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Human Decomposition and Time Since Death: Persistent Challenges and Future Directions of Postmortem Interval Estimation in Forensic Anthropology

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ABSTRACT

Estimating the time since death, or the postmortem interval (PMI), is a significant component of forensic anthropological analysis when human remains are discovered. Despite decades of research, methods for providing an estimate of the PMI with known error rates are still absent from applied medicolegal forensic work, which prompts the necessity for a critical examination of the state of the field. This review details the history of how forensic anthropology emerged from the broader discipline of biological anthropology, with a specific focus on how forensic anthropologists came to be understood as suitable experts for estimating the PMI. We describe existing PMI estimation methods and enduring barriers in advancing our knowledge. We provide an overview of the formative PMI research, then focus on a systematic review of 30 years (1993–2023) of human decomposition literature. Results of our synthesis demonstrate the two prevailing ways to estimate the PMI involve (1) the use of quantitative biomarkers and (2) macromorphoscopic observations. Results also highlight that PMI research continues to be limited by small, highly variable samples and a lack of standardized definitions of decomposition, which impedes replicability and the advancement of methods for PMI estimation. Forensic anthropologists can address these longstanding issues by ensuring the principles of Open Science are adhered to during the research and dissemination process including data sharing and transparency. Intentional research design that integrates comprehensive geospatial data and improved modeling techniques can contribute to devising methods capable of providing PMI estimates within applied medicolegal and humanitarian contexts.

1 | Introduction

Estimating the postmortem interval (PMI), or time since death, remains one of the most elusive questions asked of forensic anthropologists working within the medicolegal death investigation system in the United States and abroad. When facially unrecognizable human remains are recovered, forensic anthropological analysis provides information to further identification efforts by generating a biological profile, which includes estimates of the decedent's age-at-death, biological sex, population affinity, and stature, which can then be matched against missing person records. As a field that deals with decomposing and skeletal remains, forensic anthropologists are also frequently asked to provide insight on the PMI within these investigations. However, understanding the process of decomposition is unlike other skeletal analyses, which fit neatly within methods found in biological anthropology more broadly. The study of decomposition and PMI estimation were ultimately adopted by anthropologists as a byproduct of requests for forensic investigations in need of skeletal and taphonomic expertise. The focus here will be to examine the reasons why forensic anthropologists came to be understood as experts in estimating the PMI, existing methodologies for doing so using human remains, and how we may advance our understanding of this complex process in the future given its importance in the identification process within medicolegal death investigations.

Nearly $8 million in federal funding has been awarded by the U.S. National Institute of Justice since 2008 for research towards informing the longstanding inquiry of assessing time since death, which represents only a fraction of the research dedicated to the subject. However, reliable methods for estimating the PMI remain essentially absent from the applied medicolegal death investigation system. Accurate estimations of the PMI can inform critical legal and humanitarian questions within this system including providing a possible timeline that can be matched with missing person's records, which can lead to a successful identification of the unidentified. Furthermore, a reliable PMI estimate can contribute to reconstructing the sequence of events around an individual's death, corroborate alibis during the legal process, as well as offer families information regarding the death of their loved one.

The absence of fieldable PMI methodologies prompts the need for reflection in light of nearly half a century of human decomposition facility research meant to address this enduring question. The formation of the first facility in the world designed specifically to study the decomposition process on human cadavers was established at the University of Tennessee, Knoxville (UTK) in 1980 and has since prompted nearly 50 years of ongoing research pertaining to the complexities of human decomposition. A total of 12 human decomposition facilities exist worldwide with a unified goal of advancing our understanding of decomposition to better assist with medicolegal death investigations (Table 1). Since the mid-20th-century, forensic anthropologists have made methodological advancements regarding estimation criteria involved in the biological profile including biological sex, stature, and age-at-death (Jantz and Ousley 2005; Konigsberg et al. 2009; Ross and Pilloud 2021; Steadman et al. 2006), however, PMI estimation methods do not seem to have followed a similar trajectory. Forensic taphonomy, or what happens to the body between death and the time of discovery, was put forth in the late 1990s as a framework to advance PMI methods. While it has proven useful in helping to reconstruct the circumstances before and after deposition, interpret trauma, and identify dispersal patterns, taphonomic explanations do not translate directly into applicable methodologies for PMI estimation (Dirkmaat et al. 2008; Haglund and Sorg 1997; Wescott 2018b).

Decomposition has long since been understood to be a complex process influenced by myriad variables stemming from external components such as the environment (e.g., weather, environment, or geography), characteristics of the body (e.g., trauma or clothing) and internal, individualistic variables (e.g., body size or disease state). Although this knowledge,

TABLE 1 | Current human decomposition facilities listed by name, acronym, university affiliation, and geographical location.

Human decomposition facility	Acronym	University	Location
Anthropology Research Facility	ARF	University of Tennessee Knoxville	Knoxville, Tennessee, USA
Forensic Anthropology Research Facility	FARF	Texas State University	San Marcos, Texas, USA
The Southeast Texas Applied Forensic Science Facility	STAFS	Sam Houston State University	Huntsville, Texas, USA
Complex for Forensic Anthropology Research	CFAR	Southern Illinois University	Carbondale, Illinois, USA
Forensic Investigation Research Station	FIRS	Colorado Mesa University	Grand Junction, Colorado, USA
Forensic Osteology Research Station	FOREST	Western Carolina University	Cullowhee, North Carolina, USA
USF Facility for Outdoor Research and Training	FORT	University of Southern Florida	Gainesville, Florida, USA
Forensic Research Outdoor Station	FROST	Northern Michigan University	Marquette, Michigan, USA
Forensic Science Research and Training Lab	FSRTL	George Mason University	Manassas, Virginia, USA
Research in Experimental and Social Thanatology	REST[ES]	Université du Québec à Trois-Rivières	Quebec, Canada
The Australian Facility for Taphonomic Experimental Research	AFTER	University of Technology	Sydney, Australia
Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology	ARISTA	Amsterdam University Medical Centers	Amsterdam, Netherlands

along with facilities in which to conduct research, have prompted a diverse litany of decomposition studies over decades, issues such as methodological inconsistencies, small sample sizes, and regional limitations have hindered the discipline's ability to inform what remains a critical, unanswered question in medicolegal death investigations. To understand more fully the historical depth that accompanies the question of PMI estimation and how it came to be within the purview of forensic anthropology, it is necessary to delve into the 19th-century, through the nascent field of physical (now biological) anthropology, from which forensic anthropology emerged, and assess the last several decades of research that informs the field today.

Assessing the PMI has been of considerable interest within the discipline of forensic anthropology since its emergence and the complexities involved in providing PMI assessments have been evident since this early period. In 1878, forensic anthropology forefather Thomas Dwight describes seven questions used to assist in the identification of a human skeleton, the fifth of which is, "How long is it since death?" (Dwight 1878, 7). In addressing this question, he notes that although the forensic anthropologist may be able to reasonably approximate aspects such as the age-at-death of an individual, the practitioner is "far more helpless" and "cannot give an opinion of value" when it comes to estimating time since death (Dwight 1878, 39–40). In interpreting Dwight's remark, one must wonder how forensic anthropologists sought to overcome these hurdles.

1.1 | Historical Context: Forensic Entomology of the 19th-Century

The history of PMI estimation methods in forensic anthropology is deeply intertwined with forensic entomology, or the systematic study of the relationship between decomposition and arthropod life cycles to estimate time since death in medicolegal death investigations. Early scientific and systematic observations of human decomposition divided postmortem changes of the body into a series of typological categories, or periods of decomposition, which continue as the underpinnings of many anthropology-related decomposition studies today. Pierre Mégnin's (1894) formative publication titled "La faune des cadavres: application de l'entomologie à la médecine légale," or "The fauna of corpses: application of entomology to forensic medicine," marked a critical moment in forensic entomology as one of the first systematic presentations of a decomposition timetable, or "waves" of arthropod colonization on deceased bodies exposed to air. Megnin provided a starting point for conceptualizing the correlation between chronology and the colonization of insects on the body but never published a visual representation of his efforts. Shortly after his publication, Johnston and Villeneuve (1897) published a table of eight decomposition periods and the chronological correlations between decomposition stages and associated arthropod species based upon Mégnin's (1894) previous work in France (Figure 1). Johnston and Villeneuve (1897) were concerned with how variation in climate and environment would affect predictions of time since death after reviewing their casework from Montreal,

FAUNA OF DEAD BODIES EXPOSED TO THE AIR[1]—(Compiled from Mégnin).

	Physical Condition.	Minimum time.	Forms met with.
First Period.....	Bodies fresh...........	First three months.	(D) *Musca.** *Cyrtoneura.** *Calliphora.**
Second Period...	Decomposition commenced..............		(D) *Lucilia.** *Sarcophaga.**
Third Period.....	Fatty acids...........	3 months to 6 months.	(L) *Dermestes.** *Aglossa.* (D) *Pyophila.** *Anthomyia.*
Fourth Period...	Caseous products......		(C) *Necrobia* (*Cornytes*).
Fifth Period.....	Ammoniacal fermentations, black liquefaction..................	4 months to 8 months.	(D) *Thyreophora.* *Ophira.** *Lonchea, Phora.* (C) *Necrophorus.* *Silpha.** *Hister.** *Saprinus.**
Sixth Period.....	Dessication...........	6 months to 12 months.	(A) *Uropoda.* *Trachynotus.* *Tyroglyphus.** *Glyciphagus.* *Serator.*
Seventh Period..	" extreme...	1 year to 3 years.	(L) *Aglossa.* *Tineola.* (C) *Attagenus* *Anthrenus.*
Eighth Period...	Debris.................	Over 3 years.	(C) *Tenebrio.* *Ptinus.*

FAUNA OF BURIED BODIES.

Before Burial..................................	(D) *Calliphora**, *Cyrtoneura.*
After Burial..................................	(D) *Ophira**, *Phora.* (C) *Philontes**, *Rhizophagus.* (T) *Achorutes*, *Templetonia.*

(The genera marked * in the table are those met with by ourselves.)

FIGURE 1 | Description of physical changes of a body over time and insect succession, adapted from descriptions by Mégnin (1894), who did not create a table. Reprinted from "On the medico-legal application of entomology," by Johnston and Villeneuve (1897, 3).

Canada and clearly recognized the potential impact of different environments on the decomposition process and associated insect activity. The authors described the challenges of generalizing across regions, given that little was presently understood regarding different habits of the same arthropod species in different geographical locations (Johnston and Villeneuve 1897, 10). These authors also suggest there is little practical value of using information gleaned from animals to understand human processes, as they found much variation in the types of insects observed on humans and those observed on animals.

Like the previous authors, American medical doctor Murray Motter's (1898) study sought to determine the legitimacy of Mégnin's (1894) observations of insect activity, decomposition, and its application within legal medicine (Motter 1898, 201). Unlike Johnston and Villeneuve, Motter was exclusively focused on the decomposition rates of buried bodies and had 150 human burials exhumed in the Washington D.C. area to evaluate Megnin's observations about insect succession in this new environmental context. He noted the complexity involved in the process stating: "...from the condition of the cadaver alone, no certain knowledge of the exact date of death is to be had" (Motter 1898, 202). He noted a plethora of influential factors may affect the decomposition process including aspects such as age, antemortem illness, season, grave depth, embalming, soil chemistry, among other variables. Based on his observations, he concluded, "it is not safe to draw any conclusion at all" regarding time since death (Motter 1898, 228). Historical forensic entomology literature underscores that the complexities involved in estimating the PMI from decomposing human remains was understood well prior to forensic anthropologists' research in this area.

