaver occurred. Regardless of whether or not scavenging played a role in error within the UF PMI estimates, a formula that cannot account for scavenging during decomposition will not provide an accurate PMI and thus cannot be used in forensic investigations. Estimates of PMI must be accurate and predictable in order to be admissible in court proceedings and this method must be repeatable in accordance with region.

With this study comes new data regarding the validity of quantitative methods of estimating PMI over time, but it is important to make note of unconscious bias as well as small sample size. Within the field of forensic anthropology, it is difficult to acquire large sample sizes for research as cadavers are willed donations to few facilities around the world. This means that though the data presents as normal, the small sample size introduces greater possibility of type errors (Banerjee et al., 2009). Further studies are suggested using greater sample size as to eliminate errors such as those mentioned above as well as individual variation between cadavers (Hyde et al., 2013). Lastly, due to the nature of this study as well as the type of research, it is difficult to quantify decomposition perfectly, which was not the aim of these experiments. Therefore, before generalizations can be made, it is important to gather evidence from regions across Texas to better account for the amount of variability that exists within the field.

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Research Interests

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- Effects of postmortem ant colonization on the rate of decomposition
- Microbial succession on human cadavers in Southeast Texas

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Graduate Research Assistant — January 2022-August 2022

Undergraduate Lab Instructor — January 2021-August 2021

Courses Taught

General Biology I — 5 Sections
Introductory Genetics — 7 Sections

Poster Presentations

Texas Academy of Science 126th Annual Meeting — March 2023
"Estimation of postmortem interval in human cadavers using two different quantitative methodologies" **Molly R. Sarles**, Sibyl R. Bucheli Ph.D.

Sam Houston State University Undergraduate Research Symposium — April 2021
"The Effects of Trauma on the Coronal Suture" **Molly R. Sarles**, Stephanie Baker, Patrick J Lewis Ph.D.

Membership in Professional Societies

Texas Academy of Science 2023-Present

Entomological Society of America 2022-Present

Field Experience

- Microbial soil and human cadaveric skin swabs for microbial succession study at Southeast Texas Applied Forensic Science Facility 2021-2022
- Collection/curation of invertebrate specimen 2022
- Herpetological survey of Pineywoods Environmental Research Laboratory 2021