1.2 | The Evolving Role of the Anthropologist

Few publications focused on the PMI over the decades following these early forensic entomological advancements. This may be due to the sociopolitical climate that characterized the decades between the turn of the century and the 1950s, including the Great Depression and World Wars I and II (Hayman and Oxenham 2020, 232). However, early anthropology literature including A *Guide to the Identification of Human Skeletal Material* (Krogman 1939) and Earnest Hooton's *Medico-legal Aspects of Physical Anthropology* (1943), described that the primary expertise provided by anthropologists related to aspects of generating a biological profile from skeletal remains, with little discussion of the PMI. Hooton wrote that in his nearly 30 years as director of a physical anthropology laboratory at Harvard University, his assistance with personal identification was not requested more than a dozen times, which demonstrates how infrequently anthropologists were called upon to assist in medicolegal casework (Hooton 1943, 215). However, this began to change in the subsequent years with the increased necessity to positively identify war casualties from World War II (Kerley 1978, 161).

Shortly after the conclusion of World War II in 1945, anthropologists were deployed to assist in identification efforts of military casualties—an effort that forensic anthropologists continue today through the Defense POW/MIA Accounting Agency (DPAA) (Snow 1948; Wilson 2022). Their skeletal expertise uniquely positioned them to assist in identifying facially unrecognizable individuals. Although discussions of PMI estimations were not found in published literature from anthropologists working with the military, a 1959 manual provided by the US Departments of the Army, the Navy, and the Air Force titled *Handling of Deceased Personnel in Theaters of Operations* described the system for processing remains after disinterment. As the remains of soldiers were received, they were classified as *current* (non-decomposed, within 72-h postmortem), *flesh covered* (remains are decomposed but no bone exposure), *semiskeletal* (some flesh remains), and *skeletal* (fully skeletal without flesh) (Departments of the Army, Navy, and the Air Force 1959, 66–67). These categories determined how the identification of the decedent would proceed. For those who were not skeletal, identification efforts focused on characteristics involving soft tissue such as tattoos, scars, and hair samples, while skeletal identification required an anthropological assessment of the bones to create a biological profile and to determine the presence of skeletal fractures or anomalies (Departments of the Army, Navy, and the Air Force 1959, 67).

In a publication titled *Identification of Skeletal Remains*, early biological anthropologist Alice Brues, provided additional insight into the role of anthropologists during this era (Brues 1958). She described that most anthropologists trained in working with skeletal remains were employed as anatomists at medical schools or researchers at universities or museums. Rarely were they employed full-time as identification specialists in the medicolegal system, except in government programs involving the identification of those who died in the war (Brues 1958, 551). Brues explained that the biological anthropologist's role when assisting in forensic casework typically involved providing estimations regarding age-at-death, sex, stature, and "race." Importantly, she cautions against anthropologists offering information regarding time since death, explaining that "one of the questions often asked regarding skeletal or semi-skeletal remains is the approximate length of time since death. This is not really within the province of the anthropologist, though after some experience he may be prepared to contribute to a discussion in which various factors are considered by those investigating the case" (Brues 1958, 552). Although anthropologists were thought to be well suited to distinguish between archaeological and more recent skeletal remains, a pathologist was deemed to be more equipped to decipher the PMI based upon soft tissue changes (Brues 1958, 552).

During the mid-century, anthropologists' skeletal expertise in biological profile estimation was highlighted, however, assessments of time since death were still considered to be within the expertise of forensic pathologists. It is clear from Brues' description of casework, that she was frequently called upon to make these assessments and other taphonomic considerations, however, there were few methods available to address this aspect of the identification process (Brues 1958, 552–554). Ongoing tension between requests from law enforcement and what anthropologists could scientifically provide continued to be a recurring theme throughout the proceeding decades.

1.3 | A Turning Point: Forensic Anthropology and Time Since Death

Anthropological literature published throughout the first half of the 20th-century illustrates a period of enthusiasm for forensic anthropological advancements including the capacity for successfully identifying human remains as discussed in the first *Yearbook of Physical Anthropology* article related to forensic anthropology (Kerley 1978). In this piece, Ellis Kerley described how methods for estimating the biological profile were making considerable advancements towards human identification. He also notes that estimating time since death is the most elusive assessment that forensic anthropologists might make. Kerley highlighted two issues that prevented progress in PMI research, including the often-cited variation and influence of postmortem taphonomic conditions and that "most forensic anthropologists have not seen a large series of remains where the time since death is actually known" (Kerley 1978, 170). Unlike methods for assessing components of the biological profile, devising methods for PMI estimation require different facilities and resources outside of osteological collections, such as human donors and the capacity for conducting longitudinal studies on decomposition. Interestingly, just two years after this publication, William Bass founded the first decomposition facility dedicated to the longitudinal observation of individuals with known time since death.

Forensic anthropologist and student of Wilton Krogman, William Bass, moved from the University of Kansas to the University of Tennessee (UT) at Knoxville in 1971—a transition that spurred his interest in time since death research (Bass 2006, 181). At Kansas, Bass was largely tasked with assessing skeletal remains cases, whereas at Tennessee, he was receiving more requests regarding partially decomposed bodies. Writing in 2006 about assessing time since death, he explains that even "after 22 years there is still much to learn about this very complicated subject" (Bass 2006, 181). A defining point for his investment in

PMI research is summarized in a case study involving an embalmed American Civil War soldier.

In 1977, Bass infamously provided Tennessee law enforcement with an inaccurate PMI estimation of six months to a year for a complicated case involving the embalmed and buried remains of an American Civil War soldier who had been dead for 112 years (Bass 1984, 139). Bass was candid about his error and provided a full background on the circumstantial evidence and pressure involved in providing a PMI estimation to law enforcement while on the scene, which he explained ultimately skewed his perspective. He viewed his error as a learning experience, one that could ultimately benefit forensic anthropology, which had largely avoided tackling the issue of determining time since death. This story not only encompasses the complexity involved in accurately estimating the PMI but is also significant in that this event was perhaps one of the single most driving factors in propelling the field of forensic anthropology towards systematically investigating the question of the PMI.

The first human decomposition facility was opened as the Anthropology Research Facility (ARF) at UT, Knoxville in 1980. Bass successfully advocated with the UT administration for the creation of a human decomposition facility where researchers could methodically conduct forensic research and produce empirical data, which was unprecedented and otherwise nonexistent at the time. The initial site was roughly half an acre and later expanded to two-three acres behind the UT Medical Center. In 1981, Bass formalized an official body donation program, which continues today (Vidoli et al. 2017). After the decomposition period, all decedents are accessioned for curation in the William M. Bass Donated Skeletal Collection, which is now housed within UT's Forensic Anthropology Center (FAC). (Vidoli et al. 2017). Importantly, the creation of a facility like this was a turning point in the formalization of forensic research in providing an avenue to study human decomposition longitudinally.

The establishment of the ARF ultimately inspired the creation of 11 additional human decomposition facilities that now exist worldwide including in the United States, Canada, Australia, and the Netherlands (Table 1). Today, descriptions and mission statements found on the websites of institutions with decomposition facilities identify their primary function as informing research on time since death and further exploring the rate and pattern of human decomposition. Several facilities such as those at Texas State University (FARF), Southern Illinois University (CFAR), Northern Michigan University (FROST), and the Université du Québec à Trois-Rivières (REST[ES]) state they are imperative for documenting regionally specific patterns of decomposition. In addition to decomposition and taphonomic research, these facilities also highlight the significance of their donor program in contributing to ethically curated contemporary osteological collections. Ultimately, these facilities have become known as cornerstones for the training of students through coursework and graduate research in both forensic science and biological anthropology more broadly.

The earliest work to stem from controlled longitudinal observations of human decomposition at a facility comes from graduate student William C. Rodriguez III's master's thesis from the ARF in 1982. The following year, Rodriguez and Bass published the results of this study in the *Journal of Forensic Sciences* (Rodriguez and Bass 1983). In this study, four human cadavers were placed at ARF in different seasons to document the successional pattern of insects and provide defined criteria for determining the PMI (Rodriguez and Bass 1983, 423). This research marks a transitional period when anthropologists, using methods from entomological work, developed the underpinnings for future forensic anthropology research. Importantly, Rodriguez and Bass were influenced by earlier entomological studies involving the decomposition of dog carcasses (Reed 1958) and baby pigs (Payne 1965) and were the first to translate these methods to observations on human subjects.

Reed (1958) observed the ecological communities produced through the decomposition of 43 dog carcasses near Knoxville, Tennessee and sought to correlate arthropod species and quantity observed on the carcasses to four stages of decomposition (fresh, bloated, decay, and dry). His inspirations included early entomological literature containing similar approaches such as Mégnin (1894), Johnston and Villeneuve (1897), and Motter (1898). Reed did not report on the PMI or how his observations related to it in his publication, as his focus was primarily on categorizing arthropod species. Payne's (1965) formative study observed differences in decomposition among fetal pigs in environments with and without insect activity. For each environment, he identified and recorded different stages of decomposition. For pigs not exposed to insects, he created five stages: fresh, bloating and decomposition, flaccidity and dehydration, mummy stage, and desiccation and disintegration. When exposed to insects, six slightly different stages were identified: fresh, bloat, active decay advanced decay, and dry remains (Payne 1965, 595–599).

Neither Reed nor Payne were investigating decomposition for the purposes of further understanding the PMI. Instead, they were concerned with the entomological aspects of their investigations, but their work remains influential on anthropologists' conceptualization of decomposition as highlighted through the persistent use of dividing decomposition into stages. Following Reed's methods, Rodriguez and Bass (1983, 426) divided the decomposition process of humans into four stages (fresh, bloated, decay, and dry). Like Reed, they recorded the duration (in days) of each stage for each of the four individuals, rather than reporting on the actual time elapsed since death in relation to the stages. Rodriguez and Bass were primarily interested in the successional pattern of various insect species and suggested that carrion activity patterns were well suited to provide a better understanding of time since death. This publication laid the foundation for future observations of human decomposition to be divided into stages, which continues to influence the field today.

There was a promise that longitudinal observations of human decomposition would lead to advancements and improvements in the methods available to practitioners to estimate the PMI with greater accuracy following the opening of the ARF, but the literature reflects a nascent field navigating new terrain. For example, Rodriguez and Bass (1985) continued their explorative studies and examined the rate of decomposition among four unembalmed buried bodies to contribute to medicolegal knowledge regarding buried remains of homicide victims. A few years later, Mann et al. (1990) aimed to summarize observations on human decomposition from 150 bodies placed at the ARF from 1980 until 1990. The study highlighted several important and interrelated variables that impact the rate of decomposition, such as temperature, burial depth, carnivore and insect activity, humidity, trauma to the body, and size and weight of the individual. Although Mann et al. (1990) provided information regarding decomposition patterns, they did not report on the rate of decay or categorize their observations by stages of decomposition as seen in Rodriguez and Bass (1983). Rather, their observations included descriptions of changes in skin pigmentation, breast shape, the effects of water, among other single case observations. They concluded that "variability in the decay rate of the human body is the rule" (Mann et al. 1990, 110). Soon after their publication, Vass et al. (1992) outlined a method for PMI estimation using volatile fatty acids from the soil samples of seven donors at the ARF, stating that the PMI is "…one of the most difficult questions addressed" by practitioners (p. 1237), and one that can be reasonably assessed through observation if the anthropologist has "the expertise and experience" (p. 1237). The soil chemistry method outlined by the authors is complex and details needed for repeatability of the method were not provided in the publication.

In addition to the work occurring at the ARF, studies using larger sample sizes from forensic data were rare, with some exceptions. For example, Galloway et al. (1989) provided the first large-scale, retrospective study that examined decomposition characteristics from 189 forensic cases with known PMI information from Pima County, Arizona. Of these cases, nearly half were recovered from outdoor surfaces, one-third from within closed structures, and the remaining individuals were recovered from water or burials. The remains were classified into five decomposition stages: fresh, early decomposition, advanced decomposition, skeletonization, extreme decomposition. The first four stages were similar to those of Rodriguez and Bass (1983), who had adopted them from Reed (1958) however, Galloway et al. (1989) developed and included detailed traits associated with each proposed decomposition stage. Among the traits were characteristics of lividity, marbling, skin slippage, mummification, desiccation, bleaching, exfoliation, cortical breakdown, carnivore activity, and mold on remains or associated clothing. This thorough documentation of additional variables, or "secondary categories," associated with decomposition stages was not found in any previous publication.

This foundational period in decomposition research in the United States focused on the use of human subjects to expand understanding of the many interrelated variables that impact the complex decomposition process. Longitudinal observations of human donors were unprecedented and made possible due to the founding of the ARF. The increased case load of forensic anthropologists assisting with medicolegal death investigations in the United States also contributed to knowledge of the decomposition process across different regions and environments. Throughout these early publications, there is a clear unease with the lack of availability of reliable methods for assessing the PMI. With the exception of Vass et al. (1992), these early studies did not seek to provide an explicit method for estimating the PMI, but rather sought to offer guidance regarding the many interrelated variables that impact the PMI based upon their subjective observations of human decay in varied settings. The creation of the ARF, along with these formative studies (Rodriguez 1982; Rodriguez and Bass 1983; Galloway et al. 1989; Mann et al. 1990; Vass et al. 1992) laid the groundwork for how future research concerning the PMI would proceed in the following three decades.

2 | Three Decades of PMI Research in Forensic Anthropology (1993–2023)

A considerable amount of research regarding PMI methods has been undertaken since these foundational publications, highlighting how PMI estimation increasingly became within the scope of forensic anthropology. To more thoroughly understand the development and current state of PMI research within the field, we performed a systematic review of published English-language and peer-reviewed literature from the last 30 years (1993–2023) following methods for systematic review from Moitas et al. (2023). We used the following search queries through the PubMed database: ("postmortem interval" or "post-mortem interval" or "PMI" or "time since death") and ("anthropology" or "forensic taphonomy") and found that the first citations in this database to include these terms together was in 1993, hence our survey of the last three decades. A second search with the query ("post-mortem submersion interval" or "postmortem submersion interval") was used to identify additional related publications. The literature was evaluated first based on the title and abstract and then on the full text. Each article was assessed independently by each author, and consensus was determined through discussion among the authors. The searches initially identified 446 studies using the search queries above and were subsequently narrowed down through the exclusion process shown in Figure 2. Publications were included if a PMI estimation method was evaluated (i.e., no review articles or case studies), and if the research did not involve assessing the PMI using entomology or botanicals. We chose to focus solely on studies that investigated PMI estimation methods using humans

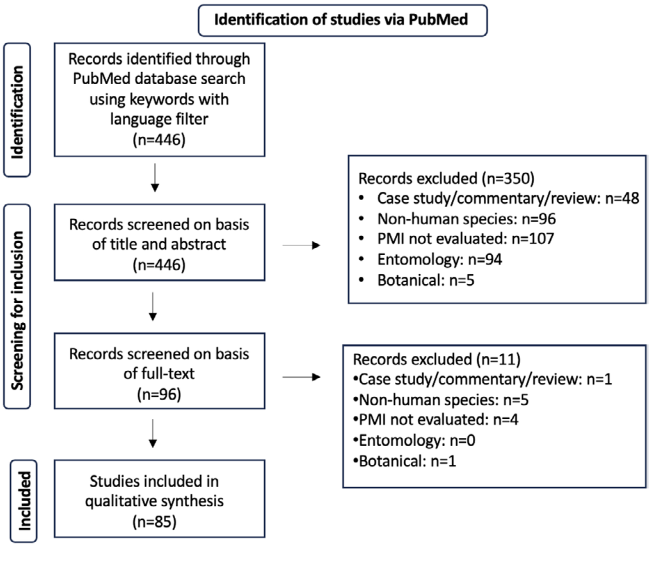

FIGURE 2 | Systematic review flow chart process (format adapted from Moitas et al. 2023).

(i.e., no animal proxies). The results yielded 85 publications for inclusion in this review (Figure 2).

We recognize this overview excludes important taphonomic research including non-English language articles, which reflects our language deficiencies. We also intentionally excluded articles that used entomology or botanicals to estimate the PMI, given the expertise these methods require fall outside of typical forensic anthropology training. Our choice to exclude research using non-humans (e.g., pig proxies) was also intentional, as studies have found that despite physiological and anatomical similarities between pigs and humans, subtle differences can impact the decomposition process in significant ways (Connor et al. 2018; DeBruyn et al. 2020; Mickleburgh et al. 2021). Other animals often used in taphonomic research such as rabbits and rats, for example, are even less morphologically and physiologically similar to humans. Animal models provide a better opportunity for controlled study, as they are adept at holding certain variables constant (i.e., diet, size, health, and cause of death) in ways that are more difficult, if not impossible, to replicate using human donors. While these controlled experiments may provide insight into the mechanism of decomposition, their PMI estimation methodology must ultimately be tested in human models to demonstrate if those assumptions can translate to human decomposition.

Undoubtedly, there are significant challenges to using human subjects for forensic research including the relatively few human decomposition facilities, especially outside of the United States, in addition to the varied and necessary legal parameters across the world that restrict and protect human donors in research. In the absence of human decomposition facilities, some studies utilize forensic casework to investigate the PMI as demonstrated by nearly 40% of the studies reviewed here. We do not seek to undermine the value in the diverse scope of research involving non-human proxies across the world, such as contributing knowledge regarding decomposition trends and patterns in a variety of regions. However, our overarching question was to investigate what PMI estimation methods exist that can adequately inform medicolegal death investigations, and we believe this inquiry is best understood through methods tested on human remains.

An overview of research between 1993 and 2023 provides insight into current PMI estimation methods and research related to forensic anthropology. Given the diversity in research methods, we divided the publications into 12 broad topics based upon how PMI was evaluated including: citrate, fluorescence, histology, microbiome, proteomics/metabolics/lipids, radiometric, scoring systems, spectroscopy, stages of decomposition, taphonomy, universal formula, and other (Table 2). We also divided the literature by case type including research conducted at decomposition facilities, forensic settings, both, or "other," which we used as an umbrella term for studies that utilized remains from cemeteries, archaeological and/or osteology teaching collections, documented skeletal collections, burials, dental extractions from the living, autopsies, and those donated to science (Table 3). Case type was further analyzed by continent, as opposed to specific countries as in Table 1, to highlight broader geographic trends. The types of samples varied across these studies and is organized into categories in Table 4 including examinations of the full body, or samples of human bone, human and animal bone, soft tissue and/or bodily fluids, soil, soft tissue and soil, textiles, human and animal full body, and human full body and soft tissue.

The examination of the literature identified two prevailing ways of evaluating the PMI: through microscopic techniques that require specialized equipment and macroscopic means that can be evaluated through visual assessment. The diverse set of microscopic techniques include quantification of biomarkers using the body, surrounding soil, or textiles. The second commonly employed method evaluates morphological variation in macroscopic characteristics associated with the decomposition process. In the following sections, we summarize the findings from the past 30 years of research involving PMI estimation methods tested on human subjects to identify trends and inform future directions.

2.1 | Quantitative Biomarker and Dating Techniques

A variety of exploratory techniques have been employed in attempts to estimate the PMI outside of traditional means of examining gross visual changes observed on the body. Estimating the PMI from badly decomposed and skeletal remains has and continues to be a challenge within forensic anthropology. Researchers have devised a series of innovative techniques involving microscopic methods in attempts to grapple with this longstanding issue including analyses using citrate, the microbiome, proteomics, radiocarbon analysis, histology, spectroscopy, chemiluminescence, among others. Broadly, these methods can be divided into inquiries that seek to define a precise timeline, as is the case for remains that retain soft tissue or byproducts in the surrounding textiles or soils (Adserias-Garriga, Hernández, et al. 2017; Adserias-Garriga, Quijada, et al. 2017; Ashe et al. 2021; Bonicelli et al. 2022; Damann et al. 2015; Hauther et al. 2015; Schwarcz et al. 2010), and those that apply techniques for sorting dry skeletal remains into those of archaeological or forensic significance (Cappella et al. 2018; Creamer and Buck 2009; Ermida et al. 2017; Hoke et al. 2013; Schrag et al. 2012).

2.1.1 | Citrate

Citrate studies operate under the assumption that citrate levels within bone decrease at predictable intervals postmortem and thus can be used to quantify the time since death for skeletal remains with reasonable accuracy, though studies have yielded disparate results (Brown et al. 2018; Schwarcz et al. 2010; Sulis and Vorobeľová 2024). The citrate method was introduced by Schwarcz and colleagues in 2010, who developed and tested the method on human and animal bones, concluding that precise and accurate PMI results could be gleaned from even small fragments of bone. Kanz et al. (2014) tested the citrate method on the temporal and femoral bones of 20 individuals stored in body bags and wooden coffins with PMIs ranging from 28 to 52 years. They suggested that the femur is better suited to retain notable citrate levels rather than bones with a thinner cortical layer and that citrate depletion may occur more slowly in buried remains. In

TABLE 2 | Results of the systematic literature review ($n = 85$) from 1993 to 2023 sorted by topic, sample type, location of study, case details, author, and publication year.

Topic	Sample type	Location	Case type details	Author
Citrate	Rib, metatarsal samples	USA	Anthropology Research Facility (ARF)	Brown et al. (2018)
Citrate	Temporal and femur bone samples	Austria	Cemeteries	Kanz et al. (2014)
Citrate	Ribs from human and pig	USA and Canada	Anthropology Research Facility (ARF)	Schwarcz et al. (2010)
Fluorescence	Femoral shafts	Italy	Archaeological and contemporary ossuaries	Capasso et al. (2017)
Fluorescence	Cranial vault (part of the parietal bone), femurs/tibiae and lumbar vertebrae	Italy	Forensic	Caudullo et al. (2017)
Fluorescence	Femora	Australia	Western Australia Museum non-indigenous skeletal collection	Creamer and Buck (2009)
Fluorescence	Clavicle	Portugal	Autopsies and remains from forensic anthropology labs	Ermida et al. (2017)
Fluorescence	Human and horse bones-long bones (femora, tibiae, humeri, and one skull)	Europe-Germany, Central and Eastern Europe	Archaeological and modern	Hoke et al. (2013)
Fluorescence	Femoral bone cross sections	Germany	Unclear-potentially historical/burial/archaeological	Sterzik et al. (2018)
Histology	Ribs	France	Donated to science-unspecific	Delannoy et al. (2016)
Microbiome	Oral swabs	USA	Forensic Anthropology Research Facility (FARF)	Adserias-Garriga, Hernández, et al. (2017), Adserias-Garriga, Quijada, et al. (2017)
Microbiome	Hard palates	USA	Forensic Osteology Research Station (FOREST)	Ashe et al. (2021)
Microbiome	Swabs from cadaver-associated gravesoil, skin of the torso, and skin of the head	USA	Southeast Texas Applied Forensic Science Facility (STAFS)	Belk et al. (2018)
Microbiome	Ribs	USA	Anthropology Research Facility (ARF)	Damann et al. (2015)
Microbiome	Caeca of cadavers	USA	Anthropology Research Facility (ARF)	DeBruyn and Hauther (2017)

(Continues)

TABLE 2 | (Continued)

Topic	Sample type	Location	Case type details	Author
Microbiome	Oral swabs	USA	Anthropology Research Facility (ARF)	Adserias-Garriga, Hernández, et al. (2017), Adserias-Garriga, Quijada, et al. (2017)
Microbiome	Proximal large intestine	USA	Anthropology Research Facility (ARF)	Hauther et al. (2015)
Microbiome	Soil samples from decomposition sites	USA	Skin swabs from donors at Southeast Texas Applied Forensic Science Facility (STAFS)	Metcalf et al. (2016)
Other/Electrical current	Full body	USA	Forensic Investigation Research Station (FIRS)	Hansen et al. (2018)
Other/Enamel	Teeth	Japan	Dental extractions from the living	Ishikawa et al. (2022)
Other/microCT	Crania	France	Body donation	Le Garff et al. (2018)
Other/teeth	Teeth	Serbia	Dental fillings from small mass grave	Zelic et al. (2013)
Proteomics/Metabolics/Lipids	Anterior midshaft of tibia	USA	Forensic Anthropology Research Facility (FARF)	Bonicelli et al. (2022)
Proteomics/Metabolics/Lipids	Textiles	Australia	The Australian Facility for Taphonomic Experimental Research (AFTER)	Collins, Stuart, et al. (2023)
Proteomics/Metabolics/Lipids	Textiles	Australia	The Australian Facility for Taphonomic Experimental Research (AFTER)	Collins, Stuart, et al. (2023)
Proteomics/Metabolics/Lipids	Tibia and innominate	USA	Forensic Anthropology Research Facility (FARF) and Anthropology Research Facility (ARF)	Mickleburgh et al. (2021)
Proteomics/Metabolics/Lipids	Muscle tissue	USA	Forensic Anthropology Research Facility (FARF)	Pesko et al. (2020)
Radiometric	Femur and rib	Australia	Australian Facility for Taphonomic Experimental Research (AFTER)	Johnstone-Belford et al. (2022b)
Radiometric	Hair, nails, and puparia	Australia	Australian Facility for Taphonomic Experimental Research (AFTER)	Johnstone-Belford et al. (2022a)
Radiometric	Vertebrae	Switzerland	Autopsies	Schrag et al. (2012)
Radiometric	Vertebrae and ribs	Switzerland	Forensic	Schrag et al. (2014)
Radiometric	Femora	Portugal	Cemeteries	Swift et al. (2001)

(Continues)

TABLE 2 | (Continued)

Topic	Sample type	Location	Case type details	Author
Radiometric, luminol, and histology	Cortical bone, cranial bone, or mandible	Italy	Forensic	Cappella et al. (2018)
Radiometric, luminol, and histology	Femur, vertebral body, occipital, parietal	Brazil	Osteological collection from cemeteries	Ubelaker et al. (2022)
Spectroscopy	Soil samples from decomposition sites	USA	Forensic Anthropology Research Facility (FARF) and Southeast Texas Applied Forensic Science Facility (STAFS)	Aitkenhead-Peterson et al. (2021)
Spectroscopy	Unspecified cortical bone	Italy	Forensic and archaeological	Amadasi et al. (2017)
Spectroscopy	Femoral and humeral bone samples	Portugal	Skeletal collection (21st-century and identified)	Baptista et al. (2022)
Spectroscopy	Left tibia	Spain	Cemeteries	Ortiz-Herrero et al. (2021)
Spectroscopy	Femora samples	Germany	Burials	Ramsthaler et al. (2009)
Spectroscopy	Teeth	Spain	Dental extractions from the living	Rubio et al. (2023)
Spectroscopy	Ribs from each donor	France	Donated to science-unspecific	Delannoy et al. (2018)
Histology and Scoring System	Liver histology and Full body	Sweden	Forensic	Ceciliason et al. (2021)
Microbiome and Scoring System (TBS)	Ribs	USA	Southeast Texas Applied Forensic Science Facility (STAFS)	Deel et al. (2021)
Scoring system (Desiccation)	Full body	Sweden	Forensic	Ceciliason et al. (2023)
Scoring system (PMSI)	Full body	The Netherlands	Forensic	van Daalen et al. (2017)
Scoring system (TADS and PMSI)	Full body	Italy	Forensic	De Donno et al. (2014)
Scoring system (TADS and TBS)	Full body	Sweden	Forensic	Alfsdotter and Petaros (2021)
Scoring System (TADS)	Full body	India	Forensic	Dalal et al. (2023)
Scoring System (TADS)	Full body	Italy	Forensic	Franceschetti et al. (2022)
Scoring System (TADS)	Full body	Scotland	Forensic	Heaton et al. (2010)

(Continues)

TABLE 2 | (Continued)

Topic	Sample type	Location	Case type details	Author
Scoring System (TADS)	Full body	Italy	Forensic	Palazzo et al. (2020)
Scoring System (TADS)	Full body	Italy	Forensic	Palazzo et al. (2021)
Scoring System (TADS)	Full body	The Netherlands	Forensic	Reijnen et al. (2018)
Scoring system (TBDS)	Full body	USA	Forensic Investigation Research Station (FIRS)	Connor et al. (2019)
Scoring System (TBS & TDS)/Microbiome/Other	Full body	The Netherlands	Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology (ARISTA)	Pittner et al. (2020)
Scoring System (TBS)	Full body	Sweden	Forensic	Ceciliason et al. (2018)
Scoring System (TBS)	Full body	Sweden	Forensic	Andersson et al. (2019)
Scoring System (TBS)	Full body	USA	Forensic Investigation Research Station (FIRS); Complex for Forensic Anthropology Research (CFAR); Southeast Texas Applied Forensic Science Facility (STAFS)	Dabbs et al. (2017)
Scoring System (TBS)	Full body	USA	Anthropology Research Facility (ARF)	Dautartas et al. (2018)
Scoring System (TBS)	Full body	USA and UK	Forensic cases and Anthropology Research Facility (ARF)	Giles et al. (2023)
Scoring System (TBS)	Full body	Sri Lanka	Forensic	Gunawardena et al. (2023)
Scoring System (TBS)	Full body	USA	Forensic	Megyesi et al. (2005)
Scoring System (TBS)	Full body	USA	Forensic Anthropology Research Facility (FARF)	Parks (2011)
Scoring System (TBS)	Full body	USA	Complex for Forensic Anthropology Research(CFAR)	Roberts et al. (2017)
Scoring System (TBS)	Full body	USA	Anthropology Research Facility (ARF)	Steadman et al. (2018)
Scoring System (TBS)	Full body	USA	Forensic Anthropology Research Facility (FARF)	Suckling et al. (2016)
Scoring System (TBS)	Full body	Australia	The Australian Facility for Taphonomic Experimental Research (AFTER)	Wilson et al. (2019)

(Continues)

TABLE 2 | (Continued)

Topic	Sample type	Location	Case type details	Author
Scoring system (TDS)	Full body	The Netherlands	Forensic	Gelderman et al. (2018)
Scoring System (TDS)	Full body	USA	Anthropology Research Facility (ARF)	Giles et al. (2020)
Scoring system (Unnamed)	Full body	Canada	Research in Experimental and Social Thanatology (REST[ES])	Ribéreau-Gayon et al. (2023)
Stages of decomposition	Full body	Panama	Cemetery excavation	Ross and Cunningham (2011)
Stages of decomposition	Full body	Canada	Forensic	Komar (1998)
Stages of decomposition	Full body	Spain	Forensic	Prieto et al. (2004)
Stages of decomposition	Full body	USA	Forensic Anthropology Research Facility (FARF)	Bates and Wescott (2016)
Stages of decomposition	Full body	Spain	Forensic	Kõrgesaar et al. (2022)
Stages of decomposition	Full body	Canada	Forensic	Cockle and Bell (2017)
Taphonomy	Full body	USA	Anthropology Research Facility (ARF)	Klippel and Synstelien (2007)
Universal formula	Full body	Canada	Forensic	Cockle and Bell (2015)
Universal formula	Full body	USA	Forensic	Maile et al. (2017)
Universal formula	Full body	USA	Anthropology Research Facility (ARF) (Knoxville, Tennessee)	Vass (2011)
Other/Collagen	Femora	Germany	Cemeteries (Historical and recent); Forensic	Jellinghaus et al. (2019)
Other/DNA	Femora	Germany	Cemeteries	Kaiser et al. (2008)
Other/Postmortem tooth loss	Teeth	USA	Anthropology Research Facility (ARF)	McKeown and Bennett (1995)
Other/Rigor mortis	Full body	Italy	Hospital, recently deceased	De-Giorgio et al. (2020)
Other/Soil Chemistry	Soil samples from decomposition sites	USA	Forensic Anthropology Research Facility (FARF)	Fancher et al. (2017)

TABLE 3 | Summary of mean sample sizes by case type and continent from the literature review (1993–2023). "Other" refers to remains from cemeteries, archaeological and/or osteology teaching collections, documented skeletal collections, burials, dental extractions from the living, autopsies, and those donated to science.

Case type	Continent	Number of studies	Sample size	Mean
Decomposition facility	Australia	3	9	3
Decomposition facility	Europe	1	2	—
Decomposition facility	North America	28	710+	26+
Forensic	Asia	2	66	33
Forensic	Europe	21	2238+	112
Forensic	North America	4	326	82
Forensic and other	Europe	1	38	—
Forensic and decomposition facility	North America	1	+	—
Forensic and decomposition facility	North America and Europe	1	377	—
Other	Australia	1	14	—
Other	Europe	13	750	58

Note: "+" indicates sample size is not clearly reported (as in: Schrag et al. 2012; Schwarcz et al. 2010; Vass 2011).

general, they found that citrate concentration did not work well to estimate the PMI in their sample beyond distinguishing between archaeological and forensic remains. Brown et al. (2018) conducted a validation study based upon the proposed methodology by Schwarcz et al. (2010) and concluded that the citrate method was not reliable. Although citrate levels did deplete over time, it did not occur in a linear manner as suggested by the original authors (Brown et al. 2018). Sulis and Vorobeľová (2024) utilized mostly non-human animal remains and two samples of human bones to test the hypothesis that citrate levels decrease linearly postmortem and could only conclude that fresher bone contained higher traces of citrate than archaeological remains and that the original formula from Schwarcz et al. (2010) was no. These studies suggest that citrate assays may be able to provide general information regarding differentiating between archaeological remains and those of medicolegal forensic significance, but the method's reliability has not been convincingly demonstrated.

2.1.2 | Microbiome

The examination of the human microbiome, specifically postmortem changes in the microbial communities of various aspects of the body (e.g., skin, mouth, or gut) or surrounding soil where the individual has decomposed, has been put forth to provide empirically-driven PMI estimates in medicolegal death investigations (Adserias-Garriga, Hernández, et al. 2017; Adserias-Garriga, Quijada, et al. 2017; Damann et al. 2015; Hauther et al. 2015; Metcalf et al. 2016). These studies rely upon the use of next-generation sequencing to track successional changes in the thanatomicrobiome that correlate with time of death, providing what some have called a "microbial clock" for PMI estimation (Ashe et al. 2021). One of the first studies to explore this idea in human donors was conducted by Hauther et al. (2015), which offered proof of concept that the gut microbiome can potentially assist in PMI estimation. Specifically, they found that three common bacterial populations within the large intestines of six donors declined at predictable rates within 20-days postmortem (Hauther et al. 2015: 1239). Another study into postmortem microbiome shifts in the human caecum of three donors identified taxa that may be related to the PMI but require further study for validation (DeBruyn and Hauther 2017).

Comparisons of microbial communities within human skeletal and surrounding soil samples have also been utilized to test methods involving the microbiome. Damann et al. (2015) examined remains from 27 to 1692 days after placement at the ARF and found that partially skeletal remains retained evidence of the gut microbiome, whereas fully skeletonized remains demonstrated evidence of microbial profiles that more closely resembled those of the surrounding soil (Damann et al. 2015). Adserias-Garriga, Quijada, et al. (2017, 388) examined microbial communities within "cadaver decomposition islands," or the soil surrounding a decomposing individual, for insight into the PMI over a 12-day period. Using three human donors from the ARF, they found that the linear abundance of the bacteria *Firmicutes* was particularly useful in assessing the PMI in the Tennessee summer climate but suggested that a larger sample size would be useful to understand the reliability of their method more concretely. A similar study by Adserias-Garriga, Hernández, et al. (2017) used these same three donors to pioneer an assessment of postmortem bacterial changes in the oral microbiome over the course of 12 days. These authors found patterned similarities in bacteria that correlated to stages of decomposition including fresh, early decomposition, advanced decay, and dry remains. Metcalf et al. (2016) utilized a Random Forests regression model to analyze data from skin and grave soil of four human donors and five mice over a maximum PMI of 143 days. They discovered that microbial succession was similar across different soil types, seasons, and between mice and humans and suggested microbial changes occur in a "clock-like manner," which can provide a precise PMI estimate (Metcalf et al. 2016, 162). Belk et al. (2018) contributed a novel idea to microbiome investigations by providing a meta-analysis

TABLE 4 | Summary of mean sample sizes by sample and case type from the literature review (1993–2023). "Other" refers to remains from cemeteries, archaeological and/or osteology teaching collections, documented skeletal collections, burials, dental extractions from the living, autopsies, and those donated to science.

Sample type	Case type	Number of studies	Sample size	Mean
Human full body	Forensic	20	2205	110
Human full body	Forensic and decomposition facility	2	377+	—
Human full body	Decomposition facility	12	268	22
Total		34	2850+	
Human bone	Forensic	5	189+	38
Human bone	Forensic and other	1	38	—
Human bone	Decomposition facility	6	170	28
Human bone	Other	12	507	42
Total		24	853+	
Human and animal bone	Decomposition facility	1	+	—
Human and animal bone	Other	2	257	129
Total		3	257+	
Soft tissue/fluid	Decomposition facility	7	58	8
Soil	Decomposition facility	2	127	64
Soil and soft tissue	Decomposition facility	1	5	—
Textiles	Decomposition facility	2	8	4
Human and animal full body	Decomposition facility	2	90	45
Human full body and soft tissue	Forensic	1	236	—

Note: "+" indicates sample size is not clearly reported (as in: Schrag et al. 2012; Schwarcz et al. 2010; Vass 2011).

of possibilities for future machine learning models to analyze microbiome data and generate PMI predictions. More recent studies involving the oral microbiome noted similar variability in the bacterial taxa present across human donors at Western Carolina's Forensic Osteology Research Station (FOREST) facility over a 28-day period but did not find a relationship between a particular bacteria type and PMI length (Ashe et al. 2021).

2.1.3 | Proteomics, Metabolics, and Lipids

In addition to exploratory techniques involving the human thanatomicrobiome, research involving bone proteomics, or the extraction of bone protein profiles for use in determining the age of skeletal remains, has gained increased interest within forensic PMI research after demonstrating promise in studies involving paleontological and archaeological remains (Mickleburgh et al. 2021). One such study utilized the iliac crests and anterior midshaft tibiae of four human donors at the Forensic Anthropology Center at Texas State (FACTS) to compare samples from the time of placement to samples collected after skeletonization, which occurred at a maximum of 872 days. They report quantifiable differences in the amount and type of proteins present by comparing the fresh and skeletonized remains, which may be informative for future studies. They caution that additional research is needed to document inter-individual and inter-skeletal element variation in order to advance the method.

In another proof-of-concept study, Bonicelli et al. (2022) presented "forensOMICS," which utilized proteomics as in the previous study but also incorporated metabolomics and lipidomics. The authors suggested that despite their small sample of tibial sections from four individuals, the combination of multiple metabolic markers (i.e., proteins, lipids, and metabolites) to provide more accurate PMI estimates, could be informative, particularly in the later stages of decomposition. Like most literature described in this section, the researchers called for additional experiments using increased sample sizes across different environments to better understand the implications of these methods for estimating the PMI.

Lipid analysis has also been employed in forensic research towards PMI estimations. A study from the Australian Facility for Taphonomic Experimental Research (AFTER) analyzed lipids absorbed into clothing worn by two donated bodies across a decomposition timeline of 0–105 days to determine if lipids are a reasonable biomarker of decomposition and to compare differences across seasons using accumulated degree days (ADD) (Collins , Maestrini, et al. 2023). The authors developed a method of lipid extraction and analysis using gas chromatography and mass spectrometry and found a correlation between 30 different lipid types and early, middle, and late-stage decomposition. A follow-up study to this research expanded their original two-person sample size by adding four pigs as human proxies (Collins, Maestrini, et al. 2023). This work investigated how ADD impacted lipid presence, with a particular focus on which lipids were best correlated to PMI estimations. Their preliminary findings yielded positive results that lipids present in textiles could relate to ADD (Collins, Maestrini, et al. 2023).

2.1.4 | Radiometric Dating

Radiocarbon dating, or carbon-14 (^{14}C) level analysis, operates under the assumption that the ^{14}C isotope, absorbed by the body from the atmosphere during life, ceases postmortem with a gradual decay rate, which can then be measured via quantifiable amounts found in bone tissue. The time depth for carbon-14 can be used to differentiate archaeological skeletal remains from those of medicolegal significance. Until recently, the method has not generally been useful for shorter timeframes that can narrow the PMI range. Atmospheric ^{14}C levels significantly increased after nuclear testing in the 1950s and 1960s, which has contributed to noticeably heightened levels of ^{14}C in humans since 1950. This spike and decline in carbon-14 levels is referred to as the bomb curve, which has led to a technique based on radiocarbon dating called bomb pulse dating that may allow for a more fine-grained determination of the PMI (Johnstone-Belford et al. 2022a; Ubelaker et al. 2022).

Bomb pulse dating has been demonstrated to potentially provide further details regarding the time since death after traditional radiocarbon analysis has confirmed medicolegal significance. Johnstone-Belford et al. (2022b) demonstrated the importance of skeletal element and bone type (i.e., cortical vs. trabecular) in these analyses. They sampled both trabecular and cortical bone from the ribs and femora of 18 willed individuals and found that rib bones contained significantly lower levels of ^{14}C than the femora and that trabecular bone from both skeletal elements had a higher turnover rate than cortical samples. The authors argued that ribs may be of better use in determining forensic relevance and estimating year of death and that bone tissue turnover time should be considered in future radiocarbon analyses (Johnstone-Belford et al. 2022b). Ubelaker et al. (2022) also found radiocarbon analysis may be useful in providing information about the year of death and provided additional findings about the variation in different skeletal elements and across age ranges in the 17 individuals and four bone elements they examined. Johnstone-Belford et al. (2022a) found promising results in determining the year of death for individuals who died between 2016 and 2018 by examining ^{14}C levels in nails, hair, and pupae from 17 human donors, although several environmental factors may have impacted their results and additional research is needed. Capella and colleagues (2018) found that in the 20 anthropological cases they examined, radiocarbon analysis provided the most accurate results for distinguishing bones of forensic significance from archaeological remains, but two quicker, more affordable methods, luminol testing and histological analysis, may provide useful screening tools with further research.

2.1.5 | Fluorescence

Various forms of luminescent analyses have also been used to evaluate the PMI, including chemiluminescence and fluorescence. Chemiluminescence involves a chemical reaction that produces a glow in the presence of luminol, often to detect blood stains. The same concept has also been applied to assess the PMI using hemoglobin present in bone, with advantages including affordability and interpretability using macroscopic means for broad PMI ranges (Ermida et al. 2017). After examining individuals from four time periods (1st–3rd century, 1878–1902, 1965–1972, 1995–2003), Ramsthaler et al. (2009) cautioned that chemiluminescence was not sufficient on its own but could serve as a starting point due to its affordability and simplicity. A study by Creamer and Buck (2009) examined 14 samples of human femora and humeri to test the applicability of the luminol technique to differentiate between remains that are > 100 years or ≤ 100 years. The authors found a correlation between reduced hemoglobin content in skeletal remains and an increased PMI. They suggested that the luminol technique is fairly consistent across skeletal elements and may be a promising method for distinguishing between medicolegal cases and those of archaeological origin (Creamer and Buck 2009).

Hoke et al. (2013) examined bone fluorescence to determine the reliability of the method for estimating time since death for remains less than 60 years old and suggested that it should never be the only criterion in ruling out sample age, as they found it falsely classified half of their forensic samples (Hoke et al. 2013). Another experiment found fluorescence to be of use in determining forensic versus archaeological (> 50 years PMI) significance (Sterzik et al. 2018). Ermida et al. (2017) tested 50 skeletal elements using chemiluminescence to determine if they could differentiate between cases that remained of criminal significance in Portugal (i.e., > 15 years) and those outside that timeframe and argued that luminol testing alone is not sufficient. They suggested future work consider taphonomic variables as well as different sample preparation procedures (Ermida et al. 2017, 1152). Caudullo et al. (2017) found that the results of luminol testing differed across skeletal element type, the integrity of the bone, and due to varying taphonomic variables thus demonstrating the method's limitation in forensic analyses of the PMI. A later study by Ermida et al. (2024) examined how taphonomic factors, including weather variables (i.e., temperature and humidity) and soil (i.e., soil type and pH), impacted the PMI estimation when using luminol techniques to determine the influence of body deposition. They buried 30 clavicles with known PMIs in vases under various conditions and found that sandier soil seemed to yield more intense chemiluminescent reactions, as did the indoor cases (Ermida et al. 2024). Burned remains, likely due to the disruption of the bone's organic matter, had no chemiluminescent reaction in their experiment. These findings emphasize the importance of considering the deposition location of the body when applying luminol techniques.

2.1.6 | Spectroscopy

Spectroscopy is a non-destructive method used to analyze a breadth of forensically significant material, including fingerprints, bodily fluids, and drugs, among others. It has also become a potential dating method for human skeletal remains, particularly for differentiating between modern and ancient contexts (Ramsthaler et al. 2009; Hoke et al. 2013). One such study used Raman spectrometry to examine the remains of 52 exhumed individuals interred in contemporary niche tombs in Spain with a PMI from 15 to 87 years (Ortiz-Herrero et al. 2021). They used applied chemometrics to analyze the data and found a correlation with spectral change and the PMI. Delannoy et al. (2016) approached the question of PMI through a micro- and macro-study on bone diagenesis using Raman spectroscopy and bone mass. They studied six ribs from three donors to compare bone mass across varied environments to understand how moisture and temperature affect bone mass over 90 days and suggested that both variables should be considered and integrated into future work. Another study developed a method to estimate the PMI of surface-discovered skeletal remains by using data from cadaver decomposition island soil and UV–Vis–NIR (near infrared) spectroscopy (Aitkenhead-Peterson et al. 2021). The model underestimated the PMI in all subjects potentially due to soil type, burial depth, body size, and scavenger activity. Baptista et al. (2022) also employed infrared spectroscopy to identify a corollary between changes to the inorganic component of bone and time since death using human femora and humeri samples from 80 individuals with a PMI from 19 to 25 years and suggested that crystallinity and carbonate ratio indices are so far the most reliable for assessing the PMI, but additional research is needed.

Amadasi et al. (2017) conducted a study to compare the practical applicability and reliability of five existing chemico-physical methodologies for PMI estimation with samples from the 1st-century BCE and samples from 2013 using analyses of spectrometry, X-ray, and luminol testing. Specifically, they endeavored to understand if any of these methods were comparable to the more preferred, albeit expensive "gold standard" technique using radiocarbon dating (Amadasi et al. 2017, 220). Although each method yielded possibilities for determining if the remains were historical or of medicolegal significance, the authors purport that additional testing with larger sample sizes is necessary to validate the methods.

2.1.7 | Other

The literature also contains several one-off studies into the PMI that did not fit into the previously identified categories. In one experimental study, Kaiser et al. (2008) applied DNA analysis to PMI determination using human femoral samples from exhumations of individuals who died between 1 and > 200 years ago. Ultimately, they concluded that additional research is needed to understand the complex relationship between DNA degradation and time since death. Hansen et al. (2018) experimental research explored the relationship between bioelectrical impedance analysis and PMI using four human cadavers with a PMI of 10–32 days, with the hope that future studies could develop equations for PMI estimations. Jellinghaus et al. (2019) tested a method involving collagen ratios in human bone samples and found differences between males and females who were buried for a total of 21–48 years and across different environmental conditions but suggested that this technique is not yet suitable for offering definitive PMI assessments within medicolegal investigations. Le Garff et al. (2018) investigated changes in the microstructure of human bone using microtomography capable of capturing 3D images of the internal architecture of bone. They sampled ten sections of a singular human cranial vault and found trabecular changes occurred within the first few weeks postmortem but note that follow-up research utilizing additional skeletal elements must be conducted before microtomography can be considered a useful tool for estimating the PMI.

2.1.8 | Summary

These innovative techniques seek to address the longstanding issue of estimating the PMI from remains in advanced states of decomposition, including fully skeletonized remains.

Many of these studies sought to find an affordable, user-friendly technique to confront the issue of the PMI (i.e., spectroscopic and chemiluminescence), while several required advanced interdisciplinary knowledge and specialized equipment (i.e., microbiome, proteomes, DNA). Despite the allure of rapid and accurate PMI estimation results, especially for skeletonized remains, much of the research seems to highlight how nascent and limited existing techniques remain.

2.2 | Macromorphological Techniques: Stages of Decomposition and Scoring Systems

Providing an estimate of the PMI using macroscopic observations of the decomposition process has been frequently utilized as it provides low-cost, quick methods that are easily used in forensic casework. An examination of the past 30 years of research highlights three common approaches for associating macroscopic observations with PMI estimations including stages of decomposition, the "universal formula," and decomposition scoring systems. Many of the macroscopic techniques use accumulated degree days (ADD) as a proxy measure for the significance of temperature on the rate of decomposition. ADD are generally calculated as the mean daily temperature summed over days to arrive at a specific calendar date associated with the amount of thermal energy that has passed based upon the observed changes on the body.

2.2.1 | Stages of Decomposition

The original method for categorizing macroscopic observations into stages was established by Mégnin (1894) and this method continues to provide an organizing principle of dividing the decomposition process. As mentioned previously, stages initially provided a way of documenting differences among insect species and colonization throughout the decomposition process (Johnston and Villeneuve 1897; Mégnin 1894; Motter 1898; Payne 1965; Reed 1958), while later anthropological studies adopted these methods (Rodriguez 1982; Rodriguez and Bass 1983). Shortly thereafter, forensic literature focused solely on observations of the body without entomological variables (Galloway et al. 1989).

Of the research reviewed since 1993 involving human subjects, six articles have used stages of decomposition to examine the PMI. Bates and Wescott (2016) used ADD and four stages of decomposition (placement, early, advanced, mummification) to categorize variation between autopsied and non-autopsied donors at the Texas State Forensic Anthropology Research Facility (FARF) and found no significant difference between the two groups in the ADD observed to reach each decomposition stage. The authors do not report on the corresponding PMI associated with the ADD values. A second study devised an eight-stage system (early and late autolysis, early, middle, and late putrefaction, and early, middle, and late skeletonization) for classifying decomposition in 121 forensic cases from outdoor surface contexts across Canada (Cockle and Bell 2017). In their study, 96 cases were recovered within one year after death and 25 were recovered after one year postmortem, although the exact distribution of the dates is unclear. These authors examined the association between the stage of decomposition at the time of discovery and ADD calculated from the date of death or the last time the individual was seen alive. They found that neither the PMI nor ADD were significantly correlated with the observed stages of decomposition (Cockle and Bell 2017). A third study used forensic cases from Spain to examine the relationship between the PMI and four decomposition stages (chromatic, bloat, colliquation, and skeletonization) in addition to considering the interplay of anthropometric and environmental variables (Kõrgesaar et al. 2022). Using a machine learning algorithm, they could reliably determine if the PMI fell into one of three groups: 1–2 days, 3–10 days, and > 10 days. Komar (1998) reported on 20 forensic cases from Canada that were recovered in advanced stages of decomposition with a PMI ranging from < 2 to 42 months. The advanced stages of decomposition were divided into four stages. The author noted differences in decomposition patterns by location of discovery, presence of clothing, and scavengers. Prieto et al. (2004) examined phases of decomposition in 29 forensic cases from Spain with a PMI of 0–75 months using a six-phase system. They reported variation across different regions of Spain, across deposition types (i.e., open air, closed structure, buried, or in water), and by scavengers (Prieto et al. 2004).

Bone weathering stages are another method used to provide rough estimates of the PMI. A method for correlating bone weathering to a PMI was provided by Ross and Cunningham (2011), who devised a five-stage system using buried remains from a tropical environment based on Behrensmeyer's (1975) study of mammal bones in Kenya. Each weathering stage described by Ross and Cunningham (2011) corresponded to broad PMI intervals from less than ten years to more than 30 years.

2.2.2 | The "Universal Formula"

A second approach for using macroscopic observations of decomposition to determine the PMI was introduced by Vass (2011) as the "universal formula." He proposed a method that used a single value to represent the percentage of soft tissue decomposition observed, along with temperature and humidity, and two constant values within the formula to derive an estimate of the PMI. According to the author, this approach for quantifying the percentage of soft tissue decomposition alleviates the lack of clear boundaries between the traditional decomposition stages (Vass 2011, 36). The method is meant to provide an immediate PMI estimate while other lab-based and time-consuming analyses occur and is based on "a large database from which visual and empirical data were collected," (Vass 2011, 35). There is no clear information about the size of the database, or the specific types of data used to develop the formulae. Two subsequent studies tested the formulae with mixed results (Cockle and Bell 2015; Maile et al. 2017). Cockle and Bell (2015) reported poor accuracy of PMI estimates in 42 surface and 22 buried cases from Canada with a PMI of 2–395 days, while Maile et al. (2017) found promising results in nine cases from Nebraska with a PMI from one to 17 days. However, these authors found relatively poor accuracy with ten cases from Hawaii with a PMI of 3–16 days. Maile et al. (2017) argued for a more regionally specific formula along with clearer and more consistent definitions for determining the decomposition value proposed by Vass (2011).

2.2.3 | Decomposition Scoring Systems: Total Body Score (TBS) and Total Decomposition Score (TDS)

The third commonly used macroscopic approach in PMI estimations involves attempts to quantify the stages of decomposition using scoring systems that produce a single value for the decomposition state, which is widely employed across forensic anthropology. The most popular method was put forth by Megyesi et al. (2005), but less known is a book chapter by Rhine and Dawson (1998), both of which assigned a point value system to the stages of decomposition proposed by Galloway et al. (1989). The method separately scores the state of decomposition in the head, torso, and limbs and sums these numbers for a Total Body Score (TBS), which ranges from 3 to 35 (Megyesi et al. 2005). TBS is then used in conjunction with ADD to account for how the progression of time and temperature influence the decomposition process. While the characteristics defined by Galloway et al. (1989) were not intended to be sequential, the TBS method assumes a sequential change representing accumulated decomposition. Megyesi et al. (2005) scored total body decomposition in 68 retrospective forensic cases with a PMI of 2–200 days from various environmental contexts across the United States and used these cases to develop and internally validate a predictive formula. This approach of quantifying the visual changes of decomposition and translating them into an easily applied formula associated with temperature provided future researchers with a new way of thinking about PMI estimation.

The method developed by Megyesi et al. (2005) has been the subject of several follow-up studies that answered the call from the original authors to test the method on geographically diverse decomposition contexts. Parks (2011) observed a single donor at the FARF and found support for the TBS method over the first ten weeks of decomposition. Another study by Suckling et al. (2016) calculated the TBS on ten donors from the FARF for 0–2500 ADD, however the exact PMI range is unclear. They reported that the method did not work well on their sample and suggested that regionally specific methods may be needed. Ceciliason et al. (2018) examined 140 indoor forensic cases with a PMI of 0.5–217 days and reported poor precision with wide confidence intervals using the TBS method. Wilson et al. (2019) used time-lapse imaging of one human donor at the Australian Facility for Taphonomic Experimental Research (AFTER) in Australia to determine if the TBS method could be improved with this technology. Images were taken every 30 min for six months, and these authors found that the TBS model worked well to estimate the PMI. In another decomposition facility study, Giles et al. (2020) examined 26 donors at the ARF from one to seven days postmortem and found that TBS was a poor predictor of PMI. Gunawardena et al. (2023) observed 13 skeletons in Sri Lanka with a PMI of 15–62 days to test the TBS method and reported no correlation between ADD and TBS.

Others have examined experimental aspects of TBS evaluations, including research by Roberts et al. (2017) using donors at the Complex for Forensic Anthropology Research (CFAR) in Southern Illinois and found that body mass was not an important variable to consider in TBS. In an interobserver error study, Dabbs et al. (2017) suggested that TBS could be reliably scored across observers using digital images of decomposition states. Dautartas et al. (2018) and Steadman et al. (2018) tested the TBS method using rabbits, pigs, and humans, and suggested that results from animal proxies are not translatable to human donors due to differences in decomposition patterns across the species.

Follow-up studies to this method include several authors who have examined the TBS equation (Megyesi et al. 2005) and demonstrated calculation errors in the original TBS formula, which impacted resulting calculations (Moffatt et al. 2016; Smith et al. 2023). Specifically, the authors found that Megyesi et al. (2005) has a rounding error in the published formula, which had a significant impact on the ADD calculation. They also demonstrated that the error calculation was incorrect in the original work and did not account for the log transformation in the formula. For example, in the original publication, a TBS of 22 has an associated ADD of 600°C ± 0°C–1376.1°C, while with the corrected formula, the same TBS has an associated ADD of 363°C ± 143°C–923°C (Smith et al. 2023).

2.2.4 | Modified and Additional Decomposition Scoring Systems

Other researchers have created new scoring systems following the model outlined by Megyesi et al. (2005). For example, Gelderman et al. (2018) created a Total Decomposition Score (TDS) using a sample of 91 forensic cases from the Netherlands with a PMI of 0–60 days. Their method is similar to TBS, with some modifications to the definitions and scoring range (values between 3 and 18, as opposed to the original 3–35 range). Gelderman et al. (2018) reported an R^2 of 0.67 for indoor cases ($n = 79$) and 0.80 for outdoor cases ($n = 12$) based on TDS and ADD. Both reported models are only internally validated, presumably due to the small sample sizes. They found that the formulae are not accurate in cases with a PMI of more than ten days and note that "…caution is advised when drawing conclusions based on the formulas developed in this research, especially when the PMI is long" (Gelderman et al. 2018, 871). In another study, Giles et al. (2023) examined TDS and ADD along with other variables, such as body position, temperature, and body mass index (BMI) in human donors from the ARF ($n = 8$) from one to 100 days postmortem. They also examined forensic cases from two medical examiners' offices ($n = 331$) using a Bayesian approach and reported that Bayesian Belief Networks (BBN) provided a promising avenue for understanding the impact of various taphonomic variables on the PMI. The model was validated on a holdout sample ($n = 38$) separate from the initial training sample. Their results demonstrated that modeling PMI estimation using BBN may provide a method to improve PMI accuracy by incorporating intrinsic and extrinsic variables into the model.

Modifications of the TBS method also include scoring systems devised for desiccated and mummified remains. Connor et al. (2019) proposed a Total Body Desiccation Score (TBDS) as a regional scoring system in Western Colorado to provide a model for developing regionally specific scoring systems in general. The TBDS was developed on 40 donors over three years and validated on 17 separate donors. The various categories identified correlations with ADD that ranged from 0.55 to 0.78, and no equation or method to determine ADD or PMI from the TBDS was provided. Ceciliason et al. (2023) observed that few methods exist for scoring desiccated and mummified remains and examined 102 indoor forensic cases with a PMI of 3–217 days to create a classification schema. They classified observed changes into leathery desiccated skin, parchment-like desiccation, and soft tissue desiccation (Ceciliason et al. 2023, 1084–1085). Their results highlighted variability in desiccation rates and time since death across their dataset, which prompted them to suggest that more research, particularly longitudinal data, is needed (Ceciliason et al. 2023). Finally, Pittner et al. (2020) examined several different methods of PMI estimation using two donors buried within 1–105 days at the Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology (ARISTA), including TBS, TDS, proteins, microbiome, and insects. None of the methods they examined provided accurate estimations of the PMI, and they suggest additional reference data are needed for buried remains. Ribéreau-Gayon et al. (2023) proposed a macromorphoscopic scoring system based upon observations of six donors from the Research in Experimental and Social Thanatology (REST[ES]) decomposition facility in Canada. The scoring system that they introduced documents characteristics observed on the body using an absence/presence scale (i.e., 0 or 1), which they suggested accounts for the overlapping nature of decomposition patterns.

2.2.5 | Formulae for Estimating the PMI From Bodies Discovered in Water

Another area of PMI research involves scoring decomposition of individuals recovered from water. A study from the United Kingdom by Heaton et al. (2010) provided a method for determining the postmortem submersion interval (PMSI) developed from 187 retrospective forensic cases with a PMI of 0–192 days and introduced a system called the Total Aquatic Decomposition Score (TADS), which generally follows the TBS method of scoring three regions of the body separately and summing the values. The authors provided a prediction formula from their observations with an $r^2 = 0.77$ based on internal validation. De Donno et al. (2014) provided a test of the TADS based on 68 individuals recovered from water from three to 118 days post-submersion. They reported that the scoring system was reproducible among different investigators; however, when comparing individuals recovered floating or submerged in shallow water versus those found amidst a deeply submerged ship in cold water, the results varied. The PMSI of individuals from shallow water were more accurate in comparison to those discovered amid the deeply submerged shipwreck. De Donno et al. (2014) concluded that TADS, like other decomposition scoring systems, provides a false sense of accuracy given the complexity of factors that impact the rate of decomposition.

Van Daalen et al. (2017) proposed a total aquatic decomposition scoring system (TADS) for bodies recovered from the water like previous work but with six possible scores per body region compared to Heaton et al. (2010) eight-stage method. The authors used 30 forensic cases to develop and validate the model and reported a significant correlation between the TADS and PMSI (van Daalen et al. 2017). Reijnen et al. (2018) tested the van Daalen et al. (2017) TADS method using 76 retrospective cases involving individuals recovered from fresh water in the Netherlands and reported a significant correlation between TADS and ADD ($R^2=0.782$) from the overall dataset. Palazzo et al. (2020) compared the two versions of TADS from Heaton et al. (2010) and van Daalen et al. (2017) using 238 retrospective cases of individuals recovered from rivers in Northern Italy between zero and 90 days postmortem and suggested that seasonality was important to consider in predictions of the PMSI. They also reported that both methods have a strong correlation between PMSI and TADS when the samples are divided by season. All cases examined were recovered within 20 days of submersion. The authors noted the advantage of the van Daalen et al. (2017) method is that the TADS can be calculated at the scene and does not require an autopsy to estimate PMSI unlike the method by Heaton et al. (2010).

Palazzo et al. (2021) performed a similar study on 61 bodies recovered from the Adriatic Sea with a PMSI of 0–80 days. They found that both of the TADS methods were correlated with PMSI, and seasonal differences were not significant in this sample. The prediction equations were developed from the study sample and not validated on an independent sample. Alfsdotter and Petaros (2021) examined retrospective cases from Sweden involving surface and water deaths with a PMI of one week to >one year using the TBS and both TADS methods. They reported overall low performance using either method and suggested that a scoring method that is not based on mutually exclusive stages may be beneficial for improving estimations. Franceschetti et al. (2022) provided a retrospective study examining 184 individuals recovered from a submerged vessel in the Mediterranean Sea. The decedents were recovered at six different times, between three to 15 months after the ship's sinking. The authors found a correlation value of 0.83 between PMSI and TADS and provided recommendations for modifications to the Heaton et al. (2010) TADS method based on their sample. Finally, Dalal et al. (2023) also tested the Heaton et al. (2010) TADS method on a sample of 53 individuals recovered from waterways in India. The authors reported a significant correlation between TADS and ADD in their sample and developed a regression formula based on the data from this region of India. Dalal et al. (2023) concluded that 50 of the 53 cases fell within the 95% confidence interval, but the TADS method tended to overpredict ADD in the reported cases.

2.2.6 | Summary

The three predominant ways in which forensic anthropologists macroscopically evaluate decomposition include dividing visual changes observed on the body into stages of decomposition, the "universal formula," and decomposition scoring systems based upon stages. These methodological approaches recognize that a general pattern of decomposition occurs in human cadavers following death. The challenge relates to how to best capture the decomposition variables that impact the process. Studies have investigated decomposition in different deposition sites including on the surface, indoors, buried, and submerged in water, along with inquiries into how postmortem and taphonomic variables (i.e., autopsy, scavengers, etc.) affect the process (Bates and Wescott 2016; Ceciliason et al. 2023; Heaton et al. 2010). Some researchers have created scoring systems in attempts to quantify and sum observed stages into a singular decomposition value meant to provide a PMI estimation (Megyesi et al. 2005; Gelderman et al. 2018). Other studies have taken this approach and modified it for use within different environmental contexts (Connor et al. 2019; Ribéreau-Gayon et al. 2023), while some reported ongoing difficulties due to the lack of uniform definitions, inconsistent methods, and the challenge of reducing decomposition into a single score, or stage (Cockle and Bell 2015; De Donno et al. 2014). The overarching goal of visual macroscopic methods is to provide quick, affordable, user-friendly, and reliable methods for informing the imperative question of time since death within medicolegal death investigations, yet this goal remains unrealized.

3 | Discussion

Forensic anthropologists working within the contexts of humanitarian crises and medicolegal death investigations have increasingly played an integral role in assessing human remains cases to provide crucial details, yet accurate PMI estimations remain an elusive component of applied investigative forensic work despite decades of research. PMI estimations, like the biological profile, can provide information that narrows down the list of potential matches with missing person records. Accurate PMI details also contribute to reconstructing events and timelines that can inform forensic investigations and corroborate alibis during the legal process. In addition to identification, PMI estimations serve the bureaucratic, public health, and government requirements for accurate reporting on death certificates and in legal proceedings (Bird and Maiers 2018; Goad 2020). PMI estimation is also included in the international guidelines for death investigation procedures such as those found within the Minnesota Protocol on the Investigation of Potentially Unlawful Deaths published by the United Nations Human Rights Commission (2017). These guidelines provide a list of minimum requirements for investigations to make all reasonable steps to determine time of death among a list of other critical elements, such as identifying the victim(s), determining cause and manner of death, and preserving evidence (United Nations Human Rights Commission 2017, 7).

In addition to informing the legal process, accurate assessments of the biological profile and the PMI serve a fundamental humanitarian need. A correct and timely identification of an unknown individual restores their identity and can provide families and friends of the decedent with information surrounding their death as well as facilitate necessary proceedings in mourning and subsequent funerary process. The impact of identification on a community functions beyond the bureaucratic level to alleviate "ambiguous loss," or the complex grief that often characterizes the experiences of those in search of a missing friend or relative (Reineke 2019, 160). Ambiguous loss is especially ubiquitous for those impacted by sociopolitical conflicts that have and continue to punctuate the globe (Gaggioli 2018; Reineke 2019).

On a local scale, the extent of the issue and the necessity for forensic anthropological expertise is highlighted among the active unidentified cases in America's National Missing and Unidentified Persons System (NamUs). A NamUs search reports that 11,282 of 15,064 unidentified individuals in the system are facially unrecognizable (NamUs, October 2024). These unidentified individuals, such as those that are severely injured or in advanced states of decomposition, could benefit from further anthropological analysis using retrospective casefiles to generate aspects of the biological profile and a PMI estimate, which could potentially contribute to advancing a case or a positive identification (Kimmerle 2014; Craig 2016; Goad 2020). While current methods for estimating the PMI are limited in addressing this need, thoughtful, collaborative, and sustained research may yet see that this goal is attained. Given the gravity of the work that forensic anthropologists are involved with, it is paramount that forensic researchers in the academic sphere be cognizant and responsive to the methodological needs within the applied context.

A review of three decades of PMI estimation method literature involving human subjects demonstrates an impressive advancement in innovative technology, but it is less clear if the application of this research extends beyond the academy and into practiced forensic anthropology. The heterogeneity of the studies reviewed points to an increased interest in novel conceptualizations for PMI research, however, the disparateness and inconclusive results suggest it is time for critical reflection if the unified goal, as most literature suggests, is to improve PMI estimation techniques for forensic cases. It is no surprise that many practitioners avoid PMI estimation altogether, especially within medicolegal contexts, given the complexity of decomposition and the plethora of experimental estimation methods. Why, then, does human decomposition research continue as it has, and why does there remain an absence of reliable, data-driven PMI estimation methods after decades of research seeking to address this question? This inquiry also stands in light of nearly half a century of decomposition facilities intended to advance knowledge regarding PMI estimation for criminal justice purposes.

Human decomposition facilities are undoubtedly at the center of the expanding corpus of taphonomic decomposition research—human or otherwise—witnessed across the globe today. These facilities are well-positioned to provide rich insights into the decomposition process while also maintaining consistency in methodology given their active donor programs involving access to full-body scientific donations and capacity for longitudinal observations of individuals with known demographic information including exact time since death. We have gained unprecedented insight into a variety of taphonomic factors including damage patterns caused by local scavengers so as to not conflate postmortem taphonomic changes with perimortem injury (Klippel and Synstelien 2007; Reeves 2009), and the impact of specific environments on the decomposition process (Connor et al. 2019; Ribéreau-Gayon et al. 2023). We are also gaining awareness into the potential effects of various unexpected intrinsic factors on the decomposition process such as recreational and therapeutic drugs, including those used in cancer treatment (Steadman et al. 2023).

In addition to this ongoing research, decomposition research facilities are an essential resource for training the future of the discipline, as they are uniquely equipped to educate forensic anthropology students and provide them with opportunities to observe the decomposition process where the PMI is known and for applied research relevant to medicolegal death investigations including taphonomy and forensic archaeological recovery. Decomposition facilities are also incredible resources for training the breadth of personnel (e.g., law enforcement, coroners, and pathologists) involved in the recovery of the deceased and subsequent investigatory process. This is particularly useful for complex forensic cases that necessitate locating and recovering individuals in clandestine burials or those in advanced states of decomposition.

As it pertains to decomposition research, specifically, there are limitations to translating what is learned from the controlled decomposition facility environment directly to medicolegal forensic cases. Practical considerations at decomposition facilities require conditions that cannot reflect the complexities of medicolegal forensic work, such as body storage, deposition site, clothing, and security parameters, among others (Miles et al. 2020). For example, donors are frequently stored in freezers or refrigerators for a period prior to outdoor placement and observation. How this intervening period impacts decomposition rates has not been fully explored and does not reflect real medicolegal casework. Donors at decomposition facilities are generally unclothed, sometimes autopsied, and placed outdoors on the ground surface to decompose, which also does not adequately mirror the breadth of situations found in forensic casework.

Facilities often utilize various forms of cages to place over a donor's body to deter scavenger activity that can damage or disperse the remains, which clearly is not representative of outdoor human decomposition encountered in medicolegal death investigations. In addition to cages directly surrounding the body, human decomposition facilities must be surrounded by tall security fences for the privacy and protection of their human donors. The extent to which this contributes to unnatural scavenging patterns through prohibiting larger vertebrates who cannot penetrate such a barrier, stands to be further explored. Additionally, the impact on the soil caused by repeated decomposing events at outdoor facilities likely affects the rate and pattern of decomposition in ways that stand to be further investigated. For instance, Damann (2010) found that bacterial communities and soil characteristics at the ARF significantly differed from surrounding areas where no decomposition had occurred. The capacity for controlled study at facilities has created unprecedented opportunities for understanding the nuances of the decomposition process, however, there are limitations when translating this knowledge to applied medicolegal contexts.

While many studies in bioarchaeology and paleoanthropology contend with small sample sizes due to the limited availability within the archaeological record, observations of decomposition are more accessible because human decomposition research facilities have active donor programs and the capacity for longitudinal study both in the field and via retrospective photographs. These retrospective photos are often the primary data collection method for documenting the decomposition process and can be used to increase sample sizes in published research. For example, Nau et al. (2023) converted 1.5 million photographs from more than 800 donors at the ARF to a web-based platform titled *Image Cloud Platform for Use in Tagging and Research on Decomposition* (ICPUTRD) to promote PMI research utilizing big data. The FARF at Texas State University reports similar numbers of retrospective photographs from human donors, but no similar database is available (Gocha et al. 2021). Millions of images documenting longitudinal decomposition exist at facilities, however, the mean sample size using full human body observations from them is only 22.3 (Table 3). The large datasets accumulated at facilities should be curated in a form they may be utilized, as exemplified by ICPUTRD (Nau et al. 2023, 2024a, 2024b), so that the field may begin to inform decomposition questions using large, documented samples.

To illustrate the importance of sample size, Weisensee et al. (2024) provide the results of a simulation study of a machine learning model for estimating the PMI to examine the importance of sample size in decomposition research. The results demonstrated that with their large dataset, sample sizes of less than 500 subjects were unreliable, while error predictions plateau around 2000 observations (Weisensee et al. 2024). A second issue with small sample sizes is that validation of the results is limited. When data are not validated on separate training samples, the results will tend to overestimate the model performance due to artificially overfitting results and are therefore not generalizable (Collins et al. 2015). For example, the often-cited TBS formula (Megyesi et al. 2005), reports an $r^2 = 0.8$ but is based on internal validation, which is an overestimation of the model's performance. Large, representative samples are necessary to create PMI estimation methods that accurately capture the variation found in the decomposition process.

Additional issues with the existing trajectory of PMI research include oversimplified methodology and conceptualization that are not well designed to represent the variation found in the decomposition process. First, stages of decomposition and scoring systems imply that decay can be ordered sequentially along a temporal continuum with no overlapping characteristics. This framework does not reflect the nuanced reality that decomposition variables can occur differently based upon myriad factors including intrinsic individual characteristics, trauma, clothing, vertebrate activity, and the environment, among others. A second difficulty is that within the scoring system framework, each body region is scored using a single value that encompasses multiple characteristics, and an individual body may not always exhibit all the variables within a singular scored category. Some researchers have noted it is unusual for all or even most characteristics in a scoring category to be observed, thus the score is ultimately a subjective “best fit” (Heaton et al. 2010, 303). The final issue is that scoring systems record decomposition scores for three regions of the body (head, trunk, limbs) and then sum the scores into a single value. For instance, in the TBS method (Megyesi et al. 2005), there are 33 possibilities for scores (3–35) used in the prediction equation, but there are 1560 different possible combinations, and this nuance is lost in the summation.

Perhaps the most troubling component of the much cited, often repeated TBS formula (Megyesi et al. 2005) is an enduring calculation error from the original formula nearly 20 years after its publication as noted earlier (Moffatt et al. 2016; Smith et al. 2023). The variation introduced by the formula error makes it difficult to assess the accuracy of the TBS in later publications. The original formula continues to be reprinted in forensic anthropology textbooks (Christensen et al. 2019, 167), although at least two publications have underscored the error (Moffatt et al. 2016; Smith et al. 2023). The persistence of unreliable PMI estimation techniques despite their unreliability is puzzling and suggests that established PMI methods should not continue to be used in the absence of a critical evaluation of methodological shortcomings.

An examination of the issues latent in modifying an existing unreliable method seems to have gone relatively unexplored. In addition to the widely used TBS method, the literature reviewed here reveal at least five different decomposition scoring methods (Table 2), some of which are intended to be applied in a very specific environment (e.g., deserts, indoors, and water) (Connor et al. 2019; Heaton et al. 2010; Ribéreau-Gayon et al. 2023). In addition to widely varying definitions of decomposition variables among the literature, it is unclear how many different regionally specific scoring systems would be necessary to provide PMI estimations across the breadth of Earth's environmental contexts and how worthwhile it would be given consistently poor results. The concept of improving PMI estimation through the development of additional regional scoring formulae (Wescott, 2018a) continues to endure despite serious methodological challenges and a dearth of literature that contends with an increasingly unpredictable global climate change that would render these formulae obsolete (Strack and Smith 2023).

Decomposition stages have been a cornerstone of forensic anthropology for 130 years, while scoring systems have endured as a standard for nearly two decades. The scoring system approach for conceptualizing the decomposition process has yet to offer consistently reliable PMI estimates, despite continued regional modifications and testing. Perhaps this reckoning can invite the discipline to rethink data collection instruments and methodology used in macroscopic decomposition observations and relegate stage descriptions to simple teaching tools for conceptualizing the process, rather than an empirical solution for providing precise PMI estimates capable of application within medicolegal settings.

4 | Looking Forward

Forensic anthropologists remain interested in questions pertaining to human decomposition and PMI estimation, as seen through an expanding body of research spanning more than half a century. Further inquiries into the topic stand to provide important insights going forward, particularly if the discipline is prepared to critically reflect for the sake of necessary improvements. Forensic researchers must be aware of developing methods that can be adopted to positively impact real humanitarian issues. This includes devising methods that are accessible and useful to everyday practitioners assisting in forensic casework. We believe that improved research design that incorporates the principles of Open Science, larger and more representative samples, and innovative technology will play a significant role in improving PMI estimation methods that can be helpful within medicolegal and humanitarian settings. In light of this focus, there are several ideas that may encourage the field to move towards this collective goal.

Open and transparent data sharing is a critical component for improving outcomes for PMI research. Open Science has been proposed as a model to alleviate concerns about the inability to reproduce published findings across scientific disciplines. Although definitions of Open Science are plentiful, the consensus is a focus on three components: data sharing and open-access publishing, critical evaluation of statistical power, and ensuring thorough documentation to allow for the replication of results (Allen and Mehler 2019). In PMI studies, data are often geographically, temporally, and contextually limited, therefore data collaboration is necessary to advance methodologies and create generalizable results. While many studies that examine the PMI do not have sufficient sample sizes to make useful inferences about the decomposition process, large datasets have been accumulated at decomposition facilities, therefore we must determine how to harness the data to increase statistical power. Moreover, the field generally suffers from inadequate methodologies to ensure replication of results and too often results are only reported in conference abstracts and graduate theses where it is difficult to fully evaluate the research. Therefore, careful consideration of both methodology and dissemination are necessary for advancing research. As Mulligan et al. (2022) outline, data sharing in biological anthropology is increasingly common as federal funding agencies and academic journals frequently require data generated to be made available in supporting materials or a data repository. Data sharing should be embraced as part of sustained efforts to fulfill the ultimate goal of devising PMI methodology capable of informing medicolegal death investigations.

Integrating novel approaches such as geospatial data can also serve to advance the field. Geospatial technology can be leveraged to capture historic weather and environmental data that can be integrated into predictive models (Weisensee et al. 2024; Karydi et al. 2024). Most researchers examining PMI methods have identified that variability in temperature, humidity, soil composition, soil pH, vegetation, and environmental contexts are significant factors in the decomposition process. Advances in geospatial technology now allow for these variables to be adopted into decomposition modeling with data available from around the world. Geospatial datasets are continually updated to allow for regionally specific understandings of decomposition across a breadth of environmental contexts without the researcher having to determine which specific PMI formula is most appropriate for a particular geographic location. The incorporation of these databases into PMI models makes them particularly adept at maintaining relevance in the future as the climate continues to change. In addition, novel approaches and innovations that could provide insights into the precise PMI of skeletal remains would be particularly useful as they are currently absent in the field.

Finally, forensic research that incorporates data science techniques including artificial intelligence, machine learning, and Bayesian approaches has demonstrated promising potential for improving PMI estimates (Johnson et al. 2016; Metcalf et al. 2017; Andersson et al. 2019; Nau et al. 2023, 2024a, 2024b; Weisensee et al. 2024). Data science tools such as machine learning can be leveraged to harness large datasets capable of interpreting patterns of decomposition in order to derive reliable PMI estimates with associated prediction intervals, while Bayesian approaches can use expert knowledge to provide an interpretable model for how patterns of decomposition occur in relation to the variety of intrinsic and extrinsic variables known to impact the process.

Research on human decomposition has an extensive history and endures as a prolific area of scientific inquiry. Ongoing research demonstrates an increasing number of interdisciplinary approaches dedicated to devising methods for estimating the PMI in novel and innovative ways. Given the significance of the question of time since death within medicolegal investigations and the clear interest of the scientific community, we are hopeful that, through collective efforts, reliable PMI estimation techniques useful for forensic casework will emerge in the coming years.

Data Availability Statement

The data that supports the findings of this study are available in the article itself.

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